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81 How Euro-BioImaging can support your research with access to the best imaging tools

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82 Optimizing FIB-based sample preparation for quantitative in-situ biasing studies of semiconductors using 4D-STEM

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83 Inversion of the Internal Electric Field in GaN/AlN Heterostructures Studied by Off-Axis Electron Holography

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84 Multi-modal correlative microscopy: simultaneous and colocalised Raman & SEM imaging

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85 Near-Ideal Direct-Electron Focused-Probe 4D-STEM Data for Open-Source Phase Reconstructions

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86 A Secondary Electron Hyperspectral Imaging characterisation of mechanochemically functionalised carbon black materials

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87 Temperature-driven in-situ TEM cation exchange at the solid state: a combined experimental and computational study

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88 Advanced electron microscopy study of Cu coated Boron Nitride sheets

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89 Second harmonic generation microscopy of femtosecond microstructured crystals

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90 Latest developments in accurate and high-throughput correlative cryo-FIB milling for cryo-ET lamella production

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91 A Novel Tool for Combined AFM, SEM, and Electrical Probing of Nanostructures

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92 TEM Investigations on the Impact of Hydrogen on Phase Transformations in Aluminum Alloys

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93 Contribution of Probiotics to the Effect of Platelet-Rich Plasma in Diabetic Rat Skin Wound Healing

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94 Asbestos Analytics In Relation To Changes Of The European Directive

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95 Quantum Wavefront Shaping with a 48-element Programmable Phase Plate for Electrons

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96 Characterisation of Skyrmion Spin Textures in CoB/CoFeB Multilayers

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97 Exploring the limits of 3D ED for accurate structure analysis at the nanometer scale

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98 Multiparametric investigation of bacterial surface structure with correlative atomic force microscopy

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99 Silicide precipitation in aged quasi-α Ti alloys

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100 Enhanced imaging for serial Cryo-FIB-SEM microscopy of biological samples with fluorescence navigation

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101 High-throughput laboratory-based scattering X-ray Tensor Tomography

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102 Achieving atomic precision 3D reconstructions through Bayesian genetic optimisation

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103 End-to-end learning of atomic resolution phase-contrast volumes from 4D-STEM measurements

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104 Mapping Orthorhombic Domains with Geometrical Phase Analysis

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105 Localized, anodic aluminum corrosion phenomena studied with electrochemical liquid phase electron microscopy

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106 In-situ SEM Correlated Nanoindentation and Electrical Nanoprobing

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107 In-situ Extreme Mechanics – Recent Innovations and Prospects

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108 MIPAR Spotlight: Shedding Light on Next-Generation Detection for Microscopists

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109 Enhancing quantifiability of S/TEM-based composition mappings through correlative techniques

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110 Confined single-molecule imaging by low-dose electron microscopy

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111 Exploring novel joining technique of Gas Actuated Bonding by utilizing In-situ Environmental-TEM

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112 3D Investigation of Lath Martensite in a Low-Carbon Stainless Steel Using PFIB-EBSD Tomography

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113 Determining alloy concentration by analyzing dynamic diffraction at strained semiconductor interfaces

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114 Electronic properties of W' twin domain walls in ferroelastic BiVO4

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115 Electron Videography and Machine Learning of Soft Matter

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116 High-resolution STEM Image Acquisition Method for Tilted Specimen Using a New Type of Aberration Corrector

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117 Simultaneous Acquisition of 4D and EELS Data by Newly Developed Pixelated STEM Detector

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118 Development of planar micro optics for ultrafast in-situ measurements in the TEM

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119 Unveiling Metal-Insulator Transitions in (V1-xCrx)2O3 through in situ Monochromatized STEM/EELS

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120 Depth Dependence of Electron Channeling Contrast Imaging in Gallium Nitride

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121 Multidimensional Characterisation and Multivariate Analysis of Cu3P Nanocages for Catalysis

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122 Universal Laser Engine:
A microscope illumination that combines FLIM and homogenized powerful wide-field lasers

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123 Quantitative chemical analysis by STEM-EDS and machine learning: Are AgAu alloyed at the nanoscale?

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124 Visualizing alloying in Au-Pd core-shell nanoparticles using in situ gas-phase transmission electron microscopy

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125 Cryogenic scanning electron microscopy of hereditary hemolytic anemias

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126 Physics-based synthetic data model for automated segmentation in catalysis microscopy

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127 Tri-layer graphene as membrane and electrode for liquid-phase electron microscopy studies of CO2 electroreduction nanocatalysts

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128 Unraveling the True 3D Structure of Colloidal Assemblies by Liquid Cell Electron Tomography

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129 Exploring solid conversion products in lithium-sulfur batteries through cryo-TEM and machine-learning-supported small angle neutron scattering

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130 A new Ion Microscope for high-resolution imaging and SIMS nano-analytics

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131 Understanding Twinning and Hierarchical Structure of Synthetic Guanine with 4D-Scanning Transmission Electron Microscopy

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132 Developing alloys presenting nanograins and spinodal decomposition: structural and compositional characterization

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133 Insights into the degradation of nanocatalysts under fuel cell conditions by 3D identical location STEM

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134 Structure analysis on the nm scale: The amorphous LiNbO3 coatings for solid state batteries

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135 Secondary envelopment of the human cytomegalovirus is a fast process, utilizing the endocytic compartment

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136 An accessible Secondary Electron Hyperspectral Imaging approach to draw meaningful insights from scanning electron microscopy

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137 High-throughput tomography correlated with light and electron microscopy for multi-scale imaging of human kidney tissue

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138 In Situ Study of Self-Organized Memristive Switching in Neuromorphic Nanoparticle Networks using Complementary SEM Methods

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139 Suppression of Stacking Faults for Stable Cesium-Formamidinium Perovskite Absorbers

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140 Using secondary electron electron beam induced current for characterization of nanoparticle morphologies

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141 Development and characterization of N2O-plasma oxide layers for high-temperature passivating contacts solar cells

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142 Characterization of aluminium diffusion in high-temperature passivating contacts by in situ scanning transmission electron microscopy

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143 Study of skyrmions in ferromagnetic metallic superlattices using in situ Lorentz magnetic methods
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144 Three-dimensional fivefold misfit in multiply twinned particles at atomic level
Assistant Professor Jihan Zhou[1], Ms. Zhen Sun[1], Mr Yao Zhang[1], Mr Zezhou Li[1], Mr Zhiheng Xie[1], Mr. Yiheng Dai[1], Dr Colin Ophus[2]
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147 Deformation mapping in Lorentz transmission electron microscopy images of magnetic skyrmion lattices
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Wenzheng Cao[1], Prof. Anjam Khursheed[1]
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151 Approaching picosecond temporal resolution in off-axis electron holography
Dr. Tolga Wagner[1], Hüseyin Çelik[2], Simon Gaebel[3], Dirk Berger[4], Christian M. Günther[4], Ines Häusler[1], Sebastian Siewert[2], Michael Lehmann[2], Christoph T. Koch[1]
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152 Synthetic data generation and Mask-RCNN for Transmission Electron Microscope Image Segmentation
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154 Oxidation states of IrOx characterised by EELS and ED-PDF
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156 Opening the third dimension to your SEM with integrated fs-laser
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161 MicroReactor for battery materials synthesis in SEM
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162 Liquid Phase Transmission Electron Microscopy
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163 EELS at Extreme Energy Losses; complementary Information to X-ray Absorption Spectroscopy (XAS) in a TEM

Dr Sorin Lazar[1], Mrs. Maria Meledina[1], Mrs. Claudia S. Schnohr[2], Mr. Thomas Hoeche[3], Mr. Peter Tiemeijer[1], Mr. Paolo Longo[1], Mr. Bert Freitag[1]
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164 Structure Prediction using Data Derived Potentials and EELS

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165 Assessing the sensitivity of 4D-STEM measurements for electric field mapping at the sub-micrometer scale

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166 In situ TEM/EELS and spatially resolved XAS/XRF analysis of CuO electrocatalyst for CO2 reduction

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167 Epitaxially-assisted Sn Crystal Phase Selectivity on Hybrid Superconductor-Semiconductor Quantum Nanowires

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168 Structure of the first isolated polinton-like virus and its host

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169 Correlative analytical scanning electron microscopy reveals periprosthetic tissue response to nanoparticles and wear debris

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170 Structural and metabolic dynamics of plant cells in context of heat acclimation

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171 Open top dual-view light-sheet microscope for live imaging of large multicellular systems

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172 Quantitative Analysis of Magnetic Spin Textures in Fe3-xCoxGeTe2 Compositions using 4D-STEM

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173 Mean inner potential variation with strain in III-nitrides studied by off-axis electron holography

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175 Nano-chemical Imaging and Spectroscopy at the Single-molecule Level

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176 The Environmental Impact of Large Scientific Infrastructure

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177 Phase detection limits in electron holography with automatic stabilisation and direct electron detection

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178 Combining SIMS elemental mapping with FIB-based imaging for multimodal analysis of biological specimen

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179 Multimodal and multidimensional EELS and EDX for investigations of catalysts at the nanoscale

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180 Development of a pixelated STEM-in-SEM detector for microstructural features characterization

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181 Measuring the Interpretability of High-Resolution Transmission Electron Microscopy Images
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182 A new EELS spectrometer, integrated with the microscope's optics
Dr. Peter Tiemeijer[1], Mr. Terry Dennemans[1], Mr. Sander Henstra[1], Mr. Dileep Krishnan[1], Mr. Sorin Lazar[1], Mr. Paolo Longo[1], Mrs. Maria Meledina[1], Mr. Sjaak Thomassen[1], Mr. Wouter Verhoeven[1]
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Ria Mitchell[1], Mr Andy Holwell[1], Dr Richard Taylor[1], Dr Eddy Hill[1]
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184 Multi-scale characterization of vitamin B2 (riboflavin) supplements using X-ray Microscopy, SEM, and automated phase analyses
Ria Mitchell[1], Dr Darragh Murnane[2], Mr Andy Holwell[1]
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185 New paradigm for ultra-low-dose high-resolution imaging through a dedicated event-driven analytical ptychography methodology
Dr. Hoelen L. Lalandec-Robert[1,2], Prof. Dr. Jo Verbeeck[1,2]
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Catherine Venien-Bryan[1], Dr Carlos Fernandes[1], Dr Dania Zuniga[1], Mr Andreas Zoumpoulakis[1], Mr Rafael Veloso[1], Dr Renaud Wagner[2]
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187 Combining in-situ synchrotron and electron microscopy techniques to study NiFe catalysts for CO2 methanation
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188 Correlative multi-spectroscopic and microscopic analyses for investigation of UV-C QDs bimodal emission
Dr. Adeline Grenier[1], Dr. Jesus Canaz[2], Mrs Névine Rochat[1], Dr. Zineb Saghi[1], Dr. Jean-Luc Rouvière[3], Dr. Edith Bellet-Almaric[2], Mrs. Audrey Jannaud[1], Dr. Anjali Harikumar[2], Dr. Catherine Bougerol[4], Dr. Lorenzo Rigutti[5], Dr. Eva Monroy[2]
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189 Identifying transient defects in exsolution of nanoparticles by semi-quantitative ABF STEM imaging
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190 Investigation of the long-range sources of contrast in momentum-resolved STEM
Dr. Hoelen L. Lalandec-Robert[1,2], Hubert Dulisch[3,4], Prof. Dr. Rafal E. Dunin-Borkowski[5,3], Prof. Dr. Knut Müller-Caspary[6,5], Prof. Dr. Jo Verbeeck[1,2]
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191 Advances in characterization of nanostructured ceramic materials by means of Scanning Electron Diffraction Tomography
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193 Nanoscale arrangement of the healthy dentin and comparison with the dentino-genesis imperfecta-affected dentin
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194 Exploring cardiac innervation by 3D light sheet imaging in horses with atrial fibrillation
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195 Operando structural and chemical imaging of lithium battery interfaces
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196 High Spatio-Temporal Resolution Differential Phase Contrast Imaging via Detector Signal Digitisation
Julie Marie Bekkevold[1,2], Dr. Jonathan J. P. Peters[1,2], Prof. Ryo Ishikawa[3], Prof. Lewys Jones[1,2]
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197 Visualising microstructural dynamics of titanium aluminium nitride coatings under variable-temperature oxidation
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198 Advanced EELS spectroscopy characterization of AlGaN/GaN materials
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199 Surface facet-dependent redox dynamics in vanadium-oxide-based catalysts

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200 TEM Methodologies for Analyzing Thin Film Polymer Structure and Polymorphism Elucidation

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201 Three-dimensional classification of dislocations from single projections

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202 Microstructural evolution of zeolitic nanocrystals for CO2 capture by Environmental in-situ TEM

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203 Unsupervised Deep Learning approach for image registration in Correlative Microscopy for the localization of Nanoparticles

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204 Distortions correction in HR-(S)TEM and low resolution TEM images: absolute size, strain and polarization measurements

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205 Correlative TEM and APT studies of metallic Mg specimens prepared and analyzed under controlled environments

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206 Determination of 3D strain fields from dynamical diffraction effects

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207 Paleopalynology of Holocene birch hybridization in Iceland

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208 Imaging MEMS motion at nano scale by time-resolved scanning electron microscopy

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209 Fine structure tuning for strongly correlated functionalities in high entropy oxides

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210 A crystallographic aspect of Li metal anodes: Understanding the functionality of lithium-ion all-solid-state batteries

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211 Dose fractionation and alternative scanning strategies for beam damage mitigation in event-driven 4D-STEM

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212 Electron microscopy characterization of king scallop (Pecten maximus) shells from low-voltage SEM to 3D-EBSD reconstruction

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213 Visualizing single-atom promotion of ultra-deep hydrodesulfurization catalysts (Pt-Co-Mo-S)

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214 Strain mapping using high-resolution electron backscatter diffraction technique: The influence of sample preparation

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216 Developing a high-temperature solid state electrochemical lab in the TEM

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217 In situ observation of dislocation evolution in cerium oxides nanocubes in an environmental TEM

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218 Deformation mechanisms of inorganic fullerenes used as lubrication additives: an in situ TEM nanocompression study

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219 Thermo Scientific Smart EPU Software: Towards autonomous screening

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220 Scanning transmission electron microscopy of quantum centers in diamond

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221 Unsupervised learning assisted secondary electron hyperspectral imaging for high-throughput cheminformatics analysis of materials

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222 In-situ observation of rheotaxy: 2D materials‘ growth on liquid substrates

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223 SARS-CoV-2 and HCV infection and antiviral treatment monitored by multimodal imaging

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224 Effect of the Applied Chemical Potential on Strong Metal-Support Interaction in Ni-TiO2 Catalysts

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225 In situ STEM investigations of defect induced memristive switching in off-stoichiometric SrTiO3

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226 Unlocking 3D nanoparticle shapes from 2D HRTEM images: a Deep Learning breakthrough

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227 Irbis: Software for 3D reconstruction and temporal analysis of 4D microscopy data

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228 Study on local indium concentration in Ga(1-x)InxN quantum wells using quantitative scanning transmission electron microscopy

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229 Gigabytes to Megabytes – Rapidly analyzing in-situ videos to track lattice spacing changes

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230 New Workflows and Innovations for Maximizing Relevance of In Situ TEM Results for Liquid-Based Applications

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231 Near isotropic, high-resolution multi-beam scanning transmission electron microscopy with iterative milling

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232 FAST-EM array tomography: a workflow for multibeam volume electron microscopy

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233 In situ TEM study of phase and electronic structure transformations in amorphous Ga2O3

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234 RUDDLESDEN-POPPER PLANAR DEFECTS IN METAL HALIDE PEROVSKITES: MECHANISMS OF FORMATION AND CONSEQUENCES FOR PHASE STABILITY

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235 Continuous EELS spectrum imaging of nano-droplet crystallization heterogeneity

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236 Electron Channeling Contrast Imaging (ECCI) of Ion Battery Cathode Materials

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237 Application of reinforcement learning to aid the alignment of an electron microscope

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238 Progress in 3D phase-contrast imaging using 4D-STEM: increased dose-efficiency and depth of field

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239 Cation Vacancies Regulate the Electron Spin Configuration of Cathode Catalytic Additives towards Robust Li-S Batteries

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240 Vacancy-driven electron spin engineering to promote Li-S redox reactions

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241 Information Transfer Improvement by Parallax Correction and Ptychography Reconstruction Applied to Large-Area 4D STEM Experiments

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242 Contrast Optimization Aided by Machine Learning Applied to Virtual 4D-STEM Images

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243 Scanning Transmission Election Microscopy Observations of Twisted Epitaxial Molybdenum Disulfide-Gold-Molybdenum Disulfide Heterostructures
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244 Deep Learning-Assisted Multivariate Analysis for Nanoscale Characterization of Heterogeneous Beam-Sensitive Materials
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245 Investigating All-Inorganic Halide Perovskite Phase Transformations via in-situ 4D STEM Heating Experiments
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246 Depth sensitivity of atomic resolution secondary electron imaging
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247 Unveiling Structural Heterogeneity and Imbalance of Gold Decahedral Nanoparticles using Four-dimensional Scanning Transmission Electron Microscopy
Oliver Lin[1], Dr. Zhiheng Lyu[1], Mr. Hsu-Chih Ni[1], Ms. Yetong Jia[2], Prof. Kejie Zhao[2], Prof. Jian-Min Zuo[1], Prof. Qian Chen[1]
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248 Investigating multiferroic phase change dynamics using in-situ electron counted spectrum imaging with synchronized holder control
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249 Maximizing Speed and Sensitivity: Simultaneous High-throughput EDS-WDS Elemental Mapping in the SEM
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250 Atomic resolution observation of zeolitic framework and captured cations using low-dose OBF STEM technique
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251 Full-field illumination ptychography with a structured electron beam
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252 Linking form and function: hyperspectral and nanostructural characterisation of photonic crystals in butterflies

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253 ORGANOIDS : UNVEILING INSIGHTS WITH VOLUME ELECTRON MICROSCOPY

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254 Effect of the content of intermetallic compounds on creep in tin-based solders

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255 Investigation of electron-beam-induced charging in a dolomite needle using off-axis electron holography

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256 OpenECCI - A Streamlined Open-Source Workflow for Electron Channelling Contrast Imaging of Crystal Defects

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257 Mapping electric fields in real nanodevices by operando electron holography

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258 Detection limits of electric field characterization at a p-n junction by 4D-STEM

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259 Studying interface charge distribution in HfO2 and Al2O3 based nanocapacitors by operando electron holography
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260 Synthesis, Characterization, and Biological Evolution Indole Molecules
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276 Holography with an Extended Reference in Transmission Electron Microscopy

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277 Fast and low dose EELS using compressive sensing

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278 TEM Investigations of Multi-Layer Selective Absorber thin films for concentrated solar plant: structure and composition

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279 Effect of Oxygen-Doping in Ferroelectric Wurtzite-type Al0.73Sc0.27N

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280 A comprehensive (S)TEM analysis of Zn_3P_2 suitability for green energy applications

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281 Characterization of LiNiMnCoO2 batteries by CT, LM, SEM and EDS for second life usability Assessment

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282 Understanding Au cluster growth through electron microscopy

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283 Creep-induced microstructural evolution of the eutectic Mo-Si-Ti alloy by correlative electron microscopy

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284 Investigating Impact-Induced Deformation in Cold-Sprayed Aluminum-Quasicrystals Composite Coatings

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285 Fast and local determination of phases in (V1-xCrx)2O3 Mott materials

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286 3D fruit microstructure characterization using micro-CT imaging and deep learning-based panoptic segmentation

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287 STEM, PED and EELS: A powerful combination for the investigation of cathode-active-materials for batteries

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288 Quantitative comparison of HRTEM and electron ptychography

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289 Multimodal OF2i-Raman – A novel high-throughput, single particle analysis method in liquids

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290 A Real Space Understanding of Short Range Order in Disordered Rocksalt Cathodes

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291 5D-ToF-STIM Hyperspectral Imaging with a keV He+ Focused Ion Beam

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292 Atomic scale evolution of Ru nanoclusters on graphitic carbon nanofibers in NH3 decomposition reaction

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293 Improving Control Signals for Interference Gating

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294 Anisotropic van der Waals Epitaxy and Sliding of CsPbBr3 Nanoplatelets on ReSe2

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295 η-Carbides (Co, Mo, or W) Nanoparticles from Octacyanometalates Precursors-Based Network

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296 Electron Microscopy of Novel Lithium Alloy Anodes for Solid State Batteries

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297 Unveiling the Optoelectronic and Thermal Properties of SnSe Polycrystals via EELS

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298 Quantitative comparison between the diffuse scattering from three-dimensional electron diffraction and single-crystal X-ray diffraction

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299 In situ electron beam irradiation of Ti3C2Tz MXenes. A STEM-EELS study

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300 In situ scanning precession electron diffraction study of chemo-mechanical interactions during Zr oxidation

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301 Sub 3Å cryoEM structure using a standard LaB6 120kV TEM upgraded with direct electron detector

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302 Microstructure characterization of CIGS/GaP/Si tandem solar cells

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303 Phonon dispersion surfaces from the electron microscope compared with calculations including dynamical diffraction

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304 Influential factors affecting the quantification accuracy of magnetic moments with electron magnetic chiral dichroism technique

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305 Structure, Chemistry, and Interface of Dispersoids in Powder Metallurgically Processed Ni based 617 ODS Alloy

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306 Mitigating radiation damage in beam sensitive battery materials by adapting scanning parameters

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307 High-resolution 3D chemical imaging of light elements by time-of-flight mass spectrometry

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308 In-situ electrical characterization of MOSFET transistors using AFM-in-SEM solution

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309 Correlative imaging of the human anterior cruciate ligament by micro-CT and histology

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310 Exploring the 3D architecture of native and stained human intervertebral discs through micro-CT

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311 Developing a multimodal imaging pipeline for molecular biochemical studies with a 3D approach

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312 3D atomic-resolution imaging of nanomaterials based on exit wave reconstruction

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313 Novel combination of integrated FLM with cryo-FIB allows targeted cryo-ET sample preparation from challenging samples
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314 Measuring electric fields with 4D-STEM: Demonstration of pitfalls by the example of GaN and SiGe
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315 Electron single pixel imaging enabled by ultrafast optical modulation of the illuminating wavefunction
Dr. Cameron Duncan[1], Beatrice Ferrari[1], Irene Ostroman[1], Maria Giulia Bravi[1], Sang Tae Park[2], Daniel Masiel[2], Jean Christophe Olaya[3], Paolo Rosi[4], Enzo Rotunno[4], Vincenzo Grillo[4], Giovanni Maria Vanacore[1]
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316 Panta rhei - tuning the radiation chemistry of silver nitrate solutions via flow in LP-STEM
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317 Glovebox coupled TEM – a new method for versatile investigations of air-sensitive samples
Dr. Walid Hetaba[1], Mr. Eugen Stotz[2], Ms. Daniela Ramermann[1], Ms. Elisabeth H. Wolf[1], Mr. Stephan Kujawa[3], Mr. Robert Schlögl[1,2], Mr. Thomas Lunkenbein[2]
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318 Atomic-scale model of the platinum (111)-water interface revealed by angstrom resolution off-axis phase shifting holography
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319 Tracking the host inflammatory response via the MAP kinase pathway
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320 Unraveling the composition of monolayer-thick InGaN/GaN quantum wells: A quantitative analysis via probe-corrected HRSTEM
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321 Nanoparticle formation mechanisms and molecular intermediates revealed by liquid phase EM and reaction pathway analysis
Ms. Jiayue Sun[1], Mr. Birk Fritsch[2], Mr. Andreas Körner[2], Ms. Lucia Morales[2], Mr. Chiwoo Park[3], Ms. Mei Wang[4], Dr. Andreas Hutzler[2], Mr. Taylor Woehl[4]
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322 Retrieving sub-angstrom resolution from low order dynamical diffraction intensities
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323 4D STEM and EELS Acquired Simultaneously with a Fast Pixelated Direct Detector with Center Hole
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324 Towards in-situ 4D-STEM observation of texture evolution in nano-crystalline thin films
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325 4D STEM in SEM with a Fast Pixelated Direct Detector
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326 Identification of inorganic fibres in workplace air by SEM-EDS
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327 Automated feedback correction of the specimen drift at the atomic scale
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328 Quantification of Dynamic Scattering Effects in Molecular Crystals using Large Angle Rocking Beam Electron Diffraction

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329 Imaging Toxoplasma gondii tachyzoite infection: tracking a single protein effector by X-ray fluorescence microscopy

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330 Fully Integrated Pixelated 4D-STEM Detector for Scanning Electron Microscopes

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331 Advanced electron microscopy of trimetallic core-shell Pt/Au_xCu_{1-x} nanoparticles

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332 Growth of Au-seeded GaAs-GaSb nanowires explored in environmental TEM

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333 Investigating the ultrafast phase transition of vanadium dioxide using time-resolved three-dimensional electron diffraction

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334 Effect of preparation and oxygen evolution catalysis on CoNiFe oxide electrocatalysts revealed by STEM-EELS

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335 L-TEM characterization of controlled skyrmion nucleation in synthetic antiferromagnetic multilayers

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336 Quantum phase modulation and retrieval in an ultrafast transmission electron microscope

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337 Hard X-ray nano-imaging of Toxoplasma gondii tachyzoites & merozoites

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338 Physico-chemical and biological characterization of Ag- and Cu-doped ZnO thin films coated with calcium phosphates

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339 Influence of titanium surface modifications on formation of composite calcium phosphates / silver nanoparticles coatings

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340 Low-voltage Secondary Electron Emission Spectromicroscopy using a Scanning Auger Microscope

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341 Impact of Electron Beam Irradiation on Carbon Black Oxidation

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342 Atomically sharp domain walls in antiferromagnetic thin films

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343 STEM-EELS studies of interfaces between perovskite oxide membranes and single-crystal carrier substrates

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344 Breaking the atomic resolution depth of field limit using multi-slice electron ptychography and tomography
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345 Strain analysis comparison in complementary and nanosheet field-effect transistor devices: nanobeam vs Bessel electron diffraction
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346 Off-Axis Electron Holography of In-Situ-Biased Highly-Doped p-AlGaAs/n-GaInP Junctions for Solar Cell Applications
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347 Multimodal and correlative imaging approaches to study early stages of SARS-CoV 2 infection
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348 Impact of physical exercise in bone healing around bio-resorbable implants imaged by synchrotron techniques
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349 Grain evolution during annealing of a semisolid Al-Cu alloy studied with lab-based diffraction contrast tomography
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350 Detection of weak ELNES signals using dose-fractionated spectrum imaging combined with direct detection
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351 Arctis WebUI redefines lamella preparation for cryo-electron tomography workflow
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352 Ptychographic imaging of nanoscale 3D objects with soft x-ray synchrotron and EUV table-top sources
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353 Unveiling Strain Fields and Plastic Relaxation in Narrow-Core GaAs/In(Al,Ga)As Nanowires with High-Resolution Electron Microscopy
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354 Quantification and denoising of atomic-resolution EDX spectrum images using a Multiscale Bayesian Approach
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355 Observation of novel ferroelectric domain configurations in $PbTiO_3$/$DyScO_3$ epitaxial films
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356 Evaluation of diabetes-associated testicular morphology and the effects of MSC secretome as a therapeutic intervention
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357 The effect of mesenchymal stem cell's secretome on hyperglycemia-related complications: focus on reproductive system disorders
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358 Microstructure and Phase Analysis of an Al-Mg-Si Alloy Produced by Laser Power-Bed Fusion
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359 Towards 3D quantitative imaging in FIB-SEM for applications in battery materials
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360 In-situ TEM study of ice nucleation and growth
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361 QCBED Measurements of Vacancy Concentrations, Lattice Contraction, and Bonding Electron Densities Surrounding Aluminium Nanovoids
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362 Transformation of Al2O3 to Cr-rich spinel during spark plasma sintering of alumina dispersed 316L steel
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363 EasyGrid: a new automated cryo-multimodal sample preparation
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364 A cryo workflow combining light, electron and soft x-ray microscopy provides targeting of unlabeled features
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365 Electron-beam-induced surface diffusion of contaminants and growth of carbon contamination
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366 CL-EBSD-TUNA Correlative Multi-microscopy Study of Grain Boundaries in Pseudo-symmetric Cu(In,Ga)S2 Solar Cell Absorber
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367 Blockwise Processing of Hyperspectral Analytical Scanning Transmission Electron Microscopy Data for Enhanced Analysis

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368 Generation of AFM cellular datasets and classification by ML

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370 Chitin in the cuticular capsule of tardigrade cysts

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371 Mapping of interstitial atoms using super-resolution and optimized machine-learning techniques

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372 Reducing user-interaction in the processing of electron energy-loss spectra

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373 Atomic Lensing Model for Atomic Scale Multi-Elemental Quantification in STEM

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374 Relativisitic EELS cross-sections for all elements

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375 Atomic transitions analogue in phased-shaped electron energy-loss spectroscopy

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376 Electrochemistry in LP-EM & Effects Induced by Irradiation of Metal Electrodes

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377 A twist to superlubric sliding in bilayer graphene uncovered by in situ TEM

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378 Combined 3D electron diffraction and nanotexture analysis for the study of planetary materials

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379 Probing the chiral behavior of nano-optical near-fields through angular momentum resolved PINEM

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380 Impact of oxidation state on copper nanoparticle stability in industrial Cu/ZnO/Al2O3 hydrogenation catalyst

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381 Damage reduction in amorphous ice by a non-conventional scan strategy in cryo-STEM

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382 Atomic-scale Defects Critical to the Performance of Perovskite Solar Cells

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383 Field emission properties of a single-crystal diamond needle under ultrafast laser illumination

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384 Electron beam imaging of extremely confined optical modes in topology-optimized dielectric photonic cavities

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385 Unveiling Atomic-Scale Defects and Optical
Properties within Quasi_2D Perovskite single crystals

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386 Modification of topological phenomena at hybrid Bi2Se3/organic interfaces

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387 AI Automation for Transmission Electron Microscope Alignment

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388 Correlating absorption and diffraction contrast tomography on earth and space materials

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389 Effect of electron microscopy sample preparation protocol on the preservation of liposomes in cell culture

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390 Thermal vibrations in inverse dynamical electron scattering

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391 Characterization of Solid Electrolyte-free Si anodes for all-solid-state batteries (ASSBs) using Cryo-STEM

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392 Direct observation of the interplay between stacking polytypes and self-intercalation in epitaxial Nb1+xSe2 films

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393 Atomic Imaging of Lattice and Electron Ordering in Tensile-Strained LaCoO3 Films

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394 Insights into the Structural Dynamics of Cu@Ag Core-Shell Nanoparticles during CO2 Reduction

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395 A Robust Toolkit to Correlate High Dimensional Multimodal Microscopy

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396 Molecular Microscopy by Thermo-Fisher-Scientific: FTIR-Imaging of a Tintoretto-Fresco and Raman-Imaging of the Strain-Distribution in Semiconductors

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397 Assessing the Precision of Local Temperature Measurement by Plasmon Energy in In-Situ Heating Electron Microscopy

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398 Enabling discovery by in-cell structural biology

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399 Low-Dose 4D-STEM Investigations of the Octahedral Network Structure in Formamidinium Lead Bromide Nanocrystals

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400 In-situ beam driven experiments by Electron time-correlation microscopy of amorphous structures

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401 Magnetic behavior of steel studied by in-situ Lorentz microscopy, magnetic force microscopy and micromagnetic simulations

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402 Thapsigargin induces non-apoptotic programmed cell death in RBL-1 cells

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403 Multimodal control of the magneto-structural phase transition in FeRh studied by TEM

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404 Defects and alloy ordering in Pt-Cu nanoparticulate electrocatalysts

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405 Whole-brain 3D quantification of alpha-synuclein spreading and toxicity in a mouse model of Parkinson's disease

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406 NP-SAM: Implementing the Segment Anything Model for Easy Nanoparticle Segmentation and Analysis in Electron Microscopy

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407 Towards advanced polymer membranes for hydrogen technologies through dose-optimised Focused Ion Beams

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408 NBED investigations of coaxial (Al,In,Ga)As nanowires

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409 Surface Polarity Dynamics and Strong Reconstruction in Partially Reduced Nickelate Films

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410 Characterization of structure and mixing in nanoparticle hetero-aggregates using convolutional neural networks: 3D-reconstruction versus 2D-projection

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412 Investigation of metal-to-metal hydride phase transformations in magnesium thin films using STEM techniques

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413 Optimizing Backscattered Electron Detection in SEM: Diode Layout and Collection Efficiency

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414 Empowering STEM in SEM: Integrative Approaches for Enhanced Detection

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415 Seeking peak precision in atomic EELS mapping with counting mode direct electron detection

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416 WhatEELS upgrade: the software tool based in Python for EELS analysis

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417 Use of prior knowlege and physics-guided NMF for improved phase segmentation of EDX datasets.

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418 3D ED for accurate structure analysis of nanoparticles
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419 Unsupervised Quantification of large EELS datasets
Prof. Dr. Jo Verbeeck[1,2], Dr. Daen Jannis[1,2], Dr. Wouter Van den Broek[3], Arno Annys[1,2], Dr. Zezhong Zhang[1,2,4], Prof. Dr. Sandra Van Aert[1,2]
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420 MAX phase-based nanocomposites for LIBs negative electrodes investigated by multi-approach TEM analysis
Irene Ostroman[1], Dr. Cameron Duncan[1], Mrs. Beatrice Ferrari[1], Mrs. Maria Giulia Bravi[1], Dr. Antonio Gentile[2], Dr. Stefano Marchionna[2], Dr. Chiara Ferrara[1], Dr. Taewon Kim[3], Dr. Changhyun Park[3], Dr. Chanhee Lee[3], Prof. Hyun-Wook Lee[3], Prof. Riccardo Ruffo[1], Prof. Giovanni Maria Vanacore[1]
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421 Many-body effects in the electron-atom ionization cross section in Quantum Electrodynamics
Dr. Ioannis Iatrakis[1], Dr. Valerii Brudanin[2]
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422 Determination of Magnetic Symmetries by Electron Diffraction
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423 Optical and acoustic plasmons in the layered material Sr2RuO4
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424 Single-crystal and pentatwinned nanorods reverse the handedness of chiral plasmonic nanocrystals: an electron tomography study
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425 Characterization of Aberration-Corrected Lorentz TEM Applying a Magnetic Field with Objective Lens

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426 Quantification of chirality from electron tomography data

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427 Femtosecond electron beam probe of ultrafast electronics

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428 BACKSCATTERED ELECTRON AND X-RAY IMAGING FOR ARRAY TOMOGRAPHY PROVIDES RAPID SPECIMEN CHARACTERISATION AND ROI TARGETING

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429 Fabrication of 2-dimensional disordered assemblies of gold nanoparticles and investigation of localized surface plasmon resonances

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430 Exploring the infection cycle of Vaccinia virus using 3D EM with the FIB-SEM

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431 The geometry of STEM tomography

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432 4D-STEM/PNBD: Fast and easy powder electron diffraction in SEM

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433 In situ size dynamics and manipulation of nanoparticle interaction under electron beam irradiation
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434 Molecular mechanism of a bacterial Retron
Researcher Arturo Carabias Del Rey[1], PhD Sarah Camara-Wilpert[2], PhD student Mario Rodríguez-Mestre[2], Academic Coordinator Blanca Lopéz-Méndez[1], Associate Professor Ivo A. Hendriks[1], PhD student Ruiliang Zhao[2], Senior Research Consultant Tillmann Pape[1,3], PhD student Anders Fuglsang[1], Undegraduate student Sean Hoi-Ching Luk[1], Professor Michael L. Nielsen[1], Associate Professor Rafael Pinila Redondo[2], Research director Guillermo Montoya[1]
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435 Data Augmentation and Innovative Machine Learning Approaches for Classifying EEL Spectra of Transition Metals Oxides
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436 Three-dimensional imaging to study early bone mineralization
Dr Elena Macías-Sánchez[1], Dr Emeline Raguin[2], Dr Nadezda Tarakina[3], Prof Peter Fratzl[2]
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437 Unraveling the original dissolution mechanism of LiFePO4 when treated for recycling
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438 Scanning electron diffraction reveals the nanoscale ordering of cellulose in a hierarchically structured hybrid material
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439 Picoseconds and nanometers; time and spatially resolved cathodoluminescence spectroscopy to characterise nonradiative defects in semiconductors.
Dr. Gunnar Kusch[1], Dr. Thomas Weatherley[2], Dr. Frank Mehnke[3], Dr. Eamon T. Hughes[4], Dr. Tim Wernicke[3], Dr. Justin Norman[4], Prof. John E. Bowers[4], Prof. Kunal Mukherjee[5], Prof. Michael Kneissl[3], Prof. Nicolas Grandjean[2], Prof. Rachel A. Oliver[1]
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440 STEM-XEDS spectrokinetic analysis of oxygen: a tool to understand redox processes in nanostructured oxide catalysts

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441 Deep orientation estimation of macromolecules in cryo-electron tomography

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442 Direct observation of Ni nanoparticle growth in carbon supported nickel under carbon dioxide hydrogenation atmosphere

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443 Development of simple image processing for in-situ TEM toward live processing

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444 In-situ TEM reduction of a solid oxide cell with NiO/YSZ and NiO/BZCY fuel electrode materials

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445 Assessing Ptychographic Methods for Maximum Low Dose Performance

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446 Electron microscopic investigation of photothermal laser printed ZnO nanoarchitectures

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447 Towards atom-counting from first-moment STEM images

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448 Multi-scale cements and concrete characterization using X-ray Microscopy, automated phase classification, and machine learning

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449 Microstructure and thermal stability of ultrafine-grained CuZn5 processed by HPT

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450 Evaluation of Conventional Adherent Cell Enumeration Methodologies alongside Image-Enhanced Flow Cytometry

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451 Structure and Function of Fructose 6-phosphate aldolase

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452 Surface microstructural evolution during Rolling Contact Fatigue of Rolling Element Bearing steels

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453 Strain mapping of a Σ5(310) grain boundary in Cu bi-crystal using scanning transmission electron microscopy

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454 CEFID: A flexible platform for spectroscopic experiments

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455 Improving Transmission Kikuchi Diffraction workflows

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456 Panta Rhei: A software platform for acquisition and processing of image and spectral data

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457 MÖNCH: A 25um hybrid pixel detector with sub-pixel resolution using deep learning

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458 Nanoscale Chemical Segregation to Twin Interfaces in τ-MnAl-C and Resulting Effects on the Magnetic Properties

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459 Overcoming the aberration-limit of a non-corrected Transmission Electron Microscope with computational ghost imaging

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460 Characterization of the crystal structure of In-Ga-Zn-O materials via Precession Electron Diffraction

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461 Investigating stability of ZIF-8 metal organic framework in operational environments: potential candidate for host-guest chemistry

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462 Denoising of 4D-STEM Dataset using Pix2Pix GAN Algorithm and Artifact Reduction Strategy

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463 Analytical electron tomography, fine structure EELS and nanoscale X-ray CT of nanoporous Copper materials

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464 M-SIS Software: Automatic tilted series acquisition for environmental (gas, liquid and temperature) multi-scale electron tomography
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465 Capturing the diffusion of individual atoms in 3D: heat-induced alloying in Au@Ag nanoparticles
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466 Unveiling the three-dimensional ultrastructure of Poly Lactic Acid (PLA) spherulites by means of electron microscopy
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467 High resolution characterization of Y(Mn,In) Blue Chromophoric Oxides
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468 Twinning in HfO2 nanocrystals
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469 Microscopy techniques show the assembly of nanovesicles around lipid droplets via the tumor protein TPD54
Doctor Sandra Lacas-Gervais[1], Maud Magdeleine[2], Christelle Boscagli[1], Doctor François Orange[1], Doctor Bruno Antonny[2]
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470 Additive effects in phase transformation of calcium phosphate to hydroxyapatite using high-throughput transmission electron microscopy
Miss Johanna Neumann Sørensen[1], Assistant Professor Murat Nulati Yesibolati[1]
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471 In situ TEM thermal study of MBE and CVD GeSn layers: cross-section and plan-view geometries

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472 Illuminating the Microscopic Realm: Application of Resin R221 for CLEM in microbes and plant tissues
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473 4D-STEM and EELS Analysis of Complex C-based Sensor Architectures
Mr Charles Otieno Ogolla[1], Mr Charles Otieno Ogolla[1], Mr Marco Hepp[1], Prof. Benjamin Butz[1], Tristan Zemke
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474 In-situ/ Opernado (S)TEM on Mass Selected Pt Clusters Deposited on CeO2 for CO Oxidation Catalysis
Ajai Raj Lakshmi Nilayam[1], Ramin Shadkam[1], Dr. Carina Maliakkal[1], Mohana Veerraju Kante[1], Prof. Horst Hahn[1], Dr. Di Wang[1], Prof. Christian Kübel[1,2]
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475 Electron microscopy and X-ray techniques correlative in situ studies in microfluidic conditions on hybrid perovskites
Doctor Claire Jolly[1], Doctor Charles Sidhoum[1], Profesor Ovidiu Ersen[1], Doctor Benedikt Lassalle[2]
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476 Atomically Sharp Domain Walls in an Antiferromagnet
Jan Michalička[1], Filip Křížek[2], Sonka Reimers[3], Zdenek Kašpar[2], Ondrej Man[1], Jan Rusz[4], Juan Carlos Idrobo[5], Peter Wadley[3], Tomas Jungwirth[1,3]
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477 Visualizing microbial interactions and CRISPR-Cas interference using FISH applied to environmental archaeal biofilms
Indra Banas[1], Dr Sarah P. Esser[1], Dr. Cristina Moraru[1], Prof. Dr. Alexander J. Probst[1,2,3]
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478 Observing Magnetic Skyrmions in Pt/Co/Cu Multilayers using in-situ Lorentz Transmission Microscopy
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479 Nanoscale T-cell membrane protein imaging across complex topography using accelerated large depth-of-field localisation microscopy

Dr Aleks Ponjavic[1,2], Dr Alex Carr[3], Dr Edward Sanders[3], Dr Mafalda Santos[4], Dr Edward Jenkins[4], Dr James McColl[3], Prof David Klenerman[3], Prof Simon Davis[4], Prof Steven Lee[3]
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480 Active focus stabilisation using astigmatism with universal objective lens compatibility and sub-10 nm precision

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481 Largely improved momentum resolution in STEM-DPC imaging of Si(110) with a segmented detector

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482 Interaction of transduction enhancing peptide nanofibrils with cells and virions assessed by complementary EM techniques

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483 Structure retrieval by parameterised inverse multislice accounting for partial coherence and thermal effects

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484 Temperature-dependence of beam-driven dynamics in graphene-fullerene sandwiches

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485 Viral replication organelles revisited by cryo-electron microscopy

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486 Influence of dynamical diffraction on DPC measurements of 2D materials

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487 Sensing the Invisible: Ultrathin (UT) Membrane Chip for In-Situ Microscopy

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488 Impact of Clostridium botulinum C2 Toxin on the ultrastructure of cells

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489 Nondestructive structural characterization of 2D materials by hybrid pixel direct detector

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490 Elucidating functionalities of N-doped carbonaceous materials by means of in-situ TEM

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491 Accessible STEM-DPC Imaging Using ADF Detector Allowing in-situ Magnetic Reversal Studies

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492 Label-free imaging of virus-cell interactions using 200 Hz ROCS microscopy

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493 The ultrastructural analysis of plant-microbe interfaces

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494 Event-responsive Beam-modulated STEM with Multi-frame and Sparse Scanning

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495 Identification of the phases resulting from the thermal crystallization of Ge-rich GeSbTe alloys using EELS

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496 Multi-scale electron tomography in liquid state and its application to the study of beam-sensitive nanomaterials

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497 In-situ nucleation of silicon particles using environmental transmission electron microscopy

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498 Heating effects in Bi-doped Cu nanowires for spintronics: atomic resolution in-situ insights

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499 Transfer of Nanomaterials for in-situ TEM with electrical Currents

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500 Zernike Phase Plates for aberration-corrected TEM in Material Science

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501 Enhanced time resolution with a room-temperature energy dispersive X-ray PIN photodiode detector

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502 Development and application of In-situ atomic-scale straining&heating&biasing platform for TEM

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503 Sn Alloying Impact on Structural and Electronic Properties of Core-Shell Ge-GeSn Nanowires: A TEM Study

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504 Cryo-ET Structures of Huntingtin-Actin Complexes Reveal that Huntingtin Organizes Actin Cytoskeleton in Neurons

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505 Ultrastructural investigation of human cytomegalovirus tegument protein UL71 and its role in secondary envelopment

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506 Strain mapping and simulation of transistor structures in a 22nm FDSOI technology

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507 Revolutionizing Electron Microscopy Through Intuitive Language-Driven Interfaces: The Emergence of the EM CoPilot

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508 Structural basis of rotavirus spike proteolytic activation

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509 Cryo electron tomography of impregnated mesoporous catalyst supports

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510 Structure-activity relationship of Pt nanoparticles during the CO oxidation reaction

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511 Advanced imaging reveals new lipid droplets dynamics in the malaria parasite Plasmodium falciparum

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512 Atom-counting for heterogeneous nanostructures using multimodal STEM

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513 Microfabricated Sample Carrier for Cryogenic Electron Microscopy

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514 Understanding phase and chemical transitions in Ge-rich GeSbTe based phase change memory: a (S)TEM tribute

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515 Mechanism of WS2 nanotube formation revealed by in-situ/ex-situ imaging and cross-sectional sequences

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516 Data clustering for chemical and structural analysis of nanostructured films

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517 In-situ TEM Investigations of the electrical Breakdown of Nanotubes made of Misfit-Layered Compounds

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518 Optimising a modern high performance FE-SEM for multimodal vEM

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519 TEM and photocurrent response of FE BTO thin films heterostructure on silicon for Neuromorphic applications

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520 Scanning electron diffraction reveals the molecular ordering of polysaccharides at the nanoscale

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521 Two-photon line-scanning structured illumination microscopy (LIL-SIM) for super-resolution imaging in deep tissue

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522 Study of nuclear materials for silicon carbide composite fuel claddings via STEM, EDX and EELS

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523 Functional chemistry of minimally altered organic matter in the meteorite Winchcombe probed by monochromated EELS

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524 Elucidation of Structure-catalytic Activity of Nickel-based Nanomaterial for Electrocatalytic Water Splitting

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525 Assessing the Accuracy of Strain Mapping using 4D-STEM

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526 Cryogenic electron microscopy for native state analysis of soft- and nano-materials

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527 PVA/PEG-based nanofibers with Au nanoparticles for treatment of chronic woundss

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528 Fine-tuning the Size of ZIF-L Nanosheets Through Controlled Synthesis

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529 How to better understand ZrCu Thin Films Metallic Glasses recrystallization: a TEM in situ characterization?
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530 Momentum resolved band gap measurement by high energy resolution electron energy loss spectroscopy
Dr Dileep Krishnan[1], Dr. Ioannis Iatrakkis
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531 Probing the Sensitive Reactions of Battery Cathodes Through (Cryogenic) Atom Probe Tomography
Dr. Mahander Pratap Singh[1], Mr. Eric V Woods, Prof. Baptiste Gault
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532 FOUR MICROSCOPY METHODS UNVEIL LIVER FENESTRATIONS: CORRELATIVE SEM, SIM, STED, AFM TARGETING SINGLE CELL TYPE
Dr Hab. Bartlomiej Zapotoczny[1], dr Karolina Szafranska[2], prof. Stefan Chlopicki[3], prof. Marek Szymonski[4], prof. Balpreet S. Ahluwalia[2], prof. Malgorzata Lekka[1], prof. Peter McCourt[2]
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533 Investigating the formation of surface reconstruction layers in Ni-rich cathode materials using STEM

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534 Breaking the limits of functional Atomic Force Microscopy imaging using Focused Electron Beam Induced Deposition
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535 Novel polymer thin film fabrication for graphitization studied by in situ transmission electron microscopy
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536 Single-cell pharmacology: atomic force microscopy and spectroscopy for multiparametric imaging of drug-induced alterations in vitro
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537 Segregation to Creep-induced Planar Faults in Ni-base Single Crystal Superalloys

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538 Polar textures in multiferroic BiFeO3-based superlattices

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539 Characterization of inorganic food additives and pearlescent pigments in sprays for food decoration by STEM-EDX

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540 Optimizing data acquisition and interpretation in atomically resolved STEM spectrum imaging

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541 Progress in Magnon EELS simulations

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542 Study of Lithiation Dynamics in Cathode Materials by in situ TEM Electrochemical Liquid Technics.

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543 Towards Quantitative Liquid Phase Electrochemistry for Understanding Electrochemical Processes

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544 Correlative cryo-bioimaging to study coronavirus replication organelles

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545 Enhancing tool performance with complex microscopy investigation of additively manufactured M2 steel and composites

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546 Leveraging gas-cell in situ electron microscopy to track atmosphere-dependent reversible transformations in reducible oxides

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547 In-situ STEM-EELS observations on heating TiO2-x nanoparticles for solar and electrocatalytic applications.

Dr Khalil El Hajraoui[1,2], Dr Leonardo Lari[2,3], Mrs Fayzah Talbi[2,3], Prof. Richard Douthwaite[4], Prof Quentin. M Ramasse[1,5], Prof Vlado. K Lazarov[2,3], Dr Demie Kepaptsoglou[1,2]
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548 ACOM characterization of phase transitions during overageing of aluminium alloys using a direct electron detector

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549 Simulations of phonon and magnon EELS/EEGS including dynamical effects and multiple inelastic excitations

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550 Direct Visualization of Temperature Induced Phase Separation of Completely Miscible Au-Pd Alloy by In-Situ TEM

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551 4D-STEM Post measurement machine learning enhanced aberration correction for amorphous and magnetic samples

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552 New insights into optical property characterization of 2D semiconductor materials through time-correlated photon/electron spectroscopies

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553 Laser particle acceleration in an ultrafast scanning electron microscope

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554 Direct Visualization of Temperature Induced Phase Separation of Completely Miscible Au-Pd Alloy by In-Situ TEM
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555 Influence of the loss function on gradient-based iterative ptychographic reconstructions in 4D-STEM
Max Leo Leidl[1,2], Carsten Sachse[2,3], Knut Müller-Caspary[1,2]
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556 The organization of organic/biominerals in the nanostructured 3D photonic crystals in insects' scales
Dr. Yin Chang[1], Dr. Hsiang-Han Tseng[2], Dr. Luca Bertinetti[1], Dr. Michaela Wilsch-Bräuninger[3], Prof. Yael Politi[1]
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557 Multi-Convergence-Angle Ptychography with Simultaneous Strong Contrast and High Resolution
Mr. Wei Mao[1,2], Mr. Weiyang Zhang[2], Dr. Chen Huang[3], Prof. Liqi Zhou[1,2,5], Dr. Judy Kim[3,4], Prof. Si Gao[2,6], Mr. Yu Lei[1], Mr. Xiaopeng Wu[1], Mr. Xudong Pei[2], Prof. Yuefeng Nie[2], Prof. Angus Kirkland[3,4], Dr Peng Wang[1,2]
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558 STEM-EELS unveils atomic stacking in 2D MoSe2 ECM memristors.
Dr Khalil El Hajraoui[1,2], Dr Andrea Capasso[3], Mr Guilherme Araújo[3], Dr Demie Kepaptsoglou[1,2], Prof Quentin. M Ramasse[1,4]
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559 Study of metal powders oxidation by means of Energy Dispersion Spectroscopy (EDS)
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560 Solid state diffusion as the driving force in the selective oxidation of 2-propanol
Dr. Thomas Götsch[1], Daniel Cruz[1], Patrick Zeller[1,2], Anna Rabe[3,4], Malte Behrens[3,4], Robert Schlögl[1,5], Axel Knop-Gericke[1,5], Thomas Lunkenbein[1]

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561 High-resolution investigation of rotavirus VLPs by cryo-EM

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562 Scale-bridging microscopic characterization of complex energy devices enabled by novel cross-sectional preparation routines

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563 Carbonaceous inclusions from the oldest sediments on Earth

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564 Probing Chirality and Topology in Ferrimagnetic Multilayer Systems

Tamer Karaman[1], Daniel Metternich[1,2], Kai Litzius[1], Timo Schmidt[3], Aladin Ullrich[3], Riccardo Battistelli[1,2], Manas Patra[1,2], Manuel Valvidares[4], Daniel Pérez Salinas[4], Jordi Llobet[4], Christopher Klose[5], Michael Schneider[5], Bastian Pfau[5], Andrada-Oana Mandru[6], Reshma Peremadathil Pradeep[6], Konrad Samwer[7], Manfred Albrecht[3], Hans Josef Hug[6], Felix Büttner[1,2]
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565 Multidimensional Electron Ptychography

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566 Simulating electron energy-loss spectroscopy and cathodoluminescence for nanoparticles located on substrates

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567 Towards Atomic Resolution of Cryogenic Ptychography Single-Particle Analysis (Cryo-EPty SPA)
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568 eCHORD crystalline orientation maps: channeling contrast at interfaces
Dr. Hdr Cyril Langlois[1], Dr. Gabriel Denhez L'Hôte[1], M. Thierry Douillard[1], Dr. Matthieu Bugnet[1], Dr. Claire Maurice[2]
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569 Coupling Clustering and Channeling Contrast in the Scanning Electron Microscope
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570 Water condensation on core-shell nanofibers studied using multiscale environmental electron microscopy
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571 Glacios 2 Cryo-TEM and Smart EPU Software streamline Cryo-EM for drug design with higher throughput
Adrian Koh[1], Michael Adams, Lingbo Yu[1], Fanis Grollios[1], Bart van Knippenberg[1], Abhay Kotecha[1], Basil Greber[2], Oliver Raschdorf[1], Julio Ortiz[3], Edward Pryor[4], Jeffrey Lengyel[4]
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572 Use of micron sized silicon particles as anode by capacity controlled cycling
Helen Estefanía Valencia[1,3], Philip Rapp[2], Maximilian Graf[2], Prof. Dr. Hubert A. Gasteiger[2], Prof. Dr. Joachim Mayer[1], Dr. Shibabrata Basak[3], Prof. Dr. Rüdiger-A. Eiche[3]
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573 Analyzing conformational and compositional heterogeneity of macromolecular complexes by cryo Electron Microscopy: Zernike3Deep and HetSIREN
Dr. David Herreros Calero[1], Carlos Óscar Sánchez Sorzano[1], José María Carazo[1]
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574 Multiscale characterization of plaster setting using operando multiscale liquid-phase Electron Microscopies
Dr Alexandre Fantou[1], Anna Wozniak[1], Dr Annie Malchère[1], Prof Karine Masenelli-Varlot[1], Prof Sylvain Meille[1], Dr Lucian Roiban[1], Dr Solène Tadier
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575 Beyond Ribosomes: In Situ Structural Biology of a Challenging Target in C. Reinhardtii
Dr Jessica Heebner[1], Sofie van Dorst[2], Dr. Ron Kelley[1], Dr. Martin Obr[1], Dr. Sagar Khavnekar[1], Dr. Xianjun Zhang[1], Dr. Saikat Chakraborty[1], Dr. Ricardo Righetto[2], Dr. Florent Waltz[2], Dr. Alicia Michael[2], Dr. Wojciech Wietrzynski[2], Dr. Grigory Tagiltsev[3], Dr. John Briggs[3], Dr. Juergen Plitzko[3], Dr. Ben Engel[2], Dr. Abhay Kotecha[1]
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576 Cryogenic large volume 3D and TEM sample preparation with multiple ion species plasma FIB
Dr. Min Wu, Haifeng Gao, Devin Wu
[1]Thermo Fisher Scientific, Eindhoven, The Netherlands
577 Formation of MgSi grain boundary precipitates with core-shell structure in fcc-Al
Dr Ali Ahmadian[1,2,3], Vahid Tavakkolisaiej[2,4], Dr. Torsten Scherer[2], Dr. Andrey Mazilkin[2], Dr. Yulia Ivanisenko[2], Prof. Dr. Christian Kübel[2,3,4]
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578 Controlling heterostructures with atomic precision in III-V nanowires using microheaters in an in-situ TEM
Mr. Christopher Røhl Yskes Andersen[1,2], Mr. Marcus Tornberg[3,4,5], Mr. Sebastian Lehmann[4,5], Mr. Daniel Jacobsson[3,4], Prof. Jonas Johansson[4,5], Prof. Kimberly A. Dick[3,4,5], Prof. Kristian S. Mølhave[1]
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579 acquiCHORD : track the rotating ROI !
Dr. Gabriel Dehnez L'Hôte[1], Dr. Joël Lachambre[1], M. Thierry Douillard[1], Dr. Hdr Cyril Langlois[1]
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580 Novel tools for the Spatio-Temporal Photocontrol of protein-protein interactions
Dr Virginia Puente[1,2,3], Dr. Jan Tonnesen[3], Dr. Matthieu Sainlos[1]
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581 Progress in STEM instrumentation: atomic-resolution SE imaging and meV-level energy resolution EELS
Dr. Ondrej Krivanek[1,2], Dr. Niklas Dellby[1], Dr. Michael Hotz[1], Dr. Joel Martis[1], Dr. Steven Quillin, Dr. Benjamin Plotkin-Swing[1], Dr. Tomas Radlicka[3], Dr. Gerardo Algara-Siller[4], Dr. Tracy Lovejoy[1]
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582 Joint Ptychographic Tomography of Frozen Hydrated Proteins
Georgios Varnavides[1,2], Dr. Yue Yu[3], Berk Küçükoğlu[4], Dr Stephanie Ribet[2], Dr Mary Scott[2,5], Dr Henning Stahlberg[4], Dr Colin Ophus[2]
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583 Iterative Phase Retrieval Methods for Weakly Scattering Signals: Transfer of Information and Efficient Regularization
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584 Electron microscopy study of energy materials growth
Prof. Yue Lin[1]
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585 Nanoendoscopy-AFM: A new technique to explore Focal Adhesions in living cells
Mohammad Shahidul Alam[1], Mr Takehiko Ichikawa[1], Mr Takahiro Fujiwara[2], Mr Clemens Martin Franz[1], Mr Takeshi Fukuma[1]
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586 Advancing Ultrafast Transmission Electron Microscopy with Dielectric Metalenses
Maria Giulia Bravi[1], Beatrice Matilde Ferrari[1], Irene Ostroman[1], Cameron James Duncan[1], Paolo Rosi[2], Payam Habibzadeh Kavkani[2], Lorenzo Viani[2], Enzo Rotunno[2], Giovanni Isella[3], Stefano Calcaterra[3], Sang Tae Park[4], Daniel Masiel[4], Jean-Christophe Olaya[5], Javier García de Abajo[6], Vincenzo Grillo[2], Giovanni Maria Vanacore[1]
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587 PFIB-preparation and STEM-characterization of electrochemically plated lithium at the interface to the solid electrolyte Li6PS5Cl
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588 Image fusion for 3D reconstruction of SEM images
Dr Turkka Salminen[1], Dr Lucio Azzari[1]
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589 Dynamics at phase boundaries of active catalyst studied by multi-scale operando EM
Prof. Dr. Marc Willinger[1]
[1]Department of Chemistry, School of Natural Sciences, TU Munich, Garching bei München, Germany
590 Phenomenology of the dealumination in Faujasite Y zeolitic catayst
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591 Development and applications of backscattered electron and X-ray detector
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592 Uranium reduction by magnetite and mechanism of UO2 formation monitored by low-dose STEM-EELS
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593 Atomic-scale microscopy of different materials by ultrashort THz-driven Atom Probe Tomography

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594 From structural evolution and interfacial dynamics to manipulation of surface plasmon resonances in Bi-Sb-Te systems

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595 Following low and high-temperature electrolysis processes with in-situ and cryo electron microscopy

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596 Real-time undersampling optimization during electron tomography of beam-sensitive samples using golden ratio scanning and RECAST3D

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597 A new approach for direct visualization of unlabeled lipid nanoparticles for intracellular pathway analysis

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598 3D visualization of in situ nanoscale dynamics in transmission electron microscopy via self-supervised deep learning

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599 Structural and mechanical adaptation of brachiopod shells

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600 Enhanced Free-Electron Wavefunction Modulation via Photon-Induced Near-Field Electron Microscopy (PINEM) with Shaped Light Fields

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601 Uncovering microscopic details of shearing mechanisms in the $L1_2$ structure by unambiguous stacking fault analysis

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602 Understanding CuO/Al2O3 Interactions during Thermochemical Redox Reactions: TEM, X-ray Microscopy, and XAS Study

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603 Operating a model Solid Oxide Fuel Cell in the Environmental Transmission Electron Microscope

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604 Prospects of nanofluidic cavities for cryo-EM sample preparation

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605 Creating free-standing nanostructures with plasmonic properties via Focused Electron Beam Induced Deposition

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606 Atomic-resolution STEM analysis of polar states in Sr1-xBaxMnO3 thin films
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607 Multi-Dimensional Data Restoration from Subsampled EBSD Data
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608 Reliable tomographic reconstructions of (sub)-nm gaps in plasmonic gold dimers for correlation to optical properties

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609 Correlative transmission electron microscopy and photoluminescence microscopy revealing enhanced fluorescence in nitrogen vacancy containing nanodiamond
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610 Electron holography of a vortex-type magnetic domain wall in a cobalt nanotube
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611 Towards atomic-resolution electron energy loss spectroscopy in an uncorrected 30kV scanning electron microscope
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612 Simultaneous acquisitions and applications of DPC/OBF STEM, EDS and EELS

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613 Imaging Atomic Processes in Catalysts using a New High-Order Imaged-Corrected Environmental-TEM

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614 Investigation of Ferroelectricity using Advanced Microscopy

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615 In-situ TEM Investigation of Degradation Process in Ni-Rich Cathodes.

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616 Identification of Chemical Segregation and Surface Twinning Structures in Electro-deposited Al Dendrites

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618 ACOM-TEM Investigation of the white etching layer formation in rail track

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619 Defect engineering of core-shell systems based on 2D transition metal dichocalgenides

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620 Electron energy-gain spectroscopy of optical excitations in integrated photonic structures

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621 Rapid and large FOV mapping of 60° grains in epitaxial MX2 with a segmented detector

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622 Stability insights of MnO2 electrocatalysts from identical location and in-situ electrochemical liquid cell electron microscopy

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623 New generation environmental in situ TEM holder for gas cell studies across multiple platforms

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624 Cryo lift-out technique to study host-pathogen interaction on cell monolayer

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625 Revealing atomic structure and composition in ultrahigh energy storage density ferroelectric thin-films

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626 Nanotubes of (SmxY1-x)S-TaS2 based on Quaternary Misfit Layered Compounds (MLCs)

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627 In situ TEM holder for liquid cell research with combined electrochemical and thermal stimuli control

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628 Self-supervised deep learning method for in-cell cryo-electron tomography

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629 Deep image prior for limited-angle electron tomography

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630 Characterisation of red fluorescent protein FLIM properties and comparison with novel StayGold live cell imaging

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631 Preparation of biological samples for cryo-electron microscopy using the HPF "Waffle" method

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632 Elemental segregation at substrate/metal interface to manipulate heterogeneous nucleation

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633 Consistency and reliability of ptychographic deconvolution approaches

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634 Atomic-resolution mapping of phonon modes across Magnéli structures in thermoelectric (Al,Nb)-doped TiO2

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635 Innovative Designs For Enhancing the Functionality of MEMS-Based Phase Plates through Numerical Simulation and Optimisation

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636 Enhanced nanoscale phase characterisation in modern steels using precession electron diffraction and energy filtering

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637 Accessible low-cost, open-source, single-shot phase imaging implemented on an openFrame-based microscope

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638 Beyond Ribosomes: In Situ Structural Biology of Diverse Targets in C. reinhardtii

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639 Quantitative analysis of single-atom support interactions by Deep Learning techniques

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640 Probing photonic resonant modes in InAs semiconductor nanostructures by STEM-EELS

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641 Application of cryogenic in situ biasing (S)TEM holder to study phase transitions in complex oxides

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642 Multiscale study of water condensation on aerosols using in-situ Environmental Scanning and Transmission Electron Microscopies
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643 In- and ex-situ implantation of helium to characterise faults in titanium beryllide
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644 Scale-Bridging Analysis of Hierarchical Mesoporous Transition Metal Foams
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645 Intralayer modification on Ti3C2Tz MXene Multilayer by Tailoring Surface Terminations
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646 Analytical Phase-Shifting Electron Holography using Fresnel-corrected holograms
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647 Effects of electron cascade and lamella preparation on InGaN quantum well recombination dynamics
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648 Magnetic layers reversal in new MRAM devices measured with operando Electron Holography
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649 Methodological development of nanoscopic defects characterization in nuclear materials: contribution of TEM-APT correlative microscopy
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650 Image Restoration from Subsampled STEM Measurements using Deep Learning
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651 Multi Element ELNES Mapping of Compounds

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652 pyEELSMODEL: python library for model-based EELS quantification

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653 Tilted multislice approach for quantitative STEM simulation
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654 Cellular in-situ Assessment of Complex Tissue Environments in 3D
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655 4DSTEM-in-SEM by placing a pixelated detector below the sample
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656 Structural and optoelectronic properties of layered halide perovskites
Dr Giorgio Divitini[1], Dr Andrea Griesi[1], Mr Andrea Cicconardi[1], Dr Sirous Khabbazabkenar[1], Dr Yurii Ivanov[1], Dr Gunnar Kusch[2], Prof Rachel A Oliver[2]
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657 Elucidating the effect of silicon on Fe-Zn phase formation in galvanized steel via advanced TEM
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658 Correlative characterization by X-ray tomography, SEM/FIB and TEM using reference markers: bridging imaging with micro-structuring

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659 Domain structures in ferroelectric epitaxial WO3 thin films
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660 Enabling atomic resolution electron microscopy at elevated temperature and beyond pressures of a few bar

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661 Deep convolutional neural networks for atomic imaging in STEM

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662 Analyzing Lithiation Dynamics in LiFePO4 Cathode : Insights from TEM Experiments and Phase Field Modeling

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663 Characterisation of 2-D chalcogenides utilising Electron Microscopy techniques and Density Functional Theory.

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664 THE INVESTIGATION OF THE PRESENCE OF TELOCYTE CELLS IN THE HUMAN OVARIAN STROMA

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665 Ptychography Optimization for Atomic Analysis of Bending Mode in Bilayer Transition Metal Dichalcogenide Translational Motion

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666 Comparative Analysis of Self-Supervised Learning Techniques for Electron Microscopy Images

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667 EDX-based annotation of biological features in large-scale EM

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668 Improving segmentation of FIB tomography data

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669 Phase formation pathways of Me2+ oxides on sapphire (α-Al2O3) substrates

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670 A new transgenic mouse model for functional tracing of circulation via albumin-tagged fluorescent probes

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671 Influence of low-pressure atmosphere in the pores formed in hexagonal boron nitride under electron irradiation

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672 Unsupervised Machine Learning-based STEM diffraction pattern denoising for enhanced grain visualization in phase change materials

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673 Investigation of Production of Boron Nanotubes by Ultrasonication

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674 On how to tame the beast: Towards a high-throughput plasma FIB pipeline

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675 Imaging the three-dimensional morphology of granular superconductors with energy-filtered TEM tomography

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676 Nanodiamond-based quantum sensing of mechanoregulated metabolic plasticity of cardiac fibroblasts

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677 FIB-SEM and automatic segmentation for investigation of mitochondrial organization in cells of urinary bladder urothelium

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678 Investigating microstructural phenomena in Additive Manufactured metals through high temporal thermal cycles in-situ heating

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679 The effects of solution processing methods on halide perovskite nanostructure

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680 Multimodality and correlative low-voltage electron microscopy: powerful tool for imaging in life and material sciences

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681 Mean inner potential change of latex sphere with temperature measured using off-axis electron holography

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682 Shedding light on the birth of hybrid perovskites by In-Situ TEM and Synchrotron X-ray scattering

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683 Ptychography at finite dose in SrTiO3

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684 Reactivity of High Entropy Nanoalloys under O2 and CO Oxidation reaction studied by in-situ TEM

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685 Attosecond electron microscopy using free-electron homodyne detection

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686 Multimodal Low Voltage Transmission Electron Microscopy Analysis of Iodine Nanoparticles for Stroke Theranostics

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687 Mitigation of beam damage on MoS2 using electrostatic beam blanking in TEM

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688 Deep Learning assisted denoising of in situ liquid STEM-movies of nanoparticle nucleation and growth

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689 In situ insights into the thermal stability of high-entropy nanoalloys

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690 Role of Miro1 adaptor protein in mitochondrial mobility between cancer and stromal cells

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691 In situ insights into the thermal stability of high-entropy nanoalloys

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692 Recent developments and future trends in time-resolved cathodoluminescence: measuring dynamics at the nanoscale

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693 Investigating the chemical oxidative polymerization of 1,8 - dihydroxynaphthalene using a correlative in-situ approach

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694 Correlative TOF-SIMS/SEM for subcellular investigation of microalgae in extreme environment

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695 Multimodal Scanning X-ray Spectromicroscopy of 2D layered Titanium Carbide MXenes

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696 Coulomb-correlated multi-electron states in a transmission electron microscope beam

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697 Operando TEM Reveals Oscillatory Surface and Bulk Dynamics of Nickel Nanoparticles in Ethylene Partial Oxidation

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698 Low dose and in situ 4D-STEM powered by real-time sparse-array analytics on an event-driven detector
Penghan Lu[1], Yucheng Zou[1], Tingting Yang[1], Janghyun Jo[1], Deli Kong[1], Dieter Weber[1], Alexander Clausen[1], Matthew Bryan[2], Dmitry Byelov[3], Giuseppe Spano[3], Thorbjörn Schönbeck[3], Rafal E. Dunin-Borkowski[1]
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699 Unveiling degradation mechanisms in layered Li-rich cathode materials using combined operando neutron diffraction and
4D-STEM

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700 In-Situ insights into Multivalent Metal Energy Storage:
A model system for calcium sulfur batteries
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701 TEM study of neutron radiation damage in tungsten
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702 Electron-photon quantum interaction enables novel ultrafast electron imaging approaches
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703 ePattern: an adaptive 4D-STEM Pattern Registration Algorithm to Optimize ACOM Pattern Matching
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704 In situ ESEM study of supported noble metal nanoparticles dynamics under reaction conditions
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705 In-Situ Charging and Charging Map for Characterization of Electronic Materials
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706 3D Operando Monitoring of lithiation spatial composition in NMC-cathode electrode by X-ray nano-CT & XANES-techniques
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707 Coherent inelastic scattering probed by holographic scanning transmission electron microscopy
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708 Improving electron tomography of mesoporous silica structures by Ga intrusion
Thomas Przybilla[1], Johannes Böhmer[1], Alexander Kichigin[1], Moritz Buwen[1], Alexander Götz[1], Dominik Drobek[1], Jakob Söllner[2], Matthias Thommes[2], Benjamin Apeleo Zubiri[1], Erdmann Spiecker[1]
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709 The significance of the phonon polarization vector in vibrational EELS
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710 Direct imaging of deformation in metallic glasses using precession nanodiffraction mapping
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711 Unveiling moiré-induced topological polar structures in freestanding ferroelectric membranes
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712 Oxygen vacancy imaging in complex oxides by a 4D-STEM optimized virtual detector

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713 Correlative cryo soft x-ray tomography and fluorescent microscopy of biological samples in the laboratory

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714 Understanding the effect of Al:ZnO coating on the structural and chemical stabilities of LiNi1/3Mn1/3Co1/3O2 electrode

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715 Momentum-resolved STEM Tomography of Gold-Silver core-shell Nanoparticles

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716 Phase Stability and Evolution of Defect Structure in (CoCuMgNiZn)-Entropy Stabilized Oxide

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717 The study of spatial relationship between Restrictor complex and RNA-Pol II through Expansion Microscopy

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718 Developing cryo-Volume Electron Microscopy using the JEOL 4700 cryo-FIBSEM to address biological ultrastructural questions

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719 Femtosecond extreme-ultraviolet imaging of magnetic domains during ultrafast demagnetization

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720 Evaluation of SXES on different kind of materials : successes and failures

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721 Nanowire Triple-Junction GaInP/InP/InAsP Solar Cells Realized
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722 Machine Learning-Enabled 3D Visualization for Microstructural Risk Assessment in Creep Strength Enhanced Ferritic (CSEF) Steels
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723 Single-Layer Transition Metal Dichalcogenides: Unveiling Excitonic Processes with Multimodal Microscopy
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724 Atomic Resolved In situ mechanical Microscopy leads to realize the negative mixing enthalpy solid solutions
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725 Ultra-fast and facile synthesis of cathodes for rechargeable batteries using an organic syntehsis route
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726 Evaluating spherical models of EBSD patterns for forward modelling indexing
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727 The role of atomic displacements in high-resolution scanning transmission electron microscopy imaging of strained nanoparticles
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728 Unravelling the collagen mineralization using multiscale in situ X-ray-scattering/Raman spectroscopy and ex situ electron microscopy/nano-Xray-fluorescence
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729 Visualization of binder mixtures in hard carbon composite electrodes using OsO4 and uranyl acetate staining
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730 Analysis of molecular packing and nanoscale atomic variation in polymer semiconductors

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731 Toward 3D imaging of the PEMFC electrode microstructure

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732 Correlating atomic-resolution structure to the properties of transition metal nitride coatings

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733 Cross-sectioning of adherent cells on thin plastic substrate for serial block-face imaging

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734 Direct visualization of chemical transport in solid-state chemical reactions by time-of-flight secondary ion mass spectrometry

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735 HRSTEM study on the phase formations and transitions in nanolaminated MAB phases

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736 Mechanics of morphogenesis – The re-invention of cell sheet folding

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737 Investigating solar degradation mechanisms of the Ta3N5 photoelectrode by in-situ transmission electron microscopy

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738 Band gap measurements of aluminum and indium doped Ga2O3 multilayers

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739 The fatigue response of the IN939 superalloy prepared by additive manufacturing

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740 Revealing the vacancy ordering in Prussian blue analogues through serial electron diffraction

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741 Characterization of 2DEG on WG semiconductors through sub-sampled 4DSTEM

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742 Scanning precession electron diffraction tilt series for orientation analysis

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743 Deep Learning assisted X-ray Microscopy Characterization of Nickel based Metal Matrix composite reinforced with TiC

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744 Cryo-FIB as a preparation tool for soft X-Ray Tomography: Analysis beyond EM

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745 A new protocol for fluorescent quantitative labeling of individual proteins for live cell internalization assays

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746 New protein fluorescent labeling methods for carbonylation and S-acylation studies in plants

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747 Supersilent AlCoFeNiCux (x = 0.6 – 3.0) high-entropy alloys

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748 Development and characterization of a laser-driven cold-field emission source
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749 Microchannel Plate-based Detector with High Pressure Operation up to 1 Pa for Scanning Electron Microscopy
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750 Solving the crystallographic phase problem by linearizing dynamical electron diffraction
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751 Structural and spectroscopic studies of Cu-doped NiO thin films
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752 From DNA Nanotechnology to biomedical insight: Towards single-molecule spatial omics
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753 Band gap shifts in Ga-doped ZnO using momentum-resolved EELS
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754 Structure and mechanism of Zorya anti-phage defense system
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755 Electron beam propagation impact on high-resolution quantitative chemical analysis of GaN/AlGaN 1 nm-thick quantum wells
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756 Structural remodeling of neural circuits through synthetic biological control of neuron-astrocyte interactions

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757 EBSD analysis of twin boundaries of prismatic calcite in oyster shells

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758 An analytical ion microscope for high-resolution imaging, nanoscale analytics and nanofabrication

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759 Benzofuran-Tetrahydrodipyrazolopyridine Hybrids: Novel Compounds for Potential Lung Cancer Treatment

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760 Gallium liquid bridge evolution on varied substrates

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761 Multiplexed 3D imaging of single-cell organization and tissue morphology in the multicellular intestinal organoid

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762 Achieving methanol photo-oxidation to hydrogen and formaldehyde over lead-free halide perovskite

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763 In-situ TEM ion irradiation studies of layered MAX phase materials

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764 Nanoscale tribology of hair fibres over large displacements

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765 Manufacture and calibration of high stiffness AFM cantilevers

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766 Solvent-induced softening of polymethyl methacrylate surfaces
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767 In-situ oxidation and reduction study of Ni/NiO by open cell environmental TEM
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768 Crystalline analysis by W-SEM using a newly developed EBSD detector
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769 Atomic-resolution investigation of 2D hematene
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770 An Improved Method for Growing Primary Neurons on Electron Microscopy Grids Co-Cultured with Astrocytes
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771 Microstructure and thermal stability of ultrafine-grained CuZn5 processed by HPT
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772 Microstructural Influence on Sodium Filament Growth in All Solid-state Batteries
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773 Cryo-EM and ED are driving structural studies at the University of Warsaw
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774 Observation of logic states of HfO2-based ferroelectric FETs using STEM-DPC
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775 Novel, low-cost hardware for 'STEM in SEM' imaging

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776 Structural phase transition induced microstructure changes in V2O3 based hybrid magnetic heterostructures

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777 In situ closed-cell microscopy study of Ti3C2Tz MXene

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778 Exploring Cell-Material Interactions at Focal Adhesion Points

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779 The effect of fixation-induced cell blebbing: how to minimize a loss of proteins from cells?

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780 Platform for the in-situ measurement of magnetic transport properties in the transmission electron microscope

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781 The development of multimodal imaging with functional silica nanoparticle

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782 Hybrid electrospun scaffolds for enhanced collagen formation in regeneration processes: electron and confocal microscopy analysis

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783 Self-assembly of amphiphilic dendrimers investigated by standard, cryo and liquid TEM

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784 Microglial contactology–mapping the connection network of microglia from the micrometer to the nanometer range

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785 Insights on the disordered nature in amorphous-based anode materials from Electron Pair Distribution Function
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786 The nature of diffuse scattering in fcc metal alloys –
investigation by 3D-ED and 4D-STEM
Hannah Cole[1], Mr James Miller[1], Mr James Hogg[1], Mr Petr Vacek[1], Mr Owain Houghton[1], Prof Howard Stone[1], Prof Paul Midgley[1]
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787 Microstructural assessment of mechanically alloyed low activation 9-Cr oxide-dispersion strengthened steels
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788 Cholesteatoma removal efficiency evaluated by Variable Pressure Scanning Electron Microscopy
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789 3D Electron Diffraction Study on the Local Structure of a doped Metal-organic Framework TCNQ@ZIF-4
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790 Structural study of perovskite-structure transition metal oxide thin film using Cs-corrected STEM: case of $LaVO_3/DyScO_3$
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792 Restructuring of silver catalysts after oxidation reactions
– looking beneath the surface
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793 Deep learning powered light optical microscopy for steel reseach
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794 High quality graphene TEM supports for high-resolution transmission electron microscopy
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795 Application of secondary electron hyperspectral imaging to the analysis of pharmaceutical materials
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796 Immunoexpression of estrogen receptor α in the ovary of mice after chronic exposure to arsenic(III)-oxide

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797 Structural recognition and stabilization of tyrosine hydroxylase by the J-domain protein DNAJC12
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798 STRUCTURAL CHARACTERIZATION OF FZD7, THE IMPORTANCE OF WATER NETWORK AND CHOLESTEROL FOR CLASS F GPCRS
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799 Towards Quantitative analysis of electrostatic potential of monolayer WSe2 using electron ptychography
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800 Functional and Structural Insights into the Modulation of the Chaperonin CCT by Small Molecules
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801 Using super-resolution imaging to understand protein organization within Z-discs of striated muscle.

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802 Determination of Ti3C2Tx Mxene few layers stacks architecture using valence EELS and diffraction

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803 Analysis of fire gilding on medieval jewellery using focused ion beam

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804 Denoising 4D STEM datasets with PCA

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805 Nanotubes of (SmxY1-x)S-TaS2 based on Quaternary Misfit Layered Compounds (MLCs)

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806 Multicore@shell nanoparticles synthesized from a multicomponent target by gas aggregation cluster source

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807 Minimising the distortive effects of diffraction on magnetic STEM-DPC imaging of monocrystalline thin films

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808 Operando Environmental TEM observations of SOEC Ni-YSZ fuel electrode dynamics
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809 Inflammatory mechanism in Diabetes – Ultrastructural investigations of endocrine pancreas using correlative electron microscopy (STEM)
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810 Structure and Composition of High-Entropy-Alloy Nanoparticle synthesized by Pulsed Laser Ablation in Liquid
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811 Towards customized in situ TEM Chips for “device-like” geometries
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812 Structure of refractory high entropy alloy and high entropy nitride thin films
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813 Fast nanoparticle passage over cell membrane identified using 3D STED cross-sectional imaging
Dr. Boštjan Kokot[1], Hana Kokot[1], Janez Štrancar[1]
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814 An examination of exit-wave reconstruction algorithms for low dose imaging at atomic-scale resolution
Jacob Svane-Petersen[1], Andreas Nymand[1], Joerg R. Jinschek[1,2], Stig Helveg[1], Joakim Kryger-Baggesen[1], Sophie Kargo Kaptain[1]
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815 Examination of the Pathophysiology of Peripheral Olfactory Dysfunction and Alpha Melanocyte Stimulating Hormone Therapy
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816 Simultaneous Indexing of Spot and Kikuchi Patterns in Scanning Electron Microscopy (SEM)
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817 Physical determinants of YAP mechanotransduction in multicellular assemblies

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818 Dual-color CLEM imaging for genetically encodable enzymatic fluorescence signal amplification method using APEX (FLEX)

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819 Multimodal mechano-microscopy reveals mechanical phenotypes of breast cancer spheroids in three dimensions

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820 Transmission electron microscopy studies of ferroelectric ZrO2 thin films

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821 Characterization of the structure and chemistry of solid electrolyte

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822 Liquid Phase 3D Electron Diffraction Combination Provides New Possibilities for Polymorphism Studies

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823 Charge density mapping of supported nanoparticle electrocatalysts by 4D STEM

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824 Correlative study of hematite-based photoanodes for solar water splitting by transmission electron and X-ray microscopies

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825 Entanglement in Bragg Scattering

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826 Three-dimensional, correlative electron microscopy and immuno-labelling revealed new principles of the Golgi complex functioning

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827 Guided growth of 1D van der Waals Nanowires for Enhanced Optoelectronic Functionalities

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828 Detailed TEM Studies of 1D Nanostructures based on Layered Cobalt Oxide

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829 Microscopic characterization of graphene derivates in life science

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830 Microscopic characterization of graphene derivates in life science

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831 3D calibration for SEM and optical microscopy - First results with next generation 3D standards

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832 Exit of different cargoes from the Golgi and their post-Golgi trafficking

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833 FIB-induced nanorod formation in 2D layered crystals

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834 Sub-nanometer mapping of strain-induced band structure variations in different semiconductor device configurations

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835 Investigating Bone Microstructure with ATUM-SEM: Implications for Pathological Conditions
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836 Automated Detection of Material Defects for High-throughput Electron Micrographs Analysis
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837 Control of inter-particle distance between nanoparticles using DNA origami
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839 Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway
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840 Analysis of epigenetic modifications with the LiveMIEL (Live-cell Microscopic Imaging of Epigenetic Landscapes) approach
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841 The EXCITE² Network: a European infrastructure providing transnational access to leading-edge imaging facilities
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842 Structural Probing of Charging Mechanism in LiNiO2 at High Voltage using Microscopy and Spectroscopy
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843 Advanced acquisition strategies for lab-based diffraction contrast tomography
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844 Thallium and arsenic incorporation in roméite group minerals
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845 Impact of improved tracking method on structure determination of perovskites from 3D ED data

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846 Effects of melatonin and alpha-lipoic acid on collagen and VEGF expression in palatal wound healing

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847 TEMsuite – A Matlab-based software platform for TEM data analysis

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848 NiTi shape memory alloy microstructure after high stress at elevated temperatures containing modulated M2 martensite

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849 Influence of the deposition parameters on properties of gold plasmonic antennas

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850 3D TEM and SEM-array tomography of Hailey-Hailey Disease human skin biopsies

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851 Precise light and fluorescent microscopy guided sequential cryo FIB lift-out

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852 Nanoscale structural and spectroscopic characterization of hard carbons for Na-ion batteries

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853 All-Micro: a Network for Sustainable Innovation in Slovenia- Italy Area

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854 Investigating nitrogen doped nanocarbon materials as potential carbon dioxide adsorbers

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855 Achieving High-Resolution Electron Nano-crystallography using HVEM and PED

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856 The mixed ionic-electronic conductors studied by advanced transmission electron microscopy

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857 The reversible phase transition of Gd-doped ceria by in situ TEM

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858 In-Situ Manipulation of Growth Mechanisms in Ni-Seeded GaP Nanowires

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859 Vitamin A affects urothelial injury and regeneration in cyclophosphamide-induced cystitis

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860 4D-STEM Optical Sectioning of Dopants in Diamond

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861 Correlative microscopy and spectroscopy of nanophotonic materials

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862 Birefringence analysis of hemozoin with surface plasmon microscopy towards malarial species distinction

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863 In situ examination of oxygen vacancy dynamics in epitaxial LaCoO3 thin films

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864 Oscillatory redox behavior of nickel catalysts observed by operando SEM

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865 Structural Characterization of MOFs using Transmission Electron Microscopy and Software Innovation

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866 Transmission electron microscopy of dermal collagen using ethanolic phosphotungstic acid staining

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867 From design to « operando » experiments in the SEM: application to battery studies

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868 High-Z Nanoparticle Tagging in Cryo-STEM for Localisation in Cryo-ET: Theory and Damage

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869 Combination of TEM, LM and micro-CT to image insects: the perspective of crop protection strategies

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870 High-resolution structural analysis of cation-mixed (Fe,Mn,Ni)PS3 van der Waals single crystals

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871 STEM-EELS and Image simulations of inelastic spin-scattering of magnons in confined geometries
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872 Microscopy of body surface sculpturing in terrestrial crustaceans
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873 Toxic buffers used in microbial sample preservation for SEM can be replaced by PBS

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874 Leveraging AutoScript for Cross-Platform Deep Learning Solutions in Electron Microscopy
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875 Exploring Role of Voltage-Gated Ion Channels in LGI1 Autoantibody-Induced Epilepsy: Insights from a Mouse Model
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876 Ultrastructural analysis of liver injury and regeneration after microcystin-LR intoxication in whitefish
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877 Effect of chloroquine and naringin on the relationship between ER stress and mitochondria in trophoblasts
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878 Understanding field evaporation sequences by in-situ correlative microscopy and simulation
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879 FTIR-Imaging of a Tintoretto-Fresco

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880 Raman-Imaging of the Strain-Distribution in Semiconductors
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881 Towards electron beam shaping in an Ultrafast Transmission Electron Microscope
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882 Deconvolution of SBF-SEM images improves quality of data in volume electron microscopy
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883 In Situ SEM at Elevated Temperature for Materials Science
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884 Quantitative Super-Resolution Methods for Cryo-CLEM
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885 Characterization of a multiphase nucleus of spheroidal graphite cast irons by transmission electron microscopy techniques
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886 Biorestoration Potential of Carbonatogenic Bacteria on Lithotypes: SEM and AFM characterization
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887 Numerical study on high orbital angular momentum vortex electron beams in hafnium dioxide
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888 Free electron spectroscopy seen through the prism of quantum optics
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889 Correlation between manufacturing parameters, properties and microstructure in 2D-C/SiC composites

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890 Analytical TEM of materials for gas sensing

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891 Three-dimensional ultrastructure of ovine pinealocytes

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892 Mass microscopy allows for high throughput, high spatial resolution mass spectrometry imaging

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893 A high-throughput compositional study of nanocrystals using a machine learning-assisted algorithm for STEM hyperspectral datasets

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894 Simulating iDPC tomography of CeO2 nanoparticles with experimentally realistic parameters and conditions

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895 Microvessel and mitochondria changes in a mouse model of Alzheimer's disease

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896 Cryo-EM Reveals RECQ5's Regulatory Role in RNAPII-Mediated Transcription

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897 Surface tension of Au-catalysed GaAs-nanowires

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898 TEM observations of threading dislocations in gallium nitride under external stimuli

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899 Deep Learning Style Transfer for Elastic Image Registration of Visually Distinct Correlative Microscopy Images

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900 Image Registration Based Navigation of Region of Interests in Volume Correlative Light and Electron Microscopy

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901 Microstructural Characterization of Electron Beam Welded Joints between EHEA and Austenitic Stainless Steel

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902 Mechanisms of microglial responses to injury related focal ATP events

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903 Complementary vEM approaches for ultrastructural changes during the development of D. melanogaster germline intercellular bridges

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904 Investigation on photocorrosion of TiO2 during photoelectrochemistry process by electron microscopy together with operando ICP-MS

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905 Advancing the real-time regime for virus self-assembly processes using liquid-electron microscopy

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906 Effects of corticosterone and amyloid beta on the corticosteroid receptors in organotypic brain slice cultures

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907 Morphological investigation of periodic structures created by focused ion gallium beam

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908 Cellulose-based Nanocomposites for Sensor Applications: Characterization and Performance Evaluation

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909 Functionalization of Single-Walled Carbon Nanotubes Analyzed by Spatially-Resolved EELS

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910 Electron beam manipulation with auto-ponderomotive potentials for interaction-free measurements

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911 Microstructure and mechanical properties of AlCu thin films in a wide range of composition

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912 Enhanced Visible Light Photocatalytic Activity of Cu-Doped ZnO ALD Films Deposited on Porous Substrates

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913 Examining dislocation and stacking faults within Hybrid MBE-Grown (110)-oriented RuO2 films

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914 On the accuracy of atomic-resolution DPC-STEM measurements

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915 Analysis of photon bunching in coherent cathodoluminescence

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916 The structural basis for energy extraction from air by Mycobacterium smegmatis

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917 Simple and efficient three-dimensional reconstruction method of plant cells using transmission electron microscope

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918 Time-resolved TEM observation of CeO2 surface with electrostatic sub-framing system

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919 Continuous-flow LHe-cooling TEM sample holder with high stability at sub-10K and in situ biasing functionality

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920 Revealing the origin of green light emission in Cs4PbBr6 by cathodoluminescence

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921 In-situ HAADF-STEM observation of phase transformation in microencapsulated Al-Cu-Si alloy

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922 Microstructural characterization of Zr/ZrN and ZrN/ZrCN hard coatings using TEM

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923 SEM Insights: Sample Temperature Evolution during EBSD

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924 Development of chopped scan control for beam blanking

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925 Analysis of the cause of imperfect adhesion of cataphoretic coating using SEM and EDS method

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926 TEM investigation of the defect structure in epitaxial corundum thin films

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927 Automated Alignment Strategies for Integrating Cryo 3D-SIM and SXT Data

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928 Reconstruction of Angstrom resolution exit-waves by the application of drift-corrected phase-shifting off-axis electron holography

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929 The effects of TiC network microstructure on the mechanical properties of spark plasma sintered Al2O3

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930 VitroJet: ice thickness control and measurement for time-efficient single particle structure determination3052

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931 Direct observation of charge modulation in nanoprecipitates by 4D STEM

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932 In-situ TEM annealing of vanadium oxide thin films

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933 Annular silicon drift detector for advanced EDS on SEM applications

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934 Automatic signal clasification of the Low-Loss Region in Electron Energy Loss Spectroscopy

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935 Is iron a hidden culprit in Alzheimer's disease?

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936 Cryo-FIB milling and Cryo-electron tomography of tissue biopsies: BBQ method

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937 Investigation of the effects of production conditions and additives on the microstructure of PVDF-HFP membranes

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938 In-situ observation and analysis of Ni-based catalysts for dry reforming of methane

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939 Combining synchrotron-based microCT with plasma FIB-SEM for targeted Volume EM

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940 Innovative Nanoanalytical Approaches for Lithium Metal Interface Analysis

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941 Mapping of phase separation of supramolecular protein assemblies by live-cell holotomography microscopy

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942 Differential axon terminal spread and synapse numbers amongst afferent neurons onto a locust's movement detector

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943 Advanced approaches for the analysis of micro- to nano-quartz particles using SEM, ESEM, FIB-SEM, SBF-SEM

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944 Enhanced sensitivity in label-free live-cell imaging using multi-pass stimulated Raman scattering microscopy

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945 Resonant inelastic light scattering micro-spectroscopy to probe inter-moiré miniband excitations in twisted 2D semiconductors
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946 Investigation of earth-abundant photovoltaic material Zn3P2 nanostructures using electron microscopy
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947 Engineering electron-electron interaction for advanced quantum metrology in electron microscopy

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948 Human Tumor Microbiome Detection using Correlative Light and Electron Microscopy
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949 Mean inner potential measurement by correlated EFTEM and phase-shifting holography
Dr. Ing. Ulrich Ross[1], Dr. Christoph Flathmann[1], M.Sc. Jonas Lindner[2], Dr. Tobias Meyer[2], Dr. Andreas Beyer[3], Dr. Jürgen Belz[3], Prof. Dr. Kerstin Volz[3], Prof. Dr. Christian Jooss[2,4], Prof. Dr. Michael Seibt[1]
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950 Atomic-scale imaging of local structure of layered Cu-Te phases
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951 Cryo-EM structure of the HD6 defensin helical assembly
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952 Temporal analysis of bone development in chick femur bone using label-free imaging
Miss Siddhi Chugh[1], Mr Jacob Kleboe[1,2], Dr Rahul S. Tare[3], Professor Richard OC Oreffo[2,3], Professor Sumeet Mahajan[1,2]
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953 Study of nanolaser optical and structural properties at the nanometer scale
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954 New strategies of TEM sample preparation for the mitigation of carbon contamination
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955 Localisation of nanoparticles in whole cells using correlative cryo soft x-ray tomography and fluorescent microscopy
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956 Sample preparation for correlative light, soft X-ray tomography, and cryo FIB-SEM imaging of biological cells
Director Imaging Applications Sergey Kapishnikov[1], Maryna Kobylynska[2,3], Prof. Roland A. Fleck[2,3], Malene Fog Lihme Olsen[4], Prof. Poul Erik Jensen[4], Dr Kenneth Fahy[1], Dr Paul Sheridan[1], Dr William Fyans[1], Dr Fergal O'Reilly[1,5,6], Tony McEnroe[1]
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957 Improved conventional TEM sample preparation exploiting the birefringence of materials
Aleksander Brozyniak[1], Karin Stadlmann[1], Philipp Kürnsteiner[1], Heiko Groiss[1]
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958 TEM studies of polarization nanodomains in (BaTiO3/SrTiO3)10 superlattices on silicon
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959 Charge Particle Optics Simulation Utilizing Hamiltonian Mechanics Perturbation Expansion and Boundary Elements Field Computation
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960 Carbon nanoribbon formation by in-situ TEM manipulation of a C59N dithiolane derivative encapsulated into SWNTs

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961 Chemical and morphological stability study of copper oxide nanocubes in controlled and non-controlled atmospheres

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962 Comparison of the FMT assay with the Cell Painting approach in healthy patient derived fibroblasts.

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963 Robustness evaluation of electric field measurements via template matching in 4D-STEM

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964 Molecular insights into the biogenesis of box H/ACA snoRNPs

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965 Structural Analysis of COPI Pathway in Chlamydomonas reinhardtii

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966 Elimination of HCV replication machinery early after antiviral treatment with DAA monitored by multimodal microscopy

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967 In-vivo DAB cytochemistry and high-pressure freezing to determine the source of the human cytomegalovirus envelope
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968 Electron energy loss spectroscopy for differentiating of minerals polymorphs
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969 Transport of Intensity Phase Retrieval in the Presence of Intensity Variations and Unknown Boundary Conditions
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970 Ultrastructural and chemical analysis of human Locus coeruleus using correlative microscopy and mass spectrometry
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971 Atomic-scale structure and defect evolution in Σ5 [001] tilt grain boundaries in copper
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972 Characterization of Precipitates in Petroleum Steels by Using Precession Electron Diffraction Technique
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973 Interpretable evaluation of STEM images of nanostructures via homology analysis
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974 Strategies for Multimodal Image Data Transformation to a Common Format for Cloud Integration and Visualization
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975 Phase-controlled formation of NixPy catalyst using environmental TEM for potential application in CO2 reduction
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976 Evaluation of inflammation and free fatty acid metabolism as biomarkers in female patients
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977 The Healing Effect Of Ferulic Acid in Monosodium Glutamate-Induced Liver Injury
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978 In situ growth and phase engineering of manganese arsenide nanostructures in environmental TEM
Hedda Christine Soland[1,2], Mikelis Marnauza[1,2], Pau Ternero[2,3], Professor Kimberly A. Dick[1,2]
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979 Advanced image analysis techniques to support and streamline cell manufacturing
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980 Ultra-low voltage SEM observation for battery materials
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981 Structural Study of Polyphasic Mixtures Using 3d Electron Diffraction:A Case Study of Oxyresveratrol
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982 Neuroprotective Effects of Bromelain in Peripheral Nerve Injuries: A Rat Sciatic Nerve Crush Injury Model
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983 Establishment of 30mm diameter milling and curtaining effect reduction by large area planar surface milling
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984 Characterization of casting inclusions in superalloys by BSE and EDS
Prof. Dr. Dragan Rajnović[1]
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985 Thin Film Phase Plates for Cryo TEM: Fabrication,
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986 PFIB and SEM engineering of luminescent centres in hBN
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987 Non-toxic clearing and labeling with fluorescent REAfinityTM antibodies for enhanced 3D visualization of tissues
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988 Deformation of steel chips due to machining
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989 Characterization of Cu doped zeolite by MicroED and electron ptychography
Mr. Masahiko Shimizu[1], Dr. Katsuaki Nakazawa[2], Dr. Kazutaka Mitsuishi[2], Dr. Hajime Matsumoto[1], Dr. Hisashi Shima[1], Dr. Takahiko Takewaki[1], Dr. Ayako Hashimoto[2,3]
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990 From volume electron microscopy datasets to segmentation models, feature quantification, and data reuse
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991 Temporal characterization of femtosecond electron pulses inside ultrafast scanning electron microscope
Kamila Moriova[1], Martin Kozak[1]
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992 Optimizing optical STEM detection for faster acquisition speeds in scanning electron microscopy
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993 HR-EBSD Analysis of High-Entropy Alloys: Understanding the Role of Alloying Elements in Mechanical Performance

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994 SEM-Cathodoluminescence Imaging and Spectroscopy - Applications in Archeology and Life Science

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995 Detection of quasicrystalline symmetries in electron diffraction data

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996 2D and 3D Oxidation State Mapping in FeO/Fe3O4 Nanocubes Using the Fe-M2,3 EELS Edge

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997 Ultrafast Microbeam Electron Diffraction at 15-keV

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998 Contribution of residual gas and surface contaminants to contamination growth on irradiated samples

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999 Measurement of electrostatic fields in Ge-doped AlGaN structures by off-axis electron holography

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1000 PolSpec – broadband cost-effective hyperspectral imaging

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1001 Dynamics of an industrial Cu/ZnO catalyst revealed by operando TEM

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1002 Structural and Electrical Characterization of $Hf_{0.5}Zr_{0.5}O_2$ Thin Films Crystallized by Rapid Thermal Annealing

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1003 Investigating the photoelectronic properties of MoS2 thin layer with ITO nanoparticles using photoluminescence and cathodoluminescence

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1004 Electron tomography of radiation sensitive nanocomposites for additive manufacturing

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1005 Hyperspectral CT allows for non-destructive elemental imaging in museum specimen

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1006 FIB-SEM/microtoming prepared Cross Section of a Stone Wool Fiber enabling (S)TEM investigation

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1007 The importance of an open camera system demonstrated with wide-ranging applications of MerlinEM detector

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1008 2D and 3D EELS Analyses on the Low-Energy Core-Loss Edges in Beam-sensitive Ryugu Asteroid Samples

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1009 Setting up fringe-free imaging for SPA on Talos Arctica and Titan Krios microscopes

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1010 Imaging ultrafast spin dynamics at the nanometer scale

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1011 Sequential tilting 4D-STEM for reliable electric field mapping across junctions

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1012 A validation methodology for size and shape measurement of nanoplastics by transmission electron microscopy

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1013 TEM 3D Spectroscopic Imaging for Catalytic Activity Evaluation

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1014 Large-volume cryoEM sample preparation for the investigation of the plant-microbiome interaction

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1015 The Development of Japanese ceramic ware (pottery and porcelain) Technology as Revealed by EPMA

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1016 Relative thickness study of TEM samples

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1017 IN-SITU SYNTHESIS OF FexPy NANOPARTICLES

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1018 Metal-Organic Chemical Vapor Deposition in a Transmission Electron Microscope

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1019 Quantitative Elemental Mapping Of Bimetallic Nanoparticles From Atomic Scale STEM-HAADF Images

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1020 Leveraging FIB-SEM with Integrated ToF-SIMS for Comprehensive Characterization of Lithium-Ion Battery Materials

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Morphological characterization of the electric field aligned block copolymers containing liquid crystal moiety

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1022 TEM investigation of AlN-Cu(O) with variable amounts of copper

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1023 FRET and TIRF microscopy for single molecule characterisation of synergistic antimicrobial peptides in artificial bilayers

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1024 Structural and Compositional Investigation of Ag-Incorporated CsPbBr3 Nanocrystal Heterostructures

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1025 A novel pipeline to elucidate the adaptation response to the G2/M DNA damage checkpoint

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1026 Differentiation of phases in phase change memory materials using 4D STEM

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1027 Imaging-based methods to identify prognostic and predictive biomarkers for Hereditary Spastic Paraplegia

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1028 Characterizing Magnetic Properties of Nanoparticle Systems: Insights from Electron Tomography and Micromagnetic Simulations

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1029 Correlative microscopy of creep cavitation in steels using image processing of SEM, FIB-XeF2 and EBSD

Dr Tomas Martin[1], Dr Siqi He[1], Ms Eirini Gallioupolou[1], Mr Peter Thomas[1], Mr Edward Horton[1], Mr Michael Salvini[1], Dr Nicolo Grilli[1], Professor David Knowles[1], Professor Mahmoud Mostafavi[1], Professor Peter Flewitt[1]
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1030 Insights into Lithium-rich Oxides from Synthesis and Characterization Studies

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1031 U-NET Enhanced 4D-STEM/PNBD: Advancing Microscopy Image Reconstruction

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1032 Anomalous effects of strain in selected area diffraction patterns due to boron doping in silicon

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1033 Van der Waals heterostructures of nanopatterned 2D materials for novel device geometries

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1034 Fabrication of electron transparent membranes and nanostructures in fluidic devices by NIL and “Flow-Through”-gas-phase deposition

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1035 Electron microscopy studies of Ni/GDC fuel electrode in solid oxide fuel cell

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1036 Assessing feasibility of detecting photogenerated charge carriers in photocatalysts via transmission electron microscopy: simulation study

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1037 Unraveling the regulation pathway of photosynthetic AB-GAPDH

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1039 Resolving short-range order in Carbon Nitride-based catalysts using EELS

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1040 Ex Vivo Metabolic Imaging for Parotid Tumors: Implications for Precise Diagnosis and Customized Treatment

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1041 Using XeF2 FIB imaging to contrast and quantify precipitation in metal.

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1042 Towards Automation of the Transmission Electron Microscope

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1043 In situ SEM of slip localization and its relation to the onset of ductile fracture

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1044 In-situ micro-mechanical tests under SEMs

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1045 Electron Diffraction on Biological Samples

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1046 Measurement of atom mobility of gold nanorods via coarse-sampling in quantitative 4D-STEM

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1047 Cryo-EM as a tool for observing alginate-based hydrogels

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1048 The effects of B4C network microstructure on the thermoelectrical properties of spark plasma sintered SiC
Dr Salih Cagri Ozer[1], Ceren Korler[1], Prof. Dr. Servet Turan[1,2]
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1049 Revealing Nanostructural Dynamics: Exploring Inelastic Scattering in Electron Microscopy

Phd Caterina Chiari[1,2], Prof. Marco Beleggia[1], Dr Vincenzo Grillo[2]
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1050 Fast mass spectrometry imaging for immunohistochemistry
Dr. Mariya Shamraeva[1], Dr. Edith Sandström[1], Kimberly G. Garcia[1], Distinguished Professor Ron M. A. Heeren[1], Dr. Ian G. M. Anthony[1], Dr. Sebastiaan van Nuffel[1]
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1051 Multiscale 3D organization of human auditory ossicles unveiled by synchrotron small-angle X-ray scattering tensor tomography
Dr Margaux Schmeltz[1], Dr Christian Appel[1], Ginevra Lautizi[2], Irene Rodriguez Fernandez[1], Torne Tänzer[1], Prof. Dr. Med. Lukas Anschuetz[3,4], Dr Meitian Wang[1], Prof. Dr. Marianne Liebi[1,5,6]
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1052 Carbonic anhydrase immobilization for microscopic investigation of enzyme activity
Dr. Zsófia Bognár[1], Jeannette de Sparra Lundin[2], Sune Christensen[2], Joerg Jinschek[1,3], Jialong Shen[4], Sonja Salmon[4], Stig Helveg[1]
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1053 Exploring Local Order/Disorder in Relaxor Ferroelectric Materials via TEM and STEM Methods
Beatriz Vargas Carosi[1], PRanjal Nandi[1], Lluís Yedra[1], Sonia Estradé[1], Angélica M. Benitez-Castro[2,3], Juan Muñoz-Saldaña[3], Bernat Mundet[4], Lluís López-Conesa[1], Francesca Peiró[1]
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1054 Structural characterisation of a phage-like bacteriocin from Pseudomonas sp. by cryo-Electron Microscopy
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1055 Structural characterization of partially relaxed hybrid radial (Pb,Sn)Te/WZ-GaAs nanowires as candidates for topological insulator nano-devices

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1056 Histopathologic Alterations of Cerebellum in the VPA-Induced Autism Model of Rats

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1057 Accessible low-cost, long range, optical autofocus module for open-source multiwell plate and slide scanning microscopy

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1059 Investigation of self-assembly dynamics of magnetic nanoparticles in liquid phase by transmission electron microscopy

Dr Malika Khelfallah[1], Dr Claire Carvallo[2], Dr Amélie Juhin[2], Dr Govind Ummethala[1], Dr Amir Tavabi[1], Peng-Han Lu[1], Dr Thibaud Denneulin[1], Prof. Dr Rafal Dunin-Borkowski[1]
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1060 3D reconstruction and temporal development analysis of dendritic spines

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1061 Fast large-area EDS characterization of additive manufactured steels

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1062 Novel nanobody-based tools for studying the synaptic vesicle life cycle

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1063 Correlative TOF-SIMS/SEM for subcellular molecular profiling of snow microalgae

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1064 Correlative TOF-SIMS/SEM for subcellular molecular profiling of snow microalgae

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1065 Innovative Microfluidic chip for Raman spectroscopy and advanced electron microscopy techniques

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1066 Electrons phase reconstruction using Kramers-Kronig relations

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1067 Compartmentalization of synaptic ER studied by correlative FLIP and FIB-SEM

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1068 Investigation of the Presence of Telocyte-like Cells in Human Patellar Fat Pad Tissue

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1069 WDS-supported Bayesian Peak Deconvolution for optimized Standardless EDS-Quantification

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1070 Visualizing atomic structure of novel three-dimensional covalent organic frameworks by 3DED and high-resolution (S)TEM

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1071 Investigation of GaAs-based nanowire heterostructures using tomography based on STEM-HAADF tilt-series[1]

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1072 Single-camera multi-color emission anisotropy optical splitter

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1073 Influence of damage dose on the defect formation in tungsten

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1074 Label-free biological composition predictions in EM images

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1075 Measurement of EELS standards and application on oxidation state determination of a MeOH catalyst

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1076 Numerical Analysis of Temperature Calibration using Plasmon Energy Expansion Thermometry

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1077 Corrosion of Alloys Suitable for Very High Temperature Systems (VHTRs) Exposed to High Temperature Helium

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1078 Optimizing Soft X-ray Spectroscopy for Silicon Anode Lithium Mapping

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1079 Operando TEM Studies of Re@Cu2O-SnO2 catalysts during CO2 reduction reaction with optimized liquid flow configuration

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1080 CAR T cell dynamics in a 3D collagen matrix: migration and interactions with cancer cells

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1081 Analysis of the local chain orientation in conjugated polymer films

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1082 Resolving single-electron and multi-electron distribution functions with event-based electron detectors

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1083 Structured illumination near-field electron ptychography

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1084 Geometric phase analysis-based characterization of skyrmion lattice equilibria

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1085 Electron microscopy characterization of grain boundaries in Nb1-xTixFeSb based half-Heusler thermoelectric materials

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1086 Interference based optical instrument for high-throughput characterization of nanoparticles in complex biofluids

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1087 Observing the in-situ formation of the lead-free piezoceramic potassium sodium niobate (KNN) with SAED
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1088 Quarantotto: a 48-segment STEM detector for enhanced STEM performance and new applications
Dr Stefano Vespucci[1], Dr Eric G.T. Bosch[1], Dr Ivan Lazić[1], Bert de Vries[1], Dr Ricardo Egoavil[1], Dr Bert Freitag[1], Dr Tim Grieb[2], Dr Florian F. Krause[2], Dr Martin Eickhoff[2], Prof Andreas Rosenauer[2]
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1089 Correlative Microscopy Analysis of Jack Hills Zircons by Photonic Atom Probe
Dr. Florant Exertier[1], Dr. Jonathan Houard[1], Dr. Martin Bizzarro[2], Dr. Rick Verberne[2], Dr. Lorenzo Rigutti[1]
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1090 Fabrication and high-resolution transmission electron microscopy characterization of nanopores in silicon nitride and 2D materials
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1091 Microstructure of light-emitting phosphor of (Sr, Ca)AlSiN3:Eu2+
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1092 Molecular dynamics simulation of the Brownian motion of biomolecules in liquid phase electron microscopy
Mr Xiaopeng Wu[1], Dr Peng Wang[1]
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1093 Multifunctional hybrid nanocomposite films: linking inorganic nanoparticles using α-synuclein as molecular linker
Prof Patrizia Canton[1], Prof Adolfo Speghini[2], Dr Emil Milan[2], Dr R Tira[2], Dr. M. D'Onofrio[2], Dr M Assfalg[2]
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1094 Quantitative EMCD analysis of Fe thin films on MgOx substrates
Mr. Sharath Kumar Manjeshwar Sathyanath[1], Dr Anna L. Ravensburg[2], Professor Vassilios Kapaklis[2], Professor Björgvin Hjörvarsson[2], Professor Klaus Leifer[1]
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1095 Laboratory Soft X-ray Microscopy for Biomedical Applications
Aurélie Dehlinger[1,2], Dr. Christian Seim[1,2], Valentina Alberini[1,2], Dr. Holger Stiel[2,3], Dr. Antje Ludwig[4,5,6], Sarah Jung[1,2], Daniel Groetzsch[1,2], Vladimir Usatikov[1,2], Dr. Johannes Tuemmler[3], Prof. Dr. Birgit Kanngießer[1,2]

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1096 Nanowire field emitters fabricated using focused electron, gallium and helium ion beam methods

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1097 Investigation of Lateral and Vertical Heterostructures of MoS2/WS2

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1098 In-Situ microscopy study on self-healing process of vitrimers

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1099 Deep learning-based single cell volume segmentation for soft X-ray microscopy data

Valentina Alberini[1,2], Aurélie Dehlinger[1,2], Dr. Christian Seim[1,2], Dr. Holger Stiel[2,3], Dr. Antje Ludwig[4], Prof. Dr. Birgit Kanngießer[1,2]
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1100 Insights into the formation of polycrystalline seed layers for the solution growth of semiconductor nanorods

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1101 SoftGrids: towards disease modelling in CryoEM

Abstract Submission Asena Oelschläger[1], Prof. Dr. Stefan Raunser[1], Dr. Sebastian Tacke[1]
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1102 Room temperature viscoplastic response of amorphous olivine films revealed by ex/in-situ TEM nanomechanical testing

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1103 Low-dose 4D-STEM cryo-tomography of biological samples
Dr. Shahar Seifer[1], Shai Kiriati[1], Dr. Peter Kirchweger[1], Prof. Michael Elbaum[1]
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1104 Ultra-low-cost, high-dynamic-range, additively manufactured CMOS spectrometers with UV, visible, and NIR sensing functionality
Mr. Hironori Kondo[1,2]
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1105 Correlative microscopy for the discovery of novel nano-grains in spintronic materials.
Dr Adam Kerrigan[1], Dr Irene Azaceta[2], Dr Ercin Duran[3], Dr Stuart Cavill[2], Dr Alex Eggeman[3], Prof Vlado Lazarov[1,2]
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1106 Hyperspectral Imaging with 'Spectromics': Label-free Chemical Mapping in the NIR-SWIR for Tissue Diagnostics
Mr Hiroki Cook Cook[1,2], Dr Anna Crisford[1,2,3], Dr Konstantinos Bourdakos[1,2], Mr Douglas Dunlop[4], Professor Richard Oreffo[2,3], Professor Sumeet Mahajan[1,2]
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1107 Simultaneous nanoscale mapping of strain and electric field in semiconductor heterostructures using 4D-STEM
Yucheng Zou[1], Dr. Lei Jin[1], Peng-Han Lu[1], Prof. Dr Rafal Dunin-Borkowski[1]
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1108 Novel In-situ TKD Nano-tensile Testing: Insights into Nanoscale Crystal Plasticity and Grain Boundary Mechanics
Dr. Tijmen Vermeij[1], Dr. Amit Sharma[1], Dr. Xavier Maeder[1], Prof. Johann Michler[1]
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1109 Physical and chemical parameters determining the formation of gold sp-metal (Al, Ga, In, Pb) nanoalloys
Prof Patrizia Canton[1], Prof Vincenzo Amendola[2], Dr Vito Coviello[2], Dr Daniel Forrer[3]
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1110 Sample holder design for TEM in-situ straining experiments on 2D materials
Carmen Rubach[1], Dr. Xin Zhou[1], Tobias Dierke[2], Robert Kammel[1], Michael Sommerschuh[1], Prof. Janina Maultzsch[2], Prof. Erdmann Spiecker[1]
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1111 Synthetic Bispecific RBD Antibody Effectively Neutralizes SRAS-CoV-2
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1112 Structure and stability of core-shell AuTiOx nanoparticles for CO oxidation

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1113 MEMS Monochromator

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1114 Structural characterization of single wall carbon nanotubes via AI assisted transmission electron microscopy

Dr. Antonin Louiset[1], Dr Daniel FÖRSTER[2], Ms. Eira MEDINA[3], Mr. Saïd TAHIR[3], Dr. Vincent JOURDAIN[3], Dr. Christophe BICHARA[4], Dr. Hanako OKUNO[1]
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1115 Investigating nanoparticle interactions with the human blood-brain barrier in vitro

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1116 Effects of swimming training on orexin receptor 2 distribution in brain damage of rats

Professor Dilek Akakın[1], Assoc. Prof. Merve Acikel Elmas[2], Phd Student Ayca Karagoz Koroglu[1], Assoc. Prof. Ozlem Bingol Ozakpinar[3], Prof. Filiz Onat[4], Professor Feriha Ercan[1]
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1117 Towards Single-Pattern Absolute High angular Resolution EBSD without using Simulated Patterns as Reference

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1118 Microscopy to discern cells behaviour on different nano/microstructured calcium phosphates ceramics

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1119 Towards direct imaging of defects in carbon nanotubes with 4DSTEM

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1120 Multimodal imaging accelerates the analysis of composition in bone implant sites
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1121 Investigation of composition and origin of the intermediate layers at the Ga2O3/AlN and Ga2O3/Al2O3 interfaces
Dr. Marco Schowalter[1], S. Raghuvansy[1], Dr. A. Karg[1], Dr. P. Vogt[1,2], Dr. D. Schlom[3], Dr. D. Jena[3], Dr M. Eickhoff[1,2], Dr. A. Rosenauer[1,2]
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1122 Cryo-electron microscopy for the study of sensitive energy materials
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1123 Automated Identification of Slip System and Twinning Activity Fields from Digital Image Correlation Data
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1124 SEM Automated Quantitative Mineralogy Method-development with Mineralogic: 200-nm-resolution Quantitative Assessment and Mineral-specific element mapping
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1125 Depositing biological segmentation datasets FAIRly
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1126 Mapping Li in thin-films and heterostructures by EELS and ptychography: case of Li7La3Zr2O12 and LiCoO3
Dr Adam Kerrigan[2], Dr Connor Murril[2], Dr Demie Kepaptsoglou[1,3], Dr Chris Allen[4], Dr Mohsen Danaie[4], Dr Frederick Allars[4], Prof Vlado Lazarov[1,2]
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1127 Spectrally resolved brightfield widefield microscopy by an ordinary digital camera

Prof. Dalibor Štys[1], Dr. Renata Stysova Rychtarikova[1], Dr. Kirill Lonhus[1]
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1128 Nanoparticle Self Diffraction in the TEM: A proposal

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1129 Tungsten nanoparticles generated in an atmospheric pressure plasma jet

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1130 Development of multiciliated cells in the respiratory and esophageal epithelium of the chicken embryo

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1131 In-situ synthesis of thin kesterite films using high voltage TEM

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1132 Morphology evolution and phase transition of $Co(OH)_2$ and Co_3O_4 investigated with STEM-tomography and in-situ XRD

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1133 FLIPs: Novel genetically encoded biosensors for polarization microscopy

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1134 Unsupervised and supervised machine learning for feature classification in atomic resolution images

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1135 Synthesis and Characterization of Ultrathin Unconventional Mixed 2H-HCP/FCC Phase Au Nanowire

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1136 Utilization of TEM in archaeology to gain in-depth information on historic artifacts

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1137 INSIHGT - Scalable, Accessible, Homogeneous Deep Multiplexed Immunolabelling Platform for 3D Spatial Biology

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1138 Nestin expression in the myocardium of normotensive and spontaneously hypertensive rats during aging

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1139 Studying nano-catalysts degradation with an identical location STEM approach

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1140 Visualisation of lepidopteran silk gland morphology using X-ray micro-computed tomography scanning technique

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1141 TEM structural analysis of photocatalytically active mesoporous single crystalline LaTiO2N particles

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1142 Correlating microscopy methods: the case of precipitation in lean, bioabsorbable Mg alloys

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1143 Imaging Chiral Spin Textures with Electron Interferometry and Polarimetry

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1144 IMG Electron Microscopy Core Facility

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1145 Interplay between microstructural properties with ionic/electronic conductivity in Na3PS4-based composite cathodes for Na-SSBs

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1146 ESEM technique for monitoring the geo-polymerization mechanism of the wet ash-cement mixture

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1147 Molecular insights into rapid cell death involving endoplasmic reticulum and nuclear remodeling p

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1148 Effects of Myricetin on Mesenchymal Stem Cells Exposed to Oxidative Stress

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1149 Impact of In Situ and Ex Situ Annealing on Al-Cu Heterogeneous Nanostructures: A Comparative Study

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1150 The effects of resveratrol on liver damage and ferroptosis in fructose-streptozotocin induced diabetic model

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1151 Multiscale characterization of Al-4Fe alloy grown by additive manufactoring

Dr Frédéric Fossard[1], Pauline Stricot[2], Simon Fritz[2], Eric Grevin[1], Maria Tsoutsouva[2], Nicolas Horezan[2], Quentin Barrès[2], Aidar Zakirov[3], Williams Lefebvre[3], Louise Toualbi[2], Yann Le Bouar[1]
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1152 Introducing a FAIR RDM infrastructure for electron microscopy and other materials science data

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1153 A silicon micro-heater chip for in-situ transmission electron microscopy

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1154 Electron Microscopy of a Gas-Atomized NiSiV Powder

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1155 Structural investigation of the 40S hnRNP particles

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1156 Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution

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1157 Xenon PFIB-milling and cryo-electron tomography of bacterial anti-phage effectors

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1158 Time ecology method

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1159 Real-Time Studies of Resistive Switching Mechanisms

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1160 Visualization of the in situ molecular architecture of tau pathology in the murine brain

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1161 Novel scan coil design for high spatiotemporal-resolution imaging in the scanning transmission electron microscope

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1162 In-situ cryo-biasing heating TEM sample holder with full-range temperature control from -170°C up to >1000°C

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1163 Spatiotemporal observation of ultrafast magnetization dynamics with 4D Lorentz Transmission Electron Microscope

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1164 Quantum Measurements using Interferometric STEM-EELS

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1165 Exploring polar ordering in lead-free K0.5Na0.5NbO3 ferroelectrics using in situ biasing and 4D STEM techniques

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1166 The use of EELS for the characterization of diamond interfaces: the ohmic contact

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1167 Large-angle Lorentz 4D-STEM for Simultaneous Magnetic and Atomic Structure Mapping

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1168 Mapping the Space Charge Region in BaTiO3 and SrTiO3 using 4-Dimensional Scanning Transmission Electron Microscopy

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Direct observation of quadrupolar strain fields forming a shear band in metallic glasses

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1170 CARS microscopy for studies in skin physiology and pharmacology
Professor Jonathan Brewer[1], PhD Irina Iachina[1], MA Lomholt[1], Kim Nielsen[2], Morten Ebbesen[1]
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1171 Exploring Melanoma Dynamics: Insights from a 3D Cell Culture Model with Vemurafenib Treatment
Rikke Tholstrup[1], Nicoine Dorothea Daugaard[1], Daniela De Zio[2], Jonathan Brewer[1]
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Miss Analle Abuammar[1], Miss Odara Medagedara[1], Mr Aaran Vijayakumaran[1], Mr Vito Mennella[1], Mr Kedar Narayan[2]
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1174 The Impact of Dietary Haemoglobin on Nymphal Stages of I.ricinus: The gut volume EM reconstruction

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1175 In-situ liquid-cell dynamic TEM observations of liquid crystal nanocomposite phase transitions
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Prof. Paul Guichard

1177 Precise targeting for volume electron microscopy, a multimodal approach
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1178 Adding Dimensions to Intravital Imaging to Better Eavesdrop on Biology
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[1]Vice President of Science Grant Programs, Chan-Zuckeberg Initiative, , , [2]Elizabeth Garrett Chair of Convergent Bioscience University of Southern California, ,
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1182 Scanning patterns evaluations towards FIB-SEM/SIMS low-dose high-speed acquisition
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1186 Geological Materials and Bio-mineral systems
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Professor Kayla Nguyen[1]
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Dr. Peter Schweizer

1198 Data analysis workflows to measure material properties and structure using 4D-STEM
Colin Ophus[1], Stephanie Ribet[1], Georgios Varnavides[1,2], Steven Zeltmann[3], Karen Bustillo[1], Alexander Rakowski[1], Benjamin Savitzky[1], Min Chen[2], Yang Yang[2], Andrew Minor[1,2], Mary Scott[1,2]
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1199 Transdifferentiation of human dental pulp-derived mesenchymal stem cells into neurospheres and transplantation into aganglionic hindgut
Msc Adam Soos[1], MSc. Emoke Szocs[1], Bendeguz Sramko[2], Anna Abbas[2], Dr. Anna Foldes[3], PhD Karolina Pircs[2], Prof. PhD. Nandor Nagy[1]
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1200 Understanding how microalgae cells remediate heavy metals using Atomic Force Microscopy (AFM)
Dr Cécile Formosa-Dague[1], MSc Victoria Passucci[2], Dr Maria Mar Areco[2]

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1205 Revealing foliar nanoparticle uptake dynamics: integrating nano-CT, confocal microscopy and LA-ICP-MS insights

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1206 Streamlining Graphene Liquid Cell Preparation: VitroTEM's Naiad

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1208 Visualizing electronic correlations in quantum materials at millikelvin temperature

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1215 HR STEM study of anion/cation exchange in colloidal lead halide perovskite heterostructures

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1217 Multi-scale characterisation of laser-induced defects in the production of heterojunction photovoltaic cells

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1218 3D imaging, visualisation, and analysis services at Lund University Bioimaging Centre

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1219 An efficient method for quantifying the degree of neurodegeneration in an insect brain

Jakub Opelka[1,2], Lucie Pauchova[1], Andrea Bednarova[1], Radka Zavodska[1,3], Michal Sery[1,3], Ivo Sauman[1,2], Hana Sehadova[1,2]
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1220 Vacuum ultra violet emission spectra observed by newly designed grating with improved diffraction efficiency

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1221 Visualization of surface plasmon propagation and emissions by cathodoluminescence

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Dr Corneliu Sarbu[1], Phd Student Marian Cosmin Istrate
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1227 Ghostbuster – a phase retrieval diffraction tomography algorithm for cryo-EM particle refinement
Mr Joel Yeo[1,2], Dr. Benedikt Daurer[3], Dr. Deepan Balakrishnan[1], Dr. Dari Kimanius[4], Dr. Tristan Bepler[5], Dr. Yong Zi Tan[1], Dr. Duane Loh[1]
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1228 Heterogeneous dissolution of Au nanoparticles under constant electrochemical potential as observed via in-situ liquid-cell TEM
Dr. Sorour Semsari Parapari[1], Layrton José Souza da Silva[1,2], Miguel Bernal[3], Daniel Torres[3], Dr. Miran Čeh[1,2], Dr. Kristina Žužek[1,2], Dr. Jon Ustarroz[3,4], Dr. Sašo Šturm[1,2]
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1230 Tracking the Ultrastructure of Life with Serial Block-face Scanning Electron Microscopy
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1231 Self-assembled nanoparticles in a thin film of water
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1232 Structural organization of the native Neisseria meningitids PilQ environment through an MS-EM approach
Dr. David Fernandez-Martinez[1], Dr. Lucienne Nouchikian[2], Sylvie Goussard[1], Prof. Pierre-Yves Renard[3], Dr. Cyrille Sabot[3], Dr. Martial Rey[2], Dr. Julia Chamot-Rooke[2], Dr. Guillaume Dumenil[3]
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1233 Parent grain reconstruction of martensitic microstructures: a comparison of different methods
Dr. Barbara Šetina Batič[1]
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1234 Interactions between Titanium Dioxide Particles and Wood Cell Wall Ultrastructurea
Petr Svora[1,2], Sylwia Krystyna Svorová Pawelkowicz[3], Petra Ecorchard[4], Jan Duchoň[1], Alena Schieberová[5]
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1235 Global BioImaging: Imaging Networks Accelerate Collaboration, Exchange and Innovation in Imaging Science

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1236 Visualization of the Endosomal Fate of mRNA-Lipid Nanoparticles Reveals the Reason for Low Escape Rate

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1239 3D imaging by Array Tomography using benchtop SEM

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1240 Electrochemical sensors for detection of benzotriazole in water

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1241 Microstructural characterization of neutron-irradiated ITER specified tungsten grades

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1244 Volume Electron Microscopy: A Powerful Tool for Studying Embryonic Pineal Organ Development in Birds

Prof. Bogdan Lewczuk[1], Dr Natalia Szyrynska[1], Dr Magdalena Prusik[1]
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1248 TEM strain measurement methods on SiGe/Si films for accuracy tests

Dr. Roberto Balboni[1], Prof. Matteo Ferroni[1,2], Dr. Fabiola Liscio[1], Dr. Anacleto Proietti[3], Dr. Chiara Mancini[3]
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1249 FlexAble Labeling of Primary Antibodies with Fluorescent Dyes and Biotin for Multiplex Experiments

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1250 Comparative Analysis of Sample Preparation and Imaging Methods for Metallographic Examination of Archaeological Silver

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1251 Phase Transformation of GeO2 Glass to Nanocrystals via High-Temperature Annealing

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1253 Computational analysis of the anisotropy of SEM images to quantify nanowire verticality and surface roughness
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1254 Cathodoluminescent and Characteristic X-ray-emissive Rare-Earth-doped Core/Shell Protein Labels for Spectromicroscopic Analysis of Cell Surface Receptors
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1255 Observation of spontaneous fluctuations in product selectivity of the acetylene hydrogenation reaction in operando TEM
Dr. Christian Rohner[1], Mr Eugen Stotz[1], Dr. Alexander Steigert[2], Dr. Daniel Amkreutz[2], Prof. Dr. Rutger Schlatmann[2], Prof. Dr. Beatriz Roldan Cuenya[1], PD Dr Thomas Lunkenbein[1]
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1256 A systematic study on PtRu alloy composition for catalytic applications
Tonya Kloos[1], Dr. Ningyan Cheng[1], M.Sc. Miquel Vega Paredes[1], M.Sc. Alan Savan[2], Prof. Dr.-Ing. Alfred Ludwig[2], Prof. Dr. Christina Scheu[1]
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1257 Correlative light electron microscopy for improved investigation of subcellular GLUT4 distribution in human skeletal muscle
PhD student, MSc Kaspar Wredstrøm Persson[1], Christian T. Voldstedlund[1], Chris Neal[3], Judith M. Mantell[2,3], Erik, A. Richter[1], Paul Verkade[2], Thomas E. Jensen[1]
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1258 Synthesis, structural and electrochemical characterization of nanostructured Ir/TiO_2 for the oxygen evolution reaction
Yeeun Bang[1], Dr. Raquel Aymerich Armengol[2], Prof. Dr. Joohyun Lim[3], Prof. Dr. Christina Scheu[1]
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1259 Insights into the mechanisms of silver phase formation vial electrochemical liquid-cell TEM
Msc. Layrton José Souza Da Silva[1,2], Dr. Sorour Semsari Parapari[1], Monica Parpal Giménez[3], Mohammed El Marini[3], Prof. Dr. Jon Ustarroz[3,4], Prof. Dr. Sašo Šturm[1,2,5]
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1260 Analysis of transcription dynamics via single cell imaging of chemically inducible system in S. Cerevisiae
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1261 Combining 3D-ED and Z-contrast imaging: crystallographic structure solution of Ca2MnO3Cl
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1262 Sub-4-Å cryo-EM structure of apoferritin from a basic 120 keV TEM with thermionic electron gun
Dr. Alexander Neuhaus[1], Dr. Anja Blanque[1], Prof. Dr. Christos Gatsogiannis[1]
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1263 Nano-characterization of Sodium-ion cathodes fabricated using economically and environmentally friendly organic routes
Dr Ana M Beltran[1], Dr. Saul Rubio[1], Dr. Francisco J Garcia-Garcia[2], Mr Julio E de la Rosa[2], Dr Eva M. Pérez[2], Dr Isabel Montealegre-Meléndez[1], Dr. Cristina Arévalo[2], Dr. Juan G. Lozano[2]
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1264 Epitaxial LiNi0.33Mn0.33Co0.33O2 thin films as a model cathode material system for Li-ion batteries
Dr. Elena Tchernychova[1], Blaž Jaklič[2,3], Jan Žuntar[2,3], Gregor Kapun[1,4], Prof. Dr. Matjaž Spreitzer[2], Prof. Dr. Robert Dominko[1,4,5]
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1265 Investigating VSV-GP replication using TEM & STEM tomography of high pressure frozen, freeze substituted samples
Johannes Georg Wieland[1,2], Rebecca Habisch[1], Dr. Eva Puschmann[1], Dr. Mareike Elvert[1], Prof. Dr. Paul Walther[2], Dr. Jorge Soza Ried[1], Dr. Martin Dass[1]
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1266 ExpertPI: A Comprehensive Tool for Automated 4D-STEM Multimodal Analysis and Method Development
Mr. Petr Mareš[1], Mr. Vojtěch Homola[1], Dr. Robert Hooley[1], Dr. Zhongbo Li[1], Mr. Branislav Hesko[1], Dr. Daniel Nemecek[1]
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1267 Fatigue fracture behaviour of high strength steels under gaseous hydrogen
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1269 Understanding of electronic structure by combination of soft X-ray spectrometer with electron energy loss spectrometer
Dr. Cheng Sun[1,2], Dr. Anton Orekhov[2], Dr. Agata Plucienik[2], Dr. Ali Rinaldi[2], M.Sc. Nico Radde[2], Dr. Elena Willinger[2], Prof. Marc-Georg Willinger[2]

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1270 Structural studies of the cross-link mutant, ABC transporter BmrA by cryo electron microscopy

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1271 Atomic Structure and Electron Magnetic Circular Dichroism of Individual Rock-Salt Structure Antiphase-Boundaries in Spinel Ferrites

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1272 Unveiling the Mechanisms of Structural Reconstruction and Magnetic Evolution in NiFe2O4 During Phase Transition

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1273 Understanding and modifying cell wall permeability to facilitate cellular nanoparticle uptake

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1274 Low energy electron microscopy and spectroscopy of 2D materials

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1275 Embryonic and postembryonic development of arthropods: imaging from the cellular to the whole organism level

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1276 Visualisation of tetrahedral Li in the alkali layers of Li-rich layered oxides

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1277 Evaluating the Efficacy of Laboratory-Based X-ray Diffraction Tomography for Crystallographic Analysis in Pure Iron

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1278 Correlative-Cryo Microscopy to Characterise Bacteria-Nanopillar Interactions: Achievements and Challenges
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1279 3D chromatin architecture by volume EM
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1280 Cryo-EM structure of wild-type Orsay virus: preliminary insights into the assembly mechanism
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1281 Nanoscale X-ray imaging to Study Bacteria-Nanopillar Interactions
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1282 Correlative X-ray and Electron Microscopy Workflow for Investigating Grey Matter Lesions in Multiple Sclerosis
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1284 AI-driven microscopy for analysis of xenotransplantation models
Dr Courtney Wright[1], Professor Deniz Kirik[1]
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1286 SEM and mCT investigations on GDEs with Copper-Nafion coating for carbon dioxide reduction to ethylene
Dr. rer. nat. Lisa Christine Ehle[1], M. Sc. Vera Ubbenjans[2], Dr. rer. nat. Adrian Mikitisin[1], Prof. Dr. rer. nat. Joachim Mayer[1], Dr. Rer. Nat. Lisa Christine Ehle
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1289 Low-Bleaching Cryo-Light Microscopy with Immersion Objectives Improves Super-Resolution STED Imaging
Niko Faul[1], Maruthi K. Pabba[2], Yujie Zhong[1], Dr. Anja Engel[1], Prof. Dr. Heinz Koeppl[1], Prof. Dr. Cristina Cardoso[2], Prof. Ph.D. Thomas P. Burg[1]
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1290 Nanosized Ti-Ni composite
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[1]Shell Energy Transition Campus Amsterdam, Amsterdam, Netherlands, [2]Shell Technology Center Houston, Houston, USA
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[1]University Of Bern, Bern, Switzerland, [2]McGill University, Montreal, Canada
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1314 Evaluation

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1331 Screening of surface Fermi level pinning governs contrast of modulation-doped n-type GaN by electron holography

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1333 Localization of SHP-1 in Natural Killer Cells Across Education

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1334 In-situ Micromechanical Testing and Optical Microscopy of Native Human Ligamentum Flavum

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1335 Annular EDS in Transmission and TKD Combined for Chemical and Crystallographic Nano-Analysis in SEM

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1339 Effect of thin 2D support layers on the catalytic properties of catalysts

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1340 Catalytically active MoS2 support for hydrogen generation from seawater

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1342 Application of dual-beam microscope with TOF-SIMS in characterization of ceramic coatings deposited on filtration membranes

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1350 Global BioImage Analysts' society: CZI funded initiative and future association

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1351 3D Spatial Transcriptomics: A MERFISH Platform for High-Resolution Molecular Imaging

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1352 Coarsening mechanisms of Ni under operating conditions of Ni/YSZ solid oxide cell using Scanning 3DXRD

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1

Scanning electron microscopy reveals how plasma differentially ablates biopolymers and modifies surface characteristics of wood

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Poster Group 2

Introduction:
Cold plasmas, including glow discharge plasma, are reactive mixtures of charged particles that can be tailored to modify the surface characteristics of materials, without producing unwanted waste. In commercial applications, plasma surface processing is mostly employed to etch silicon for the production of integrated circuits and optical devices. Plasma etching of silicon requires applying a protective mask onto the substrate, followed by subjecting the assembly to a plasma to produce different microstructure such as trenches and channels. Scanning electron microscopy (SEM) allows for high-resolution imaging of the surface morphology of the etched structures, enabling detailed examination of features such as trench profiles, etch depths, and overall surface quality. This analytical technique provides valuable insights into the effectiveness of the etching process and helps in optimizing process parameters for achieving desired patterning outcomes in semiconductor fabrication. Plasma can also etch wood, creating specific microstructures on wood surfaces. Exposure of wood to plasma was shown to be effective in modification of important properties such as surface wettability, permeability and adhesion, thereby removing restrictions on its potential end-uses. However, unlike silicon, the etching process for wood differs due to variations in microstructure and chemical composition both between and within different wood species. Wood has a hierarchical cellular structure consisting of a blend of aromatic (lignin) and aliphatic polymers (cellulose and hemicelluloses, i.e. hollucellulose), and it is reasonable to assume that differential etching of wood will occur when it is exposed to plasma. While SEM has proven to be an excellent approach for studying wood anatomy, its application in detecting plasma-induced changes in cellular ultrastructure and polymeric components at uppermost surface layer of treated wood cell walls is not straightforward. The combination of poor conductivity of wood, difficulties in satisfactory sample preparation and conservation, along with inherent variations in cell wall composition and anatomy restricts the ability to detect such subtle changes. In this research, we conducted a comprehensive scanning electron microscopy study to address these limitations and illustrate how plasma modifies the ultrastructure of cell walls by etching of its polymeric constituents, thereby influencing the surface properties of wood in various applications.
Methods:
Various softwood and hardwood species were selected, and clean defect-free surfaces were meticulously prepared and exposed for prolonged periods of time to a glow discharge plasma generated from water vapor. SEM and chromatic confocal profilometry supplemented with wet chemistry and FTIR analyses were used to examine different cell types and wall layers of wood, and to assess changes in the lignin and holocellulose-derived sugar-content. The thorough high-resolution SEM examinations were conducted to detect if there would be differential etching of wood cell walls because of variation in the susceptibility of their polymeric constituents to degradation by plasma.

We developed a method to quantify the etching of wood cell walls by combining SEM and 3D confocal profilometry, allowing us to examine how applied plasma energy affects the etching of wood cell walls. Performance of plasma-treated surfaces was evaluated by application of coatings and bleaching agents. Additionally, SEM and Environmental Scanning Electron Microscopy (ESEM) were employed to assess the efficacy of plasma in eliminating fungi from infected wood surfaces, and to in situ observe living wood-decaying fungi in the microscope.
Results:
Our approach allowed us to observe that glow discharge plasma etched the cell wall materials at surfaces of wood, commencing by creating voids through etching pits and their membranes and borders in softwoods. SEM revealed that plasma preferentially etched holocellulose (cellulose and hemicellulose), from wood creating lignin-rich surfaces, making it possible to more easily see the distribution of lignin in cell walls. The areas of wood cell walls that were more resistant to etching coincided with regions that that are rich in lignin, for example middle lamella, S3 layer of secondary wall and concentric layers in Homalium. We showed that increased porosity produced by plasma treatment was responsible for greater accessibility of chemical reagents to wood surfaces, and could improve the performance of coatings applied on wood. Furthermore, plasma treatment etched hyphal walls of a blue-stain fungus. Hyphal walls contain chitin, a fibrous polysaccharide similar to cellulose, along with melanins, which are complex, dark-colored polymers. Breakdown of hyphae's walls and removal of melanin enabled the removal of discoloration from blue-stained wood and improved the effectiveness of a bleaching agent. Moreover, in situ ESEM showed promise as a way of revealing the details on modification of wood components by fungi or enzymes at cellular levels.
Conclusions:
Combining scanning electron microscopy with precise sample preparation effectively reveals plasma-induced changes on wood cell walls. This approach permits the detection of differential degradation of wood's cell wall polymers by plasma etching featuring preferential ablation of holocellulose, and higher resistance of lignin-rich cell wall layers. The differential ablation of cell wall polymeric components by plasma, in combination with SEM, could create opportunities to study the cell wall composition and ultrastructure of different plant species or varieties. Etching of pits membranes and borders, followed by ablation of intervening cell wall materials, is the mechanism by which surface porosity of wood increases. This is an interesting phenomenon that should be explored to scale-up plasma processing of wood and other bio-based materials to industrial level.

Keywords:

SEM, Plasma, Biopolymers, Wood, Surface

Reference:

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2

Investigating Mechanically Interlocking Molecules with the btp [2,6-bis(1,2,3-triazole-4-yl)pyridine] Motif.

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Poster Group 2

The world of mechanically interlocking molecules (MIMs) has gained providence since the 2016 Nobel Prize Awards, for which Stoddart and Sauvage received the prize for their pioneering work on molecular machines. Rotaxanes are becoming a more and more prominent MIM in the world of mechanostereochemistry due to their versatility and their never-ending potential. Rotaxanes comprise a ring and a dumbbell component; these components are noncovalently bonded, but to break these components apart, a covalent bond must be broken. This project intends to synthesise novel btp [2,6-bis(1,2,3-triazole-4-yl)pyridine] rotaxanes with a twist, as the synthesised rotaxanes will include various lanthanide metals., allowing for investigations through UV visible titrations and confocal microscopy. Previously, the Gunnlaugsson Group have successfully synthesised btp macrocycles and catenanes so that this project will be building on the foundations already laid. A key element to this project is the use of Scanning Electron Microscopy along with Atomic Force Microscopy to view the topology and the morphology of our btp ligands and pseudorotaxanes. Varying our solvents for drop-casting allows for different morphologies and structures to be formed and viewed using microscopy techniques. The work being presented will focus mostly on the results to date but also on the future work and techniques which will need to be implemented to gain a wider landscape of our intended molecules so they can be utilised in day-to-day life. This project aims to produce and create novel interlocking molecule materials for use within the electronic, imaging, and magnetic industries whilst also aiming to utilise these structures in the formation of 'all organic-based redox-active materials for use in batteries.

Keywords:

Rotaxanes, Lanthanide, Smallest molecular device

3

Nanotubes and Nanostructures of VS_2, WS_2, and MoS_2: Structural Effects on the Hydrogen Evolution Reaction

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Poster Group 2

Background
Vanadium disulfide (VS_2) emerges as a remarkable functional material, boasting myriad advantages that have propelled its widespread application across various industrial sectors. Its unique electronic and optical properties render it highly desirable for utilization in electronics, optoelectronics, and catalysis. Despite these advantages, the synthesis of VS_2 in specific morphologies, particularly as nanotubes, remains a significant challenge. The complexities associated with preparing various VS_2 nanostructures stem from the intricate control required over reaction conditions, precursor selection, and the delicate balance between promoting specific morphologies and preventing unintended structural transformations. This study aims to overcome these challenges in nanotube synthesis and unlock the full potential of VS_2 in cutting-edge technologies, promising advancements in diverse fields.

Methods
This study describes the preparation of novel VS_2 nanotubes with a unique structure, offering a comparative analysis with benchmark 2D materials MoS_2 and VS_2. The unique VS_2 morphology was explored through electron tomography and compared with VS_2 nanoflowers. Catalytic activity of the various nanotubes and nanoflowers was measured, complemented by a Density Functional Theory (DFT) study to attain a fundamental understanding of the catalytic activity's origin in the materials system. Highly crystalline WS_2 nanotubes and nanotriangles, MoS_2 nanoflowers, and defect-rich MoS_2 nanotubes were synthesized and thoroughly studied for their Hydrogen Evolution Reaction (HER) activity. The synthesis of VS_2 nanostructures depended on the solvent and vanadium precursor, resulting in nanotubes and nanoflowers.

Results
Characterization techniques, including TEM, SEM, powder X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS), confirmed the structures' crystallinity and composition. Electron tomography provided 3D insights into the VS_2 nanoflower and nanotube structures. Electrochemical performance, evaluated through linear sweep voltammetry (LSV) and Tafel analysis, demonstrated that MoS_2 outperformed WS_2 and VS_2, maintaining the catalytic activity order in both acidic and alkaline conditions. Electrochemical impedance spectroscopy (EIS) was employed to evaluate charge transfer resistance (Rct), with MoS_2 nanostructures displaying the smallest Rct. Surface area analysis indicated that MoS_2 nanotubes had the highest electrochemically active surface area (ECSA), attributed to strain and defect-rich surfaces. DFT calculations provided insights into the thermodynamic stability of nanotubes and nanoribbons, with VS_2 nanotubes exhibiting a lower energy of bending than MoS_2 and WS_2. Additionally, DFT calculations on hydrogen adsorption free energy (ΔGH) suggested that VS_2 basal planes have potential catalytic activity, contrary to experimental observations.

Conclusions

In summary, this study presents a comprehensive investigation of various transition metal dichalcogenides (TMD) nanostructures for HER activity. MoS_2 demonstrated superior performance, and despite its metallic-like nature, VS_2 exhibited lower activity. The combination of experimental and theoretical approaches sheds light on the factors influencing TMD catalysis, emphasizing the importance of morphology, defects, and strain in designing efficient electrocatalysts.

Keywords:

2D, Electrochemistry, Electron Tomography, nanotubes

4

Protein imaging in body fluids to understand Alzheimer's disease progression

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Title: Protein imaging in body fluids to understand Alzheimer's disease progression
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*: Presenting author
Theme: Protein imaging
Sub-theme: Neurodegenerative diseases
Keywords: atomic force microscopy, biomarkers, Alzheimer's disease, diagnosis
Abstract:
Aims: Alzheimer's disease (AD) is a slow but relentlessly progressing neurocognitive disorders that affects memory and cognition in individuals. The two principal proteins implicated in the pathology of AD are amyloid beta (A β) and tau isoforms whose total content can be quantified in cerebrospinal fluid
(and blood using commercially available biochemical assays. Thus, aggregated forms of A β and tau proteins are emerging as strong candidates as fluid biomarkers to monitor AD onset and progression.
Yet, single particle level differe nces in morphology of these shape shifting proteins in body fluids from
oligomers, and protofibrils to mature fibrils, which are also key indicators of disease stage, remain largely unknown and hence unavailable to clinicians.
Methods: In this talk, I will present results from a proof-of-principle study conducted at Empa in collaboration with neurologists at the cantonal hospital St. Gallen using liquid-based atomic force microscopy (AFM) to resolve and quantify the morphology of protein aggregates at a single particle level adsorbed on red blood cells (RBCs) and those present in CSF from patients at various stages of decline in memory and cognition.
Results: The AFM measurements revealed patient age and stage of neurocognitive disorder dependedependent differences in size, shape, morphology of protein aggregates on RBCs, referred to as physical
biomarkers. The prevalance of pathological fibrils on RBCs when correlated negatively with CSF- A β 42/40 ratio was observed to be significantly higher in A ββ-positive patients. Dense proteins crystals were detected exclusively on RBCs in patients aged 80 years and above, which could be indicators of late stages of AD. Next, we detected direct evidence for protein fibril length to be correlated with AD severity from AFM mapping of CSF from both AD patients and age-matched healthy controls. This new information suggests that ultralong protein fibrils in CSF could be a signature of AD pathology.
Conclusion: The findings highlight the diagnostic relevance of physical biomarkers obtained using nanoscale imaging under standard laboratory conditions. I will also share the next steps in our ongoing efforts to reliably resolve protein aggregates in blood, extract their chemical signature, and

discuss the merits and challenges in validating and integrating physical biomarkers as complementary data that can be used in the clinical diagnosis of AD

Keywords:

atomic force microscopy, Alzheimer's disease

5

Diamond based quantum sensing for detecting stress responses in living cells with subcellular resolution

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Poster Group 1

Diamond based quantum sensing for detecting stress responses in living cells with subcellular resolution

A. Elias Llumbet, N. Lin, A. Mzyk, C. Reyes San Martin, Prof. R. Schirhagl, University Medical Center Groningen,

- Background incl. aims

Free radical generation plays a key role in many different biological processes including cell communication, immune responses or ageing. Since free radical generation also occurs when cells are under stress, it is elevated in many different diseases. Unfortunately, free radicals are short lived and reactive and thus the use of free radical generation as a biomarker is limited.
My group has pioneered using diamond-based quantum sensing to solve this problem.

- Methods

The method is based on NV centers, a defect in diamond, which converts magnetic signals into optical signals. As a result, one can detect signals that are equivalent to T1 in clinical MRI but by optical means with a microscope. Since these signals are easier to detect than small magnetic signals, this technique enables measurements down to the single electron level. In a cellular context that means that we have subcellular resolution at the nanoscale.

- Results

Here I will show data from free radical measurements in living cells. I will further show our first clinical data we obtained with this technique. More specifically, I will show how we can use this technique to measure synovial fluid from arthritis patients and to assess the working mechanism of certain drugs[1]. In the second part of my talk, I will show the results on germ cells where we hope to contribute to a better understanding into male and female infertility.

- Conclusions

We are able to detect free radical generation in living cells with nanoscale resolution

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Keywords:

NV centers; Relaxometry; Nanodiamonds

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Anisotropic 3-D Dirac Semimetals for Designing Terahertz Sensing Nano-Antennas

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Poster Group 2

Background: The Terahertz frequencies are increasingly becoming an important spectrum for microscopy applications, especially used widely in near-field probes for nanoscale applications. Yet, materials that can interact and enable efficient manipulation of Terahertz radiation is rare. Recently, a class of materials called "Three-dimensional Dirac Semimetals" received widespread attention for their compatibility with the Terahertz frequency regime, such as cadmium arsenide. While the bulk cadmium arsenide is just a semiconductive glass, its topological surface exhibits strong Dirac semimetal properties that has strong interaction with Terahertz electromagnetic waves. We exploit the strong terahertz response of cadmium arsenide's topological surface to design efficient Terahertz metamaterial antennas for microscopy sensing applications.
Methods: First, the topological surface of cadmium arsenide has been modelled as an anisotropic conductive surface using the Fermi-Dirac Kubo formalism. Both linear and nonlinear Terahertz response could be included into the model, as such the potential of parametric amplification microscopy could also be explored. Next, the designs of various metamaterial antenna arrays that could give the highest resonant response as well as largest frequency bandwidth possible has been explored. Geometries of circular, rectangular and triangular - all micrometers in footprint - has been chosen for the designs. Both the structural design and the material properties are input into an electromagnetic solver for simulation. The polarizing ratio between horizontally and vertically-polarized terahertz waves has been used as a performance indicator to measure the ability of the antenna array to distinguish between two different channels of signals. Finally, the nonlinear performance of the antenna arrays is investigated by raising the optical pump intensity to higher levels while observing the corresponding change in the polarizing ratio.
Results: The minimum polarizing ratio of 20 dB is established as the baseline for efficient polarizing sensors. With this standard, it is found that the rectangular-shaped antenna array element gave the best performance in both magnitude and bandwidth. Polarizing ratio of 30 dB and 4 THz bandwidth are achieved for the rectangular-shaped antenna array. As for the nonlinear performance, owing to the high nonlinear Kerr coefficient of cadmium arsenide, moderate pump intensities and power levels of about 10 kW/cm2 are sufficient to modulate the Kerr material refractive index.
Conclusions: Three-dimensional Dirac Semimetals are prospective materials to construct efficient Terahertz sensing nano-antennas for microscopy applications. We have designed Terahertz metamaterial antenna arrays using cadmium arsenide. Polarizing ratios of up to 30 dB and 4 THz bandwidth is achieved using a rectangular-shaped antenna array. On top of that, high Kerr coefficients enabled low pump intensities, which can be used for parametric amplification microscopy techniques.

Keywords:

Terahertz, Near-field, Dirac Semimetals

7

EELS Compton scattering and the electronic structure of twisted WS2 bi-layers

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PS-10, Lecture Theater 5, august 26, 2024, 15:00 - 16:00

Electronic structure is fundamental to a large class of materials phenomena, including mechanical, optical, magnetic and electronic transport properties. Momentum based spectroscopies such as angle resolved photoemission spectroscopy (ARPES), electron-positron annihilation and Compton scattering are traditionally used to measure the electronic structure. Compton scattering in the transmission electron microscope (TEM) was first explored in the 1980s using electron energy loss spectroscopy (EELS). Despite early success it did not achieve widespread use, largely due to the limitations in EELS spectrometer detector efficiencies, and artefacts arising from Bragg scattering within a crystalline specimen. However, with modern advances in EELS spectrometers as well as computational techniques for modelling dynamical scattering artefacts many of the challenges facing EELS Compton scattering are arguably now resolved. EELS Compton scattering in the TEM provides several benefits over standard X-ray and gamma-ray Compton measurements, such as a higher spatial resolution and the ability to extract useful information from even poly-crystalline materials.

In EELS Compton scattering the incident electron beam undergoes an inelastic collision with individual electrons in the solid. The Compton signal appears as a broad peak in an EELS spectrum acquired at large scattering angles (i.e. high momentum transfer). The width of the Compton profile, which can be as large as several hundred eV, is due to the intrinsic momentum spread of the solid-state electrons. The Compton profile shape is therefore directly related to J(pz), i.e. the density of solid-state electrons with momentum component pz along the scattering vector direction. J(pz) provides a 1D projection of the electronic band structure in reciprocal space. We have applied EELS Compton scattering to examine the electronic structure of bi-layer WS_2, with a twist angle of 18^o between the two layers. The twist angle and the resulting electronic structure is known to control the optical, vibrational and electrical properties of TMD bi-layers. EELS Compton scattering is particularly suitable to this problem, since the low dimensional nature of the WS_2 flakes make it difficult or impossible to analyse the electronic structure using conventional methods.

The J(pz) acquired along the 10-10 reciprocal direction indicates that the electrons are more delocalised in the bi-layer compared to monolayer WS_2. Comparison with density functional theory (DFT) simulations reveal that the delocalization is due to a small amount (i.e. 0.1%) of electronic charge accumulating in the inter-layer region (see figure; electron density is in grey, S atoms in yellow and top and bottom layer W atoms in blue and red respectively). The inter-layer charge accumulates between overlapping W atoms, thereby screening the ‘wrong’ bonds generated by the twist angle. The charge accumulation is also accompanied by a local dilation of the inter-layer spacing. The results uncover the precise nature of twist angle on the electronic and structural properties of bi-layer TMDs.

Keywords:

TMD, twist angle, EELS, Compton

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Role of phonon and plasmon inelastic scattering on Bragg diffracted beam intensities

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

The strong interaction of an electron beam with matter has enabled 3D electron diffraction of specimens that are otherwise too small for X-ray analysis. For improved crystal structure refinement it is known that the Bragg diffracted beam intensities must be calculated dynamically, rather than kinematically. Inelastic scattering, especially low energy phonon and plasmon excitations with relatively large cross sections, are also unavoidable. Bloch wave models for high energy electron diffraction can however deal with only phonon losses phenomenologically, i.e. thermal diffuse scattering (TDS) is modelled by introducing an imaginary term to the crystal potential, which has the undesirable effect of depleting the electron beam intensity as it propagates through the specimen. Furthermore, there is as yet no method for including plasmons in Bloch wave calculations.

Here Monte Carlo methods are combined with Bloch waves to calculate the inelastic scattering due to phonons and plasmons. The Monte Carlo approach treats the incident electron as a particle, and relies on computer generated random numbers to estimate parameters such as the scattering path length and scattering angles. Since it assumes particle-like behaviour, it is only valid for highly delocalised scattering events such as phonon and plasmon excitations. In such cases the periodic nature of the incident electron in the crystal (i.e. Bloch wave) will be averaged out and therefore has no effect on the scattering. The diffuse scattering is simulated by rigidly shifting the Bragg diffraction spots over reciprocal space; the direction of shift and magnitude of diffuse intensity being determined by the inelastic scattering vector and cross section respectively. Simulated phonon and plasmon diffuse scattering distributions show striking similarities with experiment. For example, the TDS exhibit Kikuchi lines and bands (see figure), while the plasmon diffuse scattering shows ‘halos’ around the Bragg beams. Furthermore, unlike the phenomenological Bloch wave model there is no loss in electron beam intensity due to inelastic scattering.

The new Bloch wave model as well as experiment are used to determine the role of inelastic scattering on Bragg beam intensities. Energy filtered diffraction patterns were acquired from a 1990 Å thick, [110]-Si specimen at single and double plasmon energy losses, as well as at ‘zero’ loss (due to the limited energy resolution energy filtering cannot remove phonon losses). The unscattered beam intensity was found to decrease with increasing energy loss, although the Bragg diffracted beam intensities remained constant within the measurement error. It should be noted that the total intensity for each diffraction pattern was normalised and that no background subtraction was performed, i.e. the diffuse scattering around each Bragg reflection was included in the intensity for that beam. The same trends were also reproduced with the inelastic Bloch wave calculations. For crystal structure refinement the unscattered beam is often ignored and only the relative intensities of the diffracted beams are utilised. Both theory and experiment indicate that the relative intensities are unchanged provided that the diffuse background is not subtracted and correctly assigned to the relevant Bragg reflection. This highlights the importance of the diffuse background in energy unfiltered 3D electron diffraction. To ensure crystal structure refinement is robust to inelastic scattering the diffuse background must be included in the intensity measurements.

Keywords:

Bloch waves, phonons, plasmons

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Electrochemical lithium intercalation & exfoliation in 2D TMDs and its in-situ studies

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Poster Group 2

We developed a lithium ion battery intercalation & exfoliation method with detailed experimental procedures for the mass production of 11 two dimensional TMDs and inorganic nanosheets, such as MoS2, WS2, TiS2, TaS2, ZrS2, graphene, h-BN, NbSe2, WSe2, Sb2Se3 and Bi2Te3, among them 3 TMDs achieved mono- or double layer yield > 90%. This method involves the electrochemical intercalation of lithium ions into layered inorganic materials and a mild sonication process. The Li insertion can be monitored and finely controlled in the battery testing system, so that the galvanostatic discharge process is stopped at a proper Li content to avoid decomposition of the intercalated compounds. The intercalation strategy can also be used to tune 2D TMDs' physical and chemical properties for various applications. For example, we developed an one-step covalent functionalization method on MoS2 nanosheets for membrane fabrication, which exhibited excellent water desalination performance. For lithium intercalation mechanism, the state-of-the-art In-Situ Liquid Phase TEM is an ideal technique for identifying the phase changes during intercalation process. With self-designed electrochemical liquid cell utilized, we can directly vapture the dynamic electrochemical lithiation and delithiation of electrode in a commercial LiPF6/EC/DEC electrolyte, such as LiF nanocrystal formation, lithium metal dendritic growth, electrolyte decomposition, and solid-electrolyte interface (SEI) formation. Combining with other in-situ techniques, such as in-situ XAS, XRD and Raman, etc, the underlying lithium intercalation mechanism in TMDs were further investigated, which render us a comprehensive understanding of the intrinsic correlation between the intercalation process and TMDs layered structures.

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Keywords:

In-situ TEM, 2D TMDs

10

Development of biological specimen preparation methods for electron microscopy using sandwich freezing technique

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Poster Group 1

[Background]
Chemical fixation has been used for observing the ultrastructure of cells and tissues. However, this method does not adequately preserve the ultrastructure of cells; artifacts and extraction of cell contents are usually observed. Rapid freezing is a better alternative for the preservation of cell structure [1, 2]. Sandwich freezing of living yeast or bacteria followed by freeze-substitution has been used for observing the exquisite natural ultrastructure of cells [3, 4]. Recently, sandwich freezing of glutaraldehyde-fixed cultured cells, glutaraldehyde-fixed animal and human tissues, and living plant tissues have also been used to reveal the good ultrastructure of cells and tissues [5, 6]. Here we report the good method to reveal the fine ultrastructure of biological specimens using a sandwich freezing technique and freeze-substitution.

[Methods]
For freeze-substitution of living microorganisms or glutaraldehyde-fixed cultured cells, they were collected by centrifugation and the small amount of concentrated pellet (~0.02 µl) was placed on a copper disk (3 mm in diameter). Another copper disk was covered, picked the disks up with tweezers, and plunged into melting propane.

For freeze-substitution of living plant or glutaraldehyde-fixed animal and human tissues, they were sliced into 0.1 to 0.2 mm thick sections with a razor blade under a stereomicroscope. Place a small drop (~0.02 µl) of 20% BSA or glutaraldehyde solution on a copper disk. Then, use tweezers to place a piece of tissue in the glutaraldehyde on the copper disk, place a 0.1-mm-thick spacer, and cover it with another copper disk. They were plunged into melting propane.

The disks were then detached to expose the specimens in liquid nitrogen, and freeze substituted in acetone containing 2% osmium tetroxide at -80ºC for 2 days. They were embedded in epoxy resin, thin sectioned, stained with uranyl acetate and lead citrate, and observed in JEM-1400 electron microscope (JEOL, Tokyo).

For cryo-electron microscopy of viruses and macromolecules, apply 2 µl of virus or macromolecular suspension (1 mg protein/ml) on the microgrids which were made hydrophilic by treating them by glow discharge (10 Pa, 400 V, 1 mA) using an ion sputter apparatus. Remove excess liquid using filter paper and rapidly freeze the microgrid by plunging them into liquid ethane. Transfer the frozen grids in liquid nitrogen, set in a cryo-transfer holder cooled at liquid nitrogen temperature beforehand, and observe under an electron microscope at low temperature.

For conventional chemical fixation of biological specimens, the sample was fixed in 2.5% glutaraldehyde, post fixed with 1% osmium tetroxide, dehydrated in ethanol series at room temperature, and embedded in epoxy resin. They were thin sectioned, stained with uranyl acetate and lead citrate, and observed in JEM-1400 electron microscope.

[Results]
Figure 1 shows ultrathin sections of chemically fixed (a) and freeze-substituted (b) Escherichia coli (bacteria). By chemical fixation (a), cell walls and plasma membranes were undulated and not

smooth; each ribosome particles cannot be distinguished; so-called DNA area was recognized; and the whole cell body became small and considered to be shrunk. On the other hand, by freeze-substitution after sandwich freezing (the present method, b), cell walls and plasma membranes were smooth; each ribosome particles can be distinguished; so-called DNA area was not present and the whole cytoplasm appear homogeneous; and the whole cell body was not shrunk and appear natural.

Ultrathin sections of living Arabidopsis plant tissues were observed by sandwich freezing and freeze substitution and was found to achieve excellent ultrastructural preservation of cells and tissues. Ribosomes, endoplasmic reticula, nuclei, nuclear membranes, plastids, plastid envelope membranes, mitochondria, vacuoles, and vacuolar membranes were clearly observed. No ice crystal formation was observed in most of the cytoplasm, but small ice crystals were observed in the matrix of the nucleus.

Ultrathin sections of glutaraldehyde-fixed mouse tissues, glutaraldehyde-fixed human tissues, and glutaraldehyde-fixed cultured cells, were observed by sandwich freezing and freeze substitution. Ultrastructure of mouse kidney, heart, liver, pancreas, human skin, white blood cells, and K562 cultured human leukemia cells was clearly observed in natural state at high resolution. They all show excellent ultrastructural preservation, and the plasma membranes, nucleus, nuclear membranes, nuclear pores, nucleolus, mitochondria, Golgi apparatus, ribosomes, microtubules, filaments were clearly observed. No ice crystal formation was recognized and membranes were smooth and not undulated.

Sandwich freezing was also useful for observation of viruses and macromolecules by cryo-electron microscopy. Ice embedded influenza virus and hepatitis B virus core particles have been observed in natural state at high resolution.

[Conclusion]

Sandwich freezing-freeze substitution of biological samples was shown to be a good specimen preparation method by the present study. A new sandwich freezing device has been fabricated and is now commercially available (MW-SFD-01, Marine Work Japan, Ltd, Yokosuka, Japan, http://www.mwj.co.jp/en/). It is useful to rapid-freeze viruses, bacteria, yeast, cultured cells, plant tissues, animal and human tissues. It can freeze plant tissues to as deep as 50 µm, and animal and human tissues to as deep as 0.2 mm if tissues are fixed with glutaraldehyde beforehand. It may be better than HPF machine since it is inexpensive and portable.

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Keywords:

sandwich freezing, freeze-substitution, ultrastructure, method

11

Digital Holographic Microscopy of Pseudomonas aeruginosa to Probe Effects of Membrane Active Antimicrobials

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LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

Background: Digital holographic microscopy (DHM) is a unique label-free imaging technique that allows interrogation of a population of individual bacterial trajectories in 3D. An inline-holography approach was taken to discern the effect of the antimicrobial compounds on the motility of the bacterium, Pseudomonas aeruginosa. Bacterial swimming motility depends on membrane-embedded stators that couple an electrochemical gradient with the mechanical rotation of the flagellum. Hence, there is a tight link between the bacterial membrane and motility. We propose that this allows us to use 3D motility changes of P. aeruginosa as a proxy of membrane damage over time, which test by following changes in antimicrobial susceptibility as a function of the bacterial growth phase. Susceptibility to antimicrobials as the upregulation of the alternative sigma factor rpoS could increase resistance as the bacteria enter the stationary growth phase. Antimicrobials were adsorbed to surfaces, exposed to bacteria, and monitored for changes in motility.
Aim: To discern the effects of antimicrobial adsorbed surfaces on bacterial motility using DHM and test whether this links to membrane damage.
Methods: To demonstrate desorption of antimicrobials (chlorhexidine (CHX), cetylpyridinium chloride (CPC), 4-isopropyl-3-methylphenol (IPMP) and formulated (f-CHX)) from the adsorbed surfaces, they were exposed to constitutive light emitting P. aeruginosa CTX::tac'-luxCDABE. Any reduction in bioluminescence is indicative of membrane damage consistent with antimicrobial desorption. The motility profiles of both exponential and stationary growth phase wild type (WT) and rpoS mutant were visualised using DHM with 1000 frame (18 s) image streams being taken every 10 min over 1 h. A 685 nm laser illumination was used and a 20x/0.75 NA objective. Bacteria were tracked using bespoke LABVIEW scripts and reconstructed using MATLAB.
Results: Bioluminescence from the constitutive light emitting P. aeruginosa CTX::tac'-luxCDABE decreased over time with CHX and f-CHX exposure for WT and rpoS cells in both the exponential and stationary growth phase. This corresponded with changes in bacterial trajectories when cells were exposed to CHX and f-CHX which showed increased numbers of sharp turns, quantified as an increase in tortuosity. Stationary WT cells only responded to f-CHX adsorbed surfaces but displayed similar changes in motility. The rpoS mutants displayed increased tortuosity with CHX at both early exponential phase and stationary phase of growth. CPC and IPMP showed no change in luminescence (no deleterious membrane impact) and no change in tortuosity at the concentrations tested.
Conclusions: CHX and f-CHX readily desorb from surfaces, dissipating membrane potential which in turn impacts on bacterial motility. The rpoS gene is important for P. aeruginosa resistance to adsorbed CHX. These motility changes could be discerned through the label-free DHM technique.

Keywords:

Holography, Microbiology, Motility

12

Single Crystal Analysis of Nanocrystals by Three-Dimensional Electron Diffraction

Dr. Zhehao Huang[1]
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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

X-ray diffraction is the predominant method used for structural determination of crystalline materials. However, challenges remain on structural analysis of nanocrystalline materials which are too small to be studied by single crystal X-ray diffraction. Three-dimensional electron diffraction (3DED) has been developed to tackle the challenges. By taking advantages of the strong interaction between electrons and matter, 3DED allows single crystal structural analysis even when the crystal sizes are down to the range of nanometers[1].
In this talk, I will give an overview of the development of low-dose 3DED method for analyzing fragile materials, where we overcome the challenges of electron beam damage to the compounds such as metal-organic frameworks (MOFs) and covalent-organic frameworks (COFs). I will talk about the high-throughput advantage of 3DED on discovery of new MOF materials among phase mixtures[2], and discovery of unknown layer stacking behavior in a 2D COF[3]. By using 3DED, I will further talk about how to probe molecular motions in MOF nanocrystals[4] and study host-guest interactions[5]. Last but not least, I will present our recent development on further reducing electron dose for studying fragile unknown compounds. We believe that using 3DED as a powerful analytical tool for discovering new compounds, and revealing their unique properties at an atomic level would help to accelerate research in physical and life science.
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Keywords:

electron diffraction; electron crystallography

13

Enhancing Nanomechanical Properties of MXene through Tailored Surface Potential: PFQNM and Kelvin Force Microscopy Studies

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

2D MXene (Ti3C2TX) possess distinct characteristics such as high electrical conductivity, adjustable surface chemical properties, and mechanical flexibility. These features make them valuable as building blocks for energy storage devices, serving purposes like electrodes, ion transfer regulation, electrocatalysts, and electrodeposition substrates. Enhancing the mechanical strength of MXenes has been achieved by combining them with 1D nanocelluloses from their stable colloidal dispersions. However, the practical application of MXene materials faces obstacles due to their tendency to swell unfavorably caused by weak interactions between nanosheet layers, convoluted pathways for ion transport channels, and susceptibility to oxidation in aquatic environments, consequently impacting the performance of EC devices. The current challenge in optimizing MXene properties is to maintain the EC properties during synthesis. Poly(ionic liquid) treated heterostructure Mexne membranes assembly with layered double hydroxide nanosheets via solution-phase showed high permeability and selectivity, the interaction with the oppositely charged surfaces accerate electrostatic crosslinking between MXene neighboring nanosheets driving the enhanced stability in colloidal phase.1 Up to this point, there has been limited focus on nano-scaled mechanical studies using quantitative peakforce nanomechanical mapping (PFQNM) using a colloidal atomic force microscopy probe to measure the localized mechanical and nano-scaled force origins of HOLO cellulose nanofibers (HOLOCNFs) during interaction with Mxenes in a colloidal phase.

The PFQNM morphology results (Fig. 1.A) revealed a uniform distribution of the HOLOCNF network nanostructure, with HOLOCNFs tightly inserted into Mxene thin sheets. The height distribution of the composite indicated that the integration of HOLOCNFs reduced the roughness of the Mxene, enhancing the overall uniformity of the composite. Specifically, the roughness of the Mxene film (1×1 µm) was measured at 56.8 nm ± 71 pm, while that of the composite was 28.4 nm ± 69 pm. The fitted elasticity modulus (Derjaguin-Muller-Toporov model) for the composite was approximately 132.8 GPa ± 0.39 GPa, compared to approximately 27.6 GPa ± 13 MPa for pure Mxene (Fig. 1.B). The Young's modulus of the composite was approximately four times higher than that of pure Mxene films. Force spectroscopy was employed to quantify the adhesion force between HOLOCNF and the Mxene surface using a HOLOCNF-modified AFM probe (Fig. 1.C and D). Statistical Gaussian fitting of the adhesion force measured during the coated probe's departure (retraction) from the Mxene surface revealed a force that was 10 times stronger (17.2 nN ± 77 pN) than the adhesion force measured for the same Mxene film when a naked probe was used (1.5 nN ± 1.2 pN). These results clearly indicate that the force interaction between nanocelluloses and Mxene originated from Van der Waals attraction, with adhesion being a long-range force at a separation distance of 150 nm. Remarkably, the negative force measured in the force-distance (FD) adhesion region during tip retraction showed a significantly larger dissipation of energy (energy loss) compared to the dissipation observed when a naked probe (solid black curve) was used. The difference in dissipation energy can be attributed to the deformation of the monolayer of HOLOCNF attached to the probe, whereas no layer existed when the Si3N3 surface of the naked probe was measured against Mxene. The force-distance curves unveiled a mechanism of force interaction between the surface of Mxene and the surface of HOLOCNF attached to the AFM probe. The revealed force origin

elucidates the notable colloidal stability of the HOLOCNF-Mxene suspension during its synthesis procedure and provides quantitative information about the strength of attraction force when Mxene film interacts with nanocellulose during the functioning of Mxene films in real time. The strong adhesion force between HOLOCNFs originated from various chemical groups covering the entire nanocelluloses, which were retained during the mild synthesis of HOLOCNF, resulting in robust adhesion force against Mxene during tip retraction. In Fig 1 (E), the localized heterogeneous catalysis mechanisms occurring at the active sites of single layer Mxene and the residual chemical groups on the nanocellulose were in situ visualized by amplitude modulated Kelvin probe force microscopy (KPFM). The decreased surface contact potential (Φ) of the Mxene (1.7 eV) measured after its interaction with the HOLOCNFs in colloidal chemical phase, compare the surface potential of Mxene (4.7 eV). This result revealed the doping mechanism the formation of Ti−O···H− hydrogen bonding between MXene and the chemical groups on HOLOCNFs, which modulated the dipolarity of the MXene surface and thus decreased the work function. The results are agreed with previous report.2 In short, the PFQNM and KPFM results revealed relationship between the structure, nanomechanical and surface chemical properties of Mxene-CNF at a single-fibril level and nanoscale. A future study aims to comprehend the mechanisms involved in electrochemical catalytic reactions in the presence of CO2 using HOLOCNF-Mxenes as electrode in electrolytes.

Figure 1. Representative results of (A) PFQNM morphology and Peakforce error of the materials; (B) PFQNM height, elastic modulus using Derjaguin-Muller-Toporov contact model, and adhesion force of the materials; (C) (left)SEM of the HOLOCNF probe;(right) Force-distance curves (curves in red were measured using HOLOCNF colloidal probe); (D) Gaussian fitted adhesion force distribution of the HOLOCNF-Mxene and naked probe-Mxene. (E) KPFM surface potential (work function Φ) maps and fitted distribution of the maps for Mxene and Mxene-HOLOCNF obtained 50 nm lift height.

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Keywords:

SPM and AFM, Force spectroscopy

14

Elimination of Human Error during CPD Process in Plant Tissue Preparation for Electron Microscopy.

Anna Walkiewicz[1]
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Poster Group 1

Critical point drying is a powerful way of removing liquid from a sample without causing damage to its structure. Thanks to the fact that at the critical point both, liquid and gaseous states can coexist, their densities are equal and the surface tension between them is zero, we can transfer molecules from the liquid state to the gaseous state without penalty of changing the appearance of the sample. Its structure will remain intact and thanks to that we can observe samples in Electron Microscopy in the way they appear in their natural state.

Although the principles of critical point drying are simple, the process itself is more complex and requires careful design depending on the sample type. Samples, also require special preparation before undergoing Critical Point Drying. That involves tissue fixation and dehydration in a sequence of solvents like ethanol and acetone.
The tissue fixation procedure is a laborious and long process thus the following step, which is Critical Point Drying, should be free from possible failure.

Wrongly conducted Critical Point Drying can easily lead to sample structure damages, from big to small like structure collapse, shrinkage or just wrinkles. This might result in a disturbance in sample examination or make it completely impossible.
Structure corruption might be avoided by performing the Critical Point Drying process in an automated way, where every step is precisely and automatically controlled. That eliminates human error and gives certainty of the outcome.

Herein we present the influence of parameters on the outcome of plant tissue Critical Point Drying and a way of eliminating human error by designing a dedicated, automated process.
Quorum QDry- an automated Critical Point dryer was used for this study. Plant tissues of a tomato leaf Solanum Lycopersicon and a garden mint leaf Mentha spicata were chosen to present the influence of the amount of solvent-liquid CO_2 exchange cycles, settling time length, heating rate and pressure release rate on the sample structure appearance

Keywords:

sample preparation, CPD, SEM, Quorum

16

SEM-micro-photogrammetry and stereo reconstruction on the Tescan Amber X electron microscope

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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Three-dimensional imaging techniques are widely demanded in various fields including science, medicine, engineering, architecture, and art [1,2]. Combining these techniques with scanning electron microscopy capabilities yields interesting results and extends the capabilities of scanning electron microscopy beyond two-dimensional imaging, opening up new applications.
In our lab, we are exploring the potential of scanning electron microscopy to investigate the three-dimensional surface morphology of a variety of complex objects, from the surface of construction materials to the spatial geometry of micromachined parts and marine organisms.
One of the methods of three-dimensional reconstruction is photogrammetry, which has been actively used in recent decades, especially in the field of geodesy. Another method under study is stereoscopy, which uses a sophisticated algorithm to calculate the surface profile from a pair of images acquired at different tilt angles.
We use automatic data acquisition on the Tescan Amber X instrument thanks to the support of Python scripts (Shark SEM Advanced). It is shown that even quite large objects are suitable for correct scanning without the need for image stitching. Further, the Structure from Motion photogrammetry methodology implemented in AliceVision Meshroom and AgiSoft Metashape software packages is used to calculate three-dimensional models. However, it should be noted that this method requires not only an accurate dataset, but also significant computational resources. Ongoing work in this area explores the use of neural network-based neural radiance fields (NERFs) for on-the-fly reconstruction [3].
The stereoscopic method reconstructs the surface profile from two photographs taken at different sample angles, typically ±5 degrees or close to it. Unlike the implemented algorithms available in the literature and well-known commercial packages, our software can use gigapixel images, allowing the characterization of millimeter-scale regions with nanometer resolution and thus meeting and exceeding the ISO 4287-1997 standard in surface topography characterization.

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2. Scianna, A.; La Guardia, M. Survey and Photogrammetric Restitution of Monumental Complexes: Issues and Solutions—The Case of the Manfredonic Castle of Mussomeli. Heritage 2019, 2, 774–786.
3. Pepe, M.; Alfio, V.S.; Costantino, D. Assessment of 3D Model for Photogrammetric Purposes Using AI Tools Based on NeRF Algorithm. Heritage 2023, 6, 5719-5731.

Keywords:

3d, surface reconstruction, photogrammetry

17

Development state of the CEOS ground-potential monochromator

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

Electron energy loss spectroscopy (EELS) is a long established analytical method to investigate the chemistry as well as the electronic and optical properties of materials. There are several kinds of EELS, namely regards the energy of the primary electrons and if the geometry is in transmission or reflection. Each method provides distinct information about the sample. In particular, EELS often is integrated into an electron microscope adding spatial information to the spectroscopic signal with about the spatial resolution of the hosting microscope. In this respect, EELS in transmission at primary electron energies in the order of several 10 keV and above gained much popularity in the last decades due to the success of (scanning) transmission electron microscopy ((S)TEM). It evolved into a widespread characterisation method being able to deliver spectroscopic information at the atomic level.

The energy resolving power in modern instruments is often sufficient for just identifying the elemental composition of a material by absorption edge onset fingerprinting, and in many cases the respective bonding states can be investigated by looking at the edge fine structure. Nevertheless, a higher energy resolution would help to find very weak signals and make the fine structure less ambiguous. More importantly, a better energy resolution gives access to very small energy loss signals that otherwise would be shadowed by the fringe of the primary electron beam signal. There, most interesting information about the physical properties of the investigated specimen can be gained.

In spectroscopy using electrons, the energy distribution of the primary electron beam ΔE_0 is a major limiting factor for the energy resolving power of the spectrometer. Reducing ΔE_0 by means of a monochromator can enhance the energy resolving power of the system accordingly. Additionally, in (S)TEM, ΔE_0 in conjunction with the chromatic aberration of the imaging system is one of the limiting factors for the maximum spatial information transfer, especially at lower energies. Therefore, a monochromator can also help to enhance the spatial resolving power of (S)TEM instruments.

There are different types of electron monochromators; crossed electric and magnetic fields, called Wien filter or trochoidal monochromator, and electric or magnetic sector fields (prisms) combined in various geometries (α- or Ω-shaped, hemispheres, or using electron mirrors), leaving out more exotic schemes like radio frequency cavities for pulsed electron beams. To date, four different types of electron monochromators for (S)TEM instruments have been commercialised. First, the simple single-stage Wien filter and second, the more advanced double-stage Wien filter, both employing crossed electric and magnetic multipole fields and a straight optical axis. Third, the purely-electro-static Ω-monochromator using inhomogeneous sector fields bending the optical axis into an Ω-shape and fourth, the purely magneto-static ground-potential monochromator combining magnetic sector fields in such a way that the optical axis follows an α-shaped trajectory. For the first three cases the monochromator is added before the accelerator at low electron energies or at high potential. The fourth implementation is different in this respect. Here, the monochromator is added after the

accelerator at high electron energies or at ground potential. The latter implementation ties in with earlier concepts from electron spectroscopy, where the energy-filtered source and the energy analyser are at the same potential. In that case, only the interaction with the specimen causes energy differences and disturbing effects from instabilities of the accelerator can be avoided. Moreover, having the monochromator, the spectrometer and the specimen at ground potential is very beneficial from a technological point of view since additional high-tension feed-through and energy transfer are not necessary. Recently, with a ground-potential monochromator implemented in a dedicated STEM, an unprecedented energy-resolution has been demonstrated and employed for phonon and aloof-beam plasmon spectroscopy. All four presently used monochromator implementations have in common, that the optical design are orthogonal systems with single-section symmetry, that is, the optical axis and the dispersive trajectories are all situated in one section. The optical axis can be curved in one section but not in two sections as long as a perfectly manufactured system without tolerances is considered.

In this contribution, we present the development state of our new design of a ground-potential monochromator based on magnetic prisms in a three-dimensional arrangement. This design abandons the energy selection at the central symmetry plane and the optical axis is not only curved in one section but in two sections. This allows for a highly symmetric and compact design with a variable energy window but no mechanically adjustable energy-filtering slits. Fixed blocking blades in combination with optical deflectors reside at distinct dispersive planes making the system robust and flexible. The practical design fits primary electron energies from 30 keV up to 300 keV and provides an energy resolving power of better than 2·10−7 with respect to the primary electron energy. Also, the resulting beam cross-over after the monochromator is free of residual spatial or angular dispersion. Very importantly, the new monochromator is designed to be potentially retro-fittable to existing microscopes, adding about 33 cm to the total height of the microscope column.

Keywords:

EELS, monochromator, TEM, STEM

18

Miniaturized material testing devices for multimodal in situ microscopic studies

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Poster Group 2

To contribute to the understanding of failure and fatigue, we examine the local microstructural strain/stress peaking as well as crack initiation/propagation by in situ methodology. Moreover, systematic errors in load/displacement of the measuring setup may be overcome by highly localized strain measurements, e.g. high-resolution EBSD in conjunction with image correlation. This requires in situ material tests, and we aim at two complementary goals. One is to contribute to alloy development and to optimize microstructure of selected alloys determining the macroscopic mechanical properties.
The second is to validate modern microstructure-based FEM simulations and to develop termination criteria for classical FEM simulations, e.g. for crack initiation. Therefore, the Mechanical Engineering Department develops in situ testing machines for static and dynamic tests. Latest developments are a portable, miniaturized 3-point bending machine for use in FIB/SEM/XRD (Fig. 1 a). Thos enable the direct observation of microstructural changes like crack initiation/propagation, as well as phase transformations, e.g. in steels. As an example, the bending machine (750g, 100x100x50mm) with independent load/displacement sensors allows for massive plastic deformation at loads up to 4kN. Our solution includes a user-friendly interface to lower the entry barriers for beginners (Fig. 1 c). In this contribution, we highlight the possibilities to observe dynamics of crack initiation/propagation, phase transformations, and the local evolution of the microstructure during testing of materials like Al alloys and austenitic steel. Electron backscatter diffraction (EBSD) is employed to in detail understand the crystallographic structure adjacent to the crack (Fig. 1 b). Direct image correlation is used to examine local strain peaking. Future plans involve establishing high-resolution EBSD instrumentation and data analysis for highly localized strain/stress/GND mapping as well as corresponding XRD data during a single experiment.

Keywords:

Multimodal, InSitu, Videomicroscopy, Material testing

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Using the YOLO DeepLearning algorithm to quantify the rAAV empty/filled ratio from Cryo-EM imaging

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Poster Group 1

Recognized as one of the most common vehicles for gene therapy, rAAVs offer numerous advantages such as low pathogenicity, the ability to precisely redistribute the genome, high tissue dispersal, high infectivity, and long-term persistence in cells [1, 2, 3]. The rAAVs are small (25 nm diameter), single-stranded DNA parvoviruses that are non-infectious for humans [4]. Their capsids are made up of an assembly of 60 viral proteins with icosahedral symmetry and contains the genome. When the rAAV enters the cell, it releases the genetic material contained in the capsid, leading to gene therapy treatment. At present, the rAAV production methods used for clinical trials inevitably lead to a mixture of empty and filled virions, present in varying proportions [5]. Consequently, empty vectors (without genetic material) or vectors with erroneous genetic information (partial or double genetic material) co-exist with vectors containing the correct genetic information (called filled vectors). These empty vectors are unable to provide therapeutic effect and acts as an impurity that can generate an immune response or inhibiting transduction, while partially/doubled filled vectors can cause a dysfunctional gene and lead to side effects. It is therefore important for pharmaceutical research to quantify the empty/filled ratio before bringing a drug to market. [6]

There are several ancestral characterization methods for integrity analyses and the quantification of empty/full ratio of rAAVs. Among these methods, cryo-EM microscopy is of particular interest for quantification, as it enables particles to be visualized without the addition of dyes. The contrast is therefore linked to the atomic composition of the particles: the presence of the genome means that filled particles are more contrasted, as empty and intermediate classes can then be determined. To use the power of cryo-EM to determine the level of empty and filled capsids, it is important to develop software tools for post-acquisition image processing. This tool development faces several challenges stemming from the nature of cryo-EM imaging. Sample purity, cryo-plunging conditions, spatial projection and acquisition parameters are all variable parameters that explain the difficulty of development of computer-aided detection methods for accurate capsid detection. As a result, many parameters such as contrast, size, gray scale values and texture must be defined by the user, given the dependence of these characteristics on image background noise and image quality.

In this study, we present a new home-made method for detecting and quantification of empty/full ratio of rAAV capsides in cryo-TEM images. First, the capsides are detected with a popular deep learning architecture, YOLO. Then, the detected rAAVs are analysed according to their image intensity projections and assigned a fullness score. The accuracy of this methodology is then compared to Relion determination, depending on various factors including sample purity, concentration, batch or even variation in plunging and acquisition conditions. These new methodological developments open the door to faster, more precise quantification of rAAVs for pharmaceutical research.

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Keywords:

rAAV, cryo-EM, quantification, deep learning

20

The behavior of additive manufactured aluminum alloys upon anodization

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Poster Group 1

Background incl. aims
Additive manufacturing of aluminum alloys, i.e. laser powder bed fusion (3D printing), introduces significant versatility and innovation to various industrial applications. Al-Fe-Zr alloys, which are suitable for additive manufacturing, exhibit mechanical properties comparable to conventionally cast aluminum alloys that are alloyed with Mg, Mn and Si, such as the 60xx series Al-alloys. Anodization of aluminum alloys is performed to enhance their chemical and mechanical performance by the formation of a protective anodic aluminum oxide (AAO) layer. The absence of those 60xx series alloying elements in Al-Zr-Fe, which typically form voids in AAOs and alter the structural integrity, suggest a distinct anodization behavior. This might lead to improved chemical and mechanical properties of the grown passivation layer.
Additionally, the overall microstructure of 3D printed alloys inherently differs from conventionally casted, processed and artificially aged microstructures of the same alloy. Here, a variety of elongated and equiaxed grains are observable along the printing direction. This disparity underscores the necessity for a comprehensive comparison of differently treated microstructures prior to anodization. Afterwards, the anodization behavior of Zr- and Fe-enriched precipitates are investigated to ascertain their impact on the chemical resistivity of the resulting AAOs.
Methods
The microstructure of the Al-Fe-Zr alloy was modified through changes in the manufacturing method, varying degrees of severe plastic deformation and adjustments to the heat treatment before anodization. Polished sample surfaces are analyzed with EBSD and subsequently anodized under galvanostatic conditions in oxalic acid solutions following a standardized protocol which allows to determine the impact of different precipitates' sizes and electrochemical potentials. All AAO-coated samples are bisected using a wire saw, which each half treated independently thereafter. The first half is directly coated with Cr/Au to enhance electron conductivity for TEM lamella preparation in FIB whereas the other half was exposed to reactive ion etching to study the chemical resistivity of the AAO.
TEM samples are prepared by employing Ga-, Si/Au- or He/Ne-FIB on randomized surface positions. The AAOs were analyzed in a Gatan Double Tilt LN2 Cooling Holder 636 using a Titan Themis 60 – 300 operated at 300 kV and at a temperature of 96 K. EDX spectra are recorded with a quadrupole SuperXG2 detector. A CCD-GIF camera is used to record nano-beam diffraction patterns (NBDPs), also denoted as 4D-STEM. Beam damage is suppressed by using beam currents as low as 10 pA during NBDP acquisition.
Results
Given the difference of current densities during anodization of various types of microstructures while maintaining the standardized protocol, the analysis of various NBDPs of different precipitates – each with a stack size of at least 10000 NBDPs – suggests a size-dependent anodization behavior of Fe-enriched precipitates and underscores the influence of the underlying microstructure and the participating precipitates on the grown AAO thickness. Using 4D-STEM and fluctuation electron microscopy, various oxide types within the AAO were distinguished and information about the

precipitates' structure and chemistry are correlated, which supports the hypothesis of size- and type dependent influence. Ga-contamination due to FIB preparation at grain and phase boundaries was diminished by changing to Si/Au or He/Ne FIB for the final polishing step. Finally, we attribute the microscopic differences in AAO growth to macroscopic properties such as chemical resistivity.
Conclusions
Based on the results, Fe-rich precipitates remain partially unaffected by anodization, depending on their size and type, while Zr-rich ones are anodized with the matrix. Unlike conventional alloys, Al-Zr-Fe alloys do not develop voids within the AAO, which leads to a distinct chemical resistivity. Moreover, the microstructure is affecting the properties of the AAO. This emphasizes the importance of selecting an advantageous microstructure tailored to the intended application of the protective layer.

Keywords:

Anodization, 4D-STEM, cryogenic measurements, precipitates

21

Local mobility and atomic structure in Pd- and Zr-based bulk metallic glasses

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Poster Group 2

Background incl. aims
Metallic glasses are a category of materials not completely understood yet. Especially the glass transition mechanisms and the origin of structure-property relations are unclear and gaining deeper insights is of great interest. With a detailed analysis of the local structure and atomic mobility in two model systems of bulk metallic glasses, the possibility of observing atomic processes that govern basic glassy mechanisms can be sought after.
Methods
The here investigated Pd- and Zr-based materials are model glass systems with high kinetic stability and superior mechanical properties, respectively. They are prepared by copper mould casting and verified for their amorphous phase by X-ray diffraction. Further characterisation in terms of thermal properties is done by differential scanning calorimetry. Microstructural investigations are based on electron microscopy techniques: In TEM mode, the method of Electron Correlation Microscopy (ECM) is used to probe the local dynamics and structural relaxation times of Pd- and Zr-based metallic glasses. ECM gives further insights into the underlying mechanisms of amorphous phases with nanometer spatial resolution. Experiments are performed both at room temperature and elevated temperatures using FIB-prepared lamella with a Protochips Fusion AX in situ heating holder. Complementary structural investigations concerning the medium range order (MRO) in glassy metals are performed by 4D-STEM fluctuation electron microscopy (FEM), evaluating the normalized variance of speckles in nanobeam diffraction patterns.
Results
ECM yields a structural relaxation time and a stretching exponent from fits to intensity correlations of dark-field images over time. The structural relaxation time is a measure of local mobility in the material and was found to be different for the two investigated material systems, attributed to their different compositions. In addition, the two material systems react differently to the electron beam during the ECM measurements, where the determined stretching exponent might help to understand the underlying dynamics mechanisms in more detail. Comparisons of ECM-determined parameters from room temperature experiments and measurements at elevated temperatures also show a difference between the two material systems, which are connected to the thermal stabilities of each metallic glass. Moreover, the MRO investigated by fluctuation electron microscopy shows different MRO sizes and volume fractions for the two material systems. A comparison of these findings with thermal parameters resulting from macroscopic measurements is performed.
Conclusion
The conducted investigations show clear differences between the two material systems in the local dynamics as well as the atomic structure. These deeper microstructural insights from electron microscopy measurements at various, also elevated temperatures complement the results of macroscopically averaging methods of thermal and mechanical investigations. Combining the findings allows to better understand the glassy state and beam-induced dynamics including underlying mechanisms.

Keywords:

Metallic Glass, Structural Relaxation, ECM

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Seeing the nanoscale dynamics in gas-based processes by using in-situ TEM

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Poster Group 1

Functional nanostructures are often transformed through gas-based processes such as restructuring, oxidation, and sublimation. This process is called plasma-less gas-phase etching and is particularly important to achieve damaged-free sub-10-nm metal recess to facilitate the integration of metal interconnects with upcoming logic or memory components, crucial for advancing future microelectronic devices towards higher performance and miniaturization. The process involves gas-phase oxidation and subsequent sublimation where the liquid phase etching usually struggles due to poor diffusion in deep and narrow trenches. Furthermore, it provides precise depth control and reduced sidewall damage, making it advantageous over plasma-based or liquid chemical etching for metal recess or etching narrow trenches in microelectronics. However, understanding the detailed dynamic pathways of these transformation processes in gas environments is lacking, yet it is crucial for optimizing such etching techniques. In this work, we tracked the details of a densely packed 32-nm-wide Mo-metal lines recess by using the gas-phase in-situ TEM method. Specifically, the nanoscale modifications in the densely packed 32-nm-wide Mo-lines induced by the gas environments were observed in real-time. In this talk, I will discuss some of this research exploring the previously unknown intermediate steps of the restructuring of Mo-lines under a gas environment. The obtained dynamic insights possess a great impact on the development of semiconductor nanodevices.

Keywords:

In-situ TEM, Nanoparticles, Metal interconnects

23

Single particle CryoEM structures using iDPC-STEM from 4D-STEM detectors at near-atomic resolution

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

CryoSTEM can image frozen-embedded biomolecules in focus without an oscillating contrast transfer function, enabling complete information transfer in each image. Using integrated differential phase contrast (iDPC)-STEM, one can obtain three-dimensional reconstructions of proteins using single particle analysis. We have recently demonstrated how iDPC-STEM can be used to reconstruct protein structures to near atomic resolution (Lazic et al. Nat.Meth. 2022), depending on the chosen convergence semi angle (CSA) of the incident beam. During scanning the electron dose rate is orders of magnitude higher compared to parallel illumination as the dose is distributed in a very small area (Ang2) and for a very short time (50 ns – 10 µs). We compare beam damage of conventional TEM and iDPC-STEM on the same frozen-embedded protein sample. We will demonstrate how this technique can be further applied to a variety of test samples and how alternative ptychographic image reconstruction methods such as single side band (SSB) can be used to further enhance the image information.
iDPC-STEM using segmented detectors is an approximation for integrated center of mass (iCOM) reconstructions. Therefore, we recorded pixelated 4D-STEM data of frozen-embedded protein molecules with a 300 kV Titan Krios G4i equipped with a fast pixelated 4D-STEM detector (Dectris Arina), derived the corresponding iCOM images and reconstructed protein structures to near-atomic resolution.

Keywords:

CryoEM, iDPC-STEM, 4D-STEM, SPA

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The Best of Both Worlds: Combining Label Free and Fluorescence Imaging with Livecyte

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Poster Group 1

Fluorescent imaging can reveal much about cellular function but can also be a double-edged sword with both phototoxic and cytotoxic consequences that can impair normal cell function or even induce cell death. Livecyte's label-free live-cell imaging is a powerful quantitative phase technique, generating high-contrast, fluorescence-like images using low powered illumination in which cells appear as bright objects on a dark background. The enhanced contrast increases the robustness of single-cell segmentation and tracking without the need for dyes, maintaining true physiological conditions over a long period of time. Additionally, Livecyte offers the best of both worlds: - intermittent fluorescence imaging reduces the potential detrimental effects and can be linked to the more frequent non-perturbing label-free images.

Our previous studies have reported the detrimental effects to normal cell division of the nuclear live-cell imaging stain SiR-DNA when compared with non-perturbing label free imaging on the Livecyte. The inclusion of SiR-DNA reduced both cell count and total dry mass in a LED power dose-dependent manner relative to non-illuminated and unlabelled conditions suggesting fluorescence altered cells natural growth and proliferation rate. Alongside this, SiR-DNA and illumination increased median cell area which correlated with changes in average dry mass per cell compared to controls. Being able to look at multiple parameters indicates a cell profile of phototoxicity with slowed division and accumulation of mass leading to a large, oversized phenotype.

Such effects will be even more enhanced when dealing with sensitive primary cells such as T-Cells and Neuronal cells. We now show how we can track neuronal outgrowth completely label free, by a unique combination of brightfield and Livecyte label free technique, and how an advanced T-Cell assay can be built in which the sensitive primary effector cells remain entirely label free.

T cell killing assays traditionally yield little or no information on the T to Target interactions and would rely on fluorescence labelling of photo vulnerable primary T cells. However, Livecyte can gain a plethora of T cell interaction kinetics with target cells including the time and number of T cell contacts with a target cell before cell death occurred. All of this without having to label T cells giving more meaningful insights on how your engineered T cells are behaving and more predictive information for in vivo studies.

Livecyte's label free imaging and analysis has also set a new precedence for neurite outgrowth assays. Neuronal cells are also more at risk of phototoxicity limiting our ability to image over long periods of time and to look at network formation dynamically. With Livecyte's advanced analysis recipe, neurite outgrowth can be quantified from the initial changes in cell morphology and migration as cells form attachments through to network formation and metrics such as the number of branching points and total neurite length, gaining a novel understanding of the effect of therapeutic drugs on the dynamics of neurite outgrowth.

There is mounting evidence revealing multiple off-target effects and phototoxicity caused by fluorescence excitation probes with reactive oxygen species being the predominant cause of phototoxicity [1,2]. Livecyte versatility enables either non-invasive QPI only or with a possibility of intermittent fluorescence imaging producing a plethora of time sensitive single-cell information where there is a strong need to protect the cells. This provides invaluable data to researchers using fluorescence to investigate subtle changes in cell behaviour and phenotype.

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Keywords:

live-cell label-free, QPI, Killing-Assay, Neurite-Outgrowth

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The Electron Bio-Imaging Centre (eBIC) at Diamond Light Source, UK

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Poster Group 2

Three-dimensional electron microscopy (3D EM) includes multiple techniques for studying large macromolecular assemblies and cellular machinery. 3D EM provides the ideal tools for discovering the structures of individual proteins and small complexes in vitro, as well as understanding their function in a larger cellular context in situ. State-of-the-art electron microscopy is often too expensive and technically demanding for individual labs to house and operate. For this reason, the Electron Bio-Imaging Centre (eBIC) was established as the UK's national centre for cryo-electron microscopy (cryoEM) following the award of a £15.6 million grant from the Wellcome Trust, the Medical Research Council (MRC) and the Biotechnology and Biological Sciences Research Council (BBSRC).

eBIC's vision and mission:
- Establish state-of-the-art facilities for cryo-electron microscopy (cryoEM) and cryo-electron tomography (cryoET)
- Provide 24/7 free user access to high-end microscopes through peer-review process
- Develop cutting-edge technology with in-house research program
- Grow and train cryo-EM user community
- Foster integrated structural biology within Diamond and beyond

eBIC is a CryoEM center providing scientists with state-of-the-art experimental equipment and expertise in the field of cryo-electron microscopy, for single particle analysis, electron tomography and electron diffraction. The location of eBIC enables scientists to combine their techniques with many of the other cutting-edge approaches that Diamond offers.

Currently eBIC houses five Titan Krios microscopes, a Talos Arctica, two Glacios, a Scios and an Aquilos cryo-FIB/SEM, and a Leica CryoCLEM.

Keywords:

cryo-EM, cryo-ET, micro-ED, community, training

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XRD and TEM study of the quasicrystalline phase in pellets consolidated using spark plasma sintering

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Poster Group 2

The production of quasicrystal powders by gas atomization has been commercialized. Among various quasicrystals synthesized, Al-Cr-Fe and Al-Cu-Cr-Fe quasicrystals are very promising materials for coatings and reinforcements in composites due to their high hardness, good wear and corrosion resistance and the relatively low cost of their constituent metals [1]. As these quasicrystals are metal based, the behavior of bonding between the quasicrystalline reinforcement phase and the metal matrix is superior to that for ceramic phase reinforced metal-matrix composites. However, long duration sintering can result in phase changes and decrease the beneficial properties of the quasicrystals in the composites.
In our study, we employed spark plasma sintering to shorten the heating duration for the Al-Cr-Fe and Al-Cu-Cr-Fe quasicrystal powders and thus minimize the chance of phase changes when fabricating quasicrystalline reinforced composites. The phases of the sintered compacts were analyzed using x-ray diffraction. The microstructure of the quasicrystalline phases was further studied using high resolution transmission electron microscopy.
Experimental results showed that even when the heating duration was as short as 30mins during spark plasma sintering at 650ºC, phase changes could still occur. X-ray diffraction patterns showed peaks from the decagonal Al-Cr-Fe phase while HRTEM images revealed that in decagonal Al-Cr-Fe quasiperiodic planes were periodically stacked along the 10-fold axis with a periodicity of about 1.2nm. Spark plasma sintering of icosahedral Al-Cu-Cr-Fe and pure Al blended powders was conducted at 450ºC for 10mins and no phase change was detected using X-ray diffraction or high resolution electron microscopy. This implies that spark plasma sintering is effective in fabricating quasicrystal reinforced Al-based composites.

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Keywords:

quasicrystal, spark plasma sintering

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In vitro genotoxic activity of leaf and flower extracts of four alien invasive plant species

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Poster Group 1

Background and aim
Alien invasive plants are rich in specialized metabolites presenting promising potential as a valuable reservoir of bioactive phytochemicals applicable to the pharmaceutical sector. A critical requirement for their practical application is their non-toxicity. This study aims to assess the genotoxic activity of leaf and flower extracts of Ailanthus altissima (Mill.) Swingle, Robinia pseudoacacia L, Helianthus tuberosus L., and Solidago canadensis L. from Istria (Croatia) by the comet (single cell gel electrophoresis) assay.
This study is performed in the framework of the project "NATURe as an ALLY: Alien Invasive Plants as Phytopharmaceuticals— NATURALLY" (IP-2020-02-6899) and "Young Researchers' Career Development Project–Training New Doctoral Students" (DOK-2021-02-3094) founded by Croatian Science Foundation. The study aims to propose a new model for exploring new invasive alien plant species in provisioning (medicinal) ecosystem services on the pilot territory of Istria (Croatia). This study represents the first of its kind in the region since it's known that the phytochemical composition and biological characteristics of plants depend on the abiotic and biotic traits of their habitat.
Methods
Plant extracts were prepared using finely minced air-dried leaves and inflorescences collected and pooled from 15 locations in Istria and solved in 2% dimethyl sulfoxide (DMSO) in Endothelial Basal Medium-2 (EBM-2, Lonza, San Diego, CA). The ability to induce in vitro genotoxic effect in human liver-derived endothelial cells (HLEC) was assessed for three extract concentrations: 1.0 mg/ml, 0.5 mg/ml, and 0.1 mg/ml. Comet assay was stained with GelRed Nucleic Acid Gel Stain, visualized with Zeiss Axioscope 5 fluorescence microscope, and analyzed using the software KOMET5 (Kinetic Imaging Ltd., Liverpool, UK). The tail DNA percentage (% tDNA) was used as the primary measure of DNA damage.
Results
The treatments with leaf and flower extracts of all four species in concentrations of 1.0 mg/ml resulted in a significant increase of % tDNA compared to the control. Additionally, the leaf and flower extracts of A. altissima and leaf extract of R. pseudoacacia in 0.5 mg/ml also increased % tDNA.
Conclusions
These findings suggest that leaf and flower extracts in concentrations below 0.1 mg/ml do not cause substantial DNA damage in human hepatocytes, indicating a promising avenue for further research regarding the potential use of these extracts in pharmaceutical applications.

Keywords:

Comet_assay, DNA_damage, plant _extracts, invasive_plants

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Examination of the Thermal and Morphological properties of Spartium Junceum Fibers for Flame Retardant Purposes

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Poster Group 2

Fibers isolated from the Spartium junceum L. – plant which is indigenous to the Mediterranean area (SJL), belongs to a group of the plant bast fibers. Taking into the account previous research, it is proven to be an excellent fibrous raw material which, among others things, can play a role as a reinforcement in the production of fiber-reinforced composite materials. Nowadays, due to the increasing environmental awareness and the need to reduce negative impact of production by-products on climate, there is an effort to develop new materials from sustainable sources. Such materials can be functionalized through targeted ecologically acceptable surface modifications. The fire risk control is a very important concept in all branches of industry, especially in the automotive where the property of reduced flammability of the materials is very desirable. In order to meet such demands bast cellulosic fibers that are easily flammable need to be modified. In this resea3rch, SJL fibers were specifically treated with silicon dioxide, aluminum oxide and titanium dioxide nanoparticles that were fixed on the fiber surface using microwave irradiation or contact heat transfer (hot press). SiO_2 (SiNPs) and Al_2O_3 (AlNPs) nanoparticles were produced by laser ablation in liquid using a high power laser which ablates the metal plates without surfactant or aggressive chemicals addition. SJL fibers were treated with 0.025% aqueous solution of SiO_2 and Al_2O_3 nanoparticles and 2% of TiO_2 (TiNPs) nanoparticles, respectively by using ultrasound technique. Examination of the thermal and morphological properties of functionalized fibers were performed using thermogravimetric (TGA), microscale combustion calorimetry (MCC), scanning electron microscopy (SEM) & energy dispersive spectroscopy-EDS (backscatter electron and X-ray technique (BEX), X-MaxN 150 detector, ULTIM Max 100 detector and Ultim Extreme detector). For the samples fixed with the contact heat transfer heat release and thermal decomposition results are exhibit same properties as untreated sample and were therefore excluded from the further analysis. The best results were achieved by treatment with Al_2O_3 and TiO_2 nanoparticles which were fixed to fiber surface with microwave irradiation. According to the MCC analysis Heat release capacity (HRC) and Peak heat release rate (PHRR) measure the amount of the heat which is generated throughout the combustion process and the maximum rate of the heat release, respectively. In these samples, a significant reduction of the heat release rate (HRR) peak is visible (From 157 W/g for untreated to 137 W/g for AlNPs treated fiber). AlNPs treated sample show a later start of the decomposition in comparison to the untreated sample at approx. 386 °C. TGA analysis shows a residual char yield increase in all treated samples confirming their better flame retardancy in comparison to the untreated sample. Untreated fiber starts to decompose at approx. 345 °C and ends at approx. 686 °C, while thermal decomposition for fiber treated with AlNPs ends at approx. 711 °C. Considering the very small amount of deposited SiO_2 and Al_2O_3 nanoparticles and the sheer size of them on the surface of the fiber, several different mapping approaches for chemical elements determination were compared. BEX technique was proven to give best results for quick inspection of large surface area due to combination of BSE imagining and high throughput EDS sensors. While for more detailed inspection, to confirm the presence of a small concentration of nanoparticles on SJL fiber surface

Ultim Extreme was proven to be the best solution that can provide enough X-ray signal even at low voltage (3kV) and low beam current (700pA) conditions necessary to image nanoparticles.

Keywords:

bast fibers, SEM, EDS, BEX

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Momentum-resolved EELS and CL investigation on 1D-plasmonic crystal fabricated by focused ion beam method

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background and aims;
Plasmonic crystals with periodic structures constructed from plasmonic materials have been widely investigated, as they exhibit attractive optical properties based on surface plasmon polaritons (SPPs). To explore the optical properties of plasmonic crystals at the nanoscales, electron energy loss spectroscopy (EELS) and cathodoluminescence (CL) techniques based on scanning transmission electron microscopy (STEM) are the two complimentary approaches; EELS gives the information on the excitation process and thus allows accessing the optical information outside the light line while CL gives information of the photon emission meaning the information inside the light line. With the angle-dependent detection, momentum-resolved spectra, i.e. dispersion relations, can be obtained by both techniques. In this study, we fabricated a one-dimensional plasmonic crystal (1D-PIC) from Al using a focused ion beam (FIB) method and thoroughly investigate their optical properties using momentum-resolved EELS and CL methods.
Methods;
1D-PIC was fabricated using a JIB-PS500i instrument (FIB-SEM system) with a focused Ga-ion beam. At first, an Al thin lamella consisting of large grains(> um) was prepared out of bulk Al to ensure the sufficient propagation of SPPs. The periodic structure was then carved with a precise control of the ion beam dose to create the terrace and trench structures using the specimen drift compensation function to prevent structural sagging. To analyze the optical properties, a JEM-ARM200F(TEM/STEM) instrument with GIF Continuum ER was used for the momentum-resolved EELS, and a modified JEM-2100F(TEM/STEM) instrument with a dedicated CL measurement system was used for the momentum-resolved CL.
Results;
Figure 1a shows TEM BF image of Al 1D-PIC, confirming the successful fabrication of the periodic structure of 500 nm periodicity with the terraces and trenches of the same width. Panels b and c in Fig.1 show the momentum-resolved EELS and CL spectra of Al 1D-PIC, where the dispersion relations of SPs are clearly visualized. The superimposed curves in panels b and c display the calculated dispersion of Al SPPs. The light lines are also indicated on images by yellow dotted lines. It is noted that EELS has the capability of accessing the information outside of light line, while the CL technique can investigate only the photon information inside of the light line. On the other hand, the CL technique has a strong advantage to achieve both nanoscopic spatial resolution and momentum resolution by utilizing the electron beam for the spatial resolution and photon detection for the momentum detection. The employed CL equipment allows acquiring the momentum-resolved CL spectrum at each electron beam position while scanning the electron beam, thus producing four-dimensional (4D) CL data set. In the EELS result, shown in Fig. 1b, enhanced horizontal lines along the momentum axis with 2.6, 5.0, and 6.5 eV are visible, which can be assigned as the localized surface plasmon modes. These local modes can be understood from the resonance conditions where the SPPs make standing waves with different orders matching the local structure of 1D PIC. The momentum-resolved CL results, as shown in Fig. 1 c, display the dispersion relation reproduced from

the upward photon emission from the specimen. Since the periodic structure is constructed on the top surface of the specimen, dispersion lines are clearly visible only in the upward emission. In addition to the dispersion line depicted in Figure c, discernible local mode around 2.6 eV, extending the intensity in the momentum direction, is also evident.

Conclusion;

We have successfully fabricated the 1D-PIC using the FIB technique out of large grains of Al. The presence of SPP and local modes on the 1D-PIC were confirmed using the EELS and CL techniques. Furthermore, through the momentum-resolved EELS and CL mapping analysis, we identified the dispersion relations of SPPs and local modes. Through the combination of momentum-resolved EELS and CL measurements, comprehensive investigations can be performed, which allows accessing the optical properties of nanostructures far beyond the diffraction limit of light.

Figure caption;

Figure.1 (a) TEM BF image of the Al 1D-PIC fabricated by the FIB method. A schematic illustration of the structure is shown in the inset. (b) Momentum-resolved EELS result. The dashed circles indicate the diffraction spot positions reflecting the period of the 1D PIC. The backgrounds of EELS signals are subtracted from the original data. (c) Momentum-resolved CL spectrum acquired in the upward direction (structured side). The CL spectrum is obtained by spatially integrating the CL signal over the 1D plasmonic crystal.

Keywords:

mometum-resolved, EELS, cathodoluminescence, CL, plasmon

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Analyses of Observed Speciation of Cobalt in NMC cathodes Using Laboratory X-ray Absorption Spectroscopy (XAS)

Andreas Bergner[1], Simon John[1]
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Poster Group 1

We report the X-ray Absorption Spectroscopy (XAS) investigation of four samples of NMC electrodes using QuantumLeap™ H2000. Co XANES show three isosbestic points. Principal component analysis (PCA) and Iterative Target Transform Factor Analysis (ITTFA) reconstruction of the experimental X-ray Absorption Near Edge Structure (XANES) show that two of the samples contain two unique species of Co. In the remaining samples, the speciation of Co can be described by the linear combinations of the two distinct species, validating QuantumLeap's synchrotron-like performance.

Keywords:

XAS ITTFA XANES battery NMC

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Observing Lithium Ion Battery Bonds During Charge Cycling Using Laboratory XAS

Andreas Bergner[1], Simon John[1]
[1]Quantum Design GmbH, Pfungstadt, Germany

Poster Group 1

X-Ray Absorption Spectroscopy (XAS) analyses of measurement results obtained with QuantumLeap H2000 of Lithium ion NMC batteries are presented. For the set of samples analyzed, Mn is largely electrochemically inactive and the octahedral coordination is retained. The Mn-O and Co-O bond lengths are shown to change minimally with cycling whereas the Ni-O bond suffers from Jahn-Teller distortion.

Keywords:

XAS battery lithium EXAFS x-ray

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In situ microscopic observations of zinc electrodeposition process in various electrolytes

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[1]Japan Fine Ceramics Center, Nagoya, Japan, [2]The University of Tokyo, Bunkyo-ku, Japan
PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

-Background and Aims-
Zinc anode batteries are expected as low-cost and high energy density batteries. While they have already been widely utilized as primary batteries, there are many problems in the application to a secondary battery [1]. In particular, the dendrite formation during the charging process is a major issue for development of rechargeable zinc anode batteries. Dendrite growth from the zinc anode is known to cause internal short-circuiting between the cathode and the anode. In order to suppress dendrite formation effectively, it is important to clarify the formation mechanism. However, electrochemical reactions in the liquid electrolytes involve many factors and they proceed through complex mechanisms. Clarifying ever-changing electrochemical reactions at the electrode (solid) – electrolyte (liquid) interface, in situ microscopic observations are advantageous. Because of such requests, we had started in situ observations of zinc electrodeposition process by using liquid-phase transmission microscope (LP-TEM). As a result, we succeeded in observing the dendrite formation at the early stage of zinc electrodeposition process [2]. However, in situ electrochemical observations with LP-TEM suffered from the problem of low reproducibility due to the severe limitation in liquid thickness. The condition of a liquid electrolyte surrounding a working electrode for LP-TEM can be changed drastically as the redox reaction proceeds. Furthermore, it was difficult for the LP-TEM method to handle a wide range of electrolytes, due to the restrictions on liquid properties.
Therefore, we devised noble setup of liquid-phase scanning electron microscope (LP-SEM) for in situ observations of electrochemical reaction in various liquid electrolytes [3]. Our LP-SEM setup for electrochemical reactions enables in situ observations in bulky liquid electrolytes and is expected to improve the reliability of reactions.
-Methods-
In situ electrochemical TEM (EC-TEM) observations of zinc electrodeposition in were carried out by combining the TEM (TITAN E-TEM, Thermo Fisher Scientific) and the liquid-cell holder (Poseidon Select, Protochips) under the accelerating voltage of 300 kV.
In situ electrochemical SEM (EC-SEM) was constructed based on atmospheric SEM (JASM-6200, JEOL). A noble electrochemical reaction vessel was created by using a commercially available electrochemical chip for LP-TEM (ECT-15WO, Protochips). Electrochemical reactions were controlled by an external potentio/galvanostat (SP-300, Bio-Logic Science Instruments) through the working electrode (WE) on the chip, metal wires as a counter electrode (CE) and a quasi-reference electrode (RE). Overview of our EC-SEM equipment shown in the figure.
-Results and Conclusions-
First, reliability of our EC-SEM setup was confirmed in a zinc electrodeposition reaction under similar conditions with EC-TEM observations. Compared with EC-TEM observations, reproducibility of zinc deposition processes and their electrochemical conditions were drastically improved. That is, EC-SEM observations can make it possible to reveal the correlation between electrochemical conditions and deposited zinc shape.
Next, zinc deposition processes in several kinds of electrolytes under different electrochemical conditions were compared by using EC-SEM observations. In comparing various constant current

conditions, shape changes of deposited zinc depending on the current density were observed in EC-SEM. For example, in a 0.5 M zinc sulfate solution, a dendric zinc structure was formed at a current density of > 2 A/cm². The shape of the deposited zinc was found to depend not only on the electrochemical conditions but also on the kind of counter anion and the concentration of zinc cation in liquid electrolytes. The details about the relationship between reaction conditions and deposited zinc shapes will be discussed.

-Acknowledgement-
This work was supported by the R&D Initiative for Scientific Innovation on the New Generation Batteries 3 (RISING3) Project administrated by the New Energy and Industrial Technology Development Organization (NEDO) (JPNP21006).

Keywords:

electrochemistry, LP-SEM, LP-TEM, liquid, batteries

Reference:

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[2] Sasaki, Y. et al. J. Power Sources 2021, 481 (1), 228831. DOI: 10.1016/j.jpowsour.2020.228831
[3] Yoshida, K. et al. Microscopy 2022, 71 (5), 311-314. DOI: 10.1093/jmicro/dfac028

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Enabling discovery with in-cell Cryo-ET & AlphaFold: Identification of a non-canonical translocation complex

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LS-08, Lecture Theater 4, august 28, 2024, 10:30 - 11:30

Background incl. aims.
Mycoplasma pneumoniae is a genome-reduced pathogenic bacterium that is the causative agent of a number of respiratory diseases and an established prokaryotic minimal cell model. While the drastic gnome reduction that Mycoplamsa pneumoniae underwent results in only about 500 proteins being expressed, approximately 20% of its genes remain completely uncharacterized. Here, I present our developed approaches aimed at advancing functional identification of previously uncharacterized proteins by in-cell structural biology.

Methods.
Mycoplasma pneumoniae was grown on cryo electron microscopy grids, frozen in vitrious ice, and tilt series was collected on the electron microscope. Particles were first segmented manually in the tomograms, where after deep neural network models were trained to further pick particles. The final cryo electron tomography map was generated by subtomogram averaging. Structures of potential candidates for the proteins were predicted using AlphaFold and rigid body refined against the map. Further proteins were identified using proteomics including, surface shaving mass spectrometry and in-cell cross-linking mass spectrometry. Structural and sequence homology searches were performed to identify functional domains in the uncharacterized proteins.

Results.
The small size of Mycoplasma pneumoniae allowed us to collect over 600, high-resolution cryo-electron tomography datasets without thinning the sample, thereby setting the stage for visual proteomics of an entire cell. In the resultant tomograms, we observed an abundant integral membrane protein complex with a large extracellular dome-like structure. We resolved the protein complex to sub-nanometer resolution by subtomogram averaging. To identify the components constituting the complex, we generated a candidate list of cell surface proteins by enzymatic surface shaving coupled to mass spectrometry, and predicted their structures using AlphaFold. The predicted structures were systematically fitted into the subtomogram averaged map, enabling us to identify three homologous lipoproteins constituting the major part of the extracellular dome. Whole-cell cross-linking mass spectrometry revealed that the three proteins form a hetero-trimeric complex. The cross-linking data also showed that an additional lipoprotein, as well as the integral membrane protein SecDF, are also present in the complex. SecDF is required for efficient translocation by the Sec translocation machinery in other bacteria. Fitting the AlphaFold models of the Sec translocon proteins secA and secYEG into the STA showed that the remaining Sec components are also present in the dome-like structure, and provide a first structural model of the conserved bacterial

translocation machinery. The three homologous extracellular lipoproteins are uncharacterized, with yet unknown functions, but are all essential for Mycoplasma pneumoniae. Structural homology searches revealed that the extracellular proteins contain a prolyl isomerase domain found in periplasmic and extracellular chaperones in other bacteria. Furthermore, only one of the three proteins retains the conserved residues important for substrate binding and catalysis. This is also the component that exhibits a prominent contact with a heterogenous substrate density observed inside the extracellular dome. Classification of the particles showed that a sub-population of the complex interacts with the membrane-associated ribosomes. The fraction of ribosome associated complexes increased when Mp was exposed to antibiotics which halt protein production, indicating stabilization of an otherwise transient interaction. In the antibiotics-treated cells, we also observed a more open conformation of the complex corresponding to the resting state where no translocation occurs. This allowed us to describe the conformational changes that take place upon translocation, especially in secDF.

Conclusions.
Our data suggest that we have discovered a non-canonical sec-translocation complex involved in protein translocation and folding in Mycoplasma pneumoniae, and demonstrates how in cell cryo ET can contribute to de novo structure determination and functional assignment of previously uncharacterized proteins.

Keywords:

In-situ Structural-Biology, cryoEM, Microbiology, Proteomics

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Chemical and structural investigations of epitaxial Fe-Cr-O thin films

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Poster Group 1

The Fe-Cr-O ternary system has been studied extensively since the mid-20th century due to its applications in various fields, such as geology, metallurgy, corrosion, and (more recently) spintronics. In this system, solid oxide phases may appear in three different crystallographic structures: the halite, the corundum and the spinel phases. The spinel structure (Fe3-xCrxO4) is a mix of divalent and trivalent cations in the cubic MgAl2O4-type structure. Multivalence cations are organized in a geometrically frustrated network of octahedral and tetrahedral sites, termed here Oh-sites and Td-sites respectively.The complexity of the spinel structure opens the way for tailoring the functional properties of these materials (magnetization, Curie temperature, electronic transport by incorporating different metal cations into the host matrix. For instance, substitution of Fe cations for Cr in magnetite converts the half-metal host ferrimagnet into either a semiconductor or an insulator, depending on the x values.
Most previous works on the Fe3-xCrxO4 series are based on bulk-like samples, while thin films have received limited consideration. Numerous studies have shown that physical and chemical properties of thin films strongly deviate from the bulk, depending on the growth method, film thickness, and surface and interface effects.
This work presents a comprehensive study of the influence of composition and structural properties of epitaxial Fe-Cr-O thin films.

Thin films were deposited by oxygen-plasma-assisted molecular beam epitaxy (O-MBE) on single crystalline α-Al2O3(0001) substrates. This method was chosen as it enables the synthesis with a perfect control of the chromium content (x) by using individual Knudsen effusion cells under a reactive atomic oxygen plasma. During deposition, reflection high-energy electron diffraction (RHEED) patterns were acquired in real time to control the crystalline structure of the oxide formed. Epitaxial growth of 15nm thick Fe3-xCrxO4(1 1 1) thin films of high crystalline quality has been obtained.
Following growth, several characterization techniques were used to obtain chemical and structural information from the thin films. The stoichiometry of the Fe3-xCrxO4 films were verified ex situ by X-ray photoemission spectroscopy (XPS) measurements. The film microstructure was investigated via high-resolution transmission electron microscopy (HRTEM) and complementary quantitative elemental analyses were performed by electron energy loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDX). X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) measurements were carried out on MARS Beamline of synchrotron SOLEIL to resolve the oxidation state and the first cation-neighbors bond distances. Magnetic hysteresis loops and in-plane electrical measurements were conducted. The Cr and Fe cation site distribution was determined by exploring the L2,3-edge X-ray absorption (XAS) and

circular dichroism (XMCD) measurements performed on DEIMOS Beamline of synchrotron SOLEIL. Multiplet calculations were used to interpret the dichroism signal.
Stoichiometric series of epitaxial Fe3-xCrxO4(111) thin films were prepared with x varying from 0 to 1.7. The film stoichiometry was first evaluated ex situ by XPS measurements. Since XPS probes only the surface, complementary chemical analyses were performed by STEM-EELS and STEM-EDX. Those analytical techniques presented similar trends with minimal fluctuations in the Fe and Cr signals throughout the layers and no composition gradient.
For x < 1.4, RHEED patterns exhibit sharp streaks and no spots, indicating a bidimensional growth mode and layers of high crystalline quality without secondary phases. However, for x > 1.4, RHEED images are blurred and the streaks are almost indistinguishable. The layer-substrate interface and structural defects were evaluated by cross-sectional transmission electron microscopy (TEM) images. Figure 1 (left) depicts TEM images of a representative Fe2.8Cr0.2O4 film studied along the [1210] direction. Low magnification TEM image shows that the film is homogeneous and has a constant thickness of about 15 nm. A perfectly flat and abrupt Fe2.8Cr0.2O4/Al2O3 interface is observed with no noticeable parasite phases at atomic scale. Misfit dislocations, which takes part on the relaxation mechanism of these films, are observed, as already mentioned for other ferrites grown on sapphire substrate by O-MBE (e.g. NiFe2O4 and MnFe2O4). When comparing HRTEM images of Fe2.8Cr0.2O4 and Fe2.3Cr0.7O4 films, the increasing Cr content seems to induce disorder in the crystalline structure of the film and many stacking defects (e.g., APBs and dislocations) are observed. To investigate further this issue, HRTEM images were acquired for Fe1.6Cr1.4O4 (figure 1 right) and buffer-Fe1.3Cr1.7O4. The increase in the amount of the crystalline nanodomains is unmistakable for both of these layers compared to the first two compositions. These results corroborate well with the decrease in quality of RHEED images. The influence of Cr content on the crystalline quality of the films can be associated to either changes in the cation distribution among tetrahedral and octahedral sublattices or modifications in the growth conditions as the deposition rate is different. For x < 0.5 the replacement of Fe3+ by slightly smaller Cr3+ in Oh-sites does not modify drastically the structure, whereas for 0.6 < x < 1.3 the displacement of larger Fe2+ into Td-sites has a huge influence. Indeed, orbitally active Fe2+ cations in Td-sites lead to tetragonal distortions due to cooperative Jahn-Teller effects. For compositions with x > 1.4, films are assumed to have the normal type of spinel structure, Fe3+ cations are replaced by smaller Cr3+ in Oh-sites.
In addition to changing the structural quality, cation disorder has a major impact on the physical properties of the films. The total magnetization and the magnetic anisotropy of Fe3-xCrxO4 thin films decrease as the x values increase. But unlike bulk materials, thin films with high Cr content (x ≥ 1.2) still show magnetization at room temperature, i.e., near (or above) the Curie temperature of bulk samples. These features were interpreted in the light of the cationic site distribution. For both bulk and thin films, the inversion parameter steadily evolves with composition, ranging from an inverse arrangement (Fe3O4) to normal ordering (FeCr2O4). Because Cr-rich thin films are still intermediate spinels, while bulk samples are normal spinels, the Curie temperature of these films were increased as they present stronger Td-Oh antiferromagnetic interactions. These results confirm that doping magnetite thin films with Cr is very effective to control the magnetic behavior of this material.

Keywords:

Magnetic materials, EELS, EDS, HRTEM

Reference:

P.V.B. Pinho et al. “Stoichiometry Driven Tuning of Physical Properties in Epitaxial Fe3-xCrxO4 Thin Films,” Applied Surface Science 615 (2023) 156354

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Electron and x-ray spectroscopy for real-space electronic structure mapping in superconducting nickelates

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Background and aims: The recently discovered superconducting nickelates are an archetypal example of how exotic or functional properties can be realized by careful materials synthesis and engineering. While many fundamental aspects of these materials remain under active investigation, it is clear that (as with many quantum materials) they are rooted in strong interactions between atomic structure, orbitals, and electronic charge. The unique sample geometry and complicated synthesis of the so-called infinite-layer nickelates, however, have not only raised questions about the origin of superconductivity in thin film specimens and generally hindered the use of many common “field-standard” spectroscopic probes. Here, I will focus on two advanced core-level spectroscopic techniques, both of which provide unique access to real-space electronic information.
Methods: At the atomic scale, highly local spectroscopic measurements are conducted by electron energy loss spectroscopy (EELS) in the scanning transmission electron microscope (STEM) with a sub-Ångström probe to enable location-specific measurements across the doping-dependent superconducting dome in nickelate thin films. Leveraging hard x-rays at high-brilliance synchrotron facilities, we harness beyond-dipole and dipole-forbidden transitions with s-orbital non-resonant inelastic x-ray scattering (sNIXS) to directly map the complete charge density [1] in bulk undoped infinite-layer nickelates.
Results: Area-selective STEM-EELS measurements reveal a distinct electronic landscape in the superconducting nickelate, particularly in regards to nickel hybridization, and it’s doping evolution across the superconducting dome which point to multi-band superconductivity [2]. Elemental and electronic investigation across the atomic substrate-film interface provide definitive evidence against the picture of possible interfacial superconductivity hosted at a strongly polar oxide interface [3]. The quantitative precision offered by bulk sNIXS measurements are used to investigate questions of nickelate “self-doping” in chemically undoped compounds through strong interactions with rare-earth states.
Conclusions: The insights offered by hyperspectral techniques, particularly those with real-space sensitivity, are crucial for building fundamental understanding of many quantum, biological, and functional materials. Both techniques described here can be further extended to in situ or operando conditions, opening the door to study phase evolution driven by temperature, pressure, fields, etc., some challenges and promising developments of which I will also mention [4,5].

Keywords:

STEM-EELS, sNIXS, quantum, superconductivity, spectroscopy

Reference:

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36

An advanced smart counting mode for pixelated direct electron detectors based on semiconductors

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Poster Group 2

Pixelated direct electron detectors identify electrons by their interaction with the sensitive detector volume. The relevant interaction is an energy deposition via electron-electron scattering. However, in applications like 4D-STEM, the user is interested in the spatial-resolved absolute number of incident electrons on the detector surface. Therefore, the goal is to count each electron exactly one time at the position it crosses the detector surface. The following assumptions were made for silicon as detector material but are transferable to other materials as well.
Low-energetic electrons (E ≈ 10 - 30keV), as used in scanning electron microscopes, typically deposit their energy in a small volume very close to the point of entry. This volume is mostly much smaller than the size of the pixel structure (e.g. for the standard pnCCD 48 µm x 48 µm).
High-energy electrons with energies > 100 keV, as usually used for transmission electron microscopes, typically do not deposit their energy locally at one specific point but deposit the energy in many steps along three-dimensional tracks through the detector volume. For electrons with a primary energy of 300 keV, the average length of these tracks is 450 µm. The shape of these tracks is caused by multiple scattering of the electrons in the detector volume and, therefore, is stochastic.
The energy deposition in the three-dimensional detector volume locally generates charge carriers that form a charge cloud. Direct electron detectors like the pnCCD collect the charge cloud at the opposite side of the entrance window. Therefore, the charge cloud drifts due to an applied electric field to the opposite side of the detector. During the drift time, the size of the charge cloud increases due to repulsion of the charge carriers themselves and due to diffusion. In comparison to the statistical behavior of the energy deposition, the drift process behaves deterministically. The collected charge carriers in each pixel of the pixel structure correspond to the binned two-dimensional projection of energy deposition, widened by the drift process. The total number of charge carriers is directly related to the amount of deposited energy.
For low primary energy, the structure of the charge cloud is mostly just influenced by the primary energy of the electron itself, and its point of entry relative to the pixel structure. Therefore, for electrons that enter the detector at the same point with the same primary energy the pixel-wise collected charge clouds look similar. For higher energetic electrons, the size and the shape of the pixelated charge cloud are mostly influenced by multiple scattering. Therefore, the distribution of the pixel-wise collected charge carriers is different for each individual electron. During the detector readout, the pixel-wise collected charge carriers are transferred into a detector response. If the measuring process of the number of individual charge carriers in each pixel is sufficiently precise, the detector response is a good approximation of the two-dimensional projection of the charge cloud. The structure of the 2D projection can be used to calculate the point of entry for individual electrons for higher energetic electrons on pixel level and for lower primary energies even on sub-pixel level. However, for some applications in TEMs and SEMs require a higher electron flux on the detector such that the measured two-dimensional projection of the charge clouds of two or multiple primary electrons overlap. This leads to three different regimes depending on the flux. The first regime is

where the detector response of individual electrons is separable. The second regime has contributions where multiple electron traces overlap but the contributions of the individual primary electrons are still visible. The third regime is dominated by a very high flux that leads to intensity images. In the third regime, the contributions of individual primary electrons are not visible anymore. Different regions in the same diffraction pattern can hold different regimes.
Our algorithm can handle the different regimes in flux on pixel level and reconstructs the number of contributing primary electrons and their points of entry. The point of entry reconstruction happens depending on the energy of the primary electrons and the flux on physical pixel level or subpixel level in real-time. In this contribution, we will show that the proposed Smart Counting algorithm significantly increases the precision in counting the number of primary electrons and their spatial position compared to a simple counting mechanism by individual discriminators on pixel level.

Keywords:

pixelated direct electron detector
pnCCD

Reference:

Ryll, H., et al, Journal of Instrumentation 11 (2016)

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Manipulating topological magnetic structures by in-situ Lorentz microscopy

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Poster Group 1

Topological magnetic structures, i.e., skyrmions, have been considered as promising carriers in future magnetic storages or logical devices since their discovery in chiral magnets from 2009, owing to their nanometer-sized dimension, high stability and low critical current density of manipulation. The topologically protected spin textures are fundamental to these excellent performance metrics over the conventional magnetic domain walls or nanoparticles. Here, we have utilized the state-of-the-art magnetic imagining techniques, i.e., Lorentz microscopy and off-axis electron holography, to visualize the magnetization or emergent field of topological magnetic objects, in order to clarify the underlying mechanism of stability and evolution. Moreover, in-situ electrical Lorentz microscopy is employed to investigate the current-driven dynamics of skyrmions in nanostructures under the nanosecond current pulses. The skyrmion velocity and skyrmion Hall angle are measured with respect to the current density. Our results prove the possibility of coding the skyrmions in prototypical devices and have immediate significance towards the skyrmion-based memory or logic devices.

Keywords:

Lorentz miscoscopy, electron holography, skyrmion

Reference:

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HeXI: The High-energy Electron Xtallography Instrument at Diamond Light Source

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Poster Group 2

The HeXI project, funded by the Wellcome Trust “Electrifying Life Sciences” grant and Diamond Light Source, aims to build a dedicated electron diffractometer to investigate the potential of Mega-electron volt (MeV) electrons for the determination of molecular structures from nanometre sized crystals. The HeXI instrument will leverage the increased penetration of MeV electrons and the high precision goniometry, cryo-sample transfer systems and sample preparation methods developed at Diamond to target crystal thicknesses between 300 nm and ~1 µm to determine the molecular structures of proteins and pharmacologically relevant molecules. The ability to acquire high-fidelity sweep and serial diffraction data from ≤1-micron thick crystals will bridge the current crystal size gap between samples amenable to electron diffraction performed on commercial Transmission Electron Microscopes (TEMs) using <300 nm crystals and microfocus X-ray diffraction of >3 µm crystals at microfocus beamlines

Keywords:

Megavolt Electron Diffraction

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Understanding strain and composition effects in fuel cell catalysts using advanced electron microscopy and DFT

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Poster Group 1

Background and aims
Hydrogen fuel cells are an important technology for decarbonising road transport and shipping. Their efficiency is limited by the oxygen reduction reaction (ORR), which requires catalysts to achieve acceptable performance for the cell. The state of the art ORR catalysts are Pt-X nanoparticles, where X is another transition metal. These nanoparticles have a core-shell structure, consisting of an alloyed Pt-X core surrounded by a Pt rich shell. They perform much better as ORR catalysts than pure Pt nanoparticles, however the origins of this activity enhancement are not fully understood. The main factors that are hypothesised to be responsible are ligand effects, where chemical differences between Pt and X modify the electronic properties of the catalyst surface, and strain effects, where changes in nanoparticle size and shape induced by the size mismatch between Pt and X lead to changes in electronic structure. Core-shell nanoparticles have been observed to have a high degree of strain at the surface which affects the d band structure at the nanoparticle surface, which is crucial in determining O binding strength and ORR catalytic activity. Complex strain states with a significant shear component have been observed in the outer layers of core-shell nanoparticles via atomic resolution scanning transmission electron microscopy (STEM); while the effect of volume changing normal strains on catalytic activity has been widely studied, those of shape changing shear strains have been largely overlooked in the literature. Our key aim in this work is to use computational modelling to understand the electronic properties of experimentally observed nanoparticle structures, and the effect of these properties on catalytic activity, as a step towards better understanding the structure-property relationship and improving fuel cell catalyst design.

Methods
Microscopy: Alloyed Pt-Co core-shell fuel cell catalyst nanoparticles on C have been provided by Johnson Matthey. Atomic resolution high angle annular dark field STEM (HAADF-STEM) with EDX and EELS has been performed on these nanoparticles to understand their structure and composition at the atomic level. Column positions relative to a reference grid which is fitted to a nanoparticle structure have been used to measure variations in strain across its extent. The compositional information allows the elastic strain to be distinguished from lattice parameter changes arising from different local alloy compositions.

Computation: DFT calculations have been performed using CASTEP 22.11 on pure Pt to measure the effect of normal and shear strains on the d band centre. All calculations were performed with the PBE functional. Equilibrium bulk and slab structures were first obtained by geometry optimisation. Strain states representative of those seen in STEM images of catalyst nanoparticles were then applied and the d projected PDOS and d band centre of these strained structures were calculated and compared.

Results

Atomic resolution HAADF-STEM measurements of catalyst nanoparticles have shown that the outer layers exhibit both normal and shear strains relative to the reference structure that was fitted to the nanoparticle. DFT calculations have shown that the shear strains typically observed in catalyst nanoparticles are unlikely to have a significant effect on their electronic properties.

Conclusions
The ability to reliably map strain at the atomic scale for nanoparticles is an important step in linking structure to activity, and ultimately determining the optimal surface structure for ORR catalysts. The independence of electronic properties on small shear strains suggests that unit cell volume is a key descriptor of catalytic activity.

Keywords:

HAADF-STEM, DFT, catalysis, strain

Reference:

Luo, X. et al. (2022), Ultramicroscopy, 239, 113561

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Array tomography enables correlative volume electron microscopy and spatial transcriptomics

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Volume Electron Microscopy (vEM) provides high-resolution data of target structures and resolves three-dimensional representations of otherwise ambiguous biological geometries. While EM provides high spatial resolution, search processes in volumes of several hundreds of square microns is tedious. Multimodal methods like correlated light and electron microscopy (CLEM) allow to bridge these scales. Among the available vEM techniques, array tomography methods like automated tape collecting ultramicrotomy (ATUM) have proven particularly powerful for targeting specific or rare biological structures as needed for correlation as they enable repetitive and large field of view imaging. Previously, we have applied diverse ATUM-CLEM approaches to reveal ultrastructural correlates of neurodegenerative pathologies. These classic correlation techniques annotate ultrastructural data with one or a few molecular targets. With the advent of spatial transcriptomics (ST), the localization of cells with specific expression profiles covering several thousands of transcripts has become accessible. So far, diverging sample preparation techniques have hindered correlated ultrastructural investigation. Here, we developed STcEM, a method that links spatially-resolved gene expression of single cells with their ultrastructural morphology by integrating ST and ATUM on adjacent tissue sections. With this method we successfully mapped microglial classes according to their transcription profile and ultrastructural morphology in a mouse model of white matter lesion. Our results offer a comprehensive view of the spatial, ultrastructural, and transcriptional reorganization of single cells after brain injury.

Keywords:

array tomography, volume EM, CLEM

Reference:

Androvic P and Schifferer M et al. Nat Commun. 2023 Jul 11;14(1):4115. doi: 10.1038/s41467-023-39447-9. PMID: 37433806; PMCID: PMC10336148.

Kislinger Georg, et al. eLife 2023 eLife 12:RP90565 https://doi.org/10.7554/eLife.90565.1

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Thermoelectric structure-property relationship establishment in TlGaSe2

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Poster Group 2

Background incl. aims
When devices, such as transistors, are under electrical load, the resistivity of the device often leads to unwanted heat generation. In many cases, this reduces the efficiency of the device and hence is detrimental both economically and environmentally due to excessive energy consumption. However, as devices approach the atomic scale, this wasted heat generation can lead to device failure. To overcome this, materials which have excellent electrical conductivity, but poor thermal conductivity are being investigated for their potential use in devices.
One such thermoelectric material which has potential use in optoelectronics is the layered ternary chalcogenide semiconductor $TlGaSe_2$. It has been shown to have a thermoelectric figure of merit, ZT, of approximately 0.8 [1], comparable with other heavily studied thermoelectric materials such as Bi_2Te_3 [2]. However, the material's layered nature means it is prone to stacking faults, which are often assumed to affect this value [1, 3], but to date, this has yet to be established. This is critical to understand if the material is to be used in future devices, such as near-IR sensors.
Methods
In this work, $TlGaSe_2$ is characterised via (scanning) transmission electron microscopy (STEM) and density functional theory (DFT) simulations. Streaking in selected area electron diffraction (SAED) patterns are correlated to simulations which indicate the presence of stacking faults in the growth direction [4].
Results
High resolution STEM confirms these stacking faults and indicates a lack of long-range order to the stacking. DFT calculations reveals a high preference for the system to include these faults. Electron transport simulations for structure in both stacking orders show a significant enhancement of thermoelectric power along the stacking direction when stacking faults are induced. These are further enhanced if the material is doped sufficiently. Phonon dispersion calculations show a suppression of several phonons modes when stacking faults are induced, indicating a suppression of thermal conductivity.
Conclusions
Stacking faults in $TlGaSe_2$ are shown to be prevalent throughout the material and they enhance its excellent thermoelectric properties.

Keywords:

thermoelectric, stacking faults, DFT, STEM

Reference:

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Nanoparticle size estimation by HR-STEM and generative AI

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background incl. aims
Scanning transmission electron microscopy (STEM) is commonly used to estimate the particle size distribution in heterogeneous catalysts. A problem with the conventional approach of manually measuring particle diameters in STEM images is that it is labor-intensive and disregards the actual particle structure, making particle size prediction with atomic precision impossible. To overcome these limitations, we propose a machine learning approach based on the CycleGAN architecture. The model learns to map between simulated and experimental images, and a subsequent network is trained to estimate the size of an imaged nanoparticle in terms of number of atoms. This technique is fully automatic and could form the basis for a modern characterization method when combined with automated data acquisition. As catalysts should ideally be studied under reaction conditions, we also explore the possibility of extending this workflow to gas-cell measurements.

Methods
Triggered by our recent experimental results, we have developed a structure generator which can generate realistic but random atomic models of Pt nanoparticles supported on ceria. These models have been used to generate a dataset of 3500 HAADF STEM multislice image simulations. A second dataset of high resolution HAADF STEM images of Pt nanoparticles supported on ceria was recorded on a probe-corrected Titan Themis operated at 300 keV. All experimental images were recorded under identical conditions. Images in both datasets are 128x128 with a pixel size in the range 10-50 pm. The simulated and experimental dataset were used to train a CycleGAN to map between the two sets. We use 2 U-Net generators and 2 PatchGAN discriminators for the CycleGAN. With the trained CycleGAN, we then send the simulated dataset through the "simulation-to-experimental" generator, thus obtaining a new dataset of 3500 fake experimental images with a corresponding particle size ground truth. Another U-Net with a fully connected layer at the end to output a scalar size prediction was then trained on the fake experimental dataset. The trained size-estimator was applied to the experimental dataset and the particle size distribution was compared with that of the manual technique of measuring the particle diameters and assuming a hemispherical particle shape.

Results
We have successfully trained a cycle-consistent generative adversarial network to map between physical image simulations and experimental HR-STEM images of small Pt nanoparticles supported on cerium dioxide. Passing raw multislice simulated images through the network yields realistic-looking experimental images with noise profile matching that of the real experimental dataset. In the same way, passing an experimental image through the network can be compared to applying a potent denoising algorithm to the image.

We apply our size estimation network to a set of 118 experimentally observed nanoparticles in a Pt/CeO2 catalyst to get the particle size distribution. Most predictions are reasonable and the particle size distribution obtained by the size-estimator network follows that of the manual hemisphere estimation technique. We find that the mean Pt particle size in the catalyst is 117 atoms with our technique, and 154 with the hemisphere estimation technique. Typical fail-cases of our model includes images of particles larger than 500 atoms and particles oriented along a major zone axis. This is likely due to a lack of large particles in the simulated data, as well as a lack of particles in zone axis in the experimental set. This could be addressed by generating more data.

Conclusion
In conclusion, our machine learning approach establishes a connection between physical image simulations and experimental images, making it possible to train supervised machine learning techniques on realistic-looking data with a known atomic model ground truth. We have showcased this by developing a size-estimation network which proves to output reasonable size estimates when applied to experimental images. Combined with automated data acquisition, we envision techniques like this will lead to more comprehensive and accurate characterization of nanoparticle-based heterogeneous catalysts.

Keywords:

Nanoparticles, Generative AI, Catalysis, Simulations

Reference:

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Khan et al., npj Computational Materials 9, 85 (2023).

Eliasson et al., Nanoscale 15, 19091-19098 (2023).

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Nanoscale phase separation in novel Zr-based metallic glasses

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background incl. aims:
Research on phase separation in metallic glasses (MGs) is motivated by a fundamental interest in the structure and properties of disordered materials and their potential for structural and functional applications. The structure and physical properties of phase separated MGs have characteristics different from those of other MGs. Recent reports have demonstrated successful achievements in the preparation of phase-separated MGs, which offer a unique opportunity to design composites or alloys with hierarchical microstructures at different length scales. The presence of a miscibility gap between the two major elements causes the formation of two-phase glassy alloys. Phase separation can occur due to the complex interplay of negative and positive enthalpies of mixing. Efforts have been undertaken to understand the origin of phase separation in MGs through mechanisms such as nucleation and growth, spinodal decomposition, yet the understanding of the mechanism is insufficient. Phase separation reported in Ce-Al (Ga) MGs is due to changes in the electronic structure of Ce-atoms. Phase separation with nano-amorphous domains plays an important role in improving the plastic behaviour of MGs and ideally suited for small mechanically challenging applications such as dental implants because of their remarkable mechanical properties. In this presentation, we will discuss the results of our recent research work on the design of new Zr-based MG composition with nanoscale phase separation for future biocompatible applications.
Methods:
Zr-based alloy ingots were prepared by arc melting under a Ti-gettered argon atmosphere. Ribbons were created by induction melting of alloys in a quartz tube and ejected onto a copper roller at a tangential velocity of 30.6 m/s under argon atmosphere. The resulting ribbons were approximately ~4 mm wide and ~40 µm thick. The structure of the melt-spun ribbons was studied by X-ray diffraction (XRD: D2 phaser-Bruker) using Co Kα radiation. Differential scanning calorimetry (DSC) measurements were done using a Netzsch DSC 404F1 Pegasus device at a heating rate of 20 K/min. Microstructural studies were carried out using a transmission electron microscope (TEM; JEOL 2100F FEG and JEOL 220FS) operated at 200 kV. APT measurements were carried out on a LEAP5000 XR (Cameca Inc., USA). Data reconstruction and analysis were conducted with APSuite 6.3 (Cameca Inc., USA) based on the voltage curve with an initial tip radius of 55 nm.
Results:
The presence of broad diffraction maxima in the XRD profiles confirms the glassy structure of the alloys. The interpretation of the DSC data suggests that the additional exothermic peak results from the presence of two distinct co-existing glasses formed through phase separation. The microstructure and thermal property analysis indicate that the glasses exhibit a typical liquid phase separation-induced two-glassy phase with droplet-like microstructures in the form of nano-amorphous domains. The microstructure of phase separated nano-amorphous domains has been confirmed by sophisticated experimental characterization techniques including transmission electron microscopy (TEM) and atom probe tomography (APT). The presence of two distinct glassy phases with distinct

contrasts has been observed in the TEM images. There is a matrix in which another phase is dispersed. The selected area diffraction (SAD) patterns displays two diffuse halos from the matrix and the dispersed phase. The formation of extremely fine nano-amorphous domains of 10 nm size can be seen in the TEM micrographs. The phase separated regions are analyzed by conducting the STEM-EDS mapping of the alloys. Bright-field (BF), high angle annular dark-field (HAADF) STEM images and the corresponding elemental mapping are compared. It has been observed that the nano-amorphous domains are uniformly distributed through out the sample. APT results give detailed information on the chemical composition and the spatial distribution of each element. The overall composition of the measured tip is found to be in good agreement with the nominal composition. The spatial atomic distribution analysis by APT revealed discontinuities for the main elements and the formation of clusters suggesting the occurrence of phase separation in these glasses.

Conclusions:

In summary, a new phase separated Zr-based MG has been successfully developed. The microstructure of phase separated nano-amorphous domains has been confirmed by sophisticated experimental characterization techniques including TEM and APT. The alloys form a two-glassy-phase structure. APT analysis revealed the formation of ~7 nm size clusters. The two-glassy-phase MG system free of toxic elements with high glass-forming ability may be used as a precursor for fabricating continuous nano-porous MGs and could be a promising candidate for implant applications.

Keywords:

Metallic glasses; Phase separation; Microstructure

Reference:

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TRIBLOCK POLY(2-OXAZOLINE)S WITH A FLUORINATED BLOCK AS A NEW PLATFORM FOR ADVANCED SELF-ASSEMBLY: CRYO-TEM STUDY

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Poster Group 2

The synthesis of defined triphilic terpolymers with hydrophilic, lyophilic, and fluorophilic blocks is an important challenge as a basis for the development of multicompartment self-assembled structures with potential for, e.g., cascade catalysis and multidrug loading. A series of fluorinated 2-substituted-2-oxazoline copolymers with hydrophilic 2-methyl-2-oxazoline, hydrophobic 2-octyl-2-oxazoline, and fluorophilic blocks were synthesized in our group previously [1-5]. The presence of the blocks with the different nature in one copolymer structure facilitated self-assembly of the copolymers in water as observed by dynamic light scattering, and cryo-transmission electron microscopy. The nanoparticle morphology is strongly influenced by the order and length of each block and the nature of solvent, leading to nanoparticles with tuning structure as confirmed by Cryo-TEM. For the polymer that has no fluorinated tail formed primarily multi-layered vesicles (Figure 1A), which is in agreement with the DLS result. In contrast, the cryo-TEM analysis of the polymer solution with shortest fluorinated block showed a mixture of aggregating rod-like micelles and little-populated single-layered vesicles (Figure 1B). However, the extension of the fluoroalkyl chain length further decreased the relative content of the vesicles (Figure 1C). This trend is continued for copolymer with the longest fluorinated alkyl chain, which only showed isolated rod-like micelles with a small fraction of agglomerates composed of rod-like micelles, while vesicles were no longer observed (Figure 1D).
Figure 1. Cryo-TEM images of the investigated polymers. (A) The non-fluorinated diblock copolymer formed single- and multi-layered vesicles, together with short rod-like micelles. (B) The C8F17 and C) C10F21 copolymers formed single-layered vesicles and aggregates of rod-like micelles. (D) The C12F25 copolymer assembled into rod-like micelles only. Scale bars: 100 nm.
The reported poly(2-oxazoline) block copolymers with high fluorine content have high potential for future development of MRI contrast agents.

Keywords:

polymers, poly-2-oxazolines, self-assembly, micelles, vesicles

Reference:

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Tunneling nanotubes provide a route for SARS-CoV-2 spreading

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Poster Group 1

Neurological manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection represent a major issue in long coronavirus disease. How SARS-CoV-2 gains access to the brain and how infection leads to neurological symptoms are not clear because the principal means of viral entry by endocytosis, the angiotensin-converting enzyme 2 receptor, are barely detectable in the brain. Here, we investigated the neuroinvasive potential of SARS-CoV-2. By applying confocal microscopy and in cellulo correlative fluorescence and cryo–electron tomography, we reported that human neuronal cells, nonpermissive to infection through the endocytic pathway, can be infected when cocultured with permissive infected epithelial cells through tunneling nanotubes (TNTs). SARS-CoV-2 and multiple vesicular structures such as double-membrane vesicles, sites of viral replication, are observed inside TNTs between permissive and nonpermissive cells. Our data highlight a previously unknown mechanism of SARS-CoV-2 spreading, likely used as a route to invade nonpermissive cells and potentiate infection in permissive cells.

Keywords:

cellulo-correlative cryo-EM
SARS-CoV-2
Neurodegeneration

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Interdiffusion-controlled phase formation at an interconnect interface during soldering

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Poster Group 2

Background incl. aims:
Soldering is a well-established method for creating permanent bonds between metal parts, often resulting in the formation of intermetallic compounds. For the purpose of soldering, various elements like Sn, Pb, Bi, Sb, Ag and Cu, or their alloys, are utilized in different compositions based on the respective field of application. The shift towards lead-free solder, driven by environmental regulations, has increased interest in Sn-based solder alloys. However, soldering interconnects involves complex processes related to material transport, phase stability, and phase transformation kinetics.
Methods:
In the present work, the interdiffusion and diffusion-controlled phase formation processes in a Sn-Sb solder alloy between a Ni-based layer with a minor Si amount and a Cu substrate were investigated using various electron microscopy techniques. Initial scanning electron microscopy analysis of cross-section samples provided an overview of the interface, including the Sn-Sb solder alloy. For detailed examination, focused ion beam techniques were employed to create electron-transparent samples for analysis using analytical transmission electron microscopy.
Results:
Examination of the untreated states of the Ni-based layer and the solder alloy allowed differentiation between the initial microstructure and changes post-soldering. The Ni-based layer exhibited a lamellar-type structure with a uniform elemental distribution perpendicular to the soldered interface, while the raw solder alloy consisted of a tetragonal β-Sn phase as a matrix and a trigonal SbSn phase. After the soldering procedure, CuSnNi and SnCu intermetallic compounds formed with various shapes, surrounded by the β-Sn matrix. The SbSn phase persisted as small inclusions. Additionally, the Ni-based layer initially shrank due to diffusive interactions with Cu and Sn, resulting in a thin film with increased Si content compared to the untreated layer, eventually leading to complete consumption of the Ni-based layer.
Conclusions:
This study highlights the intricate processes involved in Cu transport from the substrate through the solder material, emphasizing the significant role of Cu in forming intermetallic compounds. Together with Sn, Cu drives the transformation of the Ni-based layer towards complete consumption. In addition to elemental influences, microstructures play a crucial role in the Ni consumption process.

Keywords:

soldering; electron microscopy; intermetallic compounds

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Machine Learning Algorithms for Tracking Analysis of Chloroplast Avoidance Movements

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Poster Group 1

Background incl. aims:
Plants use chloroplast movements to cope with environmental changes, particularly in response to high-intensity light stress. Chloroplasts relocate within cells, minimizing energy absorption and protecting the photosynthetic apparatus [1]. Monitoring chloroplast responses to light stress requires accurate measurement of their relocation dynamics. Computerized image analysis, combined with machine learning algorithms, has proven reliable and accurate in observing organelle movements [2,3]. Our study integrates traditional microscopic image analysis with machine learning approaches to track chloroplast trajectories during their response to strong blue light.
Methods:
The model plant Lemna trisulca L. [1] was used to analyze the chloroplast avoidance reaction. After a period of incubation in the dark, the plants were treated with strong blue light generated by a led illuminator (44µE m-2s-1 for 1 minute), followed by an analysis using an LSM510 confocal microscope with 633nm and 488nm laser light. Image series in the form of time-lapse photography were collected during periods of 15 minutes of observation. Once the images were obtained, the first step was to use the open-source software Fiji [2] (which is a new distribution of ImageJ), including the plugin to automate the process of tracking chloroplasts, TrackMate [3]. The software was used to improve image quality, detect objects and perform the tracking process. The resulting data from Fiji was used in a second analysis stage created in Python 3.10.9, using the Jupiter Notebook software. The aim of the second stage was to find patterns of chloroplast movement.
Result:
Using comprehensive trajectory analysis, the movement of chloroplasts during the avoidance movement in L. trisulca cells was shown to be curvilinear. Approaches using machine learning techniques including cluster analysis allowed the separation of two groups of chloroplasts. The features that distinguished these groups were the Confinement ratio and Mean straight-line speed (these are the parameters calculated within the TrackMate plugin). The first of the distinguished groups of chloroplasts was characterized by a high efficiency of movement from the starting point to the destination. This was due to the fact that, during the avoidance response, the chloroplasts moved along a roughly constant orientation, allowing them to move more efficiently away from the start point. These observations also had high values for the average velocity in linear movement. The second group obtained had a higher number of curve sections clustered near the starting point, which looked as if the chloroplasts were "circling" longer around the starting point of the movement. This translated into lower values for the Confinement ratio and Mean straight-line speed than in the observations of the first group. Irrespective of the two main patterns of chloroplast movement that were determined, chloroplast trajectories with transient features were also observed.
Conclusion:

The application of classical image analysis methods allowed information on the trajectories of chloroplast movements to be obtained. The collected data were used to perform a cluster analysis process which then allowed the determination of chloroplast movement patterns in the avoidance response. This example demonstrates that machine-learning algorithms in the analysis of chloroplast movement trajectories can significantly extend the capabilities of classical image analysis methods.

Keywords:

ML, movement analysis, cluster analysis

Reference:

1. Samardakiewicz, S. et al. , PLOS ONE, https://doi.org/10.1371/journal.pone.0116757;
2. Schindelin, J.et al., Nature Methods, https://doi.org/10.1038/nmeth.2019;
3. Ershov, D. et al., Nature Methods, https://doi.org/10.1038/s41592-022-01507-1;

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(In-situ) 3DED and other TEM Techniques Unravel (Ca,Sr)(Mn,Fe)O3-δ and LaxSr2-xMnO4-δ Structure-Property Relations in Redox Conditions

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background
Many alternative energy applications involve inorganic crystalline solids exposed to redox conditions, e.g. solid oxide fuel cells (SOFCs) or chemical looping with oxygen uncoupling (CLOU). With the development of in-situ 3D electron diffraction (3DED) of solids at high-temperature in gas and vacuum conditions, great new potential is unlocked for unravelling structure transformations in such operational conditions, to understand the origins of e.g. high performance and degradation properties. Here, we investigated the influence of high-temperature reducing atmospheres on the structure of two such perovskite-based energy materials - CaMnO3-δ and LaxSr2-xMnO4-δ.

CaMnO3-δ is a perovskite with interesting properties as oxygen carrier in the CLOU process, i.e. a carbon capturing combustion technique that inherently separates expelled CO2 from air. The cycling stability and oxygen release temperature of this compound can be significantly improved by A or B site doping with e.g. Sr or Fe, but it has not yet been unravelled why. [1] [2]

Ruddlesden-Popper (RP) LaxSr2-xMnO4-δ are interesting electrode materials for symmetric solid oxide fuel cells (SOFCs) due to their electronic and chemical properties. Previously, they were reported from in-situ X-ray and neutron diffraction to remain stable upon heating to 700°C in H2 for $0 < x \leq 0.6$, although anomalously large atomic displacement parameters were found. Further, while all these structures had the same I4/mmm lattice, the x = 0.5 compound showed a higher conductivity in reducing environment (1.9 S cm −1), breaking the trend of the rest of the series (0.4, 0.5, and 0.8 S cm−1) which was not explained yet in literature. [3]

Methods
CaMnO3-δ, Ca0.75Sr0.25MnO3-δ, CaMn0.8Fe0.2O3-δ and LaxSr2-xMnO4-δ for x = 0, 0.25, 0.5, 0.75 and 1 were examined with in-situ and ex-situ 3DED, EDX, high-resolution HAADF-STEM and/or (mono STEM-) EELS. However, the investigation of oxides with in-situ TEM is not without obstacles. At temperatures above 700°C in reducing gas conditions, an extra silica layer is created in-situ around the studied oxide – as evidenced by EDX and EELS - due to a reaction coming from the Si3N4 of the heating chip. This shell can interfere with the reduction process, as shown from Mn valence determination by mono STEM-EELS. To overcome this problem, we performed a series of in-situ gas and vacuum experiments with LaxSr2-xMnO4-δ on graphene coated heating chips, in order to study the influence of various parameters and find the optimal conditions for in-situ TEM experiments on oxides in redox conditions, that can be directly linked to real-life energy applications.

Results
With in-situ 3DED in diluted H2 and in vacuum at 250°C-350°C, we captured the oxygen vacancy order in one of the intermediate oxygen deficient phases of CaMnO3-δ – i.e. CaMnO2.75 – and solved and refined its structure. We compared its structural transformations in H2 and vacuum high-

temperature conditions with those of the doped Ca0.75Sr0.25MnO3-δ and CaMn0.8Fe0.2O3-δ to explain the differences in performance on the level of the atomic lattice.

Also, 3D electron diffraction reveals the previously unreported structure transformations of La0.25Sr1.75MnO4-δ and La0.5Sr1.5MnO4-δ to an incommensurate 2D and 1D modulated structure respectively, but the amount of order in the crystals differs. Moreover, high-resolution HAADF-STEM shows a difference in defect structure: where the x = 0.5 compositions exhibits many higher order RP n = 2 layer defects, this is not the case for the x = 0.25 material. Those structural differences allow to explain the higher conductivity for La0.5Sr1.5MnO4-δ than for La0.25Sr1.75MnO4-δ . [4]

Further, in-situ high-resolution HAADF-STEM (in vacuum) and 3DED (in gas) at 700°C also detect the formation of a perovskite phase on the surface of both materials. From EDX, the layer is observed to be La rich, which leads to differences in thermodynamics between the surface and the bulk structure. [4]

Conclusions
Despite the additional challenges of in-situ TEM on oxides at very high temperatures in gas, the combination of in-situ and ex-situ 3DED, high-resolution HAADF-STEM, EDX and (mono STEM)-EELS offered a lot of new structural information on (Ca,Sr)(Mn,Fe)O3-δ and LaxSr2-xMnO4-δ in redox conditions. For these samples, their increased performance as CLOU oxygen carrier on doping, and differences in SFOC anode conductivity, were respectively linked to their differences in oxygen vacancy order and/or differences in defect structure. This illustrates the benefits of a combination of various in-situ and ex-situ TEM techniques to gain new insights in the structure-property relations of redox-based energy materials.

Financial support is acknowledged from FWO I003218N, University of Antwerp BOF TOP 38689 and the European Commission NanED Grant number 956099.

Keywords:

3DED, in-situ, SOFC, CLOU, redox

Reference:

[1] Galinsky, N., et al. Appl. Energy 2015, 157, 358–367.
[2] Galinsky, N, et al. Appl. Energy 2016, 174, 80–87.
[3] Sandoval, M. V., et al. Int. J. Hydrogen Energy 2017, 42 (34), 21930–21943.
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In Situ TEM Biasing and Heating of Neuromorphic Gold Nanogranular Nanofilms Showing Resistive Switching

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Background incl. aims
Nanogranular gold films, when prepared by supersonic cluster beam deposition with a thickness just above the electrical percolation threshold, exhibit highly branched structure with a neuromorphic behavior reminiscent of biological neural networks, including resistive switching and adaptation. Such characteristics position these materials as viable substrates for neuromorphic computing. Despite their potential, the understanding of the underlying phenomena has been limited to electrical measurements and theoretical speculation. The objective of this study was to visually elucidate the morphological transformations that occur at the micro- and nanoscale within these films during electrical stimulation and to assess how these changes correlate with neuromorphic functionality.

Methods
Gold nanogranular films were deposited at approximately 17 nm thickness to balance electrical conductivity with TEM imaging suitability. These films were then subjected to in situ biasing within a two-electrode configuration. High temporal resolution imaging was conducted using a CMOS direct detection camera, operating at a controlled low electron dose. The morphology of the entire area between electrodes was observed during sequential electrical stimulations—first across a voltage ramp and then at a constant voltage. Additionally, in situ heating TEM imaging was applied, observing the films under thermal stress at both high resolution and low magnification.

Results
Initial in situ biasing caused the localized de-percolation effect shown in figure [1], and characterized by the retraction of gold branches without mass loss, leading to the formation of isolated gold islands. This morphological adaptation was highly localized and accompanied by the development of small, dense gold structures. Upon global thermal stimulation, the films underwent a more extensive de-percolation, resulting in the creation of distinct, polycrystalline gold islands across the whole heating substrate. This behavior is akin to that observed in atom-assembled gold films. The in situ TEM imaging revealed the likely occurrence of extremely intense and localized hot spots, whose temperature and extent appear to grow with increased voltage and may induce localized melting, even possibly capable of disrupting the amorphous silicon nitride substrate when the highest voltage and temperature are reached [1].

Conclusions
The study presents a novel visual insight into the morphological dynamics of nanogranular gold films under electrical and thermal stimuli, suggesting that intense, localized hot spots may play a critical role in neuromorphic behavior. The findings highlight the need for further exploration of the local

temperature within these hot spots, the role of electromigration and Joule heating, and the impact of the substrate on the film's behavior. In situ TEM imaging has proven invaluable in expanding our understanding of the physical phenomena driving the unique electrical characteristics of metal nanogranular films, offering implications for the advancement of materials with neuromorphic properties.

Keywords:

in-situ biasing and heating TEM

Reference:

[1] A. Casu, Angelica Chiodoni, Yurii P. Ivanov, Giorgio Divitini, Paolo Milani,* and Andrea Falqui*. In Situ TEM Investigation of Thermally Induced Modifications of Cluster-assembled Gold Films Undergoing Resistive Switching: Implications for Nanostructured Neuromorphic Devices. Accepted by ACS Applied Nano Materials, March 2024.

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Electron holography for electrostatic potential measurement and contrast enhanced imaging of biological samples

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Poster Group 2

Background
Transmission electron microscopy (TEM), especially at cryogenic temperature, is largely used for studying biological macromolecules at high resolution and for 3D reconstruction. The main difficulty of TEM imaging of biological samples is the weak amplitude contrasts due to electron diffusion on light elements. Achieving 3D high-resolution reconstructions implies the acquisition of a huge number of TEM micrographs followed by a time-consuming image analysis. This TEM constraint could be overcome by imaging the phase shift of the electron beam resulting from its interaction with the sample.
Methods
In our work, we developed two TEM techniques, off-axis electron holography and in-line electron holography, for phase image extraction of biological samples. We mainly used unstained T4 and T5 bacteriophages as model samples which were deposited on graphene grids provided by Sara Bals's team (Antwerp University, Belgium) for room temperature experiments, while we used lacey grids for cryogenic temperature experiments.
Results and Conclusions
Thanks both to recent developments carried out at CEMES on off-axis holography [1,2] and to the direct electron detectors performances, we show on one hand that off-axis holography enables us to retrieve the phase shift information that the electron undergoes when interacting with the low-Z biological samples (figure 1 a-d). On the other hand, thanks to the in-line holography capability to be performed on any microscope, as well as the automation of a cryogenic electron microscope equipped with a direct electron detector, we show the ability of this technique to resolve high-resolution structural details (figure 1 e-f) with very low noise levels at low electron dose. We thus demonstrate the possibility of both techniques to obtain highly contrasted phase images of unstained samples both at room and cryogenic temperatures, in addition to an improved signal-to-noise ratio. Furthermore, since a change in electric charges affects the phase shift and can be detected by electron holography, we show a novel potential application using both off-axis and in-line electron holography methods, to measure electrostatic variations on biological specimens. This was evidenced in off-axis holography by measuring the phase shift changes at the capsid between phages treated with spermine, a multivalent cation that diffuses inside the capsid and neutralizes the DNA, and a non-treated T5 phages (Figure 1d). This electrostatic change was also observed locally by in-line holography by studying at high resolution the phase shift pattern of the tail, and globally by studying the phase shift signal around a full capsid and comparing it to the one around an empty one (figure 1f). More work is needed to fully take advantage of these microscopy phase imaging techniques

Keywords:

Electron Holography
Electrostatic potential
Cryo-EM

Reference:

[1] C Gatel et al, Applied Physics Letters, 113 (2018), p. 13. https://doi.org/10.1063/1.5050906
[2] M Hÿtch & C Gatel, Microscopy, 70 (2021), p. 47. https://doi.org/10.1093/jmicro/dfaa044

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Investigating the potential variation of in-operando semiconductor nanostructures in electron beam direction

Hüseyin Çelik[1], Mr. Robert Fuchs[2], Dr. Dirk Berger[3], Dr. Christian M. Günther[3], Mr. Simon Gaebel[4], Dr. Tolga Wagner[5], Prof. Dr. Michael Lehmann[1]
[1]Technische Universität Berlin, Institute of Optics and Atomic Physics, , Germany, [2]Technische Universität Berlin, Institute of Theoretical Physics, , Germany, [3]Technische Universität Berlin, Center for Electron Microscopy (ZELMI), , Germany, [4]Max-Born-Institut für Nichtlineare Optik und Kurzzeitspektroskopie, , Germany, [5]Humboldt-Universität zu Berlin, Department of Physics, , Germany

Poster Group 2

Off-axis electron holography is a well-established method for the investigation of projected potential distributions down to atomic spatial resolution. However, in the case of in-operando (electrical biasing) investigations of externally controlled semiconductor nanostructures, parasitic modulations of the electron wave occur due to long-range electrostatic stray fields [1]. In addition, a well-known problem is the alteration of the sample during preparation using a focused ion beam (e.g. ion implantation, surface amorphization or generation of conducting surfaces), which also severely influences the potential distribution within the sample [2]. Both effects have a particular impact in the direction of the electron beam as well, which makes a quantitative analysis particularly difficult. Standard approaches to resolve the entire potential distribution involve projective tilt series and their tomographic reconstruction [3], which entail a significant measurement effort (e.g. sample tracking or long-time stability) and instrumentational limitations (e.g. limited tilt angle (i.e. missing wedge), interior Radon transform or parallax displacement), in addition to extensive simulations (e.g. FEM or DEM), which are highly computationally intensive and require rarely given knowledge of the microscopic charge carrier distribution.
Here, a simple and intuitive model (SIMP) for the approximation of such potential distributions inside and outside nanostructured FIB-prepared samples of a p-n junction, requiring a limited set of parameters, is presented. The model uses only independent convolutions of an initial potential distribution (e.g. analytic textbook models) with a Gaussian kernel (see attached figure), allowing the reconstruction of the entire potential distribution from only one measured projection (electron hologram). In addition, various contacted semiconductor nanostructure samples (TEM-lamellae) are produced in a systematic approach using FIB under varying preparation parameters (i.e. currents and acceleration voltages of the ions) to evaluate the proposed model.
In comparison with FEM-simulations, representing an established simulation method, it can be shown that the self-developed model is able to accurately approximate the 3D electrostatic potential distribution of various contacted TEM-samples, whereby the computational complexity can be significantly reduced with respect to FEM-simulations (i.e. ~1000x faster with ~1/1000th of the memory usage at ~5000x more nodes). An excellent agreement can likewise be observed in comparison with electron holographic and tomographic investigations considering experimental restrictions, revealing the real potential distribution in propagation direction of the electron beam. By this, a significant reduction of the required computational power as well as a drastically simplified measurement process is achieved, paving the way towards quantitative electron holographic investigation of electrically biased semiconductor nanostructures. In particular, the latter can in turn also be used to understand the exact effects of the FIB-preparation (e.g. implantation concentration or implantation depth) on the sample, thus leading to improved preparation strategies.

Keywords:

Electron-Holography, Semiconductor-Nanostructures, 3D-Potential-Distribution, Surface-Effects, Computational-Optimization

Reference:

[1] S. Yazdi et. al., Ultramicroscopy 152, 10 (2015).
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[3] A. C. Twitchett-Harrison et. al., Nano Lett. 7, 2020 (2007).

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Differential Phase Contrast Microscopy and Atom Probe Tomography of Ferromagnetism in High-Entropy Alloys

Ms. Shabnam Taheriniya[1], Dr Harald Rösner[1], Mr. Gerhard Wilde[1]
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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background incl. aims
High-entropy alloys (HEAs) are emerging as promising materials for future applications due to their high configurational entropy and diverse local atomic environments. Among the four core effects in HEAs, the concept of the 'cocktail effect', resulting from multi-principal alloy design, is particularly intriguing as it can lead to the emergence of new properties. When elements with different structural, kinetic, and ferromagnetic properties are combined in single-phase or nanocomposite HEAs, unexpected interactions and the emergence of new properties or phases can occur, surpassing predictions based on simple mixtures. Certain equiatomic alloys, initially paramagnetic, have been found to exhibit ferromagnetic properties after undergoing deformation processes like rolling or cold-working. This unexpected behavior has been attributed to changes in the local atomic environment and the creation of defects during deformation, resulting in the formation of ferromagnetic clusters. Understanding the mechanisms behind deformation-induced ferromagnetism in these alloys is the aim of this contribution.
Methods
In this study, single phase (CoCrFeMnNi) and nanocomposite HEAs (CoCrFeMnNi and HfNbTaTiZr) were processed using high-pressure torsion (HPT), subjecting the samples to a constant pressure of 9 GPa either as a single disk or stacked disks, with the top anvil rotating at 1 rpm at ambient temperature for up to 15 revolutions. Vibrating sample magnetometry (VSM) confirmed that HPT processing induces the development of ferromagnetic properties. The distribution and orientation of magnetic domains post-deformation were examined in detail using differential phase contrast scanning transmission electron microscopy (DPC STEM), analytical TEM and atom probe tomography (APT) analysis.
Results
Analytical TEM indicates that while the nanocomposite HEA shows significant phase sep-aration due to co-deformation, its saturation magnetization measured by VSM is lower than that of the HPT-processed Cantor alloy. The pronounced phase separation in the nanocomposite HEA leads to lamellar-like alignments of NiCo-rich ferromagnetic and Cr-rich anti-ferromagnetic domains observed by DPC, reducing the overall magnetic moment. Conversely, the absence of such phase separation in the HPT-processed Cantor alloy results in comparable coercivity. APT analyses reveal that this difference arises from the deformation-induced local enrichment of ferromagnetic elements, particularly Ni, showcasing the 'cocktail effect' in HEAs where inter-element interactions lead to unique magnetic behavior.
Conclusions
Our study demonstrates that HPT processing of HEAs induces a transition from paramagnetic to ferromagnetic states at ambient conditions. Deformation-induced ferromag-netism can be explained by the 'cocktail effect' in HEAs, where the formation of ferromagnetic particles is linked to deformation-induced element-selective atomic migration and local enrichment of ferromagnetic elements.

Keywords:

high-entropy alloy; ferromagnetism; nanocomposites; deformation

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Optimizing micrograph contrast in multicomponent systems at low-voltage scanning electron microscopy

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Poster Group 2

Background incl. aims:
Scanning electron microscopy (SEM) is one of the most widely used electron microscopy techniques in science and engineering. Although the SEM was originally intended for operation at low beam acceleration voltages (BAV), it was only possible to operate at low beam energies decades after the first commercial SEM was available. Hence, most of the pioneering studies of electron-specimen interaction physics have been conducted at BAVs above 5 kV. In recent years, the reliable and easy-to-operate field emission guns (FEGs), and the improved optics, vacuum, and detectors in modern SEMs, have led to the widespread use of low-voltage SEM. However, studies in the low BAV range remain limited, and there is a lack of awareness of their importance. The purpose of this study is to investigate the effects of SEM operational parameters and specimen properties on micrograph contrast at BAVs below 2 kV.
Methods:
The modern SEMs, equipped with the in-the-column ("InLens" in the Zeiss lingo) secondary electron detector for high-resolution imaging, are flexible instruments allowing operation over a wide range of parameters. Two of the fundamental SEM operational parameters are the BAV and the working distance (WD). Here, we investigate systematically the effect of the BAV and the WD on the InLens micrograph contrast. We image a variety of specimens, consisting of conductive (carbon nanotubes) or insulating (boron nitride nanotubes) materials, or their combination, on conductive (silicon wafer) and insulating (glass slide) substrates. To include all combinations, we imaged 6 specimen types, each specimen was imaged at 3 different BAVs, where at each BAV the micrograph was acquired at 3 different WDs.
Results:
Our results show that the InLens micrograph contrast is directly affected by the BAV and the WD, and by the specimen electrical conductivity properties. We analyze the contrast changes and explain them through the changes in the secondary electron signal at different conditions. We demonstrate that choosing adequate SEM operational parameters is essential for obtaining an optimal micrograph contrast, and thus for obtaining complete information of the specimen. Moreover, in the case of insulating specimens, we demonstrate the possibility of avoiding specimen charging without using conductive coating.
Conclusions:
For proper selection of the SEM operational parameters, one should understand how micrograph contrast changes with BAV and WD, and be able to adjust them according to specimen properties. This way, an optimal micrograph with sufficient contrast but without charging artifacts can be obtained. As our study includes a variety of systems combining conductive and insulating components, its conclusions can be easily adopted for a wide range of systems and studies.

Keywords:

Low-voltage SEM, micrograph contrast

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Vibration-Corrected Electron Ptychography

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[1]Humboldt-Universität zu Berlin, Berlin, Germany, [2]Center for the Science of Materials Berlin, Berlin, Germany

IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

Background incl. aims

Far-field electron ptychography is considered to be one of the most powerful phase retrieval techniques currently available for electron microscopists. Recently it was shown that ptychography is capable to surpass the Abbe resolution limit [1] and resolve specimens features as fine as the blurring due to the vibrations of the atoms [1]. A rather simple mathematical model of coherent diffraction pattern formation standing behind conventional algorithms [2,3] does not account for any motion or superposed states [1,2,3]. In order to overcome the resolution limit caused by the lattice vibrations we utilize the mixed-object formalism initially proposed in 2013 for X-rays [3], but never applied to electron microscopy data without assuming explicit atom positions [4]. In contrast to conventional ptychography [1,2,3] that treats a single transmission function of a specimen, mixed-object ptychographic reconstruction considers a sequence of superposed transmission function states, each producing a coherent diffraction pattern via a multislice simulation [1,2]. The total diffraction pattern corresponding to the entangled system is formed as an incoherent sum over the states [2,3,4].

Methods

For the tests of mixed-object ptychography we used a 4D-STEM dataset [1,2] of a monolayer MoS2 acquired in the Nion Hermes microscope at an accelerating voltage, convergence semi-angle, scan step and electron dose of 60 kV, 33 mrad, 0.2 Å and 5.3*10^6 e-/Å^2, respectively. To evaluate the resolution of the ptychographic reconstructions we propose a novel approach, akin to the Young fringe resolution test widely applied in TEM imaging [5]. We perform two ptychographic reconstructions with random initial guess for the object, e.g. uniform prior. Then the two independent results are shifted with respect to each other and the Fourier intensity of the difference is computed. The arising interference fringes indicate the range of spatial frequencies identical in the two results, allowing to conclude how deterministic a particular reconstruction is. Further, in contrast to conventional resolution tests, e.g. visibility of the Fourier peaks [1], the proposed approach allows to include aperiodic features that are crucial for a moving specimen.

Results

Assuming a single illumination mode [1,2,3] we conducted pairs of ptychographic reconstructions in two scenarios: one involving a single-state and the other involving 10 states of the transmission function. After 500 iterations of the gradient-descent [2] the achieved resolution was estimated. As a result, two independent pure-state reconstructions appear to produce identical information up to 1.9 Å^-1, while the mixed-object reconstructions contain deterministic information up to 2.3 Å-1. Figure 1 shows a comparison between pure- and mixed-state psychographic reconstructions.

Figure 1. Comparison of pure-state (upper row) and mixed-state (lower row) ptychographic reconstructions. Panels an and d show phases of a single-state reconstruction and an average over 10 states, panels b) and e) show the Fourier intensities (averaged over the states). The panels c and f show the results of Young fringe resolution test. The information limit of 2.3 Å^-1 denoted by the

white circle was surpassed only by the mixed-state ptychographic reconstruction. The colorbar on the left side of the figure refers to the values of the phase in a) and d), the colorbar on the right refers to the Fourier intensities in b), c), e) and f).

Conclusions

Even with a monolayer specimen that is not supposed to produce a noticeable amount of incoherent scattering, we show that considering multiple entangled states of the transmission function makes the underlying model more realistic and improves the quality of the ptychographic fit. Thus, we liberate model-free ptychography from atomic vibrations, its last known resolution limit [1,2].

Keywords:

Ptychography, TDS, lattice vibrations

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Using Virtual Detectors as a Compression Tool for Electron Ptychography

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Poster Group 2

Background incl. aims

Far-field iterative electron ptychography [1,2,3] is a phase retrieval technique offering atomic scale imaging in scanning transmission electron microscopy (STEM) [4,5]. It fits an electrostatic potential of a specimen to a set of diffraction patterns collected while illuminating overlapping areas of its surface with a convergent beam [1,2,3,5]. Despite its rapid development, ptychography suffers from several practical challenges, particularly from the massive amount of data that is required to achieve a high degree of redundancy to solve the phase problem [2]. Our study addresses this limitation by proposing a lossy compression method for diffraction patterns [2] utilising virtual STEM detectors [2,4].

Methods

A 92 Å thick atomistic model of a silicon crystal containing a 60° edge dislocation dissociated into a stacking fault bounded by a 30° and 90° partial dislocation [5] was used to simulate a 4D-STEM [1] dataset with qstem [5], at an accelerating voltage of 60 kV, convergence semi-angle of 30 mrad and a scan step of 1 Å, including thermal diffuse scattering, an effective source of size 0.5 Å and a finite electron dose of 104 e-/Å^2. To perform electron ptychography we utilize an in-house written gradient-based optimization code [3] and use 4 slices of the transmission function in order to account for multiple scattering [1,2,5].

Our compression approach relies on virtual detectors [2,4]. With a single 4D-STEM detector they allow for a simultaneous recording of multiple signals, such as virtual annular dark- and bright-field (vADF and vABF), or centre of mass (COM) signals. In contrast to physical detectors, the virtual ones offer a flexibility to adjust their shapes to closely mimic actual diffraction patterns, facilitating a more efficient compression [2]. In an uncompressed scenario [1,2,3] the goal of an iterative ptychographic algorithm is to minimize the summed squared error (SSE) between the 4D-STEM dataset and the diffraction patterns generated by algorithm [1,2,3]. With a compressed data we modify the algorithm to predict the signals instead of the diffraction patterns via an additional known convolution layer [2] and aim to minimize the SSE between them and the measured signals of virtual detectors.

Results

In Figure 1 we compare three sets of virtual detectors for the bright field of the diffraction patterns: first 36 Zernike polynomials, 45 binning squares each 7x7 px wide and 15 virtual detectors generated via the Gram-Schmidt algorithm [2] from the diffraction patterns acquired at the positions indicated by the x-markers in the panel c). The dark field of the diffraction patterns was described via a single vADF detector in all three cases. For each compression option we show 6 bright field virtual detectors in panels e)-v) and compute the Fourier ring correlation with the projected potential used for the simulation of the dataset.

Figure 1 Comparison of ptychographically reconstructed projected phases of a silicon crystal from compressed 4D-STEM data. a), b) and c) show the results for first 36 Zernike polynomials, 45 binning detectors and 15 data-dependent virtual detectors, respectively. For each of three options we show 6 virtual detectors in e)-v). For data-dependent detectors we denote the scan positions used to generate them via lime-colored x-markers in c). The projected electrostatic potential used for a simulation of the dataset is presented in d). Further we compare the Fourier ring correlations between the reference potential and three reconstructions in w).

Conclusions

The diffraction patterns in the initial dataset were 500x500 px wide. Our findings reveal that through the application of virtual detectors, including Zernike polynomials, binning squares, and detectors generated via the Gram-Schmidt algorithm, we can achieve a data compression ratio [2] of 5,000 to 15,000. Most importantly, our results demonstrate that atomic resolution in ptychographic reconstructions can be maintained even under conditions of partial spatial coherence and limited electron dose. The proposed compression approach takes the specific geometry of diffraction patterns into account and retains a sufficient amount of the "intact" information [2]. No specific constraints, e.g. a regularization, were used, but as previously shown [1], this could improve the quality of the reconstructions.

Keywords:

4D-STEM, Compression, Virtual Detectors, Ptychography

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Advanced Brillouin fibre probes for in situ Brillouin imaging

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Poster Group 1

Background
Brillouin imaging (BI) has become a valuable tool for micromechanical material characterisation, thanks to extensive progress in instrumentation in the last few decades. This powerful technique is contactless and label-free, thus making it especially suitable for biomedical applications. Nonetheless, to fully harness the non-contact and non-destructive nature of BI, transformational changes in instrumentation are still needed to extend the technology's utility into the domain of in vivo and in situ operation, which we foresee to be particularly crucial for widespread usage of BI, e.g. in medical diagnostics and pathology screening. This talk reports on the progress in fibre-optic Brillouin probe design and fabrication, as well as provides outlook for fibre-based Brillouin imaging.

Methods
Two approaches in constructing Brillouin fibre probes are tested: 1) using a hollow core fibre (figures A and B) and 2) dual fibre probe with a 3D printed micro-lens system (figure C). The spectroscopic detection is done using a 6-pass scanning Fabry-Perot interferometer (TFP1, Table Stable). The results are analysed using home-build software in Matlab.

Results
The main challenge in constructing a fibre probe for Brillouin imaging is the strong back-scattered light generated in the fibre itself. Although this fibre signal appears in the frequency band that is far from the frequency region of interest (associated with biologically relevant signals), the strength of the fibre back-scattering is many orders of magnitude higher than that coming from the biological sample, thus making the detection non-trivial. Several approaches to fibre-integrated Brillouin imaging will be discussed, including: (1) single hollow-core fibre (figures A-B) and (2) two-fibre probe with a micro-lens system design (figure C). In the single fibre approach, the fibre is chosen to have a hollow core that in theory prevents background scattering. The performance of the hollow-core fibre Brillouin imaging system is evaluated by mapping a biological phantom consisting of a hydrogel spheroid and media. The scattering efficiency in this demonstration is found to be comparable with the bulk-optical Brillouin microscopy systems.
Two-fibre Brillouin probe approach can solve the challenge of the spurious scattering background due to separation of the illumination and detection paths. It, however, requires specialised solutions for light focusing that preserve both tight focusing into the sample and high collection efficiency for the scattered light. Such a solution based on a 3D printed micro-lens, specifically engineered for Brillouin imaging application, will be discussed and the scanning results using the dual-fibre probe will be demonstrated.

Conclusion
In conclusion, different strategies for achieving fibre-integrated Brillouin imaging are tested and compared. Each strategy exhibits advantages and limitations and the choice will depend on the endoscopic application in mind.

Keywords:

Brillouin imaging, fibre integration, micromechanics

Reference:

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DQE measurement for TEM detectors: from the key parameter to an ambiguous estimate

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Poster Group 1

Background incl. aims
Nowadays, the performance of CMOS electron detectors for transmission electron microscopes (TEM) are mainly compared by means of a unique parameter, the detective quantum efficiency (DQE). This parameter is largely promoted by camera providers, and is given as a unique number while it is known to strongly depend on the electron dose. In addition, the DQE calculation method may influence the result, as it will be presented in this study. Consequently, several DQE values can be found for one detector, and one should wonder if a unique DQE is the correct figure of merit for electron detectors.
One aim of this work is to demonstrate that the most commonly used method to calculate the DQE leads to strong uncertainties. To achieve this goal, measurements are conducted on an electron detector and a model is developed. Then, the second aim is to provide recommendations and trustable alternatives for detector comparisons.

Methods
The DQE depends on the spatial frequency w and is generally expressed as a component DQE(0) at the spatial frequency (0) times components depending on the spatial frequency, as in the following:
$DQE(w)=DQE(0)xMTF(w)^2/NTF(w)^2$
where MTF is the modulation transfer function and NTF the noise transfer function. MTF and NTF are well established so this work focuses on the DQE(0) calculation.
DQE(0) can be estimated by means of the full calculation of the various noise sources, including the shot noise, the gain variance "σg", the Fano noise "F", the dark current "DC" and the readout noise "NRO":
$DQE(0)=g^2/(g^2\ (1+F)+\sigma g^2+(t\cdot DC+NRO^2)/n\_i\ )$
where "n_i" is the number of incident electrons, "g" is the detector gain, "t" the integration time
The gain is extracted thanks to a comparison between the integrated electrons and the beam current measured with a Faraday cap, and the gain variation is estimated from the spatial variance of a flat field picture. While these measurements are not too complex, it is much more difficult to measure the Fano noise. It can be estimated with the standard deviation of simulated deposited energy distributions. Then, the readout noise and the dark current are measured from several acquisitions performed in dark condition.
Since several years, a simplified method has been preferred for the DQE(0) estimation, and relies on the direct measurement of the detector output noise by means of the spatial variance of a flat field image. However, in this case, the large electron hole distribution generated by the beam is spread between pixels and the spatial variance is underestimated. To solve this issue, McMullan proposed in 2009 to extract the spatial variance on binned pixels, for which a saturation is achieved if the binning number is large enough. This method is referred as the McMullan method subsequently.

Results

A Gatan-Rio-16 camera mounted on a JEOL-2100 FEG microscope is used for this study. This detector being an indirect one, the Fano noise is due to the scintillator and is estimated at 0,25. The electron dose is chosen in order to achieve the half full well capacity of the detector. For n_i=107, DQE(0)_FullCalculation=0.79 and DQE(0)_McMullan=0.62.
These two results are quite different and it is supposed that the McMullan method over-estimates the output noise and therefore gives a lower DQE(0).
With the intention to demonstrate it, a simple model is built, based on the generation of a shot noise and a gain variation leading to a similar noise distribution acquired with the Gatan-Rio-16 camera. For this purpose, the standard deviation of the noise is adapted is order to get an extracted variance identical to the measured one. In addition, a Gaussian blur is added with the intention to simulate the electron spread over the pixels and is parametrized in a way to get the same saturation of the variance according the binning number. The resulting noise distribution does not match the measured one which is much more extended and show pixels with much higher noises.
Actually, the McMullan method requires to subtract two flat field pictures in order to remove dead and saturated pixels, and the fixed pattern noise which avoid a correct variance saturation. However, it is necessary to consider other defective pixels, such as the hot pixels with a higher gain. These defective pixels are not removed by the subtraction of two flat field images and may distort the variance extraction. To demonstrate it, the previous model is modified and one population of hot pixels is introduced. The hot pixels ratio and gain are adjusted in order to obtain a noise distribution similar to the one measured with the Gatan-Rio-16. A hot pixel density of 6×10^(-4) with a high gain of 30 lead to a noise distribution comparable with the experiment, and the spatial variance extracted with hot pixels is 50% higher compared to the one extracted without hot pixels (see the figure 1).
Therefore, it is demonstrated that the commonly used McMullan method leads to an underestimation of the DQE(0) because of hot pixels. Other limitations are found and discussed, based on the fact that the noise is distributed on a statistical population of hot pixel which may give different results at every DQE measurement.
Consequently, the DQE(0) should not be used as a unique number for the detector comparison because it depends on the electron dose, on the experimental condition and on the used method. In order to fairly compare detectors, it is therefore recommended to use other parameters clearly defined and measurable: the gain for an information on the sensibility, the MTF for the spatial resolution and the dark current for the radiation hardness.

Conclusion
The DQE(0) is measured on a commercial camera with two methods and it comes that the McMullan method, largely preferred by the camera providers, leads to large uncertainties. By means of a model, it is demonstrated that detector hot pixels induce an over-estimation of the output noise leading to the DQE under-estimation with the McMullan method.
The DQE suffering from large uncertainties it is recommend to use the gain, the MTF and the dark current for fair comparisons between detectors.

Keywords:

DQE; simulation; electron detector, TEM

Reference:

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Fractal: An open-source framework for reproducible bioimage analysis at scale using OME-Zarrs

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Poster Group 2

Background incl. aims
Analyzing large amounts of microscopy images in a FAIR manner is an ongoing challenge, turbocharged by the large diversity of image file formats and processing approaches. Recent community work on an OME next-generation file format [1] offers the chance to create more shareable bioimage analysis workflows. Building up on this and to address issues related to the scalability & accessibility of bioimage analysis pipelines, the BioVisionCenter, a newly-created structure of the University of Zurich & the Friedrich Miescher Institute for Biomedical Research, is developing Fractal [2], an open-source framework for processing images in the OME-Zarr format.

Methods
The Fractal framework consists of a server backend & web-frontend that handle modular image processing tasks. It facilitates the design and execution of reproducible workflows to convert images into OME-Zarrs and apply advanced processing operations to them at scale, without the need for advanced expertise in programming or large image file handling.

Results
Fractal allows users to orchestrate the analysis of terabytes of high content microscopy images on high performance clusters. It comes with pre-built tasks to perform instance segmentation with state-of-the-art machine learning tools, to apply registration, and to extract high-dimensional measurements from multiplexed, 3D image data. We are providing a web front-end to facilitate user interactions with Fractal and streamline the submission of image analysis jobs to a slurm cluster. Finally, by relying on OME-Zarr-compatible viewers like napari [3], MoBIE [4] and ViZarr [5], Fractal enables researchers to interactively visualize terabytes of image data stored on their institution's remote server, as well as the results of their image processing workflows.

Conclusions
We present the open-source framework Fractal for FAIR image analysis at scale in the OME-Zarr format.

Keywords:

OME-Zarr, bioimage-analysis, workflows, processing, FAIR

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Light sheet imaging across scales for preclinical drug discovery research

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Poster Group 1

Whole organ 3D light sheet fluorescence microscopy (LSFM) is gaining popularity in preclinical pharmacological research to map the expression of new drug targets, characterize compound biodistribution, visualize drug induced changes in neuronal activation and to demonstrate the efficacy of pharmaceuticals to alter pathological changes in animal models of human diseases. Its broad applicability arises from the capability of imaging structures ranging from a few micrometres to centimetres, possibility of visualizing multiple fluorophores simultaneously and detecting subtle differences in their intensities. As such, LSFM bridges the gap between traditional in vivo imaging modalities and high-resolution histology and confocal microscopy of fixed tissue samples. Still, sample preparation for LSFM depends heavily on the tissue type and the desired imaging endpoints. Testing multiple parameters is often time-consuming as the protocols can take months to complete. Here we demonstrate a stepwise decision matrix to select suitable sample preparation steps for all rodent organs to characterize both compound distribution, mRNA and protein localization. We demonstrate pretreatment steps most suitable to visualize compound biodistribution, show the improved protocol to achieve uniform antibody staining in rat samples and present the workflow for 3D LSFM of hearts and other peripheral organs. Finally, we successfully expand the proposed decision matrix to include minipigs brains, a large gyrencephalic brain.

Keywords:

light-sheet microscopy
preclinical
drug discovery

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Unlocking Macroscale Solutions with Nanoscale Information: Towards Next-generation Optoelectronics

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Poster Group 2

Background incl. aims
Metal halide perovskites are a class of semiconductors that have garnered a tremendous amount of attention for application in optoelectronic devices. These low-cost materials exhibit outstanding properties, such as efficient light absorption, tunable emission, and good charge carrier transport. As an example, perovskites have accomplished a rapid rise in solar power conversion efficiency to over 25% in less than a decade, outperforming the conventional silicon solar cells. However, the fundamental understanding of their intrinsic properties and of the impact of defects on their photophysical performance and stability is lagging behind. We aim to exploit this nanoscale information to further improve the materials' applicability.
Methods and Results
Different synthesis strategies are applied to obtain perovskite nanocrystals with slight varieties in composition and structure. In this talk, I will focus on how we apply a set of advanced spectroscopic and correlated microscopic tools to detail the dynamics of the photogenerated charges and defects that govern the optoelectronic performance. Moreover, the stability of the corresponding materials will be discussed, and a novel approach to monitor device stability will be presented.
Conclusion
It will become clear that it is important to rationally micro-engineer the perovskites' chemistry, structure, and carrier dynamics toward an improved material platform for next-generation optoelectronics.

Keywords:

Hybrid perovskites
Correlated spectro-microscopy
Optoelectronics

Reference:

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Comprehensive 3D Characterization Workflow for Solid Oxide Cells

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Poster Group 2

Background

Performance and durability of solid oxide cell (SOC) electrodes depend heavily on their porous microstructure properties and evolution over time. Ga+ FIB-SEM tomography has been pivotal for reconstructing SOC microscopic structures with nanometric precision [1], albeit limited to small volumes. With the emergence of Plasma FIB-SEM (PFIB-SEM), offering access to significantly larger material volumes [2], SOC microstructure investigation has taken a leap forward.
The porous nature of SOC microstructures poses challenges during both data collection and processing, resulting in artifacts like FIB curtaining and back-pore issues. Though techniques such as pressure-filling pores with resin [3] and rocking polish [2] mitigate these, electron charging effects persist. Deep Learning (DL) methods have shown promise in addressing back-pore issues [4], but challenges remain in electron charging compensation.
Post-segmentation, serial-sectioned SEM images undergo further processing to create 3D digital representations. This involves numerical simulations incorporating morphological parameters like tortuosity and constrictivity, crucial for understanding transport properties. Various software packages aid in this 3D characterization process [5].
This contribution presents a complete workflow for SOC microstructure characterization, from 3D reconstruction to comprehensive quantification. Using a Plasma FIB-SEM platform and automated serial sectioning software, a large 3D volume of SOC electrode is obtained, enabling detailed analysis. Advanced SEM imaging techniques coupled with DL-based segmentation streamline data processing, significantly reducing workflow time.

Methods

To demonstrate the workflow (Figure 1), we characterize a fresh solid oxide fuel cell (NYDC-55-3-0). Using the Plasma FIB-SEM platform and automated serial sectioning software (Auto Slice and View 5 - ASV5), we collect a large 3D volume (180 × 150 × 30 µm³) with a voxel size of 32 × 32 × 50 nm³. The Xe+ plasma beam current of 60 nA @ 30keV serial sections the specimens with a ±3° beam rocking motion and a 25 nm cut advancement. After each odd PFIB cut, SEM images are simultaneously acquired perpendicular to the cut face using a through-lens detector (TLD) for secondary electrons (SE) and a retractable concentric backscattered electron detector (CBS) for backscattered electron images. We utilize an SEM current of 200pA @ 2keV high tension and a 5 µs pixel dwell time to exploit the charge contrast phenomenon captured by the TLD-SE detector, enabling the separation of percolated and non-percolated Ni. The backscattered electron images from the CBS detector highlight voids and YSZ grains. The activated ASV5 auto functions for SEM, including source tilt, lens alignments, stigmatisms, focus, and image matching for drift correction, ensure consistent imaging conditions throughout the ASV run. By employing auto image matching for drift correction and SEM imaging at the normal angle to the cut face, we maintain volume-lossless conditions in the 3D image stack before data post-processing.

Results
We developed automated Avizo recipes to streamline data processing, analysis, and enable consistent and reliable data quantification and 3D visualization. The image stack doublets are subjected to denoising and segmentation into Ni, YSZ, and Voids using DL models [6], which also distinguish between percolated and non-percolated phases. The recipes calculate various spatial parameters (Figure 2) such as tortuosity [7], TPBs (total length, length/volume), phase volume fractions, percentage of non-percolating for each phase, and surface area/volume for Void|YSZ, Void|Ni, and YSZ|Ni. Notably, recent advancements in SEM image post-processing and denoising using DL algorithms in Avizo software have reduced SEM imaging time by 50%, resulting in a 25-30% reduction in the total acquisition time for the entire dataset.

Conclusion
This study shows comprehensive workflow that harnesses deep-learning predictions, encompassing the entire process from PFIB-SEM-based 3D microstructure analysis to meticulous quantification, illustrated through the examination of a freshly prepared solid oxide fuel cell. Our innovations have led to a thousandfold increase in image data acquisition volume compared to previous studies, all while maintaining a consistent voxel size. To ensure robust identification of material constituents, we simultaneously acquired CBS BSE and TLD SE images, ensuring 99.99% confidence. Key focuses include precise specimen preparation, optimizing PFIB-SEM setup for serial-sectioning, SEM image acquisition, DL-based post-processing and segmentation, recipe-oriented 3D quantification of morphological parameters, and report generation. Advanced SEM imaging techniques, coupled with automation and recent advancements in SEM image post-processing and segmentation using ML algorithms, streamline the characterization process, significantly reducing workflow execution time.

Keywords:

PFIB-SEM, Deep-Learning, Serial-Sectioning Tomography, SOFC

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Optimized Bright Field STEM Imaging for Detecting Molecules absorbed within Zeolite Pores

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Poster Group 2

Background incl. aims
Zeolites, characterized by their large and regular pores, are utilized to filter target molecules from mixtures, purify effluent by removing pollutants and in catalysis. Atomic resolution imaging of small molecules in pores will contribute to elucidate host-guest interactions between small molecules and porous structures and understand diverse molecular absorption/desorption behaviors in pores. Scanning transmission electron microscopy (STEM) is a powerful technique to capture interpretable atom-scale images, facilitating localized structural analyses. Recognizing the inherent sensitivity of zeolites and molecules to electron beam exposure, low electron dose imaging techniques, including the differential phase contrast (DPC) employing segmented detectors and 4D-STEM using pixelated detectors, have been developed. Pixelated detectors employed by 4D-STEM offer superior electron collection efficiency with fine resolution and high signal-to-noise ratio (SNR) at probe positions[1]. However, pixelated detectors entail higher costs and longer dwell time compared to segmented counterparts. The integrated DPC (iDPC) technique has shown its power in imaging light elements with low-dose conditions. Recently, a newly developed optimum bright-field (OBF) STEM technique demonstrates enhanced SNR relative to iDPC[2 3]. OBF images are reconstructed by combining the images captured by segmented detectors. Each segmented image is weighted by a frequency filter for the contribution to the Fourier component of the reconstructed OBF image, where the frequency filters are built using phase contrast transfer functions (PCTFs) for the segmented detector[2]. Here, the OBF-STEM technique with a custom-made python package is utilized for the efficient detection of molecules within the pores of ZSM-5 zeolite using a four-segmented detector.
Methods
The theoretical framework of the OBF STEM image can be derived from the single side band (SSB) ptychography[2], where the specimen is weakly electron scattering. The exit wave function M(k ,Q) is given by the convolution of the entrance probe wavefunction ψ(k)·ψ* (k-Q) on the specimen with the pupil function A(k) A* (k+Q) , where Q is the spatial frequency of the probe at spaced position r on the specimen in Fourier space. Therefore, the PCTF can be calculated by T* (k)T(k-Q)-T(k) T* (k+Q), where T(k) is the aperture transfer function defined as the multiplication of the aperture function with the probe wave function in reciprocal space. In a segmented detector, the STEM image intensity of the i-th segment I_i (r) is described mathematically as ∫I(k, r) D_i (k)𝑑k, where D_i (k) is the i-th segmented detector response function in reciprocal space and I(k, r) is the inverse Fourier transform of the diffraction pattern (|M(k, Q)|^2). The OBF STEM image is reconstructed by combining the segmented images weighted by frequency filters in reciprocal space, or combining the convolution of each segmented image with a corresponding point spread function (PSF) in real space[2]. For maximizing the SNR of the reconstructed OBF image, the PCTFs in the segmented detector are optimized by incorporating real-valued functions, such as high/low pass filters[2]. In this work, a custom-made python package is developed to calculate the PCTFs and the corresponding real-valued functions for OBF STEM image reconstruction. The specimen of ZSM-5 zeolite absorbed pollutants was imaged with the convergence semi-angle of 21 mrad on an aberration-corrected FEI Titan Cubed Themis Z microscope operated at 300 kV. A four-segmented detector was employed to capture

images using a beam current around 4.7 pA and a dwell time of 2 µs per pixel within a collection angle range of 8-42 mrad, with a camera length of 185 mm.

Results

The ZSM-5 with pollutants in pores was imaged along the [0 1 0] direction under a dose rate of 856 e-/Å². iDPC image (Figure a) and OBF image (Figure b) were reconstructed using the same dataset. Compared with the iDPC image, OBF image demonstrates clearer structural details due to its higher contrast and SNR, evident in line profiles and the cropped image (Figure d) from the same area in iDPC and OBF images. The superior contrast and finer resolution of OBF image enable more distinct observation of pollutants (marked by arrows in the line profiles) within pores than that of the iDPC image.

Conclusion

OBF-STEM images were reconstructed using the custom-made python package. This package implements algorithms to compute the PCTFs and the corresponding real-valued filters for image reconstruction. The reconstructed OBF image demonstrates enhanced contrast and SNR, giving clearer discernment of structural features. Importantly, features within the pores of ZSM-5 were distinctly distinguished in the OBF images compared to those reconstructed iDPC images. These findings imply that the OBF technique, employing a high-speed segmented detector, represents a promising approach for low-dose imaging of molecules encapsulated within the pores of zeolites.

Keywords:

STEM, segmented detectors, low-dose imaging

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A Better Insight Into Battery Materials Using A Correlative Approach

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Continued demand for electrification of transport, portable consumer electronics and even grid level storage continues to drive rapid developments of battery technology. Although each use case has its own relative performance criteria there is an overall need to increase charge and power density, cell cycle lifetime and safety while continuing to decrease cost per cell. For NMC cathodes particle size and material consistency has a large impact on final cell performance. Material inconsistencies decrease lifetime and can even result in catastrophic failure. Rapidly determining consistency while performing detailed characterisation of identified irregularities enables significant improvements in the next generation of materials.
Here we show how using multiple techniques can deliver both that rapid assessment as well as detailed understanding of the material. However, it can be difficult and time consuming to know where on the sample to begin analysis. The first step is to create an overview of the sample, which can subsequently be used to identify areas for further analysis. A rapid (~2 min) survey using backscatter electron and x-ray (BEX) imaging identified areas of titanium (Ti) and sulphur (S). Where the chemistry was sufficiently divergent from NMC, that further analysis was required.
Once an interesting area with both Ti and S containing phases was selected, further detailed analysis was carried out. In this sample, it was the larger particles that were of interest to understand the chemical consistency. As some of the features identified in the BEX image were small and lithium is present, analysis at reduced electron energy (low kV) is preferred; firstly, to improve spatial resolution and surface sensitivity and secondly, to reduce beam damage of the sample. Windowless energy dispersive x-ray spectrometry (EDS) was used because the technique is designed for low kV analysis. Identifying molecules, particularly lithiated ones, is critical in beginning to understand the charging properties of the cathode. EDS is only sensitive to elements and not molecular structure, additionally Li can be difficult to detect using EDS in compounds, therefore Raman was used to identify lithiated and non-lithiated molecules. Raman can also be used to visualize where the molecules are within the NMC particles and characterise the degree of lithiation, which will dictate the electrical properties.
To understand the structure, texture and grain boundary information, which will also indicate the electrical properties and charging efficiencies, electron backscatter diffraction (EBSD) was carried out. In cathodes, grain boundaries are typically found to be a source of failure in large particles where electrolyte impregnation can cause cracking and degradation. To characterise how both structure and composition relate to the electrical properties of the cathode particles, Kelvin Probe Force Microscopy (KPFM), an electrical mode in atomic force microscopy (AFM), was used to measure localised electrical potential.
Fig.1 shows the complementary nature of individual techniques when spatially correlated. The Raman results showed that the Ti was associated with lithium titanate (LTO, teal) and the S was associated with a lithium sulphate compound (blue). The EDS was used to aid in identifying and qualifying the Raman spectra. Structurally it was found that a high percentage of the unit cells were aligning around the perimeter of the crystal. For optimum speed of charge and discharge it is preferable to align perpendicular in the 0001 direction with the particle radius, in this case it was

found to be perpendicular to the radius thus improving the motion of Li+ ions. The KPFM results showed that the small S area had a higher electrical potential than the surround particle. However, the larger S particle had zero electrical potential; this was because the S areas were damaged by the electron beam during EDS acquisition which caused bubbling of those areas, which would cause a hollow zone and would measure as zero. New regions containing S will be measured to correctly measure the electrical properties.
This work shows that a correlative workflow works well for battery cathodes and enables critical insights. Individually, the techniques deliver useful information that can help in understanding battery materials and improving processes. When the multiple techniques are correlated, they enable new conclusions, reduce uncertainty and in some cases stop the incorrect conclusion being drawn.

Keywords:

Battery, correlative, multiscale

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Quantification of Lithium in State-of-the-Art low Voltage STEM

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Poster Group 1

Background incl. aims
This paper will present new achievements and challenges in performing quantitative EDS and EELS maps of lithium-based materials in a state-of-the-art low-voltage scanning transmission electron microscope. With the energy revolution coming because of global warming, Lithium-Ion Batteries of higher energy densities and much lower cost must be developed to allow the transition of fuel-based vehicles to be replaced with electrical vehicles. This will only become a reality if we can quantify lithium at the Nanoscale to see where it becomes lost during the electrochemical cycling of the batteries.
Methods
State-of-the-art results acquired with the Hitachi SU-9000 dedicated STEM will be presented. This microscope has EELS capabilities that allow Li detection [1], being the first to be sold by Hitachi High-Technologies. It is also equipped with the Extreme EDS system from Oxford Instruments that can detect the K line of lithium [2]. The SU-9000 has a resolution of 0,22 nm in bright field STEM without aberration correctors and this allows lattice imaging. A cryogenic specimen holder allows to analyze Li based materials at 77 K.
Results
Figure 1-a shows an EELS spectrum of Li2FeSiO4 taken with the Hitachi SU-9000 at 30 keV. Even if X-ray emission was not detected with the EDS detector for this material for the same reasons stated above for Li2CoSiO4, the ionization edges are visible for Fe, Li, and Si. This shows the strong advantage of EELS over EDS (or WDS) for Li quantification. This is seen in figure 1-b which shows a Li EELS edge-to-background ratio map of an Al-Li 2099 alloy recorded with the three-detectors system on the Hitachi SU-9000 at 30 keV. This map shows the round δ' precipitates (Al3Li) of 5 to 20 nm and the T1 plates (AlCuLi) that have thicknesses between 1 to 2 nm. Results obtained with a cryo-holder to evaluate possible minimization of beam damage will be presented.
Conclusion
It is not always possible to detect Li with EDS because its 3S electron can be bonded with another atom. EELS is superior to EDS since it is always possible to detect the ionization edge if the beam damage can be minimized with a cryo-holder. The downside of EELS is the need for a transparent specimen.

Keywords:

Electron Energy Loss Spectroscopy, Lithium

Reference:

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Quantification and Control of Mass Transport in different Liquid-Phase Transmission Electron Microscopy Flow Scenarios

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background
Liquid-phase transmission electron microscopy (LP-TEM) is an emerging experimental technique which permits the monitoring of processes in liquid samples with nanometer-scale resolution. Over recent years, LP-TEM setups have evolved into sophisticated model reactors for the study of physico-, bio- & electro-chemical processes propelled by the integration of various stimuli such as temperature, electric bias, and reaction media composition. In particular, microfluidic systems have inspired numerous expectations among the community, most notably the control of supply of reagents to and/or reliable removal of unwanted (radiolytic) species from the imaging region. However, the evolution of LP-TEM into a quantitative imaging technique remains limited by several issues, most of which are related to poor calibration of mass transport dynamics in the complex LP-TEM flow channel geometries.[1] Here, we will discuss fundamental aspects of mass transport in LP-TEM flow experiments.

Methods
A general approach combining experimental and theoretic methods for the calibration of mass transport in LP-TEM flow systems will be described.[2] The experimental part relies on image contrast variation achieved by flowing a contrast agent. The theoretic part relies on finite element modelling of relevant physics, i.e. convective and diffusive transport, in realistic 3D channel geometries. Both aspects will further be implemented in a rapid prototyping procedure for the fabrication of novel LP-TEM flow reactors: a virtual prototyping step that relies on an experimentally validated model is combined with a physical prototyping step that uses microfabrication routines (lithography and wet-etching). In addition, to study the impact of the established mass transport mechanisms on beam-induced chemistry, the finite element model is further extended by reaction kinetics of radiolysis.

Results & Discussion
A comprehensive understanding of mass transport phenomena in LP-TEM flow scenarios will be provided. First, the bimodal approach is applied to understand the hydrodynamic properties of different LP-TEM flow reactor geometries. In particular, crucial geometric features of the flow channels are identified that result in either convection or diffusion being the dominant transport mechanism.[2] Second, the effect of convective transport on radiolytic reaction networks will be investigated.[3] In contrast to established assumptions, only molecular radiolytic species can be rinsed effectively. In consequence, the concentration of highly reactive species (e.g. H* and e-) follow non-linear trends and increase with increasing flow velocity. The implications of these findings for

flow-based scavenging strategies will be highlighted. Thereupon, novel LP-TEM flow setups with diffusion-optimized mass transport properties, which exceed previous constraints by ~2 orders of magnitude, will be presented.[4] Finally, the benefits of the novel setups are demonstrated through different application examples from the field of materials reasearch and electrochemistry.[5]

We anticipate that the knowledge provided will enable better planning of in situ & in-operando experiments and support more reliable interpretation of results. The rational design of flow reactors will enhance correlatability to ex situ experiments and open new fields of LP-TEM research.

Keywords:

Liquid-Phase TEM, microfluidics, in-situ experiments

Reference:

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CoCID: Compact Cell Imaging Device for Correlative Investigation of Hepatitis E Infection

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background: Hepatitis E virus (HEV) is an burgeoning, zoonotic viral infection, and is the leading cause of acute hepatitis worldwide [1]. However, the lack of robust in vitro models supporting HEV infection [2] has hindered comprehensive studies on the various stages of viral infection and its impact on host cells [3].
Methods: CoCID [4] employs novel lab-scale Soft X-ray Microscopy (SXM) [5], a technology currently limited to select synchrotron facilities worldwide, enabling rapid, label-free imaging of whole, hydrated cells. We utilized model infection systems, including HEV genotype 1 and 3 replicon-transfected human hepatocytes, to investigate structural changes during infection. Additionally, we examined large, scaffold-free, polarized 3D spheroid models infected with clinical HEV to compare these pathological changes during full virus infection.
Results: Analysis of HEV replicon-transfected cells using SXM revealed a statistically significant increase in mitochondrial elongation. This observation was corroborated by light and electron microscopy techniques. Furthermore, examination of 3D spheroid models infected with clinical HEV demonstrated notable structural changes, which were explored using Correlative Electron-Soft X-ray Microscopy (CEXM) and Correlative Light-Electron microscopy (CLEM).
Conclusions: Our study demonstrates the utility of SXM and correlative approaches in elucidating the dynamics of HEV infection. The observed structural changes in infected cells provide further understanding of HEV biology and may inform the development of targeted therapeutic interventions against HEV. Moreover, the development of a Correlative Light-Electron-Soft X-ray Microscopy (CLEXM) workflow enhances our capability to investigate viral pathogenesis.

Keywords:

Hepatitis-E, Virus, Soft-Xray, Correlative, Microscopy

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Picometer-accurate Mapping of Lattice Displacements from Unconventional Room-Temperature Bond Order in Metallic NaRu2O4

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Poster Group 1

In recent years, there has been a remarkable increase in interest in ruthenium-based transition metal compounds, especially in the fields of quantum materials and strongly correlated electron systems. The reason for this is their moderate spin-orbit coupling, which is neither too weak nor too strong compared to the Coulomb interaction U. Due to this property, the relatively heavy 4d transition metal ruthenates exhibit a number of unusual properties compared to 3d transition metals. These include unconventional superconductivity, metal-insulator transitions, high-temperature ferromagnetism, Haldane chains, and orbital ordering. The interplay between localized and itinerant electrons, together with the orbital degrees of freedom in ruthenate systems, contributes to a wider range of electronic and magnetic properties [1, 2]. Given this potential of Na-Ru-O systems to serve as a promising model for the study of electron correlations in mixed-valence 4d systems, research in this area remains surprisingly limited.

$NaRu_2O_4$ exhibits an atypical metal-to-metal phase transition with emergent charge order (CO) [3]. Typically, a CO state, dominated by the inter-site energy U, tends to confine charge carriers to specific atomic positions, resulting in an insulating phase. Therefore, metallic behavior and charge ordering are generally considered to be mutually exclusive. Through single-crystal X-ray diffraction and theoretical studies, an unconventional type of bonding has been revealed in metallic $NaRu_2O_4$, involving an additional ordering of Ru3+/Ru4+ species. Below the critical temperature (TC = 535 K), a structural transition occurs from the high-temperature (HT) phase with the space group Pnma to the low-temperature (LT) phase with emergent superlattice reflections (0, ½, 0), which cannot be explained by the Pnma space group. Instead, the structure undergoes a transformation with a doubling of the unit-cell size and the formation of Ru-Ru metal dimers through orbital-driven Peierls dimerization. The final LT crystal structure of $NaRu_2O_4$ exhibits monoclinic symmetry with the space group P1121/a. Remarkably, short Ru-Ru dimers (bond-centered charge density wave) with direct metal-metal bonding coexist with metallic conductivity [3].

This study focuses on the investigation of the atomic-scale lattice reconstruction in the metallic $NaRu_2O_4$ system. High-angle annular dark-field scanning transmission electron microscopy (HAADF STEM) with atomic-resolution and electron diffraction techniques are used to reveal the structural transition with temperature, confirming the occurrence of orbital-driven Peierls dimerization as the sample temperature drops below TC. Periodic lattice displacement (PLD) maps further visualize the atomic displacement along different zone axes. The combination of (in-situ) HAADF STEM and Fourier processing allows for the mapping of phase transitions with complex displacement patterns in the picometer range, providing direct evidence for the bonding order in metallic $NaRu_2O_4$ (Figure 1).

Keywords:

phase transition, bond order, displacement

Reference:

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Novel Insights into Atomic-Scale Interface Reconstruction during Epitaxial Growth of Metallic Delafossite Thin Films

Anna Scheid[1], Dr. Qi Song[2], Mr. Hari Pokhrel[3], Dr. Tobias Heil[1], Dr. Stephanie Ribet[4], Dr. Colin Ophus[4], Mr. Niklas Enderlein[3], Dr. Y.Eren Suyolcu[1], Prof. Dr. Philipp Hansmann[3], Prof. Dr. Darrell Schlom[2,5,6], Prof. Dr. Peter A. van Aken[1]
[1]Max Planck Institute for Solid State Research, Stuttgart, Deutschland, [2]Department of Materials Sciences and Engineering, Ithaca, USA, [3]Department of Physics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany, [4]National Center for Electron Microscopy, Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, USA, [5]Kavli Institute at Cornell for Nanoscale Science, Ithaca, USA, [6]Leibniz-Institut für Kristallzüchtung, Berlin, Germany
PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Delafossites are classified as oxides with a general chemical composition of ABO_2, containing metal ions in the formal oxidation states of A+ and B3+ [1]. Depending on the cations of choice, delafossites exhibit a wide range of interesting physical properties, including p-type or bipolar semiconductivity (when A = Cu, B = Al, Cr, Fe, Ga, Y, In), photocatalytic activity (as observed in $AgGaO_2$), and metallic conductivity ($PdCoO_2$, $PdCrO_2$, $PdRhO_2$, $PtCoO_2$, and $AgNiO_2$) [2].

The metallic delafossites crystallize in the rhombohedral 3R phase with $R\bar{3}m$ symmetry and exhibit unusually large in-plane conductivities for oxides, surpassing even most pure metals. For example, $PdCoO_2$ has a conductivity about four times that of pure Pd, approaching the level of pure Cu, Ag, and Au. Therefore, metallic delafossites such as $PdCoO_2$ are of great interest for exploring their potential in oxide heterostructures and for applications in electronics and spintronics. To account for the limited size of their single crystals, researchers have fabricated thin films of metallic delafossites using methods such as reactive sputtering, pulsed laser deposition (PLD), and molecular beam epitaxy (MBE) [2]. Delafossite thin films are typically grown along the c-axis on substrates such as Al_2O_3 (0001) and β-Ga_2O_3 (201). Despite significant lattice mismatches between metallic delafossites and substrates, e.g., a mismatch of ~6% for $PdCrO_2$ on c-Al_2O_3, high-quality c-axis oriented thin films can be successfully grown. This is possible due to an intriguing mechanism during the growth process that appears to mitigate the mismatch strain at the interface, although the exact nature of this mechanism remains unexplained. In particular, the realization of high-quality films can be attributed to the choice of CoO_2 as the first layer grown in ozone-assisted MBE [3,4].

In this study, we reveal the presence of atomic-scale reconstructions at the $PdCoO_2$ – Al_2O_3 interface by combining advanced scanning transmission electron microscopy (STEM) techniques, including electron energy loss spectroscopy (EELS) and 4D STEM phase reconstructions via electron ptychography. The reconstructed phase (Figure 1(a)) reveals the presence of atomic columns at the interface that are not consistent with the atomic model of either the film or the substrate, while EELS fine structure analyses (Figure 1(b)) indicate a Co valence reduction across the interface. Insights from the combination of spectroscopy and phase reconstruction suggest the formation of a unit-cell thick layer of CoO within the first atomic layer of the substrate. This previously undiscovered behavior has significant implications for the understanding of the mechanism of epitaxial growth of metallic delafossite thin films.

Keywords:

delafossite, interface, reconstruction, 4DSTEM, ptychography

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FFT denoising methodology through CNN for the study of WS2 vacancies

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2D materials as WS2 have become increasingly relevant and widely studied in recent years due to their electronic and optical applications [1]. One of the most effective techniques for characterizing these materials and studying their physical properties is High-Angle Annular Dark-Field (HAADF) imaging in a Scanning Transmission Electron Microscope (STEM). On the other hand, to study lighter atoms it is common to use Integrated Differential Phase Contrast (iDPC) STEM. These techniques allow researchers to study the crystallography of the material to determine the presence of defects. These images often contain noise that poses a challenge when studying vacancies. Consequently, we developed an FFT denoising technique employing a Convolutional Neural Network (CNN) with a U-NET architecture [2, 3]. The CNN was trained using more than 5000 simulated spectra from diverse materials and various orientations. After FFT denoising, we could perform an inverse FFT (IFFT) to return to real space. This results in a significantly cleaner image, rendering crystallographic analysis more accessible, as atomic positions become much more discernible. Conversely, the FFT denoising method leads to the emergence of ‘fake atoms' in locations where vacancies should exist. While this outcome may initially appear counterproductive, it was, in fact, the crucial element that enabled the execution of this study. In this way, by subtracting the original experimental HAADF-STEM image from the filtered one, we obtain a new images where the bright spots correspond to the atomic vacancies
(Figure 1).
In conclusion, the methodology employed in this study has enabled a statistically significant analysis of vacancies across multiple images. Each image has been subjected to a detailed examination of more than 3000 atomic positions, yielding robust and reliable results. This approach not only provides a profound understanding of vacancy distribution but also gives the way for automating the statistical analysis of vast amounts of images within short timeframes. This potential for automation not only enhances process efficiency but also holds promise for accelerating the pace of discovery and understanding in research fields reliant on atomic-scale image analysis.

Keywords:

CNN, FFT, iDPC, HAADF-STEM, vacancies

Reference:

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FFT denoising methodology through CNN for the study of WS2 vacancies

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Poster Group 2

2D materials as WS2 have become increasingly relevant and widely studied in recent years due to their electronic and optical applications [1]. One of the most effective techniques for characterizing these materials and studying their physical properties is High-Angle Annular Dark-Field (HAADF) imaging in a Scanning Transmission Electron Microscope (STEM). On the other hand, to study lighter atoms it is common to use Integrated Differential Phase Contrast (iDPC) STEM. These techniques allow researchers to study the crystallography of the material to determine the presence of defects. These images often contain noise that poses a challenge when studying vacancies. Consequently, we developed an FFT denoising technique employing a Convolutional Neural Network (CNN) with a U-NET architecture [2, 3]. The CNN was trained using more than 5000 simulated spectra from diverse materials and various orientations. After FFT denoising, we could perform an inverse FFT (IFFT) to return to real space. This results in a significantly cleaner image, rendering crystallographic analysis more accessible, as atomic positions become much more discernible. Conversely, the FFT denoising method leads to the emergence of ‘fake atoms' in locations where vacancies should exist. While this outcome may initially appear counterproductive, it was, in fact, the crucial element that enabled the execution of this study. In this way, by subtracting the original experimental HAADF-STEM image from the filtered one, we obtain a new images where the bright spots correspond to the atomic vacancies
(Figure 1).
In conclusion, the methodology employed in this study has enabled a statistically significant analysis of vacancies across multiple images. Each image has been subjected to a detailed examination of more than 3000 atomic positions, yielding robust and reliable results. This approach not only provides a profound understanding of vacancy distribution but also gives the way for automating the statistical analysis of vast amounts of images within short timeframes. This potential for automation not only enhances process efficiency but also holds promise for accelerating the pace of discovery and understanding in research fields reliant on atomic-scale image analysis.

Keywords:

CNN, FFT, iDPC, HAADF-STEM, vacancies

Reference:

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In situ plasma studies using a direct current microplasma in a scanning electron microscope

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Poster Group 1

Background incl. aims:
Plasmas, sometimes called the fourth state of matter and defined as an ionized gas, have applications in a variety of fields, including biomedicine, materials science, and gas conversion. A typical example of plasma technology is their use in the semiconductor industry, where they fulfill a critical role in the etching process of silicon wafers when fabricating nano-scale components that make up computer chips [1]. At the same time, plasmas are heavily researched to gain further fundamental insights, and to develop novel technologies and applications. These research areas include plasma catalysis, gas conversion, nanomaterial synthesis, and biological treatments [2].
Many of the relevant plasma processes take place at a microscopic scale. However, current diagnostics (e.g., optical techniques) are often limited to global measurements, naturally averaging out microscopic effects. Micro-scale investigations of plasma-treated materials are typically limited to ex situ studies, where post-mortem analyses are performed on samples after plasma treatment [3]. This limits the temporal resolution of current studies, while also exposing the sample to ambient conditions during the transfer from the plasma device to the diagnostic equipment.
Therefore, we aimed to develop a setup that enables the formation of a plasma inside a scanning electron microscope (SEM). Specifically, we sought to achieve true in situ SEM imaging during plasma operation and to investigate the effect of plasma on materials at high spatial and temporal resolution, while preserving the original state of the plasma-treated sample.
Methods:
We modified an environmental SEM to integrate the instrumentation for generating and monitoring a plasma within the chamber [4]. A custom flange was designed to provide a passthrough for electronics and gas supply. The gas supply is connected to a steel tube with a small orifice, which brings the gas close to the sample, and they are both placed in the field of view of the SEM. The sample is grounded, while the nozzle is connected to a DC-DC converter placed inside the chamber which is powered by a DC power supply outside of the microscope. This way, plasmas in a variety of gases were generated by supplying the nozzle with a voltage up to 2 kV. The current flowing through the sample to the ground was measured using a shunt resistor, enabling real-time monitoring of the plasma discharge. While the plasma was on, the SEM could be used in its normal high-vacuum operating mode.
The in situ setup was tested for a range of plasma and imaging conditions. Different discharge gases were used to generate the plasma, including N2, CO2, and an O2/Ar mixture. Flow rates were varied between 2.5 and 7.5 sccm, which was monitored by a mass flow meter outside of the microscope. Further, the distance between the nozzle and the sample could be varied, typically between 75 and 150 µm. This setup offers high flexibility, though a plasma discharge was not possible for all conditions (e.g., large gap distance and low flow rate) at our maximum applied voltage of 2 kV. True in situ imaging was possible with both the secondary electron and backscattered electron mode of

the Everhart-Thornley detector, while EDX measurements were performed between plasma treatments.

Results:

To characterize the plasma discharge, we acquired the voltage-current characteristics, where the plasma current was monitored while varying the applied voltage. These data were obtained for a number of gap distances and gas flow rates. These characteristics all exhibit a rising slope and the positions of the curves for varying conditions are indicative of a so-called obstructed abnormal glow discharge. This implies that a discharge should be sustainable at even lower voltages, if the gas density could be increased.

Next, we investigated how the plasma operation affected SEM imaging, showing that true in situ SEM imaging was possible during the plasma discharge. While the electron beam is deflected by the electric field needed to sustain the plasma, the presented setup is sufficiently stable to obtain high-quality SEM images. The deformation of the images by the deflected electron beam must be considered for quantitative image analysis, but regardless the images are suited for qualitative studies. In a proof-of-concept example, a video was captured where the sputtering of the sample could be monitored in real-time. The homogeneous sputtering of the sample also yielded microscopic conical structures, formed out of spherical particles or impurities that were present on the sample surface.

To further explore the SEM-plasma synergy, we employed elemental mapping to study the composition of the sample and how it is affected by various plasmas. Apart from the physical effects of the plasma (i.e., sputtering), chemical reactions were also observed. We showed that oxygen-containing plasmas, e.g., CO_2, can oxidize the Cu sample outside of the sputtered area. For the latter, the sputtering effect was dominant and exposed the pristine Cu below the surface.

Finally, we explored the effect of the voltage polarity on the plasma properties, since a negatively biased electrode accelerates the positive ions to the nozzle rather than to the sample. This yielded sputtering of the nozzle instead of the sample and subsequent redeposition of nozzle material on the sample. At the same time, the sputtering of the sample was strongly limited while the oxidation in a CO_2 plasma could be observed for a broad region.

Conclusions:

This work presents a dedicated setup to generate a plasma inside a scanning electron microscope. The setup enables true in situ SEM imaging, where electron-based images can be acquired while treating the sample with a plasma. The interaction of the plasma and the sample was studied by real-time imaging, where a pit being formed in the sample sheds light on the sputtering behavior of the setup. Moreover, microscopic conical structures were formed by bombarding spherical particles or impurities with ions. Finally, chemical effects of the plasma were observed, where a CO_2 plasma was able to oxidize the Cu sample. This setup is a stepping stone for future research, both in the direction of materials science and toward more advanced plasma diagnostics.

Keywords:

SEM, Plasma, Sputtering, In-situ SEM

Reference:

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Localization techniques in biomedical electron microscopy: good as gold

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LS-05, Lecture Theater 5, august 26, 2024, 14:00 - 15:00

Background
Combining structural information with the localization of molecules and proteins provides important information for the investigation of cellular processes especially in the context of pathology. Different strategies are available to obtain samples suitable for labeling techniques in electron microscopy.
Methods
In principle, labeling probes can be applied to a permeabilized sample before embedding and sectioning, called preembedding-labeling. Alternatively, the probes are used to localize molecules on the section by so-called post-embedding or on-section labeling. A popular example for this approach is immunolabeling of cryosections according to Tokuyasu. Apart from this, other methods are available to gain accessibility for probes to subcellular structures such as whole mount or freeze fracture replica labeling.
Results
By using mostly cryosections according to Tokuyasu we studied lipid and protein distribution in cells and tissues investigating for example the formation and composition of exosomes secreted by cultured B-cells as a mechanism of antigen presentation. Exosomes also play important roles in cell-cell communication within the nervous system. We showed that exosomes are involved in axonal support by glia cells such as oligodendrocytes which is the myelin forming cell type in the central nervous system. These properties were affected by deficiency of oligodendrocytes for specific myelin proteins. We show examples how immunoelectron microscopy provided important data complementing other investigation methods in understanding cell biology in general and axo-glia interaction in particular.
Conclusion
Different approaches for sample preparation and the different strategies in immunoelectron microscopy are available. Which of those is applied depends on the question under investigation and the properties of the sample. These techniques allow the localization of proteins or molecules in the context of the cellular or tissue environment. Localization probes require a careful validation of their specificity.

Keywords:

ImmunoEM, pre-, postembedding labeling, cryosections

Reference:

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Alternating-probe electron ptychography

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background:
Ptychography has recently become a very powerful phase retrieval method and has been investigated by a number of groups in recent years. While applying a higher dose and a perfect electron microscope seems intuitively advantageous for maximizing the precision with which the unknown parameters can be determined in this process, the reality is constrained by various limitations. Different applications are required to push their limits in different aspects; there is still quite some potential for further optimization. Much effort has been directed towards refining physical models to incorporate more prior knowledge into reconstruction algorithms, such as adding different constraints [1], using multi-probe illumination to model partial coherence [2], and using mixed objects to model lattice vibrations [3].
Recent advances in the development of a low-noise, fast-response phase plate [4] present novel opportunities for adopting a different approach to increasing the number of known parameters in the reconstruction. Probe-diverse ptychography has been proven to be capable of overcoming the problem of converging to local minima inherent in the standard single probe scheme [5]. Initial tests in X-ray ptychography came to the conclusion that two different probes would be sufficient. In this abstract, we demonstrate, using simulated data, the use of a phase plate that adds different phase shifts to the probe at different scan positions. During reconstructions, we initialize and reconstruct a single probe, but incorporate an additional step within the algorithm to introduce phase changes to the wavefront that are induced by the phase plate.
Methods:
As a proof of principle for probe-diverse ptychography, we simulated 4D-STEM data of two hexagonal boron nitride (h-BN) crystals stacked with a 10° rotation between them (see Figure, (a)-(c))). (a) represents the projected potential of the complete sample, (b) shows the projected potential of the top half of the sample with no rotation, (c) depicts the projected potential of the second half of the sample with a rotation of 10°. For different electron doses, we simulated three distinct sets of data, each generated independently. These sets include data obtained from a probe without phase shift, a vortex beam, and a beam with phase shift of π applied to half of the elements of the phase plate [4]. The simulations were all conducted with a defocus of 9 nm, a scan step of 1 Å, and partial coherence implemented by convolving the 4D-STEM data set by a Gaussian with a full-width at half maximum (FWHM) of 0.7 Å. Subsequently, we generated two additional data sets. The first combined subsets of data were obtained from the normal probe and the vortex probe. The second data set combined data from all three previously mentioned sets. These simulations aimed to evaluate the effectiveness of probe-diverse ptychography in reconstructing the sample's structure with enhanced accuracy and resolution.
We performed reconstructions of all six simulated data sets using a multi-probe approach [2]. To estimate the quality of the reconstructions, we employed the Structural Similarity Index (SSIM) between the reconstructed phase object and the original potential utilized to generate the data.
Results:
In Figure(d)-(f), SSIM versus dose for different probe configurations is presented. (d) shows the sum of the SSIM of all reconstructed slices, reflecting the fidelity of the reconstruction for the entire

structure. This plot indicates that reconstructions in extremely low-dose scenarios are not entirely successful, with relatively low SSIM values. In these cases, the benefit derived from applying diverse probes is overshadowed by noise. Upon reaching a moderate dose range (from 1×10^4 e/Å^2), despite the larger probe size associated with the vortex and pi beams, probe-diverse ptychography still yields superior reconstructions. Additionally, the dataset comprising three distinct probe configurations exhibits further enhancement. (e) shows the sum of the SSIM for the first half of the potential slices, and similarly, (f) shows the sum of the SSIM for its second half. These results reveal that the reconstruction from the dataset containing three different probes captures more detailed information along the z-axis, especially at high doses.

Conclusions:

In this abstract, we have demonstrated a novel approach to diverse-probe ptychography and provided a proof of principle for its effectiveness in electron ptychography. Our findings suggest that applying more than two probes in ptychography leads to further enhancements in reconstruction quality. Additionally, the modification of the probe is not limited to phase plate adjustments. It's worth mentioning that the aberration corrector can also achieve similar outcomes, albeit with a limited number of configurable parameters.

Keywords:

divers-probe ptychography, phase plate

Reference:

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The effect of fragrance molecules on surfactant self-aggregation in aqueous solutions studied by cryo-TEM

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Poster Group 2

Background:
The interaction of ions and hydrophobic additives with charged surfactants is a fundamental phenomenon in colloid science. This phenomenon sets the basis for industrial formulations of surfactant-based products with the addition of minor components, such as salts and fragrances, in personal care and cosmetics formulations. Although such formulations are used daily worldwide, and studies report that minor components significantly modify the surfactant nanostructure and properties, little is known about their exact effects on surfactant self-aggregation at the nanometric scale. Our research aims to elucidate the effects of additives on the self-assembled nanostructure of surfactants, thereby, serving as a basis for optimized formulations of surfactant-based products.
Methods:
We study the nanoaggregation of a surfactant system with additives by cryogenic transmission electron microscopy (cryo-TEM), a direct-imaging advanced technique, combined with careful specimen preparation to avoid nanostructural changes caused by shear. For imaging, we use the Volta phase plate (VPP) to enhance image contrast. To complement our imaging, we conduct rheological measurements to predict nanostructural changes, as the viscosity is strongly affected by the self-aggregated nanostructure of the system. The surfactant model system we use in our research is sodium laurylethersulfate (SLES), an important and widely used surfactant ("detergent") that is usually accompanied by other components, such as salts and fragrances. We study how the nanostructure of SLES aggregates changes, and thereby, their macroscopic behavior, when adding different salts (LiCl, NaCl, KCl, and CsCl) and fragrances (vanillin, limonene, citronellol, and linalool). We study the system at different salt-to-surfactant molar ratios (X).
Results:
In our study, we show the correlation between the rheological properties and the nanostructural changes of SLES with varying salt concentrations [1], and in the presence of fragrance molecules (Figure 1, showing 5 wt.% SLES with NaCl and 1 wt.% limonene, changing the nanostructures from short to elongated thread-like micelles (TLMs) to vesicles). Each additive slightly changes the zero-shear viscosity peak in terms of viscosity value and the salt concentration required to reach its maximum value. Moreover, we present the formation of different nanostructures and coexisting pseudo-phases when different types of components are added. In our recently published paper [1], we showed the above-mentioned effects on SLES in the presence of different salts, while here, we describe the extension of this study to the addition of fragrance molecules. We also demonstrate how the specimen preparation process affects the imaged nanostructures through the formation of artifacts caused by the shear of blotting during cryo-TEM specimen preparation [2].
Conclusion:
To obtain a comprehensive picture of the effects of different components on SLES, we combine rheological measurements for predicting the zero-shear viscosity peak, followed by cryo-TEM direct imaging. Salts affect the nanostructure through ion effects related to their size and affinity, while the addition of fragrances affects through complex mechanisms. Cryo-TEM is a well-suited tool to explain

and distinguish tiny differences in these systems and should be performed on a carefully prepared specimen with sufficient on-the-grid relaxation and minimal shear during blotting.

Keywords:

cryo-TEM, self-aggregation, surfactants, fragrances, rheology

Reference:

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In-Situ TEM assessment of phase transformation in VO2-based thin films with Ho addition

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Poster Group 2

Background incl. aims
We present the fabrication and characterization of VO2-based thin films with Ho addition. The thin films were prepared as so-called materials libraries by reactive co-sputtering from two targets (V, Ho) to achieve a composition gradient. The effect of Ho on the phase transformation and its functional properties, like the thermochromic effect, has not been studied yet, and the materials libraries enable a screening of a large composition spread in relatively short time. The materials libraries were characterized in a high-throughput approach regarding their chemical composition, crystallinity, and electrical resistance at different temperatures to study the phase transformation. TEM investigations were carried out on two types of samples, both selected from specific composition region of the materials library: (i) focused ion beam (FIB) prepared TEM lamella, for analysis of the present Ho-rich precipitates, and (ii) sputtered on SiN Wildfire (DENSsolution) chips for in-situ TEM heating experiments to assess the phase transformation – to avoid Ga-ion contamination and its effects on the phase transformation.[1]
Methods
The thin films were prepared by reactive co-sputtering on 4-inch Si wafer and the Wildfire chips from two targets: V and Ho2O3. The positioning of the targets results in two gradients across the thin film library (see Figure 1) the composition gradient along the diagonal from lower right to upper left with decreasing Ho-content, and a thickness gradient along the other diagonal. The substrates were heated to 500 °C and the oxygen flow was controlled using plasma emission monitoring.[2] The thin film thickness was controlled by the deposition time: 30 min (approx. 150 to 350 nm) for the materials library on Si (SEM, EDS, XRD, resistance measurements) and 5 min for the depositions on the Wildfire chips (approx. 40 nm).
Results
The materials library was first characterized regarding its chemical composition (EDS), crystallinity (XRD, Figure 2) and electrical resistance (in the range of 20 to 120 °C, Figure 4) getting the first overview of the thin films properties using high-throughput automated measurements.[3]
The preliminary investigations of the library suggested a single-phase solid solution with a monoclinic VO2 phase. The temperature-dependent resistance measurements reveal a slight shift of the transformation temperature towards lower temperatures with increasing Ho content (Figure 4).
TEM analysis of the selected composition region revealed the presence of small Ho-rich precipitates and larger VO2 crystals. The in-situ heating experiments showed that the phase transformation starts at 54 °C and it is completed at about 60 °C. The transformation is well visible in the electron diffraction but does not result in microstructural changes; grain size and morphology remain unchanged.
Future work shall focus on the investigation of the thin films functional properties and their possible applications in nanoactuators or in thermochromic coatings for windows.

Keywords:

In-situ TEM, vanadium-dioxide, high-throughput characterisation

Reference:

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SEMNet: Deep Synthetic Training for Unprecedented SEM Image Denoising and Super-Resolution

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[1]D.notitia Inc., Seoul, Republic of Korea

IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

The Scanning Electron Microscope (SEM), an essential tool in electron microscopy, excels in delivering detailed visuals by directing an electron beam over a specimen's surface. Its widespread use in material science, biology, and various industrial fields stems from its ability to provide high-resolution, in-depth focus, and three-dimensional imagery.

Despite these advantages, acquiring high-quality SEM images can be challenging. This is due to inherent resolution limitations and noise factors that impair image clarity. Addressing these issues, our study introduces a method to enhance SEM image quality through advanced deep-learning algorithms, focusing on both denoising and super-resolution (SR) to refine image details and clarity effectively.

We introduce SEMNet, a Convolutional Neural Network (CNN) specifically designed for SEM image restoration with an upscale factor of 2. Inspired by the successes of the RRDBNet [1] in real-world image restoration, our model innovatively applies this architecture to SEM imagery, overcoming typical denoising and SR challenges. Fig. 1 illustrates our SEMNet.

SEMNet's core consists of 6 Residual-in-Residual Dense Blocks (RRDBs), a sophisticated structure pivotal for meticulously refining image details. Within each RRDB, we embed 3 Residual Dense Blocks (RDBs) characterized by their densely connected convolutional layers (denoted as Conv in Fig. 1). This design ensures a seamless flow of data and gradients throughout the network, significantly boosting feature extraction performance. The RDBs each consist of 5 convolutional layers paired with LeakyReLU activation functions (denoted as LeakyReLU in Fig. 1), having a negative slope coefficient of 0.2. Overall, SEMNet comprises 94 convolutional layers and 19 LeakyReLU activation functions, considering the additional convolutional layers outside the RRDBs as well. The network concludes with an up-sampling convolutional layer (denoted as Upsample in Fig. 1) that utilizes nearest neighbor interpolation to achieve SR, enhancing the quality and resolution of SEM images.

To effectively tackle the challenges posed by diverse noise types and low-resolution issues in SEM images, we devise a degradation modeling strategy specialized to producing synthesized low-quality (LQ) images from high-quality (HQ) counterparts. Specifically, our methodology concentrates on simulating two primary noise types frequently observed in SEM images: Gaussian noise, with a mean of 0 and a standard deviation ranging from 2 to 25, and Poisson noise, with a lambda value (λ) between 10^2 and 10^4. Moreover, we integrated additional degradations into our datasets, such as Gaussian blurring effects and up-downscaling processes. This comprehensive approach is designed to bolster the robustness of our SEM image restoration model by broadening its exposure to a variety of noise and other degradation scenarios. Note that it serves as a form of data augmentation, dedicately aimed at enhancing image restoration performance.

We trained the model for 5.44×10^5 iterations using Adam optimizer, with a batch size of 4. The learning rate starts at 0.0001 and is halved when the iteration reaches a multiple of 2.0×10^5. The training utilizes the following loss functions with respective weights: L1 loss (1.0), Histogram loss [2] (0.1), and Total Variation loss (0.01). The L1 loss effectively minimizes absolute pixel differences, playing a crucial role in image restoration. Histogram loss targets the reduction of pixel distribution disparities, ensuring the restored image faithfully mirrors the statistical characteristics of the original.

Total Variation loss focuses on diminishing noise and maintaining fine details, like edges, enhancing the overall quality of restored images. Our model is trained with 1300 real SEM images and 1300 images sampled from Flickr2K [3], which is the public dataset that has high-resolution real-world images from multiple categories such as nature, cityscape, human, animal, etc.

To quantitatively evaluate the performance of SEM image SR, we employ two metrics: Peak Signal-to-Noise Ratio (PSNR) and Structural Similarity Index (SSIM). PSNR measures the pixel-level accuracy between the reconstructed and the original images, while SSIM assesses the perceptual quality by considering changes in luminance, contrast, and structural similarity. For both metrics, higher values indicate better performance. A testing set of 25 SEM images was used to calculate both metrics.

Compared to the classical restoration using bicubic interpolation (for SR) and Gaussian blurring (for denoising), SEMNet achieved an increase of 0.212dB in average PSNR and 0.1379 in average SSIM. These results suggest that deep learning-based image restoration methods surpass classical image processing techniques in enhancing the quality of SEM images. Fig.2 shows the qualitative results of classical restoration and the SEMNet. The original sizes of the LQ and HQ images are 640×480 and 1280×960, respectively. Our SEMNet demonstrates superior visual performance compared to the classical restoration method.

In conclusion, we introduce SEMNet, a new deep-learning model for restoring LQ SEM images to HQ ones. With a diverse training dataset, SEMNet effectively removes multiple types of noises and upscales the image resolution through detailed degradation modeling and specialized loss functions. It surpasses classical restoration methods in improving SEM image quality, both quantitatively and qualitatively. Our work will contribute to the field of microscopy image restoration by introducing innovative deep-learning architecture designs and training strategies, specifically tailored to handle highly complex and diverse degradations in SEM images.

Keywords:

SEM, Image Restoration, Deep Learning

Reference:

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Deep learning 3D-mineral liberation analysis with micro-X-ray fluorescence, micro-computed tomography, and deep learning segmentation

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Poster Group 2

Backgrounds
Mineral liberation has become a key step in the mineral processing industry to quantitatively determine the volume fraction of valuable commodities. Several semi-automated techniques, such as backscattered electron (BSE) images with energy-dispersive spectroscopy (EDS) marketed as commercial systems such as QEMSCAN have been developed for this purpose. However, the extrapolation of the collected 2D data to 3D volumetric remains problematic. Recent developments in X-ray microcomputed tomography (µCT) made it possible to non-destructively characterise these minerals. The problem is the densities of some geomaterials are similar, hence without additional information, it is difficult and often impossible to reliably differentiate the phases just based on µCT data. In this study, we combined the micro-X-ray fluorescence (µXRF) with µCT data and develop a workflow incorporating statistical analysis and deep learning segmentation to interpolate the 2D data into 3D volume (Deep-XFCT).

Methods
The rock used in this study was a fine-grained 24 mm basaltic andesite sample that has a clear and distinct mineral inclusion. A combination of analytical techniques was used for the mineral analysis, which include (1) XRD on powder sample to understand the mineral presence and their abundance, (2) µCT to get 3D volumetric data, (3) µXRF to get elemental mapping for the mineral labels and atomic mass percentages of discrete points for mineral verifications, (4) Raman Spectroscopy to cross check the mineral identifications through µXRF data. The data processing was performed in two steps: (1) establishing ground truth labelling of the mineral phases through µXRF elemental mapping using K-mean clustering, (2) use of the ground truth labelling to train a deep learning model that will segment the mineral phases in 3D µCT data with a specific convolutional neural network architecture specifically designed for image segmentation (U-Net model) using two commercial software Dragonfly ORS and Avizo.

Results
The XRD results provided three prerequisite information for the workflow. First, the mineral phases that exist within the sample that holds important elemental information to correlate with the µXRF data. Second, the number of mineral phases that was presence, which guided the number of clusters for the K-mean clustering. Third, the mineral phases percentage abundance which were used as the validation for the cross-referencing with the K-means clustering abundances and the accuracy of segmentation from the deep learning.

The XRD shows the presence of albite, ankerite, clinochlore, illite, laumontite and quartz. By applying the geochemical principles to the K-means clustering centroids of the µXRF maps, illite, ankerite, and clinochlore can be unambiguously identified through the highest elements concentration present in each mineral. Quartz and albite were identified through the µXRF point analysis by the highest Si and Na mass percentage, respectively. Laumontite did not have a unique elemental identifier nor characteristic combination of elements and was identified by deduction as the final element. Raman Spectroscopy was able to confirm the presence of albite, clinochlore and quartz.
The minerals assigned to each pixel of the µCT orthoslice on the top and bottom surface of the sample where a U-Net neural network model was trained to segment the µCT based on the mineral labels. From visual analysis, the model captured the major contours of the mineral phases. This includes separating the albite and illite matrix to a good approximation, despite their identical grayscale value. However, the model failed to recognise the finer details apparent in the labelled training data. The overall accuracy from all minerals for top and bottom surfaces from the U-Net model in Dragonfly ORS 2021.2 were 70.23% and 69.56%, respectively. Furthermore, the results from the U-Net model in Avizo 2021.1 were similar with 73.57% and 72.08% for the top and the bottom surfaces. To validate the segmentation from the model, the sample was cut 5mm from the top and bottom of the plug and the surfaces were subsequently mapped with µXRF to allow the mineral phases maps within the volume of the sample to be compared. In comparison to the training data, the validation data has lower but serviceable accuracy. Using the phase identification as labels, a neural network was successfully trained to segment other slices throughout the volume to obtain a 3D representation of the mineral phases in the sample.

Conclusions
Our results successfully demonstrate semi-automated multi-modal analysis with an approach that is universal to any multi-instrument approach. The blend of µXRF and µCT provides a unique opportunity for robust 3D mineral liberation analysis in both field and laboratory applications when combined with Deep-XFCT. While Deep-XFCT did not provide pixel-level accurate segmentation, it was able to provide an excellent proxy for the purposes of obtaining the presence, location, distribution, and morphology of grains dispersed throughout a core plug. It was also shown that Deep-XFCT was able to differentiate fine-grained minerals of similar density that would be impossible by manual segmentation. This technique is a valuable technique that has the potential to expedite 3D mineral liberation analysis.

Keywords:

Deep learning, Image analysis, Multi-modal

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Solving the controversy of the metal-insulator phase transition in chromium nitride thin films

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background incl. aims
CrN is a hard coating material that has applications in abrasion and wear-resistant cutting tools, bearings, and tribology applications due to its high hardness, high-temperature stability, and corrosion-resistant properties. Recently, CrN has also attracted significant interest due to its high thermoelectric power factor [1]. Much of debate in the literature is around CrN's unique and intriguing metal-insulator phase transition. While CrN bulk single-crystals exhibit the characteristic metal-insulator transition accompanied with structural (orthorhombic-to-rocksalt) and magnetic (antiferromagnetic-to-paramagnetic) transition at ~260 - 280 K, observation of such phase transition in thin-film CrN has been scarce, and the exact cause of the absence of the transition in several thin film studies is not well-understood [2].
Methods
STEM images and EDS maps were recorded with an image- and probe-corrected and monochromated Themis-Z 60-300 kV equipped with a high-brightness XFEG source and Super-X EDS detector system for ultra-high-count rates, operated at 300 kV. The spatial resolution in STEM mode was 0.7 Å. EDS maps used for atomic % quantification have well above 1 M counts, and k-factor analysis and absorption-corrected background subtraction were employed.
Results
Here, we demonstrate that the formation of a secondary metallic Cr2N phase during the growth inhibits the observation of metal-insulator phase transition in CrN thin films, see Figure 1. When the Cr-flux during deposition is reduced below a critical limit, an epitaxial and stoichiometric CrN thin film is obtained that reproducibly exhibits the phase transition. Annealing of the mixed-phase film inside reducing NH3 environment converts the Cr2N into CrN as revealed by HRSTEM imaging and EDS mapping, and a discontinuity in the electrical resistivity at ~277 K appears.
Conclusion
In summary, we show that formation of the secondary metallic Cr2N phase inside CrN during the thin film growth process inhibits the observation of its metal-insulator electronic phase transition. A high Cr-flux during the deposition process results in metallic Cr2N networks inside the CrN matrix. When the Cr-flux is reduced below the critical limit of the growth rate of 1 nm/min, a stoichiometric single-crystalline CrN thin film is obtained that reproducibly exhibits the electronic phase transition. Our results also show that when the Cr2N, inside the high-flux mixed film, is converted to CrN by annealing it inside NH3 environment at high temperature, the electronic phase transition is recovered. This strongly manifests the fact that phase pure and stoichiometric CrN films can show the metal-insulating electronic phase transition. The demonstration of the origin behind the controversy of the metal-insulator transition in CrN thin films marks significant progress for its potential applications in nanoscale devices [3,4,5].
Figure Caption
Figure 1 (a) Low-magnification HAADF-STEM micrograph of the HCF film is presented that shows the presence of both Cr2N and CrN. Inset shows the EDP of the film with cubic [001] and hexagonal

patterns. (b) STEM-EDS elemental maps corresponding to (a) show the spatial distribution and morphology of the Cr2N and CrN grains. (c) HAADF-STEM micrograph of the HCF film with bright regions that represent the Cr2N, while the darker parts showing CrN grains. (d) Cr, (e) N and (f) Cr+N STEM-EDS maps corresponding to the region (c) showing the different grains. (g) HAADF-STEM micrograph of the LCF film showing single-phase epitaxial single-crystalline CrN growth on MgO substrate. (h) Homogeneous and uniform Cr and N atomic distribution in the LCF film is demonstrated by the STEM-EDS map. (i) Atomic-resolution STEM image of the CrN/MgO interface from the LCF film is presented that exhibits cubic epitaxial CrN crystal growth on MgO substrate.

Keywords:

Chromiumnitrate, phase transition, STEM, EDS

Reference:

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[5] The authors acknowledge the facilities at Sydney Microscopy and Microanalysis at The University of Sydney.

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Ptychographic X-ray computed tomography of porous membranes with nanoscale resolution

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PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Background Incl. Aims
3D images of porous materials in high resolution have been so far obtained using transmission electron tomography or focused ion beam coupled with scanning electron microscopy. These methods require ultra-vacuum conditions, and only a small volume of the sample is visualized. Here, we demonstrate the application of ptychographic x-ray computed tomography for visualizing soft matter with a resolution of 26 nm over large fields of view. We showcase the technique's capabilities by imaging polymer hollow fiber porous membranes.

Porous materials have essential functions in nature, whether for water and nutrient transport in plants and other biological systems or for the storage of oil and water in rock reservoirs. Synthetic polymeric materials are key for chromatography and membrane separation processes. Their application is well-established in water desalination, hemodialysis, gas separation, and is expanding in nanofiltration for chemical separations. The advantages of membrane technology are low energy requirement, and low carbon footprint combined with a compact design that allows packing a large membrane area within a small volume. Effective membranes have a complex porous structure to secure selectivity, mechanical stability, and fast transport characteristics. Porosity and interconnectivity determine the membrane performance.

Thanks to the high-penetration depth of the X-ray beam, we visualize the 3D complex porous structure of polyetherimide ultrafiltration hollow fibers in a non-destructive manner and obtain quantitative information about pore size distribution and pore network interconnectivity across the whole membrane wall. The non-destructive nature of this method, coupled with its ability to image samples without requiring modification or a high vacuum environment, makes it valuable in the fields of porous- and nano-material sciences, enabling imaging under different environmental conditions.

Methods
A highly coherent x-ray beam produced by SIRIUS, the 4th generation synchrotron source inaugurated in 2020 in Campinas, Sao Paulo, Brazil, was used at a dedicated beamline for x-ray imaging - Cateretê. Ptychographic x-ray computed tomography (PXCT) is a lensless coherent x-ray technique, where the specimen is raster scanned in two dimensions by a coherent x-ray beam defined by a pinhole, and diffraction patterns in the far-field are recorded at each position. The

sample is rotated and the 2D scans are collected at different projections to obtain a 3D dataset for reconstructions.

Results
The high-performance computer reconstruction of the collected 2D projections resulted in detailed 3D images of the hollow fibers, with a resolution of 26 nm and a voxel size of 23 nm × 23 nm × 23 nm. Such a detailed model contains not only visual but also quantitative information, and it was used to evaluate the porosity gradient across the membrane walls for hollow fiber membranes. Apart from the porosity, we were able to study the evolution of the diameter size of every detected pore channel across the membrane thickness within the resolution limits (26 nm diameter), to evaluate the pore size distribution and changes across the entire membrane. We could also provide a comprehensive analysis of the interconnectivity of the pores.

Conclusions
The highly coherent X-ray beam generated by the 4th generation synchrotron source allows for the non-destructive analysis via PXCT with nanoscale resolution. The reconstruction of the X-ray coherent diffraction patterns from different projection angles, not only facilitated the visualization of complex porous morphology throughout the entire membrane wall volume but also provided a detailed 3D quantitative model of the porous network. The presented PXCT method is not limited to the measurements of porous structures but can be employed for resolving the nanostructure details in large samples within soft matter. Furthermore, thanks to the high-penetration depth of X-rays, PXCT allows for the analysis of the samples where the sectioning for electron microscopy methods is not feasible. The lack of vacuum requirements and the absence of sample modification, such as heavy metal staining, enable in situ analysis of samples in different environmental conditions. These advantages, coupled with the non-destructive nature of this technique, make it highly applicable in material science and biology for the understanding of complex porous- and nanomaterials offering nanometer-scale resolutions for large sample volumes and providing valuable quantitative information.

Keywords:

X-Ray Tomography, Ptychography, Polymer Membrane

Reference:

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How Euro-BioImaging can support your research with access to the best imaging tools

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Poster Group 2

Advanced imaging technologies are a central technology platform driving research in most disciplines of the life sciences and correlating multiple imaging approaches has become an increased need in many research approaches.

Euro-BioImaging's role in the imaging revolution is to offers all scientists open access to imaging instruments, expertise, training opportunities, and data management services beyond what is available at their home institutions or among their collaborators.

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For the technical experts at imaging core facilities, Euro-BioImaging provides a cross-European network and platform for exchange of experience, as well as new training opportunities.

Keywords:

Infrastructure, Open Access, Data Services

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Optimizing FIB-based sample preparation for quantitative in-situ biasing studies of semiconductors using 4D-STEM

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Poster Group 2

Background incl. aims

Recent advancements in four-dimensional scanning transmission electron microscopy (4D-STEM) technology have facilitated the precise quantification of electric fields and potential differences at the nanometer scale.[1] To comprehend the operational dynamics of semiconductor devices under applied voltage, operando TEM represents a logical progression. However, measurements require the sample to be properly mounted and brought into contact with the electrical chip. Since the necessary microcontacts are created by focused ion beam (FIB), a systematic study must be carried out on how the sample must be prepared, protected, contacted and thinned consequently. This creates guidelines for quantitative measurements of the amount and path of currents that must flow through the circuit. Once properly quantified, the procedure will help carry out in-situ biasing studies of semiconductor materials and simultaneously measure the built-in potential. Furthermore, this will also help do in situ breakdown study of semiconductors quantitatively.

Methods

In this study, we used a pn junction with doping concentrations and corresponding built-in potential known from electrochemical capacitance-voltage (ECV) measurements. For the characterization within the TEM, the Protochips Fusion holder was used. As a comparison criterion, we chose the IV characteristic curve for different lamella geometries, bulk material and theoretical calculations. Sample preparation leverages the combined advantages of two FIB microscopes: The Helios 5 Hydra CX PFIB and the Jeol JIB-4601F. The former has xenon plasma source which enables faster chuncking. Thanks to the advanced manipulator needle control and appropriate geometry, this microscope provides the solution for lifting out the TEM lamella and attaching it to the biasing chip without complicated intermediate steps. However, it is known that thinning of the lamella with Xe-Beam can cause curtaining.[2] In our case this would be undesirable as it could result in electrical short circuit channels. We can avoid this problem by using the second FIB microscope mentioned above with a gallium-ion source. For the electrical contacts and the protective layer tungsten is used.

Results

The electrical experiments require the smallest possible resistance between the sample and the metal contacts. It is already known from the literature that ion beam induced deposition (IBID) leads to a significantly lower resistance compared to electron beam induced deposition (EBID).[3] Accordingly, the contacts here were made using FIB.
In the following we compare different geometries of the lamellas. Graphic A shows the 'H' shape lamella. This geometry would have the advantage that the same potential can be applied to the

entire p-side. However, the distance between the positive and negative contacts is very short, which leads to a short circuit due to undesirable contamination of the chip surface. This can also be clearly seen in the IV characteristic curve. In graphic B the 'S' shape lamella is shown. This form has the advantage that the positive and negative contacts are at a significantly greater distance from each other. Contamination of the chip surface can also be neglected with this geometry, as the potential drops from the right to the left side across a cut in the chip. This geometry leads to a significant improvement in the behaviour of the IV characteristic, as can be seen in the plot. Graphic C shows the same lamella as B with additional cuts to prevent current flow to the tungsten layer on top. Although the 'S' shape geometry has an improved IV characteristic, it is more similar to a Schottky diode than a pn diode. However, this is not surprising, because the contacts are on the same size scale as the lamella. This must be considered in the theoretical calculations in order to be able to quantify what proportion of the externally applied voltage drops at the pn junction.

Conclusion

This study highlights the importance of careful sample preparation techniques for in-situ biasing studies of semiconductor materials using 4D-STEM. By optimizing the FIB-based preparation and lamella geometries, we have achieved improved IV characteristic behaviour and a lower amount of short circuits. However, the metal-semiconductor contacts must be carefully considered in the descriptive models. These results will provide practical insights for improving measurement accuracy and reproducibility in operando TEM studies of semiconductor devices and may contribute to advances in electronic device development.

Keywords:

electric_fields, pn_junction, in-situ_biasing, 4D-STEM, sample_preparation

Reference:

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Inversion of the Internal Electric Field in GaN/AlN Heterostructures Studied by Off-Axis Electron Holography

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

Background incl. aims
Wurtzite III-nitride semiconductor are renowned for their applications in light emitting devices, due to their wide bandgap and doping capabilities. One of the main challenges of these materials is managing their intense spontaneous and piezoelectric polarization fields. In order to enhance the efficiency of GaN-based emitters, it is important to design new architectures to engineer the electric field in these materials. In addition, validation of these new designs requires the development of characterization methods capable of mapping the electric fields with nanometer resolution.
In this work, we study the effect of n-type delta doping on the potential profile of AlN/GaN and AlGaN/GaN superlattice using off-axis electron holography for the quantification of the electrostatic potentials. In addition, the sample composition, and the concentration and localization of dopants are determined using correlative techniques: high-resolution electron dispersive X-Ray spectroscopy (EDX), secondary ion mass spectroscopy (SIMS) and Nextnano simulations.

Methods
The samples under study consist of 10-periods of AlN/GaN (20 nm/20 nm) (1st series: samples S1 and S2) or 12 periods of AlN0.3Ga0.7N/GaN (15nm/15nm) (2nd series: samples S3, S4 and S5) grown by plasma-assisted molecular beam epitaxy. In the first series, S1 is non-intentionally doped and S2 is heavily doped with Ge in the GaN layers, at a nominal concentration [Ge] = 2.0±0.2 × 1021 cm−3, which is close to the solubility limit. In the second series, S3 is non-intentionally doped and S4 and S5 have a delta doping of Ge ([Ge] = 1020 cm-3) and Si ([Si] = 1019 cm-3), respectively, localized in the AlGaN layers. The dopant concentrations are measured by SIMS.
The electrostatic potential of the samples are measured by off-axis electron holography performed in a double-corrected FEI Titan Ultimate TEM operating at 200 kV. The electron holograms were recorded on a Gatan OneView 4k camera. EDX-spectroscopy was performed in a probe-corrected FEI Titan Themis microscope operated at 200 kV and equipped with a Super X detector system. EDX spectra quantification was done using the Cliff-Lorimer method.

Results
Using electron holography in undoped AlN/GaN and AlGaN/GaN samples (S1, S3), we confirm the presence of internal electric fields in the GaN and AlN layers, with a sign that is consistent with Schrödinger-Poisson simulations performed with the Nextnano software. Then, comparing S1 and S2, we are able to demonstrate the effect of carrier screening due to Ge doping. However, we also observed an inversion of the internal electric field in some of the AlN layers in S2. A correlative study involving holography, EDX and theoretical calculations of the band diagram demonstrate that this inversion can be attributed to Ge accumulation at the heterointerfaces in the AlN layers [1]. These result pave the way to the use of delta doping as a design tool to modulate and even invert the electric field in polar heterostructures.

The second sample series (S3, S4 and S5) presents our attempt to reproduce intentionally the inversion of potential observed in S2 by designing AlGaN/GaN heterostructures using delta doping with silicon and germanium. In this case, we observe a systematic inversion of the internal electric field using Si doping. However, this inversion was not observed for Ge delta-doping. Further analysis using SIMS showed a migration of Ge in the GaN layer that explain this absence of inversion. This migration is consistent with our observations of a higher solubility limit of Ge in AlGaN compared with GaN [2], and is coherent with the Ge clustering observed in S2.

Conclusion

We show here that electron holography is a very powerful tool that allow the measurement of electrostatic potential with a nanometer resolution. It is particularly useful to study III-nitride materials that present peculiar electrostatic properties. We apply it here to study first the efficiency of germanium doping to screen the polarization-induced internal electric field in AlN/GaN superlattices. In addition, our identification of electric field inversion domains associated with Ge inhomogeneities paves the way for the use of delta doping as a tool in the design of heterostructures, to modulate and even invert the electric field in polar heterostructures. We have also addressed the effect of delta-doping in AlGaN/GaN, showing that inversion of the electric field is also possible using Si delta-doping, with improved reproducibility. Our studies show that the incorporation of germanium in III-nitride heterostructures remains challenging, with issues such as dopant segregation and dopant migration, but silicon delta-doping is also a very promising tool for fine-tuning of the internal electric fields in these materials.

This work, carried out on the Platform for Nanocharacterisation (PFNC), was supported by the “Recherche Technologique de Base” and "France 2030 - ANR-22-PEEL-0014" programs of the French National Research Agency (ANR).

Keywords:

Electron holography, III-nitride semiconductors, doping

Reference:

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Multi-modal correlative microscopy: simultaneous and colocalised Raman & SEM imaging

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Poster Group 1

Multi-modal sample analysis is essential to thoroughly characterise and understand our samples and materials and ultimately conduct cutting edge science.
Here we focus on 4 different modalities: Raman spectroscopy, optical imaging, SEM (scanning electron microscopy) imaging and EDS (energy dispersive X-ray spectroscopy). These techniques allow us to determine chemical composition, molecular and crystalline structure (Raman spectroscopy), collect optical images, image samples at high resolution (SEM imaging) and determine elemental composition (EDS). When combined, these techniques provide an excellent toolbox for sample characterisation.
Measurements were conducted using an inLux™ SEM Raman interface attached directly to the SEM microscope. This enabled colocalised and simultaneous SEM, Raman, and optical imaging inside the SEM chamber, making correlation between the three techniques trivial, without ever moving the sample.
Figure 1 illustrates SEM, EDS and Raman images taken from a mineral section, demonstrating a clear and accurate overlay between minerals found with three techniques. Additionally, the complementary potential of the multi-modal analysis showed species detected only with Raman (anatase) or EDS (Zn, sphalerite). The presence of anatase also shows that Raman spectroscopy is sensitive to the many polymorphs of TiO_2.
The application potential of correlative SEM and Raman imaging will also be demonstrated on batteries, polymeric and biological samples. With these examples, we illustrate how Raman and SEM can increase understanding of materials, and the power and ease of use when the techniques are combined inside a SEM.

Keywords:

Raman imaging
Correlative analysis
SEM

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Near-Ideal Direct-Electron Focused-Probe 4D-STEM Data for Open-Source Phase Reconstructions

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IM-03 (4), Plenary, august 29, 2024, 14:00 - 15:00

Background incl. aims
The availability of direct-electron cameras with high dynamic ranges and very fast detection speeds is revolutionizing the ability of scanning transmission electron microscopy (STEM) to make use of every electron for virtual imaging and advanced computational phase reconstructions. State of the art detectors can now acquire four-dimensional (4D) data at STEM pixel dwell times of over 100,000 diffraction patterns per second while counting each electron.

At the same time, the proliferation of open software packages to make use of this data has made such analyses widely accessible, and due to a convergence to the Python programming language, easy to compare in terms of computational efficiency and reconstruction quality.

Methods
The first commercially available Dectris ARINA detector [1] has been installed in the Nion UltraSTEM100 instrument in Vienna, where an ultra-stable sample stage and flexible electron optics are ideally suited to 4D-STEM. For our initial comparisons, we use an atomically focused probe (34 mrad convergence semi-angle) and choose a camera length optimized for maximum signal in the bright-field and the first-order Bragg disks.

In this contribution, we present some of the first data acquired on this new detector, namely convergent-beam electron diffraction maps of pristine monolayer graphene, which is a near-ideal dose-robust uniform atomic phase object. The ability to reliably count electrons at such speeds (the detective quantum efficiency is 0.85 at 60 keV [1]) also enables the variation in beam current to be easily measured and, if desired, corrected for, which we find has an appreciable impact on the bright-field signal and reconstructions that make use of it (most notably parallax imaging [2]).

Results
A pixel exposure time of 100 µs provided a high signal for phase reconstructions without needing to resort to multi-frame averaging. The ARINA is able to bin the native 192×192 detector array in hardware for faster acquisition, and we find that further software binning up to four times does not harm reconstructions, whereas a dense real-space sampling below 0.08 Å per pixel (512×512 px scan over the 2×2 nm² field of view) was noticeably helpful.

The graphic shows the concurrently acquired high-angle annular dark-field (HAADF, 80–300 mrad) and virtual ADF images (~40–80 mrad), as well as a range of open-source phase reconstructions from the binned 4D dataset: single-sideband (SSB) and Wigner distribution deconvolution (WDD) [3], as well as iterative differential phase contrast (DPC), parallax-corrected bright-field imaging, and batched iterative gradient descent single-slice ptychography [2]. Apart from modest scan distortions,

visual inspection of the phase images reveals deviations from the expected uniform atom contrast, and notable differences in phase magnitudes. Computational times also vary greatly depending on the algorithm and the binning.

Conclusion
The quality of the phase images is assessed by evaluating the variation of atomic phase shifts using a robust parameter-based quantification method [4] and compared to data simulated with the abTEM code [5] and reconstructed with the same algorithms. These quantitative comparisons will be presented at the meeting, where the data and code will also be provided. Further results on defocused-probe datasets and the prospects for live reconstructions will be discussed.

Keywords:

4D-STEM, graphene, direct-electron-detection, ptychography, phase-reconstruction

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A Secondary Electron Hyperspectral Imaging characterisation of mechanochemically functionalised carbon black materials

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Poster Group 2

Background
Carbon blacks find applications in composite materials for energy generation and storage, structural materials, as well as catalysis and as precursor materials. In nanocomposite applications of carbon black, the surface functionalisation of the carbon black is important in determining the structure of the composite and the function of the material [1]. Powders in this form are often characterised as a bulk material, however it is important to understand the local surface chemistry and distribution of chemistries between particles, particularly when the surface of the powder is engineered to achieve certain properties.
Such is the case with carbon black subject to surface oxidation processes. In this case, a one-step ball milling process was used to vary the particle morphology and surface oxidation [2]. X-ray photoelectron spectroscopy (XPS) can tell average oxidation. Transmission electron microscopy (TEM) can tell particle size and surface chemistry – but not for in-situ satellite particles. Therefore, a study which includes local information about particle morphology and functionalisation with process time, may offer insight into the dynamics of the process which includes fracturing, agglomeration, exfoliation and oxidation.
Secondary electron hyperspectral imaging (SEHI) proved to be a valuable technique for characterisation of polymers functionalised by plasma surface treatment [3]. Here we show the technique is applicable to a nano materials characterisation challenge to image the morphology and local surface chemistry of satellite particles in-situ produced during milling on bulk carbon black particles.

Methods
The carbon blacks characterised by SEHI were as-received carbon black and carbon black ball milled without solvent at room temperature for a total of 0h, 1h, 5h, 9h and 11h. Average spectra from 20 µm horizontal field width images were produced for the as-received carbon black and each ball milling process time. A functionalised carbon black model was fitted to spectra to obtain component peak heights using the lmfit python module (Figure 1a). Ratios of peak heights for CH (hydrogenated amorphous carbon) and OH functionalised carbon to sp^2-hybridised graphitic carbon were calculated (Figure 1b). The energy ranges 2.2-2.8 eV, 3.0-3.6 eV, 4.2-4.9 eV and 5.4-6.0 eV were used to create colour maps related to sp^2, CH, OH and CO+sp^3 surface functionalities respectively (Figure 1c). These component images were assigned to channels with hue values equidistantly spaced in the HSV colour space (Figure 1d). The composite map is a sum of these component images (Figure 1e).

Results
The CH:sp^2 ratio decreases from 1.05 to a minimum of 0.5 at 5h milling time, then increases to 1.4 at 11h. The OH:sp^2 ratio decreases from 1.3 to a minimum of 0.8 at 5h milling time then increases to a

maximum ratio obtained by processing of 1.3 at 9h. Further milling to 11h decreases the OH:sp^2 ratio to 1.1 (Figure 1b). The results indicate that the sp^2 surface content is maximum at 5h. Further milling up to 9h oxidises the surface but more milling post 9h produces more amorphous hydrogenated carbon surfaces.
At 5h, the composite colour map indicates a nanoscale 'satellite' particle morphology (see Figure 1d inset for a line profile indicating the particle size) with strongest emissions in the OH component range.

Conclusions
SEHI added to the understanding of the carbon black material by analysing the local chemistry of 'satellite' nano particles which were more oxidised versus the bulk carbon black particle. This is not clear from spatially averaged XPS analyses of the powder.
Meanwhile, a spatially averaged SEHI analysis identified components for carbons and surface carbon compounds. A qualitative comparison of the CH and OH to sp^2 ratios versus ball milling time in oxidative conditions showed a maximum of sp^2 surface chemistry by 5h before this was oxidised to the highest OH:sp^2 ratio at 9h and the highest CH:sp^2 at 11h.
Local characterisation enhances understanding of how the processing influences surface chemistry and morphology resulting from process time.

Keywords:

Carbon black; SEHI; SEM; oxidation

Reference:

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Temperature-driven in-situ TEM cation exchange at the solid state: a combined experimental and computational study

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

Background including aims
Over the past two decades, the demand for innovative nanomaterials has surged due to their remarkable adaptability across various fields such as optoelectronics, photonics, and catalysis. This has led to the development of robust synthetic techniques to produce nanocrystals (NCs) with finely tuned compositions, crystal phases, and morphologies.
Post-synthetic transformations of preformed nanostructures can be singled out among the available methods as a general approach to produce nanomaterials with features unattainable through conventional synthetic routes. Cation exchange (CE) is a prominent example this approach, because it is based on the substitution of cations within a host NC lattice, offering precise control over compositions and phases without affecting the NCs' morphology. Understanding the underlying mechanism of CE reactions is crucial for the rational design of nanostructures, but previous approaches to elucidate it relied on analyzing intermediate samples extracted during CE reactions in liquid state, and were hindered by the rapid kinetics of liquid-phase reactions and limited sampling capabilities. To overcome these limitations, solid-state observations of CE reactions were conducted using an in-situ transmission electron microscopy (TEM) approach. This technique enables continuous monitoring of CE reactions with enhanced detail over extended time frames, thanks to the slower kinetics of solid-state reactions and the rapid sampling afforded by TEM. After proving the feasibility of solid-state CE reactions, the focus was moved towards understanding CE reactions and their early stages through a combined experimental/computational approach. The experimental studies focused on in-situ TEM experiments utilizing advanced microelectronic mechanical systems (MEMS)-based heating holders for precise heating control, high-resolution scanning TEM (HRSTEM) imaging coupled with electron energy loss (EELS) and energy dispersive X-ray spectroscopy (EDS) for detailed chemical analysis, and the introduction of different crystal structures to examine variations in CE reactions. The results of these studies constituted the starting point for a comprehensive theoretical study employing classical molecular dynamics (MD) simulations, conducted to provide numerical estimations of activation energies and elucidate the factors influencing CE reaction dynamics. This combined experimental and theoretical approach offers a comprehensive framework for understanding and manipulating CE reactions, shedding light on their intricacies and potential applications in nanomaterial design.
Methods
In-situ TEM and its ancillary techniques were used for the experimental studies on CE reactions at the solid state involving Cu2Se NCs (cation donors) and CdSe nanowires (NWs) with cubic and hexagonal crystal structure (cation acceptors). The mixed populations were closely monitored against temperature using a MEMS-based in situ heating holder. HRSTEM and STEM-based chemical mapping were used to observe the initial phases of CE and analyze the local chemical compositions. MD was used for the calculations related to the atomistic CE model, which included portions of CdSe NWs

initially surrounded by Cu atoms, and a suitable force-field (FF) was adopted to replicate mutual interactions occurring between Cu atoms and CdSe matrices across both crystal structures.
Results
Two parallel studies investigating CE reactions between Cu2Se nanospheres and hexagonal CdSe NWs or cubic CdSe NWs were conducted by a combination of HRSTEM, STEM-EDS mapping and STEM-EELS-based spectrum imaging, with particular attention devoted to early-stage CE. For this reason, available copper was deliberately minimized during the experimental design to slow CE reaction rates. Both studies revealed the formation of distinct CE fronts, negligible direct influence from Cu2Se nanospheres and the presence of a thin Cu layer around the NWs prior to the actual CE. However, the activation temperatures and CE rates changed drastically from one system to the other, going from 400 °C for hexagonal CdSe NWs to 125 °C for cubic CdSe NWs. Also, an unstable regime was observed for cubic CdSe NWs up to 250°C, allowing for partial reversibility of exchanged Cu2Se domains into CdSe.
An atomistic model developed via classical MD simulations provided a theoretical framework for the experimental results, aiming to pinpoint specific local mechanisms involved in CE and to quantify their respective contributions to the overall process. The CE reaction was divided into 4 sequential steps: formation of a Cu shell around a CdSe NW; penetration of Cu atoms into the CdSe NW and subsequent creation of Cu interstitial defects; diffusion of Cu interstitial defects within the CdSe matrix; Cu to Cd substitution via a kick-off event. Each step was simulated to estimate the corresponding activation energy and interatomic interactions in CdSe and Cu2Se were modeled using a combination of Lennard-Jones and Coulombic terms, adapted from the CdSe FF to describe CdSe and Cu2Se structures. MD simulations were conducted for both cubic and hexagonal CdSe structures, revealing differences in energy barriers and migration energies. Notably, cubic CdSe exhibited lower energy barriers, suggesting a more favorable CE process. The model predictions align well with experimental observations for both systems.
Conclusions
In-situ TEM studies on thermally-activated CE reactions at the solid-state between Cu2Se NCs and hexagonal or cubic CdSe NWs were carried out employing a MEMS-based heating holder to explore the early stages of CE and how the acceptor's crystal structure influences the CE process. Notably, expelled copper forms an external shell around CdSe NWs before CE starts and it was found that CE reactions exhibit different threshold temperatures (400°C for hexagonal CdSe and at 125°C for cubic CdSe) and kinetics depending on the host matrix's crystalline phase, with an unstable regime observed for cubic CdSe NWs at low temperatures.Classical molecular dynamics simulations were employed to numerically estimate activation energies and elucidate the probabilities of the entire CE process for different CdSe crystal structures, leading to a good agreement between experimental and computational results. This combined experimental and theoretical approach not only enhances our understanding of thermally activated CE but also sheds light on the mechanisms governing CE reactions with unprecedented detail, particularly in relation to the influence of nanoparticle crystal structure on reaction dynamics.

Keywords:

In-situ heating TEM cation exchange

Reference:

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Advanced electron microscopy study of Cu coated Boron Nitride sheets

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Poster Group 1

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Background incl. aims
Since the advancement of additive manufacturing in recent years, integrating 2D materials with metal alloys has been seen as a promising route in the enhancement of thermal and mechanical performance of the printed material. However, intermixing 2D materials with metal alloys poses various challenges and nanomaterial agglomeration has remained one of them. Coating the 2D materials with metal elements could be a way to overcome such issues. In this work, we have implemented an electroless plating method to coat Boron Nitride (BN) sheets (a ceramic material) with Cu for the development of next generation metal alloys for additive manufacturing. However, evaluating the coating efficiency has seemed to be a challenging task. Hereby, we have developed a protocol of using advanced electron microscopy-based techniques to efficiently characterize the coated material and evaluate the coating efficiency.

Methods
For the study, the vacuum filtered coated powder material was glued on a Si substrate. We have employed various electron microscopy-based methods and developed a protocol to examine the coating efficiency. In-lens backscattering imaging in combination with energy dispersive spectroscopy in a Scanning electron microscope has become a primary observation tactic. Subsequently, thin lamellas were prepared from the Cu-coated BN sheets for evaluation in TEM. Additionally, High angle annular dark field – Scanning transmission electron microscopy (HAADF-STEM) was implemented at a reasonable camera length to observe the intermixed system. Further, electron energy loss spectroscopy (EELS) was performed on the Cu-coated BN sheets.

Results
Initial investigation with the in-lens backscattering detector in SEM along with EDS mapping provides a fundamental understanding of the coated material. Backscattering SEM imaging is sensitive to the material (atomic number) and Cu appears brighter as compared to the BN sheets. Followed by the initial study, Focused ion beam (FIB) was used to make cross-sectional sample and viewed (backscattering SEM) to gain information about the internal structure of the coated material. Subsequently, TEM lamellas are prepared, and high-resolution TEM images are taken which shows high degree of crystallinity of BN and Cu metal interface and provides intricate information about the plating process. Further, STEM-EELS mapping was carried out on the BN-Cu matrix that shows successful coating of the BN sheets.

Conclusion
We have presented a methodology to characterize nanomaterials coating (Cu coated BN sheets) using advanced electron microscopy techniques. The results indicate successful coating of Cu on BN

sheets and hereby confirm the efficacy of the coating procedure. The developed systematic protocol to implement advanced electron microscopy techniques to evaluate the Cu-coating of BN sheets can be used/extend to study other metal-2D material coating or multi-elemental matrix system.

Keywords:

Backscattered electron imaging, EELS, HRTEM

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Second harmonic generation microscopy of femtosecond microstructured crystals

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Poster Group 2

Background incl. aims
Second Harmonic Generation (SHG) is a non-linear parametric optical process that implies the creation of one 2ω frequency photon while destroying two photons of frequency ω. It can only happen in non-centrosymmetric materials with non-vanishing second order susceptibility χ(2), such as anisotropic crystals or fibrillary microstructured biological tissues. There is a quadratic dependence of the SHG field with the fundamental ω one, which implies that the efficiency of the process increases with laser power, and thus, to generate measurable 2ω signals, ultrashort laser pulses are the most suitable sources. Second Harmonic Generation Microscopy (SHM) is a technique based on scanning the sample with a strongly focused ultrashort laser while measuring the SHG signal that is generated at each scanned position of the sample: since the main nonlinear laser-matter interaction is strongly restricted to the focal volume, the measurements are intrinsically confocal and therefore allow for precise 3D sectioning. The SHM technique can reveal rich morphological and structural information in the micro-scale, non-linear properties and/or different chemical composition of the sample.
Methods
Ultrashort femtosecond laser pulses can nano- and micro-structure transparent solids, locally modifying with high spatial precision their index of refraction. In this work we have investigated the non-linear response of two different previously micro-structured crystals: a non-centrosymmetric crystal (β-BaB2O4, BBO) and a centrosymmetric one (Nd:YAG). A femtosecond laser system (writing laser) was used to irradiate the sample in order to produce micro-modifications inside the crystal bulk. Different sets of irradiations were carried out both in BBO and Nd:YAG samples. A homemade microscopy set-up, able to operate as a conventional optical microscope and as a non-linear microscope, was used for the SHM measurements. In the non-linear mode of operation, the sample was illuminated with 120 fs laser pulses (measuring laser) with a central wavelength of 795 nm delivered by a Ti:Sapphire oscillator at a repetition rate of 80 MHz. For this study, SH maps were obtained by scanning the sample by moving the focusing point along the laser-inscribed tracks, which results in spatial variations of the signal intensity that can be measured and precisely assigned to their XYZ point of generation.
Results
BBO is one of the most widely used non-linear crystals due to its high optical non-linearity, wide range of transparency and physical robustness. It has the possibility to phase-match the SHG process in a large spectral window ensuring efficient conversion. In this work it was demonstrated that, under certain conditions, the SHG process in the damage tracks created by ultrashort laser irradiation exhibits a local enhancement in extraordinary polarization conditions. This phenomenon is attributed to a modification, achieved with the micro-structuring, of the effective non-linear coefficients of the

susceptibility tensor, where irradiation modifies coefficients that take part in the extraordinary polarization conditions but not in the ordinary polarization situation. It can also be attributed to a modification of the Gouy phase, because the irradiation and consequent breakage of symmetry in the crystals prevents phase compensation, thus allowing SHG signal to appear.
The second crystal studied, Nd:YAG, has a centrosymmetric structure. The generation of SH signal, localized at the damage tracks, was confirmed, and its features analyzed in detail in terms of the irradiation parameters of the inscribed micro-modifications. Findings show that the SH signal increases when increasing the inscription pulse energy, and when the scanning velocity is reduced (increased pulse overlap). It can be also seen that the measurement laser polarization has a significant effect on the intensity of the SHG signal.
The micro-modifications induced in the YAG structure by the laser irradiation explain the origin of this effects. These include a locally modified crystalline state (different inter-atomic distances; presence of lattice defects) and the formation of periodic structures or nanogratings in the amorphized region, along with phase transformation that can be found at the tracks. Due to the change in the inter-atomic distances in the dislocation surfaces or to a breakage of the inversion symmetry at the boundary between the crystal and the amorphous region, both, dislocations and the presence of an amorphized region, can be responsible for the generation of SH. In this case, the laser polarization for a more efficient SH signal is the one perpendicular to the interface, since it is the field orientation that probes the damage-induced anisotropy.
Conclusions
The observed nonlinear phenomena allow the use of SHM as a powerful technique for 3D imaging and diagnosing of photonic devices micro-structured by femtosecond lasers, such as optical waveguides or micrograting. Although the technique has been extensively employed in non-linear materials, these results open the possibility of waveguide or integrated photonic devices fabrication in centrosymmetric materials that are not initially expected to have a non-linear response.

Keywords:

Nonlinear
Crystal
SHGMicroscopy
Microprocessing
Femtosecond

Reference:

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Latest developments in accurate and high-throughput correlative cryo-FIB milling for cryo-ET lamella production

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Poster Group 1

Correlative cryo-FIB milling is a powerful sample preparation technique for in situ cryo electron tomography (cryo-ET). Using this technique, vitrified cells can be thinned down and cellular components can be directly visualized in their native context at near-atomic resolution. In recent years several solutions have been proposed to simplify the technique by integrating fluorescent microscopes (FLM) in the cryo-FIB. However, it remains challenging to quickly and accurately target the region of interest (ROI). Here we present a high-quality integrated fluorescent microscope, called METEOR, that allows quick and accurate targeting of the ROI.

To ensure accurate targeting the components of METEOR were carefully selected. We ensured it is compatible with objectives with high numerical apertures (NA), the highest being 0.9. The high-end camera brings background noise to an absolute minimum and provides an exceptional quantum efficiency allowing researchers to image challenging and dim samples. The system is equipped with a filter wheel which can be filled with single-bandpass filters avoiding fluorescent bleed-through. Additionally we developed software that allows FLM imaging, sample stage control and SEM and FLM correlation.

By measuring over a 100 sub-diffraction beads we found that the resolution of the METEOR provided 385 nm lateral resolution and 1.18 um axial resolution when using a 50x/0.8 NA objective. This is in line with the theoretical resolution of such an objective. We also found that by integrating FLM and SEM correlation within the FLM acquisition software the SEM map could be used for navigation. This significantly speeds up the identification of suitable cells for milling..

The METEOR system is a high quality imaging platform that allows accurate identification of regions of interest within the challenging samples. The software provides additional benefits that speed up the workflow significantly. METEOR therefore provides an accurate and high-throughput correlative cryo-FIB milling workflow.

Keywords:

cryo-ET, Cryo-FIB, CLEM

Reference:

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2. D. Boltje et al. (2022). eLife, vol. 11
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A Novel Tool for Combined AFM, SEM, and Electrical Probing of Nanostructures

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Poster Group 1

Combining different complementary analytical methods into one instrument is a powerful technique for the contemporaneous acquisition of both structural and functional sample properties. Especially the combination of atomic force microscopy (AFM), scanning electron microscopy (SEM), and electrical nanoprobing can yield completely new insights for the study of various samples and nanostructures.
In this work, we introduce a highly integrated correlative microscopy platform, that seamlessly combines AFM and SEM within a unified coordinate system. In addition, a three-axis sample stage and a trunnion provide unique experimental capabilities such as profile view – an 80-degree tilt of the combined sample stage and AFM giving full SEM access to the cantilever tip region. This microscopy platform can easily be combined with a nanoprobing system that enables precise electrical characterization of nanoscale structures, offering insights into device functionality and performance. [1-2]
We present a variety of novel case studies to highlight the advantages of this new tool for interactive, correlative, in-situ nanoscale characterization of different materials and nanostructures. Initial results will focus on the analysis of mechanical and electrical properties of individual nanowires. The combination of SEM and nanoprobes enables easy manipulation and positioning of individual nanowires, whereas the in-situ AFM allows the characterization of topography, surface roughness, mechanical and electrical properties of the nanowire.[3-4] Using the SEM's high-resolution the AFM cantilever tip can be precisely positioned on an individual nanowire that is attached to the nanoprober and the topographical and mechanical properties can be determined (see Figure).
In addition, we will show results for electrical nanoprobing of semiconductor devices in combination with conductive AFM measurements. A semiconductor device can be electrically probed by simultaneous use of two nanoprobers while the AFM provides detailed information on the 2D conductance map of the device itself.
Based on the broad variety of applications regarding the inspection and process control of different materials and devices, we anticipate that this new inspection tool will be one of the driving characterization tools for correlative analysis on the nanoscale.

Keywords:

AFM, SEM, NanoProbing, Correlative Microscopy

Reference:

[1] M. Dukic, et al., Scientific Reports, 5 (1), 16393 (2015).
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TEM Investigations on the Impact of Hydrogen on Phase Transformations in Aluminum Alloys

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background :
This study investigates the influence of hydrogen on phase transformations in aluminium alloys, materials of critical importance to the transportation industry due to their superior strength-to-weight ratio[1]. Given the potential of hydrogen to induce hydrogen embrittlement (HE) in metals and alloys, this investigation is of paramount importance. The mechanisms behind HE involve the interactions between hydrogen and crystalline defects such as dislocations, vacancies, grain boundaries, and interfaces[2]. However, understanding these mechanisms has been challenging due to difficulties in localizing hydrogen. Phase transformation, including precipitation, is governed by nucleation sites, atomic mobility, and interface energies, all associated with crystalline defects. Utilizing advanced microscopy techniques, this work aims to indirectly gather information on how hydrogen interacts with defects by examining the influence of hydrogen on phase transformation.
Methods :
The materials under investigation were two aluminum alloys: Al-Cu, chosen for its excellent contrast in STEM-HAADF and its well-known precipitation sequence, and Al-Zr, selected for the strong interaction between zirconium and hydrogen[4]. The JEOL ARM-200F microscope, equipped with a STEM corrector, serves as the primary tool for characterizing structural changes under various conditions, complemented by Atom Probe Tomography (APT) and Thermal Desorption Spectroscopy (TDS) for elemental analysis and hydrogen trapping state investigation, respectively. Precipitates were analyzed under different zone axes to assess their shape and diameter, Additionally, Energy Filtering Transmission Electron Microscopy (EFTEM) was employed to produce thickness maps, enabling the calculation of precipitate density. The distribution of dislocations varied with each state, characterized using both bright field and dark field modes in TEM, and more precisely with the LADF detector in STEM, where dislocations predominantly aligned along (111) planes.
Results and Conclusions :
Findings for the Al-Cu alloy indicated a significant slowdown in the initial aging hardening kinetics in hydrogen-charged samples. STEM-HAADF imaging revealed a reduced precipitate density compared to uncharged samples, suggesting that while hydrogen does not alter the precipitation sequence, it does influence the growth and coalescence rates of precipitates. This effect was even more evident under high hydrogen pressure, especially in extensively deformed samples, indicating a slowdown in precipitation processes and defect recovery. In contrast, the Al-Zr alloy's hardness measurements showed no noticeable difference between samples aged in hydrogen and air. However, TEM analysis revealed that under hydrogen, the diameter of zirconium precipitates was twice as large as those aged in air, with a similar nucleation rate for both conditions. This suggests that hydrogen enhances zirconium diffusion, accelerating precipitate growth.

Keywords:

Hydrogen, Precipitation, Microscopy, Aluminium alloy,

Reference:

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Chunhui Liu, Ziyao Ma, Peipei Ma, Lihua Zhan. Materials Science & Engineering A 733 (2018) 28–38.

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Contribution of Probiotics to the Effect of Platelet-Rich Plasma in Diabetic Rat Skin Wound Healing

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Poster Group 1

Background incl. aims
Diabetic (DB) wound treatment is still an important problem due to chronic non-healing ulcers that can become infected and lead to amputation. Platelet Rich Plasma (PRP) is a Cellular Therapy (CT) product that has been frequently used nowadays. Probiotics (PB) accelerate healing through the changes they make in the intestine. This study aimed to examine the contribution of PB to the effects of PRP in diabetic wound healing.
Methods
The diabetic model was created by intraperitoneal application of 45 mg/kg Streptozotocin. A 1x1 cm2 full-thickness skin wound was created in those with fasting blood sugar (FBS) above 250 mg/dI. Mature Albino rats were divided into four group (n:5, each) as DB, DB+PRP, DB+PB and DB+PRP+PB. PRP solution prepared as 100uL was used for 10 subcutaneous 10uL applications to the wound edges. PB was administered orally at a dose of approximately 200 mg/day, once a day, starting with the experiment and until euthanasia. During the seven-day experiment, the PB group was fed by gavage every day. Formalin fixed parafine embedded sections were analysis with Hematoxylin & Eosin and Masson's Trichrom histochemical staining methods. Immunohistochemical analysis was performed with eNOS, Caspase 3, IL10, VEGF and Collagen I primary antibodies.
Results
It was observed that the diabetic wound closed significantly faster with PRP. It was determined that healing speed increased significantly and there was a better recovery with PB. It was shown that increased eNOS and Caspase 3 immunoreactivities and decreased IL10, VEGF and Collagen I immunoreactivities during injury were reversed by PRP+PB. It was found that PB application made a significant contribution to the positive effects and reversed parameters realized with PRP.
Conclusion
Preventing oxidative stress and apoptosis, increasing anti-inflammation, vascularization, numerical cell support of connective tissue and signaling pathways with PRP supplemented with PB made a significant contribution to wound healing. It turns out that PB support may be important for difficult-to-heal DB wounds. It was thought that this contribution could further improve the impaired life quality of patient's.

Keywords:

Diabetes, Wound healing, Probiotic, PRP

Reference:

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Asbestos Analytics In Relation To Changes Of The European Directive

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Poster Group 1

In 2023, the EU Parliament passed a resolution to amend the Asbestos Directive. Within a transitional period of 6 years, laboratories must either implement a detection limit of 2000 F/cm³ for the evaluation of workplace measurements and or determine fibres thinner than 200 nm (lower limit has not been defined) using electron microscopic methods (phase contrast microscopy is no longer applicable) in order to be able to apply a limit value of 10,000 F/m³.
This poses challenges for analysis. On the one hand, not every laboratory has suitable equipment to fulfil the requirements, on the other hand, the users of the equipment are usually not scientists who are fully familiar with the equipment, but users who follow laboratory instructions. The lecture or poster presents the pitfalls of SEM/EDS analysis in relation to air samples that are to be expected when detecting thin asbestos fibres.
If the detection limit is lowered, at least a semi-automatic evaluation is necessary, as otherwise the analysis times will be unacceptable.
When evaluating asbestos fibres with a diameter of less than 200 nm, two fundamental distinctions must be made. These are the visibility of fibres in the SEM and identification using EDS. The former is certainly dependent on the choice of device (device with Wolfram cathode or field emitter), but also on the choice of detector (SE or BSE detector), primary energy, working distance, brightness, contrast and other parameters. Settings at which fibres with a diameter of 200 nm or larger can be displayed may not be optically recognisable for fibres that are significantly thinner. If an acceleration voltage that is too low is selected, this can make it impossible to identify the fibres, depending on the EDS system used.

Keywords:

Asbestos, Fibres Directive

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Quantum Wavefront Shaping with a 48-element Programmable Phase Plate for Electrons

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

Similarly to Spatial Light Modulators (SLM) in optics, Electron Phase Plates (EPP) have emerged as a promising solution to several challenges in electron microscopy, such as in-focus phase contrast, adaptive tuning, and exotic beam preparation. Our proposed solution to a generalized EPP is based on an array of miniaturized Einzel lenses that can shift the electron wavefront locally. This electrostatic EPP can be positioned in the electron beam's path in a Transmission Electron Microscope (TEM). The supports and interconnections that make up the EPP will inevitably modulate the amplitude of the electron wavefront. However, the EPP also allows one to adjust the shape of the electron wavefront locally by applying a voltage to each phase pixel [1].
This work demonstrates a fully programmable electrostatic EPP of 48 phase-shifting elements mounted in a state-of-the-art TEM [2]. We prove the device's versatility by preparing a set of orthogonal probe shapes and reproducing the influence of specific geometric aberrations (e.g., defocus of a convergent electron probe). Furthermore, we show a fully automated Scanning TEM (S/TEM) routine in which the EPP corrects the aberration function without active user intervention.
Finally, we discuss the device's potential for novel imaging techniques such as electron ptychography. Typically, this technique requires scanning a convergent electron probe over a small area of the sample, which may lead to a limited field of view [3]. Recent research has demonstrated the use of a quasi-parallel beam illumination with both amplitude and phase modulation of the electron wave. By successive shifts of the specimen stage and near-field intensity recording, reconstructions could be performed with a significant expansion of the field of view, with no resolution or electron dose sacrifice [4]. Here, we present reconstruction results obtained through a similar approach for far-field propagation, using different amplitude and phase modulation strategies, as permitted by our programmable EPP design, at different applied electron doses (total dose divided by the number of phase configurations).
The figure below illustrates the far-field ptychographic reconstruction results for three incident electron doses. The illumination on the sample is structured using an array representing the EPP. The columns, from left to right, depict the amplitude mask that is used to simulate the EPP, the phase and amplitude of the object ('Ground Truth'), the results obtained through an amplitude-only modulation ('Flat'), the outcomes of amplitude plus two random phase configurations ('Random'), and the results obtained using a reduced set of two Hadamard phase configurations ('Hadamard').
To summarize, we evaluate our electrostatic EPP in various S/TEM and TEM applications. Our findings indicate that an electrostatic EPP can be integrated into any electron microscopy setup, thereby broadening the device's scope of functions.

Keywords:

Phase Plate, Wavefront Shaping, Ptychography

Reference:

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[4] Financial support provided by the Research Foundation Flanders, project G042820N. The ERC funded the ADAPTEM project (grant nr: DLV-789598)

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Characterisation of Skyrmion Spin Textures in CoB/CoFeB Multilayers

Colin Kirkbride[1], Dr Razan Aboljadayel[2], Dr Kayla Fallon[1], Ms Sara Villa[1], Dr Trevor Almeida[1], Prof. Marrows CH[2], Prof. Stephen McVitie[1]
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Poster Group 1

Background incl. aims
Developing energy efficient data storage requires the discovery of novel materials which can host a variety of high density and controllable magnetic textures. In recent years this has led to an interest in materials capable of supporting magnetic skyrmions owing to their variability in size from several nanometres to microns [1]. In addition, their discrete nature makes them attractive candidates for forming magnetic logic gates, advanced signal multiplexing and neuromorphic computing applications [2]. In this work, we analyse the field dependence of skyrmion spin textures formed in magnetic multilayers of [Ru(7)Pt(X)CoB(4.3)Ru(7)Pt(X)CoFeB(5.3)]15 (all in Å) where X is 8.5Å (S1) or 10.5Å (S2) (Fig.1a). The overall aim is to highlight the importance of Pt thickness on the established spin textures and to motivate these materials as candidates for field controllable skyrmion applications.

Methods
Throughout this work, we utilise a range of microscopic analysis techniques with a strong emphasis on Lorentz transmission electron microscopy (LTEM). All images were acquired using a JEOL JEM-ARM200cF microscope operating in either LTEM or STEM mode equipped with a CEOS probe corrector. Qualitative Fresnel images were captured within a field range of ± 130 mT. Single image transport of intensity (sTIE) reconstructions were used as a method of semi-quantitative magnetic characterisation and supported with more robust differential phase contrast mapping of the integrated magnetic induction at a range of key fields. Topographical and phase characterisation is provided through tapping zero-field MFM and bulk magnetic measurements are analysed with SQUID-VSM. Elemental mappings of multilayer cross-sections are measured using electron energy loss spectroscopy (EELS) and provide layer thickness measurements which aid magnetic volume characterisation.

Results
By comparing the magnetic states of S1 and S2 at a range of fields, we analyse the effects of interlayer coupling within the multilayer. Reducing the applied field from the saturated state above 130 mT, S1 first stabilises a ferromagnetic (FM) skyrmion lattice (SkL) phase which expands into FM domains before forming a second SkL at zero field (Fig.1b). The SkL contrast is then slowly removed as S1 enters a field stabilised synthetic antiferromagnetic phase around -40 mT before the magnetisation switches into a FM domain phase and saturates at high field. We correlate this behaviour with strong coupling between the CoB and CoFeB layers. For S2 the behaviour is markedly different on the reduction of field from saturation. We first form sparsely distributed skyrmions with weak contrast which expand into a FM domain state covering the film. We next form a mixed-phase FM state (Fig.1c) with strong and weak contrast which reduces to a purely strong contrast FM domain state at zero field. Upon field reversal, these FM domains contract to form skyrmions with strong and weak contrast below -100 mT, before proceeding towards saturation. We propose that the varying levels of contrast in S2 are caused by the thicker Pt layer forcing an effective decoupling between the

CoB and CoFeB layers allowing each to act independently in response to applied field. The formation of chiral textures is then driven by the different strengths of interfacial Dzyaloshinskii-Moriya interaction at the CoB/Pt and CoFeB/Pt interfaces [3].

Conclusion
Using Lorentz microscopy, we show that the separation between magnetic layers in CoB/CoFeB multilayers can have a significant impact on the observed magnetic textures. This has important consequences for applications, for example, if one desires a high density skyrmion state at low field (eg: for reservoir computing), then strongly coupled systems, similar to S1, would be useful. In contrast, if sparse skyrmion states are required (eg: for racetrack memories), then decoupled systems such as S2 may be more appropriate. Overall, the work serves as motivation for careful, application specific tuning of layer thicknesses during material growth, and highlights the valuable information that nanoscale magnetic imaging can provide for skyrmion stabilising systems.

Keywords:

Lorentz TEM, DPC, Magnetism, Skyrmions

Reference:

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Exploring the limits of 3D ED for accurate structure analysis at the nanometer scale

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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

Background incl. aims
The most common method for robust and reliable structure determination of materials is single crystal X-ray diffraction (SCXRD). However, it is not suitable for nanoparticles (NP) and nanodomains due to their size and geometry. When it comes to structural characterization on the scale of a few nanometers, electron microscopy offers an incomparable range of tools. In this contribution, we focus on accurate nanoscale crystallography using electron diffraction and, more specifically, precession-assisted 3D electron diffraction (3D ED), serial ED and scanning precession-assisted electron diffraction tomography (SPET). The aim is to test the limits of these parallel-beam electron diffraction methods for the accurate structure analysis of isolated NP and nanodomains measuring a few tens of nanometers or less.

Methods
For 3D ED on NPs and nanodomains, the first instrumental requirement appears to be the ability to form parallel beams well below 100 nm in size. If you want to collect single-crystal 3D ED data from a 10 nm domain embedded in a matrix, you must work with an electron nanobeam of this size or smaller. When the diffraction volume is greatly reduced, much lower diffracted intensities are expected. In this case, hybrid pixel detectors is a real asset for studying the size limit of NPs or domains for 3D ED experiments. For this work, our experimental setup is based on a JEOL F200 electron microscope equipped with a ASI Cheetah M3 hybrid-pixel detector. Beam precession movement, combined or not with beam scanning, is achieved via a Nanomegas DigiStar unit.

Results
The question of the lower limit of crystal size compatible with accurate structure analysis by 3D ED is briefly addressed with tests carried out on isolated Indium Tin oxide (ITO) and TiO2 nanoparticles around 10 nm in size. Our study provides experimental evidence that the effect of multiple scattering is still present but significantly reduced for tiny crystals. In such cases, it is possible to obtain fairly accurate structure refinements using kinematical approximation only.
Regarding nanodomains, we explore the limitations associated with their size and the extent to which we can track structural changes at a nanoscale. For this, we shall consider an approach that uses an appropriately sized parallel beam to collect ED patterns at different region of interest (ROI). One way to perform such an analysis is to combine a scanning procedure with precession-assisted 3D ED. The area of interest is selected, and the electron beam is scanned across it with the desired step size while collecting the precession-assisted diffraction patterns. Afterwards, the sample is tilted and the scanning procedure is performed again, and so on, obtaining this way a tomography of a 2D space. Eggeman et al. [1] actually exploited a similar approach for analysing the volume and orientation of domains in crystalline Ni-based superalloys, and used the acronym SPET for Scanning Precession-assisted Electron Tomography data acquisition. We extend the use of SPET for the

structure solution and accurate structure refinement of nanodomains. This implies that after the acquisition, we are able to distinguish, and sort accordingly, the diffraction patterns generated by different ROI in the sample. By doing so, several nanodomains of the specimen can be probed without the need of tracking them while tilting, and therefore to perform structure solution and accurate structure refinements of multiple ROI with a single acquisition.
We first demonstrate the usefulness of SPET for accurate structural characterizations of subtle structural changes in a 35 nm thick $PrVO_3$ thin film [2] using a line scan in a direction perpendicular to the film/substrate interface (see Graphic). Second, we take a step forward employing it for the analysis of nanodomains embedded in ceramics [3] using an area scan and show how it is possible to extract 3D ED data corresponding to single domains/ROI from the SPET data stack.

Conclusion
Regarding the possibility to reveal unknown nano-structure, we propose a shift in the paradigm of 3D ED data collection, from a "point" acquisition, focused on a single ROI, to a multidimensional data acquisition method using scanning precession-assisted 3D ED. With this approach it is possible to obtain accurate crystallographic information from multiple ROI at a scale less than 10 nm.

These results were obtained as part of the European project NanED (Electron Nanocrystallography – H2020-MSCA-ITN GA956099).

Keywords:

3D ED; serialED; Precession; nano-crystallography

Reference:

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Multiparametric investigation of bacterial surface structure with correlative atomic force microscopy

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Poster Group 2

Background incl. aims
Atomic force microscopy (AFM) has become an indispensable tool for high-resolution structural analysis of specimens ranging from single molecules to complex biological systems, while simultaneously being able to correlate topography and mechanics at near native/physiological imaging conditions. In turn, the combination with advanced/customized optics leverages the advantages of immunolabelling techniques for truly correlative microscopy. Specifically, the use of a tip-scanning AFM, as compared to a sample-scanning system, enables simultaneous high-resolution correlation experiments with advanced optical techniques. Studying certain Gram(-) bacterial species have attracted a lot of interest recently as model systems for investigating their outer membrane envelope.

Methods
We have applied fast tip-scanning AFM imaging in combination with confocal microscopy to study the surface structure of bacterial species. We have studied the surface structure of E. coli MG1655 bacteria to gain a deeper insight into the supramolecular organization of their outer membrane protein network.

Results
By additionally exploiting full-fledged sample mechanical mapping we can correlate any substructural surface analysis of the layered bacterial specimen with differences in bacterial stiffness. We demonstrate how the application of dual z-actuator tip-scanning AFM imaging can be exploited for instant high-resolution analysis of tall bacterial samples exceeding 1 µm in height. We will also discuss how the combination of high-speed tip-scanning AFM with antimicrobial agents can be used as a tool to study the bacterial envelope in real-time and potentially develop strategies for fighting microbial resistance.

Conclusion
AFM can be successfully applied to correlate high-resolution structural features and mechanical properties of the outer membrane envelope in MG1655 E. coli bacterial species in combination with advanced optical microscopy.

Keywords:

AFM, Correlative Microscopy, Bacterial Imaging

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Silicide precipitation in aged quasi-α Ti alloys

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Increasing temperature properties of materials is a major strategic challenge for the aviation industry. Titanium alloys like the so-called Ti6242 offer excellent specific mechanical strength and good corrosion resistance up to temperatures of around 550°C. On one hand, molybdenum can be added to reduce creep/fatigue effects known as dwell effect [1] but the associated decrease of alpha phase fraction is detrimental to high temperature performances. On the other hand, addition of silicon improves the mechanical properties at high temperature [2] and compensate molybdenum addition. Still, the strengthening mechanism of silicon is still under discussion.
During aging, silicon leads to the precipitation of silicides [3] which could play a role in the strengthening of the alloy. We have characterized the microstructures by complementary electron microscopies to corroborate the relationship between silicides and macroscopic mechanical properties.
In order to characterize the microstructure at different relevant scales, we used SEM and TEM imaging. Despite a good accuracy, the analysis of the planar or projected images revealed that the strong anisotropies of the lamellae microstructure prevents a good description of the silicide population. Therefore, we also performed 3D reconstructions by FIB/SEM (figure a) on the alloys in the aged state to get reliable statistics on the dimensions, shape and organisation of the silicides in the microstructure (figure b). An additional S/TEM study has been used to determine the precipitate structure and chemistry.
Thanks to FIB/SEM reconstructions, volumes containing 4000 precipitates each have been analysed. Results showed that aged Ti6244 alloys host precipitates with an elongated shape and a mean Feret radius of almost 40 nm. Moreover, when the Si content is doubled, the precipitate density increases (+55 %). The analysis of the precipitate organisation, with respect to the Ti matrix orientation, reveals that the precipitates are mainly located in the beta phase and that their nucleation is likely to occur at the interface between alpha and beta. It also showed that two directions of elongation are favored.
A careful analysis by high resolution S/TEM (figure c) reveals that the S2 silicides are semi-coherent and that their composition is close to (Ti,Zr)6Si3 which is not expected with respect to the thermomechanical treatment used and the CALPHAD calculations. The Burgers relationship between both titanium phases and the S2 silicides are clearly defined and explains the two observed variants. Additional TEM observations after creep at high temperature illustrates the role of the silicide in the dislocation anchoring (figure d).
Thanks to combined microscopy techniques, we show that the silicide precipitation plays a key role in the strengthening of the Ti6244 alloys. The semi-coherent structure and the spatial organization of the precipitates suggest that an Orowan mechanism is favored.

Keywords:

FIB/SEM tomography, titanium, silicides, HRSTEM

Reference:

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Enhanced imaging for serial Cryo-FIB-SEM microscopy of biological samples with fluorescence navigation

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Poster Group 1

Traditional methods for studying biological specimens with volume electron microscopy face two significant hurdles: the complex process of sample preparation, which includes drying and staining with heavy metals and can introduce artifacts, and the difficulty in maintaining fluorescence signals in samples set in resin. In contrast, cryo-FIB-SEM keeps specimens nearly unchanged in a frozen state, streamlining the preparation process, reducing structural distortions, and allowing easier observation of fluorescence. Yet, imaging non-contrasted biological specimens in cryo-FIB-SEM can be challenging due to low signal-to-noise ratios and charging artifacts.

Our research shows that by fine-tuning the imaging conditions, electron exposure, plasma milling parameters and the workflow management, we can greatly improve the quality of the data, speed up the rate of data collection, and capture large volumes of samples with outstanding ultrastructural clarity. This allows us to view all cellular organelles in detail, achieving resolutions as fine as 16nm. An additional advantage of cryogenic imaging is the preservation of the fluorescence signal, which helps in locating cells and guiding the initial trenches in the sample.

With the mentioned improvements, we acquired high-quality cryo-images. We demonstrate the effectiveness of our cryo-FIB/SEM method by reconstructing a segment of a pancreatic INS-1E beta cell, showcasing the workflow's capability to produce high-quality imaging of biological samples.

Keywords:

FIB-SEM, volumeEM, cryo, native, cell

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High-throughput laboratory-based scattering X-ray Tensor Tomography

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

Background incl. aims
Scattering (or dark-field) contrast X-ray microscopy methods for orientation analysis have been gaining increased attention in recent years. The primary advantage of these methods is the ability to map anisotropic structures without directly resolving them, allowing for an extended field of view (FOV). Two different technologies, scanning small-angle scattering and full-field linear grating interferometry have been applied to a wide range of materials, such as fiber-reinforced composites and biological materials [1,2]. However, both methods have disadvantages with throughput: scanning methods have significant overhead in raster scanning the 2D FOV, while gratings require linear and rotational shifts to access 2D orientation sensitivity. A recently developed design of gratings comprised of circular unit cells grid allows single-shot mapping of orientations/scattering in 2D [3]. Each unit cell of the 2D grid provides information on local scattering, emulating raster scanning, while the circular design yields 2D orientation sensitivity, all in a single frame. This is particularly important when 3D scattering tensor information is of interest, achieved by what is known as X-ray tensor tomography (XTT) [4].
Here, we present the capabilities of the state-of-the-art XTT technology based on circular gratings, commercially available on the Exciscope Polaris imaging platform. Notably, we emphasize the simplicity and efficiency of the technology in the laboratory and validate the results against the synchrotron implementation of this technique.

Methods
The sample was prepared using a free-form carbon-fiber injection molding by Addifab, ApS (Fig. 1a). Laboratory and synchrotron measurements were performed on an Exciscope Polaris X-ray microscope and at the TOMCAT beamline, Swiss Light Source, Paul Scherrer Institute, respectively. Different grating design is implemented at two setups: phase-based at the synchrotron and absorption-based at the lab. This is due to the polychromaticity and divergence of the X-ray beam of the lab setup. The latter yields geometric magnification allowing for a detector with larger FOV and pixel size. In both setups, a total of 721 sample poses were collected. A series of 8 tile images at each sample pose was acquired with the phase-grating at the synchrotron setup due to the limited size of the X-ray beam.

Results
Stitching tile images from the Tomcat dataset as pre-processing generated one projection image per pose. Subsequently, both synchrotron and lab-setup datasets were reconstructed using identical algorithms and parameters (Fig. 1b). For validation, reconstructed 3D fiber orientation volumes were registered with affine transformation. The agreement between the two volumes was calculated as an inner product of fiber orientation vectors in each voxel, where values 0 and 1 describe an orthogonal and parallel alignment, respectively. A histogram of the per-voxel inner product (Fig. 1.c) reveals exceptional agreement of the results from two experiments with a mean value of 0.95.

Conclusion

Synchrotron facilities are at the forefront of the development and advancements of 3D X-ray imaging technologies. However, limited user access and a high level of complexity hinder the general use of such large-scale facilities. In this context, we present the capabilities of the state-of-the-art XTT technology with origins at synchrotron that has been successfully transferred into the laboratory environment. The transition of XTT with circular gratings to a commercial imaging platform is presented and validated against synchrotron measurements, revealing a high level of agreement.

Keywords:

Tensor-tomography, grating, small-angle scattering, fiber-orientation

Reference:

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Achieving atomic precision 3D reconstructions through Bayesian genetic optimisation

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Poster Group 1

Background incl. aims
Accurate and precise knowledge of the three-dimensional shape of nanoparticles is critical to understanding their unique properties. High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) provides a powerful tool for quantitative analysis, allowing the extraction of structural information through atom-counting, where the intensities generated by atomic columns in HAADF-STEM are analysed to count the number of atoms with single-atom precision.
However, reconstructing the 3D structure from these counting results remains challenging. Based on the counting results and the prior knowledge of the crystal structure, we can construct an initial guess of the nanoparticle by placing the columns symmetrically around a central plane. While this can serve as an initial guess, further refinement is imperative to make claims about the structure. In recent years several refinement procedures have been proposed. Molecular dynamics and Monte Carlo methods have been used for energy optimisation but may fall short of an accurate reconstruction by becoming trapped in local minima or deviating from experimental observations. A local minima search algorithm can scan the energy landscape for the best energy minimum but does not consider the finite precision of atom counting, which strongly contributes at lower electron doses.
Methods
The implementation of the Bayesian genetic algorithm can address these issues effectively. In contrast to molecular dynamics-based reconstruction methods, where each atom is individually considered, the Bayesian genetic algorithm is a holistic approach; it incorporates all knowledge obtained from the HAADF-STEM image. The column positions are fixed to the observed locations in the image, and the thickness variations are constrained by the known finite precision of the atom-counting analysis.
The initial guess for the three-dimensional structure created based on the atom-count results and prior knowledge of the crystal structure functions as a reference in the genetic sequence. By encoding parameters such as the height offset and thicknesses of columns with respect to the initial guess, we can describe any possible 3D structure generated by the columns in the observed image. The genetic algorithm will then utilise mutations and combinations of members to optimise a cost function. By generating large populations of candidate reconstructions with genetic variety, the algorithm can effectively scan the local energy landscape for an optimal solution which balances the energy minimisation and atom-counting probabilities.
However, genetic algorithms can become prohibitively computationally expensive for large nanoparticles due to the rapid growth in the number of parameters required to describe their 3D shape. Furthermore, the simulation parameters (population size, mutation density, ...) are difficult to standardise as they may differ significantly for each optimisation problem and genetic algorithm implementation. To this end, we have optimised the algorithm for computational efficiency and proposed simulation parameters which balance the need for mutations with the convergence rate such that the algorithm can reconstruct nanoparticles of up to at least 10 nanometres in size.

Results
Though the computational cost and choice of parameters of genetic algorithms, in general, is a whole area of study by itself, we have explored the behaviour for the specific application of reconstructing the three-dimensional shape of nanoparticles. By choosing appropriate simulation parameters by balancing the mutation rate with the population size, we examine the scaling relationship of the convergence rate as a function of atomic columns in the image. A statistical analysis gives strong indications of a linear and well-behaved convergence rate.
Additionally, we used the algorithm to reconstruct a large simulated nanoparticle of approximately 10 nanometres in three dimensions with a high surface atom recovery. This demonstrates the capacity to reconstruct large nanoparticles with high accuracy and allows us to study the presence of specific surface features in such particles.
Conclusions
We have optimised and studied the performance of the Bayesian genetic algorithm for the reconstruction of nanoparticles at atomic scale with high accuracy. By doing so, we can reconstruct nanoparticles of up to at least 10 nanometres in size.

Keywords:

Quantitative HAADF-STEM, atom-counting, genetic optimisation

Reference:

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End-to-end learning of atomic resolution phase-contrast volumes from 4D-STEM measurements

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

Background incl. aims
Ptychography has been proven to have the ability to efficiently recover phase information of the specimen in the Transmission Electron Microscope (TEM). However, with traditional single slice ptychography, the multiplication assumption requires the specimen to be thinner than a few atomic monolayers. Otherwise, multiple scattering becomes significant, and the algorithm fails to recover even the basic structure. To accurately reconstruct the phase information of a thick sample, we combine electron ptychography and tomography into a single algorithm directly reconstructing a 3D electrostatic potential volume from 4D-STEM tilt-series measurements.
Ptychographic atomic electron tomography was experimentally demonstrated to be capable of imaging atoms in 3D volume, together with contrast improvements[1]. Another successful demonstration to image bulk materials was to combine the multi-slice algorithm with ptychography. This powerful combination is capable of imaging individual atoms at a resolution set by the intrinsic size of the atoms. The combination of the multi-slice algorithm and tomography have also been demonstrated using High Resolution TEM. Even with plane-wave illumination, the atoms were precisely located. Hence, with all the successful demonstration, our aim is to develop a joint reconstruction algorithm, combining the multilisce, tomography, and ptychography for bulk materials with atomic resolution. Our experimental realization is an end-to-end reconstruction process, the 3D structure of the material is recovered directly from the 4D-STEM dataset. In the result, the 3D atom positions can be clearly viewed in all 3 dimensions.
Methods
In the collection process, our method is similar to the ptychographic electron tomography experiment, a focused electron beam raster scan through the bulk specimen with adjacent scan positions overlapped. The electron beam passes through the specimen and then propagates through the vacuum. The detector, which is placed in the far-field with respect to the specimen, collects one diffraction pattern for each scan position. In the reconstruction process, we recover the electrostatic potential of the atoms, which multiplying an electron-charge interaction constant is the projected phase information of the specimen. A series of thin slices of the specimen are reconstructed for each tilt angle. The updates from all the tilt angles are applied to the same 3D volume, instead of integrating after the reconstruction. In other words, the updated volume in the current angle is used in the forward path of the reconstruction of the next angle. In Figure 1 a), the calculated volume is rotated and shifted using affine alignment (A), and then binned (B). The electron beam, with 3 modes and their exit wave shown, passes through the model and forms a diffraction pattern at each scan position. In Figure 1 b), the 4D-STEM datasets update the exit wave at the backward path at each angle, and eventually recover the potential of our model. A regularization kernel is used to reduce the binning effect. Butterworth filter and a modified L1 normalization are used to erase the noise.
Results
We used modal decomposition method to model the partial coherence of the illumination wave. The complex wave transfer function and their positions were reconstructed together with the 3D sample

volume. The sample in our experiment was ZrTe2. The reconstructed electrostatic potential of our sample clearly imaged the individual atoms. The algorithm yielded a high contrast of the core structure, which helped us separate the atoms from the vacuum. A series of hyper-parameters were tuned to test their effects. We also tested the performance of our system under low-dose condition by skipping part of the diffraction patterns.

Conclusion

In summary, we experimentally demonstrated end-to-end reconstruction for ptychographic multi-slice electron tomography is capable of 3D structure determination with atomic resolution. With a series of fine-tuned regularization tools, our joint reconstruction algorithm yields a high SNR volume with contrast improvements. With the low-dose test, the algorithm shows promising potential for imaging beam-sensitive materials such as polymers, battery materials, and biological molecules.

Keywords:

4D-STEM, multi-slice ptychography, tomography, reconstruction

Reference:

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Mapping Orthorhombic Domains with Geometrical Phase Analysis

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Poster Group 1

Background incl. aims
Perovskite oxide compounds, with the chemical formula ABO3, constitute one of the most studied family of oxides due to its large variety of fascinating properties combined with their high degree of tunability. Although the prototypical perovskite lattice is cubic, additional structural distortions emerge when the ionic size ratio (A/B) deviates from one, thus lowering its symmetry. Among all the possible resulting space groups, the orthorhombic Pbnm lattice is one of the most common ones. For instance, most Vanadates, Nickelates, Iridates and Titanates share these particular lattice symmetries. The anisotropic unit cell of such compounds is characterized by in-phase octahedral rotations about one pseudocubic axis (the long orthorhombic one) and out-of-phase rotations about the other two pseudocubic ones; plus additional relative A-site displacements that feature a zig-zag pattern along the [110]PC direction.
When growing epitaxial heterostructures, the Pbnm lattice may nucleate differently oriented depending on how the unit cell is constrained by its neighboring epitaxial layers. When different orientations are equally energetically favorable, the presence of lattice domains with distinct orientations emerge, which in turn may lead to the presence of crystal defects. Retrieving all of this information is therefore paramount when designing novel heterostructures for electronic devices, as it will affect their resulting physical properties. For instance, improper ferroelectricity in artificial superlattices requires the long orthorhombic axis to be oriented out-of-plane. However, since the three pseudocubic axes are in most of the cases very similar in length, it is typically challenging to extract this information from conventional X-ray diffraction analyses.

Methods
In this contribution, we present a very simple and fast approach devoted to map Pbnm lattice domains.1 It is based on using the conventional geometrical phase analysis (GPA) but in a non-conventional manner. Since the relative zig-zag displacements of the A-site cations double the unit cell length along the long orthorhombic axis, they feature additional half-order reflections in the Fourier Transform (FT) pattern of aberration-corrected STEM images. The strategy behind our methodology consists then on using the conventional aperture settings used for regular strain mapping (centered in the (001)PC and (100)PC or (010)PC reflections), but this time including the {(1/2) 0 1}PC or {1 0 (1/2)}PC reflections within the selected aperture area as well. When these particular aperture settings are used, additional fringes (related to the A-site relative displacements) appear in the exy and rxy maps only in those areas contributing to the emergence of these half-order reflections in the corresponding FT pattern.

Results

As a showcase of the versatility and robustness of this methodology, we further exploit this approach to determine the structural parameters that set the lattice orientation and associated domain distribution in rare-earth nickelate compounds. For this, we have analyze the domain distribution in a series of rare-earth nickelate heterostructures grown under distinct structural constraints. Although we will show that the resulting lattice orientation and domain distribution mainly depends on the in-plane normal epitaxial strain, we will also demonstrate how shear strain and interfacial structural connectivity are also very relevant, and should not be neglected when designing novel heterostructures.1-3
Finally, we will also show that this methodology is not restricted to Pbnm domain mapping, but can also be exploited to identify some specific lattice defects or domain boundaries that are otherwise very challenging to localize. Further, beyond Pbnm compounds, it can be adapted to map other types of structural periodicities from other space groups that also also render half-integer reflections in the FT patterns of STEM images.

Conclusion
To summarize, in this contribution we will first present a fast, robust and versatile methodology that is very useful to map Pbnm lattice domains. Second, we will discuss how we have applied this to identify key structural parameters that influence domain distributions in epitaxially-grown Pbnm compounds. Finally, we will also comment about other possible scenarios where this methodology can be also useful to track and map periodic structural distortions that render half-integer reflections in the FT patterns of STEM images.

Keywords:

STEM, GPA, Perovskites, strain, mapping.

Reference:

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2 L. Varbaro, B. Mundet et al., APL Materials 12, 031104 (2024)
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Localized, anodic aluminum corrosion phenomena studied with electrochemical liquid phase electron microscopy

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Local corrosion is a major concern for structural materials and it remains one of the most difficult defects to detect due to the submicron size of the local corrosion sites and the fact that they can be obscured by corrosion products [1]. The early stages of localized corrosion are the least understood and the most challenging to study as they occur stochastically at the solid-liquid interface on the nanometric scale [2]. With its combination of spatial resolution, temporal resolution, and analytical capability, electrochemical liquid-phase electron microscopy (ec-LPEM) has shown the potential to provide new insights into the understanding of localized corrosion phenomena [1], [3], [4]. Among these, Al corrosion in saline environments is a relatively well-studied phenomenon, allowing comparison of the ec-LPEM results with previous reports [2].

Commercially available transmission or scanning EM (SEM or TEM) stages were used to confine a liquid solution between two micro-electro-mechanical systems (MEMS) chips. To study Al corrosion using ec-LPEM, we have developed in-house microfabricated electrochemical chips containing one Al electrode and two Pt electrodes that can be electrically biased to perform in situ or operando electrochemical experiments. The three fabricated electrodes feature a symmetrical geometry to avoid local current density hotspots [5]. Potentiodynamic and galvanostatic electrochemical experiments were performed in situ for probing the corrosion of Al in chloride-containing electrolyte.

Linear sweep voltammetry (LSV) measurement was performed at a scan rate of 1 mV/s. The recorded electrochemical signal shows a plateau followed by a peak in the recorded current corresponding to the pitting potential. The recorded image sequence shows corrosion events occurring rapidly once the pitting potential is reached. The corrosion kinetics are found to be too fast for the time resolution of LPSEM. Thus, despite their widespread use in bulk systems, potentiodynamic techniques are not the most appropriate for recording localized corrosion phenomena using EM.

To achieve slower kinetics, galvanostatic control by means of chronopotentiometry (CP) measurements was performed at increasing current levels in the SEM. At the very low current of 1 nA, single pitting events were observed, whereas at the high end of the current range (50 nA), the corrosion was found to proceed rapidly with the simultaneous formation of a gaseous byproduct. Measurements performed at 1, 5 and 10 nA showed oscillations in the electrochemical signal, corresponding to the repassivation of the Al substrate with its naturally formed oxide. Two different corrosion shapes were observed: at low current (1, 5, and 10 nA), successive circular corrosion events occurred, whereas, at higher current densities (5, 10, 20, and 50 nA), fractal-shaped degradation occurred. This could indicate a kinetically dependent mechanism of Al corrosion.

The kinetic behavior of the anodic corrosion was calculated from the post-processed sequence of images taken over a 10-minute period. The processing consisted of 3D Gaussian blurring, median filtering, application of progressive image denoising algorithm, and segmentation of the images. This enabled the corroded area of the aluminum electrode to be determined for each image recorded.

The calculated corrosion rates in the intermediate current range of 10 and 20 nA were found to be in good agreement with the theoretically determined oxidation rate of Al within a range of ±10%, indicating the stability of the developed electrochemical system and the minimal effect of electron imaging on the corrosion mechanism.

Galvanostatic measurements at low applied current (5 nA) were also performed in the TEM. The real-time observations reveal that there is a distinct higher brightness that follows the corrosion front. This is indicative of gas formation (most likely H2) at the metal-liquid interface that is trapped during the process. It is noted that the recorded electrochemical signal did not match the one observed at the same applied current in the SEM, most likely due to electron beam-induced effects which are more pronounced at the scale of the TEM imaging.

In conclusion, we report the development of an electrochemical chip containing an Al electrode for real-time corrosion studies using ec-LPEM. Our methodology and findings can be applied to other electrochemical processes involving aluminum working electrodes, such as the cathode side of lithium-ion battery systems.

Keywords:

ec-LPEM, Al corrosion, pitting mechanisms

Reference:

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In-situ SEM Correlated Nanoindentation and Electrical Nanoprobing

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Poster Group 2

Background inlc. Aims
Understanding the electrical properties of materials and devices under mechanical stress is vital for ensuring their safe and reliable operation and optimizing their design. External mechanical perturbations can lead to significant changes in the electric response of semiconductor devices and functional materials. This presentation introduces a pioneering investigation into the dynamic relationship between mechanical stress and electrical properties, utilizing an integrated in-situ scanning electron microscope (SEM) platform for nanoindentation and electrical nanoprobing. The aim is to address the intricate interactions between mechanical stress and electrical behavior in various materials and devices, particularly focusing on those too small for traditional optical microscopy.
Methods:
The study tackles the complex challenge of probing electrical responses (typically within a current range of 10µA-10mA) during nanoindentation experiments (with typical load ranging from 100µN-4N and displacement from 0.1nm-40µm), requiring the high-resolution environment of an SEM. The methodology extends to investigate scenarios where mechanical deformations are induced by electrical biasing, offering a comprehensive understanding of the interplay between mechanical and electrical stimuli. This integrated system is versatile, applicable across diverse domains such as piezoresistive or piezoelectrical structures, nano- and micro-electromechanical systems (NEMS and MEMS), fatigue testing of integrated circuits (ICs), and failure analysis of samples.
Results:
The study showcases the versatility of the integrated in-situ SEM platform in investigating the dynamic relationship between mechanical stress and electrical properties in various materials and devices. It presents case studies including a comprehensive analysis of the mechanical stress response of PtCr metal lines and the characterization of a piezoresistive atomic force microscopy (AFM) cantilever. These case studies offer insights into material behavior under mechanical stress and provide valuable information for the design of robust electronic applications.
Conclusions:
The presented integration of nanoindentation and electrical nanoprobing within an SEM environment offers a promising avenue for advancing the understanding of material behavior under combined mechanical and electrical stimuli. By addressing the challenges of probing electrical responses during mechanical testing, this approach provides valuable insights into the behavior of materials and devices, contributing to the optimization of their design and enhancing their reliability and performance in various electronic applications.

Keywords:

nanoindentation, electrical nanoprobing, in-situ, micromechanics

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In-situ Extreme Mechanics – Recent Innovations and Prospects

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Poster Group 2

Background inlc. aims:
In situ scanning electron microscope (SEM) micro- and nanomechanical testing has become essential for materials design and fundamental mechanics research. Traditional nanoindentation techniques have expanded to include new protocols and testing geometries, allowing for the exploration of microstructure–property relationships, intrinsic material behaviors such as orientation-dependence and plasticity, fracture dynamics, and the performance of innovative micro-3D-printed metamaterials. This versatile technique contributes significantly to various scientific domains, including thin films, coatings, metallurgy, glasses, ceramics, nuclear, semiconductors, biomechanics, and architectured materials. Conducting micromechanical tests within a SEM offers distinct advantages, such as unparalleled control, stability, and positioning accuracy, as well as the ability to perform unique correlative experiments by integrating mechanical data with direct imaging or electron backscatter diffraction (EBSD) measurements and focused ion beam (FIB) techniques. This study aims to present the recent developments in instrumentation for in situ extreme mechanics testing at the micro and nanoscales. Specifically, it focuses on a testing platform capable of strain rate-dependent testing across a broad range, from 0.0001 s-1 up to 10,000 s-1 (spanning 8 orders of magnitude), while offering simultaneous high-speed actuation and sensing capabilities with resolutions at the nanometer and micronewton levels, respectively. Additionally, the study addresses the challenges and solutions associated with performing extreme micromechanical tests over a temperature range from -150°C to 1000°C, highlighting the inherent advantages of utilizing small sample volumes.
Methods:
The instrumentation discussed in this study encompasses a sophisticated testing platform designed for extreme micromechanics. It incorporates advanced capabilities for controlling strain rates and temperatures while enabling high-speed actuation and precise sensing. The methodology involves detailed experimental setups tailored to accommodate the diverse range of extreme conditions, ensuring accurate and reliable data acquisition during in situ testing within the SEM environment.
Results:
The study demonstrates the efficacy of the developed testing platform in conducting in situ extreme mechanics experiments at the micro and nanoscales. By showcasing examples of such tests, it highlights the platform's ability to explore material behaviors under various extreme conditions, including high and low temperatures, dynamic loading scenarios, and different strain rates. The results illustrate the platform's versatility and reliability in capturing crucial mechanical data with high resolution and accuracy, paving the way for comprehensive understanding and analysis of material responses under extreme environments.
Conclusions:
In conclusion, the presented advancements in instrumentation for in situ extreme mechanics testing offer valuable insights into the mechanical behavior of materials at the micro and nanoscales under extreme conditions. The study underscores the significance of employing such techniques to address diverse scientific and engineering challenges, ranging from high-temperature applications in engines to cryogenic environments in hydrogen storage. Moreover, it emphasizes the potential for future

research endeavors to further explore and expand the capabilities of extreme micromechanical testing, thereby driving innovations in materials science and mechanics.

Keywords:

nanoindentation, mechanical testing, in-situ, micromechanics

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MIPAR Spotlight: Shedding Light on Next-Generation Detection for Microscopists

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Poster Group 2

Background
For scientists and engineers across various disciplines, image analysis serves as a vital tool for gaining deeper insights into complex phenomena. Whether studying biological structures, geological formations, engineering components or materials microstructures, the ability to extract quantitative data from images is invaluable. Image analysis enables engineers and researchers to replace manual analysis and increase productivity, to gain additional data insights and improve statistics, and to advance their work by solving complex problems. By harnessing the power of image analysis, scientists and engineers can accelerate research, optimize manufacturing processes, and improve performance of products across a wide range of fields, ultimately advancing our understanding of the natural world and improving technology.

Method and results
Using deep learning and powerful image analysis engines, MIPAR (www.mipar.us) allows users to perform a fast, accurate and automated analysis of images. Through a user-friendly interface, engineers and researchers can personalize analysis to their samples as well as visualize and extract measurements - all without programming. Through five integrated applications, MIPAR offers flexibility and efficiency for 2D and 3D analysis applications. The key ingredients are in the Recipes, which include a series of analysis steps that are tailored to each application. As a result, researchers and engineers can now easily solve problems such as grain size analysis excluding the twins, particle analysis of clusters, defect identification and many more.

Seamlessly integrated within the MIPAR ecosystem, the Snap tool, powered by Spotlight, offers accelerated creation of training datasets for an efficient and streamlined model training workflow. The accurate segmentations provided by Spotlight allow for less time spent creating models and algorithms, leading to faster data collection. MIPAR Spotlight simplifies image analysis even further by limiting the need for custom model configurations and amplifying a model's ability.

Conclusion
This presentation will overview the advantages of using MIPAR Spotlight, the new cutting edge of detection, to analyse grains, particles, droplets, and defects with real industrial applications.

Keywords:

image analysis, deep learning, microscopy

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Enhancing quantifiability of S/TEM-based composition mappings through correlative techniques

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Poster Group 2

Background incl. aims
In the realm of semiconductor physics and material engineering, the precise quantification of alloy concentrations is imperative as slight variations in the concentration can have far-reaching implications for the optical and electrical properties of semiconductor devices, e.g. InGaAsN near-infrared lasers [1]. As humanity continuously advances the frontiers of nanotechnology and device sizes shrink to just a few atomic layers, material's analysis must keep up and provide sufficient precision in the determination of alloy concentrations as well as the necessary spatial resolution to resolve these tiny devices. While S/TEM based approaches provide this necessary spatial resolution, each method used to determine elemental concentrations in STEM presents its own challenges, especially when it comes to quantifiability. To reliably determine concentrations, STEM-EDX for example, relies on proper reference samples and detailed knowledge of the systems x-ray signature [2]. Diffraction based techniques, such as dark-field imaging of a chemically sensitive beam, exhibits strong dependencies on the specimen's thickness and bending, as these influence dynamic diffraction conditions [3].
The study aims to improve the accuracy of compositions mappings by leveraging the individual challenges and advantages of EDX and diffraction-based techniques against each other.
Methods
We prepare a TEM-lamella, incorporating InGaAs layers (with known In concentration) near the [100] foil normal, employing classical cross-section preparation. This specific zone axis, characterized by 4 {002} reflections, exhibits chemical sensitivity as the structure factors of these {002} reflections hinge on the difference in atomic scattering factors within the unit cell of constituent materials. To exploit this chemical sensitivity optimally, we strategically tilt the specimen, predominantly exciting one of these reflections. This minimizes dynamic diffraction effects while concurrently enhancing the relative intensity of the respective reflection.
Employing an ultrafast pixelated detector, we systematically scan the tilted specimen, capturing 2D diffraction patterns. In a complementary set of measurements, STEM-EDX signals from the layer are acquired for a comparative analysis. STEM-EDX measurements and 4D-STEM mappings are conducted on a JEOL ARM300F2 at 300kV with a 2.2 sr EDX-system. The integration of these measurements is achieved through 2D cross-correlation of the simultaneously acquired signals on the HAADF detector. Subsequently, virtual dark-field image of the (002) reflection is computed, facilitating a direct correlation with the structure factor and, consequently, revealing the corresponding Indium concentration within the layer. Results are compared to Bloch waves simulations to account for dynamic diffraction effects for the respective lamella thickness. Further investigations of the Indium segregation are conducted in high-resolution conditions analyzing the geometric phase.
Results
From a dark-field image of the (002) reflection, a segregation profile for the Indium concentration within a 5 nm layer of InGaAs, nominally possessing a 20% indium concentration, can be extracted.

It's noteworthy that, for the specified indium concentration and under strong-beam conditions, no significant disparity in indium levels, attributable to dynamic diffraction, is noticeable when comparing the (020) and (002) reflections. In Fig. 1 a segregation profile of the Indium concentration with a Muraki-Fit [4] is shown. Furthermore, for small Indium concentrations, the dependency of the concentration determined by this dark-field imaging on the specimen thickness seems to be negligible. This assertion finds support in Blochwave simulations, analyzing dynamic diffraction. For larger Indium concentrations, strain effects play an increasingly important role in the analysis of concentrations through dark-field imaging. Here, we explore the possibilities of determining concentration profiles from strain analysis, taking into consideration strain relaxation at the lamella surfaces. The obtained results are compared to STEM-EDX mappings of the same quantum well layer. Our comprehensive discussion covvers the results and their implications for the interplay with specimen thickness, Indium segregation, and how these factors collectively impact spatial resolution and quantifiability in STEM-EDX. In further investigations, we replicate the study using a set of TEM specimens prepared through a FIB TEM-lamella preparation. This replication aims to scrutinize the influence of FIB Ga-ions on the composition determination in Ga-based heterostructures, the changes to the In-distribution and amorphized layers.

Conclusion

Among the multitude of interaction possibilities of the high-energy electron beam, traditional approaches often focus on a single interaction, such as analyzing the corresponding x-ray signature of the specimen. However, scanning transmission electron microscopy techniques provide a broader perspective, as the transmitted electrons carry additional information for each scan position. This interplay becomes particularly valuable dealing with diverse measurement conditions, including specimen thickness and orientation, providing an opportunity to enhance the quantifiability of composition mappings. Importantly, this methodology transcends the limitations of individual techniques, leveraging the improved signal-to-noise ratio for thicker specimen in EDX over diffraction-based techniques, which are more effective with thin specimens. The resulting versatility not only enables precise composition determinations across a wider range of specimen parameters but also reveals additional synergies. For instance, it aids in resolving the indium concentration degeneracy in a pure analysis of the local form factor for InGaAs quantum devices. These insights not only amplify the quantifiability of S/TEM-based composition mappings but also harmonize well with novel approaches of composition determination, such as the analysis of stress relaxation for thin TEM-lamellas.

Keywords:

InGaAs, composition-mapping, Alloy-distribution, STEM-EDX, 4D-STEM

Reference:

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Confined single-molecule imaging by low-dose electron microscopy

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Poster Group 2

Single-molecule imaging with atomic resolution is a notable method to study various molecular behaviors and interactions. Although low-dose electron microscopy has been proven effective in observing small molecules., it has not yet helped us achieve an atomic understanding of the basic physics and chemistry of single molecules in porous materials, such as zeolites. The configurations of small molecules interacting with acid sites determine the wide applications of zeolites in catalysis, adsorption, gas separation, and energy storage. Here we report the atomic imaging of single pyridine and thiophene confined in the channel of zeolite ZSM-5. Based on integrated differential phase contrast scanning transmission electron microscopy (iDPC-STEM), we directly observe pyridines or thiophenes' adsorption and desorption behaviors in ZSM-5 under the in situ atmosphere. The adsorption configuration of a single pyridine or thiophene is atomically resolved and the S atoms in thiophenes are located after comparing imaging results with calculations. The strong interactions between molecules and acid sites can be visually studied in real-space images.

Keywords:

Single-molecule imaging, iDPC-STEM, zeolites

Reference:

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Exploring novel joining technique of Gas Actuated Bonding by utilizing In-situ Environmental-TEM

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background
The reliability of a critical industrial component is decided by the durability of the weakest joint or spot. For applications involving high-temperature operational conditions especially coupled with corrosive environments like fuel cell interconnections, molten metal baths, etc., the joints are the most vulnerable regions for catastrophic failure due to localized material degradation by selective corrosion leading to compromised mechanical behavior. The existing joining techniques lead to a continuous metallic phase with modified microstructural characteristics at the bridging junctures or employ a joining alloy that integrates two parent materials. These bridging phases are proving to be the weakest points of the components because they are prone to corrosion compared to parent materials. This is effectuated by the microstructural transformation as well as local compositional fluctuation incorporated in the bridging phases as a consequence of the joining techniques and procedure currently in use.
On the other hand, the semiconductor industry utilizes emerging brazing technologies built on well-established Chemical Vapor Transfer (CVT) technologies (e.g., CVD, MOVPE, PVD). Even though both oxide removal and metal migration into the gap are crucial in metal bonding, there is only one published concept for delivery of non-metallic MPD [1], and a few studies of utilizing active gases for oxide removal.

Methods
The methodology of GAB requires the joints to be made from the bulk material itself by utilizing both gaseous oxide removers (OXR) and gas phase melting point depressants (MPD). In transient liquid phase (TLP) diffusion bonding, a method that lies between brazing and diffusion bonding, diffusion is sped up by a metal MPD interlayer. The preparation of superalloys or the brazing of Al-alloys has been conducted by the oxide removing technology which is contemporarily in practice. This process resulted in the best possible corrosion resistance and strength because both the joint and the bulk have obtained a similar surface oxide and microstructure. Furthermore, by introducing melting point depressing gases on metal surfaces, GAB induces a transient liquid phase similar to chemical vapor transport (CVT) technology. The two critical rate-determining steps in GAB are the removal of the pre-existing surface oxides of the nanoparticles and metal migration into the gap corresponding to the effect of temperature of exposure and partial pressure of the MPD precursor gas.

Results
GAB of pure Cu has been studied in-situ Environmental Transmission Electron Microscope (E-TEM) from a nanometer-sized AgCu particle. Temperature was held constant at 520°C, where the particle is identified as solid. After gaseous AsH3 is allowed to flow into the chamber, the particle is observed to alloy with As and is then identified as liquid (without any temperature change). Similar trials are attempted on 316L steel nanoparticles in experiments conducted utilizing a similar setup of in-situ E-TEM wherein GAB is obtained by melting point reduction using phosphine (AsH3). Therefore, we have built upon the well-established Chemical Vapor Transport (CVT) technology to induce a

transient liquid phase that could form joints with greatly enhanced mechanical and anti-corrosion properties. This leads to a novel metal joining technology called Gas Actuated Bonding (GAB) wherein melting point depressing (MPD) elements are transported as MPD-precursor gas to the joining zones. This takes place during processing to initiate surface reactions that create a liquid transition phase. This will lead to a profound improvement in the quality and consistency of the bridging phase in terms of microstructural consistency, thermal stability, along with enhanced mechanical properties.

Conclusion
Miniature components can be effectively assembled utilizing GAB by circumventing the need for obstructive fillers through the use of MPD-precursor gases that particularly actuate the outermost surfaces. Thus, heat-affected zones and deformation are subsequently prevented. This further validates GAB as a highly suitable manufacturing technology for such small and complex components. Hence, we can observe a drastic improvement in performance as the joints and bulk will have homogeneous surface oxides and homogeneous microstructure. The GAB mechanism is thus thoroughly investigated and understood by in-situ E-TEM experiments at various partial pressures of the MPD gases and the temperature of in-situ heating.

Graphic description
Preliminary results from GAB of AgCu. a) Schematic illustration. b) Elemental mapping in E-TEM. A nanometer-sized particle of AgCu is observed to be solid at 520°C in a vacuum. When AsH3 is flowed into the chamber the particle alloys with As and melts. c) Isothermal Section of the Ag-As-Cu Ternary Phase Diagram at 500°C. The red line indicates the compositional change of the particle after introducing AsH3.

Keywords:

Insitu Environmental-TEM, Gas-actuated bonding, Deoxidation

Reference:

Lenrick, Filip, Martin Ek, Knut Deppert, Lars Samuelson, and L. Reine Wallenberg. "Straight and kinked InAs nanowire growth observed in situ by transmission electron microscopy." Nano Research 7, no. 8 (2014): 1188-1194

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3D Investigation of Lath Martensite in a Low-Carbon Stainless Steel Using PFIB-EBSD Tomography

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Poster Group 2

Background
Lath martensite in steel is well known to be tremendously complex due to a hierarchical microstructure consisting of packets, blocks, sub-blocks, and laths [1]. The complexities arising from this spatially varying and multi-scale microstructure, have effectively hindered the analysis associated with lath martensite. 3D characterization techniques have demonstrated great potential to provide important insights into the 3D morphology of complicated microstructures [2]. In this regard, serial section tomography with Xe+ plasma focused ion beam (PFIB) offers the ability to collect data from sufficiently large volumes to be statistically representative of the bulk material while maintaining sufficient spatial resolution to observe all relevant features of a complex microstructure [3]. Accordingly, this study aimed to employ electron backscatter diffraction (EBSD) technique in conjunction with high-precision and large field-of-view PFIB serial sectioning tomography in order to enable a full identification of the microstructural elements in a lath martensitic structure.
Methods
The material used in this study was an as-quenched low-carbon 13Cr-4Ni martensitic stainless steel. Block-lift-out and U-shaped trench techniques were used to prepare large cross-sectional volumes using TFS Helios G5 PFIB and ZEISS Crossbeam 350 laserFIB, respectively (Figure 1). An automated routine of serial slicing and subsequent EBSD data acquisition were then performed using the Auto-Slice-and-View 4.0 software. Reconstruction steps including the importation of the raw data, thresholding, alignment, clean-up, and segmentation were all carried out in DREAM.3D software. Following reconstruction and segmentation, the final 3D dataset was visualised using ParaView software.
Results
Figure 2(a and b), display the reconstructed 3D volume of the martensitic microstructure in the form of Euler angle and band contrast maps. Martensitic blocks were segmented manually (i.e., using the specific FeatureIDs assigned by DREAM.3D), and then reconstructed in 3D as shown in Figure 2(c-j). Crystallographic analyses revealed that each prior austenite grain (PAG) can be decomposed into clusters of martensitic blocks, namely packets, such that blocks with specific variants can adopt unique morphological arrangements in 3D. Figure 2(c-f) showcase examples of these morphological arrangements including: (i) parallel arrangement of three distinct variants sharing 60°@[011] orientation relationship, (ii) T-shape arrangement of two distinct variants sharing 10.5°@[011] orientation relationship, and (iii) Triangular arrangement of three distinct blocks sharing 10.5°@[011], 49.5°@[011], and 60°@[011] orientation relationships.
The presence of numerous blocks/platelets with various crystallographic and spatial orientations, leads to many opportunities for the martensitic features to meet at different locations and thereby forming complicated networks in 3D. Here we used the acquired morphological information to

confirm the physical contact between the martensitic blocks within the volume of the PAGs (Figure 2(g and h)). This was further combined with the crystallographic information (e.g. crystallographic misorientations between the blocks in Figure 2 (d)), to reconstruct the boundary networks in 3D. As an example, Figure 2(i and j) illustrate 3D perspectives of the boundaries corresponding to 2 different packets and their blocks delineated by a black rectangle in Figure 2(g). 3D observations suggested a significant morphological difference between the packet boundaries (strip-like morphology) and block boundaries (flat surface morphology) within the lath structure of the martensitic stainless steels (Figure 2(i and j)).

Conclusions

Martensitic features including blocks, packets, and their boundaries were analyzed in 3D. Three distinct configurations, including (i) parallel arrangement, (ii) T-shape arrangement, and (iii) Triangular arrangement, were identified for the martensitic blocks depending on their crystallographic variants. Two different morphologies, (1) strip-like morphology, and (2) flat-surface morphology, were also observed for the packet boundaries and block boundaries, respectively.

Keywords:

3D-EBSD), PFIB tomography, Lath martensite

Reference:

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Determining alloy concentration by analyzing dynamic diffraction at strained semiconductor interfaces

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Poster Group 2

Background incl. aims
In the realm of semiconductor devices, particularly in the context of quantum well lasers, the optical properties are shaped by bandgaps and band offsets derived from precisely engineered alloy concentrations in ternary semiconductors, exemplified by InGaAs quantum wells in GaAs. While S/TEM possesses the necessary spatial resolution for resolving sharp interfaces, EDX typically lacks precision in determining the alloy concentration. However, during epitaxial growth on a substrate, lattice mismatch induces stress at the interface, resulting in localized lattice deformations known as strain. When preparing a thin transmission electron microscopy (TEM) lamella of a strained interface, the stress supporting this strain lacks support on the top and bottom of the lamella, leading to stress relaxation. For a compressively strained layer, this corresponds to the lamella bulging outwards at the interface. The magnitude of this relaxation is unique to the strain at the interface cause by the lattice mismatch, and hence by the alloy concentration in the layer.
The aim of this study is to deduce the alloy concentration of compressively strained InGaAs quantum wells in a GaAs matrix through a detailed analysis of stress relaxation at the lamella's surfaces. This is accomplished by evaluating patterns inside the diffraction discs using scanning convergent electron beam diffraction (SCBED). These patterns, manifestations of multiple electron scattering or dynamic diffraction, exhibit high sensitivity to small changes in displacement in electron beam direction and consequently, to stress relaxation at the lamella’s surfaces. Alloy concentrations are determined by matching beam simulations of relaxed lamellas with varying parameters to the experimental data.
Methods
We prepare a TEM-lamella containing InGaAs layers with known In-concentration in GaAs close to the [100] zone axis using FIB. Deriving information involves detailed reproduction of measured features through simulations to infer the original lamella state. For computational efficiency, we tilt specimen along a quantum well layer into [002] systematic row conditions. Full 2D diffraction patterns (256x256 pixels) are recorded at each scan position within a 256x256 scan window. The resulting 4D dataset can be reduced to a 2D-representation, named as x-q plot, by considering the problem’s symmetry. This reduction involves focusing on one spatial coordinate x orthogonal to the layer. Additionally, only one reciprocal coordinate q along the systematic row, into which the specimen was tilted, is considered. The convergence angle is chosen to achieve non-overlapping discs.
To model the experimental data in simulations, we employ a two-step forward calculation process: Initially, we model the initial stress and the resulting relaxation by doing finite element calculations, providing the layer width, elastic constants and lattice mismatch as inputs. The resulting displacement is then incorporated into beam simulations, numerically propagating the Darwin-Howie-Wheelan (DHW) equation. The simulations are fine-tuned to align with experimental conditions, accounting for tilt, specimen bending, and lamella thickness.
Results
Leaveraging the nominal alloy concentration of a 5 nm wide In0.23Ga0.77As quantum well within GaAs, we successfully replicate dynamic diffraction features in simulations, yielding three key

outcomes, as illustrated in Fig. 1. Firstly, our measurement reveals distinct features discernible in the x-q plot, unveiling changes in dynamic diffraction, even at considerable distances of multiple quantum well widths from the interface (Fig. 1 a). Secondly, comparing these findings with DHW beam simulations that omit surface relaxation highlights the absence of these long-ranging features, conclusively attributing them to surface relaxation (Fig. 1 b, c). Lastly, by manipulating the initial lamella alloy concentration, we can modulate the influence of surface relaxation. Minimizing the discrepancy between the measurement and simulations for the Indium concentration allows us to faithfully reproduce the nominal Indium concentration. These results are validated using STEM-EDX and dark-field imaging of a chemically sensitive reflection. We further discuss the method's limitations concerning specimen thickness, quantum well width, and alloy concentration.

Conclusion

Our study successfully identifies the nominal alloy concentration of a compressively strained InGaAs quantum wells in GaAs through a detailed analysis of stress relaxation at the TEM-lamellas surfaces. This accomplishment involves precisely replicating dynamic diffraction features within diffraction discs through simulations. These simulations account for surface relaxation by incorporating the displacement from finite-element calculations of the strained interface for a given specimen thickness. The process of minimizing discrepancies between measurement and simulation by adjusting the alloy concentration opens a novel avenue of discerning semiconductor alloy concentrations at strained interfaces by analyzing the impact of surface relaxation on dynamic diffraction.

Keywords:

Strain, Relaxation, composition-determination, dynamic-diffraction, 4D-STEM

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Electronic properties of W' twin domain walls in ferroelastic BiVO4

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Poster Group 2

Background incl. aims
Topological defects in ferroic materials are important in solid state physics since they can exhibit different intrinsic properties from the bulk and act as active sites in device engineering.[1] Investigation of those defects in terms of their structural variations and relevant properties in special materials systems opens a new path for improving materials' performance and developing novel devices. Here, we aim to investigate the structural and electronic properties of the ferroelastic domain wall in a well-known visible-light catalytic material,[2] monoclinic scheelite $BiVO_4$ (ms-BVO), which may be interesting for photochemistry-relevant applications.

Methods
The BF-TEM image and SAED were obtained using a JEOL JEM-F200 S/TEM operated at 200 kV for domain wall determination. HAADF-STEM images and electron energy loss spectroscopy (EELS) were obtained using a JEOL Grand ARM300F2 double-corrected S/TEM operated at 300 kV, for atomic resolution crystallographic structure and electronic structure demonstration. A Digital Instruments Nanoscope-IV Multimode atomic force microscope equipped with a c-AFM application module (TUNA) was used for nanoscale conductivity measurement.

Results
Highly dense twin walls are found in ms-BVO single crystal and identified as ferroelastic W' wall forming along the strain direction.[3] Opposite shear strain is seen in ferroelastic twin domains (A and B) and released at the wall. The W' wall has a kink configuration, and the overall wall width (d) is approximately ~2 unit cells. The W' walls show clear electrical conductivity by c-AFM under a DC tip voltage of −9.8 V while the domains show no obvious current signal. Subsequent STEM-EELS analysis shows that the V eg state of the W' wall shifts downward of 0.12 eV, indicating a lower conduction band minimum (CBM) compared to twin domains. The larger energy splitting at V L_3 and L_2 peaks at the W' wall is consistent with the symmetry of the topological defects. The higher V L_3/L_2 peak ratio indicates a lower oxidation state of vanadium, i.e., a higher density of oxygen vacancies at the W' wall. The higher shoulder peak at ca. 517 eV (compared to V eg state) indicates the possible accumulation of small polarons at the W' wall. The lower conduction band minimum, increased amount of oxygen vacancies and small polarons may be the reasons for the ferroelastic W' wall conductivity.

Conclusions

The present work investigates the structures and electronic properties of the ferroelastic W’ wall in ms-BVO bulk crystals. The W’ wall is atomically sharp in a zig-zag shape. Compared to non-conductive domains, the W' walls show conductivity in c-AFM measurement under negative bias. The electronic structures revealed by EELS spectra show that the wall has a relatively lowered conduction band minimum, a higher density of oxygen vacancies and possible accumulation of small polarons compared to the domains, which may contribute to the increased wall conductivity. Since the photocatalytic performance of a semiconductor is highly related to its conductivity,[4] the investigation of W' wall conductivity of ms-BVO crystals may help in designing highly efficient catalytic materials.

Keywords:

ferroelastic domain walls, electronic properties

Reference:

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Electron Videography and Machine Learning of Soft Matter

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background
New methods of materials synthesis and processing with various accelerated discovery strategies have led to huge libraries of functional materials systems. Examples include morphology-controlled nanoparticles as catalysts, nanoparticle superstructures as metamaterials, polymer films with multiscale porosity as separation membranes, to composite energy storage systems. These materials are spatiotemporally heterogeneous in composition, structure, and function, which call for three aspects of advancements in materials characterization. The first is the need for real-space, non-ensemble averaged characterization methods which map the heterogeneity, at least in space, and if possible, over time to capture changes during the synthesis, application, and functioning of materials. The ever-improving toolbox of electron microscopy can meet this requirement by providing atomic to nanometer resolution mapping of the composition, structure, and phase of nanomaterials. Yet, depending on the specific materials systems, different factors still need to be considered in the imaging protocols, such as beam sensitivity, substrate effect, and in-situ stimulation. The second is the need for descriptors to describe heterogeneity. For example, low-dimensional descriptors of symmetry and lattice constants for crystalline solids do not apply for random, or mixed phase systems. Even when it comes to merely the shape (not composition or microstructure), in three dimensions (3D), irregular shapes can be hard to quantify. The third is the need for automated data analysis and reduction methods as high volumes of multi-modal experimental and simulation data are required to describe the heterogeneity and infer the structure–property relationship. Specific to electron microscopy studies, with the cutting-edge detectors, the data collected in one single in-situ experimental session can be 100,000 frames which are extremely laborious to be analyzed manually, let alone the introduction of human bias in the manual analysis process. Mapping properties such as composition and modulus at a similar resolution will only further increase the volume and dimension of the datasets.

Here we will discuss our group's efforts on developing different machine learning strategies to accommodate the data analysis requirements for two different electron microscopy methods, liquid-phase transmission electron microscopy (TEM) and electron tomography, both involving taking series of images and thus referred to collectively as "electron videography". Several materials applications will be discussed, including nanoparticle reaction, self-assembly, and polymer membranes.

Methods
We first introduced the method of U-Net based segmentation for liquid-phase TEM videos1. For liquid-phase TEM videos, they are usually of low signal-to-noise ratio, and thus make manual annotations difficult. To address this challenge, we devised a workflow to generate simulated liquid-phase TEM videos, where the noise levels were captured as dependent on the dose rates to maximally mimic the practical experimental complications. These simulated liquid-phase TEM videos were used as training datasets for the U-Net, with well-defined ground truth, and thus can enable high fidelity and high throughout training of the U-Net, to be used for practical experimental liquid-phase TEM videos.

As to quantification of complex morphologies, we defined a mathematical fingerprint function2 that considers all the coordinates in the shape contours (two-dimensional or 3D) but stays as a one-dimensional function, which can be used as input for principal component analysis (PCA) and then for machine-learning based Gaussian mixture model for grouping of different morphologies and yield analysis. This method works both for TEM images of nanoparticles and electron tomographs of polymer membranes.

Lastly, to facilitate high throughput electron tomography of complex materials3, we developed an unsupervised sinogram inpainting for nanoparticle electron tomography (UsiNet) to correct the missing wedge effects. UsiNet is the first sinogram inpainting method that can be realistically used for experimental electron tomography by circumventing the need for ground truth.

Results
For the U-Net based segmentation of nanoparticles in liquid-phase TEM videos, we were able to achieve high throughput analysis of the nanoparticle reaction kinetics, self-assembly dynamics, and internanoparticle potentials through the statistics accumulated from the liquid-phase TEM videos. For the application of fingerprint functions, they were useful to differentiate the desired tetrahedral nanoparticles from impurities, enabling yield analysis and the investigation of reaction mechanism. They were also capable of differentiating nanoparticles of different patterned polymer coatings, as well as membranes of four different shape categories, which aid the understanding of synthesis-morphology-property relationship. As to the UsiNet method, we apply it to experimental tomographs, where >100 decahedral nanoparticles and vastly different byproduct nanoparticles are simultaneously reconstructed without missing wedge distortion. The reconstructed nanoparticles are sorted based on their 3D shapes to understand the growth mechanism.

Conclusion
We show collectively the usage of machine learning to liquid-phase TEM and electron tomography for understanding the dynamics and synthesis-morphology relationships of complex materials. The experimental and analysis workflow can extend to other in-situ studies of time-series and tomography studies of complex materials, as well as the potential integration of liquid-phase TEM with electron tomography to study 3D morphological evolution of materials important for applications in self-assembly, nanoparticle synthesis, polymer morphing, and energy storage and conversion.

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3. L. Yao, Z. Lyu, J. Li, Q. Chen. npj Computational Materials 10, 28 (2024).

Keywords:

Machine learning, electron videography

Reference:

1. L. Yao, Z. Ou, B. Luo, C. Xu, Q. Chen. ACS Central Science 6, 1421 (2020).
2. L. Yao, H. An, S. Zhou, A. Kim, E. Luijten, Q. Chen. Nanoscale 14, 16479 (2022).

3. L. Yao, Z. Lyu, J. Li, Q. Chen. npj Computational Materials 10, 28 (2024).

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High-resolution STEM Image Acquisition Method for Tilted Specimen Using a New Type of Aberration Corrector

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

When acquiring scanning transmission electron microscope (STEM) images, it is necessary to align the crystalline zone axis of a specimen tilt with a goniometer and a holder. Since the backlash and drift cannot be avoided in such operations, it is necessary to wait for the drift to settle after aligning crystalline zone axis for a high-quality image acquisition. Therefore, it is desirable to operate a goniometer or a holder mechanically as less as possible. Meanwhile, advances in aberration correction technology have been remarkable and three-hexapole type STEM corrector, Large Aperture STEM Corrector (LASCOR), was recently developed by CEOS GmbH [1, 2]. It is capable of correcting aberrations up to sixth-order three-lobe aberration which had been a limiting factor in the ASCOR corrector. With the LASCOR, the Ronchigram flat area has now been extended to 80 mrad in semi-angle at an acceleration voltage of 200 kV. Thanks to this large flat area, one can align crystalline zone axis using only optical elements (beam tilt and projector shift) of a microscope without any mechanical operation. In this study, we demonstrate a high-resolution STEM image acquisition using this technique.

The procedure is described here how to acquire STEM images of a specimen with a misaligned crystalline zone axis by the proposed method. Firstly, a condenser lens aperture is inserted at the center of the optical axis, and the electron beam is tilted by the beam tilt coils in Fig. 1. This beam tilt appears as the shift of a condenser lens aperture and it can be aligned to the crystal zone axis. The beam is detilted to the center of the detector plane by the projector alignment coils. In this setup, high-resolution STEM images are acquired.

Fig. 2(a) shows an experimentally recorded Ronchigram image with dashed circles indicating the amount of beam tilts used. As the flat area is as large as approximately 80 mrad in semi-angle, the electron beam can be tilted with little influence of geometrical aberrations. Fig. 2(b) ~ (e) show high-resolution STEM images of Si[110] single crystal obtained under different beam tilt conditions corresponding to the dashed circles in Fig. 2(a). It is demonstrated that even for a specimen tilt of over 60 mrad, atomic resolution imaging is achieved by aligning the zone axis using only the optical elements. However, under the tilted conditions, STEM images were blurred in the direction of the tilts, which can also be confirmed in each FFT image. It is assumed that the blurring is due to the effects of the combination of chromatic aberration and beam tilt. Further improvement of image quality can be expected by the use of a monochrometer or a chromatic aberration corrector. Although a specimen tilt causes defocus at the edges of an image and image shrinkage in the direction of the tilt, they can be corrected by changing the defocus during the scan and by a simple image processing, respectively. The correction parameters can be directly calculated by the azimuthal and tilt angles of the beam. The technique introduced here will facilitate an automated acquisition of STEM images and is expected to have applications in the field of semiconductor where it is particularly important to acquire a large number of STEM images quickly.

Keywords:

STEM, Cs-corrector, Automation, Semiconductor, Zone-axis

Reference:

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Simultaneous Acquisition of 4D and EELS Data by Newly Developed Pixelated STEM Detector

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

In scanning transmission electron microscopy (STEM), efforts to obtain specimen information as much as possible have long been made and variety types of detectors have been developed. Pixelated STEM detector is one of these instruments for utilizing diffraction information that was otherwise partly lost in the conventional single-channel STEM detectors. Various types of applications have been introduced using the 4-dimensional (4D) dataset which include synthetic STEM image reconstruction, field map visualization and phase image reconstruction by ptychography, etc. [1,2]. Meanwhile, electron energy loss spectroscopy (EELS) is also an important and popular means of obtaining information on the composition and even on the chemical bonding inside a sample. Considering the type of necessary electron signal for both 4D-STEM and EELS, they should go well with each other in many cases: the former needs a part of transmitted electron beam and scattered or diffracted beams and the latter needs a part of transmitted beam which is complementary to the former [3]. Although simultaneous analysis using both instruments has been anticipated, making a center hole in the middle of a pixelated detector to let electron beam pass through into an EELS spectrometer was a manufacturing challenge. PNDetector is a company manufacturing high-speed pnCCD camera which can be operated at 7,500 frames per second for the 4D-STEM application. It recently manufactured a prototype pnCCD sensor with a center hole while keeping other specs the same, realizing the simultaneous acquisition of 4D and EELS data [4].

The prototype pnCCD camera was installed on a JEM-F200 (JEOL Ltd.) electron microscope with a cold field emission gun (Fig. 1). Below the camera, CEFID (CEOS GmbH) EELS spectrometer was mounted. Scan was triggered by an ELA (DECTRIS AG) camera mounted on the CEFID as a post-filter detector. Then the microscope scanning system and the pnCCD camera readout were synchronized to it. In EELS application, scanning dwell time of more than several tens of milliseconds is usually required for a sufficiently high SN ratio of an EELS mapping data. In order to synchronize this somewhat slow scanning speed with the high-speed pnCCD camera, a multiple readout signals of the pnCCD camera was accumulated at each scanning position.

Fig. 2 shows the simultaneously obtained 4D-STEM and EELS data from a semiconductor sample. Fig. 2(a) shows a diffraction pattern image obtained at a certain position on the sample. Since the sensor has a hole, there is a circular area at the center of the image where no electron signal was generated. Camera length of the microscope was set so as to let the transmitted electron beam pass through the hole while keeping the diffracted signals inside the field of view of the camera. Fig. 2(b)~(d) are synthesized STEM images from each diffracted disk in Fig. 2(a). Because all the diffracted disks were recorded at every scanning position, different image contrasts were obtained from the same field of view, thus, the 4D-STEM data acquisition was demonstrated. Fig. 2(e)~(h) show simultaneously obtained EELS elemental mapping images of oxygen, nickel, silicon and titanium, respectively. Each image has a sufficiently high SN ratio and one can clearly tell the locations of each element inside the sample.

Keywords:

STEM, 4D, pixelated detector, EELS

Reference:

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Development of planar micro optics for ultrafast in-situ measurements in the TEM

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Poster Group 1

Background incl. aims
Efforts in the miniaturization of magnetic electron optics exist for at least 10 years now, because smaller electron optics not only require less material and are potentially easier to build, but also result in other favorable scaling effects. Firstly, the small pole distances allow for magnetic flux densities in the hundreds of millitesla with a significantly reduced energy consumption and lower overall complexity regarding vacuum and cooling. Secondly, the small size translates to a low inductivity, which in turn enables switching of the optics on short time scales/with high frequencies (HF). The aim of this work is to utilize those favorable scaling effects by building miniaturized, magnetic, planar micro optics that are capable of manipulating a fast electron beam like in TEMs on sub nanosecond timescales/with gigahertz frequencies, allowing for stroboscopic, time resolved measurements in that regime.
Methods
The micro optics are produced using lithographic structuring techniques. The first layer consists of the conducting copper paths with a thickness of several hundred nanometers. The second layer consists of sputtered permalloy with a thickness of 1 µm. Through this process, many different optics e.g. dipole deflectors, quadrupoles, and higher order multipoles with various pole geometries can be produced in an easily scalable way. In order to supply the optics, a custom TEM holder capable of transmitting HF signals was engineered. The holder carries a small circuit board on which the optics are mounted and that allows for impedance matching in close proximity. To characterize the electromagnetic fields, differential phase contrast (DPC) measurements were conducted. The HF capabilities of the dipole optics were characterized in diffraction mode by measuring the amplitude of the oscillation of the diffraction disc.
Results
The optics show deflections in the single to double digit µrad range at 300 kV acceleration voltage and no major drop off in deflection up to an AC excitation frequency of 2 GHz. This corresponds to a maximum magnetic flux density of 84 mT and a possible focal length of a quadrupole of 15 cm at an acceleration voltage of 80 kV.
Conclusion
Miniaturized planar electron optics can be produced such that their diffraction capabilities especially in the HF domain are powerful enough to make them interesting for novel applications like stroboscopic, time resolved measurements on sub nanosecond time scales, imaging phenomena like the motion of domain walls with time resolved TEM. Furthermore, the miniaturization of entire CPO instruments e.g. SEMs might become possible in the future.

Keywords:

TEM, Ultrafast Microscopy, MEMS

Reference:

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Unveiling Metal-Insulator Transitions in (V1-xCrx)2O3 through in situ Monochromatized STEM/EELS

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background incl. aims
The exploration of insulator-to-metal transitions (IMTs) in Mott insulators and strongly correlated systems has revealed intriguing phenomena induced by various stimuli such as temperature, pressure, doping, or electric fields [1,2]. Despite significant advancements, the mechanisms governing electronic phase separation at the nanoscale remain elusive. Addressing this knowledge gap requires a comprehensive approach, considering structural and electronic degrees of freedom. In this context, our study aims to investigate the temperature-driven IMT in (V0.988Cr0.012)2O3 (see Figure a), employing advanced techniques capable of probing multiple features simultaneously. The Cr-doping enables us to explore transitions from the paramagnetic insulator (PI) phase to the paramagnetic metallic (PM) phase around 200 K and also from the PM phase to the antiferromagnetic insulator (AFI) phase below 180 K. Interestingly, the PI/PM transition is isostructural of the hexagonal structure (space group R-3c) while the PM/AFI transition is associated with a crystallographic symmetry breaking into a monoclinic structure (space group I2/a). There is, therefore, a strong interest in investigating both spectroscopically and structurally this material using the same in situ tool.
Methods
To achieve our objectives, we employ in situ monochromatized EELS [2] and 4DSTEM micro/nano-diffraction [3] techniques. These experiments are conducted using a state-of-the-art NION Ultra-HERMES 200 microscope, equipped with a Medipix direct detector and a HennyZ double tilt cryo-holder. This cutting-edge instrumentation allows us to unravel intricate details associated with the IMT process.
Results
Specifically, we focus on identifying variations in low-loss spectra, highlighting fingerprints of the PI, PM, and AFI phases (Figure b). By comparing structural information acquired by 4DSTEM with such spectroscopic signatures, we probe a phase coexistence at low temperatures, as depicted in Figure c [4].
Conclusion
Overall, our results highlight the instrumental capabilities of high-energy resolution EELS as a valuable tool for unraveling the intricate dynamics of IMTs, offering new perspectives on the underlying physics of these fascinating phenomena.

Keywords:

Mott-Insulators, IMT, In-Situ, EELS, 4DSTEM

Reference:

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Depth Dependence of Electron Channeling Contrast Imaging in Gallium Nitride

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Poster Group 2

Background incl. aims
Interest for Gallium Nitride (GaN) and wide band-gap semiconductors has been growing the last few decades as a green-energy solution for electronic power devices and digital transition. Their enhanced electrical properties such as reduction of power losses or lower energy consumption tends to make them more suitable for power electronics applications over silicon. However, it is unclear whether leakage currents or device failures are related to crystalline defects, such as Threading Dislocations (TD), for which defect densities in GaN are orders of magnitude higher than in silicon. Detailed characterization of the defect nature and estimation of their density is, therefore, relevant to understand and improve GaN power devices. In this context, Electron Channeling Contrast Imaging (ECCI) can provide valuable insights. ECCI is a non-destructive method that offers the capability to provide Transmission Electron Microscopy (TEM)-like diffraction contrast imaging inside a Scanning Electron Microscope (SEM) on bulk samples. ECC is generated from electrons that channel down the crystal planes. Strain and defects cause distortion of the crystal lattice, resulting in changes in Backscattered Electron (BSE) intensity, thereby producing contrast in the image.
Diffraction contrast in bulk material is widely assumed to be generated from the near-surface layers (from tens to hundreds of nanometers) by electron channeling but there is currently no consensus about the depth from which the collected information originates. Therefore, it is critical to understand the depth dependence of the method to ensure measurements are made in the layer of interest.
The present study provides an experimental measurement of the signal from which the information in ECCI arises. This estimation aims to sharpen the statistical dislocation density estimation through series of measurements at different accelerating voltage and to establish a model for information emission while imaging GaN by ECCI.

Methods
The layer of interest, the GaN layer, lies between an AlxGa1-xN barrier layer and a series of strain-relief layers called the superlattice, for which the composition differs from the first one. As such, misfit dislocations, due to lattice mismatch, stand at the interface between the GaN and the superlattice. A Focused Ion Beam (FIB) was used to create wedges of different angles across these layers to vary the GaN layer thickness. The incident electron beam energy of the SEM was varied and the final depth the misfit dislocations were visible was recorded. Comparisons between multi-beam and 2-beam diffraction conditions was also performed.

Results
First results show ECCI probing depth can be tuned as a function of incident electron beam energy and that there are discrepancies between multi-beam and 2-beam diffraction conditions as well as the theorized channeling extinction distance and the experimentally measured probing range.

Conclusion
The key expected outcomes are an increase in estimated dislocation density accuracy in GaN and a better understanding of ECCI micrographs. The dataset is expected useful for complementary dislocation related fields of study such as layer growth or electrical characterization.

Acknowledgments
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The authors would also like to acknowledge Dr. Aidan Taylor for assistance and expertise during sample preparation.

Keywords:

ECCI, GaN, SEM, Crystallography

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Multidimensional Characterisation and Multivariate Analysis of Cu3P Nanocages for Catalysis

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Poster Group 1

"Multidimensional Characterisation and Multivariate Analysis of Cu3P Nanocages for Catalysis"
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Background:
The use of nanomaterials in catalysis has consistently sparked significant scientific interest, primarily due to their high surface-to-volume ratio and the ability to finely control active sites. This study focuses on exploring the characteristics of Cu3P engineered in the shape of nanocages, with promise as a catalyst for CO2 reduction[1]. To achieve a comprehensive understanding of the system, we combined the 3D morphology obtained via electron tomography with compositional data from energy-dispersive X-ray spectroscopy (EDX), and local chemical states estimates from electron energy loss spectroscopy (EELS). The resulting understanding of the Cu3P nanocages will lead to optimal design of the structures and improvement of the catalytic performance.
Methods:
In our investigation of Cu3P nanocages, we utilised a probe-corrected ThermoFisher Spectra 300 transmission electron microscope (TEM) to define their morphological, structural, and elemental characteristics at high spatial resolution. We acquired several tilt series over a tilt range of at least 120° over isolated and assembled nanoparticles. STEM-EDX data were acquired using a Dual-X detector (collection angle ~1.7 sr), while EELS data were acquired in a Continuum GIF. Additionally, for a comprehensive examination of this nanosystem, we conducted an extensive compositional study by integrating machine learning algorithms[2] with hyperspectral EDX and EELS datasets.
Results:
Through the synergistic application of these different techniques, we gained a comprehensive understanding of structural and electronic properties of Cu3P nanocages, with an average size of 15 nm. Multivariate analysis played a crucial role in discerning compositional variations within these nanostructures. In particular, principal component analysis (PCA) and non-negative matrix factorisation (NMF) were employed on both compositional datasets (EDX and EELS core loss), allowing a deep understanding of the elemental distribution within the nanocages. This approach allowed us to uncover subtle differences in the elemental distribution of nanocages, shedding light on the intricate interplay between morphology, structure and elemental composition in these catalytic nanostructures. The analysis of the fine structure of EELS edges also enables the differentiation between oxidation states for copper through the structure. Such multidimensional analysis not only improves our understanding of Cu3P nanocages, but also shows the power of integrating microscopy techniques and advanced data analysis methods that enable comprehensive characterisation of nanomaterials. In addition, by combining electron tomography information with

compositional information, we were able to visualise the three-dimensional morphology of nanocages, providing fundamental information about the nature of these catalysts.

Conclusion:
This research contributes to the advancement of nanocatalysis and underscores the paramount importance of microscopic investigations in comprehending and refining catalytic processes. The combination of various techniques, such as EELS, EDX, and electron tomography within a TEM, presents a complete approach to studying nanocatalysts like Cu3P nanocages for CO2 reduction. These methodologies enable an in-depth examination of the structural characteristics, elemental composition, and three-dimensional morphology of catalysts. Concurrently, this study serves as a demonstration of the role played by machine learning algorithms in processing microscopy data. The synergy between machine learning and the advanced capabilities of next-generation microscopes introduces novel avenues for researchers to efficiently optimise and refine the interpretation of generated data, thereby unlocking the full potential of advanced microscopy technologies.
Keywords:

Machine Learning, Catalysis, Electron Tomography, hyperspectral imaging

Keywords:

Machine Learning, Electron Tomography, hyperspectral

Reference:

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Universal Laser Engine:

A microscope illumination that combines FLIM and homogenized powerful wide-field lasers

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Poster Group 1

The flatness of laser-based illumination and the available power have a major impact on image quality and can restrict the contrast and precision of many techniques, including the localisation precision of single-molecule localization microscopy (SMLM). In addition, different laser sets usually need to be provided for different microscope methods, leading to complicating the development of multimodal devices.

The Universal Laser Engine combines:
* a flat top-hat illumination, even within the µs-range
* a set of high-speed switching lasers
* picosecond pulsed lasers for fluorescence lifetime applications.

We have proven the applicability of the Universal Laser Engine for wide-field, confocal and TIRF microscopy. It allows to combine multiple methods for simultaneous measurements, such as alternating multi-color excitation schemes, super-resolution and lifetime approaches. Based upon the Universal Laser Engine and zero-mode-waveguides, we parallelised single-molecule and detection and implemented a 12-spot parallel FCS detection. Together with the LINCam, we achieved simultaneous STORM & wide-field FLIM recordings.

Keywords:

Illumination FLIM even-field parallel-FCS

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Quantitative chemical analysis by STEM-EDS and machine learning: Are AgAu alloyed at the nanoscale?

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Background incl. aims:

Bimetallic nanoparticles (BNPs) have attracted increasing attention in fundamental and applied sciences in recent decades. They are expected to play a central role in the energetic and ecological transition of our societies. Combining two or more metals on the nanoscale opens up a whole playground for the investigation and the fine-tuning of physicochemical properties and applications in nanoparticle-based devices. The BNP properties depend on size, shape, environment, chemical composition and spatial elemental distribution. Different chemical configurations (alloyed vs. segregated, chemically ordered, etc.) can change considerably the optical, magnetic, or catalytic properties via the electronic structure [1]. One of the most intensely studied bimetallic systems is the gold-silver alloy. Its nanoparticles have attracted much attention for their catalytic, plasmonic surface-enhanced sensing and photocatalytic properties. In the bulk, the two metals are completely miscible across the entire composition range, and their atomic radii, crystal structure, valence, and electronegativity are very similar. On the nanoscale, however, additional degrees of freedom have to be considered, namely the particle surface, as well as possible segregation and chemical ordering into structures not possible in bulk. Despite the high number of experimental and theoretical articles published on AgAu nanoparticles, the controversy about whether intrinsically one of the two metals preferentially segregates at the surface or not is still unresolved, both on the experimental as on the theoretical side [2,3]. In this context, we aim to contribute to this discussion by providing quantitative and statistically reliable chemical profiles of AgAu BNPs in the chemical ground state and thermodynamic equilibrium, using high signal-to-noise ratio and resolution hyperspectral images (HSI) obtained by Energy Dispersive X-ray Spectroscopy (EDS).

Methods:

The performance of EDS combined with Scanning Transmission Electron Microscope (STEM) has been significantly enhanced, permitting the analysis of chemical composition in nanometric objects, including bimetallic nanoparticles (BNPs) with diameters smaller than 10 nm. This breakthrough is crucial for the in-depth distinction between different chemical structures (random, gradient, or core-shell, etc.) of individual particles on a quantitative level. High-quality SNR are difficult to attain for such very small volumes, below 10 nm; besides, data acquisition and treatment methodologies, followed by rigorous error analysis, are essential. In this context, we have measured the chemical composition variations inside AgAu BNPs using EDS-STEM in a Titan-Themis Cubed and a Cold-FEG JEOL NeoARM operated at 80 kV with high solid angle X-ray detectors (≈1 sr). We have used unsupervised machine learning methods (Principal Component Analysis-PCA and Nonnegative Matrix

Factorization-NMF) to verify the existence of latent information on radial composition variations in our data. This analysis provides direct, solid statistical verification that the estimated radial composition changes represent accurate information provided by the EDS spectra [4,5]. In addition, PCA and NMF components are used for the reconstruction of the dataset for denoising and background removal, respectively.

Results:

To guarantee that the BNPs are analyzed in the chemical ground state, we protect them by a thin layer of amorphous carbon and anneal them at 300°C. Analyzing the HSIs using PCA and NMF, we are able to retrieve denoised information for quantitative analysis, but we can also evidence the Ag segregation of hundreds of BNPs by analyzing the components and abundance maps obtained by PCA/NMF, both showing compelling complementarity to EDS-HSI datasets. Using unsupervised machine learning to provide statistical validation for the information extraction, we further analyze quantitative profiles by azimuthal averaging (to attain better SNR) of isotropic BNPs in the HSI for both raw and NMF reconstructed data. Here, our results demonstrate the possibility of quantifying the chemical composition within bimetallic nanoparticles in 4-10 nm in diameter range. These findings represent a significant improvement over the widely used qualitative chemical mapping (see Fig 1.), providing essential insights into understanding the elemental distribution within small volumes. Our results reveal chemical gradients with Ag enrichment towards the surface of the BNPs.

Conclusion:

Finally, by combining well-defined model samples, robust and quantitative EDS-TEM analysis and machine learning for statistical validation we contribute to the comprehension of nanoalloy physics. We not only demonstrate the feasibility of the method but settle the ongoing controversy about nanoscale miscibility in the AgAu system. While Au and Ag are miscible in in the core of BNPs as small as 4 nm in diameter, with a core of 2 nm (<400 atoms), a slight enrichment of silver at the surface of the order of 10% is consistently evidenced. Our work furthermore paves the way for its extension to environmental and tomographic studies of multimetallic nanostructures.

Keywords:

EDS-STEM, Chemical Gradients, Bimetallic nanoparticles

Reference:

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Visualizing alloying in Au-Pd core-shell nanoparticles using in situ gas-phase transmission electron microscopy

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background
The catalytic and optical properties of bimetallic gold-palladium (Au-Pd) nanoparticles (NPs) critically depend on the atomic distribution of the Au and Pd atoms [1]. Under operating conditions, the atomic distribution is highly dynamic. Analyzing gas induced redistribution kinetics at operating temperatures is key in understanding the behavior of Au-Pd nanoparticles but requires advanced in situ characterization strategies. In situ transmission electron microscopy (TEM) allows direct visualization of changes in metal nanoparticles at elevated temperatures and/or upon exposure to reactive gases. So far, several TEM studies on alloying dynamics of core-shell nanoparticles under high vacuum have been reported using EDX (energy-dispersive X-ray) mapping, fast tomography, and atomic resolution imaging [2]. However, these methods are difficult to utilize in the presence of a gas atmosphere because 1) EDX is time consuming and requires a high electron dose rate, which can lead to undesired beam effects, 2) the fast tomography approach is not possible with the current in situ TEM holder technologies, which do not allow tilting at the needed range, and 3) high resolution studies are often limited to only one nanoparticle. Assessing the effect of a gas atmosphere on the alloying dynamics of NPs has therefore remained unexplored and requires new methodologies for extracting the alloying kinetics from in situ gas-phase TEM data.
In this work, we use in situ gas-phase TEM to directly observe the effect of a reducing or oxidizing gas atmosphere on the surface and bulk alloying kinetics of Au-Pd nanoparticles [3]. We developed a methodology to quantify the alloying dynamics of core-shell NPs from high angular annular dark field scanning transmission electron microscopy (HAADF-STEM) images acquired at atmospheric pressure without the need of EDX, fast tomography or atomic resolution imaging, imaging around 35 nanoparticles per gas atmosphere. Using this approach, we assess the NP bulk alloying dynamics from the in situ TEM data and show that the observed alloying dynamics can be predicted using a simple diffusion model based on Fick's second law.

Materials and Methods
The in situ gas-phase HAADF-STEM measurements were performed using a Protochips Atmosphere system and a Talos F200x microscope operated at 200 kV. Prior to beam exposure, the sample was heated to 250 °C in 5% H_2 in Ar for 45 minutes to ensure the removal of water and reduction of oxidized species. During the experiment, the gases (H_2 or O_2, 10% diluted in Ar) were introduced at 1 bar and 0.1 sccm flow rate. The sample was heated to 375 °C for a total amount of time of 1 h, being paused at 18 time points to perform imaging at room temperature. The impact of the temperature on the alloying kinetics was assessed by conducting an additional experiment at 350 °C. The temperature ramp was 10 °C/s and the beam was always blanked at high temperatures. The data were analyzed using custom made python scripts quantifying the intensity decrease of the Pd-shell related grey values over time, thereby indicating progress in the alloying. After the experiment, the Au-Pd NPs were analyzed using EDX mapping in vacuum.

Results and Discussion

The alloying kinetics of the Au-core Pd-shell NPs were monitored through in situ TEM in oxidizing and reducing gas atmosphere. HAADF-STEM images of around 20-25 core-shell Au-Pd NPs (23.8 ± 1.3 nm diameter, 36 ± 5 atomic % Pd) were taken in the course of 1 h exposure at 375 °C under a H_2 or O_2 atmosphere. At time = 0 min, the core-shell structure with the light grey Pd-shell and white Au-core is visible due to the different Z-contrast of Au and Pd, which is lost upon alloying (Figure 1a,b). By monitoring the intensity decrease of the grey values related to the Pd-shell (Figure 1c) and normalizing it over all NPs, we obtain an indication of the kinetics of the alloying process of the whole NP. To validate this approach, we performed an in situ heating experiment under vacuum to be able to follow the alloying process both by EDX and HAADF-STEM imaging. This confirmed that our HAADF-STEM based data analysis correctly quantified the alloying process.

We find that the temperature impacts the alloying kinetics more strongly than a change in gas atmosphere. We verified that the electron beam did not affect the alloying kinetics by imaging 10-15 NPs less frequently, and by performing EDX mapping of irradiated and non-irradiated areas after the experiment. The temperature dependence of the alloying process can be described by a simple model based on Fick's second law. The modeled diffusion behavior at 350 and 375 °C shows good agreement with datapoints derived from the in situ TEM data (Figure 1d). This validates our in situ TEM approach, and it also shows that the modeled diffusion behavior is accurate for nanoparticle systems.

Conclusions

In this work, we quantified the alloying dynamics of bimetallic nanoparticles with in situ gas-phase TEM for the first time. To do so, we followed the loss of the core-shell structure of ~35 Au-Pd core-shell nanoparticles using their difference in Z-contrast in the HAADF-STEM images. The alloying kinetics were highly impacted by a change of temperature and to a lower extend by the gas atmosphere. Quantitative insight such restructuring phenomena at elevated temperatures and in catalytically relevant gasses is critical in understanding changes in the behavior of nanoparticle catalysts under operating conditions.

Keywords:

in-situ-TEM, alloy, core-shell, Au-Pd, bimetallics

Reference:

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Cryogenic scanning electron microscopy of hereditary hemolytic anemias

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Poster Group 1

Background:
Hereditary hemolytic anemias are a heterogeneous group of genetic disorders, in which red blood cells (RBCs) are removed from the bloodstream faster than they are produced. The main hereditary conditions include RBC membrane disorders, RBC enzymatic defects, and hemoglobinopathies, such as sickle-cell disease (SCD) and β-thalassemia. Different disorders may overlap, resulting in a wide range of clinical symptoms. Imaging techniques, especially light microscopy (LM), are essential for diagnosis of hemolytic anemias. However, many essential processes such as the exact mechanism of RBC sickling are not fully understood yet. The indication of the unique structural properties of different types of cells and the evolution of their morphological changes upon specific conditions requires high-resolution methods.
Cryogenic scanning electron microscopy (cryo-SEM) combined with high-pressure freezing (HPF) specimen preparation can bring new useful information to hematological research. It allows examination of cell morphology and ultrastructure, interactions between blood components, and cell dynamics with nanometric resolution, close to their native state. Cryo-SEM is still rarely used for the evaluation of blood cells, and the aim of the work was to demonstrate the potential of this technique for hematological research.
Methods:
Whole blood samples from the patients with β-thalassemia major, homozygous and heterozygous cases of SCD, and with glutamate-cysteine ligase (GCL) deficiency and β-thalassemia trait coinheritance, were evaluated and compared with a healthy control, S trait person, GCL deficiency heterozygote, and a β-thalassemia carrier controls. The SCD blood samples were incubated with sodium metabisulfite to provide low-oxygen conditions and induce morphological changes. These samples were examined using LM, flow cytometry, and cryo-SEM at different stages of sickling. Cryo-SEM samples were prepared by high-pressure freezing (HPF). The uncoated cryo-specimens were imaged by a Zeiss Ultra Plus cryo-SEM, equipped with a field emission gun, at low beam acceleration voltage (1.2 kV). No cryoprotectants or stains were used.
Results:
Cryo-SEM reveals abnormal morphology in most RBCs from the patients. Blood specimen from a patient with β-thalassemia demonstrates evidence of cytoskeleton rupture with indicative membranal damage. Fig. B demonstrates the shell-like RBC morphology that was the most common in this patient. We followed the changes of erythrocyte morphology in the SCD patients due to the hypoxia condition, when focusing on different stages of the sickling process. Fig. C shows the typical sickled RBC characteristic of SCD patients after exposure to low-oxygen conditions. In the patient with GLC deficiency and β-Thalassemia Trait Coinheritance, we found a variety of RBC morphologies and signs of membranal damage including membranal blebbing and almost complete cell disintegration (Fig. D). These abnormalities are not seen in RBCs from carriers and healthy individuals (Fig. A).

Conclusions:
This work presents the first study of RBC morphology and ultrastructure in patients with hereditary hemolytic anemia using the novel cryo-SEM methodology. The micrographs reveal details of the erythrocytes that cannot be seen using conventional methods. We found membrane and morphological abnormalities, probably caused by oxidative damage, which is a key component of the hemolytic process in these diseases. We also proposed the mechanism for the RBC sickling process in SCD patients. This study highlights the potential of the cryo-SEM methodology for a deeper understanding of hematological disorders.

Keywords:

cryo-SEM
cryo-TEM
blood
hemolytic anemia

Reference:

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Physics-based synthetic data model for automated segmentation in catalysis microscopy

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Background:
In catalysis research, the amount of microscopy data acquired when imaging dynamic processes is typically too vast for non-automated analysis. Consistent image segmentation is a common first step to obtain data that can be correlated with time-dependent chemical observable. Developing machine learned segmentation models is challenged by the requirement of more high-quality annotated training data than is available in most cases. In our approach, we thus substitute expert-annotated data with a physics-based sequential synthetic data model.
We study environmental SEM (ESEM) data collected from the process of propanol oxidation to acetone over cobalt oxide. After raising the temperature to 350 °C during the reaction a phase transition occurs, reducing the selectivity of the catalyst towards acetone. This phase transition manifests in the µm-scale ESEM data as the formation of cracks between the pores of the catalyst surface. The aim is to generate synthetic data to train a neural network capable of performing semantic segmentation (pixel-wise labelling) of this ESEM data. Statistical analysis of this data will lead to greater insights into this phase transition.

Method:
To generate synthetic image data that approximates the observed transition, our algorithm composes ESEM images of the pristine room-temperature catalyst with dynamically evolving synthetic cracks satisfying two physical construction principles, gathered from empirical knowledge about the phase transition. First, crack growth propagates along surface paths which avoid close vicinity to nearby pores in the surface. Second, each growing path successively widens and is rendered with increasing contrast over short sequences of frames, allowing the algorithm to mimic the growth of the crack features on the surface. The synthetic dataset generated using this algorithm is then used to train a U-NET-LSTM recurrent convolutional neural network. This network architecture consists of a U-NET component with a convolutional Long Short-Term Memory cell appended.
Results:
To evaluate the quality of the neural network, and by extension the synthetic data generation algorithm, two neural networks were trained. The physics-based network was trained on a synthetic dataset generated as described above, and the random network was trained on a similar synthetic dataset, with the restriction that the crack growth paths avoid close vicinity to pores removed. These models were then benchmarked against each other using a synthetic test set to evaluate the importance of this physics-based component of the synthetic dataset. The results show that the physics-based network has a much lower rate of false positives than the random network, at a cost of a slightly lower rate of true positives. This is reflected in the resulting Dice-coefficients of 0.76 (physics-based) and 0.64 (random).

The physics-based network was then applied to the original ESEM movie, giving a fully semantically segmented dataset as the result. From this, statistics about the evolution of crack features could be extracted. Their analysis revealed that the crack feature is first visible to the ESEM about 100 minutes after the acetone selectivity loss event.
Conclusions:
By integrating physical characteristics of the features on the catalyst surface into a synthetic training data generation algorithm, we obtained a network with superior accuracy and a greatly decreased false positive rate. By taking advantage of the temporal nature of the data through the use of a recurrent Long Short-Term Memory layer, we improved the confidence and accuracy of the model further.

Keywords:

computer vision synthetic data ESEM

Reference:

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Tri-layer graphene as membrane and electrode for liquid-phase electron microscopy studies of CO2 electroreduction nanocatalysts

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Poster Group 2

Electrochemical liquid-phase electron microscopy (ec-LPEM) has been successfully applied to understand catalyst degradation during CO2 electroreduction (CO2ER).[1] However, to be able to fully reproduce full cell conditions at these highly cathodic potentials, further advances in imaging and electrode configuration are required. Herein, we have integrated tri-layer graphene to serve as a liquid-sealing membrane and a substrate electrode material and tested its applicability for studying nanocatalysts at realistic CO2ER potentials.[2]
All experiments were carried out in a dedicated scanning electron microscopy (SEM) stage that is equipped with bulk reference and counter electrodes. The fabrication of the working electrode started by patterning a single Pt electrical pad on a Si/SiNx electrochemical chip with an electron transparent window. Holes were perforated onto the 50 nm thick SiNx membrane in a cleanroom. Tri-layer graphene was obtained via PMMA-assisted wet transfer of monolayer graphene sheets. The final tri-layer graphene was wet transferred on the electrochemical chip, ensuring the coverage of the electrical contact and membrane area. To assemble the graphene electrochemical microcell, the graphene chips were treated with H2 plasma to ensure their hydrophilicity. Subsequently, Cu nanocubes (NCs) were drop-casted on the graphene region overlapping with the perforated SiNx support membrane.
First, we evaluated the inert potential range and stability of the graphene cells and the results showed that the graphene electrode has a wider inert potential range in the cathodic region than the conventionally used glassy carbon electrode. Graphene was also found to have a stable current profile for up to 10 min at a cathodic potential of -1.1 V vs RHE, which is common for bulk cell CO2ER studies. In addition, cyclic voltammetry tests with Cu2O NCs loaded on graphene allowed for the investigation of Cu oxide reduction and oxidation peaks, confirming a good charge transfer between graphene and catalyst. Overall, the bench-top experiments demonstrate the suitability of graphene as a substrate electrode for CO2ER studies. Furthermore, in situ SEM control experiments showed improved spatial resolution with the graphene membrane and also demonstrated reduced electron beam induced damage in the bare graphene region compared to the SiNx region. However, in situ SEM electrochemical tests indicated the presence of adverse synergistic effects of e-beam and applied cathodic potential on the electrochemical stability time window of graphene electrodes. These effects are shown to be mitigated by minimizing the electron beam dose (i.e. probe current) or avoiding high cathodic potentials. Optimized in situ SEM experiments on Cu NCs, performed at -1.1 V vs RHE, a probe current of 16 pA and a duration of 3 min, revealed that their degradation proceeds via a subtle dissolution and redeposition mechanism during CO2ER.
In conclusion, this work demonstrates that graphene can play the dual role of membrane and electrode for investigating the degradation mechanisms of CO2ER catalysts under realistic potentials, while increasing the spatial resolution for ec-LPEM studies.
Acknowledgment
This work was supported by NCCR Catalysis (grant number 180544), a National Center of Competence in Research funded by the Swiss National Science Foundation.

Keywords:

CO2_electroreduction, Cu_nanocubes, liquid-phase_electron_microscopy, graphene, microcells

Reference:

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Unraveling the True 3D Structure of Colloidal Assemblies by Liquid Cell Electron Tomography

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Poster Group 1

The colloidal bottom-up approach of nanoparticles self-assembling into ordered structures with tailored properties has recently attracted significant interest in various scientific fields. Therefore, their comprehensive structural and morphological characterization is essential, as the properties of these nanostructures are strongly correlated with their three-dimensional (3D) arrangement. Electron tomography is a common technique used for such nanoparticle characterization [1,2]. However, external forces that can affect the structure, such as the capillary forces caused by the common vacuum environment inside the microscope, are often neglected, resulting in misconnections between the obtained structure and the properties of the assemblies. As these materials are intended to be used in dispersion, our goal is to analyze the 3D arrangement of the assemblies in their native liquid environment. This innovative approach will allow us to establish a connection between the structure and properties of the assemblies under real working conditions.

In this contribution, we compare 3D reconstructions obtained in dry and liquid environments. We focus on two types of self-assemblies: gold NPs encapsulated in a polymeric shell and CTAB-coated gold nanorod bilayers. A commercial K-kit (Bio MA-TEK) liquid cell and a modified version with an increased angular tilt range (Tomochip [3]) were used depending on the assembly size. For larger assemblies, as the encapsulated gold NPs it is necessary to use larger window gap cells. However, this results in a larger background signal and limited tilt angles. On the other hand, smaller assemblies, such as the nanorods bilayers studied in this work, that can fit into the Tomochips, can benefit from a larger tilt range and lower background signal due to their reduced window gap.

The challenges of liquid-phase electron tomography have been addressed. Firstly, we adapted a fast acquisition approach to minimize the time and, therefore, the beam exposure of the sample, reducing possible beam damage. Secondly, we performed an interative denoise and rigid registration methodology to overcome challenges including increased background signal, low signal-to-noise ratios (SNR) resulting from scattering effects, and lengthy acquisition times required for two-dimensional (2D) projection images. Finally, we incorporate a compressed shape sensing method during 3D reconstruction to mitigate missing wedge artifacts from the limited tilt range and improve the SNR in the tilt series [4].

A comparison between similar 3D investigations performed in the native liquid environment and in dry state revealed a general decrease in interparticle distances in the latter. This effect can be assigned to the compression effect of the capillary forces generated by the vacuum environment of the electron microscope during dry characterization. These observations emphasize the importance of conducting measurements under liquid-state conditions to accurately characterize NP self-assemblies and provide essential insights into the underlying physical and chemical mechanisms that govern these structures.

Keywords:

Colloidal assembly, Tomography, Liquid TEM

Reference:

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Exploring solid conversion products in lithium-sulfur batteries through cryo-TEM and machine-learning-supported small angle neutron scattering

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Lithium-sulfur batteries (LSB) have emerged as a promising candidate for future energy storage solutions, leveraging the abundance, cost efficiency, and low environmental footprint of sulfur as well as significant theoretical capacity. These batteries feature a sulfur-infiltrated, highly porous carbon cathode immersed in an organic liquid electrolyte and paired with a lithium-metal anode. Despite their potential, the broader application of Li-S batteries is hindered by challenges such as suboptimal sulfur/lithium sulfide mass loadings, diminished rate performance, and the depletion of active materials. This depletion primarily results from the formation of mobile polysulfides, which can irreversibly react at the lithium-metal anode. A central challenge is the insufficient understanding of the sulfur-to-lithium sulfide conversion process, critical to the battery's functionality. [1]
In recent years, small-angle neutron and X-ray scattering have become popular for investigating LSB. However, interpreting the scattering data is notoriously challenging heavily reliant on the selection and tuning of appropriate models, thus introducing the potential for user bias [2]. Conversely, electron microscopy offers model-free access to structural analysis and provides local chemical resolution. Nevertheless, challenges such as a small field of view, demanding sample preparation and sensitivity to air and beam exposure complicate the probing of such samples [3].
In this work, we leverage recent advances in cryo-vacuum transfer TEM holders to enable the structural and chemical analysis of highly sensitive cathode materials, such as lithium sulfide [3]. We integrate this local information with operando small-angle neutron scattering (SANS) experiments to model the evolution of the multiphase cathode structure during cycling. Utilizing the composition revealed by cryo-TEM, we interpret the operando SANS data using Plurigaussian Random Fields (PGRF) models — a stochastic approach that provides representative 3D structures spanning several hundred nanometers. Given the computational intensity of solving this inverse design problem through PGRF, we train a generative adversarial model to expedite the simulation process.
Method
A binder-free, sulfur-infiltrated carbon cathode was dried and grinded in a mortar after discharge. A lacey carbon grid was then dipped into the powder. Next, the sample was transferred under Argon atmosphere from the glovebox to the TEM using the MelBuild Double Tilt LN 2 Vacuum Transfer Holder. During measurement, the sample was cooled down to -165°C to reduce beam damage. The samples were investigated using a JEOL GrandARM (ETHZ) operated at 300kV and a JEOL JEM 2200FS at 200kV. EFTEM and EELS measurements were conducted on the 2200FS.
The SANS data were acquired at the D-22 beamline at the Institute Laue-Langevin (ILL) using a custom-built cell. The cell, housed in a PEEK body, featured aluminum current collectors serving both as electrical conductors and neutron transparent windows. It incorporated a copper foil current collector, a Li metal anode, a glassfiber separator, a carbon black cathode, and an additional aluminum current collector. The azimuthally averaged intensity curve was fitted using a PGRF model.
Results

The cryoTEM measurements confirm a two-phase discharge product: one crystalline and the other amorphous. Similar discharge products have previously been reported in in-situ TEM cells using ether-based Li2S6 catholytes [4]. EFTEM and EELS analyses of the discharge particles reveal that the crystalline phase consists of Li2S, while the amorphous phase comprises a lower-order polysulfide (LOPS). Both primary particles are smaller than 10 nm, with the LOPS being approximately half the size of the Li2S particles. For subsequent modeling, we selected Li2S2 as the LOPS, which has been identified as a byproduct in LSB.
Building on the two-phase discharge product, we chose a PGRF model to analyze the SANS data. PGRF models are ideally suited for generating a variety of hierarchical, three-phase structures. By using a deuterated solvent in our battery, we adjusted the electrolyte's contrast to match that of the carbon matrix in the cathode, effectively reducing the number of signal sources to three: carbon/catholyte, Li2S, and Li2S2. Given the computational intensity of calculating scattering curves based on PGRF, we trained a convolutional neural network to predict the scattering curve from the PGRF model's input parameters. This model predicts a normalized curve with a mean squared error below 10^-4 and achieves a computational speed increase of three orders of magnitude (20 s reduced to 2.5 s). This acceleration enables the use of computationally intensive fitting algorithms. We opted for a Bayesian Optimization approach, well-suited for navigating through convex parameter spaces like those of PGRF. The parameters derived from fitting the PGRF model provide insight into the structural evolution of the cathode during operation, supporting the hypothesized solution-mediated conversion mechanism [2]. This mechanism involves the disproportionation of Li2S2 and its subsequent direct electroreduction to Li2S.
Conclusion
This study highlights the synergy between cryoTEM and SANS in revealing the conversion mechanism of lithium-sulfur batteries (LSB), leveraging a cryo-vacuum transfer holder for detailed cathode analysis. By minimizing user biases and enhancing model selection accuracy, we've provided a clearer understanding of the cathode's structural evolution during operation. Our approach not only advances the field of energy storage by deepening insights into LSB conversion mechanisms but also sets a methodological standard for future research on conversion-type batteries

Keywords:

cryo-TEM, EELS, batteries, lithium-sulfur, machine-learning

Reference:

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A new Ion Microscope for high-resolution imaging and SIMS nano-analytics

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

Powerful material characterization techniques excelling in terms of lateral resolution and sensitivity are needed to study nanoscopic materials and their transformation processes in 3 dimensions at the relevant spatial scales. State-of-the-art Focused Ion Beam (FIB) technologies allow not only to visualize nanoscopic 3D structures, but also analytical surface measurements with Secondary Ion Mass Spectrometry (SIMS). SIMS is a powerful surface analysis technique which uses energetic primary ions to sputter the surface of a sample and to generate secondary ions separated by a mass analyzer to record chemical information. Advantages of SIMS are the high sensitivity, the high dynamic range, the ability to differentiate between isotopes and the access to the complete periodic table. Typical SIMS analysis modes are mass spectrum recording, depth profiling, 2D, and 3D imaging. In the past, the correlation of high-resolution ion microscopy with in-situ SIMS has allowed to acquire complementary topographic and chemical information for a deeper understanding of samples in various domains, including material sciences, geology, and biology [1].
The IONMASTER magSIMS is a novel system devoted to correlative high-resolution 2D/3D imaging and SIMS nano-analysis. The system is a unique combination of a Liquid Metal Alloy Ion Source (LMAIS) [2] and a dedicated magnetic sector SIMS unit. Within the LMAIS various ion species are emitted simultaneously from a single ion source (GaBiLi [3] and AuGeSi sources available) and are separated in a downstream Wien filter within the FIB column. This allows to choose the most suitable primary ion species depending on the application and also to toggle within a few seconds between the ion species. This has for instance the advantage to use heavy (e.g., Bi+ or Au+) primary ions to delayer the sample stepwise and to use lighter primary ions (e.g., Li+ or Si2+) to image the sample at high spatial resolution. These image planes can be stacked and used to create a 3D volumetric reconstruction of the sample [4]. The SIMS unit is equipped with an insertable/retractable extraction optics to transfer the generated secondary ions through the magnet based mass analyzer onto a focal plane detector. The latter allows parallel acquisition of full mass spectra for each scanned pixel within the chosen field of view [5] which gives the user a multitude of possibilities to post-process and correlate the SIMS image data.
Further key strengths of this FIB-SIMS platform are the possibility to use application specific primary ion beams, i.e. the ability to switch quickly between reactive primary ion species to maximize either positive (e.g., Au+ or Bi+ single primary ions and clusters) or negative ionization (e.g., Li+ primary ions) of the sputtered particles. The small beam diameter of the lightest primary ions (Li+ and Si2+) allows to perform high spatial resolution imaging in SIMS (< 20 nm) . The low penetration depth of heavy Bi+ and Au+ (and clusters) primary ions into the material enables excellent depth resolution. Moreover, the combination of a LMAIS FIB, a Laser Interferometer Stage with CAD based navigation, and a magnetic-sector SIMS offers a high potential for automatized workflows.
In this contribution, we will present the key features and the working principle of the IONMASTER magSIMS system equipped with a LMAIS. We will show results on correlative 2D and 3D imaging focused on applications including CIGS solar cells (Figure 1), geological and microelectronics samples investigated on the recently developed nano-analysis system. Thus, we will demonstrate that the

new IONMASTER magSIMS paves the way for nano-analytics beyond the conventional methodology for sample analysis by combining the LMAIS technology with a stable stage and a SIMS unit for highest spatial resolution imaging.

Keywords:

FIB, SIMS, Imaging, LMAIS, Applications

Reference:

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4. A. Nadzeyka et al., JVST B 41 (2023), 062802.
5. O. De Castro et al., Anal. Chem. 93 (2021), p. 14417–14424.

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Understanding Twinning and Hierarchical Structure of Synthetic Guanine with 4D-Scanning Transmission Electron Microscopy

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PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Introduction:
Various biological systems can utilize guanine crystals in photonic structures. To synthesize structures of guanine that mimic these biological photonic systems, many factors of the crystallization need to be controlled, like the specific polymorphism, morphology, crystallographic lattice orientation, and the crystals' twinning. This requires new synthesis approaches, as well as novel characterization techniques. The possibility to acquire local crystallographic information by acquiring a whole diffraction pattern at each probed position in scanning transmission electron microscopy (STEM), a technique known as 4D-STEM, is thereby a promising technique to investigate twinning in more detail.
The aim of this study is to find synthesis approaches that can mimic guanine crystals utilized in biological photonic systems and link their properties to synthesis informed by their structure revealed by a combination of X-ray and state-of-the-art electron microscopy techniques.
Methods:
Guanine crystals were synthesized using a new synthesis approach using poly(diallyl di-methylammonium chloride) as an additive in solutions with pH levels of 13.
The resulting crystals were investigated with powder x-ray diffraction (PXRD), scanning electron microscopy (SEM), selected area electron diffraction (SAED), and 4D-STEM.
4D-STEM data was acquired at a low dose to avoid radiation damage to the guanine crystals. The resulting low signal-to-noise ratio made orientation mapping with commercially available programs challenging. Instead, the crystal orientation and lattice spacing changes were investigated with a center of mass (CoM) approach.
Results:
The synthesized guanine crystals adopted the same polymorph as those in biological photonic systems (β-guanine). They demonstrated flat and elongated structures, as also observed for crystals in biological photonic systems. Many crystals show kinks along their length, as exemplary pointed out by the arrow in the abstract figure. Via SAED, it was determined that these kinks coincided with twin boundaries in the crystals. Furthermore, diffuse scattering in the SAED pattern indicated disordered stacking of the guanine molecules. The novel CoM analysis of the 4D-STEM could relate the disordered stacking to smaller intergrown crystal domains. It indicated crystal growth by partly ordered granular nucleation. Such a growth of guanine was also observed in certain biological systems. From the CoM analysis, variations of the orientation and lattice spacing of the small crystal domains were mapped. Larger deviations from the averaged orientation and lattice spacing were found close to twinning boundaries, indicating strain minimization as the origin of twinning.
Conclusion:
The novel synthesis approach enabled tight synthesis control of guanine crystals with polymorph and morphology mimicking the polymorph and morphology found for crystals in biological photonic systems. The CoM analysis combined with an intricate understanding of the crystal structure and chemistry of guanine facilitated us to reveal otherwise inaccessible information about the crystal growth mechanism and origin of twinning in this, at first glance, seemingly simple yet biologically

extremely relevant system. The study demonstrates the strength of employing 4D-STEM, informed by crystallographic knowledge for studies of bio-inspired complex materials systems.

Keywords:

synthetic guanine, 4D-STEM, twinning

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Developing alloys presenting nanograins and spinodal decomposition: structural and compositional characterization

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Poster Group 2

Background incl. aims
The demand for reducing the emissions of CO2 in the transportation industries has, as a direct consequence, increased the need for alloys with enhanced performances. For this purpose, enabling the combination of multiple hardening mechanisms via microstructural design is a key tool. In the present study, in addition to a high Hall-Petch hardening, a different “second phase” hardening of spinodal decomposition is introduced in nanostructured materials.
Methods
Binary systems with a miscibility gap are selected (Fe-Cr and Al-Ag) [1,2]. The nanograins were produce by Severe Plastic Deformation (SPD) using High Pression Torsion (HPT). The resulting nanostructures (characterized by TKD and ACOM-TEM [3]) were aged during different holding times, allowing to establish the kinetics of the spinodal decomposition. The latter was characterized by APT applying the Radial Distribution Function in order to determine the evolution of both the wavelength and amplitude of the 3D concentration fields [4]. Micromechanical testing including micro-bending was applied to evaluate the mechanical properties evolution.
Results
Nanograins were obtained for both studied alloys after 10 revolutions of HPT, with average grain size diameter of 51 nm for the Fe-Cr alloy and about 100 nm for the Al-Ag system repectively. After ageing for 100h at 525 °C, the Fe-Cr average grain size attained 159 nm, showing relatively stable grain size.
The Fe-Cr spinodal decomposition kinetics of the deformed samples were measured to be 10 times faster compared to the non-deformed state. As for the Al-Ag alloy, it presents a non-homogenous composition even in the quenched state (cryo set-up used).
The microhardness evolution of the Fe-Cr alloy after 100h ageing shows that the decrease expected due to the Hall-Petch effect is mostly compensated by the hardening due to the spinodal decomposition. However, the bending torsion values after ageing showed an embrittment of the material.
Conclusion
Binary alloys with nanosized grains were produced by SPD. The miscibility gap in the phases diagrams was exploited to produce spinodal decomposed systems. Therefore, alloy-design considering both hardening effects is proven to be possible. The spinodal decomposition showed to be faster in the deformed nanograin conditions. With the goal of obtaining harder and tougher alloys, optimizing the process parameter is required.

Keywords:

spinodal decomposition, HPT, APT, TKD

Reference:

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Insights into the degradation of nanocatalysts under fuel cell conditions by 3D identical location STEM

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Poster Group 1

Proton exchange membrane fuel cells (PEMFCs) are electrochemical devices capable of generating electricity by oxidizing H2, reformate (H2 rich gas with carbon monoxide (CO) impurities) or other fuels. In recent times, metallic core-shell nanoparticles (NPs) (M@Pt, M=Ru, Rh…) have attracted a big interest as anode catalysts of reformate fed PEMFCs [1]. The high catalytic activity of Pt towards the HOR, together with the CO poisoning tolerance introduced by the accompanying metal make them ideal for heavy-duty applications. Furthermore, since in M@Pt NPs the less stable metal (Rh or Ru) is not directly exposed to the electrolyte, their stability is expected to be higher than in the corresponding alloyed NPs, which commonly suffer from dissolution and dealloying [2]. However, M@Pt can still suffer from degradation under fuel cell conditions by processes that are yet not fully understood, which hinders the design of more stable and durable catalysts.
We investigated the degradation behavior of Rh@Pt NPs by means of identical location-scanning transmission electron microscopy (IL-STEM). This quasi in-situ technique allows to overcome the limitations of the ex-situ techniques, in which only statistical general insights are possible, since in IL-STEM the changes of individual particles are tracked between potential cycles. In particular, we characterized the Rh@Pt NPs after 0, 1000, 4000 and 10000 potential cycles (0.06-0.8V, 0.1V/s). Furthermore, since many of the degradation phenomena take place in 3D (e.g., particle migration and corresponding aggregation), selected regions were reconstructed in 3D by means of electron tomography.
We observed particle migration on the carbon support in all the stages of the potential cycling. However, no widespread particle aggregation was observed, even after 10000 potential cycles. A slight Rh dissolution (up to 5 at.%) during the cycles was detected, which decreased as the number of cycles increased. Even though some small particles dissolved during the first 1000 cycles, the main degradation mechanism responsible for the loss of electrochemically active surface area was found to be particle detachment.
Our results indicate that the investigated Rh@Pt NPs present a remarkable stability, and show how IL-STEM can be used for studying the degradation of catalyst NPs.

Keywords:

Fuel Cells, Correlative, 3D reconstruction

Reference:

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Structure analysis on the nm scale: The amorphous LiNbO3 coatings for solid state batteries

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Poster Group 1

Background
To enhance the chemical stability of a cathode active material (CAM) in a Li ion solid state battery (SSB) a protective coating is applied. Such a coating should have - in addition to the chemical stability - a high Li ion conductivity. LiNbO3 is one material having these properties, which is already successfully applied in SSBs [1,2]. Its ionic conductivity depends strongly on the structure, amorphous LiNbO3 has higher ionic conductivity than crystalline one. This leads to the need of detailed knowledge of the structure and the morphology of the material when applied as thin coating in a battery cell and the correlation of these properties with the electrochemical performance [1].

Methods
In this work we use three different LiNbO3 coatings calcinated at 80°C, 350°C and 550°C on LiNi0.6Co0.2Mn0.2O2 (NCM622) as CAM. To get insights on the structure of the coatings various scanning transmission electron microscopy techniques (STEM) like high angle and virtual annular dark-field imaging (HAADF & VADF), energy dispersive X-Ray spectroscopy (EDS), electron energy loss spectroscopy (EELS) and scanning precession electron diffraction (SPED) were used. To further elucidate the amorphous structure of the coating the reduced pair distribution function (rPDF) [3,4], which contains information about nearest neighbor distances in the material, is calculated from the SPED diffraction patterns. These structural results are then correlated to the electrochemical performance of a battery cell with the respective coated CAM.

Results
HAADF and EDS images show that for the samples calcinated at 350°C and 550°C the coating forms a homogeneous layer covering the entire NCM622 particles. In contrast to that the coating covers only a few parts of the NCM622 particle for the sample calcinated at 80°. SPED measurements show that in the case of 550°C calcination temperature the coating is crystalline whereas for lower calcination temperatures such as 350°C and 80°C the coating is amorphous. Further quantification of the structure of these two amorphous samples through the rPDFs reveals distinct differences in the inter atomic distances. These structural differences of the three samples also influence their electrochemical performance. The battery cell with the coated CAM calcinated at 350°C shows a higher capacity after around 300 cycles compared to the cell with the uncoated CAM. For the cells with the coated CAM calcinated at 550°C and 80°C the capacity during cycling is inferior to the cell with the uncoated CAM.

Conclusion
Correlations between structural results of LiNbO3 coating on NCM622 calcinated at different temperatures to the electrochemical performance of the material in a battery cell show that the best

performance is achieved by an amorphous coating covering the whole NCM622 particles. This is achieved at a calcination temperature at 350°C. Higher temperatures lead to a crystalline structure of the coating. Lower temperatures lead to a different amorphous structures as well as an only partial coverage of the coating. 550°C and 80°C calcination temperature of the coated CAM lead then to a poorer electrochemical performance of the battery cell.

Keywords:

SPED EPDF AMORPHOUS BATTERY COATING

Reference:

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Secondary envelopment of the human cytomegalovirus is a fast process, utilizing the endocytic compartment

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background and aims: The human cytomegalovirus (HCMV), a member of the herpesvirus family, is an enveloped virus. It gains its final envelope by the critical but not well understood secondary envelopment process that takes place at the cytoplasmic viral assembly complex (cVAC). Partially tegumented capsids that were released from the nucleus and transported to the cVAC initiate a budding process into cellular membranes coated with viral glycoproteins. Previous studies present controversial results regarding the origin of the viral envelope, suggesting both, trans-Golgi and endosomal origin, as well as a similarity with exosomes (1-4). Thus, the identity of the membranes utilized for secondary envelopment remains unclear. This study investigated the role of endocytic membranes for secondary envelopment of HCMV.
Methods: Wheat germ agglutinin (WGA) either conjugated to a fluorophore or to horseradish peroxidase (HRP) were used for labelling of glycoproteins at the plasma membrane of HCMV infected cells. By this, endocytosed membrane could be visualized by fluorescence and electron microscopy (EM). Membrane marker screening was used to narrow down the potential origin of the membrane compartment used for cVAC formation and secondary envelopment. EM was used to directly visualize endocytosed membranes, HCMV capsids and their interactions. For EM, we adapted a WGA-HRP labeling protocol followed by the DAB reaction in living cells (5). Subsequently, EM samples were prepared by high-pressure freezing and freeze substitution for optimal membrane visibility and analyzed by quantitative transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) tomography.
Results: WGA was taken up into different membrane compartments within the cVAC of HCMV infected cells, including early endosomes, multivesicular bodies, trans-Golgi and recycling endosomes. TEM analysis showed budding of capsids at WGA-positive membranes and numerous enveloped WGA-positive capsids at the cVAC. Almost 90% of all budding capsids and 50% of enveloped capsids were labelled with WGA within 90 min. This indicated a rapid turnover of membranes at the cVAC in which freshly endocytosed membranes were transported from the plasma membrane into the cVAC to be utilized by capsids for their envelopment and consequent release into the extracellular space. Furthermore, we could show that 30 min are sufficient to complete endocytosis from the plasma membrane and secondary envelopment at the cVAC. STEM tomography revealed budding of capsids at various membranes, including trans-sided Golgi cisternae.
Conclusion: The endocytic compartment serves as the major membrane source for the formation of the cVAC as an optimized environment for virion maturation and consequently, as the major membrane source for secondary envelopment of HCMV. Furthermore, we show that secondary envelopment of HCMV is a fast process, in which endocytosed membranes are transported from the plasma membrane to the cVAC within minutes to be utilized for envelopment.

Keywords:

WGA-HRP, in-vivo DAB-labelling, TEM, STEM

Reference:

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An accessible Secondary Electron Hyperspectral Imaging approach to draw meaningful insights from scanning electron microscopy

James Nohl[1,2], Jingqiong Zhang[1], Lyudmila Mihaylova[1], Serena Cussen[3], Cornelia Rodenburg[1]
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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background
Hyperspectral imaging in the scanning electron microscope (SEM) commonly uses the energy dispersive X-ray (EDS/EDX) signal. The colour maps produced have become a staple part of analysis for visualising elemental heterogeneity and quantifying bulk elemental composition of hard materials. Now, automated collection and analysis routines have extended to secondary electron hyperspectral imaging (SEHI) and the ability to produce colour maps of surface chemical heterogeneity down to the nanoscale [1].
SEHI, combined with an accessible and automated data analysis tool, has yielded maps of surface chemical bonding, such as the surface functionalisation of soft materials, compounds of light elements, and crystallinity by cross-linking. As well as offering complimentary information to elemental composition, the emission characteristics of SEs offer improved surface sensitivity and spatial localisation versus X-ray imaging in SEM, particularly for softer materials.

Methods
In this implementation, SEHI data volumes are built by sequentially imaging with the through-lens-detector (TLD) with a mirror electrode which steps through a series applied voltage biases to act as a variable low-pass energy filter. Automated data collection routines control the cutoff energy of the low pass filter to acquire images quickly. Imaging is done at 1 kV accelerating voltage and < 50pA beam current to produce suitable signal to noise and SE yield close to 1, as well as to reduce SE2 background emissions, and to limit the beam dose received by the material.
The data volume is differentiated with respect to energy to plot local SE spectra and slices of energy ranges (Figure 1a) are summed to create maps which relate a colour to a chemical feature in the SE spectrum (Figure 1b-d).
The energy ranges for each colour channel can be identified without prior knowledge by a blind-source separation using a non-negative matrix factorisation (NNMF) method. The program for creating colour-SEHI images has been packaged and redistributed as a portable MATLAB application.

Results
The first demonstration of the colouring method is on a lithium metal anode used in lithium metal, lithium-sulphur and lithium-air batteries. The surface ‘platelets’ are heterogenous features in red which measure 250 nm across. Whereas lithium oxide morphologies were imaged over a 25 µm horizontal field width by windowless EDS. Ranking of surface chemical species by comparison to theoretical models was used to identify contributions from lithium hydroxide, nitride and carbon containing species and found the red regions to have a higher proportion of lithium nitrides and hydroxides while the blue range included more lithium-carbon compounds.

Applying the analysis to a spider dragline silk fibre reveals domains of nanoscale ordering due to cross-linking, which measure 45 nm across (coloured green, Figure1c). A higher proportion of cross-linking is indicated by more intense SE emissions at 1.1 eV denoting di-sulphide bridging.
At a length scale of 25 µm horizontal field width, the approach is applied to a perovskite solar cell material, which is a mix of organic methylammonium and inorganic lead iodide (ie. hard and soft) material. The automated approach to colouring identifies a two-phase material, to identify regions of lead iodide (PbI2) within an organic-inorganic perovskite.

Conclusions
This advance in SEHI analysis is an important extension of LV-SEM capability to map and identify regions of surface chemical bonding. This is achieved through an accessible data collection and analysis routine that applies hyperspectral imaging to the SE signal.
Characterisation of surface chemical bonding heterogeneity on bulk functional materials becomes accessible down to nanoscale even for soft matter, as demonstrated by mapping 45 nm wide cross-linked regions in spider silk where di-sulphide bridges are more prevalent. Light element compound distribution is identified on a lithium metal anode material as well as phase separation in an organic-inorganic perovskite solar cell material.
Having demonstrated the technique can be applied to characterise both organic and inorganic material, future studies will include the lithium metal anode post electrochemical cycling and growth of the solid-electrolyte interphase layer.

Keywords:

SEHI, SEM, surface chemistry, nanoscale

Reference:

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High-throughput tomography correlated with light and electron microscopy for multi-scale imaging of human kidney tissue

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Background incl. aims
Kidney transplantation is the treatment of choice for patients with chronic kidney failure. However, rejection of the transplanted organ is still common despite immunosuppression, with antibody-mediated rejection (AMR) providing the worst prognosis, and so improving the understanding of the mechanisms of rejection is central to developing alternative therapeutics with better patient outcomes. Current imaging methods mostly rely on 2D imaging of samples using either light or electron microscopy (EM) or extremely time-consuming 3D volume EM. In this study we wanted to utilise high throughput tomography (HiTT) to non-destructively image whole hydrated tissue biopsies in fixative using X-ray imaging[1]. With HiTT we use phase-contrast imaging, which is particularly good at imaging low X-ray absorbing material such as hydrated soft tissue. Each individual scan takes 2 minutes and 16 seconds to complete with 3D reconstructions complete in 30 seconds. The speed and quality of the images allows us to:
1. Screen and target areas of interest in the kidney biopsies for both confocal microscopy targeting specific immune cells and also serial-blockface SEM (SBF-SEM).
2. We aim to correlate all the information provided across different scales to both inform on potential changes happening in AMR but to also see if the ultrastructural information from the SBF-SEM can be correlated to differences seen at the tissue level in the confocal and HiTT imaging.
3. The final long-term aim is that these cross-correlations between the three different imaging modalities can be recognised using machine learning tools to help predict whether the kidney biopsies are showing signs of AMR.

Methods
Kidney tissue biopsies (1 mm diameter punch biopsies) taken from control and transplant rejection patients (AMR-high and AMR-low) are stored in fixative (1% para-formaldehyde (PFA)). These samples are then transported to Hamburg where they are imaged using HiTT. The biopsies are mounted in small pipette tips, still in PFA, and imaged at the P14 beamline on Petra III in Hamburg. The imaging process is repeated at four distances at an energy of 18 keV, using a 10x objective (to provide a pixel size of 650 nm), with a total exposure time of 72.4 seconds for each scan. The data are then automatically reconstructed and able to be viewed in 30 seconds. For the biopsies which are several mm long we perform a vertical stitch scan which enables the whole biopsy to be imaged. Following the non-destructive 3D imaging of the whole biopsy it is returned to the Francis Crick Institute in London, here the biopsy is sectioned and prepared for confocal imaging.
Following the confocal imaging of the vibratome slices they are processed for SBF-SEM. These heavy metal stained, resin embedded slices are then re-imaged using HiTT in Hamburg at 12.7 keV and using the 20x objective (325 nm pixel size). Each of the individual slices are imaged and reconstructed

in less than three minutes. The samples are then transported to EMBL Heidelberg where they are imaged using SBF-SEM. This will be targeted on regions of interest such as the glomeruli or peritubular capillaries using both the hydrated and resin embedded HiTT data. The HiTT, fluorescence and SBF-SEM data is correlated together in MoBIE² to allow investigation of different tissue features across multiple scales. This data is segmented with a combination of manual and semi-automated processes to help generate ground-truths for training machine learning models.

Results
Here we show that whole kidney tissue biopsies from the clinic can be imaged non-destructively in 3D, as shown in figure 1 it is already possible to observe differences between the control and AMR samples. From the X-ray data alone it is possible to identify and quantify the number of glomeruli and blood vessels present in the whole biopsy as well as observe features of disease such as inflammation. In combination with the correlated confocal data taken from the same biopsy it is possible to infer specific cell types between both modalities. This vibratome section was targeted as, based on the HiTT data it was known to contain glomeruli. This can then be registered to the SBF-SEM to show changes at the ultrastructural level on targeted structures located in the X-ray imaging.

Conclusion
The High-throughput X-ray imaging provided by HiTT has facilitated the 3D imaging of whole patient biopsies at a scale and in a time frame not seen previously. This data can then be correlated with both light and electron microscopy to reveal cross-scales changes occurring in AMR.

Keywords:

X-ray, Correlative, high-throughput, confocal, volume-EM

Reference:

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In Situ Study of Self-Organized Memristive Switching in Neuromorphic Nanoparticle Networks using Complementary SEM Methods

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Poster Group 1

Background incl. aims
Self-organized nanoparticle networks (NPNs) poised at the percolation threshold are promising for bio-inspired information processing. These networks show non-linear responses, high dimensionality and avalanche dynamics similar to those observed in the brain. The objective of this study is the imaging of the current path through the NPN to study the distributed memristive switching events.
Methods
The neuromorphic NPN was fabricated in two steps. First, an insulating base-layer, consisting of Ag NPs with a CxOyHz shell, was deposited on pre-structured Pt electrodes on a Si chip with 1 µm thermal oxide. In a second deposition step, pure Ag NPs were deposited under continuous conductance monitoring until the percolation threshold was reached. The avalanche dynamics of the NPN with features of criticality were analyzed ex situ. Ultimately, this NPN was analyzed in the SEM (FEI Helios Nanolab 600 SEM/FIB with a field emission gun operated at 3 kV) with 2 complementary methods. First, during in operando biasing a changing active voltage contrast (AVC) was observed. Second, resistive contrast imaging (RCI) revealed directly the current path after biasing steps. For these experiments, Pt electrodes below the NPN were electrically connected in situ by micromanipulators with tungsten needles to an EBIC amplifier from Kleindiek company. To gain a better understanding of the current response of NPNs, we corroborate the experimental results with kinetic Monte-Carlo simulations.
Results
After biasing, RCI was used to observe directly the current path (see graphic) revealing changes in the connectivity after successive biasing steps, while the overall morphology of NP groups persisted. AVC revealed a step-like potential distribution (see graphic) that changed during static biasing. Simulations indicated that nano-gaps between NP groups lead to the step-like potential distribution that varies due to switching events.
Conclusion
Current path imaging using AVC and RCI indicates that the current path changes steadily during biasing due to the construction and deconstruction of memristive filaments in nano-gaps between NP groups. The changing position of the nano-gaps is highlighted by strong resistive contrast. Thus, we correlated the avalanche dynamics of the NPN with spatio-temporally distributed resistive switching events within the network with the complementary combination of AVC and RCI.

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Keywords:

Resistive-Contrast-Imaging, Active-Voltage-Contrast, Neuromorphic, Memristor, Self-Organization

Reference:

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2 O. Gronenberg, B. Adejube, T. Hemke, J. Drewes, O. H. Asnaz, F. Ziegler, N. Carstens, T. Strunskus, U. Schürmann, J. Benedikt, T. Mussenbrock, F. Faupel, A. Vahl, L. Kienle, Adv Funct Mater 2024.

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Suppression of Stacking Faults for Stable Cesium-Formamidinium Perovskite Absorbers

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The technique of alloying FA+ with Cs+ is often used to promote structural stabilization of the desirable α-FAPbI3 phase in halide perovskite devices[1]–[3]. However, the precise mechanisms by which these alloying approaches improve the optoelectronic quality and enhance the stability have remained elusive. This work presents the investigation of the effect of cationic alloying in CsxFA1-xPbI3 perovskite thin-films and solar-cell devices. Selected-area electron diffraction patterns combined with microwave conductivity measurements reveal that fine Cs+ tuning (Cs0.15FA0.85PbI3) leads to a minimization of stacking faults (SFs) and an increase in the photoconductivity of the perovskite films (Figure 1). Ultra-sensitive external quantum efficiency, kelvin-probe force microscopy and photoluminescence quantum yield measurements demonstrate similar Urbach energy values, comparable surface potential fluctuations and marginal impact on radiative emission yields, irrespective of Cs content. Despite this, these nanoscopic defects appear to have a detrimental impact on inter-grains'/domains' carrier transport, as evidenced by conductive-atomic force microscopy, corroborated by drastically reduced solar cell performance. Importantly, encapsulated Cs0.15FA0.85PbI3 devices show robust operational stability retaining 85 % of the initial steady-state power conversion efficiency (PCE) for 1400 hours under continuous 1 sun illumination at 35 °C, in open-circuit conditions. These findings provide nuance to the famous defect tolerance of halide perovskites while providing solid evidence about the detrimental impact of these subtle structural imperfections on the long-term operational stability.

In the second study, utilizing a very low-dose electron beam, we observed local material loss taking place in a white striped contrast forming regularly along the SFs pathways (Figure 2a). We examined several hundreds of diffraction patterns in different leading FAPbI3-rich perovskite compositions and interestingly, the SFs domains in the cubic FAPbI3 phase were found to be predominantly oriented near the [011]C zone axis in all the perovskite films. This derived an implication that the (011) perovskite crystal planes seem to be structurally vulnerable for SFs formation and that can be a source of an intrinsic absorber instability. Based on these observations, an —asymmetric sulphur (S)— containing additive (the obtained material is named hereafter as target) was introduced into the empirically optimized Cs0.17FA0.83PbI3 perovskite precursor which manipulates the perovskite crystal growth and results in majorly oriented in-and out-of-plane (001) perovskite domains (Figure 2(b-g)). The target perovskite devices (with an active area of 0.2 cm2) were extremely stable, retaining > 93 % of their initial PCE for > 2100 hours at room temperature, and additionally, 1 cm2 active area target devices maintained T80 (the duration for the PCE to decay to 80 % of the initial value) for > 500 hours at 65 °C, all under continuous 1-sun illumination at the maximum power point tracking in ambient conditions. These findings demonstrate the critical role of eliminating SFs domains in realizing stable perovskite solar cell devices.

Figure 1. a-c) Bright-field TEM micrographs, d-f) The associated SAED patterns (The Cs content and zone axis are written on the top left and right side of the DPs, respectively), The yellow circles indicate the position of the selected-area for SAED pattern acquisition; and g) Histograms illustrating the impact of Cs content on the defect density. The estimated percentages are based on the number of diffracted domains (from all the obtained micrographs) featuring ⟦{111}⟧_C SFs or δ-CsPbI3 phases with respect to all the other different phases present in the samples.

Figure 2. a) Bright-field TEM micrograph for a FAPbI3 perovskite film demonstrating material loss manifested by white contrast arranged in stripes, b-e) 4D-STEM results showing the virtual BF images and orientation maps, where the colour legend encodes the out-of-plane orientation of the metrically-cubic perovskite domains, for both reference and target perovskite films and f-g) Schematic illustration comparison showing dominant corner-up and face-up orientations for the reference and target films, respectively.

Keywords:

Photovoltaics
Perosvkites
Stability

Reference:

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Using secondary electron electron beam induced current for characterization of nanoparticle morphologies

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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Background incl. aims
Electron tomography (ET) is an indispensable tool for determining the three-dimensional (3D) structure of nanomaterials in (scanning) transmission electron microscopy ((S)TEM). ET enables 3D characterization of a variety of nanomaterials across different fields, including life sciences, chemistry, solid-state physics, and materials science down to atomic resolution. However, the acquisition of a conventional tilt series for ET is a time-consuming process and thus cannot capture fast transformations of materials in realistic conditions. Moreover, only a limited number of nanoparticles (NPs) can be investigated, hampering a general understanding of the average properties of the ensemble. Therefore, alternative characterization techniques that allow for high-resolution characterization of the surface structure without the need to acquire a full tilt series in ET are required which would enable a more time-efficient investigation with better statistical value. Here we propose surface-sensitive secondary electron (SE) imaging in STEM employed using a modification of electron beam-induced current (EBIC) setup as an alternative surpassing electron tomography.
Methods
SEEBIC and ET experiments were performed using an aberration-corrected Thermo Fisher Themis Z TEM operated at an acceleration voltage in a range of 60-200 kV. A custom-made transimpedance amplifier with a total gain of 2 GV/A and bandwidth of 8 kHz, electrically connected to the sample via a DENSsolutions Wildfire holder, was used to convert the SEEBIC signal into a voltage signal digitized by the Attollight OUDS II scan engine. No image filtering was applied during post-processing. ET data were acquired over a tilt range of ±72° with tilt increments of 3°. Reconstructions of the tilt series were performed using the SIRT algorithm implemented in ASTRA Toolbox 1.90 for MATLAB 2022b. Scanning electron microscopy (SEM) images were obtained using Thermo Fisher Helios Nanolab 650 with nominal spatial resolution down to 0.8 nm operated at an acceleration voltage of 5 kV.
Results
We have shown that both general morphology and side faceting of the NPs can be directly observed from SEEBIC images which is less obvious from conventional STEM micrographs. SEEBIC imaging enables a gain of up to 2 orders of magnitude in data collection efficiency in comparison to conventional ET while providing necessary topographical information. The superior spatial resolution compared to SEM was demonstrated. It was shown that even though SEEBIC requires to use of high primary electron beam currents, the optimization of experimental parameters allows for the reduction of the accumulated electron dose rendering SEEBIC equally or even more dose-efficient than ET. Finally, we describe the contrast artefacts arising in SEEBIC images and discuss their origin. Next, it was shown that direct access to surface morphology obtainable on the order of minutes opens up the possibility of using SEEBIC for high-throughput analysis of the helical morphology of chiral Au nanorods (NRs). The workflow to automatically quantify the helical morphology based on

SEEBIC images and a dedicated image quantification procedure was developed and used to calculate the helicity function of the NP ensembles. We have shown that this approach overcomes the limitation of poor statistics obtained by ET, which is limited to analyzing only a few particles per sample batch. Helicity function analysis revealed a significant polydispersity at the level of surface features. We found that the average helicity values, calculated for hundreds of NRs per sample batch were in good agreement with the optical properties of the sample, confirming that helicity measurements enable linking the nanoscale morphology with the chiroptical handedness.
Conclusion
We have demonstrated that SEEBIC can be considered an attractive approach for the characterization of NP morphologies with shorter acquisition and processing times in comparison to ET and superior resolution in comparison to SEM. It was shown that the helical morphology of chiral Au NRs, with significant polydispersity at the level of surface features can be efficiently quantified using high-throughput SEEBIC measurements.
This work was supported by European Research Council (ERC Consolidator Grant 815128, REALNANO).

Keywords:

STEM, electron tomography, SE imaging

Reference:

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Development and characterization of N2O-plasma oxide layers for high-temperature passivating contacts solar cells

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Poster Group 2

Full-area passivating contacts solar cells based on SiOx/poly-Si stacks represent the key elements for the fast growing new generation of industrial silicon solar cells substituting the passivated emitter and rear cell (PERC) technology. The utilization of n-type wafers with an n-type contact at the back and a p-type diffused boron emitter has become the industry standard in 2024.
In this work, variations of this technology are explored, considering p-type passivating contacts formed via a rapid thermal processing (RTP) step on p-type wafers, which could be useful in conjunction with n-type contacts for realizing solar cells with passivating contacts on both sides. Here, a particular focus is set on investigating the influence of the applied thermal treatment on the interfacial oxide layer. Thin SiOx layers formed via UV-O_3 exposure of the wafer are compared with layers obtained through a plasma treatment with nitrous oxide (N_2O). The different steps for sample fabrication are depicted in Figure 1(a).
For both oxide types, the influence of the RTP thermal budget on passivation quality and contact resistivity is investigated, as presented in Figure 1(b). The N_2O oxide exhibits an excellent passivation quality (i.e. high implied open circuit voltage iVoc) in the range of high thermal budget, whereas the UV-O_3 oxide shows a pronounced degradation under these conditions. Simultaneously, the contact resistivity achieved with the N_2O oxide layer is comparable to that yielded by the UV-O_3 oxide. Prior research emphasised the importance of employing a higher thermal budget for contacts formed by RTP to ensure both sufficient lateral conductivity of the poly-Si layers and compatibility with localized metal contacts [1]. These results demonstrate that fabrication of the thin SiOx by N_2O oxidation can be a viable solution to obtain high passivation quality in the high thermal budget range.
To unravel the mechanisms behind the improved performance obtained with the N_2O oxide at high thermal budget, high resolution TEM is conducted on layer stacks featuring both N_2O and UV-O_3 oxides after RTP at T=860°C, as presented in Figure 2. A break-up of the UV-O_3 oxide at high thermal budget is observed, whereas the N_2O oxide is found to maintain its structural integrity along the interface. The differences are shown in higher magnification in the STEM-HAADF micrographs in Figure 2(e,f). The superposition between a region displaying contrast from SiOx and a region with a pinhole in Figure 2(f) indicates that the UV-O_3 layer is only partially disrupted.
Characterization of the same samples by X-Ray reflectometry (XRR) was also used to study the differences in the two oxide layers on a larger scale, confirming the HR-TEM results. Furthermore, chemical analysis conducted by depth-dependent X-Ray photoelectron spectroscopy (XPS) reveals that the N_2O oxide is richer in oxygen and contains a higher amount of nitrogen compared to the UV-O_3 one, as shown in Figure 3. These distinguishing characteristics can be directly linked to the enhanced stability exhibited by the N_2O oxide under higher annealing temperatures and extended dwell times, in agreement with previous research [2], [3].

This study highlights the effectiveness of the employed characterization techniques as a robust method for analyzing multi-layer stacks, particularly well-suited for nanoscale thin films. By integrating more invasive techniques such as TEM and XPS depth-profiling with the non-destructive approach of XRR, it is possible to characterize large sample areas while also resolving layers at the atomic scale. This combined approach allows the analysis of full c-Si/SiOx/poly-Si stacks after RTP, closely resembling structures used in solar cell applications, thereby providing valuable insight for further development and application of such contacts. This approach differs from the majority of similar studies in literature that primarily focus on characterization of as-deposited oxide layers [3]–[5].

Figures captions:
Figure 1. a) Sample fabrication process. (b) Implied open-circuit voltage (iVoc) after hydrogenation and contact resistivity (ρC) as a function of the RTP temperature for contacts with UV-O_3 and N_2O oxides.

Figure 2. (a,b) High resolution TEM micrographs of the c-Si/poly-Si(p) interface for the two samples with N_2O and UV-O_3 oxide layers, respectively, after RTP at 860°C. Higher magnification of the interface is shown in (c) and (d). (e,f) High resolution STEM-HAADF micrographs of the samples with the two oxide types. White arrows in (d) and (f) indicate the crystalline protrusions at the position of the SiOx for the UV-O_3 oxide.

Figure 3. XPS contour plots of signal intensity versus etching time and binding energy for Si 2p, O 1s, and N 1s spectral ranges at processing T=860°C. The intensity scale remains consistent across plots within the same spectral range for both samples.

Keywords:

Photovoltaics
Passivating-contacts
Silicon oxide
Polysilicon

Reference:

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Characterization of aluminium diffusion in high-temperature passivating contacts by in situ scanning transmission electron microscopy

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Poster Group 2

Full-area passivating contacts utilizing SiOx/poly-Si stacks are a critical component for the coming generations of high-efficiency industrial silicon solar cells, replacing the previous mainstream technology called passivated emitter and rear cell (PERC). In this work, the metallization of p-type passivating contacts formed via a rapid thermal processing (RTP) step is investigated. Metallization of poly-Si layers typically involves silver-based pastes for both sides of the cell, leading to higher production costs compared to PERC, which use Ag only on one side. Moreover, concerns over the long-term availability of silver prompt the search for more abundant alternatives to reduce costs and ensure sustainability in solar cell production [1].

The formation of Al-Si contacts, which results in the creation of µm-deep spikes within the c-Si wafer, has been well-documented in the context of PERC cells [2], but it has not been investigated thoroughly for poly-Si layers. The feasibility of utilizing aluminium pastes to establish contact with p-type poly-Si layers was explored in a prior study [3]. Characterization of the microstructure of the contacts after firing at different temperatures highlighted the numerous interactions taking place between the aluminium and the different layers in the stack (SiNx, poly-Si, SiOx and the c-Si wafer), which create a challenging trade-off between achieving a good electrical contact while retaining high passivation quality. Moreover, it was demonstrated that, for more fundamental studies, it is possible to replace the paste with evaporated Al, simplifying the analysis [3].

To gain further insight into the reaction mechanisms, this works focuses on characterization of the samples during in situ heating by scanning transmission electron microscopy and energy dispersive X-ray spectroscopy (STEM/EDX) for temperatures below 650°C. The sample fabrication steps, structure and the heating profile applied during characterization are depicted in Figure 1. The reactions observed during the heating process are represented schematically in Figure 2. The initial phase involves Si out-diffusion into the Al from the underlying layers (Figure 2(b)), followed by Al diffusion at higher temperatures. The latter appears to occur initially vertically through regions with reduced density or thickness in the parasitic SiOx layers present at the various interfaces, and subsequently laterally within the SiNx and poly-Si layers (Figure 2(c)). Here, the oxide layers at the interfaces function as diffusion barriers, promoting lateral rather than vertical Al diffusion. The nc-Si(i) layer exhibits similar behaviour, showing no discernible reaction with Al at this stage (T < 500°C), as visible in Figure 3. This is likely due to the enhanced crystallinity of the nc-Si(i) compared to the poly-Si(p) layer, which has been reported to hinder reactions with Al [4]. In the final stages of the process (Figure 2(d) and Figure 4), Al diffuses into the nc-Si(i) layer, eventually reaching the wafer interface where it accumulates at the thin SiOx and creates pinholes within the SiOx layer. These observations can explain the passivation degradation observed in previous work at relatively low temperature

(500-600°C), well below the threshold for formation of the µm-deep Al spikes. Furthermore, this study provides insights into potential avenues for improvement, such as enhancing the crystallinity of the poly-Si layer or substituting the SiOx with a layer less reactive with Al.

Figures captions:
Figure 1. (a) Fabrication process for the studied SiOx/poly-Si contacts. (b) Structure of the studied sample. (c) Temperature profile applied during in situ heating.

Figure 2. Schematic representation of a possible reaction sequence: (a) beginning of the heating process, and (b) diffusion of Si from the various layers into the Al contact. (c) Diffusion of Al through pinholes in the oxide layers, and lateral diffusion in the channel provided by the parasitic oxide layers or the nc-Si(i) region. (d) Complete reaction of the different layers with Al, and changes to the SiOx layer.

Figure 3. STEM-ADF micrograph acquired after heating up to 500°C and successive rapid cooling to room temperature, and corresponding elemental maps for Al (orange) and Si (green) (overlaid onto ADF micrograph). The different layers are indicated by the colour bar on the left side. The white arrows highlight the nc-Si(i) at the interface with the Si wafer.

Figure 4. (a) STEM-HAADF and (b) ADF micrographs of the layer stack after heating to 650°C and subsequent rapid cooling. The colour bar on the left side indicates the different layers. Elemental maps for aluminium (orange), silicon (green), nitrogen (blue) and oxygen (pink) correspond to the areas highlighted by the white rectangles in (a) and (b). The white arrows highlight the regions where epitaxy from the c-Si occurs.

Keywords:

photovoltaics
thin-films
in situ STEM

Reference:

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Study of skyrmions in ferromagnetic metallic superlattices using in situ Lorentz magnetic methods

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Background incl. aims
Magnetic skyrmions, which consist of local swirls of spins, are a prime example of topologically nontrivial spin textures. Nanoscale isolated skyrmions exist at low temperature in bulk chiral materials, while in thin films and multilayers, room temperature (RT) stable skyrmions exhibit intermediate sizes ranging between 50 nm to a few µm. Ferromagnetic metallic superlattices are materials which are promising for the development of RT nanoscale skyrmions due to the high tunability of their properties. There exist a large number of experimental studies reporting the observation of stripe domains and skyrmion bubbles in metallic multilayers, for example Pt/Fe/Ir[1] and Ta/Co/Pt[2]. It is well understood that these skyrmions and stripes are created by a combined action of the stray field effect and the Dzyaloshinskii-Moria interaction (DMI)[3]. These non-collinear spin structures can also be easily simulated numerically [2]. However, in a system with a high number of degrees of freedom, the quest remains open regarding how to optimize the parameters to obtain the most compact and stable skyrmion and the development of adequate analytical tools could lead to breakthrough and transform this quest from a random walk to a guided tour. Many experimental techniques have been used throughout the years in order to study skyrmions, including Magnetic Force Microscopy[4], soft-x-ray microscopy[1] and Magneto-Optical Kerr Effect (MOKE) microscopy [5] among others. But quantitative nanoscale studies on the conditions of skyrmion nucleation remains of primary importance. In this study we chose to study skyrmions in a classical [Pt/Co/Ta]n metallic superlattices system, n being the number of repetition of the tri-layer.
Methods
Samples were fabricated by magnetron sputtering, and studied using Lorentz Transmission Electron Microscopy (LTEM) in Fresnel mode combined with electron holography (EH). LTEM in Fresnel mode offers an easy and straightforward method for a semi-quantitative study of the local magnetization, well suited to the observation of skyrmions. We aimed to explore the magnetic phase diagrams of our multilayered magnetic system by developing an automatized process to record magnetic images with in situ application of magnetic field through the control of the objective lens and modulation of the temperature using a dedicated sample holder. In parallel, we used electron holography (EH) which allows to reach higher spatial resolution (up to 0.5 nm) and obtain quantitative magnetic measurements. Our approach is combined with MOKE, theoretical models and numerical simulations (using Mumax3).
Results
In this system, RT skyrmionic bubbles as small as 50 nm have been observed when the Co thickness was tuned close to the spin reorientation transition[2]. In our study, we fixed the Co thickness at 0.8 nm and we tuned the thickness of the total system by varying the number of repetitions n of the Pt/Co/Ta tri-layer. This allows us to tune selectively the effect of the stray field while keeping roughly constant the system intrinsic parameters (magneto-crystalline anisotropy, saturation magnetisation, DMI). We were able to image the magnetic micro-structures of [Pt/Co/Ta]n multilayers with various different number of repetitions in LTEM and MOKE (Figure 1a). We conducted an experimental study of the stripe domain structures as a function of the multilayer total thickness, see Figure 1b, and

when applying a magnetic field (using the objective lens), we studied the formation process of magnetic skyrmions, the stripe-skyrmion transition, and the presence of skyrmion lattice or isolated skyrmions in certain samples. We could correlate the magnetic contrast observations with applied field to the magnetic hysteresis obtained from VSM measurements. We also studied the influence of the temperature on the stripe pattern and on the formation of skyrmions, from which we could elaborate experimental phase diagrams coupled to theoretical diagrams, based on skyrmionic bubble model (Figure 2). Finaly, we performed EH experiments at the state-of-the-art, combining high spatial resolution with a dedicated Lorentz stage, direct electron detector with the K3 camera and long exposure time up to 4 mn thanks to the dynamical correction of instabilities. We were able to recover the magnetic phase of the Néel type skyrmions up to few nanometers of spatial resolution, and to observe the detailed stripe-to-skyrmion transition with external applied magnetic field (Figure 3). We will detail the magnetic processes which has been deduced from such observations combined with simulations.

Conclusions

Our experimental study focused on [Pt/Co/Ta]n metallic superlattices, investigating the manipulation of their properties by varying the number of repetitions (n) of the tri-layer. Through spatially resolved magnetic measurements using magneto-optic techniques and transmission electron microscopy methods combined with in situ experiments, we could explore the stripe domain structures and skyrmion formation as a function of total thickness, the applied magnetic field and varying the temperature. Our findings give new insights of the stripe-skyrmion transition and temperature's influence on these phenomena, supported by experimental phase diagrams and theoretical models. Additionally, we studied Néel-type skyrmions structure using advanced EH observations, achieving a very high spatial resolution and observing detailed transitions from stripe to skyrmion configurations under external magnetic fields. Our study provides significant contributions to understanding the magnetic behaviors of skyrmions in metallic superlattices by combining TEM magnetic imaging and MOKE, correlated to micromagnetic simulations, stripe domain and skyrmion models.

Keywords:

LTEM, EH, skyrmion, MOKE, nanomagnetism

Reference:

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Three-dimensional fivefold misfit in multiply twinned particles at atomic level

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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Background incl. aims
Fivefold symmetry is mysterious in crystallography because it lacks translational invariance and periodic structures. We cannot fill two-dimensional (2D) planes with regular pentagons or make an icosahedron with regular tetrahedra (Figure 1a-b). Multiply twinned particles (MTPs) have attracted attention since their discovery because of their excellent optical and catalytic properties, crystallographically forbidden fivefold symmetry and associated lattice misfit strain. Icosahedral MTPs composed of fivefold twinned tetrahedra have broad applications and the researchers have observed from 2D perspective for decades [1-7]. Electron tomography offers a method to image internal distortions and visualize successive twinning in three-dimensional (3D) [8-9]. However, the atomic misfit, the associated strain relief mechanism, and the bridging solid-angles in icosahedral MTPs are not yet quantitatively understood in 3D, primarily due to a lack of experimentally obtained 3D atomic structures of MTPs.
Methods
Gold MTPs, coated with a thin shell of palladium, and Pd@Pt core-shell MTPs were synthesized and deposited on thin Si3N4 film for high resolution imaging. We employed atomic resolution electron tomography to determine the 3D atomic coordinates of the MTPs. In short, the tomographic tilt series were acquired with aberration-corrected STEM in annular dark-field mode. After drift correction and denoising, we reconstructed the tomographic tilt series using the real space iterative reconstruction (RESIRE) algorithm; we then traced and classified the 3D atomic coordinates and chemical species.
Results
Here, we determined the 3D atomic structures of Janus icosahedral nanoparticles using atomic resolution electron tomography. A geometrically fivefold face (C5 side) consistently corresponds to a less ordered face (C5’ side) like two hemispheres (Figure 1c-d). We found that the 3D angular deficiency is compensated by insertion of edge dislocations and amorphization of several tetrahedra. The disordered amorphous domain with the largest solid angle is 14.6° larger than the ideal fcc domain, relaxing a large amount of strain and filling the largest angular defects (Figure 1e-h). Edge dislocations fill the angular deficiency near the axis. Our research has unveiled, for the first time, how the spatial gap of the icosahedron is compensated at the 3D atomic scale and provide a completely new insight on atomistic models for the modelling of formation mechanisms of fivefold and icosahedral twinned structures by molecular dynamics as well as computer simulations of lattice distortions and defects..
Conclusion
The novel physical insights are 1). We clearly show that the 3D angular deficiency is compensated by insertion of edge dislocations and amorphization of several tetrahedra instead of homogeneous dilatation in all the twenty tetrahedra. 2) Many structural characteristics of the icosahedral MTPs are

divided into two groups spatially distributed in two hemispheres of the MTPs, such as the bond orientation order parameters, solid angle, and the number of atoms. The twelve fivefold axes are not uniformly distorted; edge dislocations are preferably distributed in one side of the particle. Our observations provide a completely new insight on atomistic models for the modelling of formation mechanisms of fivefold and icosahedral twinned structures by molecular dynamics as well as computer simulations of lattice distortions and defects.

Keywords:

Fivefold, multiply twinned particles, icosahedron

Reference:

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Data Reduction and Clustering Approaches for a Comprehensive Phase Analysis inside Na-ion battery Cathode Materials

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

In the field of battery electrode materials, the presence of multiple phases undergoing structural modifications poses a significant challenge, as it can drastically impact battery performance and durability. These phases introduce complexity to the electrochemical processes that occur during charge and discharge cycles. As Na ions transfer between the positive and negative electrodes, structural transformations within the electrode materials take place. The spatial distribution of Na ion occupancy inside the crystal host leads to phase transitions and structural rearrangements, a phenomenon exacerbated by the co-existence of multiple phases related to multiple electrochemical activations. Each phase exhibits distinct electrochemical properties and undergoes different structural modifications during cycling, collectively influencing overall battery performance1. Therefore, it is imperative to characterize and understand the individual contributions of these phases and their spatial distribution.

For this purpose, we employed scanning transmission X-ray microscopy (STXM), offering a spatial resolution of 30 nm, coupled with X-ray absorption spectroscopy technique (XAS) at the HERMES beamline of the SOLEIL Synchrotron. Our focus was on the chemical evolution of Na3V2(PO4)2F3 (NVPF) upon charge/discharge cycles. These NVPF cathode samples underwent electrochemical cycling (versus hard carbon) processes to achieve various Na contents, specifically Na2.5V2(PO4)2F3, Na2V2(PO4)2F3, Na1V2(PO4)2F3, and Na0.3V2(PO4)2F3. NVPF is a promising material for the next generation of sodium batteries, but its complex phase behavior makes it difficult to characterize using traditional methods2. Therefore, the main objective of our study is to develop a novel approach for processing STXM-XANES data to obtain phase maps within individual crystals of the NaxV2(PO4)2F3 (x = 0.3, 1.0, 2.0, 2.5, 3.0) cathode material.

To achieve our objective, we developed a Python-based solution that leverages machine learning algorithms, i.e. non-negative matrix factorization (NMF) and the Pearson correlation coefficient (PCC). This approach enabled us to identify various sodium occupancy phases within individual Na3V2(PO4)2F3 crystals, providing detailed phase maps based on X-ray absorption edges of V and O modifying with the state of charge. With indirect observation in variations in sodium content inside individual crystals, our study offers fundamental insights into sodium-ion diffusion processes, which are essential for guiding the development of advanced cathode materials for the next generation of Na-ion batteries.

In parallel, we conducted a structural study based on electron diffraction using four-dimensional scanning transmission electron microscopy (4D-STEM) automated crystal orientation mapping (ACOM), on the same individual crystals as in STXM3. To facilitate this process, we developed the ePattern4 code to index diffraction spots and reduce the noise in large datasets. By combining ePattern with the software ASTAR (Nanomegas), a pattern matching approach for crystal orientation and phase determination, we generated phase maps based on the structural information of our sample. The close lattice parameters of the de-sodiated phases raise questions about the phase map reliability.

In this study, we developed a Python solution that allowed us to obtain phase maps and reliability maps for the STXM-XANES data, offering insights into the confidence level of our phase mapping results. These maps provide valuable information about the accuracy and reliability of the identified phases within the sample. Our study demonstrates the effectiveness of combining advanced analytical techniques with machine learning algorithms to characterize phase heterogeneities within battery electrode materials. By developing a novel approach for processing STXM-XANES data, we were able to gain detailed insights into the phase behavior of Na3V2(PO4)2F3 cathode material. These insights are crucial for advancing the development of sodium-ion batteries, paving the way for the design and optimization of electrode materials with improved performance and durability.

Keywords:

STXM, 4D-STEM, Battery material,NMF, PCC

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In and ex situ (S)TEM manipulation of 2D materials without air exposure

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Graphene—the one-atom-thick sheet of carbon—is the most famous of 2D materials due to its unique electronic properties and mechanical strength. However, its chemical inertness makes graphene also an excellent nearly electron-transparent support for other materials, nanostructures and even active individual atoms for example for single-atom catalysis. Similarly, the insulating counterpart of graphene, hexagonal boron nitride, has emerged as an interesting host for single-photon emitters realized as point defects. Unfortunately, since 2D materials consist solely of surface, they are easily obscured by the ubiquitous surface contamination that makes working with them challenging.

In this contribution, I will demonstrate that defect-engineering of graphene [1] enables its substitutional heteroatom doping [2] and growth of nanoclusters with a well-defined concentration and a narrow size distribution, as well as the direct correlation of its atomic structure and mechanical properties. I will further show that hBN can similarly be tailored with low-energy noble gas irradiation to create point-defects in a controlled manner. The defect manipulation is carried out in a vacuum system [3] that is directly connected to a Nion UltraSTEM 100 microscope, which allows sample cleaning, manipulation, and atomic-scale imaging and spectroscopy without air exposure. The defects are imaged via annular dark field imaging and the impurity atom identification is confirmed by electron energy loss spectroscopy.

The methodology employed here can be easily extended to other 2D materials, and is expected to lead to structures with potential applications in plasmonics, quantum information technology, sensorics and catalysis.

Keywords:

graphene, hBN, 2D materials, defect-engineering

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Deformation mapping in Lorentz transmission electron microscopy images of magnetic skyrmion lattices

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Poster Group 1

Background
Magnetic skyrmions are quasi-particles with a swirling spin texture. In chiral magnets, skyrmions are stabilized by the balance between the exchange interaction and the Dzaloshinskii-Moriya interaction. They have raised interest because of their potential applications in the field of spintronics, for instance in racetrack memories where they are treated as information carriers [1]. Axially symmetric chiral skyrmions usually form 2D hexagonal close-packed lattices [2]. Similarly to atomic lattices, skyrmion lattices can exhibit local deformations and crystalline defects such as dislocations and grain boundaries depending on the geometric constraints and the external magnetic fields [3]. Measuring deformations in skyrmion lattices is important to understand the interplay between the lattice structure and external influences.
In previous studies, skyrmion lattice deformations were investigated using real-space methods applied to Lorentz transmission electron microscopy (LTEM) images, which involves detecting intensity maxima or minima, finding nearest neighbors and calculating inter-skyrmion distances [3,4]. Here, we investigate the applicability of geometric phase analysis (GPA) [5]. GPA is a lattice deformation analysis technique based on Fourier transforms primarily used in high resolution TEM images. Compared to real-space methods, GPA is straightforward computationally because there is no need to detect individual maxima and perform any pixel-to-pixel operation. A Fourier transform of the image is first calculated and numerical apertures are applied to a pair of Bragg spots with non-colinear reciprocal lattice g-vectors. After inverse Fourier transform, the geometric phase term $\varphi(r)=-2\pi g.u(r)$ associated to each g-vector is retrieved, where u is the displacement vector. The deformation fields are then obtained using differentiation of the displacement fields.

Methods
Experiments were carried out using a TFS Titan TEM equipped with a Schottky field emission gun operated at 300 kV, a CEOS image aberration corrector and a 4k*4k Gatan K2-IS direct electron detector. The microscope was operated in Lorentz mode by using the first transfer lens of the aberration corrector as the main imaging lens. The objective lens was used to apply magnetic fields perpendicular to the sample which were precalibrated using a Hall probe. A liquid-nitrogen-cooled specimen holder (Gatan model 636) was used to vary the sample temperature. The Digital Micrograph software and a GPA plugin were used to calculate deformation maps. A TEM lamella of FeGe was prepared from a bulk crystal using focused Ga+ ion beam sputtering in a scanning electron microscope (FIB-SEM) FEI Helios dual-beam platform.

Results
Figure (a) shows an example of Lorentz TEM image of a skyrmion lattice in a 150 nm thick FeGe lamella recorded at 230 K with a defocus of 800 µm and in the presence of an external magnetic field of 145 mT. Normally, each skyrmion has 6 neighbors in a hexagonal lattice. However, it is common to observe dislocations formed by pairs of 5 and 7-coordinated skyrmions. Such a dislocation is present

in the middle of the image and it is magnified in Fig. (b) where the heptagon and the pentagon have been drawn. A Burgers circuit has also been traced to show the Burgers vector b. Figure (c-f) shows the experimental deformation fields calculated using GPA and Fig. (g-j) shows the corresponding theoretical deformation fields calculated from linear elastic theory. The horizontal deformation εxx, along the direction parallel to the Burgers vector, shows a butterfly shape with negative (compressive) deformation in the top part of the image and positive (tensile) deformation in the bottom part. The vertical deformation εyy shows a three-fold symmetry with alternating negative and positive deformations around the dislocation core. The shear deformation εxy and the rigid-body rotation ωxy show primarily loops oriented along the horizontal direction, parallel to the Burgers vector. Visually, the shape of the experimental and theoretical deformation fields is in good agreement even though the experimental images show some random fluctuations. To provide a quantitative comparison, Fig. (k-n) shows the difference between the experimental and theoretical deformation fields. The standard deviation δ was calculated and is indicated on each image. The region in the vicinity of the core (approximately 150 nm around the core, as indicated by the dotted square) was excluded from the measurement. The small values of δ ≈ 1% or 1° indicate a good quantitative agreement.

Conclusions

We have studied deformations in skyrmion lattices in a sample of FeGe by applying GPA to Lorentz TEM images. Deformation fields measured around a single dislocation were found to be in good agreement with deformation fields calculated from linear elastic theory. In the conference presentation, we will also show rotation fields measured at the boundaries between crystal grains and compare them with angles calculated from the density of dislocations. We will also discuss the evolution of deformation fields in applied magnetic fields series of LTEM images and explain how to calculate an orientational order parameter to study the evolution of the disorder. The influence of potential artifacts such as geometric distortions and large defoci will also be discussed.

Keywords:

skyrmions, Fresnel, deformations, lattice, GPA

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Characterization of functional nanoparticles applied in face masks by STEM-EDX

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background incl. aims
The unique properties of nanomaterials are being leveraged to create textiles with enhanced fabric properties, a trend exemplified within the production of face masks since the onset of the Covid-19 pandemic. The use of silver (Ag) is advertised in several commercially available face masks because of its biocidal properties, claiming a higher form of protection to the wearer. The silver biocide can present itself either in ionic form, nanoparticle (NP) form or as part of a composite material, however, this is not always correctly advertised[1]. In addition, the presence of titanium dioxide (TiO_2) nanoparticles, acting as a whitening and mattening agent, in synthetic fibres applied in face masks has been demonstrated[2], even when no specific information was provided on their packaging. Since TiO_2 is a suspected carcinogen (IARC 2B) and potential negative health effects are reported for nano-silver[3], their use in masks raises health concerns because of the potential inhalation exposure to nanoparticles. This emerging area of concern lacks methodological development regarding the assessment of inhalation exposure to nanoparticles during mask use. To assess the potential of particle release and associated risks, at first a detailed characterization of the localization and the form of nanomaterials within the mask is necessary. This work applies scanning transmission electron microscopy (STEM) coupled with energy-dispersive x-ray spectroscopy (EDX) on a set of 10 face masks. The method is evaluated in terms of ability to distinguish different nanoforms, to measure particle properties (size/shape/agglomeration state) important in the context of risk assessment in line with the European Union's regulatory framework and to assess potential release of NP.
Methods
A set of 10 face masks, including community, surgical and FFP2 masks, was characterized. 6 masks, where the application of biocides (5 Ag, 1 unspecified) was advertised, and 4 masks, demonstrated to contain TiO_2 (2 of them also had Ag advertised) were selected to assess the form, localization and measurement of Ag (and other possible biocides) and TiO_2, respectively. Since the masks usually consist of multiple layers of fabric, at first the layer with the highest Ag or TiO_2 content was identified using inductively coupled plasma assisted optical emission spectroscopy or mass spectroscopy and selected for subsequent STEM analysis. The mask was disassembled and a piece of 1 mm x 5 mm was cut from the selected layer. The preparation of TEM specimens from the pieces of mask followed the methodology described in Wouters et al.[4] Ultra-thin sections of the textiles were prepared by embedding them in epoxy resin, followed by ultra-thin sectioning using ultramicrotomy. Subsequent STEM-EDX analysis was carried out on a Talos F200S transmission electron microscope equipped with a high angle annular dark field detector and a Super-X EDX detector (Thermo Fisher Scientific, Eindhoven, The Netherlands). Imaging and image analysis was done using Velox software (V3.11, Thermo Fisher Scientific).
Results

In 5 face masks, a form of (nano)silver could be identified. In each of those masks, the silver biocide presents itself under a different form: (i) a silver-silica NP composite, (ii) a reaction mass of silica, titanium dioxide and silver chloride NP (see graphic), (iii) a silver-zincoxide NP composite, (iv) nanoscale silver precipitation on a mineral complex and (v) NP containing a combination of silver and sulfur or silver and tin. In 4 out of 5 cases, the identified biocide form is not matching the advertized description, which is often ambiguously reported or lacks the mention of a 'nanoform'. In cases (i) and (iii) the silver biocide is embedded within the fibres, while in the other three cases it is at least partly present on the surface of fibres. The amount of silver-containting particles detected in each sample is too low to build a statistically relevant size distribution but their sizes range from 5-200 nm and they have a spheroidal shape.
Even though only 4 masks were selected for evaluation of incorporated TiO_2 particles, TiO_2 was detected innine masks. This was notmentionedon the packaging or in the provided technical information. The TiO_2 appears in the form of aggregates and agglomerates of spheroidal (nano)particles which are embedded within the fibres, and some are also present on the edge of fibres. A preliminary quantitative analysis of TiO_2 in selected samples, reveals an average aggregate/agglomerate size of 186 nm and an average constituent particle size of 100 nm in terms of the minimum Feret diameter, in agreement with the typical textile grade TiO_2[5].
Three masks are found to consist of fibres with a (partial) coating of NP. In one mask this coating consists of a mix of TiO_2 and AgCl NPs embedded within silica (see graphic). The mask with unspecified biocide consists of fibres with a partial coating of agglomerated silica NP with constituent particle sizes on the order of a few tens of nm. And lastly, one mask, which applies a photocatalytic activation of the biocide, consists of a mix of polymer fibres and silica-containing, fibre-like structures. The latter ones have agglomerated TiO_2 particles attached to them, with constituent particle sizes varying from approximately 10 to 50 nm, i.e. smaller than the textile grade TiO_2 reported before. These three masks are considered to be most prone to partice release, based on the localization and amount of observed nanomaterial.
Conclusion
STEM-EDX proves itself as a valuable methodology to allow identification of the type of biocide, the localisation of the (nano)particles and their size measurement within fibres of face masks. Our analyses reveal that packaging labels are often not accurately reporting the type of biocide or nanoparticles applied within the product, highlighting the need for regulatory control. The presence of NP on the fibre's surface presses for investigations of potential release of particles under realistic usage conditions, which will be explored in the next stage of the project.

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Keywords:

Nanoparticles, biocide, masks, ultramicrotomy, STEM-EDX

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Strain Sensitivity of STEM-GPA-based Strain Map

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background
Strain engineering, one way to improve the performance of semiconductor devices is to apply strain intentionally to improve device characteristics. In addition, as the size of semiconductor devices decreases to improve the integration density, it has become important to analyze the strain distribution in localized areas that directly affect device performance. The GPA-based strain analysis method is widely used to analyze the strain, but there has been no clear numerical verification of the strain detection limit. In this study, we clarified the empirical limitations of the GPA method and proposed optimal experimental conditions for its application to real devices.

Methods
We used artificial STEM-ADF images with in-plane strains of 1%, 0.1%, and 0.05% to compare the strain values detected by changing the pixel resolution of each image with the actual strain values. Each image was tested up to a data binning factor of 2n (n = 1 to 6) in the original image. In addition, we considered Poisson noise to mimic the real STEM imaging conditions. Finally, we used STEM-ADF images obtained from a silicon device with various pixel resolutions and compared the strain mapping results.

Results and Conclusions
The strain analysis results using images containing 1%, 0.1%, and 0.05% strain without considering Poisson noise showed that the detected strain is 1 ± 0.001%, 0.1 ± 0.002%, and 0.05 ± 0.01% respectively for images with a pixel resolution of 22 pm or less. When considering Poisson noise, a comparison of the standard deviation (SD) of the strain values showed that for spatial resolutions of 22 pm/px or higher, strains of 1% and 0.1% can be detected with a reliability lower than 0.02% of SD. In the case of 0.05%, the strain value is detectable up to a spatial resolution of 22 pm/px, but the strain precision deteriorates from 0.04% to 0.7%. Interestingly, as the binning factor increases, a periodically repeating local strain undulation was observed on the strain map. This is due to the difference in distance between atoms and periodicity between pixels, resulting in a Moiré pattern that degrades the accuracy of the strain map. In this presentation, I will additionally share the results of strain maps obtained through GPA on real devices and will compare strain maps obtained by 4D-STEM.

Figure1. (a) Simulated Si (Z.A = [110]) STEM-ADF images containing 0.05% in-plane strain with various binning factors. Corresponding in-plane strain maps (b) without and (c) with Poisson noise.

Keywords:

GPA, strain sensitivity, strain limit

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A retractable, compact Secondary Electron Energy Spectrometer attachment for Scanning Electron Microscopes

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Poster Group 1

Background:
Energy spectrometers, such as the EDS (Energy Dispersive X-ray Spectroscopy) attachment in a Scanning Electron Microscope (SEM), have been widely used in material science for quantitative elemental analysis and contamination identification. However, EDS typically requires a minimum accelerating voltage of 6 kV and a high current primary beam to generate sufficient characteristic X-rays for accurate analysis. Unfortunately, these high voltages and currents can lead to charging effects in non-conductive materials like semiconductors and biological samples, hindering the analysis process. Additionally, EDS provides only microscale resolution due to X-rays being generated from a relatively large volume. As an alternative, Secondary Electron Energy Spectroscopy (SEES) has emerged as a promising method for achieving nanoscale quantitative material analysis using a low-voltage SEM (LVSEM) [1,2].
Methods:
A novel electric sector compact Secondary Electron Energy Spectrometer was designed and optimized using direct ray tracing of electrons through a Finite Element Method (FEM) field distribution. Based on the optimized design, a prototype was constructed and tested within a commercial SEM using a 1 to 2 KV low-energy primary beam. Various materials, including gold, copper, and silicon, were examined to assess the spectrometer's elemental identification capabilities.
Results:
The simulation results predict that the spectrometer achieves a relative energy resolution of 1.54% for a polar entrance angular spread of 20°, azimuthal angular spread of 150°, and a working distance of less than 10 mm. Experimental results confirm that different elements can be easily distinguished based on their secondary electron energy spectra FWHM and peak position, as shown in the graphic, in which test experimental SE energy spectra of gold and copper samples are presented. Furthermore, due to its compact size, the spectrometer can be conveniently installed or removed.
Conclusions:
A compact/retractable electric sector secondary electron energy spectrometer attachment for SEMs has been designed, and is predicted to have high energy resolution and high collection efficiency. This new attachment can be seamlessly integrated into the SEM chamber, greatly enhancing the capabilities of the SEM system with its quantitative elemental analysis and material science ability. Furthermore, 2D mapping and dopant profiling are also expected in the near future.

Keywords:

SEM, Energy spectrometer, elemental analysis

Reference:

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Approaching picosecond temporal resolution in off-axis electron holography

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Poster Group 2

In the course of new technological and instrumental possibilities, time-resolved investigations in transmission electron microscopy are currently gaining particular relevance [1]. For phase-resolving methods, however, there are hardly any realizations for the investigation of dynamic processes on ultrashort time scales, although these are of great importance, especially for in-operando investigations of dynamic potential distributions in nanostructures (e.g. switching semiconductor or magnetic topological nanostructures).
Recently, interference gating (or iGate) has emerged as a simple, but robust method to address this circumstance [2]. It is based on the intentional destruction of the interference pattern for a large part of a measurement, utilizing short undisturbed time periods (called gates), which are synchronized to a periodic process under investigation, to generate time-resolved interferometric information. In particular, the simple technical realization of the method (only a two-channel signal generator, two coaxial cables, an dedicated aperture holder and an electrical biasing sample holder are required) enables low-barrier access to the investigation of ultrashort phenomena on existing instruments [3]. Through continuous improvements in sample preparation, automated measurement and reconstruction routines, control signal design, and the necessary electron-optical components, the time resolution achievable with iGate has recently broken through the picosecond barrier. By an application to silicon diodes, switching at repetition rates in the MHz range, variations of the local capacitance C(x,y,t) could be observed and the cause of the anomalous (MOS-cap-like) switching behavior of a defective semiconductor nanostructure was revealed [4]. A schematic representation of the experiment is illustrated in the accompanying figure. The nanostructured diode sits in between two extended metallic contacts, forming an overarching electrical system (e.g. parallel connected capacitances in vacuum C(x,y) and the diode C(x,y,t)).
In addition, the method itself could already be implemented in transmission electron microscopes in further laboratories in very short time frames (less than a work day), whereby the FPGA-based conversion of a JEOL JEM-2200FS into a versatile phase-resolving instrument with sub-nanosecond temporal resolution is discussed here in detail. The presented results will highlight the advantages of image-based investigations of projected dynamic potential distributions with nanometer and sub-nanosecond resolutions and their application to materials science problems, and pave a new path for decoding dynamic processes and their physical causes directly at the point of their occurrence.

Keywords:

electron-holography, time-resolved, ultrafast, semiconductor-nanostructures, interference-gating

Reference:

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Synthetic data generation and Mask-RCNN for Transmission Electron Microscope Image Segmentation

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Background incl. aims
The rapid advancement of neural networks (NN) has led to a diverse array of innovations, including autonomous driving, image and text generation. Notably, within image processing, object detection and classification has been significantly improving with a variety of NNs leading the way, particularly U-Net, Fast-RCNN, Mask-RCNN. A significant challenge while training a NN is the substantial volume of training data required to achieve satisfactory results. ImageNet or COCO are typical datasets, with 14M and 200K labelled images (image and ground truth pairs), respectively, used to train NNs to detect everyday objects, people and animals within images. The Transmission Electron Microscope (TEM) field stands to benefit from machine learning image analysis techniques, which can reduce the time and effort of researchers analysing images, especially for particle size and morphology. Furthermore, automation of image analysis will lead to an increase in reproducibility when compared to manual analysis, however, there is a lack of publicly available data which is segmented and labelled appropriately for such applications. Here we present an alternative approach using synthetic data as a method for overcoming the lack of segmented and labelled data, which differs from simulated data and is a pictorial representation rather than a specifically generated simulation of a TEM image and use Mask-RCNN for instance segmentation, a form of image segmentation that detects individual objects in an image. The application shown here is for nanoparticle analysis. The principles can be generalised to all image data produced in a transmission or scanning electron microscopes.
Methods
Our research introduces an innovative approach to generating synthetic images for training machine learning algorithms in nanoparticle detection and classification. Unlike traditional simulation methods (multislice, Bloch waves), our method utilises Python packages to generate images, bypassing the need for costly computing resources. We illustrate this approach through two distinct examples: the creation of polylatex spheres and silica particles on holey carbon substrates with ultra-thin continuous carbon layers and expand to gold nanoparticles. By randomising various parameters such as magnification, particle size, illumination, and contrast, we generate synthetic data for training an instance segmentation machine learning algorithm.
Specifically, we employ a Mask-RCNN model pretrained on the COCO dataset and refine it using our synthetic transmission electron microscopy (TEM) images, a technique known as transfer learning. This approach significantly improves the model's performance by leveraging knowledge from a broader dataset for a specialised task. Subsequently, we apply the trained model to segment experimental TEM images sourced from Certified Reference Materials (CRMs), which are industry standards used for analytical validation and microscope calibration within the PAT4Nano standardization project. To enhance the algorithm's segmentation accuracy and reduce false positives, we explore augmenting the training data with synthetic particles overlaid on experimental TEM grid substrate images. The efficacy of this approach is demonstrated through comparative analysis and visualisation.

Expanding our data generation efforts, we simulate various scenarios including continuous carbon structures and diverse shapes of gold nanoparticles. Additionally, we adjust intensity values to fall within the 1-99 percentile range, further enriching the dataset. This augmented dataset is utilised to train a secondary Mask-RCNN model, which is then deployed for predicting segmentation of experimental images.

Results

Our evaluation of the models was conducted separately, utilising the Mean Average Precision (mAP) metrics. The Average Precision (AP) is quantified as the area under the precision-recall curve (PR curve), where precision denotes the ratio of true positive predictions to all predictions, and recall represents the proportion of correct positive predictions to all true positive cases. The mAP aggregates all AP values across various classes or categories. The initial model, trained on the polylatex/silica dataset with approximately 500 synthetic images, achieved a mAP of 0.40 when tasked with predicting 90 experimental images. Subsequent augmentation of the training dataset with an additional 500 synthetic images of particles overlaid on experimental backgrounds resulted in a notable improvement, raising the mAP to 0.80 for the same set of 90 experimental images. In parallel, the second model, trained on the gold nanoparticles dataset, attained a mAP of 0.84 when predicting 19 experimental images. Both model predictions were subjected to a minimum detection confidence threshold of 0.9. The graphics section visually depict the predictions generated by models 1 and 2, respectively, providing tangible insights into their performance.

Conclusion

Our research demonstrates the successful training of an instance segmentation algorithm using synthetic data generation, with notable enhancements are achieved by incorporating experimental backgrounds into the training process. Additionally, our findings illustrate the adaptability of the algorithm across diverse datasets characterised by varied backgrounds and particle shapes. Moving forward, our focus will centre on refining particle edge detections to achieve pixelwise accuracy and advancing nanoparticle measurement techniques. Furthermore, we aim to develop an open-source, user-friendly interface for the generation of synthetic data adaptable to a wide variety of transmission electron microscopy (TEM) data. This interface will include a built-in trainable model which can be tuned and refined with user generated synthetic data, facilitating broader accessibility to automated image segmentation for researchers with electron microscope data.

Keywords:

ML, Instance Segmentation, Synthetic Data

Reference:

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Probing chemical pathways in polymer membranes despite electron beam damage

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Poster Group 2

Reverse Osmosis (RO) membranes are used for seawater desalination in the oil and gas industry. These membranes are made of a polyamide (PA) 'active' layer, around 100-500 nm thick, a polysulfone (PSf) support and a polyester backing layer. The PA layer is thought to control ion flow through the membrane yet very little is understood about how that is achieved at the nanoscale. A number of techniques have been used to try to characterise the PA layer but none of them have the spatial and energy distribution necessary for nanoscale chemical mapping. Here we test whether scanning transmission electron microscopy EELS (STEM-EELS) techniques can be used to map chemical functionality across flat RO membranes with nm-scale resolution. We argue that, despite the high electron flux required during acquisition, careful comparison to dose-managed control experiments on standards together with multivariate analysis post-processing techniques can be used to extract useful information about the distribution of functional chemistry from spectra acquired from damaged PA.

Practically, commercial RO membranes are very rough making them difficult to characterise. For this reason, in-house prepared 10 nm thick PA membranes prepared via an interfacial polymerisation technique, were used for this study. STEM EELS studies were carried out on a Nion UltraSTEM 100MC 'HERMES' monochromated transmission electron microscope at SuperSTEM (Daresbury, UK). The microscope was operated at 100 kV with a probe size of 0.9 Å and convergence and collection semi angles of 31 and 44 mrad respectively.

First, chemical maps of C, N and O K-edges, the main components of the membranes, were obtained. From the nitrogen signal the exact width of the membrane could be established as N is only present in the membrane itself. Control beam damage studies were carried out on large areas (800 nm x 800 nm) of plan-view samples so that the degree of damage on the C K-edge could be assessed. The parameters required to avoid detection of beam damage effects were impractical for obtaining chemical information with a pixel size of 2 nm. However, with higher electron fluences (e.g. 3-10 x 107 e/Å2 per probe size or 6-10 x 103 e/Å2 per pixel size), whilst damage occurred to the carbon bonds, the dominant features in the fine structure of the C K-edge were in fact still present and variations could be mapped across the PA membrane with a spatial sensitivity of 2 nm. Application of multivariate analysis to the raw high electron fluence data made it possible to assign peaks related to specific co-ordination environments by comparison with the control data fine structure.

This work shows that despite severe electron beam damage evident by the loss of fine structure of the C K-edge, information about functional chemistry within the PA can still be achieved. This suggests that spectra obtained where the electron flux has been high, may still be interpreted provided care is taken to assess the extent of possible beam damage and carry out control experiments. With continued advancement of technologies such as stable cold stages, high frame

rate cameras and direct electron detection capabilities it should become easier to map functional chemistry across radiation sensitive soft materials with nm-scale high spatial and energy resolution.

Keywords:

polymers
EELS
beam-damage
chemical pathways

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Oxidation states of IrOx characterised by EELS and ED-PDF

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
The global interest in hydrogen as a primary fuel has been fostering fundamental research of IrOx (Iridium oxide) - the most stable catalyst known for water-electrolysis to produce hydrogen. The presence of Ir(IV)/Ir(III) redox coupling is crucial for Oxidation Evolution Reaction (OER) activity of IrOx while metallic Ir only plays a little role in the aspects of stability and conductivity [1,2]. However, spatial distribution of Ir(III) with respects to Ir(IV) in IrOx catalysts has never been demonstrated previously. This kind of distribution would not only be a clear picture showing how Ir(III) can work together with Ir(IV) in an active and stable catalyst but also provide guidance for chemical engineering in tuning the oxidation state configuration for higher performance-to-mass ratio of the catalyst material.
Methods
In this talk, we will present the oxidation states of IrOx characterised by STEM-based EELS and Electron Diffraction based Pair Distribution Function (ED-PDF). The EELS method is based on measuring the profiles of electronic excitations from 5p and 4f to 5d and above the Fermi level (O and N edges). The EELS experiments were designed to minimise the effect of thickness variation (that can alter the plasmonic background) while improving signal/noise and energy resolution for detailed electronic-structure profiles.
Beside EELS, Electron diffraction pair distribution function (ED-PDF) [3] is another capable technique for characterisation of Ir-O bonding and IrOx atomistic structures, especially amorphous IrOx [4].
Results
O&N-edge profiles for different Ir oxidation states are shown in Figure 1(a). As shown in Figure 1(b), the microscopic distribution of Ir(IV), Ir(III) and Ir-metal can be mapped thanks to the differences seen in the corresponding O&N-edge profiles. Complementary to the Ir O&N-edges, EELS of O K-edge can give information about the atomistic structures of different IrOx phases. Figure 1(c) shows that amorphous IrOx, hollandite-type IrOx and the rutile-type structure of IrO2 can be distinguished clearly by the hybridisation profile in the O K-edges.
Ir-oxide (both amorphous and crystalline) and Ir-metal can also be characterised by ED-PDF. Figure 1(d) shows different distribution profiles of interatomic distances (particularly Ir-O bonds and Ir-Ir metallic bonds) for rutile-type IrO2, amorphous IrOx and Ir-metal.
Conclusion
This work shows the powerful capability of EELS and ED-PDF for characterisation of IrOx. The importance of EELS combined with EDPF will also be discussed in the presentation.

Figure 1. (a) EELS O&N-edges of different Ir oxidation states; (b) EELS mapping of Ir(IV), Ir(III) and Ir(0)-metal; (c) EELS O K-edge of amorphous IrOx, hollandite-type IrOx and rutile-type IrO2; (d) ED-PDFs of amorphous IrOx, rutile-type IrO2 and Ir-metal.

Keywords:

EELS, oxidation state, ePDF, IrOx

Reference:

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Atom Probe Tomography experiments performed in a (Scanning) Transmission Electron Microscope

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background
Atom Probe Tomography (APT) is intrinsically a 3D characterization technique that provides tomographic reconstructions of materials with a near atomic scale resolution. For APT, specimens must be prepared as sharp needles. This is most of the time realized using FIB SEM and before analysis in APT, specimens can easily be characterized in a TEM or STEM using specific holders or protocols. Hence, correlative analysis by (S)TEM and APT gives access to a wide range of information about the specimen. Unrivaled spatial resolution of (S)TEM, availability of 4D-STEM or diffraction with the combination of the 3D composition fields accessible by APT hence leads to a better description of materials by the correlation of structural, physical and chemical characterization.
To make correlative analysis by (S)TEM and APT more accessible, efforts have been foreseen and made to join both techniques in a single instrument [1-4]. This work presents the main achievement of an instrumentation project started at GPM in 2014, which is the implementation of an Atom Probe in a commercial JEOL F2 (Scanning) Transmission Electron Microscope.

Methods
Specifics TEM holders were designed first for a JEOL 2010 TEM, on which they were tested, and then transferred to a JEOL F2 installed at University of Rouen in 2022. These holders offer the possibility to perform APT experiments either at room temperature or cryo temperature (78K measured at the tip). They accept APT specimens which can be polarized with a positive voltage up to 8 kV. Electrostatic pulses are superimposed to trigger field evaporation with a pulse repetition rate of 20 kHz. The APT detector consists in an advanced-delay line setup, mounted on a port facing the goniometer of the microscope.

Results
The figure below shows the setup realized in the framework of this project. The ion sensitive and time resolved spatial detector is mounted behind the column (on the left in the image) and APT specimens are loaded classically in the goniometer, using home designed holders. All imaging conditions available with the microscope can be applied to characterize APT specimens. Here, an illustration is given with the automated crystal orientation mapping method. A nearly equiatomic Fe-Cr alloy with an ultrafine grain structure has been submitted to a heat treatment leading to spinodal decomposition. The visualization of grain boundaries in the APT specimen allows to localize unambiguously the location of APT analysis in the specimen.

Conclusion
This new instrument, combining APT with (S)TEM, opens up new prospects for unambiguously correlating the characterization of structural defects (such as grain boundaries, precipitates or

dislocations) identified in TEM with the chemical characterization, atom by atom, accessible in tomographic atom probes.

Keywords:

Atom Probe Tomography, STEM, instrumentation

Reference:

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Opening the third dimension to your SEM with integrated fs-laser

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Poster Group 1

Background
Scientists and engineers in materials research laboratories performing sample preparation using focused ion beam (FIB) or plasma focused ion beam (PFIB) instruments face challenges in accessing deeply buried structural features and preparing large cross-sections and trenches at micro- and mesoscale sizes. Such preparation by FIB or PFIB is time-consuming and ineffective, as it occupies an expensive microscope that could be used for more efficient processes: high-quality imaging, high-precision analysis, and sample preparation for atomic-resolution imaging. There is a need for an effective solution for the rapid preparation of site-specific micro- and mesoscale samples.
Methods
In response to these challenges, the authors present a laser scanning electron microscope (LaserSEM) as a new Zeiss solution for fast and cost-efficient high-quality site-specific sample preparation. The Zeiss LaserSEM is a field-emission scanning electron microscope (FE-SEM) with an integrated fs-laser for large 3D volume sample fabrication. This solution enables to achieve a fast site-specific preparation from the meso- to the microscale. The Zeiss LaserSEM accelerates workflows such as 3D tomography on huge sections, preparation of arrays of pillars for micromechanical testing, or even multi-modal workflows e.g. correlative experiments between X-ray microscope (XRM) and scanning electron microscope (SEM).
Results
The examples of rapid access to undersurface features by fs-laser and Cut2ROI Workflow with the ability to identify, access, prepare, and analyze deeply buried sites with precise navigational guidance using the correlation between X-ray and electron microscopes from ZEISS are presented. The fs-laser processing recipes for different materials and their application for a large cross-sectioning with further energy dispersive spectroscopy (EDS) and electron back scatter diffraction (EBSD) analysis, as well as pillar preparation for nanoindentation, compression tests, and nanoCT, are presented.
Conclusion
Integration of fs-laser into SEM allows rapid access to deeply buried structures and fast large-volume material removal and achieves high resolution and contrast imaging with the benefits of Zeiss Gemini optics. ZEISS LaserSEM is a site-specific cross-section and micro/mesoscale fabrication solution that rapidly prepares samples by accurately removing millimeter volumes of material using integrated FE-SEM imaging to achieve accuracy.

Keywords:

LaserSEM, sample preparation, SEM

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Unique in-situ characterization workflow of Cathode Components using AFM-in-SEM

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
As the demand for efficient and sustainable energy storage solutions grows, a lot of effort is dedicated to comprehending the battery components' functionalities. Not only exploring new configurations but also new materials are essential for improved efficiency, safety, lifetime, etc. of the new-generation lithium-ion batteries [1]. However, such materials require precise sample surface preparation and handling to avoid unwanted reactions or surface changes which then influence understanding of the battery component features.
Methods
In this work, we focus on the in-situ study of Cathode Active Materials (CAM) and present a unique measurement workflow that preserves the sample surface from oxidation without compromising sample preparation quality, moreover, enabling valuable insights into chemical and electrical properties. The CAM tape samples are prepared in a glovebox on a proper holder, cross-sectioned using Broad Ion Beam (BIB) polishing, and further moved to the Atomic Force Microscope in a Scanning Electron Microscope (AFM-in-SEM) for electrical and chemical characterization. The sample transfers between all instruments are realized using a safe sample transfer system under a controlled environment, so the sample is never exposed to air or humidity, and thus, the surface remains clean and preserved from chemical reactions.
Results
With such a workflow, we studied Nickel-Cobalt-Manganese (NCM) tape with solid electrolyte, see Fig. 1. We determined the electronic conductivity using Conductive AFM (C-AFM) and distinguished the individual elements using Energy Dispersive X-ray Spectroscopy (EDS), all under in-situ conditions. We see that the NCM grains are well conductive, while the solid electrolyte remains non-conductive, which is correct, as the solid electrolyte only has an ionic conductivity (the dark part of Fig. 1c). The most conductive seems to be the secondary particles interface (the brightest part of Fig 1c), which perfectly correlates to the carbon additive observed in EDS map (Fig. 1a). Such precision would be unlikely possible or very challenging without the in-situ analysis.
Similarly, we observed a CAM tape with different grains, Nickel-Cobalt-Aluminum (NCA) and NCM, in a liquid electrolyte. The connection of the EDS map with electronic C-AFM shows that the conductivity of the grains differs 1000 times. From understanding such grains and their conductivity, the difference should be much lower. As we see in the SEM image, the NCM grain had probably been delaminated from the collector, and the conductive connection with the NCM particle was established via the inter-particle interface through the NCA particle.
Conclusion
These results emphasize the need for advanced measurement workflow and instrumentation, which could greatly help in understanding the cycling effects, durability, or lifetime of both primary and secondary particles of battery components. The above-mentioned characterization workflow is convenient, time-efficient, and suitable for a proper understanding of failure analyses and quality control of the battery components. Our findings were obtained using AFM LiteScope, which is

compact enough to be used in SEM, in this case, Helios 5 Hydra DualBeam, CleanMill for fine polishing, and CleanConnect for sample transfer [2].

Keywords:

in-situ, batteries, AFM, SEM, conductivity

Reference:

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2. We would like to thank Libor Novák and Petr Zakopal from Thermo Fisher Scientific for their assistance with sample surface preparation.

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Breaking barriers: Innovative methods for 4DSTEM diffraction data acquisition and processing in SEM

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

Background incl. aims
Electron diffraction is a reliable tool to obtain information about the crystalline structure of nanoscale materials. With the recent emergence of more sensitive detector technology (Direct electron detectors e.g., Timepix and Medipix) the requirement for the dose applied to the sample in experiment drastically decreases.
Diffraction requires a far simpler setup as compared to conventional transmission electron microscopy as the diffraction pattern can be obtained without any lenses. This lends itself ideally to an implementation in a conventional SEM tool having several advantages such as providing more space for sample stimuli and manipulation, lower cost, and much smaller footprint. Typically, the acceleration voltage in SEM is lower compared to TEM. However, in the past years the tendency for particles' size decrease can be observed and SEM could become an attractive alternative to TEM for such materials.

Rather than taking a single diffraction pattern, the combination of a SEM tool and a fast direct electron detector allow for the efficient acquisition of 4DSTEM diffraction data, having a diffraction pattern for each probe position in a STEM scan.

There are however a few challenges:
- A lower acceleration voltage compared to TEM leads to a decrease of maximum sample thickness that can be used.
- The field of view in a SEM is much larger than in TEM. On the one hand, this is advantageous to gather statistical information on a much higher number of particles. On the other hand, a large part of this field of view is often empty resulting in long recording times with large portions of the data being empty.
- Distinguishing diffraction reflexes from the background can become challenging at the reduced acceleration voltage. This reduces the intensity of the reflections and results in poor averaged diffraction patterns which are dominated by the background signal.

To negotiate these issues new methods for data acquisition and processing should be developed. In the current work we aim to push the limit of transmission electron diffraction in SEM, mitigate beam damage and sample contamination, to make SEM a versatile tool for a high-resolution electron diffraction structure investigation.

Methods
Experiments made on a Tescan Mira FEG SEM. Diffraction data collected via an Advacam AdvaPix. Sample manipulated by custom stage of 3 Xeryon linear piezo motors in XYZ configuration mounted on rotation stage for tomography. Data processing is done in Hyperspy, controls are made with Python, image denoising is achieved with a convolutional neural network, Edge detection is realized with OpenCV.

Results
To reduce the acquisition time and avoid empty areas in the 4DSTEM dataset we use a fast overview scan from which we detect all particles positions and shapes with computer vision. In a second step we determine a scan pattern to visit only those areas which likely to give diffraction patterns. We call this approach ROI 4DSTEM: each detected particle is marked with a bounding box. We then use the assumption that particles or agglomerated particle sets tend to be much thinner on their outer boundaries as compared to their centers. These boundaries are therefore the ideal positions to get good quality diffraction data from if the assumption holds that the core of the particles is the same crystal structure as its perimeter area. We call this method Edge Detected 4DSTEM. By utilizing image denoising via a modern convolutional neural network and edge detection algorithms we were able to create with high precision a mask of scan positions that is later used to position the incident beam only at those areas that most likely will lead to good quality diffraction patterns.
This approach allows us to perform 4DSTEM in SEM in a much more effective manner. Indeed, for a standard 4DSTEM raster scan of 1024x1024 scan positions we would get 1,048,576 diffraction patterns, or approximately 182 GB of data to store and 18 minutes to collect all this data at 1 ms exposure time. In our ED4DSTEM method, we only collect 23,000 positions, resulting in 4 GB of data and 23 seconds of total recording time for the same 1ms exposure time. This is almost 50 times faster/more efficient as compared to conventional raster scanning resulting in a significant reduction of beam damage and contamination on top of the benefit of pre-filtered diffraction data of much higher average quality.
By processing each individual diffraction pattern and storing only the peak positions and their intensities we can obtain a massive data reduction that considerably simplifies the following data analysis steps. This reduced dataset can then be used for any kind of diffraction analysis. In our case we are collecting “virtual” ring patterns as a radially integrated histogram of the scattering angle of all observed peaks, weighted by their intensity. This method has the advantage of suppressing the background in the diffraction pattern that originated from substrate/noise/amorphous content in the sample that otherwise would dramatically deteriorate the quality of a position averaged diffraction pattern. An added benefit is the sub-pixel position accuracy that can compensate for beam convergence and pixel size effect.

Conclusion
In this work we present methods that allow for more effective 4DSTEM diffraction data acquisition on dispersed crystalline nanoparticles at low acceleration voltages as well as methods for more reliable data processing. Our method, compared to conventional 4DSTEM raster scan, drastically decreases the total 4DSTEM acquisition time, beam damage and contamination while providing a dramatic data reduction in the process that could significantly simplify follow the data analysis. These approaches are also amenable to TEM and could be especially useful in case of beam-sensitive objects.

Keywords:

4DSTEM, SEM, TEM, EdgeDetection, ImageRecognition

Reference:

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[5] The authors acknowledge the financial support of the Research Foundation Flanders (FWO) project SBO S000121N

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Unraveling point defects and moiré patterns in liquid exfoliated PtSe2

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Poster Group 2

Background: The successful synthesis/exfoliation of Transition Metal Dichalcogenides (TMDs) covered the need for 2D materials with an energy bandgap, which is essential for transistor applications. PtSe2 belongs to Noble-Metal Dichalcogenides, which is a subcategory of TMDs, consisting of metals from group 10 of the periodic table. It exhibits layer-dependent electronic properties, allowing it to be employed either as a semiconductor or semimetal. PtSe2 has a broad range of applications in sensing, optoelectronics, and photonics [1], while properties can be tuned through defect engineering. The occurrence of magnetism in PtSe2 has been attributed to the presence of point defects [2] while stacking sequences different than the 1T, can also enhance its performance as a piezoresistive sensor [3]. However, it is still unknown which defects in ultra-thin PtSe2 layers are caused by ultrasonication, during liquid-phase exfoliation, and how they affect the properties of the system.
Methods: All samples were exfoliated using the LPE method including eco-friendly solvents. The structural characterization of exfoliated samples was performed using the FEI Titan 80 – 300kV FEG S/TEM operated at 300 kV and the Nion UltraSTEM operated at 60 kV. Electronic properties of defected PtSe2 were calculated using FHI-aims all-electron code, while geometries were optimized using FHI-vibes. Multislice STEM imaging simulations were accomplished with abTEM code [4].
Results: Selected area electron diffraction testified the 1T structure of PtSe2, while bright-field TEM and HRTEM imaging confirmed the exfoliation into thin flakes with an average diameter higher than 600 nm. STEM-EDX mapping confirmed the presence of all involved elements in exfoliated samples. Low-voltage Z-contrast imaging was chosen to minimize the generation of defects due to beam irradiation effects. Multiple type point defects, including vacancies and antisites, as long moiré patterns, have been observed. The presence of these configurations was validated by comparing experimental with simulated STEM images, using the multislice method. The symmetry breaking due to the coupling of multiple point defects results in modified band structures and properties.
Conclusions: In this work, PtSe2 was exfoliated with liquid-phase exfoliation, and different point defects and moiré patterns were observed with low-voltage aberration-corrected STEM imaging. These different structural configurations were testified with multislice STEM imaging calculations. The effect of these structural configurations on electronic properties was estimated with ab initio calculations.

Keywords:

Aberration-Corrected STEM, DFT, Defects, PtSe2,

Reference:

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Structural characterization of the active and inactive conformations of the vasopressin V2 receptor using cryo-EM

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background:
The antidiuretic hormone arginine-vasopressin (AVP) binds to the kidney V2 receptor (V2R) triggering Gs protein coupling and thereby activating adenylyl cyclase. First, the AVP-V2R-Gs signaling complex regulates body water balance and solute transport in mammals, and exhibits physiological effects throughout the body. In addition, arrestins also interact with this G protein-coupled receptor (GPCR) to stop G protein activation and to initiate other key signaling pathways.
Dysfunctions in this GPCR lead to clinical disorders, ranging from dysregulation of water balance such as the syndrome of inappropriate antidiuretic hormone secretion (SIADH, associated to many forms of cancer), congestive heart failure, hepatic cirrhosis, to urine disorders (incontinence, nocturia). Moreover, V2R loss-of-function or constitutively active mutations lead to two rare genetic diseases: (a) the congenital Nephrogenic Diabetes Insipidus (cNDI) characterized by excessive urine voiding, and (b) the nephrogenic syndrome of inappropriate antidiuresis (NSIAD) characterized by excessive water loading and hyponatremia. Unfortunately, despite the global prevalence of kidney-related diseases, there is currently no safe prescribed drug on the market for V2R-related disorders. The nonpeptide antagonist Tolvaptan approved for treating hyponatremia (SAMSCA®) and recently for polycystic kidney disease (PKD, the most frequent Mendelian inherited disorder affecting million people worldwide), has hepatotoxic effects. Discovery of therapeutic compounds based on structural information may have a direct impact on millions of citizens. To that purpose, determining both the active and inactive conformations of the V2R is crucial for a comprehensive understanding of the complete conformational changes responsible for the receptor activation or inhibition. Defining the molecular mechanisms occurring in the core of the V2R during these changes is essential for a complete understanding of its function, and to ultimately propose innovative drugs based on rational and structural data.

Methods:
By combining biochemistry and structural biology, we have successfully purified the V2R in complex with either Gs protein or βarrestin1, and structurally characterized the complexes using cryo-EM. Determining structures of the inactive states remains a challenging endeavor due to intricate biochemistry, their dynamic nature, their small size and sophisticated activation mechanism. Given this, the V2R has been modified by introducing a BRIL (cytochrome b562 RIL, 15 kDa) module in the third intracellular loop (ICL3) of the receptor. It is a promising strategy, as a Fab anti-BRIL and an anti-Fab nanobody have been designed to significantly increase the size of the receptor for cryo-EM (~100 kDa).

Results:

We have recently reported the cryo–electron microscopy (cryo-EM) active structures of the wild-type AVP V2R in complex with either Gs protein and βarrestin1. First, the cryo-EM characterization of the AVP-V2R-Gs allowed us to describe three structures, mostly based on the positioning of the Gs relative to the receptor. Those structures reveal an original receptor-Gs interface in which the Gαs subunit penetrates deep into the active V2R. The structures help to explain how V2R R137H or R137L/C variants can lead to cNDI and NSIAD. Then, the AVP-V2R-βarrestin1 structure reveals an atypical position of βarrestin1 compared to previously described GPCR-arrestin assemblies, associated with an original V2R/βarrestin1 interface involving all receptor intracellular loops. Phosphorylated sites of the V2R carboxyl terminus are clearly identified and interact extensively with the βarrestin1 N-lobe, in agreement with structural data obtained with chimeric or synthetic systems. Finally, in the core conformation, the βarrestin1 finger loop inserts into the intracellular cavity of V2R and overlaps the same binding space than the Gs α5 helix, explaining the desensitization mechanism of the V2R.

We are actually characterizing the V2R into its inactive state, using different types of antagonists. They fully antagonize cAMP signal, arrestin recruitment and MAP kinase phosphorylation associated to V2R activation. Preliminary cryo-EM data demonstrate the potential of the V2R-Bril construction to reach high resolution for the inactive state of the receptor. Resolving the cryo-EM conformation of the antagonist-V2R complex will constitutes the first characterization of an inactive structure of the V2R.

Conclusion:
Elucidating high-resolution structure of different states of the receptor using cryo-electron microscopy will allow to apply structure-based drug design to discover effective innovative drug compounds using computational approaches. In addition to bring those receptors to drug design initiatives, our data will provide new insights into GPCR's function.

Keywords:

Cryo-EM, V2R, structure-based drug design

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MicroReactor for battery materials synthesis in SEM

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Poster Group 1

Background incl. aims
In-situ SEM can help with understanding and optimization of processes forming (nano-)materials usable in electronic devices and energy storage systems. The high-temperature synthesis of these materials often happens in reactive, explosive, or harmful environments.
This study aimed to prove the functionality of the MicroReactor device for in situ SEM imaging during experiments requiring reactive gases (such as pure hydrogen or hydrogen sulfide) that result in the synthesis of the currently used and possible future battery materials.

Methods
MicroReactor device [1] equipped with a MEMS heating & biasing chip [2] was used in Helios G4 UX and Scios DualBeam systems. The MicroReactor's low-volume reaction chamber, coupled with a retractable lid enabling work with reactive gases during SEM operation and open access for sample preparation by FIB, assures improved process cleanliness and safety compared to an environmental SEM. In-lens detectors of the SEM column as well as STEM or 4D STEM detectors placed below the transparent MEMS chip can monitor changes in the processed sample. Real-time gas analysis of reaction products is possible on the outlet line connected to the reaction volume. After gas-assisted processing, EDS and EBSD analyses can follow while the sample remains heated up to 1200°C. In the latest design, the MicroReactor sample holder can be transferred in argon between a glove box and a FIB-SEM chamber.

Results
Three novel applications of MicroReactor will be presented.
1) The capacity and lifetime of NMC cathode in Li-ion battery depend on the parameters of the high-temperature lithiation process. The MicroReactor can be used to follow morphological changes of the NMC particles which indicate the state of lithiation. An example of a possible way of lithiation process, that uses LiOH as the source material, is shown in the graphics. A pure oxygen environment was selected in the MicroReactor to remove the hydrogen from the final cathode material.
2) The growth of tungsten suboxide W18O49 nanowhiskers from WO3-SiO2 nanofibers was studied in the MicroReactor [1]. Reduction of the WO3 in nanofibers was possible at 800°C in a hydrogen environment (100 Pa). The tunability of redox properties, thermal stability, and large surface area make tungsten suboxides promising materials for applications in energy storage systems.
3) Tungsten disulfide is a 2D material with unique properties applicable in many fields including electronics, catalysis, and energy storage. A modified pumping system of the Scios DualBeam equipped with MicroReactor enabled the injection of hydrogen sulfide & hydrogen mixture which was essential for the successful sulfidation of a sample containing W18O49 nanowires. Using a combined in-situ SEM and ex-situ TEM analysis, it was possible to explain the mechanism of WS2 synthesis from tungsten suboxides for the very first time [3].

Conclusion

The three presented examples demonstrate novel applications of the MicroReactor in SEM. The second example proved process cleanliness sufficient for sample reduction at high temperatures. The creation of this abstract proves the possibility of safe work with dangerous gases in the third example, which at the same time demonstrates the possibility of exploring the fundamental behavior of chemical processes.
In addition to the conclusions summarized in [1] and [3], it can be stated that in situ SEM equipped with the MicroReactor is a useful complement to the more widespread TEM in-situ techniques, which can bring new insight with several benefits. SEM excels in capturing reaction kinetics at a larger scale and provides topographical information in the surface-sensitive signal of secondary electrons. The presented technique can further be combined with ex-situ TEM analysis or with ETEM, both on the same MEMS chip that is used in SEM.

Keywords:

In-situ SEM, MicroReactor, batteries, WS2

Reference:

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Liquid Phase Transmission Electron Microscopy

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Poster Group 2

Background
Liquid phase transmission electron microscopy (LPTEM) is a small field in the realm of microscopy. However, its significance appears in understanding various scientific domains. However, measuring the main driving force in many liquid-based processes, e.g., in electrochemistry, is still yet to be fully explored. Similarly, understanding the liquid itself is key in quantum mechanical models for our understanding of how liquids behave.
Electron holography (EH) has emerged as a new tool to measure both the electrostatic- and magnetic potentials of a specimen. As the electrons from the incoming electron beam undergo elastic scattering, a phase shift is presented in the exit wave [1]. In EH, the phase shift is measured by measuring against a reference wave, which has not been shifted in phase. By computing the phase shift between the two waves, the object and reference wave, the electrostatic and magnetic potential are retrieved by post-mortem analysis.
It, therefore, begs the question if it is possible to combine EH with LPTEM in order to measure those potentials, which may be used in more delicate systems in order to study liquids and liquid-based processes where the potential is the main driving force, e.g. in any electrochemical experiment. We coin the new method of Liquid Phase Transmission Electron Holography (LPTEH).
Methods
In LPTEH, we make use of two primary chips, which have previously been used in LPTEM to examine nanoscale liquid-based processes. The first type of chip used, the nanochannel chip, is a chip comprised of two bonded silicon wafers with nitride as the window material, see Figure 1(a). The thickness in the nanochannel chip is much better defined, given that the channels have a width of < 2 µm, where bulging is less compared to the clamped liquid cell with a width of > 100 µm. However, these chips have not yet been fabricated with electrodes. Thus, the nanochannel chip is primarily used via LPTEH to determine the intrinsic properties of the liquids.
The other type of chip, the clamped liquid cell, is an LPTEM cell where the liquid is squeezed between two electron transparent windows, see Figure 1(b). The thickness of the liquid is defined by how thin a liquid can be squeezed by squeezing on the surrounding O-ring. Additionally, we have in our clamped liquid cell fabricated Au electrodes, where we start to examine how to use those chips for any electrochemical experiments. Lastly, EH itself is done using a TEM. There are two main methods for EH, namely in-line and off-axis EH. For our experiments, we use off-axis EH, since the TEM at DTU (the FEI 80-300 kV E-TEM) has a standard ThermoFisher biprism (a quartz filament) installed in the SAD plane. We carry out experiments on a single biprism setup, where the biprism voltage determines both the interference width and the fringe spacing.
Alignment of the object and reference wave can be done in different ways for both chips. For instance, if one were to examine the liquid itself on the nanochannel chip, the reference wave is usually passed through the bonded nitride between the channels, as that defines a common 0 rad phase shift. If features in the liquid are instead studied, the reference wave can be passed through the liquid, thus only the phase shift elastic scattering with the feature is measured.
Results

We show our latest results of both systems and some of the features studied with LPTEH. First and foremost, we extend an earlier paper on the study of the mean inner potential (MIP) of water [2], where our updated results show how the MIP can be understood as a refractive index for high energy electrons by studying the change in MIP of glycerol:water mixture, see Figure 1(c). By increasing the volume fraction (f_g) of glycerol, it can be seen that the MIP of the mixture increases linearly shown by the dashed black line in Figure 1(c). This linear increase with solute concentration can be thought of as an analogy to how the optical refractive index increases linearly with solute concentration, which was also how the MIP was first described by H. Bethe [3]. We compare our experimental results to a first-order approximation of the MIP, known as the independent atom model, shown as the dashed red line in Figure 1(c), and compare our results of pure MQ water with [2] shown as the blue data point. Evidently, it can be seen that the MIP is the refractive index of high-energy electrons, and it behaves similarly to the optical refractive index.
In our second example, we show how LPTEH can be used to measure the potential from the solid-liquid interface, see Figure 1(d). This study shows how LPTEH can be used to measure the potential of the electric double layer, which is key in any electrochemical process. Its potential profile indicates that of a screened potential, which is understood as the screening of free ions in the liquid. Such a measurement of the potential is the first of its kind, as most techniques use an almost ideal non-polarizable reference electrode. However, the e-beam used for EH/LPTEH is ideally non-polarizable, as it does not create its own double layer contrary to any solid reference electrode. It could, therefore, be speculated that the e-beam can be used in the future as an ideal reference electrode and be able to measure the driving force in any electrochemical experiment.
Conclusion
In summary, we have shown two studies of how LPTEH can be used to measure the driving force and intrinsic properties of a liquid. By combining two TEM-based methods, LPTEM and EH, we have shown how we may start to use LPTEH as a new tool to understand liquids in a deeper understanding. We note that this is the first of its kind, and thus, there is still much to improve in terms of spatial resolution to better capture the innermost layer of the double layer, where our results only provide evidence of the outermost layers of the double layer.

Keywords:

LPTEM, Electron Holography, MIP, EDL

Reference:

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EELS at Extreme Energy Losses; complementary Information to X-ray Absorption Spectroscopy (XAS) in a TEM

Dr Sorin Lazar[1], Mrs. Maria Meledina[1], Mrs. Claudia S. Schnohr[2], Mr. Thomas Hoeche[3], Mr. Peter Tiemeijer[1], Mr. Paolo Longo[1], Mr. Bert Freitag[1]
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Poster Group 1

Energy electron loss spectroscopy is already an established technique to study structure and property relationship required to optimize materials and make break-through inventions in material science. So far EELS lacks performance at energy losses higher than 2 keV to obtain bonding or coordination state information via ELNES or EXELFS with high lateral resolution [1]. We used a new optical setup designed to optimize the coupling between spectrometer and post-specimen optics for high energy resolution and high collection efficiency. The new optical setup allows to minimize the acquisition time (minutes) and the beam current to avoid sample damage or drift artifacts and to achieve results beyond the earlier attempts reported in literature [2].
The real application benefit in EELS is its spatial resolution which is much better than the micron resolution that XAS can achieve. A challenge lays in analysis of elements in low concentration due to the signal strength. For this reason we have made a comparison between XAS and EELS data from a sub-micron size precipitate on the Zr_L23 edge in high-strength, translucent glass-ceramics in the system MgO-ZnO-Al2O3-SiO2-ZrO2 with only 5.8% Zirconia in the compound [3]. The comparison of ELNES with XANES reference data clearly reveals that the coordination is CN=8.
For a more quantitative comparison the setup was used to acquire Cu_K edge at 8.98 keV energy loss from metallic Cu, CuO and Cu2O. The EELS data is benchmarked against XAS data in XANES and EXELFS and relevant information is extracted from the EELS data using similar analysis as done for XAS data. It is noteworthy to mention that the results show an excellent match of EXELFS and EXAFS and the EELS data allows extracting the bond length with very good accuracy as it will be shown in this work.
In order to further assess the optimization of the entire EELS optics including the S/TEM column we have acquired a series of K-edges at extreme energy loses namely the Mo K-edge at 20 keV, Ag K-edge at 25.5 keV and Sb K-edge at 30.5 keV as presented in figure 1 (from left to right: Cu K-edge, Mo K-edge and Ag K-edge) and for some of the materials the bond length has been calculated from the EELS data.
With this work we will also discuss the limitations of the current technology and how they are mitigated by the deep integration of the filter in the optics on the new platform.
The integrated approach optimizing the entire EELS optics including the S/TEM column enables this increase in performance. The examples presented show the power of correlative studies involving X-ray absorption spectroscopy (XAS) and high loss EELS, which opens new applications to combine the advantages of both techniques.

Keywords:

XAS, EELS, TEM

Reference:

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Structure Prediction using Data Derived Potentials and EELS

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Background

(Scanning) Transmission Electron Microscopy ((S)TEM) coupled with Electron Energy Loss Spectroscopy (EELS) is a powerful and information rich technique capable of providing information about composition and local bonding environments at a high spatial resolution. However, the Energy Loss Near Edge Structure (ELNES) of core-loss EELS peaks can be difficult to interpret due to the large number of factors which can influence the ELNES shape. Traditionally, spectra collected from known standards have been used to interpret ELNES, or spectra have been simulated from structures obtained from databases such as the Materials Project or the Inorganic Crystal Structure Database. This work presents a workflow for using Ab-Initio Random Structure Searching (AIRSS) accelerated by Ephemeral Data Derived Potentials (EDDP) to predict the structures of compounds after measuring their composition using quantitative EELS, then using CASTEP and OPTADOS to predict the ELNES of these structures. The workflow has been tested on silicised boron nitride, a system important in high temperature ceramic matrix composites.

Methods

TEM lamella were prepared from single crystal SiC and silicised BN. A TEM sample of commercially pure BN was obtained by dispersing the BN nanopowder on a carbon grid. The TEM samples were analysed using STEM-EELS, and elemental maps, thickness information, and ELNES datasets were obtained.

These EELS datasets were fitted with the model fitting functionality of Hyperspy, and these models were used to calculate the compostitions of the samples.

After calculating the composition of the silicised BN sample, a unit cell of this composition was generated containing multiple formula units. The cell was then used to train a data derived potential, capable of calculating the energy of an arrangement of atoms given their coordinates. This was performed with the EDDP code, which generates random sensible structures and calculates their energy using the plane wave pseudopotential Density Functional Theory (DFT) code CASTEP with the Regularised Strongly Constrained and Appropriately Normed (rSCAN) functional and the Many Body Dispersion (MBD) semi-empirical dispersion correction to account for long range van der Waals forces. These atomic coordinates and energies are used as the training data for an ensemble neural network.

The initial potential learned by the system will have a large error, but is sufficiently robust enough to allow for structure searching, by generating a random sensible structure and relaxing it to a local minimum using the learned potential. DFT is then used to calculate the actual energies of the minima of this initial structure search, and expand the training data for the learned potential. The network is retrained on the expanded training dataset.

This process is repeated 5 times until a robust and accurate potential is formed. This potential can then be used for rapid random structure searching and can predict low energy structures of the unit cell defined by the measured composition.

The lowest energy structures are then relaxed further using DFT to ensure they are at true energy minima, and the ELNES spectra for each of the atomic environments present are calculated using CASTEP and OPTADOS.

These calculated spectra can be used as reference spectra in a library and can be compared directly with ELNES measured in STEM-EELS experiments.

Results

The validity of the model fitting functionality in Hyperspy for determining the composition of silicised BN was tested using the pure BN nanopowder and semiconductor grade SiC, and the method could predict the compositions of these known compounds, proving that the quantification method is suitable for systems containing Si, C, B and N.

Initial EDDP calculations involved the Si-B-N system. A unit cell containing 3 Si, 6 N and 5 N atoms was created, and this composition was chosen based on literature values of the typical composition of silicised BN. An ensemble neural network containing a single layer with 5 nodes was produced, and this resulted in a potential with an error of less than 30 meV / atom on the testing set.

This data derived potential was used for a random structure search of over 10,000 structures. The lowest energy structures were selected, and a stricter geometry relaxation was performed using DFT. The EELS spectra for these low energy structures were calculated with CASTEP and OPTADOS before being compared with experimental spectra from silicised boron nitride. This allowed for information about the local bonding environment to be understood.

Conclusions

The EDDP method has enabled rapid random structure searching of systems which were previously too large to search efficiently. This has opened up a workflow in which EELS can be used on a wider range of materials, including those which do not have high quality reference standards or even known crystal structures in large databases.

The usefulness of this workflow was demonstrated on silicised BN, and further work could use a similar workflow to understand how the composition and ELNES of these materials changes as a result of environmental degradation.

Keywords:

EELS, DFT, ML, Structure Prediction

Reference:

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Assessing the sensitivity of 4D-STEM measurements for electric field mapping at the sub-micrometer scale

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Poster Group 2

Four-dimensional scanning transmission electron microscopy (4D-STEM) can provide quantitative information about electric fields in materials and it is currently a technique of choice for such measurements. However, mapping electric fields over large (micrometers) field of view with a very high sensitivity and a nanometer spatial resolution remains challenging [1]. Herein, we perform a systematic study to evaluate the expected achievable precision of 4D-STEM measurements for electric field mapping in vacuum.
All experiments were performed on a Thermo Fisher Scientific Titan Themis TEM operated at 300 kV in micro-probe configuration and equipped with a MerlinEM detector. First, the effect of microscope parameters was investigated by acquiring 4D-STEM maps in vacuum while varying one parameter at a time such as convergence semi-angle, electron beam current, dwell time or camera length. To assess the accuracy of the electric field mapping, a coplanar capacitor was fabricated by focused-ion beam (FIB) deposition of Pt on a MEMS biasing chip (DensSolutions). The center of mass (COM) method was used to analyze the shift of the transmitted beam, which was achieved by subtracting the COM map obtained at an applied voltage (object map) from a COM map in free field conditions (reference map) [2]. The reconstructed electric field was then compared with the results of finite element method (FEM) simulations using COMSOL [3].
The electric field sensitivity was evaluated in terms of measurement error. The results show that reducing the convergence semi-angle increases linearly the sensitivity but has a negative effect on the spatial resolution. Electron beam current and dwell time are also shown to have a direct effect on the field sensitivity, as directly controlling the electron dose. In practice, low electron dose values mean low sensitivity, which follow the root square behavior of the Poisson noise. Finally, the camera length study shows that this parameter has no influence on the sensitivity except for extremely small values when it strongly deteriorates. Overall, the experimental trends are in good agreement with previous theoretical studies [4]. The microscope parameters that optimize the measurement, including a convergence semi-angle of 370 µrad, dwell time of 2 ms, electron beam current of 150 pA and camera length of 1.45 m, were then used to quantify the stray electric field of the capacitor induced by an applied difference of potential between the plates. The integral of the in-plane component of the stray electric field over the electron path was directly compared with a FEM model where the simulated electric field was integrated along the z direction, assumed to be the direction of electron propagation. This comparison revealed good agreement for applied voltages in the range of 1 to 10 V, corresponding to a maximal projected field in the capacitor gap of 2 to 25 V, respectively. Finally, the electric field reconstruction was repeated using the template matching (TM) method and the results showed that proper choice of template is essential for comparable trends.
In conclusion, this study highlights the effects of microscope parameters on the sensitivity of large field of view electric field measurements using 4D-STEM and provides guidelines for optimized measurements. The full quantification of the electric stray field induced by an applied external potential is demonstrated as an example.

This work was supported by the Swiss National Research Foundation (SNSF) under award number 200020_204240.

Keywords:

4D-STEM
Electric_field_mapping
Finite_element_simulations

Reference:

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In situ TEM/EELS and spatially resolved XAS/XRF analysis of CuO electrocatalyst for CO2 reduction

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Poster Group 1

Background
Electrochemical devices such as fuel cells and electrolysers enable chemical and energy transformations that are required to help achieve net-zero targets by 2050. One of the emerging technologies is electrochemical reduction of CO2 into value-added products, such as carbon monoxide, formic acid and ethylene. [1] The best performing cathode catalyst for producing ethylene and other C2+ products in a CO2 electrolyser is oxide-derived copper (OD-Cu), which is metallic coper formed in situ via reduction of a copper oxide-based electrode in the electrolyser. The activity of OD-Cu tends to be significantly higher than that of similar Cu catalysts that have been reduced ex situ prior to being incorporated into the electrolyser, which suggests that additional or superior active sites are generated during the in situ electrochemical reduction [2]. In addition, the catalyst can experience significant structural changes under CO2 electroreduction conditions [3]. Obtaining structure-performance relationships of copper requires in situ characterization, which is due to the surface oxidation and possible restructuring the metal experiences when exposed to open circuit potential and air during disassembly of the electrochemical cell. In situ characterisation imaging techniques such as XAS and XRF are therefore a crucial step to capture the active catalyst formation and the morphology dynamics under electrochemical conditions. These techniques suffer from lower spatial resolution, which limits the level of understanding of the underlaying dynamics for reactions involving gases or liquids. This motivated our investigation of the hypothesis that specific conditions of thermal reduction experiments can led to the Cu structures formed under electrochemical conditions. In this way, a higher spatially-resolved analysis could be done by extrapolation from gas-phase investigation.
Methods
In this work we present in-situ TEM gas phase thermal reduction experiments of CuO samples and correlate them with in-situ liquid phase electrochemical reduction of CuO samples carried out on the i14 nanoprobe beamline (Diamond Light Source). Synchrotron-based spectroscopy techniques, such as X-ray absorption spectroscopy (XAS) and X-ray fluorescence spectroscopy (XRF) are very powerful methods to study the chemical nature of the catalyst under relevant conditions [4]. Combining the i14 nanoprobe beamline(down to 50 nm resolution) with aberration corrected electron microscopy (Å resolution) allows to use spatially resolved XRF imaging to study morphology and dynamics in a liquid biasing environment and link it to gas-phase experiments on the nanoscale.

Results/Conclusion
We will show the benefit of combining these techniques to reveal the effect of the thermal reduction on the CuO particles (morphology / oxidation state) through a combination of in-situ STEM imaging and EELS versus the electrochemical reduction as revealed by the in-situ liquid cell i14 XRF/XANES experiment (Figure 1). We will also address the challenges with liquid biasing experiments such as beam-induced damage, especially to the ionomer that is commonly present in catalyst layers (e.g. Nafion), and challenges involved with sample preparation.

Keywords:

CO2-reduction, Cu, in-situ, TEM-synchrotron,

Reference:

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Epitaxially-assisted Sn Crystal Phase Selectivity on Hybrid Superconductor-Semiconductor Quantum Nanowires

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background incl. aims
Hybrid systems of semiconductors connected to metals/superconductors provide interesting platforms to explore exotic topological properties for topological quantum computing purposes. In particular, Sn is a promising material as it can present two main structural phases when deposited on III-V semiconductor nanowires (NWs): semimetallic α-Sn (cubic) and metallic β-Sn (tetragonal) which also presents superconductivity below 3.7 K. In addition, α-Sn can turn into a topological insulator under strain [1] and β-Sn has the potential to turn into a topological superconductor when hybridized with a strong spin-orbit coupling 1D semiconductor, as In-V (V=As/Sb) nanowires (NWs) [2]. However, few studies focus on the deposition of Sn in NWs, and coexistence of α-Sn and β-Sn on InSb NWs has been reported. Therefore, achieving Sn phase selectivity on In-V NWs remains challenging.

Methods
In this work, we have deposited Sn on different In-V compounds including zincblende (ZB) InSb and InAsSb and wurtzite (WZ) InAs and we explore the role of epitaxial relationships between Sn and the NW in crystal phase selectivity based on Atomically Resolved Scanning Transmission Electron Microscopy.

Results
After the optimization of different growth conditions, our studies reveal that the main parameter determining Sn phase is the crystal lattice of the NW, which acts as a template assisting the Sn crystal arrangement. InSb acts as a template for the growth of a monophase α-Sn shell that evolves to β-Sn rich shell with increasing β-Sn density when increasing layer thickness, and so, the accumulated strain. In a second step, poorly lattice matched NW templates of InAs WZ were employed for Sn growth, where selective β-Sn could be grown by enhancing interface energy between α-Sn and the NW. At the same time, our structural characterization is complemented with device fabrication and electrical measurements, where superconductivity yield in the measured NWs is in agreement with the atomic structure of Sn.

Conclusions
As a summary, our studies reveal that Sn phase control is mostly driven by the structural arrangement of the nanowires and we are able to selectively tune the Sn phase formation by employing the desired NW template. Therefore, this work provides a key to control Sn phases on hybrid NWs to permit the tuning of the topological properties by unveiling the insights of Sn growth.

Keywords:

epitaxy III-V Sn nanowires hybrid

Reference:

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Structure of the first isolated polinton-like virus and its host

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Poster Group 2

Viruses in the PRD1/Adenovirus (AdV) -like lineage infect bacteria, archaea and eukaryotic organisms and build their capsids with proteins with beta-jelly rolls orthogonal to the capsid surface (1). It has been suggested that Polintons (dsDNA transposons) are members of this lineage, because they encode genes very similar to viral proteins involved in replication, assembly, maturation, and possible structural proteins with jelly roll fold. These observations prompted the hypothesis that Polintons may have evolved from a PRD1-like ancestor (2). Subsequent evolution would have resulted in the "polintovirus" elements splitting into two different ways of life: the transposable, capsid-less integrating elements, and the bona fide viruses. Marine metagenome analyses have revealed a group of putative polinton-like viruses (PLVs) in eukaryotes. PLV genomes contain genes for single and double jelly roll proteins and a packaging ATPase, but lack the protease and integrase genes present in the polintons (3). Therefore, PLVs could represent a minimal version of the PRD1/AdV-like lineage in eukaryotic hosts. The study of this kind of virus could reveal the adaptations necessary for the jump from a bacterial to eukaryotic host. The first isolated PLV, TsV-N1, infects the unicellular eukaryotic algae Tetraselmis striata (4)

We have used cryo-electron microscopy to solve the structure of TsV-N1, and FIB-SEM volume electron microscopy on resin-embedded cells to analyse the structural changes induced by infection in the host. The high-resolution capsid structure (2.7 Å) corroborates the placement of TsV-N1 in the PRD1/AdV-like lineage and reveals similarities with those of bacteriophages in this lineage, although TsV-N1 infects a eukaryotic host. These similarities include: triangulation number T = 21d, size, genome arranged in concentric rings. However, the main differences with bacteriophages in this lineage are the absence of an inner membrane, the major capsid protein fold being more similar to other eukaryotic viruses, a complex cementing protein network and a high packing fraction, more similar to those of HK97-like viruses. TsV-N1 induces large changes in the host during the infection. Although this virus assembles in the nucleus, other cellular structures are modified too. Particularly striking is the disappearance of starch grains and the cell wall, suggesting that the virus hijacks energy sources for propagation. More studies must be carried out to understand how this virus reaches the nucleus. However, its similarities with AdV (no membrane, nuclear assembly) suggest that similar genome delivery mechanisms may be involved.

Keywords:

Polinton-like virus, cryo-EM, cellular-sections, volume-EM.

Reference:

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Correlative analytical scanning electron microscopy reveals periprosthetic tissue response to nanoparticles and wear debris

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Poster Group 2

Background
One of the leading factors in aseptic loosening, osteolysis, and subsequent failure of implants used for total joint replacement is the production of wear debris and the associated reaction in periprosthetic tissue [1]. Nanoscale wear particles (0.2-0.8µm) are a critical component in the activation of macrophages and inflammatory response [2]. Particle composition, identified using electron microscopy and energy dispersive x-ray spectroscopy (EDS), can determine their site of origin [3] and is a critical factor in macrophage cytotoxicity and the activation of pro-inflammatory cytokines [4]. However, EDS can only provide part of the information required to understand the molecular interactions between patient tissue and wear debris. Organic/inorganic molecules and their interactions are not detected by EDS alone.
Methods and aims
Here we present a correlative study incorporating a fast compositional imaging method for rapid identification of regions of interest (backscattered electron x-ray, BEX, imaging) across large tissue areas, combined with higher resolution EDS and Raman spectroscopy. Samples were prepared following methods presented by Xia et al. [3] with modification for scanning electron microscope (SEM). The uncoated resin-embedded tissue samples were imaged in a Zeiss Gemini460 SEM in variable pressure mode at 10kV using a Unity BEX imaging detector combined with an Ultim Max 100 EDS detector, followed by analysis with RISE (Raman Imaging Scanning Electron). RISE imaging was conducted using a 785 nm laser, tuned to 25 mW using the TruePower module. The multimodal imaging datasets were colocalised and overlayed using the Relate software package (version 9.3.10333).
Results and conclusions
We investigated the ultrastructure, chemical and elemental properties of tissue interacting different wear particles. We observed particles of titanium accumulated into large clusters (50 to 500µm) surrounded by tissue that had distinct density difference. High density areas of the tissue were characterised by an elevated weight percentage (wt%) of calcium (Ca) and phosphorus (P) (Figure 1 BEX). RISE analysis of the same region shows spectra indicating two phosphate populations: one spectrum was characteristic of hydroxyapatite, the second was bound to tissue stained with lead citrate with the presence of lead confirmed using both Raman spectroscopy and EDS. A third spectrum peak in the carboxylate region, overlays with the low-density tissue observed by BEX (Figure 1 RISE, RISE spectrum, BEX + RISE overlay). Similar results were observed in samples containing much smaller (<0.8µm) cobalt nanoparticles.
The multimodal and correlative approach combines the speed of BEX screening with high-resolution characterisation (EDS and RISE) of tissue reactions to nanoscale wear particles. The proposed correlative workflow can generate detailed structural and molecular data for studying the effects of metal nanoparticles from implants and may provide a fast screening technique to facilitate diagnostic analysis of pathological features in the future, in particular the molecular mechanism that triggers host allergic and inflammatory response to orthopaedic implants.

Fig 1. Overlayed BEX and RISE images show 3 distinct tissue phases characterised by different electron density (BEX), chemical (RISE, RISE spectrum) and elemental compositions (BEX, BEX+RISE overlay). BEX images display Uranium (U) and Lead (Pb) in cyan, Titanium (Ti) in yellow, Calcium (Ca) in pink and Phosphorous (P) in blue, electron image in greyscale. RISE images and spectra: Hydroxyapatite in red, resin substrate in blue, metal bound Phosphate in green.

Keywords:

Correlative, EDS, Raman, BEX, SEM

Reference:

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Structural and metabolic dynamics of plant cells in context of heat acclimation

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LS-07 (1), Lecture Theater 4, august 28, 2024, 11:30 - 12:30

Background incl. aims
In order to survive sub-lethal heat stress, plants possess the ability to acclimate to moderately elevated temperature. They are altering their physiology in many different ways to maintain cellular homeostasis despite the change in environmental conditions. The carbohydrate metabolism is important for energy storage and biomass production and is also involved in regulation of heat acclimation response. To accurately resolve subcellular metabolic fluxes and compartment specific metabolite concentrations in the model organism Arabidopsis thaliana, it is necessary to combine ultrastructural and metabolic data.
Methods
A non-aqueous fractionation procedure to determine subcellular metabolite concentrations was combined with 3D imaging of leaf tissue by serial block-face scanning electron microscopy, which resulted in two datasets of control and heat treated samples.
Results
We were able to generate a dataset of subcellular volumes of A. thaliana leaf tissue. This allowed us to calculate effective metabolite concentrations in three compartments of the cell, namely cytosol, vacuole and chloroplasts. Applying a kinetic model of carbohydrate metabolism, subcellular fluxes were estimated which revealed metabolic heat acclimation strategies of plant metabolism and provide evidence for a tightly regulated metabolic network.
Conclusion
The combination of microscopic techniques with metabolic and photosynthetic measurements allows a more holistic interpretation of plant heat acclimation and can help preventing misinterpretation of the individual datasets.

Keywords:

heat acclimation arabidopsis carbohydrates SBF-SEM

Reference:

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Fürtauer, L., Weckwerth, W., & Nägele, T. (2016). A benchtop fractionation procedure for subcellular analysis of the plant metabolome. Front Plant Sci.
Seydel, C., Kitashova, A., Fürtauer, L., & Nägele, T. (2022). Temperature-induced dynamics of plant carbohydrate metabolism. Physiol. Plant.

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Open top dual-view light-sheet microscope for live imaging of large multicellular systems

Franziska Moos[1,2], Simon Suppinger[1,2], Gustavo de Medeiros[1,3], Koen Oost[1], Andrea Boni[3], Camille Rémy[3], Sera Weevers[1,2], Charisios Tsiairis[1,2], Petr Strnad[3], Prisca Liberali[1,2]
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Poster Group 1

Background
Visualizing single cell dynamics shaping complex tissues and understanding the underlying mechanisms is an overarching goal in biology. However, these processes often cross large spatiotemporal biological scales, are affected by sample-to-sample variability and are rather sensitive to light. Light-sheet microscopy is due to its high spatiotemporal resolution and low phototoxicity a method of choice for imaging of living samples. Multi-view light-sheet microscopy has been designed for imaging of large specimens by acquiring images from opposing directions using several objectives or rotating the sample. However, these techniques are limited in throughput. For multi-sample imaging, open top, inverted or single objective approaches have been developed, where the sample is supported from the bottom while allowing accessibility from the top. These microscopes can not provide imaging from opposing detection sides. We aimed at developing an open top, dual-view and dual-illumination light-sheet microscope, combining the advantages of the multi-view and open top design enabling multi-position 3D live imaging of large multicellular systems.
Methods
The presented open top, dual-view and dual-illumination light-sheet microscope uses two opposing detection objectives to image samples from two directions and two illumination objectives tilted from the horizontal. This arrangement of the four objectives creates an unobstructed linear space for a custom designed sample holder which has space for up to four interchangeable sample chambers enabling multi-positional imaging, manufactured from FEP by a thermoforming process. Water is used as immersion medium and is placed in a reservoir filling the space between the objectives. The objective area is equipped with environmental control (humidity, temperature and CO2).
Results
We demonstrate the capabilities of the light-sheet microscope by long term live imaging of murine intestinal organoids, hydra, liver organoids, gastruloids, murine salivary gland organoids and human colon cancer organoids for more than 8 days. Moreover, with the multi-chamber design we show parallelized biochemical perturbations on murine intestinal organoids. To demonstrate its potential to extract quantitative features across biological scales, we developed a custom light-sheet data processing and analysis pipeline. Single cell tracking and segmentation analysis is performed during intestinal organoid and gastruloid growth. We could reveal cell shape changes and an increased cell motility induced by a Wnt activation pulse, suggesting a partial epithelial to mesenchymal transition in gastruloids.
Conclusion
In conclusion, we developed a dual-view and dual-illumination open-top light-sheet microscope designed for long-term, multi-position imaging of diverse samples at single-cell resolution. The image quality is suitable for cell segmentation and tracking throughout the entire organoid. We achieved sample-specific and flexible mounting by utilizing a thermoforming process to produce sample

holders of various shapes. The developed light-sheet data processing pipeline serves as the starting point for the implementation of a new data analysis framework for light-sheet data.

Keywords:

Microscopy, Light-sheet microscopy, Organoids

Reference:

Huisken et al., Science 2004
Tomer et al., Nature Methods 2012
Yang et al., Nature Methods 2022
Strnad et al., Nature Methods 2016

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Quantitative Analysis of Magnetic Spin Textures in Fe3-xCoxGeTe2 Compositions using 4D-STEM

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Poster Group 1

Magnetic spin textures are becoming increasingly important in the field of spintronics due to their potential uses in devices such as racetrack memory. The discovery of skyrmions in Fe3GeTe2 (FGT), has triggered a great interest in studying spin textures in 2D-van der Waals (2D-vdW) magnets. These textures arise from the interplay between the Dzyaloshinskii-Moriya interaction (DMI), exchange interaction and the zeeman interaction in the presence of an external magnetic field [1]. Controlling these textures can further be achieved through factors such as thickness of the material, the temperature and structural defects [2]. Recently, it has been reported that the level of substitution of the Fe within the material by Co can change the ordering temperature and thus the behaviour of the material Fe3-xCoxGeTe2 or (FCGT) [3]. Understanding how these factors affect the behaviour of the skyrmions including the size, mobility and stability is crucial, as these features will be important when considering the materials used in potential spintronic devices such as racetrack memory or in probabilistic computing applications [4].
Here we use transmission electron microscopy (TEM) as well as four-dimensional scanning transmission electron microscopy (4D-STEM) to quantify the behaviour of FCGT as the composition of the material changes. Additionally, we simulate the textures using micromagnetic simulation software mumax3 [5].
In this work we utilize Fresnel imaging in Lorentz TEM (LTEM), a technique where the objective lens of the microscope is turned off to reduce the magnetic field so that the sample is not uniformly magnetized by the magnetic lenses. The beam is then defocused to the order of 0.1 mm so that Fresnel fringes appear caused by the magnetic potential of the sample revealing the spin textures in the sample as seen in Figure 1(a-b). These images can then be reconstructed using transport-of-intensity (TIE) equations to obtain the magnetic field information of the sample, an example of which is demonstrated in Figure 1(c). Chemical vapour transport and standard focused ion beam lift-out procedure were used to prepare the sample. During TEM imaging, the samples were cooled to liquid nitrogen temperatures and then heated to the appropriate temperature desired. The residual magnetic field within the TEM was calibrated using a custom-built in-situ Hall probe holder. The composition mapping was studied using energy dispersive x-ray (EDX) methods. Additional investigations into the material's bulk magnetic properties were carried out using magnetic force microscopy (MFM).
Using the methods described we have studied a range of stoichiometries of FCGT by varying out-ofplane (OOP) magnetic fields and temperatures and have demonstrated how the domain structure and temperatures at which these domains occur vary depending on the composition of the material. We have also confirmed the level of cobalt doping in FGT using EDX methods to quantify how this doping affects the behaviour of the materials, as well as investigated whether the elements are uniformly distributed throughout the material and how this affects the spintronic behaviour. Using the techniques described and through controlling the temperature and OOP magnetic field we have investigated the spin texture the material occupies at any given phase. Micromagnetic simulations

were done to estimate the behaviour of the materials at different thicknesses to verify results as well as to improve the speed of workflow when working on time intensive processes such as MFM and LTEM where the sample has to be cooled before imaging.
We have observed magnetic spin textures in a range of compositions of FCGT, showing that the behaviour of the materials depends directly on the levels of iron and tellurium as well as on any cobalt doping. Work has begun to further quantify the behaviour of these systems more accurately using 4DSTEM methods such as centre of mass measurements (COM) and ptychography, the methodology of which is shown in Figure 1(d).
Figure 1: Experimental setup of Fresnel TEM and 4D-STEM data acquisition. (a) Schematic of Fresnel TEM set up in overfocused condition. (b) Fresel TEM image of FGT showing skyrmions. (c) Reconstruction magnetic data. Here showing TIE reconstruction but other methods such as COM or ptychography would obtain a similar result. (d) Schematic of 4D-STEM experimental configuration.

Keywords:

Skyrmion, Ptychography, 4D-STEM, Fe3GeTe2, LTEM

Reference:

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Mean inner potential variation with strain in III-nitrides studied by off-axis electron holography

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Poster Group 2

Background incl. aims
Wurtzite III-nitride semiconductors are well known for their intense spontaneous and piezoelectric polarization fields. A deeper understanding of these properties is relevant for applications in various fields such as optoelectronic or power electronic devices. Off-axis electron holography is a powerful tool to measure directly and with a nanometer resolution the total electrostatic potential in these materials. However, to be able to study the polarization fields in III-nitrides, we need to remove the contribution from mean inner potential (MIP), which corresponds to the volume average of the Coulomb potential in the specimen. In the literature, MIP is traditionally derived for bulk materials within density functional theory (DFT).1,2 Evaluating the MIP using electron holography, we noticed disparities with values from the literature. One explanation that we propose is that the calculation for bulk material does not reflect the reality of heterostructures that are used in devices, with for instance the importance of strain. We propose here a deep analysis of the MIP evaluation for III-nitrides, comparing electron holography results with DFT calculation of the MIP that include the variation of strain.

Methods
MIP was computed through ab-initio simulations performed using GPAW code with plane wave basis-set of 300 eV cutoff energy, PBE functional, and 8x8x1 k-point sampling. The calculations were performed in a slab containing 11 atomic layers terminated in nonpolar m-{10-10} facets, centered in a 6.2-nm-long vacuum region with periodic boundary conditions. The MIP was obtained by averaging the Coulomb potential in the center 7 atomic layers, to prevent surface effects. The effect of strain has been modelled considering a biaxial strain configuration for layers grown along the <0001> direction.
From the experimental point of view, we study here 4 different samples. The first structure consists in a 6-period non-polar AlN/GaN (13nm/16nm) on 60 nm of AlN deposited on a (1-100) SiC substrate. The second sample is a 10-period polar AlN/GaN (20nm/20nm) superlattice grown by plasma-assisted molecular beam epitaxy (PAMBE) on 100 nm of GaN deposited on 1-µm-thick AlN-on-sapphire templates.3 The third and fourth samples consist in 5-period GaN/InGaN multi-quantum wells (MQWs) grown on a 2 µm-thick GaN layer that is itself grown on a 220-nm thick AlN buffer layer on patterned Si(111) templates using low-pressure metal organic vapor phase epitaxy (MOVPE). In one of the samples, the MQW consists in 5 periods of In0.13Ga0.87N (2 nm)/GaN (12 nm). In the other one, the indium content in the barriers is higher, between 14 and 16%.4 These samples have been studied using off axis electron holography performed in a double corrected FEI Titan Ultimate TEM operating at 200kV. Electron holograms were recorded on a Gatan One View 4k camera. TEM specimen were prepared by in-situ lift-out focus ion beam (FIB) using a FEI Strata 400.

Results

III-nitride heterostructures are usually grown along a polar axis, resulting in strong polarisation-induced internal electric fields that contribute to the total potential measured in electron holography. Additional contributions come from dopants, which can partially screen the internal electric fields, and from the MIP. Our first experiment consist in an attemp to measure MIP in an AlN/GaN heterostructure. To avoid the presence of internal electric fields, the heterostructure was grown along a non polar axis, and we verified that the lattice was fully relaxed. We obtained a difference of MIP between GaN and AlN of 1.53 V which is very different from the generally accepted 1.01 V, calculated by Schowalter et al. [1], and the 2.77 V that we obtain from our DFT calculations for relaxed materials. This opens a question about the real value of MIP for these compounds.
We used then a polar superlattice of AlN/GaN (second sample) to repeat the same measurement. In this case, the MIP difference between GaN and AlN, extracted from the electron holography result, was 1.3 V, i.e. slightly lower than in the non polar sample. We assumed that the difference could be attributed to a different strain in the structure. However, keeping in mind that AlN is under tensile strain and GaN is under compressive strain, the expected result is rather an increase of the MIP difference, which might reach up to 3 V. In summary, we conclude that current uncertainties in the AlN MIP value make it extremely difficult to extract precise information about the potential profile field in GaN/AlN heterostructures.
We have extended this work to InGaN/GaN, now obtaining more reliable results. The TEM specimen from the third sample was prepared with a gradient of thickness, so that there is a different degree of surface-induced relaxation in the 5 quantum wells. In this sample, we can show experimentally the variation of MIP with respect to strain, with the MIP of GaN and InGaN being almost equal for a completly relaxed sample. The experimental results match closely our DFT computation, including the strain dependence.
In the last sample, also InGaN/GaN but with different indium concentration, we could further confirm the effect of strain on the MIP, including a clear evaluation of the MIP difference between GaN and In0.16Ga0.84N.

Conclusion
The understanding and computation of MIP is highly important for a correct interpretation of the potential profiles measured in electron holography. In this work, we provide a theoretical and experimental evaluation of the MIP in III-nitrides. We outline the important disagreement between theory and experiments in the case of AlN/GaN heterostructures, even taking the strain into account. In contrast, we were able to provide reliable values of MIP for InGaN/GaN, showing excellent agreement between experimental and theoretical estimations, and confirming the effect of strain predicted by the calculations.

This work, carried out on the Platform for Nanocharacterisation (PFNC), was supported by the “Recherche Technologique de Base” and "France 2030 - ANR-22-PEEL-0014" programs of the French National Research Agency (ANR).

Keywords:

Holography, III-nitride, Mean inner potential

Reference:

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Micro LED performance - a cathodoluminescence study

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Poster Group 2

Background & aims
Gallium Nitride (GaN) microLEDs have received a lot of attention in the field of visible light microdisplays due to their interesting properties, such as high luminosity, wide wavelength range and robustness. However, their miniaturization poses a challenge due to the decrease of LED performance as the perimeter-to-surface ratio increases, a phenomenon that has not yet been fully understood. Studying carrier dynamics in these devices is crucial for understanding how recombination mechanisms affect their performance. The lateral diffusion length and carrier lifetime are therefore a major subject of interest for understanding and improving the characteristics of pixelated mesa LEDs.
In this work, we show the impact of Light Extraction Efficiency (LEE) and Internal Quantum Efficiency (IQE) independently as a function of mesa size and shape. Furthermore, lifetime measurements provide insights about the origin of IQE decrease near the mesa edge, showing the importance of diffusion coefficient and lifetime measurement techniques.
Methods
Multiple quantum well (MQW) LED structures (nGaN/5x(2.5nm InGaN/8nm GaN)/AlGaN EBL/pGaN) were patterned into 2.5, 5, 7.5 and 10 µm square and round mesas. These structures were analyzed using steady-state hyperspectral cathodoluminescence (CL) measurements at 10K and room temperature. The impact of mesa size and shape on LEE versus IQE has been explored. Additionally, Time Resolved Cathodoluminescence (TRCL) was performed with a streak camera at room temperature across a mesa to determine QW carrier lifetime and wavelength variation of the emitted light with the distance to the mesa edge.
Results
The maximum CL intensity at the center of the MQW mesas exhibits a non-monotonic trend with increasing mesa size, showing an optimal emission for the intermediate sized mesa of 7.5 µm, for both circular and square geometries. This behavior is explained by a competition between IQE (which decreases with mesa size reduction) and LEE (which increases with size reduction, with different trends for square and circular geometries), separated using the low and room temperature CL measurements. These results highlight the difficulty in properly interpreting CL intensity maps in terms of the impact of non-radiative recombination from the sides on LED mesa efficiency. However, the importance of IQE cartography at the nano scale to explore the impact of the mesa side remains significant. As already demonstrated in numerous papers, IQE decreases near the mesa wall. This decrease is mainly attributed to non-radiative recombination at the mesa edges and strongly affects smaller mesa performances. TRCL lifetime measurements across a mesa indeed show that carrier lifetime decreases near the mesa side. Furthermore, we observed a CL intensity reduction up to 3.5 µm from the mesa edge, which determines the optimal mesa size for emission. Thus, the diffusion length of carriers in the QW is one of the key parameters to minimize the impact of sidewall damage on LED efficiency. This means that a smaller diffusion length will reduce the impact of non-radiative recombination on the sidewalls on the LED efficiency.

Conclusion
This study investigates the influence of mesa size and geometry on external quantum efficiency, highlighting the significant impact of non-radiative recombination at the mesa edge. Moreover, a competition between IQE and LEE is observed, elucidated through measurements at both low (10 K) and room temperature. This competition reveals an optimal LED size for two distinct mesa geometries, offering valuable insights for the design of microLED arrays. Furthermore, our findings provide clear indications for enhancing LED characteristics, such as mitigating surface recombination at sidewalls to reduce non-radiative recombination, or minimizing diffusion length to mitigate sidewall non-radiative impacts on small mesa sizes. Since diffusion length is intricately linked to the square root of the diffusion coefficient and radiative lifetime, characterizing the diffusion coefficient becomes critical. Both steady-state and time-resolved cathodoluminescence techniques are proposed to address these parameters effectively at the mesa scale.
This work, carried out on the Platform for Nanocharacterisation (PFNC), was supported by the “Recherche Technologique de Base” and "France 2030 - ANR-22-PEEL-0014" programs of the French National Research Agency (ANR). Manufacture of the samples was fund in the frame of the Important Project of Common European Interest (IPCEI) for microelectronics and as part of Nano 2022IPCEI.

Keywords:

cathodoluminescence, quantum well, microLED

Reference:

P. González-Izquierdo et al, Influence of Shape and Size on GaN/InGaN µLED Light Emission: A Competition between Sidewall Defects and Light Extraction Efficiency. ACS Photonics 2023, 10, 4031−4037
S. Finot et al, Surface Recombinations in III-Nitride Micro-LEDs Probed by Photon-Correlation Cathodoluminescence. ACS Photonics 2022, 9 (1), 173–178

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Nano-chemical Imaging and Spectroscopy at the Single-molecule Level

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Background
Biological processes rely on a wide class of biomolecular and macromolecular machines that have nanoscale physical dimensions and whose function emerges from a correlation between their chemical and structural properties. A fundamental objective of modern analytical methods is the comprehension of how physical-chemical properties and heterogeneity of single biomolecules underlie their role in cellular function and disease. While innovative nanoscale imaging methods have been developed to characterise biomolecules, imaging microscopies are to the most part chemically blind; thus, hampering the characterisation of inhomogeneous and complex systems.

Methods
The introduction of photothermal infrared nanospectroscopy (AFM-IR) has revolutionized the field of nano-chemical analysis in a wide-open range of fields, including biological, material and polymer sciences. Here, we will present an overview of our latest development and application of AFM-IR in combination with advanced spectroscopic analysis and chemometrics, as a real breakthrough for the analysis of heterogeneous (bio-)molecular systems and materials down to the single molecule level.

Results
To illustrate our path towards single-molecule AFM-IR, we first show the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power, and short pulse ORS-nanoIR. [1] This approach enables the accurate determination of the secondary structure elements of single proteins and amyloids in the amide band I region. We will then showcase the application of this unprecedented single molecule sensitivity to: i) unravel molecular structure and interactions of protein and organic molecules [2]; ii) origin of chirality in click chemistry polymers [3]. Finally, we illustrate the application of this sensitivity to probe the surface and structural properties of functional materials, such as artificial model membranes [4] and functional protein self-assemblies [5].

Conclusion
Overall, our aim is to expand the capabilities of analytical nanoscience to shed light on the structure-activity relationship of biomolecules and functional materials design.

Keywords:

Nano-Imaging;Spectroscopy;Atomic Force Microscopy; Supramolecular Assembly

Reference:

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[2] Ruggeri, Nature Comm., 2021.
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The Environmental Impact of Large Scientific Infrastructure

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Poster Group 1

Standardised energy labelling is routinely used on many of the consumer goods we interact with daily. These aim to inform customer purchasing and usage behaviours and are used by regulators to incentivise the replacement of older equipment with more environmentally-conscious choices. Currently, there is limited sustainability data available for large scientific infrastructures (such as magnetic resonance imagers, electron microscopes, and research furnaces, among many others). As a result, no scheme currently exists to fairly compare energy usage across different devices or between families of devices.

Energy usage is often not easily characterised for such infrastructure due to their diverse and multi-faceted needs; aspects may include but are not limited to, electrical draw, air compressors, water cooling, laboratory air conditioning, cryogenic gas and liquid purification/storage, as well as compute power. Usage patterns must also be considered, and optimised for equipment that is required to always be on. Some effort has been made in characterising energy consumption of hospital equipment such as MRI and CT scanners that show some parallels with scientific equipment [1-3].

The left of Figure 1 shows thermal images taken in the Advanced Microscopy Lab, Dublin of a JEOL-2100 transmission electron microscope, the ventilation output for one of the building's chillers, and a building air compressor. In the latter two, this energy is currently vented directly to the environment with no heat recovery implemented. In a cooler country such as Ireland, this means waste heat is being exhausted from instruments while central heating systems are used to heat the building's working spaces.

Datacentres occupy an increasing slice of global energy demand and associated CO2 emissions, and this is expected to grow significantly [4]. While much of this consumption is due to sectors unrelated to scientific research, modern science is driven by huge quantities of data generation and the storage and processing of this data can still result in significant energy usage. In microscopy, the data generated by modern imaging techniques can reach 200TB per hour (Figure 1, right), and will likely increase further in the future [5].

The scientific community has increasingly moved towards open data initiatives, and to support this we will present and share a fully open access tool for calculating the complex energy usage of large scientific infrastructure. Through collaboration with facility managers on a global scale, we hope to provide an open database from which a sustainability labelling system can be created, sourced entirely from community input.

With increasing global concern both with energy usage and energy efficiency, it is vital that the footprint of modern scientific research equipment be assessed and quantified to enable new auditing and labelling to be carried out. This can inform decisions made by facility managers to reduce energy consumption, leading to both lower environmental impact and financial cost. Eventually, we hope this will lead to enhanced policy direction and apply pressure to manufacturers to be more mindful in the future design and manufacture of what is today wholly unlabelled hardware.

Keywords:

Research infrastructure, energy labelling, sustainability

Reference:

1. T. Heye et al., Radiology 295 (2020), p. 593-605.
2. M. Martin et al., Health Services Research and Policy 15 (2018), p. 1385-1393.
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Phase detection limits in electron holography with automatic stabilisation and direct electron detection

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Electron holography can measure local electric, magnetic and elastic strain fields. Furthermore, the incident wave can be structured with the aid of a biprism in the illumination system for specialised interference configurations [1]. In all cases, the measurement precision is directly related to the precision with which the phase of the holographic fringes can be determined. Random errors from shot noise are related to the fringe visibility and the electron dose but also depend on the transfer characteristics of the detector and the mask used in the Fourier reconstruction:

$$\sigma(\varphi)=\sqrt{(2/N)}\times 1/V\times(MTF(q))/\sqrt{(DQE(q))}\times\sqrt{a} \quad (1)$$

where N is the number of incident electrons per pixel, V the observed visibility of the fringes, MTF(q) and DQE(q) the modulation transfer function and the quantum efficiency, respectively, of the detector at the spatial frequency corresponding to the hologram fringes and “a” a factor depending on the Fourier mask size [2]. We will test the limits of the theory and present two ways of maximising sensitivity: acquiring holograms over very long exposure times through automation [3] and with detect electron detection.

Methods

Automatically stabilised holograms were acquired on the I2TEM (Hitachi HF3300-C) microscope, operating at 300 kV and equipped with a cold-field emission gun (C-FEG), double stage, image aberration corrector (B-COR, CEOS), condenser biprism and three post specimen biprisms. In particular, two series were acquired: one on a conventional scintillator-coupled CMOS camera (OneView, Gatan Inc.) and the other on a direct detection device (K3, Gatan Inc.). Holograms covered the field of view of the detector and were adjusted to have indentical fringe spacing (5.5 pixels) using two post-specimen biprisms. Hologram phases were monitored in real-time using HoloLive! v1.1 (HREM Research Inc.) and dynamic automation was implemented through customised scripts [3]. Holograms were acquired continuously over 15 minutes (900 s) at comparable dose rates (< 9 e-/pixel/s), the cumulated intensity output every 4 s (Fig. 1a) so that the progression could be monitored. Automatic stabilisation also allows the choice of the exact position of the hologram fringes. Systematic errors were investigated by recording holograms with different initial phase values [4].

Results

The phase error, measured by the standard deviation in a region of vacuum, diminishes over time as expected by the theory (Fig. 1b). Indeed, direct detection provides a spectacular improvement in the precision of the phase. However, in both cases, the phase error reaches a plateau, with little improvement beyond a certain dose. We will show the cause of the systematic errors and how they can be corrected.

Conclusions

The theoretical limit for the phase precision can be obtained using dynamic automation and direct electron detection. We will show how systematic errors can be removed by controlling the incident illumination for an ultimate precision of 1 mrad at nanometre spatial resolution.

Acknowledgements
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Keywords:

Electron Holography, Automation

Reference:

[1] T Denneulin, F Houdellier, MJ Hytch, Ultramicroscopy 160, 98 (2016).
[2] SLY Chang et al, Ultramicroscopy 161, 90-97 (2016).
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[4] C Gatel, J Dupuy, F Houdellier, MJ Hÿtch, APL 113, 133102 (2018).
[5] M.J. Hÿtch and C. Gatel, Microscopy 70, 47 (2021).

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Combining SIMS elemental mapping with FIB-based imaging for multimodal analysis of biological specimen

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Poster Group 2

Background: The generation of chemical maps of sub-cellular structures in biological samples is one important tool to identify and localize accumulating ingested particles, drugs or metal tagged biomarkers. By correlating this chemical information obtained by spectroscopical methods like x-ray spectroscopy, etc. to an ultrastructural image obtained by light or electron microscopy the underlying physiological processes can be investigated. However, the better the lateral resolution of the chemical mapping, the lower the signal detected per voxel, oftentimes leading to inaccessibility of certain markers for chemical imaging in cells and tissues. In this regards, secondary ion mass spectrometry (SIMS) is a powerful technique for analyzing surfaces at high spatial resolution, owing to its ability to detect all elements from H to U and to differentiate between isotopes, its excellent sensitivity and its high dynamic range. Our group focuses on the development of multimodal imaging platforms giving access to correlative investigation approaches by directly linking SIMS data with data obtained by other analytical (such as Energy-Dispersive X-Ray Spectroscopy) or imaging techniques on the same instrument (e.g. secondary electrons (SE), back-scattered electrons (BSE) or images of the transmitted ion or electron beam (STIM, STEM)). The possible combinations are dependent on the sources and detectors available on these platforms.
Methods: Currently, several focused ion beam platforms with or without an additional electron column are available and are equipped with at least a secondary electron detector and LIST's magnetic sector SIMS system (FIB-SIMS with < 15 nm spatial resolution). These instruments use various primary ion beam species, such as Ga [1], He / Ne [2-3], Cs [4] as well as Si / Au / Li / Bi [5]. Our magnetic sector SIMS system is equipped with a continuous focal plane detector technology allowing parallel detection of all masses for each single pixel in the scanned region of interest. One instrument is also equipped for imaging the transmitted beam information and can be operated at RT and under cryogenic conditions facilitating the analysis of beam sensitive samples and frozen samples.
Results: A number of examples from different application fields (nanotoxicology, environmental pollution, water organisms etc.) will be presented.
Conclusion: The performance of the different instruments will be discussed with a focus on new developments, different SIMS acquisition modes (mass spectra, depth profiling, 2D and 3D chemical imaging) and their applicability for biological samples including frozen-hydrated specimen.

Keywords:

focused-ion-beam-microscopy, SIMS, correlative microscopy, cryoanalysis

Reference:

[1] Anal. Chem 94 (2022) 10754
[2] Rep. Prog. Phys. 84 (2021) 105901
[3] Anal. Chem. 93 (2021) 14417–14424
[4] Microscopy Microanalysis 27_S1 (2021) 24–25
[5] Microscopy Microanalysis 29_S1 (2023) 536–537

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Multimodal and multidimensional EELS and EDX for investigations of catalysts at the nanoscale

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Poster Group 2

Ceria-based materials find their applications in various fields. Due to their oxygen deficiency at the surface the ceria nanoparticles are especially attractive catalysts [1]. By introduction of dopants into the ceria lattice the catalytic performance of ceria nanoparticles can be even further increased. To fine-tune the catalytic performance though the insights into the structural-properties relationships in the materials advanced TEM with spectroscopy should be applied. 2D and 3D multimodal STEM is of crucial importance to understand the structure and chemistry of doped catalysts at the nanoscale and to enable the targeted approach to the materials development.

In this work we combined EDX, EELS and electron tomography to investigate the local chemistry of Cu-doped ceria (Cu:CeOx) catalyst exhibiting superior performance for low temperature CO oxidation with respect to the undoped CeOx material. EELS both in 2D and 3D evidenced the elegant double core-shell structure of the undoped CeOx nanorod (see the figure), most likely related to the large amount of voids inside the particle: a shift of the Ce M4,5 edge onset as well as the white line ratio variation was clearly observed in the EELS spectrum allowing to distinguish between Ce4+ and Ce3+. In contrast to the previous work [1] where to gain the similar insights the monochromated 120kV setting was applied in the present study the experiment did not require the use of the excited monochromator and the lowered HT making the experimental work easier. Simultaneous EELS-EDX investigation was performed to study the Cu:CeOx material. Even though the CuL2,3 edge is visible in the EELS spectrum, its overlap with the fine structure of Ce inhibits the reliable STEM-EELS mapping of Cu in Cu:CeOx. EDX in this case clearly located Cu locations within the nanorod while STEM-EELS evidenced the Ce3+ oxidation state of cerium, highlighting the high amount of oxygen vacancies which can be correlated to the superior performance of the doped ceria catalyst.
To sum up, both EELS and EDX giving the access to the local chemical information, in combination with the electron tomography, giving the access to the 3D information, are of great help while investigating catalytic nanoparticles. We highlighted here the unique insights of the multimodal and multidimensional approach by an example of doped ceria catalyst investigation.

Keywords:

EELS, EDX, Multimodality, Catalysis

Reference:

[1] Maria Meledina, Stuart Turner, Vladimir V Galvita, Hilde Poelman, Guy B. Marin, Gustaaf Van Tendeloo F, Nanoscale 7 (2015), p. 3196-3204. https://doi.org/10.1039/C4NR06060A

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Development of a pixelated STEM-in-SEM detector for microstructural features characterization

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Poster Group 1

Background incl. aims:
Microstructural investigations provide engineers with information to computationally predict the performance of components and electron microscopy is a well-versed technique for analyzing micro/nanostructural features. Two types of electron microscopes are commonly used and provide complementary information: Transmission Electron Microscope (TEM) at the micro/nanoscale and Scanning Electron Microscope (SEM) at the macro/mesoscale. Historically, Scanning Transmission Electron Microscopy in Scanning Electron Microscope (STEM-in-SEM) techniques were predominantly used by biologists due to their user-friendly nature and their ability to analyze specimens at low accelerating voltages (30 kV or below). This made STEM-in-SEM techniques complementary to conventional TEM ones as the latter generally use high accelerating voltages (~200 kV). Recent advances in electron guns and electromagnetic lenses have sparked growing interest in STEM-in-SEM techniques across various scientific disciplines. However, researchers often face challenges in establishing the precise imaging conditions required to obtain comprehensive micrographs, thus limiting their interpretation and analysis.
Methods:
In this study, we propose a novel STEM-in-SEM approach, which aims to overcome the practical limitations of traditional techniques for microstructural characterization in thin foils. The technique leverages an advanced detector designed to replicate TEM-like characterizations while minimizing the presence of artifacts that commonly hinder comprehensive analyses. In addition, this STEM-in-SEM technique benefits from On-Axis Transmission Kikuchi Diffraction (On-Axis TKD), thus allowing diffraction pattern acquisition with a high signal-to-noise ratio.
Results:
First results already show the possibility to acquire diffraction patterns and to image dislocations under modulable bright-field and dark-field conditions.
Conclusion:
By implementing this pixelated STEM-in-SEM technique, we anticipate significant advancements in the field of microstructural characterization by obtaining TEM-like and TEM-complementary information. Ultimately, the developed technique should facilitate studies to obtain detailed information about thin foil specimens, thereby contributing to a broader range of scientific applications.

Keywords:

Electron microscopy, STEM-in-SEM, On-Axis TKD, Microstructures characterization

Reference:

Brodu, E., Bouzy, E., Fundenberger, J.-J., Guyon, J., Guitton, A., & Zhang, Y. (2017). On-axis TKD for orientation mapping of nanocrystalline materials in SEM. Materials Characterization, 130, 92-96. https://doi.org/10.1016/j.matchar.2017.05.036

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Measuring the Interpretability of High-Resolution Transmission Electron Microscopy Images

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background
Nanoparticles find uses in a plethora of applications ranging from food preservation and pharmacology to industrial catalysts. Obviously, the first question which is asked when it comes to nanoparticles is the size. However, several aspects of nanoparticles dictate their shape and function. This includes morphology, chemistry, crystallography, oxidation state and environment.
When it comes to providing answers on these topics, transmission electron microscopy (TEM) has been the go-to tool. Modern microscopes easily provide answers on all these topics. However, there are three main challenges. 1) the structure of nanoparticles is not always unaffected by the exposure to high-energy electron beams; 2) to provide statistically relevant information, a large number of nanoparticles should be analyzed; 3) the environment inside the TEM is most often not the same as that where the nanoparticles are used. To accommodate the beam sensitivity, we can lower the electron dose rate to levels where the effect is negligible or otherwise easy to accommodate for. To improve the statistical significance, we can count large numbers of particles and use automated data analysis approaches. Lastly, to image the particles under realistic conditions, we can control the environment in which we image them.

Methods
Here we use environmental transmission electron microscopy to monitor the size, shape and dynamics of nanoparticles relevant for catalytic applications. We focus particularly on gold nanoparticles on cerium dioxide which catalyzes the oxidation of carbon monoxide. Using a CMOS based OneView camera from Gatan, we have acquired data series at varying electron dose rate and under different environments. Using these datasets, we evaluate the signal to noise ratio (SNR) based on different models. Furthermore, we estimate the usability of the structure similarity index measure (SSIM) to detect structural changes of the gold nanoparticles and how prefiltering and adjusting can help our data analysis. We compare these results to measurements performed using data analysis by convolutional neural networks trained on simulated data [1, 2].

Results
Using three models of the signal-to-noise ratio we estimate the interpretability of HRTEM images and rate the measured SNR against the output of data analysis based on convolutional neural networks. SNR(I) is the traditional method of calculating SNR. SNR(II) uses the standard deviation of the nanoparticle and background region separately. SNR(III) takes the high intensity variation of phase contrast images into account. Figure 1 top pane shows images extracted from sequence acquired at increasing dose rate. The bottom pane shows the SNR calculated based on different models showing data from the entire image series. We have further pursued the analysis of Au nanoparticle structure as a function of the environment.

Conclusions
Determining the SNR of an image can tell us something about the interpretability and if it makes statistical sense to continue a more thorough analysis. Here we have attempted alternatives to the traditional method of determining SNR which may not be well-suited to estimate the interpretability of HRTEM images. We have correlated this with data analysis based on convolutional neural networks.

Keywords:

Nanopareticles, TEM, Interpretability, Low-dose

Reference:

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2. Leth Larsen, M.H., et al., Quantifying noise limitations of neural network segmentations in high-resolution transmission electron microscopy. Ultramicroscopy, 2023. 253: p. 113803.

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A new EELS spectrometer, integrated with the microscope's optics

Dr. Peter Tiemeijer[1], Mr. Terry Dennemans[1], Mr. Sander Henstra[1], Mr. Dileep Krishnan[1], Mr. Sorin Lazar[1], Mr. Paolo Longo[1], Mrs. Maria Meledina[1], Mr. Sjaak Thomassen[1], Mr. Wouter Verhoeven[1]
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Poster Group 1

The Selectris energy filter was introduced in 2020 [1]. This filter comprises a large tapered prism and ten multipoles most of which are implemented as dodecapoles. These enable stable and superior correction of non-isochromaticy and image distortions. Now, we are developing an EELS spectrometer based on the technology of this filter.
Compared to energy filtered imaging, the optics of EELS has the additional challenge that a broad range of electron energies (up to several thousands of eV wide) must be simultaneously transferred through the microscope and through the spectrometer, from specimen to detector, without introducing chromatic blur or chromatic distortions. This is a non-trivial task, especially when we desire that such transfer is maintained across different microscope settings (different camera lengths) and across different spectrometer settings (different dispersions and different spectrum offsets). Maintaining this can only be ensured by closely integrating the optics of the microscope and of the spectrometer, to keep the chromatic defocus in the microscope matched with the focus of the spectrum. Otherwise, the superior performance offered by the tapered prism and ten multipoles could only be fully enjoyed at (one or) a few specific experimental settings, and would be lost when switching to other camera lengths or other dispersions or other energy offsets.
Compared to the Selectris energy filter, our EELS spectrometer has the following additional functionalities: (i) above mentioned integration between the optics of the TEM and the spectrometer, (ii) a dedicated detector optimized for fast read-out, low-noise, and radiation hardness, (iii) fast electrostatic elements (shutter, bias tube, deflector) for near-simultaneous acquisition of multiple (dual, triple, ...) regions of the EELS spectrum, and (iv) fully automated EELS tuning routines.
Figure 1 illustrates the optical performance of our spectrometer. It shows triple-EELS for $DyScO_3$ taken at 60kV, monochromated, 30mrad semi-convergence angle, 5mm spectrometer entrance aperture, and at five different camera lengths. The top panel shows data acquired without correction for the chromatic effects: clearly, the chromatic blur leads to resolution loss at the higher energy losses and at the smaller camera lengths. Bottom panel shows data acquired with on-the-fly correction of the chromatic effects: resolution is ensured across all settings, even for the challenging case of ΔE=1300eV at 60kV, and semi-collection angle of 49mrad (CL=25mm).
The stability of the Selectris, the full automation of the EELS tuning, and the integrated correction for chromatic effects greatly improve the ease-of-use and productivity of the new spectrometer.

Keywords:

EELS spectrometer optics automation

Reference:

1] T. Nakane et al., Nature 587 (2020) 152, Single-particle cryo-EM at atomic resolution.

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Materials characterization of recycled batteries (black mass): automated quantitative phase classification in 3D

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Poster Group 1

Background incl. aims
Recovery of valuable raw materials from spent batteries (e.g., Cu, Al, Fe, Mn, Ni, Co, Li, P, etc), is important in the shift away from environmentally damaging raw material extraction. The black mass production process starts with the collection and disassembly of the battery. The procedure that follows is akin to the processing of a mineral ore. The disassembled components are shredded to produce the black mass powder, containing the valuable metals. Most processes then treat the black mass with reagents to separate the individual metals. The efficiency of any such process depends on the knowledge of the composition of the black mass, of any impurities it may contain, and how said impurities interact with the reagents to passivate the recovery of the metals. Therefore, the efficient recovery of metals from black mass is dependent on the detailed understanding of its composition. Here, we present novel bulk phase analytical techniques for the 3D characterization of black mass. The use of automated phase classification via X-ray microscopy highlights details of the chemistry and morphology of its constituents non-destructively in 3D, therefore improving the characterization process immeasurably.

Methods
We present a combined workflow using 3D X-ray imaging via a ZEISS Xradia 620 Versa X-ray Microscope (XRM), and advanced reconstruction techniques to enhance contrast, reduce noise, and resolution recovery using the ZEISS Advanced Reconstruction Toolbox (ART). Finally, we use an automated 2D and 3D quantitative phase classification approach in the Scanning Electron Microscope (SEM) and XRM using Mineralogic 2D and 3D, respectively. This combination of approaches in a correlative workflow contribute towards our better understanding of the black mass.

Results
We identify a variety of components of different densities, including foils, separators, and both liberated and non-liberated cathodes containing electrode particles (Figure 1). To enhance the data further, we use modules of the Advanced Reconstruction Toolbox: firstly, ZEISS DeepRecon Pro software, a machine-learning based reconstruction option for advanced de-noising and enhanced contrast. Reconstructing data in this way allows us to collect fewer projections meaning scans are faster and more samples can be imaged. In the black mass, there is a marked improvement in the quality of the data: noise is significantly reduced, and contrast is improved so individual electrode particles are identified with more clarity. Secondly, we apply ZEISS DeepScout software. DeepScout is an AI powered method to upscale scans to a larger field of view whilst preserving high resolution, a form of Resolution Recovery. We increase the field of view by over 2x, enabling us to observe electrode particles at high resolution over a larger area. Both approaches lead to segmentations and quantification representative of a larger sample area without compromising resolution. To quantify the different components within the black mass, we use two complementary approaches: ZEISS Mineralogic 3D and 2D. Mineralogic 3D classifies materials in the 3D XRM data using deep learning to execute automated mineralogy and phase identification. The materials classifier uses the XRM data

histogram following DeepRecon Pro reconstruction and identifies two types of battery (LFP and NMC) and two types of foil (Al and Cu), as well as graphite and Al polymer separator. Therefore, we can quantify each of these components and visualize them in 3D to identify the location of valuable retrievable elements in the black mass. This is further supported by complementary analyses via ZEISS Mineralogic 2D. This is obtained using an SEM with the assistance of the chemical SEM-EDS data, allowing for quantitative phase analyses rather than singular chemical elements alone. In this approach we are also able to quantify the different phases, and understand the location and quantities of recoverable elements such as lithium, manganese, cobalt, aluminum, copper, and iron. Both results indicate that in fact two different battery types are present in this sample, as well as two different types of foil when it was thought that the foils were removed during an earlier part of the black mass production process.

Conclusion
Our results show that it is easily possible to identify and quantify recoverable phases from black mass, which will help towards the battery recycling process. These advanced characterization techniques are imperative to ensure the black mass production process is reliable and exact in a world where valuable constituent recovery from batteries will become essential going into the future.

Keywords:

battery recycling, automated mineralogy, tomography

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Multi-scale characterization of vitamin B2 (riboflavin) supplements using X-ray Microscopy, SEM, and automated phase analyses

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Poster Group 1

Background incl. aims
Riboflavin is a water-soluble micronutrient that is essential for the reproduction of cells and growth in the human body, as well as helping to prevent inflammation and ageing. It naturally occurs in a variety of foods including fruit, vegetables and meats, however it is regularly incorporated into vitamin or dietary supplements to bolster healthy lifestyles. Recently, it has been found to potentially be a natural alleviator of migraines due to its preventative effects of migraine symptoms such as neuroinflammation, and now many migraine-relief specific versions of the nutrient exist and can be purchased 'off the shelf'. However, while most pharmaceutical products are highly regulated, dietary supplements such as these have less stringent regulatory assurances. The most popular cost-effective method of their production is from fermentation, where the active nutrient is subsequently mixed with other components such as anti-caking agents, fillers, and lubricants to homogenize the mix and ensure enough of the vitamin enters the body. However, different manufacturers include differing components in variable quantities, and there is variation in their manufacture. Therefore, a full multi-scale characterization of these supplements is important to (i) understand the spatial distribution and morphological characteristics of the different components, (ii) to quantify the active components, and (iii) to understand quality assurance based on the variable manufacturing processes involved with production of these supplements.

Methods
Here, we have studied two different vitamin B2 supplement capsules from two different manufacturers to characterize and quantify the different components in 3D. Capsule 1 contains riboflavin (active ingredient), silicon dioxide (likely as anti-caking agent), and Mg stearate (likely as lubricant), and capsule 2 contains riboflavin (active ingredient) and Mg stearate (likely as lubricant) only. We use X-ray Microscopy (XRM) to image the powders non-destructively in 3D, and apply various advanced reconstruction approaches to enhance visualization of the data: DeepRecon Pro, a machine-learning based reconstruction approach to reduce noise, improve contrast, and shorten scan time/increase throughput; and PhaseEvolve, which uses AI algorithms to enhance the data so that it uncovers different material contrasts uniquely revealed by XRM. Finally we use an automated phase classification and segmentation method to quantify the different components in 3D. This approach not only introduces the benefits of using 3D imaging to study pharmaceuticals, but sheds light on the structural morphology of the active components in vitamin supplements. Additionally, we use SEM in a correlative workflow for 2D imaging and chemical analyses of individual components.

Results
Both capsules' powders were imaged in 3D at the multi-scale using a ZEISS Xradia Versa 520 XRM. Initial observations reveal that the morphology of each capsules' powders are indeed very different, with large morphological and structural differences, suggesting that the powders were likely produced by two different production methods: spray drying and granulation. DeepRecon Pro

significantly improves the quality of the data compared with tradiaitional reconstruction methods (e.g., filtered back projection) by reducing noise and improving contrast. PhaseEvolve further enhances the contrast between the different powder components, and we are able to identify an additional 'binder' phase which is not identifiable from filtered back projection data alone, therefore contributing towards a more accurate segmentation of phases and data. Complementary analyses via Mineralogic 3D for phase classification indicates that this new binder phase is Mg stearate which is the 'glue' holding riboflavin particles together as agglomerates, and allows us to segment and quantify this phase fully in 3D. Further, using Mineralogic 3D we identify a filler (calcium phosphate) in capsule 1 which is not listed on the ingredients.

Conclusion

The results presented here show that non-destructive 3D imaging via X-ray Microscopy (XRM) and associated advanced reconstruction techniques are a useful tool for understanding the composition of vitamin supplements. We find previously unobservbable phases using these techniques, and are able to identify and quantify components which are not listed on the ingredients. This approach could be extended to other pharmaceutical materials to maintain regulation.

Keywords:

Pharmaceuticals, Tomography, materials characterization, AI

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New paradigm for ultra-low-dose high-resolution imaging through a dedicated event-driven analytical ptychography methodology

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Poster Group 2

Recently, significant advances in scanning transmission electron microscopy (STEM) have made it a viable solution for the high-resolution imaging of beam-sensitive specimens such as 2D materials, ordered nanoporous particles, viruses or protein crystals. In particular, the development of dose-efficient computational imaging approaches such as analytical ptychography, e.g. in the form of the Wigner distribution deconvolution (WDD) and side-band integration (SBI) methods, have permitted the direct retrieval of the projected electrostatic potential with the highest achievable signal-to-noise ratio [1]. Furthermore, with the introduction of event-driven detectors (EDD), the ultra-low-dose measurement (<100 e-/Å2) of the far-field electron intensity was enabled as well [2].

In this work, we present developments of WDD and SBI aiming to improve their performances in imperfect recording conditions, e.g. by including geometric aberrations, partial coherence and the modulation transfer function (MTF) of the camera. We also introduce a scan-frequency partitioning algorithm permitting the computationally heavy implementation of those methods in a RAM-limited device, such as a commercial graphics processing unit (GPU). Moreover, we evaluate the capacities of analytical ptychography to transfer a range of frequency information, with dependence on both the scan sampling and the illumination parameters, on the basis of the Cramér-Rao lower bound [3]. As such, an updated metric for the probe overlap requirement of electron ptychography is presented.

Additionally, we establish the guided progressive reconstructive imaging (GPRI) method, which constitutes a new framework to perform WDD and SBI through the direct treatment of a stream of electron events provided by an EDD. This process is purely cumulative, as each count leads to the addition of a single contribution to the result, selected from a set of pre-calculated libraries. GPRI should thus permit reaching the best achievable dose-efficiency and processing speed in live ptychography, in particular through a direct implementation in the field-programmable gate array (FPGA) unit of the EDD and thus with no need for a prior transfer of the event data to a separate computer, which currently represents the main bandwidth limitation in other approaches. This method can be furthermore supplemented with recording strategies optimized for damage mitigation [4,5].

The methods developed in this work are tested by means of multislice frozen phonon simulation, allowing the exploration of different experimental parameters, such as dose, probe focus, specimen thickness, source size and focus spread. Preliminary experimental demonstrations are carried out as well.

Figure: examples of analytical and iterative ptychography results to recover the projected potential μ or a transmission function T (complex exponential of μ). The simulated STEM scan was performed on a single-layer MoS2 specimen. The chosen semi-convergence angle α was 30 mrad and the acceleration voltage U was 60 kV. Two cases were investigated: the use of a perfectly focused probe

and of an overfocused probe, with focus f equal to 50 nm. Dose limitation was included by realistic representation of single counts, whose total number per frame was determined by Poisson statistics. Note that, for the focused probe case, an average number of 4 counts per scan position leads to a dose of about 164 e-/Å2 (i.e. ratio of total electrons used by scanned surface).

This work received funding from the Horizon 2020 research and innovation programme (European Union), under grant agreement No 101017720 (FET-Proactive EBEAM).

Keywords:

Ptychography, Low-dose imaging, Event-driven detection

Reference:

[1] C. O'Leary et al. ; Ultram. 221, 113189 (2021)
[2] D. Jannis et al. ; Ultram. 233, 113423 (2022)
[3] C. Dwyer and D. M. Paganin ; arXiv:2309.04701v2
[4] A. Velazco et al. ; Ultram. 232, 113398 (2022)
[5] D. Jannis et al. ; Ultram. 240, 113568 (2022)

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Cryo–electron microscopy unveils the gating mechanism of the human Kir2.1 channel

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Poster Group 1

Background
Inward-rectifier potassium (Kir) channels are a group of integral membrane proteins that selectively control the permeation of K+ ions across cell membranes. The small outward K+ current through Kir channels controls the resting membrane potential and membrane excitability, regulates cardiac and neuronal electrical activities, couples insulin secretion to blood glucose levels, and maintains electrolyte balance1. All Kir channels are tetramers and share characteristic structural features. They have a canonical pore-forming transmembrane domain (TMD) made of two transmembrane helices separated by a K+ ion selectivity filter and a large cytoplasmic domain (CTD) containing both N and C termini. The CTD extends the ion conduction pathway and provides docking sites for regulatory ions, proteins, and ligands2. The strong inward-rectification mechanism results from a block on the cytoplasmic side of the channels by endogenous polyamines and Mg2+ that plug the channel pore at depolarized potentials. The blockers are then removed from the pore when the K+ ions flow into the cell at hyperpolarized potentials. In addition to being inwardly rectifying, the gating of Kir2.1 channels are selectively activated by the lipid phosphatidylinositol 4,5-bisphosphate (PIP2). The physiological importance of these channels is underpinned by the fact that mutations in these proteins cause a wide range of pathologies. In previous published work, we used cryo–electron microscopy (cryo-EM) combined with image analysis to elucidate the structure of a human Kir channel, Kir2.1 in the closed state3. Furthermore, computational investigations reveal crucial conformational movements, including compaction of the structure and opening movements at the interface between the TMD and CTD, which could facilitate the binding of PIP2. In order to understand the gating mechanism, both structures of Kir2.1 in the closed and open state are needed.
Methods
A total of 9944 micrographs were collected on a Titan Krios G4 microscope operated at 300 kV equipped with Falcon4 and SelectrisX image filter. After a visual inspection to remove poor-quality micrographs, the movies were motion-corrected and dose-weighted (MotionCor2) and contrast function parameters were estimated (CTFFIND4) on 6961 selected micrographs. A total of 781,076 particles were automated picked (SPHIRE-crYOLO). The extracted particles were subject to one round of 2D and 3D classifications (RELION). A map containing 187,153 particles were subjected to 3D Auto-Refine, CTF-refinement and particle polishing. The polished particles were submited to a 3D non-uniform refinement (cryoSPARC)
Results
Here we present the cryo-EM structure of the human Kir2.1 channel complexed to PIP2 in the open state (Fig.). and provide the final cryo-EM map of the Kir2.1/PIP2 complex at 2.85 Å resolution. Preliminary atomic structure of Kir2.1/PIP2 complex was build from the cryo-EM map and revealed the PIP2 binding site.
Conclusion

Comparative structural analysis of the Kir2.1/PIP2 complex (open state) with apo-Kir2.1 (closed state) and in silico studies reveal the structural changes that lead to channel opening. Moreover, the structure of the Kir2.1/PIP2 complex highlights the role of secondary anionic binding site for channel opening. These data will help to understand the pathological mechanisms associated with mutations in the Ki2.1 channel

Keywords:

Potassium channel, gating, in silico

Reference:

1. Hibino et al. Physiol. Rev. 90 291–366 (2010).
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3. Fernandes, Zuniga, et al. Sci. Adv., 8, eabq8489 (2022)

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Combining in-situ synchrotron and electron microscopy techniques to study NiFe catalysts for CO2 methanation

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Poster Group 1

Background
Nickel-based catalysts on various supports have been widely studied for CO2 methanation due to higher activity, nickel's natural abundance, and relative low cost. However, most Ni-based catalysts suffer from deactivation due to carbon deposition, oxidation, sintering, and chemical poisoning. Recently, significant progress has been made in the exploitation of novel/modified Ni-based catalysts for CO2 methanation. As far as the support is concerned, the effect of various supports (CeO2, ZrO2, TiO2, MgO and mixed oxides) on Ni-based catalysts was studied with the goal to increase metal dispersion as well as induce an electronic effect for improving the selectivity towards methane over CO. In our collaborative study we investigated the effect of promoters of both support and active metal on hydrotalcite derived Ni/Al2O3 catalysts. From our catalyst screening experiments, we were able to select TiO2 as best support modifier and Fe as an active metal promoter. Amongst the prepared novel catalysts for CO2 methanation Ni-Fe/Al2O3 and Ni/CeO2-TiO2-ZrO2-Al2O3 were found to be exceptionally active and relatively more stable than commercially available catalysts (Ni/Al2O3). We apply multi-length-scale structural characterization in operando to these systems to gain mechanistic insights of general validity.

Methods
In this contribution we will combine in-situ TEM gas phase experiments with in-situ hard X-ray experiments to reveal the changes of the electronic structure the catalysts undergo under reduction and methanation conditions. We will present a comparative analysis of the following systems: Ni/Al2O3, Ni-Fe/Al2O3, Ni/CeO2-Al2O3 and Ni/CeO2-TiO2-ZrO2-Al2O3. In-situ TEM experiments will be complimented by in-situ gas phase experiments carried out on the i14 nanoprobe beamline utilizing an adapter which allows us to study the sample under the same experimental conditions [1]. This capability allows us to carry out multilength scale in-situ experiments by combined TEM and nanoprobe XANES, thereby linking dynamics observed on a nm- scale with those at the um scale. Mass spectrometer data acquired during the experiments will be used to correlate structural changes with catalytic activity.

Results
Figure 1 shows, as an example, the changes Ni-Fe/Al2O3 undergoes from its fresh form, during reduction at 500°C in H2/He and during reaction conditions at 300°C in a mixture of CO2, H2 and He. The distribution of Ni metal and oxide species as well as the changes in Fe oxidation state as function of the experimental conditions are obtained by in situ EELS mapping. Two regions can be identified: Metallic Ni nanoparticles are exsolved during the hydrogenation step (blue square in Figure 1 a, c), which contain a small amount of Fe; Highly dispersed NiO species are collocated with Fe(II)/Fe(III) species (black and red squares in Figure 1a, c and corresponding EELS spectra in Figure 1 e)). In addition, operando XANES unveil the presence of metallic Ni and Fe species, more so in the core of the particle (Figure 1 f, g).

Conclusion
The comparative analysis of these systems allows to clarify the role of the heteroatoms on the electronic structure of Ni. We will show that similarly to Fe, Ce acts as a sacrificial O-scavenger, maintaining Ni in a metallic state during the methanation thereby favouring methane formation over CO. We will also clarify the role of TiO2 in preserving a high dispersion of Ni.

Keywords:

in-situ, TEM-synchrotron, multilengthscale, CO2-methanation, catalysis

Reference:

[1] J. Synchrotron Rad. 2022, 29, 431-438.

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Correlative multi-spectroscopic and microscopic analyses for investigation of UV-C QDs bimodal emission

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background and aims
There is a strong interest in the development of efficient solid-state UV-C sources in the 220-270 nm range due to their powerful germicidal properties. Light emitting diodes (LEDs) based on the AlGaN semiconductor system are the most promising approach, but the early stage of development of the AlGaN technology still presents challenges, such as inefficient p-type doping and resistive ohmic contacts, resulting in low efficiencies. An approach that circumvents these material limitations is the use of electron beam pumped lamps; with a UV-emitting material (anode) housed in a vacuum tube. High-energy electrons are emitted from a cold cathode and accelerated towards the semiconductor anode, generating free carriers. Unlike LEDs, electron beam pumped lamps don't require p-type doping or contacts. To further enhance device performance, the use of AlGaN quantum dots (QDs) as radiative recombination centers is very promising. The three-dimensional (3D) carrier confinement within QDs prevents carrier diffusion towards non-radiative centers, leading to improved radiative efficiency even at high temperatures. However, samples emitting at wavelengths shorter than 270 nm present broad or bimodal spectra. It is crucial to minimize bimodal emission or emission line width to achieve highly efficient devices for disinfection.
In this work, we investigate the origin of the bimodal emission in Al0,14Ga0,86N/AlN QD superlattices designed for electron beam pumped UV emitters. We propose a comprehensive analysis combining 2D-3D scanning transmission electron microscopy (STEM), atom probe tomography (APT), cathodoluminescence (CL) spectroscopy associated to 3D simulations of the electronic structure.
Methods
The sample consists of 100 periods of Al0,14Ga0,86N/AlN self-assembled Stranski-Krastanov QDs deposited on commercial 1-µm-thick (0001)-oriented AlN-on-sapphire templates by plasma assisted molecular beam epitaxy (MBE) [Fig.1(b)]. 2D and 3D structural properties of the samples were studied by bright field (BF) and high-angle annular dark field (HAADF) STEM imaging using a probe-corrected Titan Themis (Thermo Fisher Scientific) operating at 200 kV. Compositional information of the sample was measured by laser-assisted atom probe tomography using a CAMECA FlexTAP instrument. Cathodoluminescence measurements were carried out using an Attolight CL-dedicated SEM microscope. The electronic structure of the QDs was modelled using the Nextnano3 8-band k·p Schrödinger-Poisson equation solver with AlN and GaN parameters and experimental dimensions and composition as inputs parameters.
Results

The top-view CL [fig.2(a)] presents a predominant line at 290 nm and a secondary emission at 305 nm. The SEM (scanning electron microscopy) image associated to the λ-filtered CL map [Fig.2(b)] evidences that the emission at longer wavelengths is linked to pits.
To gain deeper understanding of the structural and optical effects associated with the pits observed at the surface, we performed CL and transmission electron microscopy (TEM) characterization on the same TEM sample. Figure 3(a) presents a λ-filtered CL map of a zoomed region with two wavelength emission domains superimposed to a HAADF-STEM image of the same region. The associated HAADF-STEM image [Fig. 3(b)] shows that the longer wavelength emission appears associated with 3D conical structures.The zoomed BF-STEM image ([fig.3(c)] of a conical structure shows that it appears at the interface between the AlN buffer layer and the superlattice with inclined facets around 30° with the (0001) plane at the edge. To gain further insight into the nature and compostion of the conical domains, electron tomography has been performed and shows the propagation of the shallow pit profile through the superlattice [Fig.4(a)], with a brighter chemical-related contrast indicating a higher Ga content compared to the surrounding regions. In addition, HAADF-HRTSEM has allowed to determine the dimension of QDs in conical and homogeneous (non conical) emission regions.
APT was performed to gain combined 3D structural and quantitative compositional information within the conical structure. Figure 4(b) displays an APT Ga map; which enables the visualization of the QD planes, as they are those containing Ga. The upper part of the tip exhibits a homogeneous region with flat QD planes. However, a significant portion of the tip contains a threading defect which corresponds to the faceted step present at the boundary of the cone-shaped extended defect. Focusing on the APT analysis of 5 QD planes [Fig. 4(c)] located on the lower part of the sample, the Ga-atom planes exhibit a bending angle of approximately 30°, corresponding to the behavior observed in bright field (BF) STEM [Fig.4(d)]. Projected side and top view Ga map density for the 5 QD layers are presented in the figure 4(e) and (f), respectively. We see that the QD layers exhibit a Ga enrichment towards the 30° facet. We choose to quantify the composition of the QD-SL in the defect and homogenous region by the comparison of the Ga density. Considering that the non-defective regions contain Al0.14Ga0.86N QDs (nominal concentration deduced from growth conditions), we deduce that the faceted region should contain Al0.07Ga0.93N QDs, which should red shift the emission wavelength.
To investigate whether the chemical and morphological changes of the QD population within the entire structure are responsible for the emission bimodality, we conducted 3D simulations of the electronic structure of AlGaN/AlN QDs by solving the Poisson and Schrödinger equations using Nextnano3 within the conical and homogeneous regions. Dimensions and composition in (i) homogeneous [Fig.5 (a)], (ii) conical area [Fig.5 (b)] and (iii) at the frontier of the defect area with 30°-facet [Fig.5 (c)] are used as inputs parameters for simulations. The direct comparison between simulated and experimental wavelength in both regions [Fig. 5(d)] confirms that the bimodality emission is due to the different size and chemistry of the dots within the defect and at the defect boundary.
Conclusion
The bimodal emission is attributed to the presence of a secondary population of quantum dots located within cone-like structural defects. These defects emerge at the initial interface between the AlN buffer layer and the QD superlattice and propagate vertically. They are associated to a dislocation featuring a strong strain field, thereby facilitating the generation of a shallow pit characterized by 30° faceted boundaries.

Keywords:

UV-QDs, bimodal emission, correlative microscopy

Reference:

[1]: J. Canaz et al., under review, ACS NANO.
[2]: This work, performed on the Platform for Nanocharacterisation (PFNC), was supported by the “Recherche Technologique de Base” and "France 2030 - ANR-22-PEEL-0014" programs of the French National Research Agency (ANR).
[3]: This project received funding from the French National Research Agency via the ASCESE-3D and FUSSL projects (ANR-21-CE50-0016 and ANR-22-CE09-0024).

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Identifying transient defects in exsolution of nanoparticles by semi-quantitative ABF STEM imaging

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Poster Group 1

Background
Exsolution enables direct growth of transition metal nanoparticles embedded in metal oxide supports, which are attractive for various electrochemical and catalytic applications for energy conversion and storage. In exsolution, the transition metal is first incorporated into the metal oxide matrix, and exsolve as metal nanoparticles under reducing conditions by diffusion, reduction, nucleation and growth. Foreign cations in perovskites usually substitute the A- or B-site depending on their relative size with respect to the host species. Small transition metals are thus commonly expected to prefer the B-site. However, the exsolution process is observed to be relatively fast, which is unexpected based on the low diffusivity of the B-site with strong covalent bonds to the oxygen octahedra. In similar perovskite systems, it has been suggested that small transition metals favor an interstitial position with square planar coordination adjacent to A-site vacancies [1], as illustrated in fig (a). In this work, the Ni position in A-site deficient La-doped $SrTiO_3$ in the initial stages of exsolution is explored using semi-quantitative STEM. The knowledge obtained will support the development of a microscopic model of the exsolution phenomenon, which can be applied for optimized process strategies for energy conversion and storage applications based on the anchored nanoparticles.

Method
Powders with nominal stoichiometry $La_{0.2}Sr_{0.7}Ti_{0.9}Ni_{0.1}O_3$ were synthesized by solid-state reaction. Exsolution of nickel metal particles were performed by reduction under 5% H_2 in Ar atmosphere at 800 °C for 30 minutes. The relatively large difference in atomic number (Z) between O (Z=8) and Ni (Z=28) is exploited in the STEM images by projecting the matrix along the <100> orientation to isolate the potential interstitial Ni position on the oxygen column. A FEI Titan G2 60-300 instrument with a DCOR Cs probe corrector was operated at 200 kV with a convergence angle of 33.1 mrad to obtain an ABF image from collection angles of 8-34 mrad, suitable to image the lighter element columns. The Z-dependency was identified using the multislice method as implemented in the abTEM package [2] by simulating STEM images with the experimental conditions for a thickness-defocus series of an ideal $SrTiO_3$ structure, and for an atomic model with an interstitial Ni and A-site vacancies. The experimental column intensities were determined by fitting 2D Gaussians using the Atomap package [3]. A statistical-model based approach based on Akaike and Bayesian Information Criterion (AIC, BIC) was utilized for fitting the intensity histogram to identify the most likely independent intensity contributions. The semi-quantitative approach proposed by Aarholt et al. [4] was adopted to identify the point defect from the relative intensities of the columns.

Results
As a rule of thumb, the intensity of ABF images is proportional to Z^a with a = 1.3 which originates from a balance between elastic and thermal scattering that are most dominant for lighter and

heavier atoms, respectively.[5] However, changes in collection angle, thickness, defocus, and atomic number may alter this Z-dependency. By simulating a thickness-defocus map of ABF images of the ideal <100>-orientated $SrTiO_3$ structure with the employed collection angles, a linear Z-dependency is identified from the ratio between the intensities of the Ti- and Sr-columns, as seen in fig 1(b). From the simulated ABF image of the atomic models with interstitial Ni located on the O-column in fig (c), a split in a histogram of integrated intensities of the columns is expected to be observed in the experimental ABF image.
In fig (d), the experimental ABF image of the exsolved sample on the <100> orientation is presented with the inset illustrating the location of the 2D Gaussians fitted to the atomic columns. A statistical-model approach based on the integrated classification likelihood is adopted to avoid under- and overfitting the histogram of intensities, and to avoid biased interpretations. From fitting an increasing number of Gaussians, one calculates a score based on maximizing the log-likelihood function of the fit while penalizing the number of components and their complexity, which in our case, was done according to AIC and BIC. The log-likelihood function has a negative pre-factor, meaning a lower score indicates a higher likelihood of a valid physical description of the experimental data by the model. Both criteria agree on a three-component model for the optimized fit of the histogram of the integrated intensities of the 2D Gaussians of the O-column, seen in fig (e) and (f). There is, however, no guarantee of a physical validity of the model despite a good fit, but from the interpretation of the ratio of the mean value of component 2 and 3, the ratio becomes 1.23 ± 0.14 which results in an average Z of 9.83 ± 1.11 given a linear Z-dependency and interpreting component 2 as the O-column. The calculated value of 1 Ni for every 10 O provides an average Z of 9.82. While the absolute quantification of the number of Ni on the O-columns is challenging, the results indicate the presence of a foreign species on the columns which may be Ni. The first component can be interpreted as O-columns with a high number of oxygen vacancies generated due to the reducing environment.

Conclusion
A semi-quantitative approach utilizing the open-source packages abTEM and Atomap was adopted to identify the defect position of Ni in A-site deficient La-doped $SrTiO_3$ upon exsolution under reducing atmospheres. Simulated multislice images show a linear Z-dependency for the experimental conditions used. The intensity distribution of the O-columns in the experimental ABF image is best modelled using three Gaussians. The component of highest intensities holds a relative mean value to the main component that agrees with a calculated intensity ratio for the presence of Ni using linear Z-dependency. The interstitial defect position of Ni is expected to play an important role in the diffusion step of the exsolution process.

Keywords:

Point defects, Quantitative STEM, Open-source

Reference:

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Investigation of the long-range sources of contrast in momentum-resolved STEM

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Poster Group 2

Scanning transmission electron microscopy (STEM) is a technique consisting in the illumination of a specimen with a convergent beam of electrons. Through the recording of the far-field intensity pattern generated for a variety of scan points, it is then possible to track specimen-induced changes in the position-dependent momentum distribution of the electrons. Such momentum redistributions are the result of the subtle interplay of repeated elastic and inelastic scattering events occurring throughout the propagation of the electron wave within matter, which leads to the appearance of both thickness-dependent Bragg reflections and diffuse backgrounds, including thermal. Such scattering can generally be understood as characteristic of the high-frequency components of the interaction, being then useful for atomically resolved investigations of crystals. Conventional examples of those include the high-angle annular dark field (HAADF) imaging mode, comprising in large part of thermal diffuse scattering (TDS), and the center of mass (CoM) of the far-field pattern, which informs on the gradient of the electrostatic atomic potential of the specimen [1]. In addition to those high-frequency sources of contrast, the CoM signal is sensitive to long-range features, such as the Lorentz interaction with stray and internal electromagnetic fields [2] or the influence of the shape envelope of the specimen [3,4], which can otherwise be thought of as a refraction effect along the optical path of the electrons.

In this work, we make use of the microprobe set-up of the microscope, where the semi-convergence angle α is kept equal to a few mrad at most, to investigate the long-range sources of contrast in a CoM-STEM image. In this condition, the numerical aperture remains small enough to not include the high-frequency components (i.e. the probe is larger than inter-atomic distances), and lower frequencies become dominant. As is confirmed here, this strategy still does not permit a full elimination of diffraction effects in the result, since repeated scattering makes it possible for electrons to be scattered back to the central beam. As a result, electrons with a large variety of path length within the specimen end up contributing to the result, which then creates a dependence of the apparent long-range contrast to the underlying atomic lattice, i.e. low- and high-frequencies are not independent anymore. This effect nevertheless only accounts for a small fraction of the CoM signal extracted, and a low value of α indeed permits a direct visualization of particle shape in momentum-resolved STEM.

Moreover, we introduce a simplified quantitative STEM simulation approach where the specimen potential is modeled as a continuous shape envelope function, thus with no atomic structure at all, multiplied by a known mean inner potential. Such an approach permits a major acceleration of the

calculations needed to computationally reproduce an electron microscopy measurement, thanks to the lower pixelization of the simulation window, and can then be used for a semi-quantitative analysis of long-range contrast. Here, the results provided by the continuous object simulation method are compared to those based on an atomistic specimen, as well as to experiments, for further confirmation of the dominance of shape effects in a CoM image. By additionally including electromagnetic fields in the model, it becomes achievable to reproduce this supplementary source of contrast in (S)TEM images, for instance in the cases of electron holography or differential phase contrast [5]. What makes this prospect particularly interesting is that, even now, most of the analysis done in the framework of those methods still rely on the phase object or weak phase object approximations, and thus do not include the influence of propagation.

The present work is performed on the basis of experimental data collected from Au nanoparticles as well as large amorphous latex spheres. Data analysis is supplemented with an extensive multislice simulation study, including with the newly introduced continuous object model, which is also used to explore further material cases and future prospects.

Figure 1: Comparison between experimental (left) and simulated (right) CoM-STEM micrographs of Au facetted nanoparticles. The vectorial quantity is depicted using a colour wheel representation, with the brightness representing the modulus and the hue the orientation. The simulation shows qualitative agreement with the experimental data within the marked region of interest (white doted square). The model used for the atomistic multislice simulation is visible in the bottom right corner of the figure.

This work was supported by the Initiative and Network Fund of the Helmholtz Association (Germany), under contracts VH-NG-1317 and ZT-I-0025, and by the Horizon 2020 research and innovation programme (European Union), under grant agreement No 101017720 (FET-Proactive EBEAM).

Keywords:

Momentum-resolved STEM, Centre of mass

Reference:

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[2] M. Lohr et al. ; Ultram. 117, 7-14 (2012)
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Advances in characterization of nanostructured ceramic materials by means of Scanning Electron Diffraction Tomography

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Background incl. aims
Copper rich sulfides have attracted significant attention in the last few years as potential thermoelectric materials. These materials exhibit a rich microstructure with the existence of order/disorder domains or coexistence of different crystallographic phases, which plays a crucial role on the thermoelectric properties of copper-based sulfides. In here, the case study of a Cu2.3Mn0.7GeS4 nanocomposite is reported. It is characterized by two different phases (enargite and stannite), which form interconnected domains at the nanoscale. By means of scanning electron diffraction tomography, our aim is to accurately characterize the crystal structure of the nano-sized different domains with a single data acquisition.

Methods
As a first reported example, Scanning Precession Electron Tomography (SPET) is presented here as a tool to characterize ab initio more than one region of interest (ROI) with a single acquisition. Eggeman et al. exploited a similar approach for analyzing the volume and orientation of domains in crystalline Ni-based superalloys. This technique combines a scanning procedure with the already widely used Precession-assisted Electron Diffraction Tomography (PEDT) routine, avoiding the need of an accurate tracking system. The area of interest is selected, and the electron beam is scanned across it with the desired step size while collecting the diffraction patterns. Afterwards, the sample is tilted and the scanning procedure is performed again and so on, obtaining this way a tomography of a 2D space. SPET was performed with a Jeol F200 on the nanocomposite cut by FIB in a TEM lamella. We also tested the feasibility of this approach on a TESCAN TENSOR analytical 4D-STEM to similarly acquire diffraction tilt series on different ROIs of the sample under test.

Results
From the SPET measurements made on the Jeol F200, we show how it is possible, by appropriately sort the acquired diffraction patterns, to perform structure solution and refinements of more than one nanodomain at the same time. This also allows to compare in a reliable way the obtained structures, being the experimental conditions equivalent. In this case, the sorting was performed with NMF (Non-negative Matrix Factorization).
On the TESCAN TENSOR, the acquisitions were performed with a parallel beam at different tilt angles on a ROI using a large grid. 3D ED tilt series were then extracted and used for a structure refinement of the two phases (Fig. 1). Secondly, using a fine grid at a single tilt angle, precession-assisted 4D-STEM was used to determine the distribution of enargite and stannite, including their orientations, in the same ROI. This identification is based on templates matching generated directly from the actual crystal structure identified by 3D ED.

Conclusion

These results illustrate how these electron diffraction tomography approaches can allow us to better understand the internal structure of this type of nanocomposites and to establish a link between their physical properties and their (micro-)structure, allowing this way to customize them for obtaining the desired performances.

These results were obtained as part of the European project NanED (Electron Nanocrystallography – H2020-MSCA-ITN GA956099).

Keywords:

SPET, nanodomains, thermoelectrics, 4D-STEM

Reference:

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TEM Exploration of High-Performance Non-Noble Metal Catalysts for OER

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Within the current global energy crisis scenario, the oxygen evolution reaction (OER) has emerged as a pivotal process in vital systems for sustainable energy generation, conversion and storage. However, the demanding 4-electron transfer mechanism of the reaction requires exceptionally high activity levels, currently only achieved by noble metal-based nanocatalysts (1). This limitation impedes the widespread economic adoption of OER systems, restraining advancements in green energy technologies. Consequently, recent research has focused on non-noble metal-based systems such as cobalt tungstate ($CoWO_4$). $CoWO_4$ has emerged as promising and cost-effective noble metal-free catalyst for OER, thanks to its remarkable physicochemical properties (2). Nevertheless, a profound up to the atomic scale comprehension of the catalytic system's structure, underlying reaction mechanisms, and potential degradation and alterations in composition and morphology under harsh OER conditions, requires the use of powerful and sophisticated characterization tools (3).

In that regard, (scanning) transmission electron microscopy ((S)TEM) stands out as an outstanding solution, integrating high spatial resolution imaging with spectroscopy techniques for chemical analysis. Moreover, TEM can be adapted for in-situ experiments, allowing the direct observation of dynamical effects in catalytic systems within their natural or operational environment. Furthermore, additional synchrotron X-ray absorption spectroscopy (XAS) served as a powerful complement to the comprehensive (S)TEM characterization.

In this work, (S)TEM was employed to effectively examine the catalyst's structural and compositional changes during activation, such as the formation of vacancies on its surface. These vacancies are crucial contributors to the observed high activity and stability of the system (4). The results yielded unique information about the catalyst's morphology, which would have been inaccessible through other methods. Furthermore, employing in-situ TEM allowed for initial observations of the catalyst's response to electrochemical bias. Finally, XAS helped to establish a cohesive correlation with the insights gained from TEM analysis.

In summary, the exploration of highly active catalysts for the OER not only unlocks valuable insights into catalyst activity and stability but also correlates it to the system's morphology and composition. This structure-property relationship is crucial for advancing the development of catalytic materials with exceptional efficiency and enduring stability in OER applications, contributing significantly to the progress of cost-effective green energy technologies.

Keywords:

(S)TEM, OER, nanoparticles, catalysis, noble-metal-free

Reference:

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Nanoscale arrangement of the healthy dentin and comparison with the dentino-genesis imperfecta-affected dentin

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Poster Group 2

1. Introduction/Background
The tooth, mainly composed of dentin, which lies between enamel and pulp, is the hardest organ in the human body. Dentin is a mineralized collagen framework comprising 70% carbonated hydroxyapatite, 20% organic matrix (mainly type I collagen forming collagen fibrils), and 10% water. The tooth's necessary mechanical resistance without causing it to fracture comes from its particularly complex micro- and nano-structural organizations. Dentinogenesis Imperfecta (DI) is a rare genetic disease that causes severe dentin hypomineralization and damage to its structure (de la Dure-Molla, 2015). In some cases, DI is caused by a type I collagen mutation (Yamaguti PM, 2023), which leads to the modification of dentinal tissue organization at the nanoscale and microscale. Clinically, it can lead to rapid attrition of the dentin tissue, eventually leading to tooth loss, probably due to lower mechanical properties. In this study, we present an original analysis of the nanostructure of the healthy dentin compared to dentin affected by DI. A focus on collagen fibrils and hydroxyapatite entanglement in both samples has been performed from transmission electron microscopy (TEM) techniques.
2. Methods
Both permanent and deciduous teeth with no visible pathological signs were prepared for the control samples. In contrast, a deciduous molar was chosen for pathological dentin from a patient diagnosed with DI exhibiting damage to type I collagen. All samples were sectioned from the crown to the root and mechanically polished using abrasive Si-C paper with decreasing grain size. The samples were then dehydrated progressively through a series of increasing ethanol concentrations. TEM samples were then prepared in the different samples at different location in teeth by using a FEI ThermoFisher Helios Nanolab 660 focused ion beam. The obtained sections were observed with a FEI ThermoFisher TITAN3 G2 80-300 transmission electron microscope equipped with a Cs probe corrector given a 0.07nm spatial resolution and a SuperX detector for energy dispersive X-rays spectroscopy (EDX) analyses.
3. Results
Using HR-(S)TEM techniques, the microstructure of the healthy dentin has been studied at different scale close the enamel and in the middle dentin. Collagen fibrils and hydroxyapatite crystals are completely tangled. While fibrils looks like long cylinders with sizes over than hundreds of nanometers, crystals are usually smaller than 5 nm in length and it seems that they are roughly aligned with the direction of collagen fibrils.
Let’s first focus on collagen fibrils. The analysis of their diameter revealed that collagen fibrils tends to shrink as they are close to the enamel. Their distribution in the intertubular dentin also appears to be different close the enamel and in the middle dentin. The collagen fibrils are organized in a more isotropic way near enamel than in middle dentin where they are lying in planes perpendicular to the tubule axis. In those planes perpendicular to tubule axis, bundles of collagen fibrils and mineral are oriented in preferential directions, creating a woven structure.

Concerning the hydroxyapatite crystals, we observed that they are organized in S-shaped structure composed of individual HAP crystals and parallel to the adjacent fibrils. The individual crystals are mainly elongated along their c-axis but can also be elongated along a- and b- axis. This organization looks similar regardless of the location in the dentin.
In comparison, DI dentin look very different. The density of collagen fibrils drastically drops compared to the one in the healthy dentin. At the opposite, hydroxyapatite crystals are much denses and their distribution looks homogeneous. Looking at individual crystals' size, we did not observe any statistical difference between healthy dentin and dentin affected by DI. With the EDS analysis, we found Ca/P ratios approximatively at 1.67 (ideal hypothetical value in hydroxyapatite crystals), with a tendency to lower values in dentin affected by dentinogenesis.
4. Conclusions
At the nanoscale, dentin shows a complex and anisotropic organization involving a mineral phase made of hydroxyapatite crystals and an organic phase mainly made of collagen fibrils. In this work, we firstly analyzed precisely the microstructure of dentin according to the location in the tooth and with cross-sections parallel or perpendicular to the tubular paths thanks to the combination of FIB and TEM. The comparison of the healthy dentin and the DI one brings new insight into the tooth formation.

Keywords:

bio-minerals, architectural materials, (S)TEM

Reference:

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Exploring cardiac innervation by 3D light sheet imaging in horses with atrial fibrillation

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Poster Group 1

Background incl. aims
Local hyperinnervation plays a pivotal, yet poorly understood role in the initiation and maintenance of atrial fibrillation (AF). Changes in the intricate 3-dimensional (3D) network of nerves are difficult to characterize using traditional histological methods and AF has been challenging to study in preclinical rodent models. Non-destructive imaging techniques capable of visualizing larger tissue samples from large animal models are crucial to understand innervation changes in AF. Here, we aimed to investigate the feasibility of 3D light sheet fluorescence microscopy (LSFM) in equine atrial tissue and characterize the autonomic cardiac remodeling in a horse model of experimentally induced chronic AF.
Methods
Biopsies from the anterior descending ganglionated plexus were harvested from horses after 4-months of induced AF (n = 9), from horses with naturally occurring AF (n = 3) and healthy control horses (n = 3). Immunostaining with two neuronal markers (Tyrosine hydroxylase and Neurofilament) in parallel with a vascular marker (Transgelin) was performed to determine the local density of nerves and vasculature by computational image analysis. Whole-mount immunohistochemistry and clearing was optimized for equine heart samples by testing different depigmentation, permeabilization and imaging protocols.
Results
We present a protocol for 3D imaging of nerves in large equine atrial samples, spanning several centimeters in size. Optimized sample preparation with stepwise chemical and enzymatic extracellular matrix loosening and digestion enabled uniform sample labelling with antibodies against neuronal markers. Customized autofluorescence bleaching, sample clearing and imaging parameters facilitated high resolution imaging of cardiac innervation across the entire tissue sample. Computational analysis of atrial innervation permitted quantitative analysis in the study groups to demonstrate spatial changes occurring in atrial fibrillation.
Conclusion
3D LSFM in large animal models can improve our understanding of the mechanisms of diseases. This newly developed sample preparation protocol is well suited for single-cell resolution imaging in dense cardiac biopsies. We show the applicability of the method in characterizing innervation changes in an equine model of AF.

Keywords:

3D LSFM, AF, equine model

Reference:

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Operando structural and chemical imaging of lithium battery interfaces

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Lithium metal is a very promising anode material to develop batteries with higher energy density due to its high specific capacity and low redox potential. However, the widespread use of lithium as anode is hindered by the safety concerns stemming from the formation of dendritic structures at the electrode-electrolyte interfaces during battery operation [1]. Hence, understanding the fundamental mechanism behind the formation of these dendritic structures is crucial for the utilization of lithium metal in producing batteries with enhanced energy density and safety. Traditional ex-situ and in-situ analysis techniques are not sufficient to understand the transient processes involved in the nucleation and growth of these dendrites. Thus, operando techniques, which allow analysis while the battery is functioning, are essential for gaining deeper insights. The primary aim of this study is to devise a methodology for capturing the nanoscale structural and chemical changes occurring at the electrode-electrolyte interface during battery operation.
Methods
Our approach utilizes a dual-beam focused ion beam-scanning electron microscope (FIB-SEM) equipped with an in-house developed magnetic sector secondary ion mass spectrometer (SIMS) for operando structural and chemical imaging [2]. Secondary electron (SE) imaging helps to visualize the structural changes and SIMS imaging depicts the chemical alterations at the battery interfaces. Here SIMS is used as it is an ideally suited technique for high-sensitivity chemical analysis and imaging of all elements, including light elements like lithium. Thus, SIMS enables unequivocal detection of the lithium rich dendrites near battery interfaces. A special operando analysis setup has been developed through which the charging-discharging of the battery can be performed inside the FIB-SEM-SIMS instrument using an external potentiostat, while maintaining the necessary sample bias conditions to allow secondary ion extraction for simultaneous SIMS analysis.
Results
Using the FIB-SEM-SIMS instrument, all solid-state symmetrical lithium cells with garnet type solid electrolyte lithium lanthanum zirconium oxide (LLZO) were analyzed both before electrochemical cycling and after short-circuit [3]. Phases with darker contrast were identified in the gallium ion induced SE images of the electrolyte after short-circuit failure, SIMS imaging showed higher lithium-ion signal in those phases (Fig. 1e,1f). Thus, the presence of lithium rich dendrites in LLZO after short-circuit was confirmed by SIMS. The development of the operando setup enables us to perform SE and SIMS imaging of the battery interfaces during cycling. Therefore, it is now possible to detect and image the progression of lithium dendrite growth at different stages of cell operation. Furthermore, this operando setup allows direct correlation between the alterations in structure and chemistry of the electrode-electrolyte interface with the evolution of interfacial resistance by combining electrochemical experiments such as impedance spectroscopy with the SEM-SIMS analysis.
Conclusion
In summary, our operando FIB-SEM-SIMS setup allows real-time observation of the nanoscale processes at the interfaces linked with dendrite growth in solid-state lithium metal batteries. This

helps in acquiring a more complete understanding about the phenomenon of dendrite formation in lithium metal batteries, paving the way for development of batteries with improved safety as well as electrochemical performance.

Keywords:

Operando imaging, lithium dendrites.

Reference:

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High Spatio-Temporal Resolution Differential Phase Contrast Imaging via Detector Signal Digitisation

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Poster Group 2

Imaging of the functional properties of materials using phase contrast techniques is increasingly in-demand. The high sensitivity of phase techniques to weak phase objects makes them excellently equipped to study weakly scattering and beam sensitive materials in STEM. To study beam sensitive materials, the beam current must be significantly reduced to avoid damaging the sample. However, this often means that high dwell times are used to collect a high enough signal for the image to be interpretable. This requires the sample to be stable and unchanging over the significant time needed to acquire the frame. A different approach to collect enough signal is acquiring many subsequent frames as a lower dwell time and then later average the signal to get the final interpretable image. This has the added benefit that it fractionises the dose resulting in lower beam damage to the sample[1]. However, at fast scan speeds a scintillator detector is often too slow to keep up, resulting in streaking artefacts in the image due to single electron events lasting longer than the pixel dwell time[2].
In this work we investigate the practicability of in-hardware digitisation of scintillator detector signal in the ABF region for differential phase contrast (DPC). Digitisation retains the precision of electron detection events in time, such that every electron is detected in the correct pixel only[3]. Still, the occurrence of two electrons too close in time to be separated into two digitised events increases at collection angles within the semi-convergence angle because the arrival rate of electrons to the detector is high. This coincidence loss problem reduces the practicable beam current for digitisation imaging in the ABF region. Using segmented detectors for DPC imaging reduces the coincidence loss, since each segment is digitised separately.
Experimentally using live digitisation of four segments from an annular segmented detector, we demonstrate that atomic position precision can be retained at incredibly high scan speeds. With a low beam current of 5.2pA, to reduce loss of signal due to coincidence loss, and multi-frame imaging, the atomic column positions in an STO sample have been recorded at dwell time as low as 50 ns. The analog images captured with the same parameters show significant loss of information in the fast scan direction due to image streaking. At this high-speed scanning, binning of the multi-frame data stacks allow us to gain time-resolution as well. Thus, we may sacrifice some of our spatial resolution for time-resolution – paving the way for in-situ materials characterisation with DPC.

Keywords:

DPC, scintillator digitisation, multi-frame imaging

Reference:

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Visualising microstructural dynamics of titanium aluminium nitride coatings under variable-temperature oxidation

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Background incl. aims
Titanium-aluminium nitride (Ti1-xAlxN) coatings are used to achieve high oxidation and wear resilience in coated metal cutting tools. For the highest performance, the coatings' composition and microstructure are carefully controlled. However, it is difficult to obtain knowledge how these factors effect of the oxidation processes at the microstructural level because studies are most commonly performed: (1) post mortem and cannot observe the structural dynamics underlying the oxide formation in real-time; or (2) averaged over a large volume of the sample (and use powdered materials rather than actual coatings) and lack the spatial resolution to form connections to microstructure. Here, we describe the use of ETEM for visualizing the oxidation process at high spatial and temporal resolution, using technically relevant materials, to provide important insight into where oxidation initiates and how it proceeds to determine the performance of the coating.[1]

Methods
Industrial style Ti1-xAlxN coatings were grown on WC-Co substrates with x = 0, 0.18, 0.44, & 0.67. Samples were prepared through focused ion beam milling (fig. 1a), transferred to MEMS heating chips (Norcada Inc., fig 1b), and finally imaged in ETEM (Hitachi HF3300-S, operated at 300 kV) during heating in an O2 environment (ca. 1 Pa). The samples were observed over a temperature range from 200 to 1000 °C and energy dispersive X-ray spectroscopy (EDX) with pre- and post- elemental mapping by EDX.

Results
The high-frame-rate ETEM movies show that oxidation in TiN proceeds at the grain boundaries and cracks formed during the heating process; in contrast, Ti1-xAlxN coatings transform from large as-deposited grains into oxide nanoparticles. The onset temperature increase with increasing Al content up to x= 0.44, as shown in fig. 1c-s. Moreover, high-resolution ETEM imaging show the presence of anatase TiO2 at the early stages of oxidation across all compositions. Above ~850 °C, the oxide nanoparticles grow through crystal merging, diffusion and recrystallization to form rutile TiO2. The EDX elemental maps coupled with secondary electron imaging reveal a uniform TiO2 sublayer decorated with increasing coverage of Al2O3 particles for x = 0.18 to 0.44. In contrast, coatings with x = 0.67 reveal a complete in-plane phase separation of Al- and Ti-oxides.[1]

Conclusion
The trend with increasing onset temperature for the oxidation with increasing Al content is consistent with the resilience of the corresponding coatings during cutting operations. The in-plane separation observed for the highest Al content (x = 0.67) exposes the softer TiO2 at the surface, and the TiO2-Al2O3 grain boundaries can act as a conduit for oxygen to reach further into the coating;

together, these results can rationalise the poorer long-term oxidation resilience resulting from excessive Al.[1] Finally, the study provides insight into the real-time structural dynamics underpinning the oxidation resistance of Ti1-xAlxN coatings, illustrating how ETEM can uniquely complement other in situ techniques that have recently been applied to these materials.[2,3]

Keywords:

ETEM; TiAlN; coatings; oxidation; tooling

Reference:

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Advanced EELS spectroscopy characterization of AlGaN/GaN materials

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background incl. aims
The combination of STEM with electron energy-loss spectroscopy (EELS) and Electron-Loss Near-Edge Structure (ELNES) has gained great interest in the microelectronics industry. Indeed, ELNES can provide additional information on local atomic and electronic structures of materials compared to the already commonly used XEDS method for chemical quantification. This project intends to investigate High-Electron Mobility Transistor (HEMT) devices based on AlxGa(1-x)N semiconductors for high-power and high-frequency applications.
Methods
The HEMT device presented in this work [See Fig.1 (a) and (b)] is composed of a GaN channel and an AlGaN barrier structure separated by a few-nanometer AlN spacer grown to improve the performance of the 2-Dimensional Electron Gas (2DEG) formed at the interface [2]. The wider bandgap and stronger polarization of AlN compared to AlGaN provides higher breakdown fields and enhances the electron mobility and sheet density in the 2DEG while limiting alloy scattering. Due to significant lattice mismatch between GaN and Si, an AlN nucleation layer is grown first on 300 mm Si wafers. Successive AlxGa(1-x)N buffer layers with decreasing amounts of Al are then needed to accommodate stress in the structure. This buffer stack allows the growth of an ideally crack-free GaN channel as shown in Fig. 1 (a), assuring optimal electron mobility in the device. Layers are grown using MOCVD deposition method on Si (111) wafers. The main goal of this project is to investigate the comportment of materials in this device, especially at the active AlGaN/AlN/GaN interface through EELS analysis with “ultimate” resolution. TEM experiments are performed on a probe Cs-corrected JEOL Neo-ARM 200F equipped with a Cold-FEG and a GIF (Gatan Image Filter) Continuum spectrometer.
Results
Preliminary work has been conducted to investigate the variation of the plasmon peak in the low-loss region (≈ 10-30 eV) along the buffer stack which demonstrated a linear dependency between the plasmon energy peak (Ep), and the fraction of Al in the ternary alloys, xAl [Fig. 2 (a)]. Likewise, the acquisition of the N K-edge ELNES structure along the buffer layer stack has been conducted at 200 kV, highlighting the transition between layers [Fig.2 (b)]. The most significant difference is observed for the first 3 peaks of the spectra (≈ 400-410 eV) where the first and third peaks gradually gain in intensity over the second as the AlN mole fraction increases in the ternary alloys. According to literature [3], these peaks should result from N-cation anti-bonding interactions and the intensity re-distributions is mostly due to the valence d-electrons of the GaN. These experimental N K-edges are consistent with calculated wurtzite AlxGa(1-x)N spectra obtained in the previous studies [4,5].
Conclusion
Based on these first results, additional work has been carried out to optimize TEM sample preparation for EELS analysis, especially using Plasma-FIB Xe and PIPS II Ar ion polishing to successfully prevent Ga implantation experienced with standard Ga FIB thinning as illustrated by Fig.3 (a) and (b). Further experiments will be performed to investigate electron beam irradiation as well as the contribution of lower acceleration voltage (i.e. 80 and 60 kV) on the N K-edge ELNES analysis.

Keywords:

EELS, ELNES, AlGaN/AlN/GaN, HEMT

Reference:

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Surface facet-dependent redox dynamics in vanadium-oxide-based catalysts

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background incl. aims
Oxide materials play an important role in heterogeneous catalysis, either as functional nanomaterials in their own rights or as supports for other active nanostructures. The reactivity of oxide materials is often attributed to oxygen deficient surface sites. However, the surface motifs can undergo substantial rearrangements that are difficult to predict. A prototypical example of such complex behaviour is VOx/TiO2 catalysts, which are widely used for e.g. selective catalytic reduction (SCR) of NOx emissions. VOx undergoes a redox cycle during the SCR reaction [1], where the possible effect of the supporting TiO2 surface has been much debated. The debate reflects that earlier investigations have not provided definite information on local variations in structure and oxidation state of VOx under catalytic reaction conditions. To overcome this challenge, we present in situ atomic-resolution electron microscopy and spectroscopy observations of VOx/TiO2 catalysts under reducing and oxidizing reactions conditions.

Methods
The investigations focus on a VOx/TiO2 catalyst prepared by impregnation of commercially available TiO2 nanoparticles [2]. The morphology of a single catalyst particle is shown in Figure 1a. The catalyst was investigated using a Titan ETEM instrument [3]. High-resolution transmission electron microscopy (HRTEM) and electron energy loss spectroscopy (EELS) data, for structural and oxidation state analysis respectively, were recorded using low-dose-rate approaches [2,3]. The oxidation states were measured both as an average over multiple particles [2] and locally at individual facets by means of scanning TEM (STEM) [4].

Results
The integrated oxidation state of the VOx/TiO2 catalyst could be reversibly tuned in situ through the V(V)-V(II) range by suitable choice of gaseous environment. For conditions relevant to the SCR reaction (excess O2 with traces of NH3 and NO) an average oxidation state between V(V) and V(IV) was measured, consistent with operando spectroscopic and theoretical investigations [1]. However, the STEM-EELS measurements illustrated in Figure 1b,c revealed that the {001} facets retained oxidation states closer to V(V), whereas {101} facets on the same nanoparticle were almost fully reduced to V(IV). This difference rationalizes previous suggestions of enhanced reactivity for the {001} facets. The difference was consistently found for all nanoparticles investigated [4].

Increasing the reducing potential of the gaseous environment further revealed also reversible structural alterations in the outermost atomic layers of the VOx/TiO2 catalyst; the VOx surface transformed from an ordered to a disordered state concomitant with a reduction to the V(IV)-V(III) range in response to oxygen exchange at the surface. Surprisingly, the restructuring was found to depend on the supporting facet, with {001} facets demonstrating a greater ability to accommodate

large changes in oxidation state through its high surface cation mobility, as shown by the changing contrast at the marked atomic columns in Figure 1d [2]. The high-index {10l} showed even more extensive restructuring, forming rock-salt VO with V(II); this localized highly reduced phase must be accounted for to understand the overall redox behaviour of the supported VOx.[5]

Conclusion

The enhanced reactivity of the (001) facet terminations of anatase TiO2 have been extensively studied for photocatalytic reactions. Whether such properties extend to VOx/TiO2 catalysts, e.g. in relation to low temperature NH3-SCR of NO is addressed here for the first time at the atomic level. Varying cation coordination and density were found to be important factors for explaining differences between facets. This work shows that ETEM/STEM/EELS can be used as a unique tool to discriminate between different structural motifs at the atomic level, even on commercial catalysts.

Graphic

Figure 1. A) TEM overview image of VOx/TiO2 particle. B) EELS show that {001} facets better retain V(V) states. C) HRTEM shows distinct structural rearrangements for the facets.

Keywords:

ETEM; catalysis; SCR; vanadia; anatase

Reference:

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TEM Methodologies for Analyzing Thin Film Polymer Structure and Polymorphism Elucidation

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Poster Group 2

Background incl. aims:
The properties of polymeric materials, such as their strength, flexibility, and durability, are deeply influenced by their crystalline structure. Understanding this structure at a microscopic level is crucial for the fabrication of materials with specific properties for various applications. High-resolution transmission electron microscopy (HRTEM) is a powerful tool for visualizing the arrangement of polymer chains and crystalline regions within polymeric materials. However, due to the sensitive nature of polymers to electron beams, achieving high-resolution images for insights into polymer polymorphism without damaging the sample has been a challenge.

Methods:
In our research, we tackled this challenge by employing a technique called low-dose focal series reconstruction (LD-FSR)[1] to conduct high-resolution TEM imaging of thin films of polycaprolactone (PCL), a commonly used biodegradable polymer. LD-FSR allowed us to capture detailed images of polymer crystals with sub-molecular resolution while minimizing damage to the sample caused by the electron beam. Additionally, we utilized scanning nanobeam electron diffraction (NBED) with the 4D scanning transmission electron microscopy (STEM) technique to map the crystalline domains at both micro- and nano-scales[2] within the PCL samples. Furthermore, to broaden our understanding of polymer polymorphism, we investigated Langmuir–Blodgett thin films[3] of another polymer with multiple crystalline phases, polyvinylidene fluoride (PVDF).

Results:
Employing LD-FSR combined with 4D STEM, we directly observed interacting polymer chains in the crystal lattice, elucidating the crystal structure with a high degree of precision, including lattice deformations in PCL. In addition, by using 4D STEM in conjunction with nano Fourier-transform infrared spectroscopy (nano-FTIR) we were able to identify the presence of a partially crystalline phase alongside the well-known α-PVDF crystal phase. These findings, supported by bulk measurements such as FTIR and X-ray diffraction (XRD), and other techniques like scanning electron microscopy (SEM) and atomic force microscopy (AFM), represent a significant advancement in our understanding of PVDF polymorphism.

Conclusions:
Our combined real space imaging and nano-diffraction approach provides a unique opportunity to investigate nanoscale polymer crystallinity with high resolution, revealing distortions in the polymer structure on the local scale. Similarly, it allows for the identification of new polymeric phases based

on previously imaged different morphologies. Our methodology represents a powerful tool for the structural science of polymers in view of complex and variable polymer crystallinity, resulting from differences in preparation and processing.

Keywords:

TEM, polymer, 4D STEM, polymorphism

Reference:

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Three-dimensional classification of dislocations from single projections

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Poster Group 2

Background incl. aims
Many material properties are governed by dislocations and their interactions. Examples range from the strengthening of metals and alloys to efficiency in semiconductor laser devices. Thus, knowledge of the three-dimensional topology of dislocation networks is crucial for material engineering. A two-dimensional projection of dislocation networks can be readily obtained by conventional (scanning-) transmission electron microscopy (S/TEM) images. Here we show a way to reveal the three-dimensional location of dislocations and simultaneously classify their type from a single 4D-STEM measurement [1].
Methods
Under systematic row conditions, where the systematic row is oriented non-parallel to the dislocation's Burgers vector, the 4D-STEM dataset can be represented by a reduced 2D dataset, with a spatial dimension x perpendicular to the dislocation line and a diffraction dimension q in the direction of the systematic row. Due to dynamical diffraction effects, such (x, q)-datasets exhibit sufficient information to uniquely identify the dislocation type and its three-dimensional depth within the S/TEM lamella. This identification is facilitated by comparison of the experimental (x, q)-data to a database with calculated (x, q)-data, where the relevant parameters Burger's vector, dislocation depth, lamella thickness, and beam tilt were varied. The column approximation is sufficiently accurate for the calculation task, which allows a numerically efficient calculation of the database by a forward propagation of the Darwin-Howie-Whelan equations [2].
Results
For an exemplary dislocation the results are shown in the figure. The investigated dislocation is a threading screw dislocation with a line vector of [0001] found within a GaN buffer grown on a sapphire substrate. The data was obtained under (0002) systematic row conditions. In the left-hand panel the experimentally obtained (x, q)-data for this dislocation is displayed. The panel in the middle shows the best matching calculation for the data, which was obtained for a Burgers vector of [000-1], a lamella thickness of 132 nm, and a depth of the dislocation core of 55 nm behind the entrance surface. The right-hand panel shows the mean squared error evaluated during the comparison, which is the average squared difference between the experimental data and the individual (x, q)-data from the database. Even though local minima are found for several specimen thicknesses and dislocation depths, the global minimum can be easily identified.
Conclusion
The ability to three-dimensionally locate a dislocation within a specimen and simultaneously classify its type provides an extremely powerful way for the investigation of dislocation networks. A 3D classification is possible from a single 4D-STEM measurement within the limitations of the g·b criterion. In comparison to tomographic methods a single 4D-STEM measurement can be performed much faster and with less dose, which enables the investigation of dislocation networks in beam-sensitive materials or in dynamic conditions, like during an in-situ heating/cooling experiment.

Keywords:

4D-STEM, Dislocations, Dynamical diffraction

Reference:

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[2] A. Howie, Z. Basinski, Phil. Mag. 17 (1968), 1039

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Microstructural evolution of zeolitic nanocrystals for CO2 capture by Environmental in-situ TEM

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

In the current context of demographic evolution resulting in the considerable increase in greenhouse gas emissions, the academic and industrial community allocates more and more resources to the development of new solutions for capturing, storage and recovery of these undesirable products, the main component of which is carbon dioxide (CO2). Micronsized zeolites such as FAU, LTA or TiSi are currently the most widely used for CO2/CH4 separation, but they have disadvantages such as reduced accessibility of pore volume, slow CO2 kinetics, low regeneration and low cycling, and high costs of organic templates used for their synthesis. In this context, functionalized nanosized zeolites (RHO type) with fully accessible active internal and external surfaces and high crystalline yields were synthesized [1]. Considering the ultimate atomic resolutions (<1 Å) achieved in a TEM, and the in-situ capabilities available nowadays, the in-situ environmental TEM is the only approach able to allow the real-time exploration of the impact of specific parameters such as the gas flow, pressure, temperature on the nanosized crystals evolution upon reactions. It allows the real-time analysis of parameters such as crystal size, the proportion of open/closed pores and/or crystalline facets.
In this study, we focus on and the microstructural changes of nanosized RHO zeolites analyzed by the MET in-situ Environmental under a CO2 flux under high temperatures and pressures.

The STEM-HAADF 2D and 3D observations were carried out on a double corrected Analythical JeolARM 200CF equipped with a Jeol Centurio EDS detector and a Quantum GIF. The in-situ experiments were performed by using an Environmental-Cell from Protochips [2] which has been employed under 1 bar of CO2 flow and for temperature increased from 25 to 900 °C with a heating rate of 10 °C/min. Owing to the high sensitivity of zeolites upon electrons irradiation, the STEM observations were carried out at well-defined temperatures (the beam was turned off during the heating steps).

The RHO nanozeolite was initially heated under Ar at 200 °C, and images under these conditions were taken as references. Maintaining the temperature at 200 °C, CO2 was then contacted with the sample followed by heating and imaging at 700°C, 800 °C and 900 °C. No significant lattice expansion occurs between 200 and 700 °C, when the RHO nanosized zeolite is exposed to CO2. However, the visible expansion of the crystals at 800 °C is consistent with the structural flexibility behavior under air where we observed a substantial increase of the lattice parameter (0.2221 Å) from 700 to 800 °C due to the change in symmetry of the crystalline structure from non-centrosymmetric to centrosymmetric [1]. Superposition of the very same nanozeolite crystals recorded at different temperatures revealed distinct differences in the size of the discrete nanocrystallites. Specifically, the images recorded at 800 °C [3] and 900 °C superimposed with the reference images taken at 200 °C under CO2 show a clear difference in the size of the nanosized crystals, corresponding to an expansion of the particle-matrix by 3 nm and 4.8 nm, or 9% and 15% of the average particle size, respectively. The increase in the volume of RHO crystals was evaluated on the basis of 2D micrographs and corroborated with the exploration of the volume of nanocrystals obtained by

electron tomography. The particle expansion between 800°C and 900°C is accompanied by a sharp change of the nanocrystal's microstructure. The crystals morphology remains stable up to 1000°C. This original study highlights for the first time the flexibility and the microstructural stability of RHO nanosized zeolite at high temperatures under CO2 exposure by in situ HRTEM.

Keywords:

In-situ ETEM-CO2, Flexible RHO zeolites,

Reference:

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Unsupervised Deep Learning approach for image registration in Correlative Microscopy for the localization of Nanoparticles

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Background incl. aims
Various microscopic methods such as scanning tunneling microscopy, atomic force microscopy, electron microscopy, and confocal laser scanning microscopy are already present in the ongoing research scenario and provide a lot of information to researchers. Each of these microscopic methods provides different information about the local structure of the sample under investigation, depending on the interaction of the probe with the sample. In general, the information obtained from two different microscopy methods is complementary. Correlative Light- and Electron Microscopy (CLEM) is the potential tool for determining the localization and identification of biological images combining different fluorescence labelling image with an electron microscope image. Automated Multi-modal registration with reference to the prediction of landmarks is a very challenging problem. We demonstrate the image registration of correlative light and electron microscopy (CLEM) images with the precise localization of landmark positions using a training-free, unsupervised deep learning approach.
Methods
Microscopy imaging with Deep Learning (DL) have transformed the field of biological sciences and is expected to be capable of providing relevant features of images to perform complex analysis tasks. The imaging problems include image segmentation or classification [1], object detection and image resolution are experimentally performed using Deep Learning methods. Deep learning techniques such as deep neural networks (DNNs) have produced ground breaking performance for computer vision and image classification. Image registration is the very first and crucial step for intracellular studies. This is achieved by aligning two different images of the same object taken by different image sources [2]. Images with different modalities can provide additional and unique information about the localized structures [3] e.g. cells, tissues, proteins, etc. Till now, various methods are proposed and even implemented either based on neural networks or supervised approach but they all are manually driven, require lot of time and high accuracy for landmarks. So, we implement a DL based architecture to achieve the multi-modal image registration without using any landmark assumptions or modeling of the images.
Results
CLEM technique is used to get information at different length scales. The electron microscopy provides high resolution with detailed information of the sample to the nano level and the fluorescence microscopy highlights the different regions of interest using fluorescent labels. We apply feature mapping is achieved through a deep neural network and we optimized the homography hyperparameters using different learning rates at each hidden layer of neural network and regularly optimize for the minimized neural estimation loss (MINE) and generates the transformation matrix. With the calculated transformation matrices, the fluorescence channel can then be overlaid on the EM image quite easily with pixel accuracy for CLEM output. The obtained CLEM micrograph can be viewed in the graphic figure.
Conclusions

We are presenting an unsupervised DL approach for registering CLEM datasets with automated unsupervised localized landmarks. The DNN was trained by using training free MINE network with matrix exponential. It is time efficient and can handle CLEM data up to an image size of 5120 x 5120 pixels (even RGB images) and, due to the generated transformation matrices, registration of multi-channel images are also possible. The bottleneck of our neural network depends on the estimation that how fast and accurately the network optimized itself. Our DNN identifies the required features as precisely as the manual landmark based methods and promises to provide better optimized results. For training DNN, we do not require any image dataset and it is optimized in approx. 5 hours and after that, it does not require any further optimization for linear image pairs. Thus, the computing time is reduced to a few seconds per image pair and therefore, it is possible to process a large number of image datasets in a very short time even without human supervision or training.

Keywords:

Correlative microscopy, image registration, DNN

Reference:

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3. A Nan et al., Medical Imaging with Deep Learning. PMLR (2020)

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Distortions correction in HR-(S)TEM and low resolution TEM images: absolute size, strain and polarization measurements

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[1]CEMES-CNRS, Toulouse, France

Poster Group 2

Background incl. aims
TEM and STEM imaging are widely used techniques for visualizing and quantifying shape, size, density, composition, strain and structure in solid state materials at micro-, nano- and subnanometer scales. Such images are subjected to different types of distortions and calibration errors. For the purpose of fully quantitative analysis, these distortions should be removed. We propose here several methods for the correction of low magnification TEM, high-resolution TEM (HR-TEM) and STEM (HR-STEM) images, followed by their quantitative analysis.
Methods
For the correction of low magnification images taken at a basic conventional TEM we apply the technique named “Moiré by specimen design” (MoSD) [1] which allows to measure deformation and displacement in single-crystal structures, in cross-sectional and plan view geometries, with nanometric resolution over a micrometer field of view. The method is based on a specific sample preparation of a stack of two superimposed rotated lamellas of known single crystal that provides the formation of “controlled” moiré images. The deviation of experimentally obtained pattern from the anticipated one can be quantitatively mapped in form of a displacement field by reciprocal space treatment. We developed a method which allows to correct any other image for these distortions.
For HR-(S)TEM images, we present a method named “Absolute strain” (AbStrain) [2]. It allows for quantification and mapping of interplanar distances and angles, displacement fields and strain tensor components with reference to a user-defined Bravais lattice and with their corrections from the image distortions. AbStrain goes beyond the restriction of the existing method known as geometric phase analysis by enabling direct analysis of the area of interest without the need for reference lattice fringes of a similar crystal structure in the same field of view.
For the case of a crystal composed of two or more types of atoms, we also present a method named “Relative displacement” [2] for measuring relative atom displacements of the sub-structures in the unit cell, the quantity being in link to the intrinsic polarization.
Results
We applied the first method for the correction of low-magnification TEM images taken with old generation Jeol2010 TEM. The images contain layers of different thickness, nanoparticles and dislocations, which require a statistical analysis of their size, density and volume fraction. Before correction, the relative errors in the measured values are estimated of 5%, 10% and 60%, respectively. The correction allowed a drastic enhancement in the accuracy of measured values by an order of magnitude.
We demonstrated a successful application of AbStrain and Relative displacement to HR-STEM images of functional oxide and semiconductor heterostructures. BaTiO3/SrTiO3 ferroelectric heterostructure grown on silicon and InGaN 2D layers and 3D islands embedded in GaN will be of particular concern.
Conclusion
The first method proposed here enhances the precision in quantification of nanomaterials dimensions by using low magnification TEM images, that paves the way for elucidating their growth laws based on justified numbers. The 2 other methods extend the range of possible crystalline

materials that can be analyzed by the treatment of their HR-(S)TEM images. For example, nanoparticles embedded within different types of matrices, free-standing nanowires, high-angle grain boundaries, multiple stacks of heterostructures of complex oxides and semiconductors, anti-phase domains and defects inducing long range displacements, constitute just a part of the list of such materials.

Keywords:

HR-(S)TEM, distortions, strain, polarization, Abstrain

Reference:

Figure: Abstrain corrected HR-STEM-HAADF image of the BaTiO3/SrTiO3/Si structure decomposed into images of Ba+Sr and Ti atoms; out-of-plane strain with reference to the BTO Bravais lattice (colored background) and the relative displacement between the Ti and Sr+Ba atoms (arrows).

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Correlative TEM and APT studies of metallic Mg specimens prepared and analyzed under controlled environments

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Poster Group 2

Background
Successful correlative transmission electron microscopy (TEM) and atom probe tomography (APT) studies on metals require a surface and subsurface with minimal defects. The potential of these powerful analytical techniques can be hindered by surface damage during material processing, implanted Ga during focused ion beam (FIB) specimen preparation, or environmental degradation during specimen transfer in an ambient environment. Therefore, specimen preparation is a critical component of successful correlative analyses. We present a sample preparation and analysis workflow under controlled environments using broad Ar ion beam milling of the bulk sample and post-FIB condensed Ar ion beam milling of APT specimens. The aim is to differentiate the sub-surface properties of a metallic sample related to Ga diffusion by FIB preparation and by post-FIB condensed Ar ion beam milling using a controlled environment workflow; APT specimen yield from ambient versus controlled environment sample preparation also will be compared.

Methods
Under controlled environments, bulk Mg foil ribbons (hot rolled and deformed) were prepared using a broad Ar ion beam milling system [TrionMill, Fischione Instruments] to remove surface artifacts. The bulk sample was then transferred in a protected environment to a FIB system for imaging and electron backscattered diffraction (EBSD) analysis. Specific regions on the Ar ion beam milled sample were selected (Fig.1, areas 1 and 2); from these regions, APT specimens were prepared. Areas with multiple grain boundaries were selected to elucidate the distribution of Ga in the Mg specimen during specimen preparation [1]. FIB specimen preparation of the APT specimens followed by post-FIB condensed Ar ion beam milling [NanoMill® TEM specimen preparation system, Fischione Instruments] and subsequent TEM and APT characterization were performed in protected environments. APT characterization involved the use of an environmental transfer hub (ETH) station [2] to transfer the APT specimen to a local electrode atom probe system [LEAP®, CAMECA].

Results
Surface damage and oxides on the bulk were successfully removed by broad Ar ion beam milling based on the resulting pristine surface (Fig. 1a), which is polycrystalline with sub-micron grain sizes that average 600 nm. EBSD analysis of an area near the crack region (Fig. 1a, pink region) found smaller grains; multiple grain boundaries (marked in Fig. 1b) were identified on the band contrast image and inverse pole figure (IPF) map. The acquired TEM image (Fig. 1c) showed the presence of the FIB Pt cap layer (Fig. 1a, area 1), while the post-FIB condensed Ar ion beam milled specimen (Fig. 1a, area 2) showed the removal of the Pt layer from the TEM image (Fig. 1e). The Ga APT reconstructions displayed a significant amount of Ga at 18.7 atomic %, especially on the specimen's surface following Ga FIB milling (Fig. 1d), which is reduced to 3.9 atomic % following post-FIB Ar ion beam milling (Fig. 1f). The dark contrast on the bright field TEM image of the post-FIB condensed Ar ion beam milled specimen (Fig. 1e) correlates to the Ga-rich area marked on the Ga APT reconstructions. Using this feature, the position of the grain boundary decorated with Ga along the

vertical axis of the APT specimen was identified (Fig. 1f). For both APT specimens after Ga FIB and Ar ion beam milling, low amounts of O and H (Fig. 1g,h) within the Mg-rich region were observed. At the interface of the Mg and Ga-implanted region, parallel trends of increasing amounts of O and Ga from the Ga FIB specimen (Fig. 1g) were detected. In contrast, the post-FIB condensed Ar ion beam milled specimen showed a constant amount of O at 0.50 atomic % across the specimen. Additionally, diffusion of Ga via the grain boundaries of the polycrystalline specimen occurred during FIB preparation as evidenced by the Ga-rich line along the APT specimen (Fig. 1h, inset).

Conclusion

The broad Ar ion beam milling, Ga FIB, and condensed Ar ion beam milling workflow – all under controlled environments – removed specimen surface damage, oxidation, and Ga implantation from the specimens. Consequently, the metallic nature of the Mg sample was conserved. Ga diffused to the grain boundary of the post-FIB Ar ion beam milled specimen, which correlates to previous study [3]. The formation of Mg oxide on the FIB-prepared specimen possibly occurred due to FIB-induced thermal effects [4]. APT compositional analysis showed that surface oxidation was suppressed by controlled environment transfers.

Keywords:

magnesium, controlled environments, specimen preparation

Reference:

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Determination of 3D strain fields from dynamical diffraction effects

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background incl. Aims
Strain fields have a large influence on many material properties. Examples range from electronic properties like the band gap or charge carrier mobilities in semiconductors to structural properties like nucleation energies of extended defects. Several techniques for a nanometer-resolved measurement of strain fields within the S/TEM are available, like nanobeam electron diffraction, geometric phase analysis, or dark field electron holography (DFEH). However, these methods typically only reveal strain field variations in directions perpendicular to the electron beam and assume a constant strain field in the beam direction. Within these methods, dynamical diffraction effects are often seen as a nuisance since they often complicate the interpretation of the data. However, strain fields are seldom purely two-dimensional and often also exhibit inhomogeneities along the electron beam. Here we show that dynamical diffraction effects can be leveraged in several ways to obtain information about the 3D strain field.
Methods
We use the scanning convergent beam electron diffraction (SCBED) with non-overlapping disks to capture the dynamical diffraction data, where for each scan point a CBED pattern is obtained. The scan coordinates add spatial context to the individual CBED patterns, which enables their comparison with numerical strain field models.
We demonstrate the validity of this approach by investigating the dynamical diffraction effects originating from an epitaxially grown layered structure, where the layer exhibits a 45° angle with respect to the specimen surface. The inclined layer creates a well-known inhomogeneity of the strain field in the beam direction. Due to the 45° inclination of the layer, the depth of the inhomogeneity is constantly changing with the lateral position of the beam. The specimen is investigated under systematic row conditions with reflections in the direction perpendicular to the layer. The SCBED data then can be fully represented by a reduced two-dimensional (q, x)-dataset, where both the spatial dimension x and the diffraction dimension q are oriented in the direction perpendicular to the layer.
Additionally, we performed DFEH measurements of the specimen. As DFEH requires parallel illumination, a beam tilt series is acquired to achieve similar (q, x)-datasets. In contrast to the SCBED data, the DFEH datasets also contain the phase of the diffracted beam [1].
Results
The experimentally obtained (q, x)-data are compared with simulated (q, x)-data, which are calculated from a strain model using numerical multi-beam propagation of the Darwin-Howie-Whelan equations. A very good agreement between the experimental and simulated plots is found. The results show that the inclusion of surface relaxation effects into the strain model becomes mandatory for matching the simulation to the data, which further demonstrates the high sensitivity of the dynamical diffraction data to the 3D strain field.
A striking feature of these (q, x)-data is a mirror symmetry with respect to the specimen's midplane, which is also expected theoretically [2]. This mirror symmetry forbids discrimination between the case where the strain inhomogeneity is located at a certain distance behind the entrance surface of the specimen from the case where the inhomogeneity is located at the same distance before the exit

surface. However, this midplane symmetry occurs only in the intensities of the diffracted beams, not in the diffracted beam's phases. The phases recorded in the DFEH tilt series do not exhibit this ambiguity and allow even the differentiation between these cases and thus the unique determination of the depth of the inhomogeneity.

Conclusion

Within dynamical diffraction effects information about the 3D strain field is encoded. Using techniques like SCBED or DFEH tilt series allows to record dynamical diffraction data obtained from small variations of the incident beam tilt together with spatial information. This data can be quantitatively compared with numerical strain field models. The determination of the 3D strain field enables several new applications: for instance, the depth and Burgers vectors of dislocations can be obtained from a single 4D-STEM measurement [3]. Another example is the determination of the thickness and concentration of layers in semiconductor heterostructures by detailed measurements of the strain relaxation at the specimen surfaces.

Keywords:

4D-STEM, DFEH, Strain, Dynamical diffraction

Reference:

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Paleopalynology of Holocene birch hybridization in Iceland

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Poster Group 1

Betula L. (birch) is a genus of about 50–60 tree species distributed throughout the temperate, boreal, and arctic regions of the Northern Hemisphere. Birch woodlands are an integral component of the tundra biome, and birch is one of the key broad-leaved tree species of the circumpolar boreal forest. Birch woodlands in Iceland, which cover around 1.5% of the total land area, are composed of 80% birch shrubs less than 2 m tall. Similar birch shrubs, often referred to as mountain birch, are found in Fennoscandia, northern Russia, European mountain regions and beyond. Previous botanical, cytogenetic and molecular genetic studies have shown that shrub-like birch in Iceland is the result of introgressive hybridization between the two co-existing Betula species, the arctic dwarf birch (Betula nana) and the European downy birch (B. pubescens). As dwarf birch is a diploid species and downy tree birch a tetraploid species, their hybrids are triploid. In the introgression process, triploid hybrids, which are partially fertile, can backcross with the parental species, producing progenies comprising introgressed diploid, triploid, and tetraploid plants. Triploid plants produce both normal triporate pollen (with three pores) and abnormal, aborted pollen, due to dysfunctional meiosis. The type of pollen abnormality that can be detected and quantified is non-triporate pollen (with four or more pores in the pollen wall). The presence of non-triporate pollen was therefore used in the study resented here as a marker to trace birch hybridization in the past. In this study fossil pollen in samples from peat sediments was examined. The peat monoliths were extracted from three geographically diverse locations in Iceland, from Grímsnes (S), Eyjafjördur (N) and Thistilsfjördur (NE Iceland), and throughout the Holocene epoch. Ages were calibrated based on known volcanic tephra layers and by radiocarbon dating. By measuring the size of individual pollen grains, we were able to differentiate pollen of the shrub-like downy birch (B. pubescens) from its closely related dwarf birch species (B. nana). The results revealed an establishment and a rapid expansion of birch woodland predominated by shrub birch B. pubescens soon after the retreat of the Icelandic Pleistocene icesheet. Non-triporate pollen grains were detected in samples from all three locations and throughout the Holocene, but with different frequencies. The results showed peaks of intense hybridization following woodland expansion in the initial period of the Holocene, from about nine thousand years ago, and again in the warming period of the mid-Holocene Thermal Maximum, the period between five and three thousand years ago. Triploid hybrids that were produced during the intense period of hybridization could potentially backcross with the dwarf birch or the downy birch, allowing gene flow by introgression between the two species, presumably making birch more adaptive to environmental changes. Thus, climate warming in the current era is expected to promote this introgressive hybridization resulting in a significant change of landscape of the birch woodland in Iceland. Birch woodlands are likely to become more widespread. Furthermore, introgressed shrub birch is likely to be more competitive over dwarf birch as average summer temperature rises.
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Keywords:

Betula, birch, palynology, pollen, triploids

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Imaging MEMS motion at nano scale by time-resolved scanning electron microscopy

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Poster Group 2

Background
Reconfigurable micro-optics and on-chip integrated photonics often rely on micro-opto-electro-mechanical-systems to provide dynamical optical processing such as beam steering and focusing or optical coupling and phase tuning. In the process of design and fabrication of micro-electro-mechanical-systems (MEMS), and to assess their quality, a local and direct measurement of their motion on a point-by-point basis would provide unprecedented supporting information. Such a technique must be fast enough to track the MEMS dynamics with sub-micron resolution.
Methods
We introduce and discuss the implementation of dynamical imaging of MEMS by time-resolved scanning electron microscopy (TR-SEM). MEMS resonators are actuated in-operando close to their resonance frequencies, and a synchronized comb of electron pulses is used to image stroboscopically the device at a controlled time delay with respect to the beginning of its oscillation period.
Results and conclusions
We demonstrate the acquisition of stroboscopic movies by a proper sequential acquisition of secondary electron signal. Unprecedented information about local trajectory is provided, in the microsecond scale and at tens of nanometer lateral scale. In-operando nonlinearities in the response of the system, interpretable as related to system hardening are brought into evidence. We also discuss strategies to reach the ultrafast time scale.

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Keywords:

MEMS, SEM, Dynamics, Nanoscale, Strain

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Fine structure tuning for strongly correlated functionalities in high entropy oxides

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Poster Group 1

Background incl. aims
Strongly-correlated phenomena, such as, colossal magnetoresistance (CMR) and metal-insulator transitions (MIT), exhibited by perovskite manganites are accompanied and reinforced by coexistence of competing magneto-electronic phases [1]. Such magneto-electronic inhomogeneity is governed by the intrinsic lattice-charge-spin-orbital correlations, which are conventionally tailored via chemical substitution, charge doping or strain engineering. Alternately, the recently discovered high entropy oxides (HEOs), owing to the presence of multiple-principal cations on a given sub-lattice, exhibit indications of an inherent magneto-electronic phase separation encapsulated in single crystallographic phase. In this abstract we present the structure characterization at atomic resolution for a series of single-phase orthorhombic HE-manganites, (Gd0.25La0.25Nd0.25Sm0.25)1-xSrxMnO3 (x = 0 – 0.5), which combines high entropy (HE) concept with standard property control by Sr2+ (hole) doping, with the aim to extend the HE based approach to design strongly-correlated systems. High resolution scanning transmission electron microscopy (HR-STEM) and integrated differential phase contrast (iDPC) imaging have been used to determine the crystal structure variations in HEO introduced by different Sr concentrations. Electron energy-loss spectroscopy (EELS) has used to reveal the Mn valence change with Sr doping levels. We aim to correlate the atomically resolved geometric and electronic structures to the finely tuned electromagnetic properties of the series of Sr doped HEOs.
Methods
The powder samples were synthesized using nebulized spray pyrolysis (NSP) technique. The details of the synthesis procedure can be found elsewhere [2]. A double aberration corrected transmission electron microscope Themis Z (Thermo Fisher) equipped with a Super-X energy dispersive X-Ray detector and Gatan GIF Continuum 970 HR + K3 IS camera (operated at 300 kV) were used to examine the specimens.
Results
From the high-angle annular dark-field (HAADF) STEM images, the strong orthorhombic distortion can be identified for x = 0 (Figure 1a). The distortion becomes weak with increasing the Sr concentration. For x = 0.5 system (Figure 1b), the crystal structure is very close to pseudo-cubic. However, the higher symmetric phase shows noticeably higher density of different types of lattice defect, including twin boundaries and Ruddlesden-Popper faults, as reveled by iDPC and HAADF images in Figure 1c along [01-1] and [001] zone axis, respectively. Figure 1d and 1e present the HAADF STEM image and the corresponding atomically resolved elemental distribution maps for x = 0 and x = 0.5. A homogenous distribution of the rare-earth cation (and Sr for x = 0.5) on the A-site (RE) sub-lattice along with presence of Mn on the B-site sub-lattice, without any observable segregation

at the atomic length scales, can be confirmed. The change in the oxidation state of Mn can be evaluated from the integrated area ratio of the Mn L3 to L2 edges (L3/L2 ratio) and the energy difference (ΔE) for the O K-edge features between the Mn 3d feature (~ 530 eV) and the RE 5d/Sr 4d feature (~ 536 eV). The changes in the Mn L3/L2 ratio and ΔE as a function of Sr2+ doping (Figure 1f) unambiguously confirms that increasing amount of Sr2+ doping results in change of Mn valence state from ~3.1+ for 0%Sr to ~3.6+ for 50% Sr. The quantitative EELS analysis indicates that the separation of the antiferromagnetic ferromagnetic phases mainly depends on the ratio of the Mn3+ and Mn4+. More Mn4+ enhances the double-exchange interaction between Mn3+ – O – Mn4+, which increases the ferromagnetic component.

Conclusion

This initial study signals excellent potential to achieve complex magneto-electronic phase diagram with unique temperature dependencies that stem from competing magneto-electronic interactions. The unique subtle properties can be tuned by the merger of the high entropy-based design approach with the strongly correlated electron systems. The tunable electromagnetic properties are attributed to the structure changes by the high-resolution imaging and EELS analyses showing an increase in the amount of Mn4+ and a corresponding decrease in Jahn–Teller effect and the degree of orthorhombic distortion upon increasing amount of Sr2+ doping [3].

Keywords:

high entropy oxide, STEM, EELS

Reference:

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A crystallographic aspect of Li metal anodes: Understanding the functionality of lithium-ion all-solid-state batteries

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Poster Group 1

Background

Enabling the use of Li metal as an anode in all-solid-state batteries (ASSB) promises higher energy density and safer operation when compared to current Li-ion batteries due to the integration of nonflammable solid-state electrolyte. Increasing demand for safe electrical energy storage, especially in the transportation sector, has resulted in rapid and continuous growth of ASSB research [1].
It is undeniable that both cathode and anode microstructure have a direct impact on battery cell performance. The coulombic output, charge and discharge rates, and life cycle depend on grain size, boundary characteristics, and crystal orientation (texture). The small grain size tends to increase electrical resistivity, which degrades battery performance. High-angle grain boundaries are the most resistive due to corresponding strain fields, which are the result of high dislocation densities. Crystal orientation can promote intercalation, prohibit intercalation, or increase instability [2]. For example, closely packed crystallographic planes (grain facets) are favorable for higher ion mobility. Considering the many microstructural parameters and relationships between them, it is critical to evaluate the best candidate for Li metal anode before battery fabrication.
However, there is little work reported on the microstructural characterization, which allows for the evaluation and comparison of various lithium metal sources.
Li is highly sensitive to oxygen, nitrogen, water, and carbon dioxide, which makes sample preparation for microstructural characterization impossible in ambient atmosphere. Lithium is also a very soft material, and its crystallographic structure can be easily damaged by mechanical interaction. The inherent characteristics of lithium metal demand a controlled environment workflow from sample preparation to structural investigation in a scanning electron microscope (SEM). In the past few years, considerable effort has been devoted to developing accurate sample transfer technology [3].
We demonstrate a workflow that allows the evaluation and comparison of different lithium anode sources. The workflow consists of cryogenic broad Ar ion beam milling sample preparation, cryogenic sample transfer to the SEM, and microstructural characterizations. We will discuss the challenges of cryogenic observations in the SEM. All results are supported by energy dispersive X-ray spectroscopy (EDS) and backscatter electron diffraction (EBSD) measurements.

Methods

All samples [pure Li metal ribbons, Sigma-Aldrich] were prepared using a broad Ar ion beam milling system [TrionMill, Fischione Instruments]. The samples were transferred from the ion mill to a Ga FIB system [Scios DualBeam, Thermo Fisher Scientific] using cryo transfer [Actively Cooled Transfer device, Quorum Technologies Ltd. / Fischione Instruments]. The Scios DualBeam is equipped with a PP3004 airlock and PP3005 SEM/FIB cryo stage [Quorum Technologies Ltd.] All SEM analytical measurements were done using an X-Max 150 mm2 EDS detector coupled with Aztec software [Oxford Instruments] and e-FlashFS EBSD detector combined with Esprit software [Bruker Nano Analytics].

Results and conclusions

Three lithium sheets (Li-1, Li-2, and Li-3) were compared. Figure 1 shows the inverse pole figure (IPF) color-coded EBSD maps for the three samples. The average grain size is 100 µm, 150 µm, and 1150 µm (Li-1, Li-2, and Li-3, respectively). Figure 2 represents corresponding pole figures. Note that multiple maps acquired from different sample regions were used for average grain size and texture determination for each sample. Sample L1-1 is characterized by {110}<001> texture, sample L1-2 has strong rotated cube texture {100}<011> in the rolling (RD), transverse (TD), and normal (ND) reference directions, while sample Li-3 represents random texture. If one of these materials was integrated as an anode material to ASSB, the normal direction would be aligned with the Li+ mobility direction between the anode and cathode.
The {110} crystal facets have the lowest surface energy and the closely packed atom arrangement for body-centered cubic lithium structure; these characteristics contribute to high Li diffusivity, which improves cycling stability and battery lifetime [4]. Large grains are more desirable for high coulombic output, while small grains are not [5]. Therefore, larger grain sizes combined with a {110}<001> texture would be the best choice for a Li metal anode because it is likely to provide the best battery performance.
Accurately evaluating lithium anode microstructure is critically important to developing superior battery performance.

Keywords:

Li-ion battery, texture, microstructure, BIB

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Dose fractionation and alternative scanning strategies for beam damage mitigation in event-driven 4D-STEM

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Poster Group 1

The introduction of event-driven direct electron detectors, e.g. those based on the Timepix3 chip (Amsterdam Scientific Instruments CheeTah T3, Advacam AdvaPIX TPX3), has removed a major constraint in scanning transmission electron microscopy (STEM), making it possible to achieve momentum-resolution by recording the far-field intensity at each scan position with no dwell time or dose penalty. In contrast, most of the popular frame-based detectors still impose a minimum acquisition time of a few tens of microseconds. Such recording times are at least an order of magnitude larger than what can typically be achieved by the microscope's scanning system. Additionally, frame-based representations are inefficient regarding data size and processing speed, especially when using large or repeated scans. Consequently, event-driven detection has significant advantages for fast 4D-STEM data acquisition, including electron ptychography.

The development of user-programmable scan engines allows another layer of optimization, particularly with the introduction of alternative scan patterns, which have been shown to permit mitigation of the overall specimen deterioration during an experiment. This mitigation can be related to the accumulation of damage sites (e.g. charge carriers induced by radiolysis or atomic defects created by knock-on displacement) when the electron incidence is strongly localized, and the naturally occurring diffusion of those defects. The more the total electron dose is homogenized in time and space, the better the damage distribution spreads out, and the less likely local accumulations of damage sites become, thus preventing irremediable destruction of the specimen structure. When combining an optimized scan strategy with an event-driven detector, one can obtain the information richness of 4D-STEM, combined with favorable beam damage behavior, as often praised in conventional TEM.

Here, an experimental set-up that routinely allows fast dose-fractioned event-driven 4D-STEM with custom scan strategies is described. A software toolkit controls and synchronizes the scan engine and detector, performing in-line processing of the event data stream for live visualization. Initial experimental results will be presented, demonstrating the interest of dose fractionation in microprobe investigations of beam-sensitive metal-organic frameworks and high-resolution imaging. Additionally, a more fundamental and theoretical analysis of electron-induced damage diffusion statistics is performed by using a simple split operator approach, thus modeling the damage distribution function in space and time, within a given STEM recording. The cases where a fractionation of the scan window is performed or when an overfocused or time-dependent probe is employed are investigated, and conclusions are drawn regarding their potential to lower beam damage while keeping the dose constant.

Keywords:

Event-driven detection, 4D-STEM, Beam damage

Reference:

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Electron microscopy characterization of king scallop (Pecten maximus) shells from low-voltage SEM to 3D-EBSD reconstruction

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Poster Group 2

Background
The shell of the bivalve Pecten maximus, also called the king scallop, was previously found to be detrimentally affected by the presence of metal contamination, in particular Cu, Pb, and Zn originating from mining activities on the Isle of Man [1]. In addition to a reduction of shell thickness, scallop shells from the contaminated area exhibit a sharp break line in the mineralization within the foliated region of both the top and bottom valves. Our data suggest that these mineralization break line caused reduced fracture strength compared to pollution-free scallops, which results in increased mortality due to predation and during the process of dredging. These break lines have already been referred to as being of aragonitic prismatic structure [2].
To shed light on the possible impact of metal contaminations on the growth and strength of scallop shells and in particular on the 3D morphology and microstructure of the scallop, from contaminated and uncontaminated sites, we used characterization tools at different length scales (from cm to µm) using electron and X-ray probes to determine areas of interest in contaminated and healthy (uncontaminated) shells to finally realize crystallographic orientation map using EBSD technique and localized 3D reconstruction by focused ion beam techniques (FIB). The aim of this study was to combine these techniques to produce a full 3D EBSD map of the crystallographic orientations of the critical zones.

Methods
The scallop samples were cut using a diamond cut-off wheel (South Bay Technology Low Speed Diamond Wheel Saw MODEL 650) to extract a large cross-section of contaminated and healthy scallop to study the whole shell section. The cross-sectioned samples were then polished with lapping pads to a felt disk with a 50 nm colloidal silica suspension, to obtain a perfectly flat surface for future SEM observations and EBSD analysis.
We first applied 3D X-ray microscopy using micro-computed tomography (microCT) scanning with a ZEISS Xradia 520 Versa X-Ray microscope, at different resolutions to locate potential structural defects or the mineralization break line on contaminated shell and healthy shell.
The same samples were then analyzed by scanning electron microscopy (SEM) coupled with elemental analysis by energy dispersive X-ray spectroscopy (EDS) to detect differences of microstructure or orientation on the shell cross section. The equipment used for this purpose is a Hitachi field emission gun (FEG)-SEM SU8230 with low voltage analysis (3KeV) by secondary and backscattered electrons (PD-BSE) imaging, Bruker XFlash EDS detector for elemental quantification point analysis, and Bruker Flat Quad detector for EDS mapping analysis. A 10 nm layer of carbon coating was necessary to be able to run long EDS mapping acquisition.

Electron Backscattered Diffraction analyses were conducted for both types of samples on a SU3500 SEM from Hitachi under VP-SEM mode at 20keV on main areas detected by the previous SEM analyses.
The FIB tomography is performed using a Hitachi Ethos NX5000 FIB-SEM to obtain a stack of images and the Dragonfly software from Comet [3] to build the 3D volume samples from each type. In addition, the FIB-SEM NX5000 is equipped with a Symmetry S2 EBSD camera from Oxford Instruments that can allow us to obtain stacks of EBSD maps to build 3D volumes with crystallographic orientation information.

Results
MicroCT analyses carried out at different resolutions (from 30 µm to 2.5 nm pixel size) have shown that the break line visible in the contaminated shell is also present in healthy shells, but less marked and finer than that observed in the contaminated shell. These lines extend across the entire shell surface, although their point of initiation remains a mystery.
SEM analysis enabled us to observe the variation in microstructure across the entire cross-sectional area and to determine the zones of interest for the different microstructures observed in each type of shell. The zone we call the "break line" presents a prismatic microstructure as expected, different from the microstructure observed in the main parts of the shell, largely composed of elongated and foliated grains. In addition, a detailed analysis of the samples revealed the presence of a main prismatic layer, which was found to be thicker in the contaminated samples, and we were also able to observe several thinner prismatic layer in both types of sample.
EBSD analyses, carried out on various zones of interest selected from SEM observation, confirmed that the foliated microstructure is calcite. We detected that the prismatic layer was indeed clearly well-crystallized aragonite for the disruptive line. EBSD maps obtained in regions containing thinner disruptive layers reveal that there is no detection of specific crystallographic orientation.

Conclusion
The combination of microscopy techniques using electron probes and X-rays at different length scales has enabled us to gain a better understanding of the structure of scallop shells and the impact of metal contamination from mining on the Isle of Man.
After a detailed study using microCT and low voltage SEM, targeted EBSD analyses revealed that the break lines present over the entire shell surface in all shells are prismatic in structure and are associated with the aragonitic myostracial layer, while the rest of the shell is predominantly composed of calcite with a foliated microstructure. This aragonitic layer, well crystallized in its thickest zone according to EBSD results, shows no detectable crystallization in its thinnest zone. A more in-depth study of these areas would help determine the cause of this lack of identification.
EBSD mapping acquired with the FIB-SEM NX5000 will enable us to obtain 3D volumes with crystallographic orientation information for healthy and contaminated shells, allowing us to compare growth and development of each type of scallop and detect defects in shell crystallization depending on their environment.

Keywords:

Scallops, SEM-FEG, EBSD, 3D-FIB, 3D-EBSD

Reference:

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Visualizing single-atom promotion of ultra-deep hydrodesulfurization catalysts (Pt-Co-Mo-S)

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Poster Group 2

Background incl. aims
The shift towards environmentally friendly fuels has led to stricter regulations on transportation fuel purity, necessitating more efficient catalysts for high-quality, low-sulfur diesel from both fossil and renewable sources. Co-Mo catalysts, supported on alumina, are used to remove sulfur from organosulfur molecules in mineral oil in industrial processes conducted under high hydrogen pressure. However, a significant challenge remains in extracting sulfur atoms from certain sulfur compounds, such as 4,6-dimethyldibenzothiophene (4,6-DMDBT), due to steric hindrance in the molecular reaction pathway.
Over the last four decades, our understanding of Co-Mo catalysts has greatly improved, largely thanks to the Co-Mo-S model introduced by Dr. Henrik Topsøe and his team [1]. According to this model, the active phase of the catalyst is MoS_2 nanocrystals with Co atoms attached at the edges [1,2]. Similar detailed atomic-level imaging of industrial catalysts has remained a challenge since the catalysts are prepared by incipient wetness methods on high-surface area (oxide) supports. However, recent advancements in aberration-corrected electron microscopy, which now allow for single atom detection, have extended this understanding to industrial-grade catalysts supported on high-surface area (conducting) materials, such as graphite, by imaging atom-by-atom the Co-Mo-S structure, even in 3D [3,4]. This has been available due to a combination of ultra-thin supports for single atom contrast optimization and low-voltage (60kV) and low-dose imaging schemes to suppress beam damage.
These breakthroughs provide a powerful set of tools for designing and characterize new active edge and corner structures in hydrotreating catalysts. Here, we present such an approach to enhance a commercial alumina-supported Co-Mo catalyst with small amounts of Pt [5]. This has significantly increased the catalyst's hydrodesulfurization (HDS) activity, boosting the process of removing sulfur from diesel distillates, including 4,6-DMDBT molecules, by +46% in a pilot test unit under industrial conditions. The tested catalysts are analyzed using atomically resolved scanning transmission electron microscopy (STEM) to precisely pinpoint the location of the Pt promoter atoms. These findings are compared to DFT studies based on the well-established Co-Mo-S model. Furthermore, the desulfurization process of 4,6-DMDBT molecules will be modeled to shed light on the catalytic role of Pt.

Methods
The Pt-Co-Mo catalysts were synthesized by incipient wetness impregnation on a mesoporous shaped alumina carrier, resulting in catalysts with metal loads of 16 wt% Mo, 3.5 wt% Co, and between 0 and 1.9 wt% Pt [5]. The catalysts were tested for their HDS activity in a pilot unit using a diesel oil feed containing 1.22 wt% sulfur. After the HDS process, the catalysts were cleaned with the solvent dichloromethane to remove any remaining oil residues. The catalysts were then characterized by scanning transmission electron microscopy (STEM) using two different microscopes;

a FEI Talos F200X for element mapping (EDX), and a probe-corrected JEOL ARM-200F for high resolution imaging, operated at 200kV with a probe size of ~1Å for single-atom detection. The interpretation of these results was aided by STEM image simulations conducted in QSTEM, and density functional theory (DFT) calculations of Pt and Co-Mo-S interactions to visualize edge motifs of the Pt-Co-Mo-S catalyst. The nature of Pt was further justified by Pt L-edge X-ray absorption near edge structure (XANES).

Results
Here we present the synthesis of platinum-cobalt-molybdenum (Pt-Co-Mo) catalysts and their application in the hydrodesulfurization (HDS) process. The resulting catalysts showed a significant increase of +46% in HDS activity when tested in a pilot unit under industrial conditions. Surface science studies using scanning tunneling microscopy (STM) and density functional theory (DFT) have provided a detailed understanding of the catalysts' edge-motifs in Co-Mo-S structures at the atomic level [2]. These model studies, prepared on planar Au substrates under ultra-high vacuum levels, suggest that the cobalt atoms act to increase the concentration of sulfur vacancies on the MoS_2 edges and that corner sites provide favorable geometries for adsorption of specifically the sterically hindered 4,6-DMDBT molecules. Therefore, we focus on characterizing the Pt-Co-Mo catalysts at the atomic level using Pt L-edge XANES and high-resolution STEM. The results revealed a tertiary transition metal sulfide nanostructure, Pt-Co-Mo-S, with Pt attached to edges and corner sites of the Co-Mo-S structure. The study also utilize DFT calculations to further understand the catalytic working principle and the promotional role of Pt in ultra-deep hydrodesulfurization, especially the desulfurization of refractory 4,6-DMDBT molecules.

Conclusion
This study reveals that a Pt-Co-Mo-S nanostructure, with Pt strategically positioned at the edges and corners of the Co-Mo-S structure, significantly enhances the desulfurization process, particularly for bulky 4,6-DMDBT molecules. The introduction of Pt in these tertiary structures appears to break the conventional scaling relations, modifying the behavior of corner and edge sites in the Co-Mo-S catalyst. These single-atom level insights into complex edge motifs in industrial catalysts elucidate the role of promoter elements and provide a new perspective on how to tailor nanostructures for more efficient catalysts in the future.

Keywords:

HDS catalyst, single-atom promotion, HRSTEM

Reference:

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Strain mapping using high-resolution electron backscatter diffraction technique: The influence of sample preparation

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Poster Group 2

Background

When developing a new metallic alloy, one of the critical parameters is how it will resist expected loads without failure. Understanding how materials deform is important when doing research and development in fields such as aerospace, where public safety is the top priority. An emerging strain distribution measurement technique is based on cross-correlation analysis of high-resolution electron backscatter diffraction (HR-EBSD) patterns. The technique measures lattice distortion-related differences between EBSD patterns obtained within the same grain. The HR-EBSD technique was applied to elastic strain, evidence of plastic strain, and geometrically necessary dislocations (GND) measurements [1]. However, aside from intrinsic instrumentation limitations, HR-EBSD is very sensitive to diffraction pattern quality. Therefore, sample preparation factors prominently into the accuracy and precision attained in HR-EBSD strain analyses.
However, it is not easy to distinguish between the residual real strain present in a material and the strain induced by sample preparation. To be able to persistently illustrate the effect of stress on the microstructure during sample preparation, we have chosen the phenomenon of dynamic strain-induced transformation (DSIT) of austenite to surface martensite [2, 3]. The DSIT can occur during sample preparation by mechanical polishing (MP) or while using ion beam-based sample preparation techniques: focused ion beam (FIB) or broad ion beam (BIB) [4, 5]. We prepared a series of austenitic precipitation hardening grade steel samples using MP, BIB, and FIB techniques and exposed an austenitic precipitation hardening grade steel to Ga ions at a variety of beam energies and sample geometries. Energy-dispersive X-ray spectroscopy (EDS), electron backscatter diffraction (EBSD) and high resolution (HR)-EBSD techniques were used to assess structural damage induced by sample preparation.

Methods

The samples were mechanically polished by colloidal silica on a grinder/polisher tool [MultiPrep™ system, Allied High Tech Products]. Then the samples were prepared by broad ion beam milling [TrionMill, Fischione Instruments]. Finally, the samples were exposed to Ga ions at a variety of beam energies and milling geometries in a FIB system [Scios DualBeam Ga FIB system, Thermo Fisher Scientific].
After each sample preparation technique, the samples were analyzed using:
- a Ga FIB system equipped with an EDS detector [X-Max 150 mm2 EDS detector with Aztec software, Oxford Instruments] and an EBSD detector [e-FlashFS EBSD detector with Esprt software, Bruker Nano Analytics], and
- a scanning transmission microscope [S-4700 SEM, Hitachi] equipped with an EBSD detector [NordlysNano EBSD detector with Aztec software, Oxford Instruments].

EBSD data were processed using HKL Channel 5 software [Oxford instruments]. High HR-EBSD measurements were done using Open X-Y software [Brigham Young University].

Results and conclusions

Figure 1 shows HR-EBSD average strain measurements and geometrically necessary dislocations (GND) of steel samples after mechanical polishing (Fig. 1a, 1c) and BIB milling at 5 keV, 2 keV, 1 keV, and 3° milling angle (Fig. 1b, 1d). No DIST phase transformation of austenite to martensite is observed in the sample prepared by BIB milling. In the sample prepared by MP, 20 % strain-induced martensite is observed. Figure 1a shows an HR-EBSD average strain map of partially transformed martensite to austenite grains. Strain accumulation of up to 10-2 of austenite grains around martensite can be observed. This strain gradient can be correlated with the high GND densities shown in Fig. 1c. Compared to the sample prepared by a BIB mill, the GND density is higher – up to two orders of magnitude. No strain is observed in the BIB milled sample. The BIB milled sample with no presence of DIST of austenite to martensite was exposed to Ga FIB at different ion beam energies. The area affected by the Ga ion beam had a very poor quality EBSD pattern (Kikuchi bands shift and overlap). The EDS analyses demonstrate strong Ga ion implantation. Figure 2a shows the HR-EBSD average strain map of regions both exposed and not exposed to a 1 keV Ga FIB. In contrast to the MP sample, no strain gradient correlated with GND density is found. Figure 2b shows {100} crystal plane orientation facing the Ga ions bombardment. The part of the grain exposed to the Ga FIB shows high strain accumulation. This suggests that structural damage is dependent on grain crystallographic orientation. Structural damage and strain accumulation result from FIB-induced atom displacement and vacancies, which leads to strain-induced austenite deformation to martensite.

Keywords:

HR-EBSD, GND, strain, BIB, FIB

Reference:

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Developing a high-temperature solid state electrochemical lab in the TEM

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background incl. aims
Conducting both electrochemical and structural/compositional analysis simultaneously and on a nanoscale level enables a direct correlation between electrochemical performance and the material's properties, tracking their changes over time and different operational conditions1. The aim of the presented work is to establish a solid method that combines high-temperature solid state electrochemical analysis with (S)TEM2,3. Such a method is crucial for advancing solid oxide electrolysis and fuel cells (SOEC/SOFC) and can be relevant to other solid-state electrochemical technologies such as solid-state batteries and thermoelectric devices.

Methods
A method development is presented in which MEMS chip-based heating-biasing TEM holders are combined with environmental TEM (ETEM) and a potentiostat for electrochemical impedance spectroscopy (EIS). Various preparation methods and TEM sample geometries are included, such as model solid oxide cells prepared by pulsed laser deposition (PLD), along with an advanced FIB-SEM sample preparation procedure. Operando high-temperature electrochemical (S)TEM experiments are conducted.

Results
The feasibility of conducting electrochemical cell tests in the (S)TEM is demonstrated, including reliable EIS measurements of full solid oxide cells at temperatures up to 800 ˚C in gases such as O2 and H2/H2O and with applied electrical polarization1,2. The presentation will discuss challenges and necessary requirements for performing such experiments2. Examples of EIS analysis in the TEM will be presented1,2. Additionally, results from experiments are presented where polarization-induced degradation at an electrode-electrolyte interface in an SOEC was indicated by EIS analysis and confirmed by electrochemical operando STEM observation.

Acknowledgements
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Keywords:

Operando (S)TEM, electrochemistry, SOEC, SOFC

Reference:

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In situ observation of dislocation evolution in cerium oxides nanocubes in an environmental TEM

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Poster Group 1

Characterizing the mechanical properties and deformation mechanism of ceramic materials at the nanoscale is of much interest to improve their processing and properties. At the nanoscale, plastic deformation have already been observed in alumina and magnesium oxide nanoparticles at room temperature. Cerium oxide, as one of the most important ceramic materials, is widely used in many applications, such as in solid oxide fuel cell electrodes, catalysis, or gas detection. However, there are few experimental pieces of evidence of the evolution of defects, which have significant impact on many material properties, especially on mechanical behavior. TEM observations of CeO2 nanocubes demonstrated a beam sensitivity with reduction under high electron dose. Reduction may have an impact on the mechanical properties. The development of TEM nanocompression experiments inside an environmental transmission electron microscope (ETEM) is of much interest to study the mechanical properties of ceramic particles and the effect of the reduction on them. High resolution imaging may also be very useful to fully study the defects formation (dislocations, stacking faults…) during compression. In situ nanocompression under gas and at high resolution in an ETEM were developed on CeO2 and are reported in this work.

Cerium oxides nanocubes (20-50nm size) were compressed using a dedicated Hysitron PI 95 sample holder in an environmental transmission electron microscope (ETEM). By controlling the electron dose and by using a small pressure of air in the ETEM, different CeOx structures (x between 1.5 and 2) were tested to compare their mechanical properties and their deformation mechanisms. Displacement-controlled in situ compression tests were performed in a Titan ETEM 80-300 kV microscope equipped with a Bruker PI95 picoindenter. The loading direction was [100]. Different imaging techniques have been used to identify the slip systems in CeO2. High resolution imaging has also been performed during compression of Ce2O3 nanocubes to observe dislocations, stacking fault or nano-twin formation. Simulation calculations (DFT, DM) are very useful to complete/understand the in situ nanocompression experiments and were developed on CeOx material, even if computing time might be long due to the complexity of the structures

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CeO2 has a fluorite structure (space group Fm-3m). Their reduction under the electron beam creates oxygen vacancies in the structure, which leads to a fcc bixbyite structure (space group Ia-3) with a double cell parameter compared to fluorite. In the case of CeO2, deformation mechanism is found to be similar to those observed in other fluorite structure, with <110> {111} slip systems. The evolution of the yield stress with the nanocube size reveals to be similar to the one already observed for other fcc structures. In the case of Ce2O3 bixbyite structure few studies have reported the deformation mechanism and the slip systems so far. Oxygen vacancies may play a role in defect formation. To observe the deformation mechanisms accurately, high resolution imaging could be performed during in situ nanocompression. Dissociation of perfect dislocations into partials and stacking faults has been observed, which is unusual in oxides. The results obtained for both structures are discussed and compared to simulations works. Compression cycles carried out on the

same nanocube under different irradiation conditions (thus either in the fluorite or in the bixbyite structure) will be shown to highlight the differences between both mechanisms.
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In situ nanocompression experiments have been used to study and compare the deformation mechanism of CeOx nanocubes. On single nanocubes, different mechanisms have been evidenced depending on the irradiation conditions and thus the crystallographic phase. When compressed along the <100> direction, fluorite deforms at room temperature via <110> {111} perfect dislocations and the effect of the nanocube size on the yield stress is similar to what is observed in other fcc materials. In bixbyite, the dislocations dissociate and form stacking faults. This process is reversible.

Keywords:

In-situ
Nanocompression
ETEM
Oxides

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Deformation mechanisms of inorganic fullerenes used as lubrication additives: an in situ TEM nanocompression study

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Energy saving is a crucial point to hinder environmental issues. Lubrication of systems, especially engines, have been improved but further developments are still mandatory. Energy loss and wear of moving parts have to be decreased. Inorganic fullerene-like – IF- nanoparticles made up of metal disulphides MoS2, WS2 are considered as promising candidates for anti-wear and anti-friction additives. It was first thought that their round shape will allow them to act as small ball bearings inside the contact but an exfoliation mechanism has been observed [1,2]. This exfoliation leads to the formation of lubricant sheets in the contact area. TEM nanocompression experiments allowed to observe this exfoliation in situ [3]. Depending on the synthesis conditions, fullerenes can present a well-defined onion-like structure with a hollow center or can be composed of not well aligned MoS2 sheets without hollow center. This difference of structure leads to different tribological results. To better understand this discrepancy of behavior, in situ TEM nanocompression/nanofriction experiments were performed on both types of structures to mimic what can happen in the tribological contact. Furthermore, the chemistry of the IF nanoparticles may have an impact. In situ testing experiments were performed under vacuum and under oxygen to monitor the effect of oxygen on their behaviour inside the tribological contact.

MoS2 nanoparticles with controlled sizes and structures were prepared by exposing sheelites MoMO4¬ nano particles to a H2S-CCl4-Ar gas mixture [4]. After dispersion in a solvent by sonication, they were deposited on a silicon substrate mounted on a copper pallet attached to the Bruker/ Hysitron PI-95 TEM holder. In situ compression and friction behaviour of MoS2 nanoparticles with different level of crystallinity (poor, medium, high) under vacuum and oxygen atmosphere were performed, in an aberration corrected environmental TEM microscope (FEI Titan ETEM). Poorly crystalline fullerenes present a structure with a random orientation of MoS2 sheets. Medium and highly crystalline fullerenes present an onion-like structure, highly crystallinity having less defects. The structural changes of nanoparticles under compression and/or during friction were imaged in TEM bright field mode with a 25 frames/s acquisition rate. A precise processing, by following the change of the particle shape and the presence of shear, was determined to fully characterize and understand the deformation mechanism. Observations were linked to the force-displacement curve.

Comparison of compression experiments for the different crystallinities present different deformation mechansims. For the low crystallinity sample, orientation of the sheets perpendicular to the compression axis is observed during compression and shearing (see figure). For onion-like medium crystalline particles, buckling of the inner shell initiates the deformation, while it is not observed in highly crystalline ones. The buckling may be due to the presence of defects. The onion-like morphology leads to a large amount of elasticity and their plastic deformation to orientation of planes perpendicular to load direction. The amount of elasticity, the mechanism of deformation

initiation and the forces differ according to morphology, while at the end MoS2 sheets align to the perpendicular direction of applied load.

In situ nanocompression experiments in ETEM are useful to observe the exfoliation mechanisms of fullerenes. It was already known that the lubrication mechanism of fullerenes is based on their exfoliation. The interest here is to be able to observe the exfoliation mechanism and to compare the behavior of fullerenes with different crystallinity. A different behavior has been observed depending on their crystallinity, with both mechanisms leading to the formation of MoS2 sheets in the lubricated contact.

Keywords:

In-situ
ETEM
Nanocompression
Nanofriction

Reference:

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Thermo Scientific Smart EPU Software: Towards autonomous screening

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Poster Group 2

Background incl. aims
Over the years, the Cryo-EM community has anticipated the achievement of software automation that would simplify the Single Particle Analysis (SPA) data acquisition workflow without requiring any user setup. This advancement would enable researchers, regardless of their expertise in applications or microscopy, to effectively utilize high throughput cryo-electron microscopes, particularly in industrial settings or entry-level laboratories where efficiency and user-friendliness are crucial barriers to adoption. Notably, one of the primary challenges in SPA is the automation of screening. Thermo ScientificTM Smart EPU Software currently offers a workflow that enables automated screening of multiple samples. Here, we are expanding the capability of Smart EPU to make SPA screening a fully unattended and robust process.

Methods
By utilizing EPU, EPU Multigrid, EPU Quality Monitor (EQM), Embedded CryoSPARC Live, a novel set of AI/DL algorithms, and CryoFlow we can now demonstrate a workflow that allows users to screen an entire cassette of samples on an autoloader system without any manual interventions.
To achieve such a prominent level of automation, various aspects of the workflow have been optimized. User workflows for screening often involve acquiring and analyzing small datasets from multiple grids under different conditions to identify optimal ice thickness and particle behavior. These tasks are laborious, as users must load each grid, select grid squares with varying ice thickness, identify foil holes, select foil holes with varying ice thickness, curate initial selections, and perform data analysis on acquired high-resolution images to determine favorable conditions for further collection of large datasets. Smart EPU presents a set of innovative AI/DL plugins that automate those steps that hitherto needed manual actions. To increase robustness, we also introduced two new set of features: one to access foil hole selection by ice thickness deterministically, using plasmon imaging, which is particularly useful when working with gold grids; secondly, when the image analysis of a screening session reveals a particle preferential orientation issue, Smart EPU fully supports the session setup for automated acquisition on tilted specimens.

Results
Experimental results indicate that the autonomous algorithms perform comparably to an experienced user with a significant gain of time. Furthermore, we combine these innovations with a redesigned user interface that allows users to initiate the setup process with just one click. This screening solution is connected to EQM and Embedded CryoSPARC Live, enabling real-time acquisition adjustments and image analysis.

Conclusion
With these advancements, users will be able to load their samples, quickly initiate the screening process on site, and visualize remotely the results on the web portal of our data integration platform,

CryoFlow, while still acquiring or after completion of the screening session. This new strategy allows users an easier and efficient process for a more comprehensive understanding of their samples and better decision making about further data collection.

Keywords:

acquisition software; automation; AI methods

Reference:

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Scanning transmission electron microscopy of quantum centers in diamond

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Poster Group 1

Background incl. aims
Quantum centers in diamond have been a topic of extensive research for over two decades. Various dopants found naturally in diamond or introduced into the lattice artificially have been studied computationally and experimentally, investigating their properties and potential use as quantum technological devices such as qubits or quantum sensors. The most prominent example is the nitrogen vacancy center, known for its extraordinary photoluminescence qualities. Similar photoluminescent properties have been found for the silicon vacancy, germanium vacancy and erbium vacancy center, among many others. However, the atomic structure of such quantum centers hasn't been fully uncovered via scanning transmission electron microscopy (STEM) imaging so far, as these centers have been rarely imaged. Due to this, it would be important to know how these centers could look like when imaged in STEM.
Methods
In this work, STEM annular dark field image simulations of various quantum centers (N, Si, P, Ge, Er, Ni, etc.) in diamond will be discussed first, including the predicted visibility of the dopants in the diamond lattice in dependence to factors such as sample thickness, defocus, and dopant placement. The results indicate that quantum centers with dopants of atomic number higher than 50 are viable to study with annular dark field STEM imaging. Based on this, diamond samples doped with Er were prepared and imaged with annular dark field STEM for the first time, also investigating the effects of two different doping methods, with dopants induced in growth via chemical vapor deposition vs. dopants induced via ion irradiation, and varying electron beam acceleration voltage.
Results
The experimental data was found to be in agreement with the simulation results. Interestingly, it was observed that the dopants do not remain static in their positions, instead moving between images, similar to the results of a previous study on STEM of Bi in silicon samples. Stacks of consequent images of up to hundred were acquired, where the motion of the dopants can be followed. Automatic dopant tracking thus becomes a good choice for the analysis of such data, which is used to uncover the relations between the dopant movement, imaging and sample conditions.
Conclusion
This study offers new insights into the imaging of quantum centers in diamond, demonstrating that resolving their atomic structure requires special care due to their dynamic behavior in the imaging process.

Keywords:

Quantum centers, STEM, Diamond,

Reference:

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Unsupervised learning assisted secondary electron hyperspectral imaging for high-throughput cheminformatics analysis of materials

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Background
As a powerful instrumentation tool, scanning electron microscopy (SEM) has been increasingly used to perform analysis on a wide range of minerals, metals, biological specimens, nanostructured materials, polymers, composites, and electronic components [1]. By integrating hyperspectral imaging techniques into SEM, secondary electron hyperspectral imaging (SEHI) technology is able to offer detailed and comprehensive spatial-spectral information of the materials, making it versatile for analysing chemical properties and surface morphology at micro- to nano-scale [2].
However, effectively visualizing spatial-spectral information can still be challenging, especially when changes in spectra are subtle due to spectral mixing or occur only in a very small percentage of the area analysed. To address this problem, we propose a novel analytical workflow for SEHI data using unsupervised learning, which can automatically identify chemical bonds or elements present in the imaged materials and additionally segment the materials surfaces into corresponding chemical groups.
Methods
The proposed automated analytical workflow includes the following steps:
(1) Microscopy image data is processed by traversing the whole field of view of the image, through small block-based or pixel-wise methods. For block-based processing, the entire image is divided into smaller, predetermined units (e.g., blocks of 3*3 pixels).
(2) For each small block, the peaks in the corresponding spectral curve, also known as spectral peaks, are identified and gathered. This allows for the collection of the overall distribution of all spectral peaks across the image.
(3) The distribution of spectral peaks is learned by using unsupervised clustering approaches. In this work, the Gaussian mixture model (GMM) approach is adopted to perform probabilistic clustering. The centroid of each GMM component reflects the location of the corresponding spectral peak, which can be used to deduce the associated chemical bonds or elements in the material sample.
(4) Image blocks that fall into the same cluster are then identified. Accordingly, the spectra of these image blocks from the same cluster are extracted. These extracted spectral signatures then act as reference spectra, or like "endmembers" in spectral unmixing processes.
(5) By evaluating spectral similarity using spectral angle mapper (SAM), the image regions, sharing similar spectral properties with these reference spectra obtained in (4), are distinguished.
Results
We implemented this framework into analysing a complex metal alloy (palladium & silver, PdAg) and carbon film, imaged using a Helios Nanolab G3 UC microscope [3]. Firstly, the raw hyperspectral image slices are registered through a template-matching algorithm [4]. By dividing the entire image into smaller blocks and identifying the localized spectral peaks from these blocks, we obtain the distribution of spectral peaks. To figure out the predominant spectral peaks within this distribution,

unsupervised clustering by the GMM is applied. As shown in Fig.1, the GMM outcomes reveal 5 components with peak locations at 0.83, 1.98, 3.57, 4.75, and 5.60 eV, respectively. According to the literature, the spectral peaks at 0.83 and 1.98 eV are likely attributed to metals Pd and Ag [3]. The peaks observed in 3-6 eV range are thought to be linked to the contributions from sp^2-like, a-CH and sp^3-like carbon bond types. Intuitively, image blocks that fall into the same cluster, primarily contribute to a particular spectral peak. Thus, the reference spectra, or endmembers-like spectra, can be extracted from the image blocks belonging to the same cluster. The SAM is utilized to assess spectral similarity against these reference spectra for image segmentation.

Conclusion

Conventional and manual microscopy data analysis methods, due to their limitations in processing efficiency and accuracy, could hinder the applications of SEHI in advanced characterization of materials. Machine learning-based approaches, especially unsupervised learning, offer promising automated analytical solutions for tackling these challenges effectively. The automated analytical workflow proposed here well identifies the chemical bonds and elements present in the imaged materials. The unsupervised clustering method, GMM, perform well in modelling the overall distribution of all spectral peaks and uncovering the chemical bonding types. According to the clustering outcomes, the materials surfaces can then be segmented into the corresponding chemical groups. This novel workflow can facilitate comprehensive cheminformatics analysis of materials, particularly complex carbon material systems.

Keywords:

SEHI, clustering, material cheminformatics, segmentation

Reference:

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In-situ observation of rheotaxy: 2D materials‘ growth on liquid substrates

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims
The isolation of graphene in 2004 raised an enormous excitement in the community, bringing to light a fundamentally new class of low-dimensional materials. Soon after, the graphene's insulating cousin – hexagonal boron nitride – was introduced, initiating a burst of papers speculating about a new electronics era. Process scalability, which is necessary for the utilization of 2D materials on a larger scale, excludes the mechanical exfoliation and significantly narrows the choice of suitable growth techniques. In this regard, the most promising approach is that of chemical vapour deposition (CVD), in which the precursors are delivered from a gas phase to the substrate, where they react and form the growing layer. However, in contrast to exfoliated layers, those prepared by CVD exhibit a significantly higher concentration of various structural defects, which are detrimental to many quantum effects and device functionalities the physicists and engineers search for. The defect family in 2D materials includes domain boundaries (GBs) and twin defects, together with many types of vacancies and anti-sites. GBs were identified to play a critical role in many aspects. The mitigation strategies include specific sample preparation techniques or the use of single-crystal substrates. Developing a more general strategy to avoid the formation of GBs within a footstep of the intended 2D-material-based device (and, preferably, at a wafer-scale) is desirable. Rheotaxy (growth on a liquid substrate) has been proposed as a viable method to achieve domain ordering and self-assembly already in 2012 for graphene [1]. However, it remains poorly explored due to a lack of in-situ experimental techniques that confirm or disprove hypotheses raised concerning the formation mechanisms [2]. Only recently, experimental studies started to pop-out [3].

Methods
We have utilized two modified microscopes for observing the graphene growth on liquid : a microReactor [4] in SEM and ultrahigh vacuum SEM. We observe graphene growth behaviour in situ, allowing to record movies of graphene flake behaviour on liquid gold and copper. We combine our electron microscopy observations with in situ atomic force microscopy (AFM) measurement of the contact angle of graphene on liquid gold, together with ex situ Raman characterization of the final graphene layer.

Results
We have been able to demonstrate some aspects of the graphene growth on liquid substrate, that have been hypothesized so far, namely movement and ordering of the graphene flakes on liquid surface (Cu and Au), via real-time movies under various reaction conditions. The flakes seem to assemble into regular patterns on liquid surface, however, this pattern is prone to disturbances, caused by e.g. hydrodynamics of the liquid (Fig. 1). Additionally, we obtained kinetic data of graphene growth, allowing us to directly compare solid and liquid growth rates. These data show significant increase of reaction constants on liquid metal. Last, we will show in situ AFM data,

discussing the possible presence of meniscus between the graphene flake and the liquid metal. Altogether, we frame these observations within a simple physical model.
Fig.1 shows behavior of graphene islands on undercooled liquid gold captured by the SEM, at temperature 1040 °C and C2H4/H2 partial pressure of 40 Pa. The movement is oscillatory (a) and the islands rotate and align with respect each other, which causes the distortion of the image and appearance of “vibrations” because of the scanning beam. Coalescence happens slowly, except for cases when the islands’ distances decrease below a certain value or after stimuli such as solidification (b). The scale bar is 1 µm.

Conclusion
In this contribution, we will show a quantitative analysis of in situ experimental data of graphene flakes assembly on liquid metal under growth reaction conditions. We combine in situ electron microscopy in different vacuum environments at extreme temperature with atomic force microscopy to show that the choice of the liquid metal is vital for achieving regular assembly of graphene and seamless stitching. We corroborate experimental observations with simple physical models of 2D solids floating on a liquid surface and explore weak-points of the synthesis, giving directions for further research towards scalable growth of 2D materials.

Acknowledgements
This work was supported by the project Quantum materials for applications in sustainable technologies (QM4ST), funded as project No. CZ.02.01.01/00/22_008/0004572 by OP JAK, call Excellent Research.

Keywords:

In-situ, liquid metals, graphene, growth

Reference:

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SARS-CoV-2 and HCV infection and antiviral treatment monitored by multimodal imaging

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Background
A common feature among positive strand viruses is that they alter cellular membranes to generate replication complexes. Although the origin, nature and structure of these membranous compartments are not identical, they constitute a characteristic feature of these viruses and are observed in yeast, plants and higher eukaryote (+)-strand RNA viruses (3).
HCV infection provokes a rearrangement of intracellular membranes, designated membranous web (MW). This term referred to compact vesicle accumulations embedded into a membranous matrix (2).
In SARS-CoV-2, the expression of the viral proteins results also in a profound remodeling of the infected cell cytoplasm, with the characteristic membranous compartment, generically denominated viral replication organelle (VRO). One of the salient characteristics of the coronavirus VRO is the presence of double-membrane vesicles (DMVs), where various nsp and double-stranded RNA (dsRNA) have been shown to colocalize and where active RNA-dependent RNA synthesis has been shown to occur. Thus, DMVs are the structures where coronavirus RNA replication is thought to occur. While the nature and origin of the membranes may differ, DMVs are also the putative RNA replication organelle for hepatitis C virus (HCV).
In our research we study the morphology of the membranous rearrangements induced by HCV and SARS-CoV-2 infection in near-native conditions (1).
These infection alterations in HCV could be reverted by the clinically approved direct-acting antivirals (DAAs) for the treatment of chronic HCV infection. The availability of DAA drugs against HCV provides a unique opportunity to revert this process and to define the ultrastructural events that follow viral replication blockade short after antiviral treatment (4).

Methods
In this study we have performed infrared microscopy, confocal immunofluorescence and correlative cryogenic light-soft X-ray tomography (CLXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by HCV and SARS-CoV-2 infection and after antiviral treatments in near-native conditions.

Results
Our results compare the HCV and SARS-CoV2 replicating structures. SARS-CoV-2 infected cells display DMV structures similar to those found in other coronaviruses or hepatitis C virus infection. Our studies provide a wider cellular context in which these membranous alterations occur and point at the formation of compact perinuclear structures where viral antigens are concentrated by constriction within intermediate filaments, as determined by confocal microscopy. This perinuclear structure is formed by a tightly juxtaposed tubular membranous network reminiscent of a highly modified endoplasmic reticulum. This structure is virtually devoid of normal mitochondria and

adjacent mitochondria display clear ultrastructural signs of stress. Finally, late stages of the infection indicate deformation of the cell nucleus in areas close to the viral factory and an overall cytoplasmic retraction of the infected cell.
Analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment. CLXT studies confirmed the rapid elimination of the viral machinery, and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments (5). A general survey of control cells and HCV replicons indicates that HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase is located in pleomorphic, high-absorption contrast organelles in DAA-treated cells.

Conclusions
Overall, our cryo-SXT data provide an additional piece of the puzzle in building a precise map of the ultrastructure of SARS-CoV-2-infected cells by providing insight into the overall structure of the viral replication organelle and the cellular context within which these changes occur.
Our results suggest that HCV replication compartment is constantly recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in a transient activation of the endo-lysosomal system to achieve rapid viral machinery removal. Our results also constitute a proof of concept for the use of cryo-SXT at ALBA synchrotron and at lab-scale soft X-ray microscope (SXM) as a platform that enables determining the potential impact of candidate compounds on the ultrastructure of the cell that may assist drug development at a preclinical level.

This study was funded by ALBA Synchrotron standard proposals 2022065884, 2021024899. AJPB, GC, VC, EP and PG are part of the CoCID EU project, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No.1010171162. And KM is funded by CLEXM, a Marie Sklodowska-Curie Doctoral Networks Action (MSCA-DN), funded by the European Union under Horizon Europe

Keywords:

SARS-CoV-2, HCV, DAA, cryo-SXT,
cryogenic-light-soft-X-ray-tomography(CLXT)

Reference:

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Effect of the Applied Chemical Potential on Strong Metal-Support Interaction in Ni-TiO2 Catalysts

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Background incl. aims
Strong metal–support interaction (SMSI) refers to metal nanoparticle coverage by suboxides that are generated from the support under reducing conditions. SMSI may serve as a powerful tool to tune the activity, selectivity, and stability of a catalyst in diverse reactions. Since the pioneering work of Tauster et al. on SMSI in Pt group nanoparticles (NPs) supported on a TiO2 support,1 there have been plenty of studies about SMSI for different catalyst systems, pretreatments and conditions. Recent studies showed that SMSI depends on many parameters, such as temperature and gas composition. For example, Matsubu et al. found that after treatment in 5% H2/N2 at 550 °C for 10 min, a TiOx SMSI crystalline bilayer was formed for Rh-TiO2, while after treatment in 20CO2:2H2 at 250 °C for 3 h, an amorphous SMSI overlayer was induced.2 Frey and co-workers reported that SMSI-induced encapsulation of Pt-TiO2 observed under reducing conditions is lost once the system is exposed to a redox-reactive environment containing O2 and H2.3 Very recently, Monai et al. found that thin TiOx bilayers formed on 111 facets of Ni-TiO2 catalysts during 400 °C reduction, which were completely removed under 1CO2:3H2. Conversely, after 600 °C reduction, the amorphous TiOx overlayers was only partially removed under 1CO2:3H2.4 It is currently still unknown how overlayers evolve with temperature. Moreover, it is yet to be studied systematically how the applied chemical potential affects the SMSI. Herein, through in situ ambient pressure TEM, we studied the effect of the applied chemical potential, including partial pressure of H2, temperature, and temperature rate, on SMSI in Ni-TiO2 catalyst systems.

Methods
Ni catalysts supported on TiO2 (Degussa P25, S.A. = 60 m2/g) were prepared by homogeneous deposition precipitation (HDP) with urea. The system was kept at 95 °C for 20 h under vigorous stirring to induce Ni precipitation via the hydrolysis of urea. Then, the system was washed by centrifugation with water until the pH of the supernatant was neutral. The resulting powders were dried at 60 °C overnight, followed by further drying at 120 °C for 24 h. In situ scanning transmission electron microscopy (STEM) studies were carried out using an aberration-corrected ThermoFisher Scientific Titan Cubed electron microscope operating at 300 kV. The gas and heating nanoreactor comprised two electron-transparent Si3N4 windowed chips and a gas cell holder (climate G+, DENSsolutions). The prepared powder Ni(OH)x/TiO2 was dispersed in ethanol and deposited on the lower climate chip before constructing the climate holder and sealing the nanoreactor. The reduction was performed for 4 h in a 5% H2/He, 50%H2/He or 100% H2 at 400 °C and following the increasing temperature step by step to 600 °C.

Results
The reduction process in 5% H2/Ar is shown in Figure 1a-c. Almost no changes were observed after more than 3 h reduction at 400 °C. When the temperature was raised to 600 °C and held for 48 min,

Ni(OH)x was reduced to Ni (as shown in the red dashed square in Figure 1c). Some of the TiO2 support decomposed to small TiOx NPs. Figure 1d-f showed the reduction in 50% H2/He. Ni NPs were obvious at 400 °C only for 37 min. At 600 °C, the Ni NPs sintered significantly and the TiO2 support was not stable (marked by red dashed square in Figure 1f). These results indicate the importance of the partial pressure of H2 during the reduction and SMSI formation in supported Ni catalysts. Atomic resolution HAADF-STEM images are shown in Figure 1g-j. As we increased the temperature step by step from 400 °C to 600 °C, TiOx bilayers survived at even 600 °C. These results demonstrated that the temperature ramp also has significant effect on the structure of SMSI layers in Ni-TiO2.

Conclusions

Using in situ ambient STEM, we studied the effect of the applied chemical potential, including partial pressure of H2, temperature, and temperature rate, on SMSI in Ni-TiO2 catalyst systems. We found that partial pressure of H2 affects the reduction of Ni(OH)x and the type of the SMSI encapsulation. Although SMSI bilayers survived with temperature increasing, Ni NPs still sintered.

Keywords:

SMSI, Ni-TiO2, sinter-resistant, chemical potential

Reference:

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In situ STEM investigations of defect induced memristive switching in off-stoichiometric SrTiO3

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Background
In the pursuit of neuromorphic computing, which seeks to emulate the human brain's intricate computational capabilities, the transition of digital memory to an analogue state is crucial. A central scientific question revolves around directing materials to incorporate synaptic plasticity. Among the most promising and technologically advanced strategies for achieving this are resistive random access memory (ReRAM) devices. Traditional ReRAMs operate through the stochastic formation and breakage of conductive filaments within an insulator storage medium, making control challenging. By deliberately introducing an A-site cation (Sr) deficiency in $SrTiO_3$ of up to about 16% through metal organic vapor phase epitaxy (MOVPE), we have successfully realized resistive switching without the need for filament formation, achieving on/off ratios as high as 10^3 [1].
In this study, we integrated various techniques of in situ scanning transmission electron microscopy, including differential phase contrast (DPC) imaging, integrated-DPC imaging, energy-dispersive X-ray spectroscopy (EDS), electron energy-loss spectroscopy (EELS), precession electron diffraction, along with I-V curve measurements. This comprehensive approach enabled us to gain insights into the underlying mechanisms and holds potential for developing more controllable memristive materials for ReRAM devices.
Methods
The theoretical crystal and electronic structures were calculated by the Heyd-Scuseria-Ernzerhof (HSE) hybrid functional method within density functional theory (DFT). The STEM simulations were performed with the structure concluded from the HSE calculations and with the simulation code adapted from Kirkland[2]. The high-resolution STEM imaging and spectroscopy were acquired from a probe-corrected Thermo Fisher Scientific Spectra Ultra operated at 300 kV, while the precession-assisted 4D STEM were obtained on Tescan Tensor STEM operated at 100 kV.
Results
As shown in the figure, a reversible switching of electrical field direction was observed from iDPC and DPC imaging of the off-stoichiometric $SrTiO_3$ TEM sample upon in situ switching between positive to negative bias. Reducing the voltage to 0 V results in a stable state, albeit with the polarization erased, returning the local film to a high-resistance state. The results from EDS and EELS mapping tells that approximately 16% of the Vsr sites are occupied by Ti. The observed resistive switching phenomena can be attributed to trap-assisted tunneling through Ti antisite defects, which induce a switchable polarization characterized by negative spherical aberration imaging (NCSI).
Additional details, including scanning precession electron diffraction, which maps nanoscale strain and electric field with high precision, EELS mapping, which discerns different valence states of Ti, and a few others, will be presented on site.
Conclusion

The correlation between structure, composition, bias and electric field distribution from in situ STEM biasing experiments gives direct evidence of antisite defects induced memristive switching in the off-stochiometric $SrTiO_3$ sample. The findings offer bottom-up perspective on the promising prospects for future neuromorphic computing applications. The workflow presented here facilitates new insights in developing similar materials.

Keywords:

Resistive switching,antisite defects,DPC,in-situ biasing TEM

Reference:

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Unlocking 3D nanoparticle shapes from 2D HRTEM images: a Deep Learning breakthrough

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Poster Group 2

Background incl. aims
Nanoparticles (NPs) are typically observed and analysed using High Resolution Transmission Electron Microscopy (HRTEM) for highly precise structural studies at the atomic scale. However, determining their 3D shapes from 2D HRTEM images is a tedious process. Indeed, this type of analysis is based on manual post-processing which suffers, among other issues, from experimental noise or human bias performed at post-experimental stage. In this context, the integration of artificial intelligence (AI) methodologies into data acquisition and analysis protocols is a very promising approach [1]. To tackle the problem of identifying the 3D shape of NPs, we developed a Deep Learning (DL) model to automate this task ensuring reliable statistical analysis of a large number of NPs many of which cannot be identified by conventional methods.
Methods
For this purpose, we extend an approach we had developed to identify the structure of carbon nanotubes from their Moiré patterns obtained from HRTEM images [2]. More precisely, the DL model, leveraging Convolutional Neural Networks (CNNs), is trained on datasets of simulated HRTEM images of NPs, labelled according to their shapes, ranging from 4 to 8 nm. A critical point of this study was generating a representative and optimised dataset. To accomplish this, we constructed atomistic 3D models of NPs deposited on an amorphous carbon substrate, subjecting NPs to random rotations to encompass all potential observed orientations. Furthermore, we simulated the amorphous substrate using realistic carbon membrane derived from a tight-binding framework and noise models, to mimic experimental conditions [3]. Finally, HRTEM images were simulated using the Dr Probe code [4] based on the multi-slice method with parameters consistent with aberration-corrected transmission electron microscopes.
Results
The objective of generating an optimal training dataset was attained through comprehensive studies evaluating the impact of various parameters, including amorphous carbon, resolution, focusing conditions, NPs' size, and NPs' orientations, on DL model predictive accuracy.
Conclusion
This approach has resulted in the development of an efficient and accurate DL framework for predicting 3D NP shapes from 2D HRTEM images, validated across both simulated and experimental datasets (see figure).

Keywords:

DL; nanoparticle; TEM; atomistic simulation

Reference:

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Irbis: Software for 3D reconstruction and temporal analysis of 4D microscopy data

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Poster Group 2

Background:
Irbis is a newly developed software aimed at analyzing 3D and 4D microscopy image data. It is particularly useful for live-cell super-resolution microscopy experiments, enabling users to accurately reconstruct structural details, segment the model into relevant substructures and monitor those over time. The primary focus of Irbis is on the analysis of dendritic spines, although it seeks to be applicable to a wider range of biological structures in future developments.
Methods:
The software puts emphasis on multimodality with three main data types supported: bitmap images (such as confocal z-stacks), localizations (from single molecule localization microscopy experiments) and trajectories (from single molecule tracking experiments). The raw data is transformed into a 3D model, segmented, and the objects are subsequently registered in time so that their temporal development can be studied.
To transform raw data into a 3D model, Irbis uses Marching Cube (1) and Poisson surface reconstruction (2) algorithms. The primary model is subsequently segmented into individual substructures. The segmentation of dendritic spines is performed via a customized process that begins with solving the shortest path problem to identify the dendrite's center line, followed by the creation of the dendritic shaft's 3D outline using an adjusted active contour algorithm. This structure is then used to separate the protruding spines from the centre of the dendrite.
Results:
Irbis produces detailed 3D models from the analyzed data. It enables identification and analysis of substructures, such as synaptic spines, over time. Moreover, if more channels with different structures are present in the microscopy data, the dynamics of these structures can be related to each other. This can be used, for example, to study the behavior of synaptic signal relative to the structural information from dendritic spines.
Conclusions:
Irbis contributes to the field of microscopy image analysis by offering a tool that supports detailed 3D reconstruction and the temporal analysis of biological structures. While it currently focuses on dendritic spines, its methodology and features will be expanded for broader application in future development. Compared to other spine analysis tools, Irbis emphasizes accuracy in data reproduction and the analysis of structure development over time, within a user-friendly interface designed for accessibility to researchers without programming skills.

Keywords:

3D-reconstruction, super-resolution, live-cell, neuronal spines

Reference:

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Study on local indium concentration in Ga(1-x)InxN quantum wells using quantitative scanning transmission electron microscopy

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Multiple quantum wells (MQW) and single quantum wells (SQW) of Ga(1-x)InxN on GaN are promising candidates for nanooptical light emitters due to their high quantum efficiency. The band gap of Ga(1-x)InxN can be controlled over a wide wavelength range by tailoring the variation of the indium concentration [1]. In addition, the epitaxially grown quantum well structures can be easily modified using chemical etching for the formation of a pyramid containing a quasi quantum dot structure. As the dimension of the quantum structure shrinks, the variation of the local indium concentration impairs the optical properties. Therefore, a robust characterization technique is required to understand and optimize such complex structures on the atomic scale.
Methods
In this study, an ultra-thin single quantum well of Ga(1-x)InxN between GaN layers on an Al2O3 substrate was grown using metal organic vapor phase epitaxy (Fig. 1). The cross-sectional specimen was prepared using focused ion beam, including the final low kV milling. The prepared specimen was characterized using a double-aberration corrected JEOL NeoARM 200F equipped with a cold field emission gun. For a precise intensity and local lattice parameter analysis, sequential high-angle annular dark-field (HAADF) imaging was utilized with a reasonably short acquisition time (1 sec/frame). The entire image stack was aligned by the rigid registration algorithm and averaged out to obtain a single drift-corrected HAADF image (Fig. 2(a)).
Results
The fractional intensity (normalized by the incident electron beam intensity) of the HAADF image was analyzed based on frozen phonon multi-slice calculation results [2]. The local thickness was determined by the mean intensity of the Ga atomic columns in the GaN layers and additionally verified by the acquired position averaged convergent beam electron diffraction (PACBED) pattern (Fig. 2(e,f)) [3]. The local indium concentration of the single quantum well of Ga(1-x)InxN was finally derived from the fractional intensity of the HAADF image (Fig. 2 (d)), showing the mean indium concentration of 13 at.% within the single quantum well. In addition, the local lattice parameters were analyzed based on the individual atomic column positions obtained from the drift-corrected HAADF image (Fig. 2(g)). The local indium concentration was then derived using a modified Vegard's law including biaxial elastic strain effects (Fig. 2(h)). The mean indium concentration within the single quantum well from this analysis was 10 at.%. This result was reasonably comparable with the local indium concentration obtained from the quantitative HAADF intensity analysis. Moreover, this local composition information was supported by the quantitative electron energy-loss (EEL) spectroscopy analysis at the unit cell level, confirming the mean indium concentration of about 16 at. % (Fig. 3).
Conclusion
The combination of HAADF imaging, PACBED, and spatially resolved EEL spectroscopy techniques provided detailed structural and chemical information of Ga(1-x)InxN quantum well structure. The

relationship between atomic structure and composition could be thoroughly studied. These robust combined scanning transmission electron microscopy (STEM) techniques will ultimately reveal the effect of altering indium concentration on complex quantum dot structures.

Keywords:

GaInN, Quantitative STEM, PACBED, EELS

Reference:

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Gigabytes to Megabytes – Rapidly analyzing in-situ videos to track lattice spacing changes

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Poster Group 2

Background incl. aims
Recently, counted direct detection cameras which are virtually noise-free have enabled very faint diffraction spots to be detected at high spatial frequencies. These same cameras provide sharp detail, enabling spots to be precisely located. This combination of a large range and high resolution in reciprocal space requires large images to be captured. When samples are also changing over time, the capacity to capture an entire video dataset of the dynamic behavior is also valuable. This leads to three-dimensional datasets with billions of pixels that may be tens of gigabytes in size. These may be information-rich, but are impossible to visualize or analyze without some processing. In this work, we use a Python script to process each diffraction pattern in an in-situ video dataset to produce a series of radial profiles over time. This reduces the dimensionality, making them more amenable to visualization, easier to analyze, and significantly smaller. It also provides a more directly interpretable result in minutes that can be used to determine whether good data has been captured while the user is still sitting at the microscope.
Methods
The Python script used in this work processes each diffraction pattern in an in-situ video dataset to produce a series of radial profiles over time. It is freely available online[1] and runs within the Gatan DigitalMicrograph software, which facilitates immediate application of the processing to diffraction datasets just collected at the microscope. The script does not merely calculate a radial average, since this will tend to miss faint spots, especially those at large spatial frequency, where there may be thousands of pixels at a specified radius, but only a few with real diffraction signal. Even if the detector was perfect, contributing no noise to the pattern, shot noise from the diffuse scatter in the pattern at that radius would often contribute more to the average than the real signal. Instead the script calculates a radial maximum, and subtracts the radial average, reducing the impact of any uniformly distributed background intensity. It also masks the center of the pattern and optionally filters it with a median filter prior to calculating the profiles. The script applies this processing to every frame in an in-situ video dataset captured by a Gatan in-situ camera, and produces two equivalent outputs. The first output is a 1D profile over time, which can be played back with the same In-Situ Player used to play back the original data, and is automatically synced with the original data if this is also displayed. The second output, as shown in Figure 1, is a 2D visualization where the horizontal axis is time, and the profiles are displayed vertically with the center of each pattern at the top. While this abstract is focused on the application of this script to diffraction patterns, it should be noted that a similar approach can be applied to high resolution TEM videos by first computing the FFT of each frame.
Results
This script reduces the dimensionality of datasets from 3D to 2D, reducing data size and facilitating visualization and analysis. For a diffraction dataset with 1k x 1k patterns (1 million pixels) a single radial profile with minimal loss of resolution is 500 pixels, so the reduction in data size is roughly a factor of 2000. If the same dataset has 1,000 frames with a bit depth of 32, the data size will be reduced from around 3.7 GB to 1.9 MB. This takes about 3 minutes on the Gatan camera computer,

making it possible to run during a TEM session. In many cases, this can immediately inform the user about whether they've collected the data they need. For almost all datasets, it is easier and faster to perform further processing or analysis on the 2D data, rather than the full 3D dataset.
We have applied this diffraction processing to 2 classes of in-situ videos. First, this has been applied to monitor the damage of beam-sensitive materials over time, including metal-organic frameworks (MOFs) and zeolites. If the electron dose rate is known, this can rapidly quantify the amount of dose required to degrade or destroy the sample. Second, the same script has been applied to in-situ videos of reversible phase changes, making it easy to determine the exact timing of the phase change, and to correlate this with the in-situ conditions.
It should be noted that not all changes observed in diffraction patterns over time can be attributed to beam damage or phase changes. Sample drift in x-y or in the z direction, or rotation of small crystals can lead to changes in the pattern which are difficult to distinguish changes that are the result of more interesting phenomena. Care must be taken by the user to correctly interpret the changes observed, and a full discussion of this is outside the scope of this work.
Conclusions
In-situ diffraction data captured by modern cameras is information-rich, but can also be very large and must be processed. Rapid processing and visualization can be performed using Python in DigitalMicrograph to reduce dimensionality and data size prior to performing detailed analysis. A freely available script has been written to perform this analysis, and applied to video datasets of beam damage and phase transformations.

Figure: Examples of 2D visualizations of in-situ diffraction datasets. For each dataset, the profiles go from the center of the pattern at the top toward the edge of the pattern at the bottom. Left: Datasets recording the effects of beam damage for 2D MOF-2 and for ZSM-5. The MOF sample was damaged in seconds, while the zeolite dataset was recorded for over an hour. Right: Datasets recording temperature-induced reversible phase transformations: Sn nanoparticles being heated repeatedly above and below the melting point, VO2 heated above and below its metal-to-insulator transition, with varying ramp rates.

Keywords:

Diffraction
Python
Processing
In-Situ
Continuous

Reference:

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New Workflows and Innovations for Maximizing Relevance of In Situ TEM Results for Liquid-Based Applications

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Poster Group 1

Background
Over the last 20 years, in situ (scanning) transmission electron microscopy [(S)TEM] has become an established experimental technique, driving innovation in material design and supporting new applications-based research strategies [1]. Its unique combination of ultra-high resolution imaging/analysis and relevant in situ sample environments has opened a new window into understanding material performance and degradation over time [2-3]. The goal of in situ TEM research is understanding the relationship between the structure of a material and its resulting function. Once commercial in situ TEM systems became available, a new set of experimental challenges revealed themselves: creating reproducible sample preparation that does not alter material behavior, understanding how to properly scale an experiment from bulk to nanoscale, keeping accurate records of the tens or hundreds of changing parameters utilized during an experiment, understanding electron beam effects, and managing/visualization of large amounts of data. While the ability for the in situ TEM system to produce pertinent environmental conditions is incredibly important to the relevance of the results, it is equally important to have a reliable means of streamlining all steps of the experimental workflow surrounding the in situ TEM experiment itself. Here we describe recent advancements supporting the entire in situ TEM workflow, from sample preparation to in situ conditions to data publication, that increase the reproducibility and relevance of the results while also providing compliance with FAIR principles [4]. Examples to be discussed are related to the global push for more efficient, cost-effective, and environmentally friendly means of energy storage and generation: batteries, electrocatalyst, and nanomaterial chemistry and growth.
Methods
All results discussed use Protochips in situ TEM solutions equipped with AXON machine vision software. Protochips in situ solutions are flexibly designed to create the most relevant in situ environments for a variety of materials in different research areas while being optimized for important analytical techniques such as EDX, EELS, iDPC, 4D-STEM, and others. Sample supports are MEMS-based silicon chips, called E-chips, that are designed and fabricated in-house at Protochips with input from scientific experts in the community. Functional measurements are done with high-sensitivity, low-noise potentiostats and highly sensitive source measuring units (SMUs). Liquid-phase electrochemical experiments are performed with Poseidon AX Liquid Cell system and newly added capabilities will be shown, such as standard reference electrodes, flow management E-chips, and others. The electron beam is calibrated and dose/dose rate are measured and recorded using the AXON Dose module. Stabilization of dynamic samples is done live with automated physical drift correction from the AXON Synchronicity module. Visualization and export of results are done offline with the AXON Studio module.
Results
Data from a wide array of research areas such as liquid-based batteries, electrocatalysts, nanomaterial synthesis chemistry, and corrosion will be discussed. The in situ TEM workflow involves

multiple different facets: sample preparation onto E-chips, experiment optimization outside the TEM, data collection of dynamic materials, production of in-situ conditions that are relevant to the material's operational or native environment, and alignment of structural data (TEM) with functional data (mass spectrometry, cyclic voltammetry, etc.) for trend identification (Fig. 1). Each part of the integrated workflow will be discussed, describing the recent innovations that support proper scaling from bulk to nanoscale, accelerating productivity in and outside the lab, and fostering collaboration and discovery with local and global collaborators. Utilizing these new innovations will significantly improve experimental reproducibility, strengthen the relevance of results to applications-focused communities and enable more publications in application-specific journals, as well as lower the learning curve to in situ TEM for new researchers.

Conclusions

In situ TEM is clearly gaining traction as an important technique for application-focused research strategies alongside traditional bulk, macro, and mesoscale techniques. Relevant in situ TEM results can provide the most direct window towards innovations for improved materials. Increasing the relevance of the results not only lies in creating new environmental capabilities and in situ stimuli, but in innovations for all the steps involved in the scientific workflow. From reproducible sample preparation to data management and visualization, the entirety of the workflow needs to be considered when planning for and incorporating in situ TEM into applications-based research.

Keywords:

innovation, new technology, in situ,

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Near isotropic, high-resolution multi-beam scanning transmission electron microscopy with iterative milling

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Volume electron microscopy is the only technique to date that provides both sufficient resolution (<20 nm) and sufficient field of view (>100 µm) for the dense reconstruction of neuronal wiring diagrams. Currently, there exist two systems that have already delivered mm³-sized synaptic resolution electron microscopy stacks: Multi-beam scanning electron microscopy [1, 2] (mSEM) and Gridtape-based automated transmission electron microscopy [3, 4] (Gridtape-TEM). Both techniques currently rely on collecting and imaging thousands of ultrathin serial sections (30 - 40 nm) being cut with a diamond knife on an ultramicrotome and collected on a tape, silicon wafer or gridtape. However, serial collection of ultrathin sections is delicate and inherently prone to failures and artefacts such as section loss, folds, cracks or knife marks. More than 50% of the errors of today's state-of-the-art automated neuron segmentation algorithms can be attributed to missing information due to serial sectioning. Consequently, more than 40 hours of manual segmentation proofreading by human experts are currently required to reconstruct a single cortical pyramidal cell accurately.

Gas Cluster Ion Beam Scanning Electron Microscopy (GCIB-SEM) [5] and Broad Ion Beam Scanning Electron Microscopy (BIB-SEM) [6] methods have been proposed to address the section loss issue. These methods involve the collection of thicker sections, in a range of 100-10000 nm in thickness, onto a silicon wafer, offering a more robust approach for section collection. The collected volume is then iteratively sub-milled using either GCIB or BIB techniques to match the desired resolution in the Z-axis and then imaged using an SEM.

Here, we adopt the same principle of collecting semi-thin sections with subsequent sub-milling and, to have an imaging time advantage, we propose using a commercially available multi-beam scanning transmission electron microscope - mSTEM (FAST-EM Delmic, Netherlands), and use BIB for iterative sub-milling. We will refer to the combination of the two as BIB-mSTEM. In contrast to mSEM, mSTEM forms the image from high-energy transmitted electrons that are much less sensitive to local electromagnetic fields and milling-induced irregular surface topography than low-energy secondary electrons. Additionally, mSTEM offers the advantage of being more cost-effective compared to mSEM, making it an attractive option for large-scale imaging projects.

As proof of the method, we first collected serial sections of 250 nm thickness directly onto a scintillator plate using the commercially available ultramicrotome (Leica, Germany). Subsequently, we iteratively imaged the sections with FAST-EM and sub-milled the volume using BIB, producing a series of iteratively milled TEM projection images for each section. We then used those projections to computationally reconstruct a high-resolution 3D stack of each section. We used a deconvolution

procedure to reconstruct or deconvolve virtual reslices (Fig. 1B+D) from the series of projection images (Fig. 1A+C). The data shows that the proposed method increases the Z-resolution sufficiently to capture small changes in the ultrastructural appearance of neurites, mitochondria and synaptic vesicles (Fig. 1D) [7].

Fig. 1: Data from an iteratively BIB-milled and FAST-EM imaged 250 nm thick section. A STEM projection images of the iteratively milled section. B Corresponding deconvolved/reconstructed slices. C, D Zoom-in onto a dendrite in A and B, respectively. Scale bar = 1 µm.

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Keywords:

volume electron microscopy, connectomics

Reference:

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FAST-EM array tomography: a workflow for multibeam volume electron microscopy

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Background
Emerging volume electron microscopy (vEM) techniques are providing unprecedented insights into the nanoscale 3D structure of biological specimens. However, the limited sustained throughput of electron microscopes hinders large sample handling (1). Multiple approaches, including camera-array transmission EM, beam-deflection transmission EM and multibeam scanning EM, have been developed to improve throughput and are now delivering first results (2, 3). We present a workflow for multibeam volume microscopy, FAST-EM array tomography. FAST-EM is a commercial multibeam scanning transmission electron microscope which speeds up acquisition by scanning the sample with 64 beams in parallel (4).

Methods
In FAST-EM array tomography, biological samples are prepared by chemical fixation, heavy metal staining, dehydration and resin embedding (Figure 1A). Serial sections are produced by ultramicrotomy and deposited on a cerium-doped yttrium aluminum garnet (ce:YAG) scintillator crystal, coated with molybdenum. FAST-EM employs optical scanning transmission electron detection (OSTEM), where transmitted electrons are converted into photons by the scintillator, which are then collected and descanned onto a detector array. The sections are imaged sequentially using stage translations. The volume is reconstructed from 2D images by finding point-pair correspondences in the overlap region between individual images. The 3D reconstructed data is then segmented using manual or automatic techniques, after which data analysis is conducted.

Results
Several biological samples, including cultured cells, organoids, as well as tissue samples were imaged with FAST-EM. To demonstrate the workflow, a 265.000 um3 volume of ~70 cultured MCF-7 cells was reconstructed from 72 100nm thin serial sections resolving the mitochondrial cristae and membrane structures (Figure 1B). The data quality and alignment is consistent throughout the volume (Figure 1C-D). To further demonstrate the applicability of analysis tools developed for other electron detection techniques to FAST-EM and OSTEM detection data, all mitochondria were automatically segmented using MitoNet, a convolutional neural network for 3D segmentation (5). MitoNet applied to FAST-EM datasets demonstrated performance metrics similar to benchmark datasets obtained using other vEM modalities.

Conclusion
We implemented a workflow for multibeam volume transmission electron microscopy capable of imaging large regions of interest with feasible acquisition times, while providing images with high

resolution and contrast. FAST-EM potentially expedites EM for comprehensive sample scanning and statistical analysis across multiple conditions. This creates opportunities for novel applications, such as examining the inter-cellular variability in a large population of cells. Further increase of throughput is possible by optimizing the signal generation and collection, reducing overhead and increasing system autonomy.

FIGURE LEGEND:
Figure 1: Volume EM reconstruction of cell culture with FAST-EM array tomography. A: FAST-EM array tomography workflow. B: Overview images of sections, showing a zoom in on a single section, a single multibeam field-of-view and a single beam image respectively. C: Aligned volume reconstruction from 54 100nm thin serial sections showing the orthogonal reslices through the red lines (xz and yz). D: Volume rendering of the full (continuous) stack. Inset shows smaller sub volume at 8nm/pixel resolution.

Keywords:

vEM, SEM, FAST-EM, STEM, OSTEM

Reference:

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In situ TEM study of phase and electronic structure transformations in amorphous Ga2O3

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

Background
Amorphous semiconductors have ignited considerable interest in technological explorations owing to their distinctive electrical and optical properties stemming from their disordered structure and thermodynamic metastability. Thermal treatments have the potential to enhance or activate certain unique properties by reorganizing their atomic and electronic structures. Establishing precise correlations between thermal processes, structure evolutions, and electrical properties is essential for understanding fundamental mechanisms and achieving high-performance semiconductor materials. Gallium oxide (Ga2O3) is an advanced semiconductor material characterized by an exceptionally wide bandgap ranging from approximately 4.5 to 4.9 eV, depending on its concrete crystal phase and doping level. This wide gap renders Ga2O3 well-suited for high-power and high-frequency electronic devices.
Herein, we will present our in situ TEM work in this scenario by visualizing the atomic and electronic structure evolutions of amorphous Ga2O3 annealed in an O2 atmosphere.
Methods
High-quality morphous Ga2O3 films, ranging in thickness from 100 to 200 nm, were deposited on gas cell chips from Protochips using molecular beam epitaxy (MBE). Under the ambient pressure of an O2 environment, the as-grown films were heated from room temperature to 1000 °C at a heating rate from 1 to 1000 °C/s. Time series of selected area electron diffractions (SAED) and images were acquired with an image aberration-corrected TEM of FEI Titan 80-300 during the heating process. Furthermore, in situ STEM images and electron energy loss spectra (EELS) were collected on a probe aberration-corrected Thermo Fischer Spectra Ultra equipped with GIF Tridem 863 from Gatan to elucidate the atomic and band structure changes.
Results
Figure (a) and (b) depict the bright field (BF) TEM images and corresponding SAED patterns at four typical stages during the heating process of the amorphous Ga2O3 from room temperature to 1000 °C at a heating rate of 1 °C/s. Figure (c) were SAED patterns processed by CrysTBox software with background eliminated, direct beam removed, and intensity averaged. Gold and red arcs are superposed in SAED patterns to represent Debye-Scherrer rings referred to {hkl} reflections of the cubic spinel and monoclinic phases, respectively.
As shown in the figure, a circular cubic spinel phase of Ga2O3 (γ-Ga2O3) with a size of approximately 100 nm is formed at the initial stage. Subsequently, these structures evolve into dendritic morphology, accompanied by the generation of the monoclinic phase (β-Ga2O3) and finally undergoing a full transformation to a polycrystalline monoclinic phase.
This transition can be identified as a reconstructive disorder-to-order phase transition mediated by the exchange of cations to the next nearest neighbor sites, as shown in Figure (d). The figure provides

a schematic description of the atomic structural evolution and energy barriers needed to overcome for disordered to ordered phase transition upon heating [1].
Further results, such as the complete time-series of BF images and SAEDs, the in situ low loss EELS reveling the band gap shift, and more, will be presented on site.
Conclusion
Our in situ TEM work provides a correlated perspective on the thermal treatments, phase transformations, and band gap shifts involved in the pure Ga2O3. These findings have significant implications for understanding the fundamental mechanisms and are pivotal for the design and engineering of wide bandgap semiconductors in photoelectric applications.

Keywords:

Amorphous Ga2O3, in situ TEM

Reference:

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RUDDLESDEN-POPPER PLANAR DEFECTS IN METAL HALIDE PEROVSKITES: MECHANISMS OF FORMATION AND CONSEQUENCES FOR PHASE STABILITY

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Background: Metal halide perovskites (MHPs) are promising candidates for the next generation of optoelectronic and photovoltaic devices. This is mainly due to their wide-gamut emissions, high color purity (<35 nm spread) and remarkable photoluminescence quantum yield of more than 90%. However, their commercial application is still hindered by fast degradation under real conditions. The main cause of the CsPbI3 instability is its transformation from the black perovskite phase (photoactive) into the yellow non-perovskite (inactive) phase under ambient conditions, where water molecules decrease energy barriers of phase transition. Strategies towards more phase-stable CsPbI3 include interfaces, ligands as well as composition engineering. The last one is frequently associated with the formation of Ruddlesden-Popper (RP) planar defects, as shown for doped CsPbBr3. However, the underlying reasons and pathways of formation of the RP phase remain undiscovered. Therefore, the aim of this study is to understand how modification of CsPbI3 with Zn2+ and Cd2+ helps to prolong phase degradation by exploiting local techniques such as quantitative transmission electron microscopy (TEM) combined with molecular dynamics (MD) structure simulations.
Methods: In this work, we utilize different TEM techniques such as High Angle Annular Dark Field Scanning Transmission Electron Microscopy (HAADF-STEM), 4D STEM and Energy Dispersive X-ray Spectroscopy (EDX) under low-dose conditions to retrieve local structural changes which might explain stability improvement upon doping. These experimental investigations were complemented by quantitative analysis using StatSTEM software. Moreover, to demonstrate single-phase 3D structure as well as the structure of RP/perovskite interfaces, MD simulations were used along with TEM image simulations through the MULTEM software.
Results: Cd2+ and Zn2+ precursors were introduced into the reactive batch during hot-injection synthesis of CsPbI3 nanocrystals. As-prepared modified material contained ~2.1 at.% of Zn and ~2.5 at.% of Cd uniformly distributed along the nanocrystals. To understand the mechanism of nanocrystals degradation, a series of ex situ TEM experiments was performed for pristine and modified samples. It was established that the degradation pathway includes the agglomeration stage and the phase transformation. Here, both modified samples have demonstrated prolonged stability after agglomeration up to 5 days for Cd-containing and 10 days for Zn-containing CsPbI3. Ruddlesden-Popper planar defects in a specific configuration were identified for each modified sample by means of advanced electron microscopy. Using high-resolution EDX we showed that both transition metals are mainly distributed in these defects which are present along the diagonals of the square-like projection of the nanocrystals. Detailed analysis showed that Cd2+ doping results in ½ lattice shift in one direction, whereas Zn2+ doping provides ½ lattice shifts in two perpendicular directions. This difference can be ascribed to the ionic radii difference between Cd (1.09 Å) and Zn (0.88 Å). Combining these observations with molecular dynamic simulations, Zn2+ presumably occupies interstitial positions in RP regions with only changing Cs and I content slightly to balance the charges, whereas Cd substitutes Pb atoms. Moreover, the RP phases include CsI layers of different

length in-between perovskite structure, providing a smooth transition from RP into the gamma-phase along [110]CsPbI3/[001]RP direction. The process of RP phase formation includes the drifting apart of neighboring [PbI6]4- octahedra as a result of strain in the lattice due to Zn and Cd incorporation. Afterwards, additional Cs and I atoms have to enter the created void, which is followed by a shift of the two perovskite parts in one or two directions for the Cd- or Zn-modified samples, respectively. We associate an improvement in phase stability for these materials with the presence of as-described RP defects. In the α-phase of CsPbI3 a corner-sharing structure is preserved with Pb-I-Pb bond angles of 180^o, while in a δ-phase the structure consists of edge-sharing [PbI6]4- octahedra with the Pb-I-Pb bond angles of 90^o. Hence, the smaller these angles are, the lower kinetic stability is, hence, black-to-yellow transformation occurs faster. According to MD simulations, the gamma-phase most likely has Pb-I-Pb bond angles of 152.7^o, while for the one-shifted RP phase, observed for Cd-modified material, the value equals to 154.2^o and for two-shifted RP phase, observed for Zn-modified material, the angle is 155.1^o. The observed tendency perfectly correlates with the phase stability test, where Zn-modified material demonstrated the highest stability in comparison to Cd-containing and unmodified sample, whereas Cd-containing sample also surpassed unmodified material. At the interface between RP/perovskite phases the angles are 154.5^o and 156.0^o for Zn and Cd-modified samples respectively, which also contributes to the enhanced phase stability.
Conclusions: We applied a combined experimental/theoretical approach to unravel the influence of modification with Zn2+ and Cd2+ on phase stability of CsPbI3 nanocrystals. From an experimental point of view, we were able to observe specific types of Ruddlesden-Popper (RP) phase defects across the nanocrystals, where Cd and Zn are mainly localized. These defects were analyzed in a quantitative manner using statistical parameter estimation theory applied to the TEM results. Combination of these experimental results with MD simulations reveals how the RP planar faults are formed in a presence of transition metals, where these atoms are positioned and explains how phase stability, as shown by series of ex situ TEM results, is related to these defects. This is why our study contributes to an enhanced understanding of mechanisms underlying perovskites stabilization and may foster its commercialization for further use in devices.

Keywords:

phase transitions, lattice shifts, defects

Reference:

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Continuous EELS spectrum imaging of nano-droplet crystallization heterogeneity

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Poster Group 1

Background incl. aims
Electron energy loss spectroscopy (EELS) is a powerful technique for characterizing nanomaterials; a number of materials properties can be extracted from the information-rich spectra. One application is nano-thermometry, where the local temperature of individual metal nanoparticles can be measured by a precise determination of the plasmon peak position [1]. As thermal expansion increases a particle's volume, the density of electrons decreases, and the energy loss of the plasmon peak shifts to lower energies. This same approach can be used to detect melting and crystallization, which result in even larger changes in the electron density. Continuous acquisition of EELS data enables precise determination of the melting and crystallization temperatures of individual particles. Using the new in-situ EELS spectrum imaging features of the Continuum GIF, a continuous series of drift-corrected spectrum images can be acquired over an ensemble of particles, and the melting and crystallization behavior of each one independently monitored. This would be more difficult or impossible with other techniques such as TEM imaging, diffraction, or 4DSTEM.
Methods
In this work, we show how a series of EELS spectrum images can be acquired and processed along with the temperature data from a MEMS-based heating holder from DENSsolutions. With modern fast detectors and spectrometers, spectrum images with thousands of spectra can be acquired in less than a second, making continuous in-situ spectrum imaging feasible. The holder temperature data is automatically synchronized and correlated with EELS spectrum image data. We also show how the entire series of in-situ EELS spectrum images can be rapidly fit using the built-in NLLS tools in DigitalMicrograph, yielding series of synchronized fit maps, as seen in Figure 1. After summing the EELS spectra over a single nanoparticle, plots of the peak position over time can be generated, and even plotted against the nominal temperature from the holder in scatterplots in DigitalMicrograph. The plasmon peak position within each particle indicates whether the Sn is melted or crystallized at that time.
Results
This new in-situ EELS spectrum imaging capability has been applied to a Sn nanoparticle sample which was oscillated above and below its melting temperature with varying ramp rates. Spectrum images were recorded at a rate of 1 frame every 1.54 s (2000 spectra/s). Watching the maps of plasmon position over time reveals that while all the Sn particles crystallized during most cycles, and most particles crystallized during every observed cycle, some particles occasionally did not crystallize even though surrounding particles did. Images and 4D STEM maps of the particles after the in-situ recording do not indicate a clear difference between a particle that always crystallized and the particles that sometimes did not. Some variation could be the result of random chance, as the nucleation and growth of a crystalline nanoparticle from a nano-droplet is governed by both thermodynamics, which would indicate that the particles should all crystallize at the melting temperature, and kinetics, which stipulates that nucleation times for an ensemble will have some distribution. However, there are only 3 particles (of 11 in the field of view) which don't always re-

crystallize, and one of them remains melted during 4 cycles, which seems unlikely to be due only to random statistical fluctuations in nucleation time.
This behavior would be significantly more difficult to observe with TEM or STEM imaging, where the visibility of lattice fringes is dependent on the orientation of the nanoparticles. It would also be challenging with standard diffraction techniques, where the crystallinity of multiple particles could not be independently and simultaneously observed. Continuously acquired 4DSTEM could be used, but this requires more data to be stored, and the analysis is more complex, especially if there are other small crystallites (oxides, etc.) which overlap the droplets, as there are here.
Conclusions
This heterogeneous and stochastic behavior at the nanoscale can only be observed with high spatial and temporal resolution. In-situ electron microscopy, and specifically in-situ EELS spectrum imaging is an excellent technique for exploring these dynamics.

Figure 1: One frame from a screen-capture video showing playback of the in-situ EELS spectrum image dataset. A) Simultaneously acquired dark field STEM images. B) Maps of the plasmon peak position. Arrows indicate 2 particles which have failed to re-crystallize despite super-cooling them to 50 °C. C) Live fit of the zero loss peak and plasmon peaks from the red “picker tool” region indicated in A. D) In-Situ Player which plays back and synchronizes all displayed in-situ dataset components. E) Automatically acquired and synchronized temperature data from the DENSsolutions heating holder used to heat the Sn above and below its melting point of 232 °C.

Keywords:

EELS
In-Situ
nanoparticle
crystallization
melting

Reference:

[1] Mecklenburg, M. et al. Nanoscale temperature mapping in operating microelectronic devices. Science 347, 629–632 (2015).

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Electron Channeling Contrast Imaging (ECCI) of Ion Battery Cathode Materials

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Poster Group 1

The microstructural characterization of ion battery cathode materials is often studied by transmission electron microscope (TEM) due to its high spatial and angular resolution which enables precise analysis of the battery materials. However, TEM studies present several challenges that might hinder their suitability for numerous potential applications. The conventional processes of preparing thin foils for TEM analysis, such as twin jet electropolishing, are time consuming and expensive. Moreover, when high-energy electron beams interact with the specimen in TEM, the studied sample may experience damage through various mechanisms, including knock-on displacement, radiolysis, and heating, leading to changes in the microstructure and properties of materials, particularly those sensitive to beams [1,2]. Here, another interesting approach for bulk specimens is presented that offers both a high spatial resolution and a large field of view at relatively low accelerating voltages: the use of ECCI in a field emission scanning electron microscope (FESEM). ECCI is an imaging method that relies on the change of backscattered electron (BSE) intensity caused by differences in the angle between the incoming electron beam and the crystallographic orientation of the lattice planes in crystalline samples. When the incoming electrons are parallel (or very close to parallel) to the lattice planes, low BSE intensity and hence a darker area can be anticipated, while with the increase in the angle, higher intensity and a brighter area are expected. ECCI technique allows us to figure out how the specimen's crystallographic orientation changes, along with identifying features like grain boundaries and cracks, as well as individual lattice defects such as dislocations [3,4].
In this study, the microstructural evolution of two different layered Li-ion based (composed of secondary particles) and Na-ion based (composed of primary particles) cathode materials in their pristine state was investigated. High resolution secondary electron (SE) and ECC images were acquired with the use of Hitachi SU8000 dedicated FESEM at a relatively low accelerating voltage of 4 kV. For this purpose, cross-sectional samples were prepared using a Hitachi IM4000 ion milling machine.
Figures 1a and b show the SE and ECC cross-sectional images of a Li-ion based cathode polycrystalline secondary particle. The secondary particle comprises many single crystalline primary particles. Primary particles are simply like the 3-dimensional grains found in conventional polycrystalline metallic materials with different crystal orientations. As can be seen in Figures 1a and b, the primary particles cannot be well recognized in the SE image (Figure 1a), while in the ECC image they are completely clear with very well-defined grain boundaries thanks to the ECCI technique (Figure 1b). Furthermore, the imperfections between the primary particles such as voids and pores are more evident with sharp edges in the ECC image compared to the SE image.
The SE and ECC cross-sectional images of a Na-ion based cathode single crystalline primary particle are represented in Figures 1c and d. The SE image displays the primary particle with an irregular shape without any distinguished lattice features (Figure 1c). However, the ECC image depicts the same particle with two clearly visible features shown by yellow arrows: I) coincidence site lattice boundary (CSLB) and II) intragranular crack along the CSLB as a preferential site for crack initiation

(Figure 1d). In an earlier study by the authors [5], the presence of CSLBs in the same specimen has been shown with the aid of Hitachi SU9000 scanning transmission electron microscope (STEM). Conclusively, this work unraveled the unique role of ECCI technique as a perfect microanalysis tool for reasonably priced and rapid investigation of the cathode battery materials.

Figure 1. a) SE and b) ECC cross-sectional images of a Li-ion based cathode polycrystalline secondary particle. c) SE and d) ECC cross-sectional images of a Na-ion based cathode single crystalline primary particle.

Keywords:

ECCI, Battery, Cathode, Grain Boundary

Reference:

[1] Y. Wen et al., NPG Asia Materials, 9 (2017), e360.
[2] J. Cui et al., Advanced Materials, 33 (2021), 2000699.
[3] S. Kaboli et al., Microscopy and Microanalysis, 19 (2013), pp. 1620-1631.
[4] D.C. Joy et al., Journal of Applied Physics, 53 (1982), pp. R81-R122.
[5] M. Naghizadeh et al., Microscopy and Microanalysis, 29 (2023), pp. 1331-1332.

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Application of reinforcement learning to aid the alignment of an electron microscope

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Poster Group 2

Background incl. aims
In both SEM and TEM, the alignment process for each experiment is unique and not replicable. If not done well this also has major implications on the stability of the experiments and the quality of the obtained data regardless of the specimen or sampling technique. It requires days of diligent practice on a variety of electron microscopes in order to become an expert and be able to collect data with the highest possible resolution consistently across experiments.
A proposed solution to this skill barrier is a digital twin for an electron microscope, with the purpose being to make experiments more reproducible, efficient and improving the overall reliability and resilience of experiments [1]. The initial steps in this is to automate the alignment process, starting with the eucentric height using machine learning (ML) techniques.
Methods
The proposed method for finding the optimal eucentric height is reinforcement learning (RL), a subset of ML where an agent learns how to interact with an environment in order to make decisions. Using a q-learning function and a reward function the agent is able to determine the optimal action in order to achieve the correct z-height. For training an agent on images at different z-heights learning a q-table is sufficient due to the small and finite action space. However, for applications on a microscope, a deep-q-network (DQN) is required, which instead of knowing the possible rewards from a trained q-table estimates the rewards and works for larger action spaces [2].
Results
Initial tests on a small state space where the environment is defined as images taken at different z-heights with intervals of 2µm ranging from 0µm to 50µm (including the eucentric height), show that the agent is able to learn a q-table within 10000 iterations. With this initialized q-table the agent is able to find the image that was taken at the eucentric height instantaneously.
Conclusion
Preliminary tests indicate that reinforcement learning holds promise as a viable solution for automating the alignment process of an EM, potentially reducing the expertise needed to conduct experiments effectively and accurately.

Keywords:

Digital Twin, Reinforcement Learning, Deep-Q-Network

Reference:

1. VanDerHorn, Eric, and Sankaran Mahadevan. "Digital Twin: Generalization, characterization and implementation." Decision support systems 145 (2021): 113524.

2. Mnih, Volodymyr, et al. "Playing atari with deep reinforcement learning." arXiv preprint arXiv:1312.5602 (2013).

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Progress in 3D phase-contrast imaging using 4D-STEM: increased dose-efficiency and depth of field

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

Scanning Transmission Electron Microscopy (STEM) is used extensively to study the atomic structure of materials at the nanoscale. So far, the dominant method for atomic resolution imaging is annular dark-field STEM, enabled by modern electron optics and popular because of its incoherent atomic number contrast. Developments in detector technology have caused the widespread adoption of fast direct electron detectors, which allow collecting a 2D momentum space image at every position of a 2D real-space scan, motivating the name 4D-STEM. Such 4D-STEM datasets allow recovery of phase information, and the high number of intensity measurements allows us to fit ever more complex models to the scattering data, increasing dose efficiency, resolution, and imaging throughput. Modern reconstruction algorithms can recover partial coherence effects, probe positions, and three-dimensional information beyond the numerical aperture limit from a single 4D-STEM dataset. However, the obtainable depth resolution in these approaches is currently more than a factor of ten away from the atomic scale needed for de novo structure determination. This motivates the collection of tilt-series 4D-STEM measurements, which allow higher dose efficiency to reach the 3D atomic resolution regime than 3D imaging approaches based on ADF-STEM.
Here, we show a series of advancements in instrumentation and 4D-STEM reconstruction methods that allow the determination of 3D atomic structure from 4D-STEM measurements and extend the sensitivity and volume limits of electron microscopy.

Methods & Results
Fig. 1a) shows an experimental demonstration of ptychographic electron tomography. We reconstructed a complex hybrid structure of a ZrTe nanowire encapsulated in a double-walled carbon nanotube [10]. We then solved the complete atomic structure from this 3D reconstruction. The nanowire comprises three unique structural motifs: an elliptic curved 2D material composed of ZrTe5 units, Te-Te chains, and a previously unobserved ZrTe2 core structure. The ZrTe2 core structure is closely related to the ZrTe5 structure, with the longer bond-length Te atoms removed and the stacking slightly modified. We also performed DFT to verify the stability of the proposed atomic structure of this nanowire [1].
In this study, we used a fast linear method for initial reconstruction and aberration parameter estimation, followed by iterative single-slice reconstruction, including partial coherence, and finally, tomographic reconstruction.
By explicitly modeling dynamical scattering within the sample using multi-slice ptychography, the accessible volume in ptychographic tomography can be significantly expanded beyond the conventional depth of field limit for 3D imaging in STEM. This allows for the retention of 3D atomic resolution in larger volumes.

Fig. 1b) shows an experimental demonstration of a sequential reconstruction approach to multi-slice ptychographic tomography on a Co3O4 nanocube. Here, we reconstruct a volume of 18.2nm side length and break the depth of field limit by a factor of >3x. The bottom panel of Fig 1b) shows cuts through the 3D power spectrum of the reconstruction, indicating that a resolution of 2Å axial and 0.7Å lateral resolution has been reached.
Reconstructing the electrostatic potential in an end-to-end fashion directly from 4D-STEM data can further increase the coupling of the voxels in 3D space, allowing the recovery of missing wedge information. The reconstruction strategies developed above can be utilized to bootstrap all parameters of such an end-to-end reconstruction efficiently.

Conclusion
We have developed atomic resolution ptychographic electron tomography to solve 3D atomic structures containing light elements and spanning volumes larger than the depth of field. Future applications include the imaging of beam-sensitive polymers, hybrid organic-inorganic structures, battery materials, and biomolecules.

Keywords:

4D-STEM, multi-slice ptychography, tomography, reconstruction

Reference:

[1] Philipp M. Pelz, Sinéad M. Griffin, Scott Stonemeyer, Derek Popple, Hannah DeVyldere, Peter Ercius, Alex Zettl, Mary C. Scott, and Colin Ophus. Solving complex nanostructures with ptychographic atomic electron tomography. Nature Communications, 14:7906, November 2023.

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Cation Vacancies Regulate the Electron Spin Configuration of Cathode Catalytic Additives towards Robust Li-S Batteries

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PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

Background
Lithium-sulfur batteries (LSBs) are promising energy storage devices due to their high theoretical energy density (2600 Wh kg-1), large specific capacity (1675 mAh g-1), potential cost-effectiveness, and environmental friendliness.[1] However, the commercialization of LSBs is limited by factors such as polysulfide migration, sluggish kinetics of the sulfur redox reaction (SRR), and poor electronic and ionic cathode conductivities.[2] The use of defect-engineered catalysts, with tunable surface chemistry and electronic properties, as cathode additives in LSBs is a promising strategy to accelerate the Li-S redox reactions and thus promote LSB performance. In contrast to earlier research that predominantly concentrated on how defects influence the electronic density of states, the present work explores the impact of defects in modulating electronic spins and how these changes in spin configuration influence the macroscopic adsorption properties and activity of the catalytic additive.
Methods
In this study, a defect engineering strategy was used to tune the electron spin state of an SRR electrocatalyst. This strategy was showcased by introducing controlled amounts of cation vacancies within ultrathin CoSe nanosheets through plasma etching. Atomic resolution aberration-corrected high-angle annular dark-field scanning transmission electron microscopy (AC-HAADF-STEM) was used to gain in-depth insights into the spatial distribution of Co vacancies. 3D atomic models were obtained by using Rhodius and the corresponding AC-HAADF-STEM image simulations obtained by using STEM-CELL software.[3-4] XRD, EXAFS, XPS, Raman spectroscopy, XMCD, DFT calculations, EPR, in situ XRD combined with electrochemical test were conducted.
Results
Vacancies were also indirectly evidenced by an increase of disorder and reduced lattice spacing, Co-Se bond length, and Se coordination number using XRD, HRTEM, EXAFS, XPS, and Raman spectroscopy. XANES and XPS spectroscopy further showed an increase in the average oxidation state of cobalt with the introduction of vacancies and EELS pointed to a higher occupation of Co 3d states in v-CoSe. Beyond modifying the structural parameters and electronic state occupation, the presence of vacancies resulted in a polarization of the 3d electron spins as evidenced by an increase in the intensity of the satellite peak in the Co 2p XPS spectra, a strong Co dichroism signal obtained by XMCD and magnetic measurements that confirmed a large moment per Co ion of 2.8 muB in v-CoSe. EPR spectroscopy further confirmed the generation of additional electrons with unpaired spins by the introduction of Co vacancies.
DFT calculations showed these spin-polarized unpaired electrons to be easily transferred from v-CoSe to polysulfide. Computational results showed that the d band center shifts toward the Fermi level with the introduction of vacancies, which involves that electrons are more prone to disperse in the high-spin configuration. The altered spin configuration results in a swift transferability of additional unpaired electrons to the polysulfide. DFT calculations also showed v-CoSe to have significantly higher lithium polysulfide adsorption energies and to decrease the polysulfide stability upon adsorption, thus enhancing both polysulfide adsorption and conversion activity. Besides,

computational results showed the modified spin configuration to result in a more favorable thermodynamic reduction process, particularly for the transition from soluble polysulfide to solid Li2S that showed a lower nucleation energy barrier.
Experimental measurements confirmed an enhanced adsorption of the lithium polysulfide on the v-CoSe surface. Symmetric cells showed v-CoSe to provide a low polarization voltage and sharper and more intense redox peaks. In situ XRD showed a very rapid and complete transformation of S8 and a high lithium polysulfide redox activity in v-CoSe. EIS analyses showed v-CoSe to be characterized by a low SRR activation energy, particularly for the liquid-solid conversion stage. Besides, the presence of vacancies resulted in promoted Li2S nucleation, reduced LSB polarization voltages, and higher Q2/Q1 ratios. v-CoSe cathodes not only demonstrated excellent electrocatalytic properties but also outstanding LSB electrochemical performance in terms of specific capacity, rate performance, and cycling stability, even at high sulfur loadings.
As a result, more uniform nucleation and growth of Li2S and an accelerated liquid-solid conversion in LSB cathodes are obtained. These translate into CoSe-based LSB cathodes exhibiting capacities up to 1089 mAh g-1 at 1C with 0.039% average capacity loss for 1500 cycles, and up to 5.2 mAh cm-2, with 0.16% decay per cycle after 200 cycles in high sulfur loading cells.
Conclusion
Overall, this study demonstrates the need for considering the electronic spin configuration in the design of electrocatalysts, particularly for developing robust LSBs. Thus, the spin engineering approach showcased here paves the way to the rational design of new generations of LSB cathodes based on defect-engineered SRR electrocatalysts, toward the development of a cost-effective LSB technology with market-ready potential.

Keywords:

AC-HAADF-STEM, battery, vacancy, spin polarization

Reference:

[1] A. Manthiram, et al., Advanced Materials 2015, 27, 1980.
[2] Z. Liang, et al., Advanced Energy Materials 2021, 11, 2003507.
[3] J. Arbiol et al., Appl. Phys. Lett. 2002, 80, 2.
[4] S. Bernal, et al., Ultramicroscopy 1998, 72, 135-164.

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Vacancy-driven electron spin engineering to promote Li-S redox reactions

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Poster Group 1

Background
Lithium-sulfur batteries (LSBs) are promising energy storage devices due to their high theoretical energy density (2600 Wh kg-1), large specific capacity (1675 mAh g-1), potential cost-effectiveness, and environmental friendliness.[1] However, the commercialization of LSBs is limited by factors such as polysulfide migration, sluggish kinetics of the sulfur redox reaction (SRR), and poor electronic and ionic cathode conductivities.[2] The use of defect-engineered catalysts, with tunable surface chemistry and electronic properties, as cathode additives in LSBs is a promising strategy to accelerate the Li-S redox reactions and thus promote LSB performance. In contrast to earlier research that predominantly concentrated on how defects influence the electronic density of states, the present work explores the impact of defects in modulating electronic spins and how these changes in spin configuration influence the macroscopic adsorption properties and activity of the catalytic additive.
Methods
In this study, a defect engineering strategy was used to tune the electron spin state of an SRR electrocatalyst. This strategy was showcased by introducing controlled amounts of cation vacancies within ultrathin CoSe nanosheets through plasma etching. Atomic resolution aberration-corrected high-angle annular dark-field scanning transmission electron microscopy (AC-HAADF-STEM) was used to gain in-depth insights into the spatial distribution of Co vacancies. 3D atomic models were obtained by using Rhodius and the corresponding AC-HAADF-STEM image simulations obtained by using STEM-CELL software.[3-4] XRD, EXAFS, XPS, Raman spectroscopy, XMCD, DFT calculations, EPR, in situ XRD combined with electrochemical test were conducted.
Results
Vacancies were also indirectly evidenced by an increase of disorder and reduced lattice spacing, Co-Se bond length, and Se coordination number using XRD, HRTEM, EXAFS, XPS, and Raman spectroscopy. XANES and XPS spectroscopy further showed an increase in the average oxidation state of cobalt with the introduction of vacancies and EELS pointed to a higher occupation of Co 3d states in v-CoSe. Beyond modifying the structural parameters and electronic state occupation, the presence of vacancies resulted in a polarization of the 3d electron spins as evidenced by an increase in the intensity of the satellite peak in the Co 2p XPS spectra, a strong Co dichroism signal obtained by XMCD and magnetic measurements that confirmed a large moment per Co ion of 2.8 muB in v-CoSe. EPR spectroscopy further confirmed the generation of additional electrons with unpaired spins by the introduction of Co vacancies.
DFT calculations showed these spin-polarized unpaired electrons to be easily transferred from v-CoSe to polysulfide. Computational results showed that the d band center shifts toward the Fermi level with the introduction of vacancies, which involves that electrons are more prone to disperse in the high-spin configuration. The altered spin configuration results in a swift transferability of additional unpaired electrons to the polysulfide. DFT calculations also showed v-CoSe to have significantly higher lithium polysulfide adsorption energies and to decrease the polysulfide stability upon adsorption, thus enhancing both polysulfide adsorption and conversion activity. Besides, computational results showed the modified spin configuration to result in a more favorable

thermodynamic reduction process, particularly for the transition from soluble polysulfide to solid Li2S that showed a lower nucleation energy barrier.
Experimental measurements confirmed an enhanced adsorption of the lithium polysulfide on the v-CoSe surface. Symmetric cells showed v-CoSe to provide a low polarization voltage and sharper and more intense redox peaks. In situ XRD showed a very rapid and complete transformation of S8 and a high lithium polysulfide redox activity in v-CoSe. EIS analyses showed v-CoSe to be characterized by a low SRR activation energy, particularly for the liquid-solid conversion stage. Besides, the presence of vacancies resulted in promoted Li2S nucleation, reduced LSB polarization voltages, and higher Q2/Q1 ratios. v-CoSe cathodes not only demonstrated excellent electrocatalytic properties but also outstanding LSB electrochemical performance in terms of specific capacity, rate performance, and cycling stability, even at high sulfur loadings.
As a result, more uniform nucleation and growth of Li2S and an accelerated liquid-solid conversion in LSB cathodes are obtained. These translate into CoSe-based LSB cathodes exhibiting capacities up to 1089 mAh g-1 at 1C with 0.039% average capacity loss for 1500 cycles, and up to 5.2 mAh cm-2, with 0.16% decay per cycle after 200 cycles in high sulfur loading cells.
Conclusion
Overall, this study demonstrates the need for considering the electronic spin configuration in the design of electrocatalysts, particularly for developing robust LSBs. Thus, the spin engineering approach showcased here paves the way to the rational design of new generations of LSB cathodes based on defect-engineered SRR electrocatalysts, toward the development of a cost-effective LSB technology with market-ready potential.

Keywords:

battery, cationic vacancy, spin polarization

Reference:

[1] A. Manthiram, et al., Advanced Materials 2015, 27, 1980.
[2] Z. Liang, et al., Advanced Energy Materials 2021, 11, 2003507.
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Information Transfer Improvement by Parallax Correction and Ptychography Reconstruction Applied to Large-Area 4D STEM Experiments

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Poster Group 2

Scanning Transmission Electron Microscopy (STEM) is currently a reference technique for high spatial resolution imaging, with wide adoption in the characterization of material science samples and with growing use in life science studies. 4D-STEM [1] approach presents a more detailed recording of the electron scattering pattern using pixelated electron detectors and extends the imaging possibilities by combining the real-space scanning and reciprocal-space scattering components. Recent improvements in the direct electron detector technology allow 4D-STEM experiments at similar speeds to STEM imaging [2], and motivates its exploration as a possible substitute to conventional imaging.

This study addresses the information retrieval from 4D STEM datasets using virtual bright field imaging, parallax-corrected phase imaging, and ptychography reconstruction. Large fields-of-view (> 500 nm) of reference samples were measured with fast 4D-STEM (10 µs dwell time), moderate defocus (~100 nm), and scanning sampling between 0.3 and 2.4 nm/pixel. The 4D STEM datasets were processed with the open-source python-based py4DSTEM package [3, 4], including the preliminary assessment and subset selection by virtual STEM images. Defocused probe parallax imaging and a ptychographic gradient descent method were used to correct probe aberrations, particularly defocus. These methods resulted in reconstructed images with effective upsampling, due to the information retrieval from both real and reciprocal spaces.

While the 4D STEM reconstruction with a virtual BF approach resulted in an image with spatial resolution limited by either probe aberration or sampling, equivalent to conventional BF STEM imaging, both parallax-corrected phase imaging and ptychography reconstruction allowed for information retrieval down to lattice level (< 0.2 nm). The findings indicate that 4D STEM reconstruction methods can yield resolution beyond real-space sampling, possibly limited by the effective electron dose used in fast 4D STEM experiments. A current challenge is to extend and optimize these image reconstruction methods to recover resolution from the full field of view of such large-area scans. However, with the increasing efficiency and accessibility of 4D STEM data analysis, the calculation and display of (near-) real-time super-resolution images is foreseen.

Fig. 1. Comparison of (left) Virtual DF image and (center) parallax-corrected phase image from a cropped 128x128 pixels region from a standard gold nanoparticles sample. scale bar = 10 nm.(right) Detail of up-sampled crop region and respective Fourier Transform indicating transfer of lattice information. scale bar 5 nm.

Keywords:

4D STEM, ptychography, parallax

Reference:

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2. M. Wu et al., J. Phys. Mater. 6 (2023) 045008. DOI: 10.1088/2515-7639/acf524
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4. G. Varnavides et al., arXiv preprint: https://arxiv.org/abs/2309.05250

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Contrast Optimization Aided by Machine Learning Applied to Virtual 4D-STEM Images

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Scanning Transmission Electron Microscopy (STEM) has revolutionized imaging due to its high spatial resolution and easy interpretation due to varied contrast mechanisms, such as atomic number (Z) contrast at high-angle scattering and phase contrast from the bright-field disk. The enhanced flexibility in terms of scattering range detection in comparison with STEM is one of the reasons that led to the increased interest in 4D-STEM [1]. Supported by the recent evolution of pixelated detectors, 4D-STEM currently allows for the recording of the complete electron scattering range at speeds commensurate with traditional STEM experiments [2]. With the possibility of flexible reconstruction of virtual STEM images with arbitrary detector shapes, contrast optimization for sample regions with different scattering cross-sections is envisioned. This study delves into the optimization of contrast in virtual 4D-STEM images, employing both user-guided and machine-learning (ML) optimization approaches.

Reference samples from semiconductor devices and supported catalysts were measured with fast 4D-STEM under experimental conditions mirroring standard STEM imaging practices, with 1024x1024 scan positions and 10 us dwell time. The resultant datasets comprised 106 diffraction patterns with 96x96 pixels each, presenting a great challenge for manual contrast optimization due to the vast data volumes and nuanced contrast differences within the areas of the specimens. Figure 1 shows manual contrast optimization applied to virtual 4D-STEM images, serving as a foundational comparison point for our machine learning (ML)-aided methodology. The left panel reveals a virtual Bright Field (BF) STEM image, with an inset in the upper left corner illustrating a typical example of electron scattering and the application of a virtual mask overlay, highlighting the initial manual approach to contrast enhancement. The center panel demonstrates a virtual STEM image that has been collected utilizing an optimized annular mask, reflecting the outcomes of manual contrast optimization efforts. In the right panel, a line profile from these virtual images is presented, with indications of contrast levels, offering a quantitative perspective on the enhancements achieved through manual methods. This figure effectively sets a benchmark for the subsequent introduction of our ML-aided approach, illustrating the initial state of contrast optimization against which the improvements facilitated by automated, ML-driven processes can be measured. By providing a clear depiction of manual optimization efforts, Figure 1 underscores the necessity and impact of transitioning towards more sophisticated, automated methodologies for contrast enhancement in 4D-STEM imaging

In the current study, we develop an innovative computational framework designed to automate the enhancement of contrast in similar regions within 4D-STEM data. Our methodology integrates the advanced deep learning architecture, ResNet101 [3], for feature extraction, followed by Principal Component Analysis (PCA) for dimensionality reduction, and the application of hierarchical clustering techniques (Figure 2). The utilization of ResNet101, distinguished for its deep residual learning capabilities, is strategically chosen to adeptly capture the nuanced, hierarchical

features inherent in 4D-STEM datasets, which are pivotal for identifying similarities across various regions. The initial phase of our analysis involves processing the 4D-STEM diffraction patterns through the ResNet101 model, which has been pre-trained on extensive image datasets. This step is instrumental in extracting comprehensive high-dimensional feature vectors that encapsulate the essential attributes of each pattern. Such a transformation of raw diffraction data into a quantitative form surpasses traditional manual feature identification methods, which are often subjective and labor-intensive, by leveraging automated, objective feature extraction. Following feature extraction, we employ PCA to transform the high-dimensional feature space into a lower-dimensional one, effectively reducing the computational complexity while preserving the variance critical for subsequent analysis. This dimensionality reduction is crucial for enhancing the tractability and interpretability of the dataset, allowing for a focused examination of the significant variances among diffraction patterns. The analysis concludes with hierarchical clustering, an agglomerative method that iteratively merges data points based on their similarity, thereby organically identifying clusters of similar regions without the need for predefining the number of clusters. This method is selected for its adaptability in uncovering the inherent groupings within the data, thereby facilitating an intuitive understanding of similarities across the dataset. The dendrogram generated in this process serves as a pivotal tool for visually determining the grouping of similar regions, thereby informing the selection of clusters for targeted contrast enhancement.

This integrated approach—merging deep learning-based feature extraction, PCA, and hierarchical clustering—presents a robust strategy for automatically enhancing the contrast of similar regions within 4D-STEM data. By doing so, it significantly advances the automation of contrast enhancement, ensuring more efficient, accurate, and objective analysis of material structures. This methodology helps to streamline the process of identifying and enhancing similar regions within complex materials.[4]

Fig. 1. (left) Virtual BF STEM image, inset (upper left) indicates an example scattering and with the virtual mask overlay. (center) Virtual STEM image collected with an optimized annular mask. (right) Line profile from virtual images with the relative contrast level calculated from normalized intensities, with an An contrast increase from 28% to 39% is observed between images reconstructed from virtual BF and with an optimized annular mask.

Fig 1. B) Workflow applied for the ML-based contrast optimization.

Keywords:

4D STEM, contrast enhancement

Reference:

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2. M. Wu et al., J. Phys. Mater. 6 (2023) 045008. DOI: 10.1088/2515-7639/acf524
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Scanning Transmission Election Microscopy Observations of Twisted Epitaxial Molybdenum Disulfide-Gold-Molybdenum Disulfide Heterostructures

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims: Confinement of atoms and molecules inside the vdW gap of 2D materials provides a possible strategy for the synthesis of nanostructures that do not evolve 2D phases by themselves. In addition, precise orientation control of the guest 2D nanostructures in this heterogeneous system could provide a promising opportunity to engineer the tunable physical and chemical properties of this heterostructure. We have found a "twisted epitaxial" growth method to synthesize nanometer thick gold (Au) nanodiscs with an average diameter of 50 nm by encapsulating Au nanoparticles within twisted bilayer MoS2 then annealing.1,2 In this "twisted epitaxy" regime, both top and bottom substrates could interact with the grown Au epilayer and influence its crystallographic registry. Transmission electron microscopy (TEM) shows that, Au aligns midway within the twisted bilayer MoS2 when the twist angle is small. For larger bilayer twist angles, Au exhibits only a small misorientation with the bottom MoS2 which varies approximately sinusoidally with the twist angle of the bilayer MoS2. The discovery of twisted epitaxy therefore provides opportunities for tri-lattice heterostructure Moiré engineering and for structure-property investigation of 2D materials with advanced scanning transmission electron microscopy (STEM). Here, we present the STEM-annular bright field (ABF) imaging of so-called "Moiré of Moiré" in twisted epitaxial MoS2-Au-MoS2 trilayer heterostructures. Multislice ptychographic reconstruction shows the atomic structure of each layer and the Moiré pattern from each interface. We have also analyzed the Au plasmon energies and MoS2 excitons by electron energy loss spectroscopy (EELS) to establish whether any new electronic or optical properties might be generated by the twisted epitaxy.
Methods: In the present abstract, the STEM-ABF analysis was carried out on a Thermo Fisher double-corrected, Spectra TEM-STEM operating at 300 kV with a convergence semiangle of 30 mrads and a collection semiangle from 5 to 25 mrads. Multislice ptychography was performed under the same condition using an electron microscope pixelated array detector (EMPAD). Monochromated EELS measurements were conducted at 60 kV with a 60 meV energy resolution.
Results: In the twisted epitaxial MoS2-Au-MoS2 trilayer sample, the twist angle of bilayer MoS2 was determined to be 6.0o by selected area electron diffraction (SAED), and the orientation of the {220} planes of Au nanodisc was measured to have a 3.0o mismatch with the {110} planes of both MoS2 substrates. Therefore, there exists three primary Moirés in this trilayer heterostructure: Moiré from bilayer MoS2 {100} planes with a spacing of 2.7 nm, Moiré from Au {220} with bottom MoS2 {110} with a spacing of 1.4 nm, and Moiré from Au with top MoS2 {110} with a spacing of 1.4 nm. The three primary Moirés are difficult to image at the same time using conventional TEM imaging as it provides the averaged information of the heterostructure along the beam direction. Instead, the STEM-ABF image shows a larger period Moiré (~ 15 nm in spacing), which is attributed to Moiré of the two Moirés from Au with the top and bottom MoS2, respectively, due to their slightly different misorientation. Multislice ptychography iteratively solves for both the electron probe and the object in three dimensions (3D), sliced layer by layer, thus enabling detailed atomic structure visualization at varying depths of view within each slice.3 Employing multislice ptychography, the Moiré patterns of

the two MoS2-Au interfaces were resolved with atomic precision. In addition, the EEL spectrum from a typical twisted epitaxial Au nanodisc (in an approxiamtely rectangle shape with a length of 55 nm, a width of 35 nm, and a height of 3 nm determined by atomic force microscopy) encapsulated in bilayer MoS2 shows a long axis dipolar plasmon peak at 0.8 eV, a short axis dipolar plasmon at 1.0 eV, and a peak at 1.5 eV at the center of the nandisc. This result is different from that of previously reported epitaxial Au island on MoS2, where it shows a long axis dipolar plasmon ~ 1.3 eV and a short axis dipolar plasmon ~ 1.6 eV, indicating a different electronic and optical property of twisted epitaxial Au.4
Conclusion: Our investigation using a double-corrected and monochromated STEM combining ABF, multislice ptychography and EELS has allowed us to delve into the intricate Moiré structure and plasmon resonance of a twisted epitaxial MoS2-Au-MoS2 heterostructure. However, this exploration marks just the starting point, as more questions remain unanswered. For instance, whether there are lattice reconstructions at the twisted Au-MoS2 interface, how the Moiré of Moiré affects the electronic and optical properties of Au, etc. This paper will discuss progress along these lines and outline potential directions for future avenues of research.

Keywords:

Twisted epitaxy, STEM-ABF, ptychography, EELS

Reference:

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2. Yi Cui et al. Science 383, 212-219 (2024)
3. Zhen Chen et al. Science 372, 826-831 (2021)
4. Kate Reidy et al. Nano Lett. 23, 1068-1076 (2023)

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Deep Learning-Assisted Multivariate Analysis for Nanoscale Characterization of Heterogeneous Beam-Sensitive Materials

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Poster Group 2

Nanoscale materials characterization often uses highly energetic probes which can rapidly damage beam-sensitive materials, such as hybrid organic–inorganic compounds. Reducing the probe dose minimizes the damage, but often at the cost of lower signal-to-noise ratio (SNR) in the acquired data. This work reports the optimization and validation of principal component analysis (PCA) and nonnegative matrix factorization for the postprocessing of low-dose nanoscale characterization data. PCA is found to be the best approach for data denoising. However, the popular scree plot-based method for separation of principal and noise components results in inaccurate or excessively noisy models of the heterogeneous original data, even after Poissonian noise weighting. Manual separation of principal and noise components produces a denoised model which more accurately reproduces physical features present in the raw data while improving SNR by an order of magnitude. However, manual selection is time-consuming and potentially subjective. To suppress these disadvantages, a deep learning-based component classification method is proposed. The neural network model can examine PCA components and automatically classify them with an accuracy of >99% and ~1s for 100 components. Together, multivariate analysis and deep learning enable a deeper analysis of nanoscale materials' characterization, allowing as much information as possible to be extracted.

Keywords:

CNN, EDX spectroscopy, PCA, STEM

Reference:

Kosasih, F.U. et al. (2023) 'Deep learning-assisted multivariate analysis for nanoscale characterization of heterogeneous beam-sensitive materials', Microscopy and Microanalysis, 29(3), pp.1047–1061. doi:10.1093/micmic/ozad033.

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Investigating All-Inorganic Halide Perovskite Phase Transformations via in-situ 4D STEM Heating Experiments

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background incl. aims
Halide perovskite materials, in particular Cesium lead-based halides (CsPbX3, where X = Br or Cl), are known in the halide perovskite family due to their promising optoelectronic properties. Understanding any phase transitions in CsPbX3 that would occur under typical device operating conditions is crucial for assessing device thermal stability and structural integrity [1, 2]. This work focuses on elucidating phase transitions in CsPbX3 that occur as a result of thermal variations by utilizing in-situ four-dimensional scanning transmission electron microscopy (4D STEM). This approach captures their structural evolution across a temperature range from sub-ambient to the melting point.
Methods
Energy-filtered 4D STEM data were acquired using a JEOL ARM300F microscope outfitted with a GIF Continuum® and post-GIF K3® detector from Gatan. DigitalMicrograph® version 3.6 and eaSI™ technology were used for in-situ 4D STEM data acquisition. Digital Temperature modulation of the samples was achieved using a DENSsolutions heating holder controlled within DigitalMicrograph and a Gatan LN2 holder for cooling.
Results
The phase diagram of CsPbBr3 predicts multiple phase transformations as the material is heated from room temperature until its melting point at 500°C. The material begins in an orthorhombic (Pnma) phase and begins a phase transformation to a tetragonal (Cmcm) phase at 88°C, followed by a 2nd transformation to a cubic (P4/mbm) phase at 130°C that persists until the melting point. CsPbBr3 was heated from room temperature until its melting point and initial in-situ 4D STEM data shows temperature-dependent variation in the averaged diffraction patterns, seen in Figure 1. Furthermore, virtual bright field images derived from the in-situ 4D STEM data show dynamic reaction interfaces and evolving contrast during heating. These results emphasize the presence of structural evolution of CsPbBr3 about 60°C, 130°C, and above 500°C.
Conclusion
In-situ 4D STEM data acquisition of CsPbX3 during heating is a powerful methodology for quantitative analysis of the phase evolution of halide perovskites from ambient temperature until their melting point. The data collection tools available with in DigitalMicrograph link 4D STEM data with the experimental temperature profile from the heating holder, providing a detailed chronology of the phase evolution. This allows for a more concrete understanding of the stability ranges of the observed intermediate phases during heating and deeper insight into the material behavior under simulated operational temperatures. Adding complementary techniques such as in-situ electron energy loss spectroscopy (EELS) should provide important chemical information to further assess the structural behavior of CsPbX3 and its consequences on optoelectronic properties.

Keywords:

Halide perovskites, in-situ, 4D STEM

Reference:

1. M. Keshavarz et al., Tracking Structural Phase Transitions in Lead-Halide Perovskites by Means of Thermal Expansion. Adv. Mater. 2019, 31, 1900521. https://doi.org/10.1002/adma.201900521

2. Erik Fransson, J. Magnus Rahm, Julia Wiktor, and Paul Erhart. Chemistry of Materials 2023 35 (19), 8229-8238. DOI: 10.1021/acs.chemmater.3c01740

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Depth sensitivity of atomic resolution secondary electron imaging

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Poster Group 2

Background incl. aims
Recent advances in spherical aberration-corrected electron microscopes have enabled us to observe not only the projected structure of a sample using transmission electrons but also the surface structure with atomic resolution using secondary electrons (SE) emitted from the sample [1,2,3]. Atom-resolved SE imaging of surface structures is one of the key challenginges. The first attempt was made by Ciston et al. for 2 x 6 reconstruction on the surface of SrTiO3 (001) [4]. This report showed that SE signal has a potential capability to detect surface structures uniquely formed only on the topmost surface of the material with a high spatial resolution. In this study, the depth resolution of the SE imaging is investigated using twisted layers of MoS2, which are the thinnest system composed of a surface layer and substrate. The spatial resolution and elemental contrast of the SE images are also evaluated.
Methods
Monolayer MoS2 was grown on SiO2 thin film formed on a silicon substrate by chemical vapor deposition (CVD). The monolayers were peeled off from the substrate and transferred to a carbon support film for TEM observation. Some of the MoS2 monolayers were stacked on the other monolayer MoS2 by rotating 30 deg. to fabricate twisted bilayer MoS2. An aberration-corrected scanning transmission electron microscope equipped with a cold field emission gun and Everhart-Thornley SE detector (Hitachi High-Tech HF-5000) was used to acquire atom-resolved SE and ADF-STEM images at an acceleration voltage of 200 kV. The convergence semi-angle of the incident beam was set to 23 mrad, and the acceptance angle of the ADF detector was set to 40-200 mrad.
Results
Figures 1(a) and 1(b) show ADF-STEM and SE images simultaneously taken from a region containing both monolayer (upper left) and bilayer (lower right) MoS2, respectively. The ADF-STEM image of the monolayer region shows Mo and S sites as bright spots with different intensity, or Z-squared contrast, confirming the projected structure of MoS2 [0001] incidence shown in Fig. 1(c). The SE image of the monolayer region is noisier than the ADF-STEM image, but shows signals at positions corresponding to the locations of Mo and S atoms, forming the six-membered rings of the MoS2 structure. Mo and S show a similar intensity, indicating that the observed signal is not due to the backscattered electrons but due to SEs emitted from the illuminated atoms and their surrounding regions. The spatial resolution of the ADF-STEM and SE images, which are estimated by the outermost peaks in the FFT patterns, are 0.1 nm, and 0.16 nm, respectively.
The ADF-STEM image of the bilayer region shows wheel-shaped atom clusters with 12-fold symmetry as indicated by the white circle in Fig. 1(a). The 12-fold wheel-shaped clusters are formed by stacking two MoS2 monolayers rotated by 30 deg. to each other. Figure 1(d) shows an atomic arrangement of the projection structure of the wheel-shaped cluster. The central bright dot of the wheel-shaped cluster corresponds to the superposed two Mo atoms and surrounding 12 dots correspond to 12 Mo atoms in the surface and second layers, 6 Mo atoms are in the surface layer and the other 6 Mo atoms are in the second layer. SE images of the bilayer region do not show the projection structure of

bilayer MoS2 but show the six-membered rings as observed in the SE image of the monolayer region. In other words, SE images selectively visualize only the surface monolayer. This result indicates that atomic-resolution SE imaging has an extremely high surface sensitivity and is very effective for the determination of the surface structure.
The SE image of the bilayer region is about 1.5 times brighter than the monolayer region. An FFT pattern of the SE image of the bilayer region shows 12 spots around the central spot, which are composed of 6 intense spots alternate with 6 weak spots. The FFT pattern can be understood as an overlap of two 6-fold patterns rotated by 30 degrees with different intensities. This confirms the fact that the SE image visualizes one of the monolayers more intensely than the other. The reason why the second layer is not visualized in SE imaging is considered to be because SEs emitted from the second layer and directed toward the detector are significantly attenuated as they pass through the surface layer.
Conclusion
In the present study, we found that high-resolution SE imaging selectively shows atomic arrangement of a surface monolayer, even though the monolayer is located on other materials. The present results indicate that high-resolution SE imaging has a high surface sensitivity of a monolayer level. We also confirmed that the transverse spatial resolution of the SE images does not differ significantly between the monolayer and bilayer MoS2, achieving at less than 0.2 nm. The thickness dependence of the SE yield should also be examined to clarify whether the SE production by energy transfer of excited states produced by primary electrons leads to the enhancement of the SE production beyond volume effects. A further study will be conducted on the formation mechanism of layer-number-contrast in SE imaging of atom layer materials.

Keywords:

atomic resolution secondary electron imaging

Reference:

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[3] H. Inada, et. al. (2011) Ultramicrosc. 111: 865.
[4] J. Ciston, et. al. (2015) Nat. Commun. 6: 7358.

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Unveiling Structural Heterogeneity and Imbalance of Gold Decahedral Nanoparticles using Four-dimensional Scanning Transmission Electron Microscopy

Oliver Lin[1], Dr. Zhiheng Lyu[1], Mr. Hsu-Chih Ni[1], Ms. Yetong Jia[2], Prof. Kejie Zhao[2], Prof. Jian-Min Zuo[1], Prof. Qian Chen[1]
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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background incl. aims
Multi-twinned structures have been observed in technologically important crystal systems, for example diamond cubic and face-centered cubic (FCC) lattices, that include materials such as diamond, silicon, a wide range of noble metals, and their nanoscale counterparts. Beyond atomic building blocks, the special arrangements also occur in the self-assembly of nanoparticles (NP) and µm-sized colloidal particles and occupy parts of their phase diagrams. Spanning a wide range of length scales, the universality of the structures arises when the systems attempt to achieve multi-twinned structures by overcoming geometric misfits during minimizing surface energies with entirely {111} or close-packing facets. While it is fundamental to understand how strain is sustained upon twinned structures and symmetry breaking, the knowledge will be paramount in practical aspects such as guiding and controlling the thin film growth, anisotropic NP growth, and self-assembly of NPs. Au decahedral (Dh) NP, as the most prevalent multi-twinned model system, fits five tetrahedral motifs into a circle by sharing an axis resulting in a geometric misfit angle of 7.35°, or a disclination with power of -7.35°. Postulating how Au FCC lattice adopts the misfit, theoretical models have been developed to address the underlying lattice symmetry and inhomogeneous strain distribution separately. Yet, experimental reports regarding the former have been limited due to the relatively large X-ray beam sizes that do not fit the sizes of NPs. On the other hand, though the latter has been widely adapted in the thermodynamics of small (<10 nm) multi-twinned nanoparticles, previous literature has shown that, at edge length of 17 nm, the theory's is invalidated by shear strain that is observed in a defect-free Au Dh NP by high-resolution transmission electron microscopy (HR-TEM) imaging. Though the advancement of aberration-corrected scanning transmission electron microscopy (AC-STEM) imaging and ab initio calculation techniques brings new opportunities, along with challenges in complicated image analysis and limitation in particle size (usually below 10 nm), the gap between nanoscale and mesoscale has never been extended to gain insight from atomic system with straightforward interaction potentials.
Methods
In this work, we performed strain mapping using four-dimensional STEM (4D-STEM) on Au Dh NPs with edge lengths between 18 and 50 nm at single particle level. In 4D-STEM, a nm-sized electron probe (at a convergent angle of 0.6 mrad) offers nm spatial resolution and decouples the complex diffraction patterns (DP) of the entire five-fold twinned structure into single-crystalline patterns corresponding to each tetrahedral grain. As such, NP's lattice plane directions can be easily identified and aligned with strain components. Diffraction disks were tracked by circular Hough transformation for sub-pixel accuracy. DPs are acquired on an Electron Microscope Pixel Array Detector (EMPAD) to reduce step size and acquisition time. Further integration of AC-STEM is utilized to confirm the absence of large-scale defects, such as additional twin boundaries and disclination, in the NPs and to focus on the geometric effects, excluding other known pathways of strain relaxation.
Results

The resulted strain mappings across various particle sizes exhibit values and distributions agree with previous Dh models: ~ 2% of tensile strain along {220} direction concentrated at particle edges. From reciprocal space, we also observed ~ ±0.5% of shear strain on two sides of twin boundaries. Other results, such as strain along {002} direction, also match with finite element analysis (FEA) simulation based on the closing of geometric misfit. Additionally, since the crystallography of local lattice is being tacked across a particle, it is possible to map out local symmetries of Dh NP at nm spatial resolution for the first time. We have uncovered the particles we analyzed are mostly in body-centered tetragonal symmetry, yet as particle size increases, lattices near twin boundaries shift towards FCC symmetry. Furthermore, with the help of the small step size only made possible with EMPAD, our mappings show structural heterogeneity among tetrahedral grains and among particles we surveyed. The detailed features lead us to study how imperfect Dh geometry, such as particle edges, tip truncations, etc., contributes to variations in strain values and distributions. With FEA simulation, we associate the heterogeneity with the shifting of disclination away from the center of a particle because of statistical fluctuation of energetics during the fast growth of multi-twinned particles.

Conclusion

Using 4D-STEM strain mapping with an advanced pixelated detector at high frame rate, we have unveiled new information of Au Dh NP at particle sizes that were experimentally inaccessible before. The diffraction-based technique provides richness in the crystallographic information of nanostructure at high spatial resolution as well as approaches to in-depth data analysis beyond atomic imaging. The results help experimentally bridge a variety of previous theoretical concepts, including lattice symmetry, disclination shifting, and heterogeneity associated with particle growth energetics. The understanding of imbalanced geometry has future implication of precise control over anisotropic NP shapes and additional over-growth or etching. Fundamentally, the extension of length scale shows novel insights into mechanisms that nature choses to accommodate the geometric misfit by simple atomic potentials. We believe it will inspire assemblies at different length scales that leverage complex interactions.

Keywords:

4D-STEM, multi-twinned nanostructure, strain mapping

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Investigating multiferroic phase change dynamics using in-situ electron counted spectrum imaging with synchronized holder control

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Poster Group 1

Improper ferroelectrics have strong potential for use in low power domain wall nano-electronic devices, as the formation and motion of conducting domain walls in such materials is governed by strain as opposed to their electric polarization [1,2]. Multimodal STEM spectrum imaging performed in the (scanning) transmission electron microscope (S)TEM is ideal for characterization of the ferroelectric domain dynamics in improper ferroelectrics, as the technique enables correlation of local chemistry and bonding information, with crystallographic and strain information determined from identical specimen regions at micro to (near) atomic scale.

The ferroic phase changes of the improper ferroelectric: Co-Cl, Cu-Cl and Fe-I based boracites ($M_3B_7O_{13}X$) are complex, with the materials undergoing multiple phase transitions from low temperature up to Tc. For example, Co-Cl boracite undergoes a trigonal to monoclinic transition at 296 K, monoclinic to orthorhombic transition at 500 K and orthorhombic to cubic transition at 673 K. No memory effect is apparent through the paraelectric to ferroelectric transition though memory effect from trigonal to monoclinic phases are observed [3].

MEMS based heating-biassing and cooling-biassing holders can be used to investigate phase change dynamics in these materials as a function of applied temperature and bias, though manual holder control becomes impractical if high stimuli resolution is required, due to the large number of temperature steps required for meaningful analysis in combination with the large number of individual biassing steps required at each temperature.

Here we present a holder automation strategy that takes advantage of the high-level communication Python library, ZMQ, to generalize external stimulus control within the DigitalMicrograph Python scripting framework. Control commands from the embedded in-situ SI data acquisition routine are sent via a generalized modular framework, enabling synchronized control of any external device supporting Python and ZMQ.

In-situ spectrum imaging was performed on Co-Cl, Cu-Cl and Fe-I based boracites ($M_3B_7O_{13}X$) with a 50-80 pA probe at 300 kV, using a counted mode EELS / energy filter system (GIF Continuum K3, Gatan) and flexible scan control system (Digiscan3). Domain wall dynamics were investigated as a function of applied bias at variable temperature using MEMS based in-situ heating-biassing, and cryogenic cooling-biassing holders (Lightning & Lightning-Arctic, DENSSolutions). Holder control and synchronization to data capture was performed using Python scripting in the DigitalMicrograph and DENSSolutions Impulse software packages. This scripting allowed multiple pass in-situ spectrum image (SI) data acquisition with all SI passes acquired at fixed holder stimuli conditions. Full voltage sweeps were applied at each temperature step in a temperature series. All data acquisition and holder control was fully automated . Data processing was performed using a combination of DigitalMicrograph (EELS, 4D STEM) and the Py4DSTEM (4D STEM) software packages.

Gatan's eaSI platform and DigitalMicrograph 3.60, allowed multipass single electron counted SI data capture with zero dead time between passes, at SI pixel rates of up to 3000 pix/s with continuous drift tracking in real time. A software screenshot showing the results of an in-situ energy-filtered 4D STEM data acquisition from Co-Cl boracite is shown in figure 1. Heating was applied in 10 ^{o}C steps from 270 ^{o}C – 340 ^{o}C. Biassing was applied at each temperature step in increments of 1 V from 0 V to Vmax = + 3.0 V and Vmin = -3.0 V and back to 0 V. The in-situ dataset comprises three primary data objects. An ADF image time series, a simultaneously acquired energy-filtered (EF) 4D STEM time series, and a holder auxiliary data plot. Measured temperature is shown in the auxiliary plot in pink. Voltage (holder bias) is shown in red. Both profiles are stepped, indicating SI passes are acquired at fixed holder stimuli conditions. A summed EF-CBED image from the highlighted region is also shown. In spite of the low probe current, the SNR of this image is high due to the high sensitivity of the counting detector. Two HOLZ rings are clearly visible.

Python scripting was successfully utilized to customize in-situ spectrum image capture, allowing synchronized control of a remote application using the ZMQ Python library. and a client-server design pattern. This design strategy was found to be a powerful means of leveraging the strengths of the native software application such as zero dead time multipass scanning, sub-pixel scanning, continuous drift tracking, etc. while also enabling holder control options not yet natively supported. In the case of Co-Cl, Cu-Cl and Fe-I based boracites ($M_3B_7O_{13}X$), this allows the setup of complex heating and biassing conditions at high temperature and bias resolution that would be unachievable if a manual approach was adopted. Such setups allow exploration of the FE memory affect at specific phase transitions in addition to complex analyses such as the correlation of strain mapping with local chemical and coordination environment changes at micro and macro domain walls in these materials.

Keywords:

ELNES, 4DSTEM, InSitu, Ferroelectric

Reference:

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[4] M.C. acknowledges funding from Royal Society Tata University Research Fellowship (URF\R1\201318), EPSRC NAME Programme Grant EP/V001914/1&Royal Society Enhancement Award.

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Maximizing Speed and Sensitivity: Simultaneous High-throughput EDS-WDS Elemental Mapping in the SEM

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Poster Group 1

Background incl. aims
High-throughput Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS) elemental mapping ensures the acquisition of multiple elemental maps simultaneously (hyperspectral mapping) within minimal timeframes. This process requires a high beam current on the SEM side to generate high input of X-ray counts, coupled with fast electronics of the Silicon Drift Detector (SDD) to operate at short pulse processing times, thereby efficiently converting incoming X-rays into output X-ray counts. However, shorter pulse processing times may lead to a low energy cut-off, as the noise level becomes comparable to that of the signal from low energy X-rays, presenting a challenge for high-throughput EDS mapping of light elements. While Wavelength Dispersive X-ray Spectroscopy (WDS) exhibits lower throughput than EDS at equivalent beam currents, its superior sensitivity and higher peak-to-background ratio enable better detection of light elements that may be difficult to observe using EDS alone. Therefore, simultaneous WDS mapping of light elements and high-throughput EDS mapping of higher-Z elements can facilitate rapid and accurate determination of element distribution for both light and higher-Z elements.
Methods
To demonstrate this capability, a boron steel sample was mapped using an EDAX Octane Elite Super EDS detector and an EDAX Lambda Plus WDS detector (Gatan inc.) in a Field Emission SEM. The beam current was set at 75 nA to achieve approximately 250,000 counts per second (cps) on the EDS detector. Capitalizing on advancements in SDD efficiency [1], a pulse processing time of 0.48 µs was used, resulting in an output of approximately 190,000 cps. An 80 Å diffracting crystal combined with polycapillary optics in the WDS detector were utilized to map B K. The region of interest was simultaneously mapped using EDS and WDS for a duration of 2 hours in the EDAX APEX 3.0 software (Gatan inc.). Drift corrections by the software were applied to compensate for sample drift induced by the high beam current.
Results
The SEM image (Figure 1a) unveils grains clusters segregated by relatively large melt-like pockets ranging from 5 to 25 µm in size. Orientation contrast highlights individual grains approximately 5-10 µm in size, with melt-like features as small as 1 µm developed at triple junctions. The resulting elemental maps are 512x400 pixels. The Co K EDS map (Figure 1b) illustrates a higher Co concentration within the grains in comparison to the melt-like pockets, while the Fe distribution depicted by EDS (Figure 1c) displays an inverse correlation with Co levels. Leveraging the superior sensitivity and high peak-to-background ratio of WDS, the WDS map of B K (Figure 1d) distinctly reveals a significantly higher concentration within the melt-like pockets and a depletion within the grains. However, due to the superfast pulse processing time utilized, the EDS map of B K (Figure 1e) fails to show the pronounced concentration contrast between melt-like pockets and grains.
Conclusion
In summary, simultaneous EDS-WDS mapping at a high beam current successfully delineated the element distribution for both the light element boron and the transition metals in a boron steel sample. By harnessing the benefits of both techniques, simultaneous high-throughput EDS-WDS

mapping enables the acquisition of high-quality X-ray map data within the shortest possible timeframe.

Keywords:

EDS-WDS, mapping, high-throughput, light elements

Reference:

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Atomic resolution observation of zeolitic framework and captured cations using low-dose OBF STEM technique

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Zeolite is a common porous material with a unique framework structure and periodically aligned nanosized pores. Zeolites have a wide range of industrial applications such as gas separation, catalysis, and ion exchange. In these applications, the material properties emerge from the interaction between the host zeolitic framework and guest materials, such as molecules or ions. However, zeolites are very weak to electron irradiation, and it is difficult to analyze this interaction at the atomic scale using electron microscopy.
Recently, we developed an optimum bright-field scanning transmission electron microscopy (OBF STEM) technique for low-dose observations [1]. OBF STEM uses a segmented-type STEM detector and can achieve a dose efficiency that is two orders of magnitude higher than that of annular bright field (ABF) STEM, a conventional phase-contrast-based STEM technique. Furthermore, the OBF STEM images can be acquired in real-time in sync with the probe scanning, which makes low-dose data collection much easier. It was shown that low-dose OBF STEM can visualize the atomic structure of zeolitic frameworks for both T (=Si or Al) and O sites, even in lattice defects such as twin interfaces. Furthermore, the Na-captured LTA-type sample, which is one of the most beam-sensitive zeolites, was observed using OBF STEM, which visualizes extra-framework Na sites with a lower occupancy of approximately 1/4 [2].
We then applied the low-dose OBF technique to the Cs-exchanged LTA-type zeolites. Cs exchange is a very important application for the removal of hazardous chemicals, and understanding how Cs species are captured inside this material is of great importance. The atomic structures of the Na- and Cs-captured LTA samples were investigated using high resolution TEM (HRTEM) in the literature [3]. However, because of the higher Al content inside the LTA framework (Si/Al=1), which decreases the electron illumination resistance, the attainable spatial resolution remains limited. In this study, we observed Cs-LTA zeolites using the OBF STEM technique with high spatial resolution (~1 Å) under low-dose conditions, with the aim of revealing the interactions between host zeolitic frameworks and captured guest cations.
We prepared Cs-exchanged LTA-type zeolite samples using the ion-exchange procedure of a commercially available Na-captured LTA zeolite sample with a CsCl aqueous solution. The Cs-LTA sample was gently crushed using an agate mortar and then dispersed onto a TEM grid with a carbon support film. We then observed the Cs-LTA sample using the OBF STEM technique with aberration-corrected STEM equipped with a segmented detector. To suppress the irradiation damage, the probe current was set to 0.16 pA, which is more than two orders of magnitude lower than the usual STEM observation conditions.
Figure 1(a) shows the OBF STEM image of the Na-LTA zeolite sample. The LTA-type has a cubic shape framework, and eight-membered rings (8MRs) can be observed along the [100] zone-axis. In Na-LTA-type zeolites, the Na cations captured inside the 8MRs split into four sites, with an occupancy of approximately 1/4 [4]. In addition to the clear visualization of the T and O atomic sites inside the LTA

framework, the OBF image shows a slight contrast around the center of 8MR, corresponding to the captured Na cations [2]. We then observed the Cs-captured sample from the [100] zone-axis using OBF STEM, as shown in Figure 1(b). The OBF image contrast at the center of the 8MRs changed into a distinct sharp one compared with the Na-LTA case, indicating that the captured Cs cations were rigidly confined into the LTA framework instead of splitting into multiple sites with low occupancy. This observation agrees well with the literature using HRTEM and density functional theory (DFT) calculations [3], showing the capabilities of OBF STEM to directly observe captured cation sites inside zeolites at the atomic scale.

We applied a low-dose OBF STEM technique to the Cs-exchanged LTA-type zeolite samples. The atomic structure was clearly visualized for not only the LTA framework structures but also the captured Cs sites, identifying the different behavior of the captured cations in comparison to the Na-LTA case. This result shows that the OBF STEM technique is promising for the atomic-scale analysis of zeolites, including extra-framework cations.

Keywords:

OBF-STEM, low-dose, zeolite, counter-cation

Reference:

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Full-field illumination ptychography with a structured electron beam

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background incl. aims
Coherent diffractive imaging (CDI) is a technique for reconstructing the complex wavefield from far-field diffraction pattern. CDI has advanced rapidly with the improvement of recent detectors and computing performances, and among them, ptychography has been attracting great interest. One of the key challenges in ptychography is accurate phase reconstruction for large structures. The diffraction intensity carrying the information of large structures is generally concentrated in the low scattering angle region and is often saturated beyond the dynamic range of the detectors. To overcome this obstacle, near-field ptychogrpahy [1][2], which utilizes the Fresnel diffraction pattern for the reconstruction, has been proposed in recent years. In this study, we propose a novel configuration for near-field ptychography with full-field illumination using a structured electron beam, aiming at the accurate and efficient observation of large structures.

Methods
The proposed method is configured on the conventional TEM setups. A series of in-line holograms observed in the Fresnel region below the specimen are obtained at different beam positions by scanning the illumination beam. Then, both the wavefield of the illumination beam and the complex transmission function of the specimen are reconstructed from the obtained holograms through a ptychographic procedure. This method does not require a spatial restriction on the specimen plane as a constraint, and instead introduces an irregular structure into the illumination beam that works as an alternative constraint. The structured beam is generated by using a film with random openings, placed in the condenser lens system above the specimen. The diameter of the incident beam is adjusted so that the entire field of view is always illuminated during the scanning. Full-field illumination prevents error accumulation when reconstructing a large field of view compared with conventional ptychographic reconstruction, which “concatenates” small patches of local structures. Full-field illumination provides uniform redundancy over the field of view since the entire field of view of each hologram is fully overlapped. These contribute to accurate and robust reconstruction of large structures. The use of random openings for beam structuring also suppresses coherence degradation of the illumination beam due to inelastic scattering, thereby improving the accuracy of phase determination.
The performance of the proposed method was first evaluated using simulated holograms. Then, its experimental feasibility was evaluated using MgO particles.

Results
Graphic 1a shows one of the in-line holograms obtained below the specimen. The wavefield of the illumination beam and transmission function of the specimen were reconstructed from the obtained holograms. The reconstructed amplitude (Graphic 1b) and phase (Graphic 1c) of the specimen transmission function show good agreement with the given structure. The phase reconstruction error

was 1/12,097 and 1/3,485 of the wavelength for the fine (20 nm) and large (400 nm) structures, respectively. Both are comparable to other accurate phase measurement methods [3].
Graphics 2a and 2b respectively show the amplitude and phase components of the transmission function reconstructed from the experimental holograms of MgO particles. The reconstructed phase component shows a periodic phase contour at the edge of the particle, and also shows a uniform phase in the constant thickness region of the particles with faces oriented toward the beam direction. These results are consistent with the particle shape, indicating the validity of the method.

Conclusion
In this study, we proposed a new configuration for near-field ptychography using full-field illumination with a structured electron beam. A simulation study demonstrated phase reconstruction with an accuracy of 1/3,485 of the wavelength, a performance comparable to other phase measurement methods. An experimental study using a MgO crystal also showed reasonable reconstruction consistent with the specimen structure. These results suggest that this method can be adopted for accurate and efficient observation of large structures.

Keywords:

Wavefield reconstruction, Structured illumination, Ptychography

Reference:

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Linking form and function: hyperspectral and nanostructural characterisation of photonic crystals in butterflies

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Structural colour is a phenomenon whereby colour results from underlying nanostructures. For example, green colouration in the butterflies Erora opisena and Parides sesostris is a result of light interference with photonic crystals, which arrange in a single Gyroid geometry. Formation of these complex geometries and evolution of the resulting optical signals is not fully understood.

We have developed an in vivo hyperspectral microscopy technique to detect and measure optical signals emitted from wing scales of living P. sesostris pupae as they develop. Our aim is to couple these in vivo hyperspectral data with ex situ high resolution electron microscopy (SEM/TEM) and 3-dimensional imaging (3-D FIB-SEM, TEM tomography) data in order to link photonic properties to underlying nanoscale geometries of developing gyroids, over time.

Optical signals resulting from the formation of Gyroid photonic crystals begin to be emitted from the wing scales of developing pupae approximately 13 days after pupation. At this stage, a small signal peaking at ~635 nm (red) can be observed and electron microscopy confirms the onset of gyroid formation within a cellular environment. Over the next few days, the emitted spectra then increase in reflectance and the peak shifts towards shorter wavelengths by approximately 10 nm. Electron microscopy of wing scales fixed 15 days after pupation show well defined gyroid nanostructures still within a cellular environment. On emergence at ~20 days after pupation, the adult butterfly wing scales are visibly green, with gyroid formation now complete and existent in a dry state.

Due to the complex nature and length scales of these developing gyroid structures, 3-D information at the nanoscale is required to better understand their formation and development. Both FIB-SEM and TEM tomography methods offer the potential to reveal 3-D information across the nano- and micron-length scales relevant to our geometric features of interest. Preliminary FIB-SEM data reveal that we can visualise gyroid structures within wing scales of developing pupae that have been prepared using heavy metal staining and resin embedding techniques. We explore the suitability of these methods to investigate gyroid geometries in developing wing scales at time points where key shifts in optical properties have been identified with hyperspectral imaging (e.g. 13-20 days after pupation).

This multi-modal approach allows us to move toward validating mathematical modelling of simulated changes in structural geometries and functional (photonic) properties against actual structural and spectral data from developing gyroids in butterfly wing scales, over time. Such knowledge will contribute to exciting developments in fields such as synthetic biology, biomimetics, and drug delivery applications.

Keywords:

Gyroid, bicontinous structures, FIB-SEM, tomography

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ORGANOIDS : UNVEILING INSIGHTS WITH VOLUME ELECTRON MICROSCOPY

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Poster Group 1

Background:
In recent years, the adoption of three-dimensional (3D) multicellular models, such as organoids, spheroids, and organs-on-chips, has surged in biological research due to their superior physiological relevance compared to traditional cell culture methods. These models, cultivated from stem cells or organ progenitor cells, emulate the intricate structure and functionality of real organs, providing researchers with invaluable insights into organ development, disease mechanisms, and drug responses within a controlled laboratory environment. They offer a versatile platform derived from various tissues, including the brain, liver, kidney, intestine, and pancreas, holding immense promise for advancing biomedical research, personalized medicine, and drug discovery endeavours.
Understanding the complex structure of these 3D multicellular models poses a significant challenge, especially when aiming for high resolution. However, recent advancements in electron microscopy (EM) have introduced techniques enabling comprehensive imaging of these samples. Volume electron microscopy (vEM) has emerged as a powerful tool for analysing their overall structure and potential variations corresponding to different states, offering nanoscale resolution imaging capabilities.
While cells and tissues have been extensively studied in two-dimensional (2D) settings, successful machine learning and deep learning solutions for detection and classification have been developed. However, transitioning to 3D complex structures introduces novel complexities. Developing effective deep learning models is hindered by the limited human capacity to annotate large volumes of 3D data accurately and reliably. Additionally, the availability of 3D algorithms significantly lags behind those for 2D, creating an annotation bottleneck that hampers progress and stifles the development of innovative 3D solutions.

Methods :
This study focuses on cancer cell organoids (HCT116) under different conditions, imaged using a Serial Block Face (SBF) Katana microtome within a Scanning Electron Microscope (SEM). The SBF-SEM technique involves a miniature ultra-microtome attached to the motorized stage of an SEM, where a diamond knife repeatedly removes thin layers from a sample block. After each section is removed, the exposed block surface is imaged with a back-scattered electron detector. This automated in-situ method enables the acquisition of a series of images throughout the depth of a large sample. From this stack of 2D images, 3D morphology can be reconstructed, and various organelles of interest can be segmented using developed deep learning models.

Results:

Our study demonstrates the suitability of SBF-SEM for imaging organoids, allowing for the imaging of large volume samples at a relatively fast acquisition speed. The field of view is comparable to Array Tomography (up to 3 mm) but surpasses that of Focus Ion Beam-SEM (less than 100 µm). With this technology, we successfully imaged the entire organoid within a reasonable timeframe, with sufficient resolution to segment cells, cellular compartments, and organelles of the organoid. Furthermore, we are utilizing manual annotation and deep learning on the image dataset to identify structural changes in cells based on the conditions of the organoid and to correlate with 3D imaging with others technics, like confocal microscopy.

Conclusion :
However, the utility of these models necessitates tools for imaging and analysing the large quantity of complex 3D images required for comprehensive analysis. Convolutional neural networks trained on this extensive dataset can precisely detect and quantify subcellular and multicellular features, including mitotic and apoptotic events, multicellular structures like rosettes, cells, and organelles. The integration of high-resolution 3D microscopy techniques with machine learning approaches enables quantitative descriptions of organoid morphogenesis correlated with phenotypic characterization, facilitating the deciphering of mechanisms involved in morphogenesis and human physiopathology, as well as responses to extreme conditions.

Keywords:

3D, Organoids, volumeEM, Deep learning

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Effect of the content of intermetallic compounds on creep in tin-based solders

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Poster Group 2

Background incl. aims

Sn-Ag-Cu (SAC) alloys are commonly used in lead-free solder joints in electronic interconnection applications, where joint reliability can be limited by creep. Sn-3Ag-0.5Cu wt.% (SAC305) has become widespread solder joints in thermal cycling. Sn-1Ag-0.5Cu wt.% (SAC105) is often selected for its superior drop impact reliability. SAC solder alloys have more than 95% of the β-Sn phase in a microstructure consisting of β-Sn dendrites and eutectic regions after solidification with Cu6Sn5 and Ag3Sn embedded within the β-Sn matrix. These solders demonstrate creep susceptibility even at room temperature (298 K is T/T_M ~ 0.6) and intermetallic compounds (IMCs) within the solder matrix play an important role in reliability. This study aims to explore the role of the IMC content to address how strain accumulates and how the microstructure evolves with respect to IMC content. This investigation is expected to promote the development of a new understanding concerning the mechanisms of microstructural deformation of solder alloys.

Methods

The creep behaviour of bulk solder samples with different compositions, e.g. high-purity tin (99.99% Sn), SAC105 and SAC305 is investigated under constant stress at room temperature (~298 K). The deformation mechanisms are investigated using electron backscatter diffraction (EBSD) and strain heterogeneity is identified by two-dimensional digital image correlation (2D-DIC).

Results

At the macroscopic lengthscale, the IMC content controls the appearance of heterogenous deformation, the size and the extent of the neck. At the microscopic scale, the deformation initiates in the β-Sn phases around IMCs for the IMC-containing samples subject to low strain rates in the secondary stage creep, resulting in local recrystallisation. Large recrystallised grains form and propagate into the β-Sn matrix at higher creep strain in the tertiary stage creep. An obstacle-controlled and thermal-activated dislocation creep is the dominant mechanism for these solder samples with different IMC contents.

Conclusion

Both macroscopic and microscopic heterogeneous deformation have been observed for large-scale Sn-based samples during uniaxial creep at room temperature and constant stress. The creep performance of the samples shows dependence on the IMC content for HP Sn, SAC105 and SAC305 samples. SAC105 samples have been observed to have the best resistance to creep at constant stress

and room temperature, and it therefore would be sensible to optimise the IMC content subject to various load conditions.

Keywords:

Microstructural evolution, Recrystallisation, EBSD, DIC

Reference:

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Investigation of electron-beam-induced charging in a dolomite needle using off-axis electron holography

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Poster Group 2

The phenomenon of electron-beam-induced charging of specimens in the transmission electron microscope (TEM) is attracting increasing attention, both for fundamental reasons and because it has a strong influence on image contrast and quality, in particular when studying insulating and (cryo-)biological specimens [1], in which it is responsible for the bee-swarm or Berriman effect [2-4]. Electron-beam-induced charging is generally believed to result from secondary electron emission from specimen surfaces as a result of primary electron bombardment. However, the underlying physics is not yet fully understood.

Recent developments in off-axis electron holography in TEM offer the possibility to directly map charge density distributions in materials in real space [5, 6] as a function of parameters such as dose (rate), applied voltage and specimen temperature.

Figure 1 shows a measurement of electron-beam-induced charging of a needle-shaped specimen recorded at 300 kV using off-axis electron holography in the TEM at an electron dose rate of approximately 0.9 e-/Å2/s. The needle contains an apex of insulating dolomite (CaMg(CO3)2) and a W base. These two regions are separated from each other by Pt, which was deposited in a focused ion beam scanning electron microscope. The phase contour map shows that the needle is charged at its apex. The contours suggest the presence of a dipole-like charge distribution along the length of the needle. Asymmetry in the phase contour map results primarily from the influence of the perturbed reference wave. There is neither a dose-rate nor a total dose dependence of charging in this needle (not shown).

Surprisingly, a decrease in the reconstructed amplitude is visible in the vacuum region around the apex region. Although this observation is suggestive of a time-dependent charging and discharging process, no double exposure effects are visible in the recorded holograms in the form of Moiré fringes. Secondary electron emission alone may not be sufficient to interpret this result. Coincidence measurements between secondary electrons and electron energy-loss spectra may help to determine the physical origin of secondary electron emission in such samples. Parameters that include the distance to surrounding regions of the specimen and microscope, the presence of surface contamination, the specimen temperature, the presence of residual gas in the TEM column and the voltage applied to the specimen should be varied systematically in such experiments, in order to understand their influence on electron-beam-induced charging and to unravel the underlying physics of charging during illumination by high-energy electrons (and photons) [7].

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Figure 1 Electron-beam-induced charging of a needle-shaped specimen recorded using off-axis electron holography. The needle contains dolomite (CaMg(CO3)2) in the apex region, a W base and Pt in between. The electron dose rate is 0.9 e-/Å2/s. Upper panel: Electron holographic phase contour map obtained from reconstructed phase images. The phase contour spacing is 2π radians. Lower panel: Amplitude image reconstructed from off-axis electron holograms. Each image is stitched together from results obtained from 5 aligned off-axis electron holograms.

Keywords:

Electron holography, specimen charging

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OpenECCI - A Streamlined Open-Source Workflow for Electron Channelling Contrast Imaging of Crystal Defects

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background
Quantitative analysis of crystal defects, such as dislocations, stacking faults, etc., has traditionally been exclusive to transmission electron microscopes (TEM) for decades [1]. This technique involves stereo-tilting of a crystal to the “two-beam” diffraction orientations with the assistance of transmission Kikuchi pattern projected on a phosphor screen or a camera. Recent years, Electron Channelling Contrast Imaging (ECCI) techniques, including accurate ECCI [2], controlled ECCI [3] and rotational ECCI [4], have gained increasing interest for quantitative defect analysis in bulk specimens. Unlike TEM, ECCI in SEM offers easier, less intrusive sample preparation, and fewer sample-prep induced artifacts. Additionally, ECCI enables examination of larger polished surfaces, yielding more statistically representative results. Nevertheless, several challenges hinder its widespread adoption:

- High spatial resolution accurate ECCI requires modified SEM column alignment for orientation control [2].
- Controlled ECCI requires proprietary software and additional hardware for precise orientation control [3, 5].
- There is a lack of reliable method for measuring and controlling the electron beam convergence angle inside SEM.
- Absence of a quantitative method for assessing sample stage tilt/rotation accuracy and repeatability for ECCI.
- For controlled ECCI, coordinating the identification of a feature between EBSD map and SEM image, and applying stereo-tilt without losing the feature of interest could be challenging.

Hence, our current work aims to address these issues by leveraging the open-source software community to develop a more accurate and user-friendly ECCI workflow.

Methods
In this study, we present a refined controlled ECCI workflow using electron channelling patterns with moderate spatial resolution for automated orientation calibration. Utilizing open-source packages such as EMsoft, Orix, and Kikuchipy, we developed an interactive Python package to streamline the ECCI workflow. This package accurately predicts the required sample stage tilt and rotation to achieve "two-beam" orientations based on Kikuchi pattern simulations. Additionally, SEM stage tilt/rotation accuracy and repeatability have been quantitatively assessed by comparing experimental electron channelling patterns with simulations. Furthermore, an automated routine for measuring the electron beam convergence angle has been tested based on OpenCV package. Lastly, we have developed a routine for correlating EBSD maps with SEM images (or specimen stage position) to facilitate stereo-tilt without losing the region of interest.

Results

Fig. 1 illustrates a brief summary of the workflow. Fig.1A shows a blended image of a EBSD map overlaid on the corresponding BSE backscattered electron image. The EBSD map is homographically transformed to correct the scan distortion induced by 70 deg stage tilt, so that all the feature positions on EBSD map and BSE image are well aligned. The interactive plot allows users to click on any point within the map to obtain SEM stage position and corresponding Euler angles from EBSD. The RKP reflected Kikuchi pattern from the point can also be simulated from the Euler angles, i.e. Fig. 1B, 1C. Fig. 1B is an overview Kikuchi pattern with relatively large angular range showing neighbouring poles and bands as a guiding map for stereo-tilt. Fig. 1C provides a zoomed-in region (blue box in Fig. 1B) for finding suitable "two-beam" orientations. By clicking on points within the pattern, users receive recommended stage rotation and tilt for orienting the point to the electron beam incident direction. Once a suitable orientation is achieved, ECC images can be acquired. For example, Fig. 1D and 1E depict ECC images acquired at 20kV, showing dislocations and stacking faults, respectively. Fig. 1D shows an array of dislocations piled up at a grain boundary. Most of the dislocations have dissociated into partial dislocations with Burgers vectors of ±1⁄2[10¯1]. Fig. 1E shows a few stacking faults on (1¯1 ¯1) close to a (11¯1) twin boundary. By analysing the beat pattern (oscillating intensity) across the stacking fault. The Effective extinction distance can be estimated ξ_g^eff = 13.7 nm. The total visibility depth of ECCI contrast at 20kV is approximately 80nm.

Conclusion

The OpenECCI package and the developed workflow offer a reliable and convenient method for ECCI experiments. Notable achievements include:

- With the stage accuracy and repeatability assessment routine, it has been demonstrated that many native SEM stages are accurate enough for ECCI without high precision substages.
- Providing reliable guidance on setting ECC imaging conditions through the measurement of electron beam convergence angle
- Streamlining the workflow for ECCI acquisition and data post-analysis through an interactive software interface that correlates EBSD maps, SEM images, and Kikuchi pattern simulations.
- OpenECCI is freely accessible to all researchers. Facilitated by its reliance on open-source packages, it could democratize the access to ECCI technique.

Acknowledgements

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Keywords:

EBSD, ECCI, diffraction, defect-analysis, open-source

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Mapping electric fields in real nanodevices by operando electron holography

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background
The development of nanometer-scaled electronic devices with reduced dimensions, involving new materials or new architectures such as Magnetic Random Access Memories (MRAM), memristors or Phase Change Memories (PCM)1, requires a deeper understanding of their operating properties. While electrical and physical characterizations are widely used to monitor and evaluate both the device performance and the quality of the layer stack, there is a lack of knowledge on how the electromagnetic fields are precisely mediated along devices at the nanoscale level. Correlating electric fields mapped at the nanoscale across a chosen device with its structural properties and chemical composition would give new insights on the local electrical properties such as resistivity, polarisation, and charge traps.
Off-axis Electron Holography (EH) is an interferometric technique that allows quantitative mapping of electrical potentials inside and around the specimen, as well as the measurement of charge distributions2. However, operando EH has been rarely used, in particular on real devices3,4, because of key issues such as specimen preparation, surface damage layers, stray field and electron radiation. We will present the methodology that we have been developing for mapping the electrical properties of nano-electronic devices extracted directly from production lines without using a probe-based approach. Taking the example of phase change memory cell, we will show how electron holography can be used to measure in situ the resistivity distribution within the active layer after changing its state.

Methods
Phase-change materials exhibit a huge change of electrical resistivity between the amorphous (high resistance) and crystalline phase (low resistance). The electrical resistivity encodes the state of the bit, denoted SET for the crystalline state (low resistance) and RESET for the amorphous state (high resistance). To read the device, the resistance is measured by biasing the top and bottom electrode connected to a thin metallic filament called the “heater”. To write, a high-amplitude current pulse is injected, whose exact form depends on whether the operation is to stabilize the crystalline phase (SET) or the amorphous state (RESET). Current passing through the heater and GST layer causes localised Joule heating; the associated rise in temperature in turn causes the phase change. To SET, a relatively long pulse allows gradual crystallisation of the amorphous state whilst for RESET, a short pulse induces rapid melt-quench of the crystalline to amorphous state.
In situ biasing TEM experiments necessitate a specific and complex sample preparation that minimizes preparation artifacts whilst ensuring the electrical functionality of the nanodevice itself. The specimen-device was extracted from production lines before being thinned by focused ion-beam (FIB) and contacted to a chip with predefined electrical contacts, similarly to our previous work on electrostatic fields.5 An important part of the preparation was to preserve the encapsulation layer of insulating material all around the heater and GST layer. Special care was also taken to avoid any electrical discharges which would destroy the device. Holograms were recorded during long exposure

time using dynamical automation for compensating instabilities under bias, and while measuring the total current. Experimental phase images were then compared to additional numerical simulations using finite element modelling (FEM) including factors such as specimen geometry, stray fields and local resistivity.

Results

The GST layer within the device is initially crystalline (Initial state) and holograms were first recorded under a DC bias as if the memory was to be read. The layer was then switched to an amorphous state (Final state) by sending a sequence of pulses. Remarkably, the thin lamella survived the injection of peak currents of several hundreds of µA during the pulse. The overall resistance, measured under a biasing of 0.5 V during the holography experiments, changed radically between the initial and final states, increasing 5-fold after switching.
The corresponding phase images in initial and final states are presented in Figure b and e, respectively. We can clearly see the difference of the potential distribution between both states, with a dome-like shape in spanning the GST layer in the final state after amorphisation. The electrical information, in the form of isophase contours, has also been superimposed onto the conventional TEM images (Figure a and d) to help visualise the region involved. We can see, notably, that the phase contours seem to radiate outwards from the tip of the heater into the GST layer.
We obtained a very good agreement between experimental and simulated phase images using finite element modeling (Figure b and Figure e). This agreement gives access to the distribution of the electric potential within the device, and therefore to the local resistivity with the Ohm's law. From the reasonable assumption that the conductivity is radially symmetric around the heater tip and using the experimentally measured total current flowing through the device in situ, we determined the local map of the conductivity. A small seed of highly resistive material around the heater tip was revealed in the initial crystalline state and for the final state, the crystalline to amorphous transformation occurred at a certain radius, about 10 nm, from the heater tip.

Figure. Conventional bright-field TEM image, Initial state (a), Final state (d) with isophase contours (values on the right) extracted from experimental phase maps, Initial (b), Final (e). Simulated phase maps, Initial (c), Final (f). In dotted line the dome-like shape where the phase change occurs. Scale bars are 20 nm.

Conclusion
We developed a new methodology to map the local resistivity across the active area of an individual device in operation. This method, based on operando electron holography, has been applied on a PCM cell and directly highlights unexpected features. On switching the device by electrical pulses, we demonstrated that electrical resistance is inhomogeneous near the heater. This study shows the power of our methodology for studying the local electrical properties of active devices and can be applied in a general way.

Keywords:

Phase change materials, in situ

Reference:

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Detection limits of electric field characterization at a p-n junction by 4D-STEM

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

The emergence of fast pixelated direct electron detectors over the last years is allowing experiments in four-dimensional scanning transmission electron microscopy (4D-STEM) that would previously be too slow or noisy to be feasible, for example enabling the assessment of internal electric fields with high spatial resolution and precision[1–4] However, the electric field strength of long range built-in electric fields present in semiconductor devices, for example those associated with p-n junctions, is typically three orders of magnitude smaller than atomic electric fields, making 4D-STEM experiments in such systems challenging. It is therefore interesting to assess the detection limit and signal to noise that can be expected depending on the TEM operation conditions.

In this presentation, we describe how the quantification, sensitivity and spatial resolution of electric field mapping in a silicon p-n junction are influenced by the acquisition parameters in a momentum-resolved 4D-STEM experiment [3,5] comparing two different TEM setups differing in brand and in age by 10 years: the first setup, a TFS Titan Ultimate, was installed in 2012; the second, a Jeol Neo Arm in 2022. Both instruments provide 4D STEM capabilities using a Merlin camera based on medipix technology from Quantum Detectors. We have used the center-of-mass approach to analyze the diffraction patterns, obtained on a focused ion beam prepared silicon sample containing a p-n junction.

It will be shown that the success of the quantitative measurements depends not only on the correct estimation of the dead layer, but also on the acquisition conditions, most importantly the electron beam convergence angle.

The detection limit, defined as the three-sigma standard deviation of the field value away from the field containing region, was evaluated both as a function of beam semi-convergence angle as well as TEM setup. It was observed that the electric field precision improves by decreasing the semi-convergence angle [5]. For example, at a convergence angle of about 200 µrad, the detection limit was as good as 0.01 MV/cm, and it can even be lowered to 0.007 MV/cm in the more recent TEM setup. While such a low convergence angle setting gives the best detection limit and signal to noise ratio of the electric field (SNR 10 and 20, respectively), the increased probe size (estimated around 10 nm) can lead to an underestimation of the electric field value if the field containing region is of similar size as the electron probe. This was the case in the sample under study, where the field containing region was about 50 nm wide. Increasing the convergence angle to about 1 mrad avoids the underestimation of the field since the probe size is smaller (3-4 nm), at the expense of an increased detection limit. With about 1 mrad convergence angle a precision of 0.087 and 0.030 MV/cm with SNR 1.8 and 4.7 were achieved, respectively. As an example, maps of the modulus of the electric field are shown in Fig. 1 together with electric field profiles integrating either over 12 or 90 nm at three different convergence angles, where the Jeol Neo Arm is operated in Lorentz STEM mode.

The effect of electron dose is an important parameter, in particular in relation to beam sensitive materials. The influence of dose was evaluated and invariable electric field profiles were obtained using an electron dose as low as 24 e-/Å. Of course, the precision of the measurement does depend on dose. It was shown that for an average electron count of about 25 -75 counts per px, the detection limit does not further decrease for increased exposure times. Above this number of counts, the detection limit seems no longer limited by statistical Poisson noise on the detector, but by experimental instabilities, such as fluctuations on the high tension or lens currents or irregularities on the sample such as surface roughness.
In addition, in-situ electrical biasing coupled to momentum-resolved 4D-STEM measurements were performed, investigating the study of the degree of ideality of the junction abruptness, allowing the detection of phenomena like dopant segregation or interdiffusion [4].

This work paves the way for the development of advanced STEM based techniques able to provide imaging and quantification of built-in electric fields, potentials and charge densities in semiconductor devices with high spatial resolution, providing crucial feedback to improve growth/device fabrication processes.

Figure 1: 4D STEM maps at different convergence angle: The local modulus of the electric field is represented using three different Lorentz STEM modes of the Jeol Neo Arm, with three different convergence angles. Profiles are made along 12 nm, top row, and along 90 nm, bottom row.

Acknowledgements: This project received funding from the European Research Council under the European Union's H2020 Research and Innovation programme via the e-See project (Grant No. 758385). Experiments have been performed at the Nanocharacterisation platform PFNC in Minatec, Grenoble as well as at TEM facility JEOL NEOARM co-financed by the European Union under the European Regional Development Fund (ERDF, contract n° RA0023813).

Keywords:

4D-STEM, electric field characterization, biasing

Reference:

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Studying interface charge distribution in HfO2 and Al2O3 based nanocapacitors by operando electron holography

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Background
Among dielectric materials, HfO2 and Al2O3 are widely studied for micro and nanoelectronic applications. They present very interesting properties for several types of devices such as a higher dielectric constant compared to SiO2, high thermal stability and a compatibility with complementary metal oxide semiconductor (CMOS) technologies. For instance, resistive random-access memory (RRAM) devices [1, 2] or memristor devices [3] containing high-κ HfO2 and Al2O3 dielectrics are promising candidates in neuromorphic computing. However, the exact location of the charge stored in such devices is subject of debate and hence the mechanisms for charge trapping remain unclear. Particularly, the influence of interface charges has rarely been addressed because of the lack of suitable characterization techniques.
Whilst dielectric properties can be readily measured macroscopically using electrical methods, the task is difficult at the nanoscale. What is missing is a local and direct measurement of the electrical potential distribution within such devices upon biasing. Indeed, the phase of the electron hologram can be directly related to the electrostatic potential encountered by the fast electron along its trajectory. Recently, we were able to perform operando electron holography experiments in the same region under different biases and to successfully map the electric potential across a working SiO2-based MOS nanocapacitor with unprecedented sensitivity [4]. We observed an unexpected charging of the dielectric at both interfaces with the electrodes, over a distance extending up to few nanometers, a value much larger than the structural or chemical width of each interface [4]. In this study, we aim to take one step further to quantify the trapped charge at several interfaces, including insulator-insulator interfaces and metal-insulator interfaces, for which we have designed two thin tri-layer nanocapacitors: TiN/HfO2/Al2O3/HfO2/TiN (T-1) and TiN/Al2O3/HfO2/Al2O3/TiN (T-2) as shown in Fig. 1(a) & (b).

Methods
The tri-layer nanocapacitors were fabricated by atomic layer deposition (ALD). Each HfO2 or Al2O3 layer has a nominal thickness of 20 nm. TiN layers were deposited by sputtering before and after the insulating layer to serve as top and bottom electrodes during electrical biasing. In situ biasing TEM experiments necessitate a specific and complex sample preparation that minimizes preparation artifacts whilst ensuring the electrical functionality of the specimen itself. Specimens were prepared by FIB (FEI Helios 600i), on a Hummingbird chip-based biasing holder. Holography experiments were caried out using a Hitachi HF-3300 (I2TEM) microscope operating at 300 kV in Lorentz mode enabling a large field of view with a spatial resolution of 0.5 nm. Holograms were acquired during long exposure time using dynamical automation for compensating instabilities under different applied bias [5]. Finite element method (FEM) modelling was performed using COMSOL software to interpret

the phase profiles quantitatively, including factors such as specimen geometry, preparation artefacts and stray fields around the sample device.

Results

Figures (a) and (b) show the studied regions for T-1 and T-2, respectively while the corresponding phase maps extracted from holograms acquired under a bias of 6V are given in Figure (b) and (d), respectively. The phase map has been corrected by the reference hologram at 0 V, by which the experimental artifacts were removed and the remaining signal can be attributed to the applied electrical biasing. The artifacts comprise the possible variable lamella thickness, damage layers, diffraction contrast, and electron-beam-induced charging.
The amplitude of the measured phase profiles across the tri-layer insulators (Figures (e) and (f)) extracted from phase images rise with increased bias, and appear symmetric when switching the sign of the bias, for example, 6 V and -6 V. However, the potential distributions as well as the measured electrical fields are quite different from those expected theoretically. Considering a relative permittivity of 7.4 for Al2O3 and 18 for HfO2, both measured by C-V tests at 100 kHz for their macro devices, the electric field should be 2.44 times higher in Al2O3 than HfO2, which consequently should result in a 2.44 times higher potential drop in Al2O3. In contrast, the experimental phase profiles highlight a homogenous electric field in all the layers for both T-1 and T-2 due to the appearance of charged layers at insulator-insulator interfaces as well as metal-insulator interfaces. Combined with extensive FEM simulations, we successfully quantified the charge densities of each interface for both capacitors as a function of the applied bias responsible of the homogenous electric field. These charges densities linearly vary with the applied bias and present a thickness between 3 and 5 nm width. Scanning TEM electron energy loss spectroscopy (STEM-EELS) measurements have been carried out to understand the origin of these charged interfaces.

Figure. (a) Device structure for T-1; (b) phase map of projected electric potential obtained by electron holography for T-2; (c) Device structure of T-2; (d) phase map of T-2. Here, (b) and (d) correspond to the same regions as (a) and (c) for T-1 and T-2, respectively. Scale bars are 20 nm in (a) and (c). (e) and (f) are the phase profiles extracted from the green arrows as a function of applied biasing for T-1 and T-2, respectively.

Conclusion
We used operando electron holography to evidence and quantify charges occurring at the different interfaces in tri-layer HfO2/Al2O3/HfO2 and Al2O3/HfO2/Al2O3 nanocapacitors with TiN electrodes. An equivalent uniform electric field distribution throughout the whole dielectric stack in HfO2 and Al2O3 is observed even though HfO2 has a much larger dielectric permittivity than Al2O3. The electric field distribution originates from the presence of charges trapped at each interface. It is revealed that there is a linear relationship between the surface charge density and the applied bias, and, at each bias, the interfaces between HfO2 and Al2O3 have an equivalent trapped charge density. With unprecedented sensitivity and spatial resolution, the information gained with operando electron holography on the location of the trapped charges and on their densities provide unique insights on the functioning of nanocapacitors. Further work could be devoted to the interface charging behaviour in ferroelectric-dielectric devices.

Keywords:

Electric field mapping, electron holography

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Synthesis, Characterization, and Biological Evolution Indole Molecules

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Poster Group 2

Aims: Lung cancer is the most prevalent and mortal cancer in the world. The percentage of 85 of lung cancer incidence belongs to non-small cell lung cancer type (NSCLC). Therefore, it is crucial to develop reliable and efficient curing approach and therapeutics to treat this cancer type in order to suggest life prolonging and pain ameliorative option to the sufferers [1]. Heterocyclic compounds are within the carbon ring structure; contain hetero atoms such as Sulfur, Nitrogen, Oxygen, Selenium and Silicon into the ring. Hetero aromatic compounds form the basic structure of many drug molecules. Nitrogen-based heterocyclic compounds constitute an important and unique class among the application areas of chemistry and organic chemistry. Nitrogen-containing hetero aromatic molecules have contributed to the development of many organic synthesis protocols and different application areas in the last two decades. N-heterocyclic compounds found in nature, in addition to having physiological and pharmacological properties, constitute the basic building blocks of many biologically important molecules, including many vitamins, nucleic acids, pharmaceutical products, antibiotics, dyes and pesticides. In addition, N-heterocyclic compounds form an integral part of many pharmacologically active molecules, base pairs of DNA and RNA (guanine, cytosine, adenine and thymine) as well as purines, pyrimidines, etc. It consists of N-heterocyclic compounds such as. These nitrogen-containing heterocycle molecules with diverse properties and applications have gained importance in the field. Furthermore, electron-rich Nitrogen-containing heterocycle-containing organic molecules not only readily accept or donate a proton, but also have a variety of weak bond interactions; It can also easily establish some of the intermolecular forces such as hydrogen bond formation, dipole-dipole interactions, hydrophobic effects, van der Waals forces and π-stacking interactions of Nitrogen organic compounds [2-3].
Methods: In this work, New indole compounds were designed, which is crucial for synthetic organic chemistry and medicinal chemistry. Novel indole derivatives with NO2 have a great deal of potential for biological activity. Many pharmaceutical compounds with indole in them are on the market today. Thus, the synthesis and characterization of indole derivatives are critical to the identification of novel therapeutic compounds. The biological activity of the new synthesis molecules obtained in this study was obtained on lung cancer cells (A549) by MTT cytotoxicity test.
Results and Conclusions: The IC50 values obtained as a result of the Mtt test were found to be 4.62µm in the molecule coded EH-303 for 24 hours at the lowest concentration, while the lowest value for 48 hours was 8.17µm in the molecule coded EH-300.

Keywords:

Lung cancer, compounds, organic synthesis,

Reference:

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Texture analysis of radiation sensitive organic films: Comparative study by electron diffraction tomography and GIWAXS

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Poster Group 2

Background incl. aims
Texture describes the preferred orientation of grains in crystalline materials. For characterization of texture, the reciprocal space is conventionally investigated in diffraction experiments using electrons, X-rays or neutrons as probes. In polycrystalline thin films texture is an important characteristic of the microstructure determining anisotropic properties like, e.g., charge carrier transport in bulk heterojunction (BHJ) organic solar cells (OSC). Gracing incidence wide angle X-ray scattering (GIWAXS) is a well-established method for texture analysis of OSCs, however, real space information is only indirectly accessible as averaging measure (e.g. grain size or coherence length). In contrast, electrons as probe in transmission electron microscopes (TEM) provide direct access to both real and reciprocal space while additionally delivering analytical signals for local chemical analysis. However, due to the challenge of radiation damage, electron diffraction is not well explored for OSCs. In this work we show that electron diffraction tomography (EDT) can be used to investigate the reciprocal space of polycrystalline thin films in 3D, providing information on texture comparable to results obtained with GIWAXS.
Methods
We chose two radiation sensitive BHJ OSC active layers as model sample systems. Both OSCs contain $PC_{71}BM$ as fullerene acceptor, but differ in that the donor component is a small molecule (DRCN5T) in one case and the classical polymer P3HT in the other. Large-area plan-view samples of the active layer were prepared by floating off the spin-coated film from the substrate and transferring it to a TEM grid. Real space information on the nanomorphology of the donor and acceptor phases was obtained using STEM-EELS or EFTEM as exemplary shown for the DRCN5T/$PC_{71}BM$ system in Fig. 1a. For EDT we acquired tilt series of zero-loss energy filtered SAED patterns across a tilt range of ±70° using a single-axis tomography holder (Fig. 1b). Energy filtering is necessary to suppress inelastic scattering background in particular at small scattering angles, which is critical to reveal crystallographic information on the small molecule/polymer crystallites with their nm-sized unit cells. To minimize effects of radiation damage, each diffraction pattern was acquired at a fresh sample area with electron dose well below the evaluated critical dose. From the SAED tilt series the 3D diffraction space was reconstructed with a home-developed code for 3D reciprocal space filling which is based on polar transformations (Fig. 1c). Furthermore, azimuthal averaging of the volume around qz axis was performed to obtain qr-qz reciprocal space maps for direct comparison with GIWAXS data.
Results

The qr-qz reciprocal space maps derived from EDT and GIWAXS are exemplarily depicted side-by-side in Fig. 1d for the DRCN5T/$PC_{71}BM$ film. They show excellent agreement, indicating that not only GIWAXS but also EDT is perfectly suited for texture analysis of such radiation sensitive organic films. Moreover, direct comparison of different line profiles extracted from the reciprocal space maps reveal even quantitative matching of EDT and GIWAXS data. Interestingly, the EDT data show a better signal-to-noise ratio, in particular regarding the in-plane reciprocal information, which is difficult to access with GIWAXS. From the reciprocal space maps, it can be seen that the π-stacking of the small molecules in the DRCN5T crystallites exhibit preferred edge-on and face-on orientation in the fibre like nanomorphology revealed by STEM-EELS. Quantitative evaluation of the diffraction peak characteristics yields comparable structural information in terms of crystal coherence length, mosaicity, etc..

Conclusion

In summary, we demonstrate that the texture of radiation sensitive OSC thin films can be investigated using EDT yielding results in quantitative agreement with GIWAXS. Combining EDT with real space characterization using high-resolution and analytical TEM methods provides great opportunities for a more comprehensive and in-depth analysis of OSC thin films. Furthermore, the application of the presented methodology to state-of-the-art, non-fullerene OSC systems consisting of PM6:Y6 is in progress and will be covered in the conference contribution.

Keywords:

Thin-film texture, EDT, OSC, GIWAXS

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Two-dimensional few-atom noble gas clusters in a graphene sandwich

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims
Van der Waals atomic solids of noble gases on metals at cryogenic temperatures were the first experimental examples of two-dimensional systems [1]. Recently, such structures have also been created on surfaces under encapsulation by graphene, allowing studies at elevated temperatures through scanning tunneling microscopy [2,3]. However, for this technique, the encapsulation layer often obscures the arrangement of the noble gas atoms. When atoms are introduced into the van der Waals gap between the layers of suspended multilayer graphene the resulting structures can be directly observed at room temperature via transmission electron microscopy [4].
Methods
In this work low-energy ion irradiation has been used to irradiate suspended bilayers and double layers of graphene and hBN. Implantation parameters for noble gases from Ar to Xe were experimentally determined. Trapping occurred for Ar between 20 and 30 eV, for Kr between 25 and 60 eV, and for Xe between 15 and 65 eV. Irradiation can be carried out within the ultra-high vacuum system directly connected to a scanning transmission electron microscope [5] where the samples can be transported to a Nion UltraSTEM 100 microscope, capable of atomic-scale imaging and electron energy loss spectroscopy, without exposure to air.
Results
The atomic structure of small Kr and Xe clusters was studied via aberration-corrected scanning transmission electron microscopy [4]. We show that small crystals ($N < 9$) arrange on the basis of the simple non-directional van der Waals interaction (Fig. 1). Larger crystals show some deviations, possibly enabled by deformations in the encapsulating graphene lattice. We further discuss the dynamics of the clusters within the graphene sandwich and show that although all the Xe clusters with up to $N \approx 100$ remain solid, Kr clusters with already $N \approx 16$ turn occasionally fluid under our experimental conditions (under a pressure of ~0.3 GPa).
The figure shows the structure of small clusters. Filtered STEM-ADF images of Kr (top row) and Xe (bottom row) including all the cases where the experimentally observed structures correspond to that with the lowest energy according to the simulations for Kr (middle row) are shown.
The encapsulated clusters show dynamic behavior such as "jumps" between different positions and occasional cluster growth and shrinking. Molecular dynamics simulations were used to understand this behavior. The trapped clusters also exhibit interesting phase behavior. While all small clusters remain solid, larger clusters exhibit either solid- or liquid-like structures depending on their size, chemical element and local microscopic environment.
Conclusion
We image noble gas atoms, their cluster formation, stability and phase in two dimensions. Due to their chemical inertness, they would not condense under normal conditions, however, when trapped between two graphene sheets the atoms are forced together by the external pressure that leads to the formation of clusters [4]. This results in a simple two-dimensional van der Waals solid that can be described to a good degree simply with the Lennard Jones potential. This opens a way for the

unexplored frontier of encapsulated two-dimensional van der Waals solids with exciting possibilities for fundamental condensed-matter physics research.

Keywords:

graphene, hBN, noble gas, irradiation

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Design of a momentum scanning electron microscope

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Poster Group 1

Background
As nanostructured materials become more sophisticated, there is an urgent need to develop experimental methods that can map electronic band structure information on the nanoscale. The techniques that are widely used today, such as angle-resolved photon emission spectroscopy (ARPES), typically operates with a spatial resolution in the hundreds of micron range. State-of-the-art instrumentation at synchrotron radiation facilities have been developed for the purpose of improving the spatial resolution performance, such as the ARPES photoemission electron microscope (PEEM), also known as the momentum microscope, however, its band structure information still lies in the micron range. This paper presents a proposal to transform the scanning electron microscope (SEM) into a momentum microscope capable of capturing band structure information on the nanoscale. It sets out to achieve this by designing a spectrometer attachment that can capture both the energy and momentum distributions of the scattered secondary electrons (SEs) that leave the sample inside the SEM specimen chamber. SEMs at present, in their normal imaging mode of operation, function by forming a single bulk signal from the SEs that leave the specimen, and discard any information that may be contained in their energy and momentum distributions. The recent success however of acquiring accurate valence band DOS distribution information inside a commercial SEM by using a secondary electron energy spectrometer (SEES) attachment demonstrates that there is a considerable amount of under-utilized quantum state information embedded into the SE energy spectra [1]. This suggests that a further step of creating an angle-resolved SEES attachment to acquire band structure information can in principle be taken, where the SE emission angular distribution captured by a 2D image detector placed after the energy spectrometer can be used to provide wave vector information, in addition to SE energy spectra, in a similar manner to the ARPES-PEEM momentum microscope.
Methods
A direct tracing simulation method of plotting electron trajectory paths through numerically solved field distributions will be used in order to design the angle-resolved SEES energy/momentum spectrometer attachment. The simulation will investigate how best to combine the SEM's primary beam optics with the SE energy/momentum dispersion optics. The main aim will be to find viable energy/momentum spectrometer designs that can maximize the predicted image resolution, while at the same time maximizing the energy and wave vector resolutions for the band structure. The simulation will need to incorporate a viable means of acceleration of the SEs together with momentum dispersion, since 2D electron image detectors usually function by being biased to voltages of 10 kV or higher.
Results
Preliminary simulations for two different compact electric sector SE energy spectrometer designs have been made, one is a 40° deflection first-order focusing geometry, while the other is a toroidal 225° second-order focusing design. Viable acceleration/momentum dispersition unit designs in both cases incorporate two intermediate focal points before the SEs reach a 10 kV image detector, where the electrode voltages inside the acceleration unit need to change as a function of the energy

spectrometer pass energy. The predicted geometrical aberrations at the detector plane were found to be dominated by the SE energy spectrometer's exit geometric aberrations. In the case of the 40° deflection spectrometer design, the relationship between initial polar angle and detector position is predicted to be non-linear, while for the 225° toroidal design, it is predicted to be linear. However, the 40° deflection spectrometer unit design has the advantage of being simpler and smaller than the 225°toroidal spectrometer, allowing for a working distance of less than 10 mm. Further simulation details as well as other viable designs will be presented at the conference.

Conclusion

Preliminary electron optics simulation results predict that there are viable electron energy/momentum spectrometer attachment designs capable of enabling a SEM in principle to map band structure information on the nanoscale. Further considerations beyond electron optical design will be presented at the conference, such as vacuum level and signal to noise requirements.

Keywords:

Scanning electron microscopy, energy spectroscopy

Reference:

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Triggering and tracking grain boundary phase transformation at atomic resolution

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background incl. aims
Phase diagrams and crystallographic defects are essential pillars for modern materials design. In recent years, increasing numbers of defect phases (also known as “complexions”) have been identified, and there are evidences on their phase transformations. Nevertheless, due to the vast variety of defects (for example, grain boundaries have five degrees of freedom), it remains challenging to study their thermodynamics in a systematic way, eventually requiring the construction of defect phase diagrams [1].
Methods
We developed a quasi in situ approach to trigger phase transformations of a single grain boundary. The same defect was monitored by atomic-resolution scanning transmission electron microscopy during various steps of triggers. We also developed automatic pattern recognition to distinguish different grain boundary structural units, and applied ab initio simulations to understand their thermodynamics.
Results
We demonstrate the phase transformation of a Mg grain boundary triggered by local Ga alloying using focused ion beam, as well as the structural evolution with time. As shown in Fig. 1, successive steps of Ga incorporation lead to phase transformations of a Mg [0001] tilt grain boundary [2, 3]. There are two aspects of the phase transformations: 1. Structural transformation from T-type (highlighted in green in Fig. 1a) to A-type (highlighted in red in Fig. 1b) structural units; 2. Formation of chemically ordered grain boundary phases as shown in Fig. 1c, d.
Conclusion
The discovered grain boundary phases and their transformations observed quasi in situ enabled us to construct a phase diagram for this grain boundary. Our developed methodology including atomic resolution imaging, automatic pattern recognition, and ab inito simulations formulates a blueprint to develop defect phase diagrams systematically and propel a new paradigm for materials design.

Keywords:

Grain boundary complexion, phase transformation

Reference:

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Ex situ observation of ferroelectric domain evolution in wurtzite-type AlScN thin films

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Poster Group 1

Background
Since the discovery of ferroelectricity in solid solutions of AlxSc1-xN in 2019, the new class wurtzite-(W)-type ferroelectrics have raised huge expectations for the introduction of ferroelectric functionality into novel transistor structures, e.g., with integrated memory. Although most of the challenges targeting the materials integrability, e.g., temperature stability, thickness scaling and epitaxy with GaN have been overcome through the past years, a fundamental understanding of the switching mechanisms has been mainly approached by theoretical studies describing the local atomic origins for switching, the switching kinetics and possible pathways. However, while visualizing local polarization switching with the scanning transmission electron microscope (STEM) seems impressive,[1] an overview onto the established polar domain structures within w-type ferroelectric thin films in support of the theoretical models remains elusive; a fact which is strongly coupled to the crystalline quality of sputtered films, whereas the accessibility of monocrystalline films grown by MBE remains limited.
Methods
We exploit annular-bright field STEM, differential phase contrast (DPC) and 4D-STEM methods to visualize the local atomic structure, polarization direction and large-scale distribution and strain of polar nano-domains.
Results
Recently, we reported on the analog switching capabilities achieved in sub-5 nm thin films and demonstrated for the first time the realization of a multiple domain state within a single grain of AlScN linked to the presence of horizontal polarization discontinuities.[2] Moreover, here we report on the first large scale observation of ferroelectric domain patterns in monocrystalline-like epitaxial AlScN films enabled by the realization of 250 nm thin ferroelectric AlScN films grown on GaN/Sapphire templates by metal organic chemical vapor deposition.[3] The analyses reveal cone-like shaped pinned interfacial domains of opposite polarization present for the “fully” polarized states even after wake-up and 400 x cycling and their spiking extension into the bulk after further biasing. The lateral dimensions of pinned and spiking domains suggest that lateral growth is complicated by the vertical grain boundaries.
Conclusions
The results will be discussed with respect to the proposed domain propagation models for w-type ferroelectrics. The existence of pinned domains with opposite polarization serve as nucleation sites which mainly reduce the overall required energy to domain wall motion rather than the formation of new nucleation sites. These results provide the next step in understanding of the ferroelectric switching mechanisms of epitaxially grown wurtzite-type ferroelectrics driving research on ferroelectric memory, power electronics and innovative computing applications.

Keywords:

STEM, ferroelectric, AlScN, thin films

Reference:

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Extending 4D-STEM based strain mapping to polycrystalline materials

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
Four-dimensional scanning transmission electron microscopy (4D-STEM)-based techniques for elastic strain mapping have advanced significantly, offering a robust tool to investigate deformation mechanisms in materials at the nanoscale. In single crystalline materials, measurements of the reciprocal lattice vectors allow to accurately determine the full in-plane elastic strain tensor [1]. This technique exhibits high sensitivity, enabling the detection of stress fields associated with individual dislocations. In amorphous materials, like metallic glasses, 4D-STEM based strain mapping techniques have also been successfully utilized to determine nanoscale strain states in materials [2]. In contrast, for polycrystalline microstructures, lying between the two extreme cases of single crystals and amorphous materials, a technique for local nanoscale strain mapping is lacking. Although global strains can be inferred from the ellipticity of diffraction rings in selected area diffraction (SAD) patterns, this method provides only average values across multiple grains, lacking insights into the stress state within individual grains or at grain or phase boundaries. This study aims to close this gap and develop a new routine to map the nanoscale strain in polycrystalline materials, facilitating the measurement of strain fields and grain rotations during in-situ deformation of polycrystalline materials with a resolution of 2 nm.
Methods
To demonstrate the technique a 50 nm thick nanocrystalline gold (Au) thin film is used. The sample was produced by DC magnetron sputtering and subsequently underwent heat treatment at 360 °C to reduce the dislocation density present after deposition. The thin film was mounted on a push-to-pull MEMS device and in-situ tensile testing was conducted using a Hysitron PI 95 nanoindenter holder capable of recording the load-displacement data during deformation. The tensile test was intermittently halted during deformation to acquire full 4DSTEM datasets. Precession electron diffraction was employed to enhance the quality of diffraction patterns. By precessing the electron beam the experiment is shifted towards more kinematical diffraction conditions vastly improving the quality of the diffraction patterns. On the one hand, inner intensity distributions are reduced, making peak detection more precise, and on the other hand, more diffraction disks are revealed thus improving on the crystal orientation determination. The utilization of a direct electron detector and an in-column energy filter further refines the dataset quality.

Results
The resulting strain and orientation maps, obtained during in-situ deformation, affirm the feasibility of this study's objectives. Only small changes in crystal orientation were observed, consistent with the low plasticity exhibited by the nanocrystalline thin film. The limited thickness of the Au film, consisting of a single layer of grains, might also lead to less pronounced grain rotation. Nonetheless, orientation changes within individual grains resulting in subgrain structures could be detected and quantified. At the same time, the precise determination of elastic strain states in polycrystalline materials during in-situ deformation was demonstrated. Under loading, a discernible shift towards higher tensile strains was observed, with a histogram analysis revealing a gradual increase in the tensile strain spread with increasing applied load. This clearly indicates, that even in the elastic

regime dislocations are generated. Following fracture, the average strain go back to zero, however an even larger spread is measured. This shows that the increase in dislocation density and thus the broader strain distribution is not due to a measurement artifact but an actual mechanism in the material that can be quantified.

Conclusions
Atomic-level elastic strains in a nanocrystalline thin film were determined by combining concepts from single crystalline strain mapping and automated crystal orientation mapping techniques. This paves the way for future in-situ deformation experiments on industrially relevant polycrystalline structural materials, thereby enhancing our understanding of their deformation mechanisms.

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2. H. Sheng, D. Şopu, S. Fellner, J. Eckert, C. Gammer. Mapping Shear Bands in Metallic Glasses: From Atomic Structure to Bulk Dynamics. Physical Review Letters 128 (2022) 245501.
We acknowledge support from the Austrian Science Fund (FWF):Y1236-N37

Keywords:

In-situ, strain mapping, 4D-STEM, ACOM-TEM

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From Tarquinia's Necropolis to biorestoration: the role of the characterization in a multidisciplinary research context

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Poster Group 2

Background incl. aims
Moonmilk is a secondary calcite deposit often found in karst caves, formed by nanofibers of biogenic origin, mostly on calcarenite surfaces, in high humidity and low temperature conditions. Recently, in the ancient Etruscan hypogean tombs of the necropolis of Tarquinia, moonmilk has been identified in a white patina covering the wall paintings. The presence of bacteria, promoting the calcium carbonate deposition, has opened the way to their potential use as a new and promising way for biorestoration. Our aim is to optimize this process for a successful and stable calcite deposition, because the conservation of the hypogean environment is a real challenge for restorers and conservators, and it requires a strong characterization work, both from a biological side, for the selection of the best bacteria producers of calcite, and from the applied methods, microscopies or spectroscopies, to establish a safe protocol for the evaluation of the deposited calcium carbonate and its application as an innovative restoration material in cultural heritage.

Methods
Optical microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to characterize the calcite morphology, while its crystallinity was assessed using X-Ray Diffraction (XRD), Raman spectroscopy. Metagenomic studies were carried out to identify the complete bacterial community present in the moonmilk, while the choice of the most promising species capable of rapidly depositing calcium carbonate under specific conditions was performed by selecting single strains through culture-dependent method.

Results
Our research has covered all aspects of moonmilk biogenesis, from structural to microbiological studies. In our previous work we have described how Moonmilk formation occurs not only on the outer surface of the rock, but also in the inside, and how the interaction between bacteria and the rock substrate can influence the final morphology of the calcium carbonate precipitates, showing structures at both the nano- and micro-scales. Moonmilk can also be seen as a biosignature in the field of astrobiology, while for biotechnological applications, the study of the microbial community has allowed the selection of different species capable of rapidly producing calcium carbonate, making them suitable for bioconsolidation intervention.

Conclusion
Although the chemical reactions that elongate moonmilk nanofibers have yet to be determined, their origin is certainly biogenic, characterized by the presence of an entombment structure produced by a bacterial strain that detoxifies calcium ions by producing calcium carbonate. Environmental

conditions and rock substrates also have a strong influence on the final shape of moonmilk crystals. Nevertheless, the analysis of the microbial communities in the Etruscan hypogean tombs has demonstrated their very fundamental role, which can be used as a green and innovative source for bioconsolidation interventions.

Keywords:

Moonmilk, calcium carbonate, biorestoration

Reference:

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Operando ETEM study on solid oxide cells

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PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

Background incl. aims
Solid oxide electrolyzers and fuel cells (SOEC/SOFC) are crucial for reducing global carbon emissions. Understanding their degradation processes is key to making them more durable and cost-effective for widespread use [1]. Usually, researchers examine these cells after electrochemical tests, which limits insights into the ongoing degradation process. Our study introduces a new method that makes it possible to observe the cells in operation. This method combines the TEM characterization and the electrochemical test and correlates the structural and compositional evolution with electrochemical performance.

Methods
The presented method combines a TEM holder with a heating-biasing MEMS chip and an environmental TEM capable of introducing relevant reactive gases such as O_2, H_2, and H_2O, mirroring the operational conditions of SOEC/SOFC. We developed and utilized a special sample preparation procedure to apply three types of stimuli—heat, reactive gas, and electrical bias—directly to the SOEC/SOFC within TEM [2, 3]. The tested cells have lanthanum-strontium-cobalt oxide (LSC) electrodes and an yttrium-stabilized zirconia (YSZ) electrolyte. To study cell degradation, those cells are tested with polarization in an atmosphere of 2.7 mbar O_2 at 700 °C.

Results
STEM imaging revealed that degradation feature development in the negatively polarized LSC electrodes varies with polarization voltage. Crack formation was also observed at the interface between the positively polarized LSC electrode and the YSZ electrolyte. EELS analysis conducted simultaneously showed a Co valence state change, with lanthanum's state remaining constant. Subsequent analyses, including EDS, SAED, and HRTEM, suggested that the newly observed features in negatively polarized LSC are the result of LSC decomposing into Co_3O_4, La_2O_3, and La_2CoO_4. This decomposition process was particularly pronounced near the YSZ interface, indicating a direct correlation between structural changes and electrochemical performance.

Acknowledgements
This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No 850850)”

Keywords:

Operando; ETEM; SOEC; SOFC; degradation;

Reference:

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[2] Ma, Zhongtao, et al. "Experimental Requirements for High-Temperature Solid-State Electrochemical TEM Experiments." Small Methods (2024): 2301356.

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Dissolving alloying additions inside precipitates of lightweight alloys to promote phase transformations

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
Microalloying elements are commonly added to high-strength aluminium alloys, mainly to promote the formation of key strengthening precipitate phases. Classic examples include Sn, Cd and In at the 100 ppm level [1], which significantly accelerate the nucleation of the strengthening phase θ' in Al-Cu alloys, thereby improving the precipitation hardening response. More dramatic is the effect of Ag additions to Al-Cu-Mg and Al-Cu-Li alloys, where new phases (Ω and T1) form in high number densities, leading to ultra-high-strength alloys typically used in the aerospace industry. In all these cases, the microalloying additions either segregate at the precipitate-matrix interfaces or precipitate first as a well-defined crystalline phase before acting as heterogeneous nucleation sites [1].
An unusual case is that of Au additions to Al-Cu, where the precipitation hardening response is enhanced and accelerated through Au dissolving inside the strengthening precipitate phase θ' [2]. This observation leads to two questions which this work aimed to address:
(1) What is the mechanism by which Au enters the precipitates and promotes their formation?
(2) Can other elements behave in this way?
Answering these questions would be useful not only for improving one's fundamental understanding of phase transformations, but also to provide a potential way of immobilizing contaminants.

Methods
This work employed a combination of aberration-corrected scanning transmission electron microscopy, density functional theory (DFT) simulations and classical nucleation theory (CNT) calculations. The microscopy was performed on a FEI Titan3 FEGTEM and a Thermo Fisher Scientific Spectra-ϕ FEGTEM, both double-aberration corrected and operated at an accelerating voltage of 300 kV in scanning transmission electron microscopy (STEM) mode. Al-1.7at%Cu-0.02at.%Au alloys having undergone different ageing treatments were examined. More details about the experimental and computational procedures can be found in Refs. [3-4].

Results
In order to understand how Au atoms are incorporated into θ' precipitates, the alloy was characterised at the very early stages of ageing. It was found that the overwhelming majority of θ' precipitates at the earliest stages of precipitation were 80-100 nm long and only 1 to 1.5-unit cell high, with ~2nm long θ" precipitate regions at the semi-coherent interfaces (see Fig. 1). The 1c-high configuration has never been observed in pure Al-Cu alloys. Chemical analysis indicated the presence of Au atoms within the structure. An atomic-scale mechanism is proposed that involves the transformation of the plentiful coherent θ" precipitates into 1c-high θ' through the incorporation of Au solute and vacancies. This mechanism is supported by DFT and CNT calculations [3,5].

The intriguing possibility that other solute elements may dissolve into the θ' phase was also examined through DFT simulations.

Conclusion
An unusual mechanism through which a microalloying element (here Au) promotes the formation of a strengthening precipitate phase (here θ') by dissolving inside it, was investigated at the atomic scale. This was found to lead to sub-nanoscale thick precipitates of aspect ratio >100. This direct transformation mechanism may also be at play in other important aluminium alloy systems, such as Al-Cu-Li with Ag additions.

The authors acknowledge the Monash Centre for Electron Microscopy, a Node of Microscopy Australia, and the Australian Research Council.

Keywords:

STEM; aluminium; precipitates; phase transformations

Reference:

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Fe/Pt spintronic bilayers: Tailoring structure for enhanced THz emission

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Poster Group 2

Background incl. aims
Driven by potential applications in spintronics, layered ferromagnetic/non-magnetic (FM/NM) nanostructures have attracted significant interest, particularly due to efficient spin transport across epitaxial interfaces in Fe/Pt bilayers [1,2]. This renders Fe/Pt bilayers as a promising candidate material system for THz spintronic devices [3]. However, the crucial role of interface quality in these bilayers cannot be disregarded, as it significantly affects spin current transmission. Optimization of the FM/NM interfacial structure and defect density is thus a potential approach for manipulating the performance of spintronic THz emitters [4]. Here, we explore this concept by investigating structural modifications of the Fe/Pt interface, obtained by tuning growth and annealing temperatures of the bilayers. The structural properties of the resulting configurations with modified interfaces were then characterized to establish a correlation between interfacial features and the emitted THz signal.
Methods
Fe/Pt bilayers with a nominal thickness of Fe(12nm)/Pt(6nm) were deposited on MgO(100) substrates using electron beam evaporation. To maintain a constant total thickness across all samples, only the Pt layer's growth temperature was varied, to induce modifications in the Fe/Pt interface. At first, a 12 nm thick Fe film was deposited on the MgO substrate at 300°C, followed by annealing at the same temperature for 30 minutes, in all samples. Subsequently, 6 nm of Pt were deposited on top of the Fe layer at various growth temperatures, namely 300°C, 450°C and 600°C. After Pt layer deposition, an annealing process similar to the Fe layer was performed at the corresponding growth temperature of each sample. The terahertz (THz) emission properties of the resulting heterostructures were characterized using a standard THz time-domain spectrometer (THz-TDS). Initial structural investigations were conducted using X-ray diffraction (XRD). Nanoscale structural and chemical analysis was then performed using a combination of transmission and scanning transmission electron microscopy (TEM/STEM), energy-dispersive X-ray spectroscopy (EDXS), high-resolution TEM (HRTEM), and Z-contrast high-resolution STEM (HRSTEM) imaging, along with Z-contrast HRSTEM image simulations.
Results
The largest THz emission, nearly double the amplitude observed for the Fe/Pt(300°C) sample, originated from the Fe/Pt(450°C) sample, while the Fe/Pt(600°C) sample displayed negligible signal [5]. XRD analysis revealed an almost strain-free Fe/Pt configuration for the 300°C sample. In contrast, the presence of an additional $L1_0$-FePt peak in the 450°C sample suggested interfacial modifications. Furthermore, the disappearance of Fe and Pt peaks and the emergence of new peaks corresponding to the face-centered-tetragonal (fct) lattice of $L1_0$-FePt in the 600°C sample, suggested a complete transformation of the bilayer into the ordered alloy.
TEM observations confirmed distinct Fe and Pt layers with nominal thicknesses for the 300°C sample, exhibiting epitaxial growth on the MgO(100) substrate following the Bain orientation. In the 450°C sample, HRTEM analysis revealed the formation of a 2 nm thick interlayer (IL) at the Fe/Pt interface

(graphic). This IL displayed a characteristic periodic intensity modulation, indicative of a superlattice structure with high chemical ordering. Further HRTEM imaging confirmed the IL's tetragonal symmetry and identified the ordered $L1_o$-FePt structure. Moreover, the interplanar spacings of resolved lattice planes in MgO/Pt and Fe nearly matched the corresponding values of their bulk structures, implying a strain-free configuration and an epitaxial growth of the IL on the Fe layer. Z-contrast HRSTEM observations and simulations confirmed the IL's chemical ordering, with the observed intensity modulation matching a periodic arrangement of single-element Fe and Pt atomic layers in the $L1_o$-FePt structure. STEM-EDXS analysis further corroborated this, revealing interdiffusion of Fe and Pt elements confined solely at the Fe/Pt interface. For the 600°C sample, TEM observations showed complete interdiffusion and merging of the Fe/Pt layers into a single layer comprising large grains slightly misoriented to each other. The latter finding can be attributed to the presence of uneven atomic size steps and terraces on the MgO surface. HRTEM imaging revealed a periodic intensity modulation along the growth axis, indicating the formation of a chemically ordered FexPt(1-x) alloy throughout the entire layer. STEM-EDXS analysis confirmed Fe and Pt intermixing across the entire FexPt(1-x) layer, with the Pt composition decreasing from the top of the film towards the MgO/FePt interface. Notably, the $L1_o$ structure with fct symmetry was preserved regardless of the non-uniform stoichiometry.
In summary, the significantly enhanced THz emission concurred with the modified Fe/$L1_o$-FePt(2nm)/Pt interface in the Fe/Pt(450°C) sample compared to the Fe/Pt(300°C) bilayer. Conversely, the absence of the non-magnetic Pt capping layer at the Fe/Pt(600°C) sample, and despite the $L1_o$ ordered alloy formation, resulted in the suppression of the THz signal.
Conclusion
The interplay between Pt growth temperature, structural features, and THz emission in Fe/Pt spintronic bilayers on MgO substrates was investigated. High-quality Fe/Pt bilayers were achieved at 300°C. Distinct structural modifications were observed at elevated Pt growth and subsequent annealing temperatures, which were used to engineer the Fe/Pt interface, namely a 2 nm thick $L1_o$-FePt IL formed at 450°C, while a fully intermixed FexPt(1-x) alloy with ordered fct structure and alternating pure Fe and Pt-rich atomic layers superseded the individual Fe and Pt layers at 600°C. The presence of the ordered IL in the Fe/Pt(450°C) sample significantly enhanced THz emission by facilitating interface transmission. Conversely, complete intermixing and the absence of the non-magnetic Pt capping layer in the Fe/Pt(600°C) sample suppressed the THz signal. The observed enhancement in the Fe/$L1_o$-FePt/Pt trilayer configuration opens exciting possibilities for spintronic THz emitters. Furthermore, the ability to control (FM/NM) interface properties through engineered interlayers offers significant flexibility for designing future spintronic devices.

Keywords:

Fe-Pt heteroepitaxy, TEM-STEM-EDXS, $L1_o$-ordering, THz-emission

Reference:

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Polar Discontinuity Governs Surface Segregation and Interface Termination: a case study of LaInO3/BaSnO3

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Poster Group 2

Background and aims.
Interfacial polar discontinuities are a unique way to manipulate charge states at interfaces and to create novel two-dimensional electron states. The polar-nonpolar interface between ABO3 perovskite oxides offers new degrees of freedom to tune interface states by the accessibility of mixed-valence states. The commonly accepted model for the formation of a two-dimensional electron gas (2DEG) is based on the concept of charge transfer between the layers terminating the polar-nonpolar interface. To realize a 2DEG or a two-dimensional hole gas (2DHG), control of the interface is a prerequisite. When growing the heterostructures, it is commonly assumed that the surface termination of the non-polar layer controls the interface. In this work, we provide experimental evidence that polar discontinuity compensation can drive surface segregation and thus control the interface

Methods.
We study the surface termination of BaSnO3 and the interface formation between the cubic wide bandgap semiconductor BaSnO3, and orthorhombic LaInO3, by negative cs imaging and direct spectral imaging of the LaInO3-BaSnO3 interface using Scanning Transmission Electron Microscopy Energy Dispersive X-Ray Spectroscopy (STEM-EDS) with the Thermo Fisher Spectra Ultra electron microscope equipped with Ultra X EDS detector. In parallel we perform Integrated Differential Phase Contrast STEM (iDPC-STEM) technique to study the oxygen octahedral tilt at the interface, providing further insights into the compensation of polar charges at that interface. Density functional theory (DFT) calculations are used to rationalize our experimental results. The samples were grown by plasma assisted molecular beam epitaxy on DyScO3 substrates at 835°C using a mixture of Sn and SnO2 as a SnO source.

Results.
While TEM experiments of BaSnO3 bulk crystals and DFT agree that BaO is the most stable surface termination of BaSnO3 over wide range of chemical potentials, we find by EDX spectral imaging the interface between BaSnO3 and LaInO3 is terminated by SnO2 (Fig. 1). This is consistent with the presence of a 2DEG but also with our DFT calculations which show this interface to be the most energetically favorable. STEM EDX shows the presence of BaO on the surface of thin LaInO3 films indicating Ba surface segregation. Based on our DFT calculations we find that the driving force for Ba segregation is the compensation of the polar discontinuity at the interface. This compensation is an effect the gradual reduction of the octahedral tilt from the orthorhombic LaInO3 to the cubic BaSnO3 and the polar and non-polar distortions at the interface as evidenced by the evaluation of iDPC images (Fig. 2). In the case of the n-type SnO2 interface, this leads to an expansion of the out-of-plane lattice spacing at the interface which most efficiently compensates for the discontinuity. At the BaO terminated p-type interface it leads to non-polar distortions in the BaSnO3, while polar distortions remain mainly in the LaInO3 which compensate the polar discontinuity less efficiently.

This shows that in Perovskites in addition to surface energy and strain, the response of the system to compensate for the polar discontinuity must be considered as an additional driving force for segregation which may control the interface termination.

Keywords:

EDX, EELS, iDPC, Perovskites, 2DEG

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Measuring hybridisation in Van der Waals heterostructures using momentum-resolved EELS

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background

Van der Waals heterostructures have become omnipresent in materials research today as they offer sheer endless opportunities to tune properties by stacking [1]. In a Lego-like fashion, researchers combine a range of two-dimensional (2D) materials to create ever-more complex structures. However, regarding the properties of stacked 2D materials, the question arises: do we make something new or do we have a simple sum of its parts? We are still often lacking a means to study these complex stacked materials. One particular challenge that is frequently faced is that the phenomena under investigation might lie in an energy-momentum space that is impossible to access using optical methods.

Methods

Here, we show that using modern electron microscopy (EM), the energy-momentum space that becomes available can cover several Brillouin zones (BZ) in the momentum direction and several electron-volts in the energy direction. EM therefore represents an excellent opportunity to study stacked heterostructures. We combine the resolving power of the Nion HERMES instrument with the detection capabilities of a direct detector camera (Dectris ELA) to map phenomena across the BZ. Using this approach, the behaviour of phonons, excitons and plasmons can be mapped across the entire BZ. Results were compared to time-dependent density functional theory (TD DFT). To demonstrate this technique, we present the findings from a heterostructure commonly used in nanoscience research today. Hexagonal boron nitride (hBN) is the most common encapsulant for nanoelectronics and nanodevice fabrication as it is thought to not affect the properties of the TMDCs, due to its insulating character. It is known to even enhance the excitonic intensities and to decrease the excitonic bandwidth of TMDCs [2].

Results and Conclusions

Momentum-resolved EELS (q-EELS) was employed to obtain ωq maps of hBN, WSe_2 and a hBN – WSe_2 heterostructure along the high symmetry directions ΓM and ΓK of the crystal structure and the results are presented here.[3] Figure 1, a shows q-EELS as acquired of the hBN – WSe_2 heterostructure. The spectra are shown for steps in q= 0.0106 x |Γ-K-M'-Γ'| to cover the |q| distance from 1.6 to 2.2 of |Γ-K-M'-Γ|. This partially shows the detection challenge at hand. There is a significant difference in intensities at q=0 at Γ and Γ' of excitonic and plasmonic peaks compared to the finite q intensities between the Γ points. Figure 1, b shows the peak evolution between the Γ

points. This time the spectra of the same data set are shown for steps in q= 0.0106 x |Γ-K-M'-Γ| for |q| from 0.01 to 0.85 of |Γ-K-M'-Γ|. Both, excitons and plasmons and their hybridisation were studied using this approach. When comparing the energy-momentum dispersion of the main plasmon peaks, clear signs of hybridisation in the hBN – WSe2 heterostructure were observed when compared to the individual components of the heterostructure. The π- π* and the π- σ plasmon peaks were found to shift to energies that matched neither WSe2 nor hBN. Aside from the plasmon peaks, hybridisation in excitons was also investigated. hBN represents a particular challenge due to the presence of excitons that have a highly directional q dependence and lie at high q and high in energy. The presence of the fine structure at the M point as shown in Figure,b is originating from the hBN itself and is also found in hBN alone. The peak at the M point is thought to originate from vertical e-h transitions between the k points belonging to the ML line in the band structure.[4] This fine structure is absent in the ΓM direction. These findings are in excellent agreement with BSE calculations [4] as well as non-resonant inelastic x-ray scattering data from a synchrotron facility [5]. It shows that EM combined with modern direct detector technology can at least rival the results from x-ray facilities with the added benefit that the EM brings improved spatial resolution. Our results regarding the plasmon hybridisation also show that great care has to be taken when predicting properties of Van der Waals heterostructures.

Keywords:

momentum-resolved-EELS, excitons, plasmons, TMDC, hBN

Reference:

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Beam damage and dynamics modelled with equivariant neural networks

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Background incl. aims
Molecular Dynamics (MD) simulations require a model for the interatomic interactions that is both accurate and computationally inexpensive. Density Functional Theory (DFT) offers good accuracy and decent computational performance for small systems, and ab-initio molecular dynamics (AIMD) is now possible based on DFT. However, many simulations require system sizes and/or simulation times that makes AIMD impossible.

Machine learning potentials can be used to generate inter-atomic potentials for specific systems, with accuracy close to that of DFT and computational efficiency close to that of classical interatomic potentials [1]. While such machine learning potentials have been used to extend the size and time scale of MD simulations to obtain adequate sampling of structures and thermodynamical properties, they suffer from the need of very large training sets and depend on the quality of the rotationally invariant descriptors used as input to the neural networks [2]. Equivariant Neural Networks change this dramatically [3], cutting down the amount of training data by several orders of magnitude while giving better accuracy. In an equivariant network, vector quantities can be used directly as input to the network and are then processed in a rotationally equivariant way.

We apply these methods to two different scientific problems. The first is heating of supported metallic nanoparticles by the electron beam in HR-TEM. The second is thermal vibrations in supported 2D nanoparticles, where recent HR-TEM studies show significantly increased vibrations near the edges of the nanoparticles [4].

Methods
We develop a simulation protocol for training Equivariant Neural Network Potentials (ENNPs) on a limited number of DFT simulations, based on the NequIP package [3]. We gradually expand the training set based on Molecular Dynamics simulations, where we use an ensemble of ENNPs to identify configurations where the uncertainty is large. DFT calculations are then added to the training data, gradually improving the ENNPs. After a few generations, we have a stable ENNP, but can still use the ensemble method to monitor the accuracy, also when simulating systems beyond the size limitations of DFT.

We simulate the heat transport between gold nanoparticles and TiO_2 support. From this we make a simple model for the temperature of the nanoparticle based on the heat deposited by the electron beam and the heat transport into the substrate. Parameters for the energy deposition by the beam are extracted from electron energy-loss spectroscopy (EELS), whereas the heat transport parameters come from the MD simulation.

Results
We simulate the heat transport between gold nanoparticles and TiO_2 support with molecular dynamics and ENNP potentials. We see that thermal transport is limited by the interface between nanoparticle and support, due to the mismatch in phonon frequencies. From this we make a simple model for the temperature of the nanoparticle based on the heat deposited by the electron beam and the heat transport into the substrate. Parameters for the energy deposition by the beam are extracted from EELS data, whereas the heat transport parameters come from the MD simulation [5].

We also simulate thermal vibrations in nanoparticles of the two-dimensional material MoS_2, supported on graphite. We use the simulated vibrational amplitudes as input to HR-TEM image simulations using the multislice algorithm and the frozen phonon formalism for vibrations. We find that edge phonon states significantly increase the vibrational amplitude not just at the edge of the nanoparticle, but several lattice constants away from the edge. This matches well what has been found experimentally by Chen et al. [4], where exit wave reconstructions were used to gauge vibrational amplitudes in HR-TEM image series of supported MoS_2 nanoparticles.

Conclusion
Equivariant Neural Network Potentials make it possible to use molecular dynamics simulations on systems with tens to hundreds of thousands of atoms, with accuracy close to that of DFT, but without the computational cost of DFT, where even a thousand atoms are a challenge. The accuracy of the potentials can be monitored with ensemble methods, and problematic configurations can be used to retrain the ENNP. The method can be directly applied to study effects of the electron beam in HR-TEM, and the role of realistic atomic vibrations in imaging.

Keywords
Equivariant Neural Network Potentials, HR-TEM, image simulation, molecular dynamics, nanoparticles.

Keywords:

Molecular Dynamics, HR-TEM, Machine Learning

Reference:

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PHOSPHO1-mNeonGreen reporter cells are a robust model to study matrix vesicle biogenesis during osteoblast-driven mineralisation

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Poster Group 1

Background and aims: Bio-mineralisation is an essential process in which bone cells initiate the laying down of mineral within the extracellular matrix (ECM) to form an organic-inorganic composite material that is both tough and stiff to support the physiological demands of bone. The delicate balance of inorganic phosphate and pyrophosphate concentrations is a major determinant of the rate of ECM mineralisation. This process is under the control of tissue non-specific alkaline phosphatase (TNAP) and PHOSPHO1, key phosphatases found in a specific set of extracellular vesicles termed “matrix vesicles” which are postulated to drive mineralisation. The biogenesis of matrix vesicles remains unclear, in part due to lack of imaging options available for such small vesicles, and the delicate nature of calcium phosphate mineral. This study aims to develop a robust imaging system that will allow us to study the mechanisms underpinning matrix vesicle biogenesis, and the process of mineral deposition within the ECM.

Methods: Mineralisation was studied in the osteoblast cell line MC3T3. The calcium-binding compound, Alizarin red was used to confirm mineral deposition, and gene and protein expression assessed via qPCR and protein across the time course. Key matrix vesicle phosphatase PHOSPHO1 was tagged with mNeonGreen and live imaging performed with pseudo-super resolution (LSM880) and super resolution (ELYRA) microscopy in live and fixed cells. Reporter cell lines were generated using Fluorescence-activated Cell Sorting (FACS) with a Bigfoot Spectral Cell Sorter, and validated for matrix mineralisation capability and fusion protein expression. Organelle markers and immunofluorescence allowed the localisation of PHOSPHO1-mNeonGreen to be followed throughout the cell.

Results: PHOSPHO1-mNeonGreen expressing cells are a robust model for imaging the intracellular stages of matrix vesicle production and biogenesis by osteoblasts. Using fluorescent PHOSPHO1 as a marker, we have followed the trafficking pathway of MV precursors in the endoplasmic reticulum to the trans-Golgi network; a process involving RAB8 vesicles and driven by microtubules. This suggests a RAB GTPase-dependant method of intracellular vesicle packaging and subsequent MV secretion by the osteoblast. Furthermore, confocal imaging shows PHOSPHO1 positive matrix vesicles appear to be released at actin-rich sites at the osteoblast cell membrane, possibly linked to focal adhesion sites. Time-lapse confocal imaging of cells surrounding mineralised nodules produced in vitro shows small PHOSPHO1-mNeonGreen objects present and interacting at the mineral-cell membrane boundary, suggesting evidence of matrix vesicle activity. Preliminary evidence with a TNAP-mKate2+PHOSPHO1-mNeonGreen double reporter line shows co-localisation of both matrix vesicle markers on intracellular objects, with object size and localisation as supporting evidence for potentially an MVB driven method of MV release.

Conclusions: PHOSPHO1-mNeonGreen expressing osteoblasts are a valuable and robust model for studying the process of bone mineralisation in vitro and allow our first insights into the intracellular steps of matrix vesicle biogenesis.

Keywords:

Super-resolution microscopy, cell matrix mineralisation

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Electron channeling pattern imaging – a novel approach for the determination of wafer offcut angles

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Poster Group 2

Background incl. aims
The epitaxial growth of semiconductor multilayers can benefit from the use of wafer substrates with different offcut angles. The offcut angle of a wafer refers to the angle between the wafer surface normal and the chosen zone axis. These offcuts can be precisely controlled on monocrystalline wafers such as silicon or sapphire according to the growth needs. A possible application consists in tuning the strain in the epitaxial stacks by growing the layers on single-crystal wafers with precisely controlled offcuts. However, improper wafer offcuts can lead to the undesired formation of grains, anti-phase boundaries and uncontrolled growth modes. These offcuts must therefore be accurately controlled. To characterize wafer offcuts, methods based on high-resolution X-ray diffraction (XRD) [1] and electron backscatter diffraction (EBSD) [2] can be used to characterize the wafer offcuts with sub 0.1° angular resolution. However, such measurements are complex and time-consuming (several hours). Therefore, the availability of a fast and accurate method for wafer offcut angle determination is highly desirable to enable efficient quality checks of incoming wafers and to support the growth of high-quality materials for advanced semiconductor devices. In this contribution we propose a novel workflow based on applying electron channeling pattern imaging (ECPI) techniques and successfully utilized this method to measure wafer offcut angles.

Methods
ECP is based on the diffraction of backscattered electrons (BSE) inside crystalline materials [3]. The variation of the BSE yield as a function of the incident beam direction results in a distinctive band pattern on the sample surface. This generates a set of electron channeling bands (ECB) appearing as dark lines, which forms the ECP superposed on the BSE image. For monocrystalline samples, the ECP is determined by the crystal structure and orientation. While ECP represents the reciprocal space projection onto the detector plane, the sample rotation causes the respective rotation of the ECP. The beam center can be determined by the sample rotation. Having a sample with an offcut angle deviated from the zone axis means that the ECP rotation center is not fixed to the nearest zone axis. Upon sample rotation, the ECP center forms a circular trajectory as the sample is rigidly fixed with the zone axis. As a result, the precise wafer offcuts can be directly measured from the analysis of ECP images.

Results
In this work, we successfully applied this method to Si(001) wafers with and without offcut angles. After sample loading into the chamber, the sample surface normal is first properly aligned to the primary electron beam. For the zone axis (offcut angle < 3°) present on one ECP image, a rotation series is needed to determine the wafer offcuts. During the sample rotation the whole ECP including the zone axis moves around the surface normal along a circular path. The resulting radius of the zone axis trace is determined as the wafer offcut angles. In case the zone axis is not present in the field of view, additional tilts are required to determine the zone axis position and thus the wafer offcut.

Following this approach, the achieved angular resolution is 0.1°, which agrees well with reference XRD results. With the routine procedure established in this work, the results can be obtained within 1 hour. Compared to the XRD and EBSD methods, it is faster and provides an acceptable accuracy, without requiring any specific sample preparation.

Conclusion
We demonstrate a new approach based on ECP images to determine wafer offcuts. The wafer offcut angle is characterized rapidly with an angular resolution of 0.1º. As a fast, accurate, and easy-to-use solution, the technique has wide applications, especially in the semiconductor field.

Keywords:

Wafer offcut, misorientation, ECPI

Reference:

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Holography with an Extended Reference in Transmission Electron Microscopy

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Background
Electron holography, first introduced in 1948 by Gabor [1], is one of the most widely used techniques for the retrieval of phase information in a transmission electron microscope (TEM). Conventionally a hologram is formed by interference between a scattered wave and a reference wave, for either an inline or off-axis experimental configuration. Holography is possible with an extended reference, arising from compact support in the specimen plane, for use in lens-less X-ray and laser applications as a dose-efficient method with a fast reconstruction process [2, 3]. In such approaches the reconstruction of the exit wave requires just one inverse Fourier transform of the recorded far-field diffraction pattern (Fig. 1a), with pre-weighted spatial frequencies. For a compact support defined by a polygonal aperture, sharp corners and edges provide innate scattered reference waves to interfere with the object wave (Fig. 1b), which can greatly simplify the mathematical form of the reconstruction process [2].
Methods
In this work, we experimentally implemented this method within a TEM operating at 300kV, choosing a square aperture as the compact support. To create effective reference waves, it is essential to ensure sharp edges and corners and to have a binary compact support defined by a sufficiently opaque aperture. To this end, a focused ion beam was used to accurately mill a micron sized square hole in an aluminum foil that was originally not electron-transparent at standard TEM operating voltages. Some residual material was left at the corner as an effective test object, the shape of which was imaged in the TEM by observing absorption contrast at low magnification. A diffraction pattern was recorded at the far field diffraction plane and used for the reconstruction.
Results
The shape of the test object was successfully reconstructed from the inverse Fourier approach using a single diffraction pattern, which provided six independent reconstructions (Fig. 1c-1d). Beyond that, we explored the possibility of applying a scanning parallel beam to overcome the limitations in the relative position and size of the object to the aperture. We also explored the comparative challenges against other instruments by considering the short wavelength of accelerated electrons, the quality of the fabricated corners, lens aberrations, centering of the diffraction patterns, far-field requirements, and detectors used. At this meeting, we will also discuss the use of a square probe forming aperture to provide optimum electron-optical configuration for this technique, as well as facilitating its implementation in a scanning mode.
Conclusions
To the best of our knowledge, this work is the first implementation of this deterministic extended reference Fourier holography method in TEM. Compared with other phase retrieval methods in TEM, the fast, real-time reconstruction process makes it promising for in-situ imaging techniques of

magnetic materials. It also shows the feasibility of determining exit wave phases from just a single diffraction pattern, with potential applications in beam-sensitive materials and biological samples.

Keywords:

holography; phase retrieval; non-iterative reconstruction

Reference:

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4. This research used equipment funded by Australian Research Council grants LE0454166 and LE0882821 at the Monash Centre for Electron Microscopy (a node of Microscopy Australia).

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Fast and low dose EELS using compressive sensing

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Poster Group 1

The scanning transmission electron microscope (STEM) can capture a multitude of signals corresponding to the structural and chemical properties of a material. Examples of these methods include bright/dark field imaging, energy dispersive x-ray spectroscopy (EDS), or electron energy loss spectroscopy (EELS) [1]. EELS is of particular interest due to its sensitivity to low-mass elements and ability to determine their oxidation states, chemical bonding, and spatial distribution. An EEL spectrum is challenging to acquire due to low signals, energy spread of the beam, and the sensitivity of the detector. Furthermore, the stability of the sample is compromised due to the acquisition speed, which is a combination of signal limitations and camera read-out speed. One solution to overcome these limitations is the use of probe sub-sampling, whereby only a subset of probe locations is acquired with respect to the typical scanning grid. This has been shown to work for various STEM techniques such as 2-D imaging, EDX, and 4-D STEM [2,3]. Our goal is to apply these same strategies to EELS acquisition to increase speed whilst maintaining the structural and chemical analysis of the material.

A focused electron probe is aligned and the scan coils are connected to a scan generator to allow for a customised scanning pattern. The electron probe is then positioned at the sub-sampled probe locations and the EELS spectra acquired. For real time imaging, a subset of energy losses can be inpainted using a GPU implementation of the Beta Process Factor Analysis (BPFA) algorithm [4] on-the-fly to allow for finer alignment of the probe. For offline analysis, the data is reshaped to form a 3-D dataset where the first two dimensions correspond to probe locations, and the final dimension is a specific energy loss. This data is then inpainted using the BPFA with a 3-D patch. This process is depicted in Fig. 1.

To test this method, we simulated a sub-sampled EELS experiment using a silicon carbine sample with graphene grown on the carbon face [5]. The dataset contains 17x104 probe locations with a scan step of 0.13nm, and an energy width on the camera of 0.25eV (2048 channels). The dataset was tested using only 25% of the original data. The results (given in Fig.1) show that the data can be recovered to achieve functionally identical results to that of the original, full-sampled dataset.

This work has shown that atomic resolution EELS can be achieved with far fewer measurements of the sampling grid. By employing these methods, STEM-EELS can be faster, lower dose, and importantly accessible for potentially beam sensitive or dynamic samples. In this talk, we will present experimentally acquired data using a JEOL GrandARM2, with the goal of demonstrating practical implementation of this method.

Figure 1: Method for acquiring probe sub-sampled EELS data (left). The PC determines the scanning pattern which is connected to a scan engine to change the scan coil voltages. The EELS spectra is formed by integrating the camera signal column-wise, an this spectra is then used to form energy filtered images which can be inpainted using SenseAI's inpainting algorithm. In this case, an example of reconstructed EELS data and corresponding spectra are given. EELS spectra with zoom in at carbon π^* and σ^* characteristic peaks (top right). Example of integrated EELS maps at 285-286 eV energy loss for 25% probe sub-sampled data (bottom right).

Keywords:

STEM, EELS, compressive sensing, sub-sampling

Reference:

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TEM Investigations of Multi-Layer Selective Absorber thin films for concentrated solar plant: structure and composition

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Poster Group 2

Background and Method
The solar energy resource is still relatively under-exploited although it could cover most of our energy needs. Beyond the development of photovoltaics, there is a worldwide challenge to deploy large-scale concentrated solar thermal power plants. To address this issue, materials with high absorption in the visible range, low emissivity and good temperature resistance are required. To this end, multi-layered optical thin films produced by plasma processes are developed in the NANOPLAST project (nanoplast-project.cnrs.fr, ANR-19-CE08-0019). The stack encompasses an anti-reflective top-layer based on tantalum oxide or oxynitride, a selective absorber in the W-Si-C ternary system and a tungsten Infra-red reflective layer, denoted [Ta-O-N]/[W-Si-C]/[W]. These films are deposited by plasma-assisted technique: first Direct Current sputtering from a W target for the IR reflector, second dual reactive magnetron sputtering and Plasma Enhanced Chemical Vapour deposition, from a W target combined with different amounts (5% to 28%) of TMS-Si(CH3)4 diluted in Ar and finally reactive magnetron sputtering by adjusting O2 and N2 for Ta-O-N top layer[1].

Results
TEM characterisations were carried out to get insight into the structure and composition of the absorber, the anti-reflective top films and then the annealing of the complete system. Particular attention was paid to the influence of sample preparation. FIB (Focused Ion Beam) and ultramicrotomy were carried to get insight in the homogeneity along the growth direction of the multi-layer. In this work, we want to highlight the complementarity of both sample preparations for TEM. Actually if FIB is now well-established and allows to precisely select the region to be analysed, in the present case due to the non-equilibrium deposition conditions, FIB could also induce artefacts due to differential sputtering and/or re-deposition of elements (such as Ga) that may lead to misinterpretations. Hence by observing cross-sections prepared by ultramicrotomy, we were able to dismiss possible impacts of FIB on the observations thanks to the adhesion of the whole stack. Therefore, for the absorber, depending on the tetramethylsilane (TMS-Si(CH3)4) fraction in the Ar discharge, the quantity of Si introduced in the W-SiC:H material can be tuned with the objective to explore the possibility to prepare nanocomposite thin film composed of W nanoparticles dispersed in a dielectric SiC:H matrix. In dedicated conditions (5 and 8% TMS), FIB preparation and ultramicrotomy confirmed that W nano-crystallites incorporating C atoms could actually be identified by coupling STEM-HAADF (Figure 1.a) (Scanning Transmission Electron Microscopy - High-Angle Annular Dark Field) images, EDS mapping (Figure 1.b) and High Resolution imaging. In other conditions (20 and 28% TMS), amorphous layers are observed.

Next, the annealing of the layers was studied, first the absorber alone and then on the complete multilayer device (Figure 1.c multilayer before annealing). We thus highlighted the influence of the annealing mode (air or vacuum) and its duration on the structure, composition and therefore properties of the thin film.

Conclusion
These STEM analyses provided crucial information on the structure of the active layer and on the evolution of all layers after thermal annealing. Such information is crucial for optimizing the deposition process, especially concerning its intended application. In addition, this study illustrates the complementarity between sample preparation by FIB and ultramicrotomy. Selecting regions of interest for the FIB preparations can bring more interesting results compared to random slicing by ultramicrotomy whereas the latter allows ascertaining results observed on FIB cross-section thanks to its less damaging conditions.

Keywords:

STEM-HAADF, Multilayer coating, FIB, Ultramicrotomy

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Effect of Oxygen-Doping in Ferroelectric Wurtzite-type Al0.73Sc0.27N

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Poster Group 2

Background and aim
Al1-xScxN is a group III-N based wide-bandgap wurtzite (w)-type ferroelectric. The material has been proven to be highly temperature stable as well as thickness scalable towards ultra-thin range.[1], [2] Most importantly, it is also compatible to both CMOS and GaN technologies. Therefore, ferroelectricity in Al1-xScxN is highly promising for applications such as non-volatile memory devices, neuromorphic computing, high-electron mobility transistors and even harsh environment electronics.[3], [4] However, ferroelectricity in w-Al1-xScxN is affected by high-leakage current which can become a limiting factor for its successful integration into devices.[5] In the past, standard approaches such as doping or strain was used in semiconductors to alter their properties, especially conductivitiy. Therefore, in this work we address this issue via elemental doping with Oxygen (O). During sputtering of 200 nm thin Al0.73Sc0.27N on Pt/Si templates, we introduced O via gas source into. This allowed us to tune the overall O-content in the bulk thin-film and study its underlying effect on the material structure and the ferroelectric properties of Al0.73Sc0.27N.

Methods
The structure of the doped samples was examined using X-ray diffraction, scanning transmission electron microscopy (STEM). The surface imaging and topography was performed via scanning electron microscopy and atomic force microscopy.

Results
Our results show that all the O-doped Al0.73Sc0.27N films have an overall lower leakage current density compared to the undoped films. This applies to the films even with significantly high O-concentrations ≥ 8 at.%. STEM analysis showed that the columnar Al0.73Sc0.27N crystalline grains remained unhampered with no local phase or O segregation in the bulk. In addition, as O-dopant increases, the overall polarity of the film gradually changes from entirely nitrogen to metal polarity. This modification has no significant impact on the overall (0002) crystalline texture of the film, hence allowing for better control of the as-deposited polarization state of the material.

Conclusion
Our study shows that O-doping could be a viable remedy for the leakage current compensation in w-Al1-xScxN and possibly for all other wurtzite-type ferroelectrics as well.

Keywords:

Wurtzite-type, Ferroelectric, doping, Oxygen

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A comprehensive (S)TEM analysis of Zn_3P_2 suitability for green energy applications

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Poster Group 2

There has been an increasing demand for the development of innovative and sustainable energy conversion devices, aiming to replace conventional and often non-renewable or intermittent energy sources. The motivation for this drive lies in the quest for sophisticated devices, propelling efforts to discover innovative nanostructured materials pivotal in enhancing performance and safeguarding the environment. Understanding a material's compositional and structural traits at the nano and atomic levels, including the configuration of active atoms, crystalline phases, and defects, is paramount in establishing the structure-performance relationship crucial for designing new materials and enhancing existing ones. Transmission electron microscopy (TEM) is a valuable technique employed in characterizing functional nanomaterials, offering insights into crystal structures at a local scale. When combined with techniques like Electron Energy Loss Spectroscopy (EELS) and Energy-dispersive X-ray spectroscopy (EDS) it provides a comprehensive map of elemental information and atomic arrangements, facilitating insights into various promising applications. This work provides an overview of the benefits of utilizing these characterization tools for mapping layer growth, thickness, morphology, and defects of nanostructured Zinc Phosphide (Zn_3P_2) thin films and nanoislands grown by Molecular Beam Epitaxy (MBE) on substrates such as InP and 6H SiC/graphene, with a focus on solar energy applications. Atomic resolution HAADF STEM images of Zn_3P_2 thin films on InP substrates present homogeneous growth and epitaxial relationship between substrate and layer. GPA analysis displays misfit dislocations and rotated domains, and STEM EELS and XRD results indicate composition varying from 60-70% of Zn, in contrast to 30-40% of P. In the case of Zn_3P_2 nanoislands grown on 6H SiC/graphene, atomic resolution HAADF STEM images and GPA analysis reveal that some of the Zn_3P_2 grains are randomly oriented with respect to the 6H SiC/graphene substrate and also, between each other. Rotation maps reveal that, in some areas, there is a rotation of up to 1.6° of the Zn_3P_2 layer with respect to the substrate, which can be justified by the Van der Waals epitaxy between the Zn_3P_2 and graphene. These findings are corroborated by EBSD results, that indicate a most prominent orientation of the grains, and a potential small population of islands with different orientations. Rotated domains are observed, and STEM EDS attest the stoichiometry of the Zn_3P_2 grains. Samples of Zn_3P_2 on different substrates have been analysed to determine the ideal growth conditions for an effective final device. Atomic resolution STEM images have given powerful information on the crystallinity defects present on the samples. These results give the key for growth Zn_3P_2 in an optimal way and go beyond the state-of-the-art in the field of solar cells.

Keywords:

Solar cells;Zinc Phosphide(Zn3P2);HAADF STEM;Electron Microscopy.

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Characterization of LiNiMnCoO2 batteries by CT, LM, SEM and EDS for second life usability Assessment

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Poster Group 1

Background incl. aims
The most commonly used batteries at present and in the near future are Li-ion-based systems. Reliable characterization methods are the basis for improving battery properties and evaluating ageing phenomena. Our major goal is the creation of a damage catalog in which the results of the analytical investigation of aging phenomena are summarized. In this damage catalog, the damage patterns are further correlated to electrochemical data. In this way, the usability of aged cells can be classified based on the electrochemical analysis.
Methods
The analytic workflow goes from the macroscopic to the nanoscopic scale by using computer tomography (CT), light microscopy (LM), scanning electron microscopy (SEM) for imaging and Energy-dispersive X-ray spectroscopy EDS, secondary ion mass spectrometry (SIMS) for chemical analysis. Due to the reactivity of Li with air, cell opening, sample preparation and transportation from the glove box to the SEM was performed in argon atmosphere with a newly designed direct shuttle device developed for the Zeiss airlock system. The studied batteries were commercially available high voltage LiNiMnCoO2 round cells (LG21700 M50T, LG18650 HG2, Samsung INR18650 20R) aged within and outside the specifications. EIS measurements were carried out to monitor the aging process and the state of health (SOH) was determined before cell opening. In addition, cells from the same cell series were examined that were used in unknown devices with unknown applications and come out of a recycling process. This allows us to determine the degradation phenomena of batteries in daily use.
Results
CT enables to detect non-destructive macroscopic changes like, an open current interruption device (CID) created by gas formation, delamination of battery active material and cracks (Fig 1 a-d). The SEM images in Fig. 1 e, f show cross sections prepared by mechanical polishing of a cathode-side aging (e) reference cell and (f) cell out of the recycling process (LG18650 HG2). The layer thickness of the altered cathode decreases => alteration process: loss of active cathode material. The most significant change is the loss of the particle agglomeration of the battery active material. This degeneration can be assigned to high cycling rates [1]. The chemical composition of the cathode material was determined with energy dispersive X-ray spectroscopy (EDS). We obtained Ni:Mn:Co ratio close to 811. This fits to the chemical composition, which was also obtained for the LG21700M50T cell. There we performed a round robin test to proof the reliability of quantitative EDS analysis and used alternative sample preparation like Au-coating, C-coating, Ar-ion milling, transfer shuttles and yield relative deviations between the methods, Institutions and preparation techniques less than 10% for Ni and Co and less than 15% for Mn content [2]. A challenge for the analytic characterisation of Li-ion batteries is the Li detection. There the windowless Oxford Extreme EDS detector open new opportunities for qualitative and quantitative analysis. Fig. 1 (g) shows a top view SEM image of an anode of a reference cell (LG18650HG2), with marked EDS spectra positions and (h) element distribution image. The phases 1. C-rich, 2. Si-rich, 3. O-rich are visible. With the correlated EDS spectra (i-k) the Li-rich phase P3 can clearly be identified. A persistent limitation for chemical

quantification of Li in Li-ion batteries by EDS is that bonded Li cannot directly detected. Our idea is to determine the Li amount indirectly as the missing element fraction of not normalized quantitative EDS analysis. This was exemplary applied on the quantification of the Li-amount in discharged batteries cathodes. That results in a mass fraction of 6,01 ± 0,94 % for the (LG18650HG2) cell. For the alternative sample preparations of the (LG21700M50T) cell mass fractions are: shuttle: 6,0±0,5 %; air: 6,1±0,8%; Argon Ion milling: 6,4±0,6% [2]. These results agree nicely with the expectation of 6,4% for a 100% lithiated cathode material after the first formation cycle.

Conclusion

The correlative analytical workflow with CT and electrochemical characterization enables safe opening of the cell, rather artefact free sample preparation and a clear classification of the aging phenomena. This provides reliable results for the assessment of usability in the second life.[3]

Fig. 1 CT, SEM and EDS analysis. (a-d) CT images of (a) a not and (b) a released CID, (c) cell coil deformation and (d) cracks marked with red arrows. (e, f) SEM images of a cathode cross-section of (e) reference and (f) aged cell, (PE: 5keV, Mag:500, AsB detector, LG 18650 HG2). (g) SEM top view image SEM of a anode with the positions of the EDS spectra, (h) element distribution image, (i-k) comparison of the EDS spectra (PE: 3.5keV and Magnification 1k).

Keywords:

battery, CT, LM, SEM, EDS

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Understanding Au cluster growth through electron microscopy

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Poster Group 2

Background incl. aims
Metal nanoparticles are the key active component in a wide array of heterogeneous catalysts. Specifically, Au nanoparticles are highly active catalysts for oxidation reactions, most notably the oxidation of CO [1]. The structure of Au nanoparticles in the size range less than 5 nm is crucial for their catalytic performance, with specific facets and edge-sites key to maximizing activity and selectivity. Understanding the formation of different Au nanoparticle morphologies is therefore highly important to predicting and controlling their use at catalysts.
In this study we have employed aberration-corrected scanning transmission electron microscopy to observe the structure of Au nanoparticles formed via growth from individual sputtered atoms on amorphous carbon films. Understanding of the specific structures formed from surface-growth at different nanoparticle sizes is vital to predict catalytic performance. We aim to determine whether structures are two-dimensional or three-dimensional, whether they are spherical or hemi-spherical and whether they are amorphous or take on a distinct crystalline morphology (e.g. single crystal fcc or a twinned structure such as icosahedra or decahedra).
Methods
Gold atoms were sputtered onto a carbon film using a sputtered atom source (SAS), which produces an Ar+ beam using a cold cathode ion gun (Scienta Omicron ISE 5) typically operated at 5 keV. The ion beam strikes the gold target (99.999% pure gold) at 45 degrees, producing a secondary flux of gold atoms onto the holey amorphous TEM carbon substrate. The gold atoms were deposited at thermal energies, thus ensuring surface deposition, not implantation. Subsequently, the sputtered atoms form gold clusters on the surface of a holy carbon film (on a copper TEM grid). Enough material was sputtered to observe a wide range of cluster sizes, from single atoms to clusters consisting of >1000 atoms.
High-angle annular dark field (HAADF) images were acquired on a probe-corrected JEOL Grand ARM scanning transmission electron microscope (STEM) operated at 300 kV at the ePSIC facility at Diamond Light Source. A convergence semi-angle of 26 mrad, a beam current of 25 pA and a detector inner semi-angle of 58 mrad were used for all the data collection. Quantification of numbers of atoms in each nanoparticle was performed using a calibrated intensity measurement for single Au atoms.
Results
Au nanoparticles of size < 100 atoms were found to contain a mixture of 2D and 3D structures. In sizes < 50 atoms, approximately one third of structures were two-dimensional. This is contrary to structure predictions from DFT simulations of free-standing nanoparticles (i.e. without the influence of any support), which typically predicts three-dimensional structures are energetically favourable in structure of size > 15 atoms [2]. In novel DFT simulations of Au structures supported on a 2D carbon support, we find that 2D structures compete in energy with 3D structures up to approximately 60

atoms in size. This cut-off size of 60 atoms from DFT simulation corresponds well with our experimental data, where the largest 2D structure observed was approximately 80 atoms in size.
As nanoparticles grow to larger sizes (between 100 and 1000 atoms), they all adopt a three-dimensional morphology. This morphology was found to be approximately spherical in shape, indicating a weak interaction between the Au nanoparticle and the carbon film. The sphericity of the nanoparticles was determined through plotting of number of atoms vs diameter (as shown in the attached Figure). This result is in contrast to previous results that indicated a mixture of spherical and hemispherical structures of Au nanoparticles on a carbon film [3], which we hypothesize may be due to differences in growth rates due to different atomic deposition fluxes.
In the surface-grown nanoparticles of size 100 – 1000 atoms, we also determine the proportion of amorphous and crystalline structures at different sizes. The proportion of amorphous/glassy structures decrease as the size of nanoparticle increases (79% in the range 100-228 atoms, 54% in the range 229-435 atoms, reaching 0% in sizes above 700 atoms). The proportion of different crystalline structures also varies with size of nanoparticle, with icosahedral structures preferred at small sizes but a clear increase in the proportion of decahedral and single-crystal structures as nanoparticle size increases. The proportion of each crystalline structural isomer at different sizes closely matches previous studies of nanoparticles produced from gas-condensation cluster sources [4,5], demonstrating that nanoparticles produced using these two different methodologies are highly comparable.

Conclusion

Nanoparticles grown from single sputtered atoms on an amorphous carbon film show clear trends in morphology. 2D structures are supported up to sizes of approximately 80 atoms due to interactions between nanoparticles and support. At sizes greater than 100 atoms, nanoparticles form approximately spherical structures with no clear influence of support interactions. Surface-grown nanoparticles in the size range 100 – 1000 atoms show a distribution of crystalline structures that closely matches the distribution found in nanoparticles produced using a gas-condensation cluster source, although greater proportions of amorphous nanoparticles are observed in surface-grown samples.

Keywords:

Gold nanoparticles, AC-STEM, nanoparticle growth

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Creep-induced microstructural evolution of the eutectic Mo-Si-Ti alloy by correlative electron microscopy

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Poster Group 2

Turbine blades are critical parts of turbine engines because they experience the highest temperature in the combustion zone. The thermodynamic efficiency of the turbine engine directly depends on the turbine entry temperature (TET) of hot gases; the material's temperature capability dictates the operable temperature. The current state-of-the-art, single-crystal nickel-based superalloys operate close to 80 to 90 % of their melting temperatures. There is a continuous need to design newer high-temperature materials that can withstand higher operating temperatures above 1100 °C to improve efficiency and thus reduce CO2 emissions [1].
The Mo-Si-Ti system has been explored as a potential alternative to the existing high-temperature materials because of their lower density and high melting temperatures. The eutectic Mo-Si-Ti alloy showed pesting resistance and optimal creep behavior. It has a two-phase microstructure: a body-centered cubic (bcc), solid solution consisting of Mo, Ti and Si (Mo,Ti,Si)ss and hexagonal silicide (Ti,Mo)5Si3 [2]. The creep mechanism of this alloy is yet unknown and needs further investigation to understand how the creep strain accumulates in response to the applied stress and subsequent microstructural evolution at meso- and nano-scales in the two phases.
Compressive creep tests were performed under vacuum at 1200 °C and true stress of 100 MPa to understand the creep curve with characteristic minimum and creep rate acceleration after that. To understand the microstructural evolution, the samples were crept to true strains of 1.3, 10, 20, and 40 %. Electron backscatter diffraction (EBSD) was performed to study strain distribution in two phases and identify the region of interest for transmission electron microscopy (TEM) specimen preparation by focused ion beam (FIB). The dislocations were imaged using the diffraction contrast of the selected two-beam conditions in scanning transmission electron microscopy (STEM) mode. The invisibility criterion was used to determine the acting slip planes and the character of dislocations in both phases.
The deformation of silicide was observed in 0.2 % crept samples from EBSD-kernel average misorientation (KAM) maps. Both phases undergo deformation by dislocation plasticity. The following slip systems were identified: {110}, {-121} and {-213} planes with Burgers vector b = 1/2 <1-11> in the solid solution and basal slip {0001} or prismatic slip {1-1 00} both with Burgers vector b=1/3<-1-120> in the silicide [3]. During creep deformation, the growth and coarsening of (Ti,Mo)5Si3 precipitates in the solid solution, and the nucleation and growth of solid solution precipitates in the silicide occur [3]. STEM-energy dispersive spectroscopy (EDXS) was performed to determine the local chemical composition of the precipitates in the crept samples. Figure 1a shows numerous dislocations originating from the interface in the solid solution and the interaction of gliding dislocations with (Ti,Mo)5Si3 precipitates. Whereas Fig.1b shows the formation of low-angle grain boundaries in the silicide phase, confirming dynamic recovery. The microstructural findings

were correlated with the creep curve, and the dominant creep deformation mechanisms (dislocation climb controlled creep and diffusional creep) were identified in the alloy.

Keywords:

Creep, dislocations, Mo-Si-Ti, (Ti,Mo)5Si3, precipitates

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Investigating Impact-Induced Deformation in Cold-Sprayed Aluminum-Quasicrystals Composite Coatings

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Poster Group 2

Introduction

From the perspective of engineering metallic coatings, it is typically challenging to meet diverse requirements using single-phase coating materials, which often fall short in satisfying industrial needs. With metal matrix composite coatings, however, it becomes feasible to meet these diverse needs effectively. Cold spraying (CS) is a solid-state coating deposition and additive manufacturing technology that is promising for several industrial applications. It is effective in the deposition of a variety of materials, including metals, alloys, polymers, and ceramics, but also it stands out because of its compatibility with heat and oxygen-sensitive materials and capacity to create composite deposits. The CS process in general involves accelerating solid feedstock particles using pressurized and preheated gas, resulting in supersonic particle impact on a substrate. This impact leads to coating formation from deformed particles. During coating processing, impacted metal particles undergo high strain rate deformation, triggering phenomena such as grain refinement and phase transformation. These microstructural changes within CS deposits significantly influence their local mechanical properties. At the micron and submicron scale, these alterations can either enhance or compromise coating performance. Notably, phenomenon such as dynamic recrystallization occurs at interfaces—between particles and between particles and substrates—due to significant plastic deformations and adiabatic shear instability conditions during solid-state deformation. Consequently, heterogeneous microstructures might emerge at these critical locations, accompanied by localized variations in mechanical properties. However, despite numerous efforts to have a clear understanding of such featured developed in CS alloys, the precise relationship between local microstructure and mechanical behavior remains elusive for cold-sprayed composite coatings containing multiple ingredients. To engineer coatings with specific mechanical characteristics, a comprehensive understanding of microstructural features is essential. Investigating how variations in grain size, phase distribution, texture, and defects influence mechanical properties is crucial. Bridging this knowledge gap can optimize cold-sprayed coatings for diverse applications, ensuring reliability and advancing materials science.
Aluminum alloys are in high demand in light-weight structures; however, it has the potential short fall of tribological and mechanical properties. Our research team has made significant strides using cold spraying (CS) to produce compact and well-incorporated aluminum alloy (AA6061)-quasicrystal composite coatings (referred to as Al-QC). The coatings have shown superior tribological characteristics and increased hydrophobicity compared with conventional Al-based coatings and bulk metallurgical counterparts.

Materials and methods

Expanding on our previous works, our current research explores the detailed microstructural intricacies of CS Al-QC composite coatings, particularly emphasizing bonding states and particle-

particle interfaces and micromechanical properties. To achieve this, we sprayed the composite coatings using a high-pressure CS system with optimized parameters, utilizing pressurized nitrogen (N2) as the propellant gas. Additionally, we fine-tuned process settings to accelerate a limited number of particles, facilitating particles impacts on the substrates to study the deposition mechanism. Analytical scanning and [scanning] transmission electron microscopy (SEM) and (S)TEM) played a pivotal role in evaluating the microstructure of our specimens and forming scenarios regarding deposition behavior. Electron backscatter diffraction (EBSD) technique (by conventional and transmission Kikuchi diffraction TKD) was also used to collect crystallographic data from cross-sections of cold-sprayed coatings and to narrate the state of deformation. In-situ nanoindentation mapping (+1600 indents) was performed on the top surface of composite Al-QC coating, embracing both Al and QC phases, to provide detailed micromechanical insight completing the production/microstructure/properties chain.

Results and Conclusions
While QC particles mostly underwent brittle fracture and shattering upon impact, their contribution to in-situ hammering of the coating structure significantly contributed to densification and elimination of pores in the structure. Impact-induced grain refinement close to the exterior of impacted particles and alternation of texture and pattern quality due to high degree of deformation in Al-based matrix was found to be significant. Evidently, these features can endow a reliable bonding between dissimilar constituents in the composite coating structure. Presence of QC fragments turned out to provide enhanced bonding to Al particles within the composites. In addition, hardness and elastic modulus variations were found to be consistent with the heterogeneity of the microstructure induced by particle impacts and their deformation. However, intimate bonding at the interface of Al-QC and formation of interlayer enhanced the coating integrity. The findings regarding the bonding state can potentially justify the enhanced mechanical and tribological properties of composite coatings, and extended retention of the reinforcing phase in the structure under load, as observed in our previous works.

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Keywords:

Electron Microscopy, Aluminum-Alloys, Quasicrystals, Microstructure

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Fast and local determination of phases in (V1-xCrx)2O3 Mott materials

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Poster Group 2

Background incl. aims
The Flash memory technology is getting close to its limit of downscaling and many researches have focused on alternative non-volatile memory technologies [1]. Mott memories, a special case of the broader class of resistive memories, are characterized by localization of their valence electrons owing to strong electronic correlations [2]. An electric field well above the avalanche threshold creates a nonvolatile conductive filament within the Mott insulator such as $(V1\text{-}xCrx)_2O_3$ [3]. Recent µ-XRD analysis of this filament shows that it is made of the same material but with smaller cell volume [4]. With the desired miniaturization of devices, the question of the actual and minimum size of this filament is vivid and requires techniques with nanometer scale resolution. Such resolution is achievable in STEM providing that a spectroscopic technique sensitive to the conductive state of the compound is used. The objective of this communication is to demonstrate that STEM-VEELS, by carefully studying the conditions and limits of the technique, indeed fulfils these requirements.
Methods
Measurements were performed using a Themis Z 80-300 kV (S)TEM equipped with a Gatan 969 EELS spectrometer. TEM lamella were prepared with a Focused Ion Beam (ZEISS, Crossbeam 550L) on V_2O_3 and $(V0.95Cr0.05)_2O_3$ single crystals (Figure a). In order to obtain data on the AFI phase appearing at low temperature in $(V1\text{-}xCrx)_2O_3$ compounds, a cryo sample-holder was used and data acquired at 95 K.
EELS spectra in the low-loss region were acquired at 300 kV with 2-10 nm pixel sizes and 0.2 ms per pixel.
The STEM-VEELS-SI were Fourier-Log deconvolved, carefully processed with PCA and the plasmon peak at around 27 eV was fitted on a limited energy range with a Drude-Lorentz model (Figure b). The precision of the method (<0.1 eV) was checked with respect to the influence of the local thickness, the crystal orientation and the possibility of presence of ice at low temperature.
Results
Our numerous experiments carried out on V_2O_3 single crystals showed that the use of the plasmon peak energy was precise enough to distinguish between the room temperature paramagnetic metal (PM) and the low temperature antiferromagnetic (AFI) phases (Figure c-d). The paramagnetic insulator (PI) and AFI phases of (V0.95Cr0.05)2O3 can also be analyzed with this method. The shift of the plasmon peak can be at least partially explained by the change in the volume density of electrons consecutive to the increase of the cell parameter in the AFI phase at low temperature. The existence/absence of a gap in the electronic structure is proven to have a minor influence on the measurement.
Other experiments on MIM devices show that the plasmon peak energy can be determined at a 5 nm resolution establishing this technique as very suitable to the visualization of (conducting) nanofilaments suggested to explain the resistive transition in these Mott-insulators.

Polycrystalline thin films and MIM devices were also studied before/after resistive switching to gain a better knowledge of the phenomenon when applied to technology relevant systems. The analysis of the considerable changes observed at the local scale will be presented.

Conclusion

By carefully setting-up parameters and experimental conditions, we demonstrated the usefulness of STEM-VEELS spectrum imaging to distinguish, at the nanometer scale, PM, PI, AFI phases in $(V_{1-x}Cr_x)_2O_3$ compounds. These temperature dependent and the ex situ characterizations of the electrical switching are prerequisites to assess which changes to expect for future in situ measurements and consequently progress on this still partly elusive mechanism.

Acknowledgments.

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Keywords:

Mott-insulators, Resistive transition, STEM-VEELS, plasmon

Reference:

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3D fruit microstructure characterization using micro-CT imaging and deep learning-based panoptic segmentation

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Background
Metabolic processes in plants involving transport of water, metabolic gasses, and nutrients are controlled by the three-dimensional (3D) microscopic morphology of the plant tissues. However, imaging and quantifying this microstructure, including the spatial layout of cells, pores (intercellular spaces) and vascular bundles, is a challenging task.
X-ray micro-computed tomography (micro-CT) has been proposed for 3D plant tissue imaging. This advanced imaging technique requires little preparation and resolves easily pore and cell phases due to differences in attenuation. X-ray micro-CT also covers a large field of view compared to other microscopy techniques, thus rendering more representative volumes of interest.
To quantify plant tissue morphology, the tomographic images require extensive image processing, which can become time-consuming and labor-intensive. Cell segmentation in particular is a difficult task because of low contrast in X-ray attenuation at cell-to-cell interfaces.
Deep learning (DL) is increasingly being used for complex image processing tasks in various fields. DL models can be trained to predict semantic labels where each pixel or voxel is assigned to a class label. Cell segmentation, however, is an instance segmentation task, where pixels or voxels of the same class are assigned to separate instances.
This work aims to speed up and improve 3D plant tissue microstructure characterization using X-ray micro-CT imaging and DL-based models for panoptic segmentation, which combines semantic and instance segmentation tasks. The method was developed and validated for pome fruit tissue samples.
Methods
An X-ray micro-CT dataset was collected to develop panoptic segmentation models. This dataset consisted of pairs of conventional and contrast-enhanced X-ray micro-CT images of the same tissue sample. Pear and apple fruit were sampled at three different radial positions. Different cultivars were compared. X-ray projections were acquired using a UniTom HR micro-CT system (Tescan XRE nv, Ghent, Belgium) with voxel resolution of 3 µm for apple and 2.5 µm for pear. For the contrast-enhanced scan after the conventional scan, the tissue sample was carefully unwrapped and incubated in a 10% (w/v) cesium iodide solution for 1 (all pear cultivars and ‘Jonagold’) or 2 h (‘Braeburn’ and ‘Kizuri’) while agitating every 15 min.
After reconstruction, the corresponding 3D images were registered. From the conventional scan, the binary of the cell matrix and pore space were extracted using Otsu’s thresholding. From the contrast-enhanced scan, the individual cell labels were extracted using a semi-automated cell segmentation workflow; the vasculature and stone cells were semi-manually segmented if present. Labeled images were used as ground truth for training the DL algorithm. Data was split into test, training and validation sets making sure datasets were from different fruit. Following the state-of-the art method for cell segmentation, the marker-based watershed algorithm was applied to the binary of the cell matrix as benchmark. As additional benchmark, an instance segmentation model trained on 2D data and enabling 3D prediction by averaging the 2D predictions in all orientations was included, to evaluate the cell segmentation accuracy.

Results
The panoptic segmentation model was able to segment following semantic labels: pore spaces, cell matrix, vascular bundles and clusters of stone cells (brachysclereids, only in pear tissue) and at the same time to predict intermediate representations of the instance labels, i.e. the cells, that allow cell reconstruction in a post-processing step. Thereto, it exploited the 3D extended version of the public domain Cellpose instance segmentation model, which in this study was adapted to a panoptic model after optimizing instance segmentation performance. The original instance segmentation model uses a 3D U-Net architecture to predict gradient map representations of cell instances. Following changes to this network architecture improved the instance segmentation accuracy: addition of long skip connections with direct summation, replacement of the standard building blocks with residual blocks whereby two consecutive residual blocks were implemented per layer, resulting in double the depth of the original 3D U-Net architecture, and retrieval of a style vector using global average pooling on the convolutional maps of the smallest dimension. The 3D model achieved Aggregated Jaccard Indices of 0.788 ± 0.061 and 0.889 ± 0.030 for pear and apple tissue, respectively, compared to 0.732 ± 0.075 and 0.861 ± 0.028 for the 2D model and 0.631 ± 0.134 and 0.715 ± 0.034 for the watershed-based benchmark.
The 2D instance segmentation model was able to recognize vascular bundles and stone cell clusters and exclude them from the volume-of-interest. This demonstrated the potential to expand to panoptic segmentation combining semantic and instance segmentation tasks for the 3D model. However, prediction of the semantic labels was difficult as the dataset was highly imbalanced. From the 810 training samples, 308 contain vasculature and/or stone cell clusters and if these labels were present, the occurrence based on the amount of voxels was much lower compared to the cell matrix and pore space labels. Focal loss was the most appropriate loss functions that learned the model to focus on the vascular bundles and stone cell clusters.
Conclusion
The 3D model succeeded in improving the cell segmentation accuracy over the 2D model and watershed-based benchmark. Cell segmentation remains more difficult for dense pear tissue compared to apple, but the 3D model showed greater improvement for pear tissue segmentation, reducing the difference between tissue types. The prediction of semantic labels is hindered by the large imbalance in the data. Therefore, further training wit data augmentation techniques have yet to confirm how much the focal loss can improve segmentation accuracy of vascular bundles and stone cells.

Keywords:

X-ray micro-CT
Image processing
AI

Reference:

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STEM, PED and EELS: A powerful combination for the investigation of cathode-active-materials for batteries

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Poster Group 1

Background incl. aims
With the ever-increasing demand for high-capacity and high-power energy storage systems, solid-state batteries and their components are subject of intense research [1].The Cathode Active Material (CAM) is the main component contributing to the battery's specific energy as well as its cost [2]. State-of-the-art CAMs are transition metal oxides of the form Li (Ni_(1-x-y) Co_x Mn_y) O_2 [3]. To increase the specific energy and simultaneously decrease the cost of the CAM, high Ni contents above 95% are desired [4]. However, these Ni-rich CAMs suffer from rapid capacity fade due to degradation during cycling, forming a rock-salt-like NiO phase [5]. To detect and understand these degradation processes at the micro- and nanoscale, we investigate $LiNiO_2$ (LNO) sintered at different temperatures as a model system for Ni-rich CAMs utilizing transmission electron microscopy techniques. Here, we demonstrate the effectiveness of combining direct imaging, diffraction, and spectroscopy for investigating LNO secondary particles across various length scales. High Angle Annular Darkfield Scanning Transmission Electron Microscopy (HAADF STEM) can provide high-resolution images at atomic resolution. However, the data acquisition is locally confined to an area of a few hundred nanometers and the differentiation between similar structures of different phases may not always be unambiguous. Complementary, Precession Electron Diffraction (PED) offers detailed information about the phase and orientation of entire CAM particles with a local resolution in the nanometer range, but this information relies on previous knowledge about the expected phases as input. Thus, unknown inter- and mixed phases cannot be identified, which necessitates validation by other measuring techniques. Electron Energy Loss Spectroscopy (EELS) provides insight into the elements present in the CAM and their bonding environment, which effectively complements PED and STEM measurements.

Methods
The lamellae for TEM investigation were prepared by focused ion beam milling with a JEOL JIB 4601F dual-beam system. A beam of Ga ions with varying energies from 30 kV to 5 kV was employed to thin the samples to a thickness below 100 nm. HAADF STEM images as well as the EELS spectra were recorded on a double Cs-corrected JEOL JEM 2200FS microscope operated at 200 kV. The PED data sets were recorded on a JEOL JEM 3010 operated at 300 kV. Scanning and precession of the beam were realized by employing the NanoMEGAS ASTAR and Topspin System.

Results
During our investigation of LNO primary particles using STEM, we observed several regions that cannot unambiguously be assigned to either the layered $LiNiO_2$ phase or the degraded rock-salt-like NiO phase solely based on the high-resolution HAADF STEM images. One example is depicted in the attached Figure. On the bottom, the layered LNO phase in (210) orientation is easily recognizable. In

the center of the micrograph, a structural transformation occurs, resulting in a different structure on the top. However, from the HAADF STEM image alone it is unclear whether it is the layered phase in (211) orientation or the NiO phase in (210) orientation, as both structures exhibit nearly identical atomic positions of transition metals, which are mainly detected in HAADF STEM. In this context, a PED dataset, which correlates recorded diffraction patterns with computed diffraction patterns of the expected phases, can provide valuable information regarding the phase and orientation of the measured particle. A PED orientation map of the investigated primary particle is presented in the attached Figure. Regions of the same color exhibit the same crystallographic phase and orientation. The diffraction patterns of the layered phase better match than those of the NiO phase, confirming that the layered LNO phase is not degraded but is present in a different orientation. However, in addition to the two prominent orientations highlighted in green and orange on the PED orientation map, a thin purple region appears at the surface of the particle close to the vacuum, matching the NiO rock-salt phase. Since the sample's thickness affects which diffraction spots appear in the PED pattern, it is essential to verify whether the recorded pattern results from the presence of a different phase or from a thickness effect at the grain's edge. Moreover, the PED algorithm always matches one of the pre-input phases with the recorded data, leaving uncertainty regarding whether the proposed phase truly exists in the sample or if it resembles one of the matched phases but is actually a new, unknown phase. In this regard, spectroscopic methods such as EELS can complement PED measurements. As EELS is sensitive to the oxygen bonding environment, LNO and NiO exhibit different O EELS signatures. Particularly, the oxygen pre-peak at 527 eV is characteristic of transition metal layer oxides and absent in NiO, serving as a reliable indicator for phase determination. In the observed LNO particles, the O pre-peak is evident in the bulk but absent at the surface of the investigated grain, consistent with the PED results. Representative EEL spectra from both bulk and surface regions are provided in the Figure. By combining locally resolved STEM measurements with PED datasets of the entire secondary particle and spectroscopic measurements, we conclude that degradation of the pristine layered LNO particles occurs not only at the surface of the secondary particle but also at the grain boundaries of the primary particles within the secondary particle. The degree of degradation correlates with the sintering temperature, whereas a higher sintering temperature leads to more severe degradation.

Conclusion

We combined the results of HAADF STEM, PED, and EELS measurements of LNO secondary particles sintered at different temperatures as a model system for the investigation of degradation processes in Ni-rich CAMs. These techniques complement each other exceptionally well, providing a comprehensive understanding of the material's properties beyond what can be achieved through individual measurements alone.

Keywords:

STEM, PED, EELS, CAM, Battery

Reference:

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Quantitative comparison of HRTEM and electron ptychography

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background incl. aims
Electron ptychography in the scanning transmission electron microscope (STEM) has been demonstrated to be capable of providing low-noise phase images of beam sensitive materials at low dose [1]. For such materials, in particular biological samples, conventional high-resolution transmission electron (HRTEM) is the most widely used approach - usually cryo-TEM. The question then arises of whether ptychography or HRTEM offers the most dose-efficient imaging approach. While resolution can be a useful measure for comparing imaging techniques, it is dependent on the electron dose. For some electron microscopy techniques, a phase contrast transfer function (PCTF) can be defined to quantify the technique's performance with respect to spatial frequency. However, the PCTF also does not account for the dose used and is not uniquely defined for common electron ptychography techniques like the Wigner distribution deconvolution (WDD) method. In this work we introduce the detective quantum efficiency (DQE), applied to electron microscopy as a dose independent and sample independent measure of technique performance.
Historically, the DQE has been used as the ultimate performance measurement of linear systems [2] ranging from electron detectors to medical imaging systems. If the incoming noise is pure Poisson noise, it can be calculated by dividing the signal to noise ratio of the system output squared ($SNRout^2$) by the signal to noise ratio of the system input squared ($SNRin^2$). SNRin also represents the signal to noise ratio of an ideal imaging system at the same dose. In this work the ideal TEM is defined as fully coherent HRTEM phase contrast imaging with an ideal Zernike phase plate. The SNRout represents the signal to noise ratio of the various electron microscopy techniques studied. Even though the signal to noise ratio is dose and sample dependent, the DQE is not. The DQE can be thought of as the fraction of incoming quanta contributing to the image.
Methods
For the calculation of the empirical DQE, simulations were performed using the MULTEM package [3]. All 4D-STEM simulations assumed an 80keV beam energy with a probe step size of 0.15 Å and a semi-angle of convergence angle α of 22.5 mrad on a 301 by 301 grid of probe positions. A detector size of 128 by 128 pixels was assumed. Reconstructions were performed using the single sideband (SSB) method, the Wigner distribution deconvolution (WDD) method and the integrated centre of mass (iCOM) method. As a comparison, simulations were also performed for high resolution transmission electron microscopy (HRTEM). The reconstruction methods were evaluated on coherent and partially coherent datasets containing 500 noise realisations each at a dose of 4.4M e/Å². Partial coherence was simulated through the introduction of a chromatic envelope with Cc = 1.1mm and an energy spread of 0.4eV leading to a defocus spread of 5.5 nm.
The ground truth was defined as the image from a fully coherent, aberration free HRTEM phase contrast image. For the purpose of calculating the empirical DQE of the different methods, a single carbon atom served as a sample because it provided a continous Fourier transform.
Results
The SSB and WDD electron ptychography methods reach a maximum DQE of around 23% as shown in the figure. The HRTEM reaches a maximum DQE of 100% at low spatial frequencies. However,

through the introduction of a defocus spread of 5.5 nm, a rapid decay in the DQE of the HRTEM is observed. The DQE of the HRTEM decays to almost 0% at spatial frequencies above 1α while the DQE of SSB and WDD remains substantial up to 2α.
It can also be observed that the DQE of both SSB and WDD shows almost no change through the introduction of the chromatic envelope.
The DQE of iCOM follows the same shape as that of SSB and WDD. However, across all spatial frequencies it is 3-5% below that of SSB reaching its maximum at around 18%. It is interesting to note that even with partial incoherence introduced SSB outperforms fully coherent iCOM.
Conclusion
In this work we have successfully defined a dose and sample independent framework in which HRTEM and electron ptychography can be compared against each other using the DQE. We showed that in the absence of partial incoherence, an HRTEM can achieve a DQE of 100% while SSB and WDD ptychography have a maximum of around 23%. However, the introduction of partial coherence shows the weakness of the HRTEM. Considering a defocus spread of 5.5 nm the DQE of HRTEM remains close to 100% at low spatial frequencies but decays rapidly at higher spatial frequencies.

Keywords:

Ptychography, HRTEM, 4D-STEM

Reference:

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Multimodal OF2i-Raman – A novel high-throughput, single particle analysis method in liquids

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Poster Group 1

Background incl. aims
The efficient detection and analysis of micro- and nano-particles is a topic of increasing importance, both due to their wide spread use in industrial process and the increasing issue of environmental pollution, especially with micro- and nanoplastics [1]. We specifically focus on the analysis of micro- and nano-particles in liquids, most commonly water. Here we aim to combined the advantages of microscopy based methods, which typically measure on the “single-particle” level, but require sample preparation and can be cumbersome to use for the measurement of a larger number of particles, with the low sample preparation requirements and high-throughput of ensemble based methods [2]. To this end, we combine OptoFluidic Force Induction (OF2i®) with a Raman microscopy based setup. OF2i® uses a laser beam to trap particles inside a flow cell and determines both particle sizes and concentration by observing the scattered light and acceleration of single particles through the measurement cell with an ultramicroscope setup. Raman microscopy can determine the composition of both organic and inorganic particles (but not metals), by observing the inelastically scattered light of a laser focused onto a particle. A multimodal OF2i®-Raman setup can therefore provide the best of both worlds, allowing for high-throughput “flow-through” measurements with little to know sample preparations, whilst providing analytical information on the single particle level.

Methods
The multimodal OF2i-Raman setup is based on a conventional OF2i instrument (BRAVE B-Curious). The conventional OF2i consists of a 2D optical trap in a microfluidic flow channel and an ultramicroscope observing the elastically scattered light perpendicular to the laser propagation direction [3]. The ultramicroscope is modified into two beam paths (elastic and inelastic scattering), with the elastic path functioning as a regular OF2i and the inelastic path is coupled to a Raman spectrometer. The Raman spectrometer is operating in a mode similar to “line-illumination” imaging mode in Raman microscopy, thus allowing for the simulations Raman measurement of particle spectra along the particle flow direction. For the optical trap a linearly polarized laser (wavelength: 532nm/power up to 2W) is used either as a Gaussian beam or a doughnut shaped Laguerre-Gaussian.

Results
We have consturcted a OF2i-Raman setup prototyp and have achieved a prove of principle of the setup for the detection of both organic (polystyrene, see graphic) and inorganic (TiO2) particles. In order to achieve the highest possible sensitivity in the Raman measurements we had to modified the standard OF2i-approach, which uses a laser beam propagating in the flow direction to accelerated the trapped particles, to one where the laser beam is propagating against the flow direction, thus slowing down the particles and allowing for longer accquisition times in the Raman. For challaning particles (small particles, small Raman crosssection) this approach also allows us to fully trap specific particles, in the focus of the Raman-setup. Furthermore, the modified OF2i-Raman-approach has been used for hyphenation with ICP-MS [4].

Conclusion
With our novel approach combining OF2i and Raman microscopy, spectroscopic analysis of micro- and nanoparticles, on the single particle level, is possible with high-throughput, directly in water (or other liquids) and minimal sample preparation. We are thus convinced that this approach can contribute to the growing research into environmental pollution with microplastics as well as other fields of research on the topic of micro- and nano-particles. In addition, the potential of our new approach for further hypenation with complementary analytic techniques has already been demonstrated [4] and we are convinced that combing different analytical techniques in a seamless way is the way forward towards a universal analytical tool box for nanoparticles.

Keywords:

Multimodal, Raman, Particle analysis, Microplastic

Reference:

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[5] The authors are deeply grateful to project FFG Bridge 895429 Nano-VISION for funding.

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A Real Space Understanding of Short Range Order in Disordered Rocksalt Cathodes

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Poster Group 1

Background
Disordered rocksalts (DRS) have a rocksalt structure, with the disordered nature relating to compositional disorder, predominately on the cation sublattice, which generally contains lithium and some combination of transition metals. In some instances, disorder can also be present in the anion lattice when fluorine is added to the composition.
They have emerged recently as an alternative to layered cathode structures in Li-ion batteries. These materials have attracted commercial and scientific interest because of their potential to enable fully cobalt-free, high-energy density cathodes [1]. DRS cathodes do not have a well-defined layered structure through which lithium can move in and out of the material as in most well-established commercial materials. Instead, lithium is understood to percolate through the material by hopping from one octahedral site to the next, via the tetrahedral interstitials. The energetics of the tetrahedral interstitials are proposed to be related to the surrounding ions [2]. This process can only occur when a network of such sites is formed on a length scale matching the particle size. Short-range order (SRO) and its inherent correlation with local arrangements of atoms play a critical role in determining the ability of lithium to migrate reversibly through the material [3]. This study aims to demonstrate the capability of STEM imaging methods to provide insights into the local arrangements of atoms associated with the SRO.

Methods
The presence of highly structured diffuse scattering in electron diffraction patterns, such as that observed in Figure 1a, has been widely used to indicate the presence of SRO in these materials [3]. Still, to the best of our knowledge, no precise understanding of the nature of this ordering has been achieved. Diffuse scattering of a similar nature has been observed in other materials understood to contain SRO; and is widely discussed as being related to correlations of lattice sites associated with polyhedral geometries, such as the octahedral cluster depicted in Figure 1b. These polyhedra are understood to correspond to three-dimensional loci in reciprocal space, from which the electron diffraction of a particular reciprocal lattice plane can be understood as the intersection of the lattice plane with the three-dimensional loci, illustrated in Figure 1c [4]. However, electron diffraction does not give precise local information about the distribution of the ordering within a particle nor does it identify the composition of the correlated clusters. The Z-contrast nature of annular dark field (ADF) imaging is demonstrated to be sensitive to the SRO by the presence of the diffuse scattering features in the Fourier transform of ADF images (Figure 1d and 1e).

Results
In this study, we develop a method of understanding these features by suppressing contrast from the Bragg reflections in the lattice from ADF images which show intensity variations in neighbouring atomic columns. Extending on this, we demonstrate a real-space method based on calculating the inverse Fourier transform of the square modulus of the Fourier transform, which we refer to as a

correlograph, to identify the nature of the correlations in real space, as demonstrated in Figure 1f. The inset region in Figure 1f shows the correlograph and identifies both positive and negative correlations on a filtered image where the lattice contrast has been suppressed.

Conclusions

The method proposed in this study allows distinct differences in the SRO to be interpreted from the ADF images that arise from composition variations. These differences in terms of the homogeneity of the SRO and composition of the clusters can't be distinguished from the diffraction data alone. This method enables us a new understanding of the SRO and, in turn, a link between the structural units of the SRO and the ability of the materials to sustain lithium percolation.

Keywords:

Disordered-Rocksalts
STEM
Energy-Materials
ADF

Reference:

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5D-ToF-STIM Hyperspectral Imaging with a keV He+ Focused Ion Beam

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Scanning ion microscopy in combination with transmission ion energy-loss spectroscopy (IELS) for low-energy ions is a promising field of study, which seeks to provide complementary information to that of more conventional techniques like electron energy-loss spectroscopy (EELS) in scanning transmission electron microscopy (STEM). In contrast to electrons, ions are capable of both capturing and losing charge, allowing for charge exchange and neutralization processes [1]. In addition, while primary electrons are capable of exciting plasmonic and electronic states, as well as ejecting core-shell electrons, ions are further capable of temporarily merging their electron orbitals with those of the sample's atoms (potentially ejecting ion-induced Auger electrons), as well as ejecting sample atoms (measurable using mass spectrometry), creating unique signals not available to electron beams. The energy lost by the ions during these sample interactions can be measured as an increase in the time-of-flight (ToF) from the sample to the detector. Furthermore, the scattering pattern seen on the detector contains detailed information not only about the crystal structure, but also the trajectories of the ions within that structure [2]. By combining spatially resolved ion energy-loss and scattering information, we can thus create a rich 5D dataset: 2D position on the sample plane, 2D position on the detector plane, and finally ToF information for each ion or neutral collected. He+ ions are chosen for this work as they are particularly well suited to low-energy transmission ion microscopy due to their relatively small scattering cross-section (allowing for thicker samples) and their relatively low damage to the sample.
Our work at LIST focuses on low-energy (up to 30 keV) scanning transmission helium ion microscopy (STIM) coupled with ToF to analyze the angular and energy distribution of the transmitted helium ions and neutrals. Two prototype instruments are being developed: one based on a commercially available gas-field ion source (GFIS), which can achieve sub-nm probe sizes, and a second based on a commercially available DuoPlasmatron source with a probe size of <300 nm. Each prototype is equipped with a high-sensitivity microchannel plate (MCP) with a delay-line-detector (DLD), capable of measuring the exact location and timing of single ion or neutral impacts. However, due to the complexity of modifying the GFIS column to perform beam pulsing, it is not yet capable of TOF measurements (i.e. only capable of 4D datasets), while the DuoPlasmatron source comes with pulsing/blanking plates. The DuoPlasmatron based prototype also has a high flexibility in the instrument configuration, allowing for post sample ion deflection to determine the fraction of ions neutralized within the sample, as well as the ability to vary the post-sample flight distance.
Using a previous iteration of the DuoPlasmatron system, we have been able to create images by generating contrast from the total counts, the ratio of neutrals to ions, the scattering angle, and the energy loss (other criteria could also be envisioned with such a rich dataset). As each pixel in the sample image contains a full ToF spectrum and a scattering pattern, we have developed python software based on matplotlib and pandas with a graphical user interface for interactive plotting of sample images, detector images, and ToF spectra based on flexible combinations of ROI selections, in

order to intuitively explore the 5D dataset. We have also compared our experimental scattering angle and energy-loss results from this instrument to SRIM simulations [3].
In parallel with the development of these instruments, we have also performed stationary-beam transmission ion experiments at the mature time-of-flight medium energy ion scattering (ToF-MEIS) beam line at Uppsala University, where extremely fast blanking speeds (below 1 ns) and higher accelerating voltages (up to 350 kV) are possible (Rev. Sci. Instrum. 83, 095107). Here we have seen differences in both the scattering pattern and ion energy distribution based on the direction of the beam passing through a thin single-crystal Si film with an amorphized layer on one side. In the case where the ions pass through the amorphous layer and then the crystalline layer, we observe a distinct crystalline scattering pattern, but a relatively broader energy distribution. For the inverted sample, where the ions instead pass through the crystalline layer first, we observe an apparently random scattering pattern, but a relatively narrower energy distribution. Ion channeling simulations performed with IMSIL [4] at TU Wien corroborate the dependence of both the energy and scattering distributions on the orientation of the sample with respect to the beam. These results are also in agreement with the simulation and experimental results of Holenak et al. demonstrating that a disordered surface layer can redirect incident ions into planes and channels, instead of randomizing their trajectories [5].
The authors acknowledge funding from the Luxembourg National Research Fund (FNR) AIMSTHIM2 (C21/MS/16215748) and STHIM (C16/MS/11354626), for the experimental work performed at LIST, as well as funding from the EU Horizon 2020 programme through the RADIATE project for the experimental work performed at Uppsala University.

Keywords:

Time-of-Flight, Ion Beams, Energy-Loss Spectroscopy

Reference:

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Atomic scale evolution of Ru nanoclusters on graphitic carbon nanofibers in NH3 decomposition reaction

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Abstract
Background incl. aims
Ammonia dehydrogenation is the key process for completing the ammonia-based clean energy production-utilization cycle, and Ru/CeO2 has been known as one of the most active catalysts for ammonia decomposition reaction. Here, the catalytic activity of Ru/graphitic carbon nanofibers (GNFs) not only shows higher activity compared with Ru/CeO2 under the identical condition, but also continues to increase gradually over the time. Understanding the evolution of Ru nanocluster on GNFs (Ru/GNFs) is necessary for developing highly active and durable catalysts for ammonia decomposition reaction.
Methods
Loading of the catalyst onto the Transmission Electron Microscopy (TEM) grid for the Identical Location Scanning TEM (IL-STEM) experiments was carried out as follows: suitable GNFs was drop-cast on a holey carbon-coated Au grid (Agar scientific, H7 finder grids), where Ru nanoclusters were then deposited onto GNFs coated Au grid by magnetron sputtering. Samples are imaged by a JEM-2100F TEM (JEOL, Japan) operated at 200 kV, which is equipped with a spherical aberration (Cs) probe corrector for STEM (CEOS, Germany). The probe convergence angle is 19 mrad and the collection angle range of the annular dark field (ADF) detector is set from 31 to 82 mrad. aberration-corrected STEM (AC-STEM) images are captured with an electron probe size of 8 C and a pixel dwell time of 38 µs with a scanning area of 1024 × 1024 pixel. The bright field (BF) detector is also used in parallel. X-ray photoelectron spectroscopy (XPS) data was collected using a Kratos AXIS Ultra DLD instrument using monochromated aluminium Kα emission at 120 W and a Thermo Scientific K-Alpha X-ray spectrometer with a monochromated aluminium source at 1486 eV.
Results
A series of AC-STEM images of Ru nanoclusters at the identical location GNFs under different conditions have been recorded to track the evolution of the Ru nanoclusters. It clearly shows the sizes of Ru nanoclusters are relatively small and there are some single atoms exist in as-prepared material. After reduction process, the fraction of single atom increased, and the nanoclusters grown with increased size variation. After 3 hours of the ammonia decomposition reaction, the single atoms seem absorbed into clusters. To quantify the evolution of Ru nanoclusters, the images have been analysed with a custom python program. The average size of the Ru nanoclusters increases after the reduction process, from around 1.04 (as prepared) to 1.34 nm, and after 3 hours of the ammonia decomposition catalysis it still maintains around 1.34 nm. The size evolution suggests that the Ru nanoclusters agglomerate during reduction process to reduce the surface energy. Additionally, the

nearly identical size of Ru nanoclusters after reduction and under ammonia decomposition reaction indicates that the Ru nanoclusters are stabilized by GNFs.
After reduction, the Ru nanoclusters show hexagonal close-packed (hcp) structure, which is maintained after 3 hours catalytic process. The development of the crystallinity of the Ru nanoclusters during the heating treatment is also validated by IL-STEM observation. The crystallinity of diffuse, almost amorphous Ru nanoclusters increases only slightly after the reduction process, and significantly more after 3 hours of catalysis. To quantify the crystallinity of the clusters, relative intensities of the strongest reflection spots in FFT of the images of different nanoclusters are analysed. The intensity is found to be the strongest for the crystalline, medium for semi-crystalline and low for amorphous forms of the clusters. The crystallinity of Ru nanoclusters during the reduction treatment has shown a general tendency of increasing and further enhancement after 3 hours of catalysis.
As the catalysis of the ammonia decomposition reaction proceeds for a longer period (12 hours), the ratio of single atom decreases further compared with 3 hours, while the average diameter of nanoclusters has not increased further after a longer catalysis time. The development of the hcp structure of the Ru nanoclusters after 12 hours is also validated by AC-STEM observation.
The strong binding energy of nitrogen on metals means that the rate-determining step in the ammonia decomposition reaction is recombination N atoms to N2, while the hcp Ru exhibits the lowest activation energy for the N2 desorption reaction, so it possesses the highest activity compared to other structures for low temperatures ammonia decomposition reaction. By activating the Ru/GNFs catalyst during the reaction process, the Ru nanoclusters evolve into faceted hcp structures, which lead to increased density of B5 sites (catalytic active sites with favourable electronic and geometric properties for rate-determining step). The electronic density on the Ru nanoclusters also increases during the catalysis, which has been indicated by the clear evidence of N present in the spent catalysts.
Conclusions
The Ru/GNFs, where Ru nanoclusters are deposited onto GNFs by magnetron sputtering, has shown as a highly active and self-improving catalytic behaviour for low-temperature ammonia decomposition reaction. A series of AC-STEM images of Ru nanoclusters at the identical location GNFs at different stages of the reaction process have been recorded to track the evolution of the Ru nanoclusters at the atomic level. The data shows that activation of the as-prepared Ru nanoclusters on GNFs at 450 °C induces a structural change from amorphous nanoclusters to the faceted hcp morphology, increasing the density of B5 sites, which are known to serve as active sites and exhibit high activity in ammonia decomposition reaction. This study demonstrates that GNFs stabilize the Ru nanoclusters and B5 sites in hcp structure which is crucial role for the enhanced catalytic activity. These results may open a path for designing highly active and durable catalysts for ammonia decomposition reaction.

Keywords:

STEM; Ruthenium; Nanoparticles; Catalysis; Carbon.

Reference:

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Improving Control Signals for Interference Gating

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Poster Group 2

Background incl. aims
Time-resolved methods in transmission electron microscopy have become increasingly important in recent years. For off-axis electron holography, one of the most established phase-resolved methods in electron microscopy, interference gating (iGate) [1], has proven to be the most effective implementation for dynamic studies, achieving a time resolution of 25 ns [2]. It is based on the targeted elimination of interference patterns for a large part of the period, synchronized with a periodic process to be investigated. By disturbing the interference pattern with uniformly distributed phase shifts between object and reference wave, the interference pattern is smeared, while the measurement remains undisturbed for a short time (gate), allowing a time-resolved interference pattern to build up.

Methods
Based on theoretical approaches to describe the contrast-influencing properties of gating signals, a novel approach for square-wave-based control signals is developed and presented. The basic idea is that to cancel the interference it is sufficient to superimpose two identical interference patterns, which are shifted only by a constant phase shift of exactly pi to each other. This can be realized by simple square wave-based signals, thus avoiding the signaling problems associated with complex signal shapes (e.g. noise-based control signals) as used so far. The main advantages of this approach are, on the one hand, that such signals can be realized with common electronics (e.g. fast square wave generators) and, on the other hand, that the improved control signal requires a significantly lower amplitude to cancel the interference pattern. The presented adaptations are tested by means of time-resolved electron holography on a nanostructured coplanar capacitor at the FEI Titan 80-300 Berlin Holography Special.

Results
Compared to the noise-based control signal, the developed square wave-based control signal provides matching results when sampling a sawtooth signal applied to the capacitor at a time resolution of 100 ns. Due to the advantages mentioned above, it is already possible to achieve a time resolution of less than 2 ns using a conventional digital pattern generator (DG2040). With this it is possible to investigate the switching behavior of the capacitor at a repetition rate of 50 MHz. The attached figure shows the slope of the time-resolved phases (orange). Compared to the expected switching behavior (dashed green line), these reveal overshooting, which is most likely caused by signal reflections.

Conclusion
According to the results, the square wave-based control signal proves to be superior to the previous noise-based control signals and will be indispensable in future implementations of interference

gating, particularly due to its ease of implementation. Time resolutions in the picosecond range are now within reach thanks to this innovation.

Keywords:

Electron Holography, Temporal Resolution, iGate

Reference:

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Anisotropic van der Waals Epitaxy and Sliding of CsPbBr3 Nanoplatelets on ReSe2

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Poster Group 2

Background
Dimensionality and size profoundly influence material properties, driving diverse functionalities and applications. Current nanomaterials research focuses on specific dimensionalities like fullerene (0D), carbon nanotube (1D), graphene (2D), and graphite (bulk 2D), each offering distinct traits. Combining materials with different dimensionalities can unlock new functionalities, leading to "mixed-dimensional heterostructures." The crucial role of interfaces in these structures often lacks precise control.
We aim to utilize two-dimensional layered materials as substrates for growing nanostructures through van der Waals (vdW) epitaxy. This approach targets mitigating strain and stress issues inherent in conventional covalent epitaxy.[1] By creating well-defined mixed-dimensional heterostructures, we aim to enhance the heterostructure functionalities.[2] Here, we present the study of the growth of $CsPbBr_3$ perovskite nanoplatelets on low symmetry layered $ReSe_2$.
Methods
The mixed dimensional heterostructure of $CsPbBr_3$ on two-dimensional $ReSe_2$ was synthesized in a home built chemical-vapor-deposition system composed of a three-zone tube furnace. The structural and chemical characterization of the heterostructure was done by scanning and high-resolution electron microscope (Sigma 500 SEM, Zeiss and HRTEM-Themis Z, respectively). Band alignment characterization was done by X-ray photoelectron spectroscopy. Nanotribology studies were performed using AFM and in-situ combined AFM-SEM system.
Results
Mixed dimensional heterostructure of $CsPbBr_3$ platelets on $ReSe_2$ shows the growth of well-aligned and elongated $CsPbBr_3$ nanoplatelets. The optoelectronic properties the mix-dimensional were characterized, revealing type-I band alignment. In addition, tribological studies[3] were conducted by monitoring the force required to push the nanoplatelets along the surface. This revealed an anisotropic sliding effect when pushing rectangular platelets along their long and short axis. This effect is correlated with the commensurability of the two lattice structures in each direction, as revealed by HRTEM analysis. Sliding along the surface can also be hindered by atomic steps and defects, which are easily detected by scanning electron microscope. Therefore, sliding experiments were performed in situ in a combined AFM-SEM system and were compared to an ambient AFM system. These comprehensive experiments enabled a detailed view of the surface and $CsPbBr_3$ platelets before and after sliding, providing a rapid overview of the sample.
Conclusions
The mixed dimensional heterostructure of $CsPbBr_3$ platelets on $ReSe_2$ shows well-defined epitaxial relations. The heterostructure interface was probed to study the charge transfer mechanism and the epitaxial relations. The anisotropic growth of the platelets manifested itself in both TEM characterization and tribology experiments, revealing an anisotropic sliding effect of the $CsPbBr_3$ over the $ReSe_2$. Overall, this work presents a route for guided growth of mixed-dimensional

heterostructures using vdW epitaxy and characterizes the vdW bonding nature within the heterostructure.

Keywords:

Mixed-dimensional hererostructures, vdW epitaxy, nanotribology

Reference:

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η-Carbides (Co, Mo, or W) Nanoparticles from Octacyanometalates Precursors-Based Network

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Poster Group 2

Noble metal nanoparticles such as Au and Pt are well known and widely used for their catalytic properties. Because of the cost of these noble metals, different strategies are pursued to find surrogates to noble metals in catalysis. Emerging and promising approach is to play with NPs of transition metal carbides. Among the candidates for these substitutions are WC NPs [1] ,Mo2C nanoparticles [2] or Co6Mo6C2 combined with graphitic carbon for oxygen reduction. The aim of this study was to develop a route for synthesizing bimetallic carbides NPs in two-step process and the originality of this approach lies in the choice of the metallic precursors.
Octacyanometalate-based networks (OCMs) are 3D metal networks in which the metals are linked together by CN bridges. A water-based surfactant-free synthesis was shown to be applicable for the synthesis of PBAs [3]. It is versatile enough to get networks composed of the combination of any transition metals of our choice. The desired 3D compounds are obtained by mixing a solution of cyanometallates involving one kind of metal (M' = Mo or W) with a solution of another metallic salt of the second metal (M = Co). To turn the metalate network into metallic carbide, the OCMs undergo heat treatment, under inert atmosphere as the procedure used by Aparicio et al [4].
In bimetallic particles, different types of η-carbides-type structures were evidenced in our sample. Co3Mo3C, Co6Mo6C, and Co2Mo4C from the Co-Mo-C system. Co3W3C (fig 1), Co6W6C, Co4W2C, and Co2W4C from the CO-W-C system. These compounds were identified by combination of Transmission Electron Microscopy, high resolution STEM/HAADF imaging of individual NPs (fig A), EDS analysis (fig B-C), and compared simulated (fig D) and experimental HAADF-STEM image (fig E). The size of these carbide nanoparticles is 5 nm in average. Along with η-carbides, the presence of monometallic carbide nanoparticles such as Mo2C and WC has also been observed. The pyrolysis temperature is an important factor in the formation of nanoparticles. Indeed, a treatment at a too low temperature, < 700 °C for Co-Mo system and 800 °C for Co-W system will not enable the complete transformation of the precursors. Beyond these temperatures, η-carbide nanoparticles account for the majority of the nanoparticles observed (> 60%).
Co3M'3C, Co6M'6C, Co2M'4C (M' = Mo or W), and Co4W2C, metallic carbide nanoparticles were successfully synthesized by thermal decomposition of Cs-Co-M' precursor octacyanometalate networks. Their presence in significant proportions in a definite temperature range attests for the efficiency of the synthesis route by thermal decomposition of OCM. The strength of this method also lies in the wide choice of metals that can be used to fabricate octacyanometalate-based networks and thus potentially the wide choice of carbides nanoparticles that could be made easily and rapidly accessible

Keywords:

eta-carbides, STEM/HAADF, catalysts

Reference:

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Electron Microscopy of Novel Lithium Alloy Anodes for Solid State Batteries

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
All-solid-state lithium-ion batteries offer higher capacities and improved safety compared with batteries that make use of a liquid electrolyte in part because of their potential to utilise a lithium metal anode and a solid ceramic electrolyte. Changing to a solid-state electrolyte removes many of the safety concerns surrounding lithium-ion batteries that are associated with the volatile and flammable liquid electrolytes currently used. An initial lack of understanding of the mechanical and diffusion properties of lithium metal means attempts to use pure lithium metal as the negative electrode (anode) have failed at the high currents and rates required for powering electric vehicles (EV). In this work we aim to solve these issues through alloying lithium to produce anodes with novel microstructures that can achieve the currents and rates required for EV application. Understanding how these new materials function as anodes requires characterisation of the behaviour of lithium entering and exiting the material, requiring microscopy up to atomic resolution with spectroscopy [1]. We study three lithium alloy systems (Li-Ca Li-Sr, and Li-Ba) for which no transmission electron microscopy has so far been reported in the literature.
The overarching aim of this work is to develop a deep understanding of the materials properties and mechanisms behind the premature short-circuiting and failure of solid-state batteries. A proposed route for initiating dendrite growth that leads to short-circuit failure is a non-uniform electric current at the anode-electrolyte interface caused by void formation during stripping [2]. We propose an alloy anode material as a solution that on stripping will retain full interface contact between the anode and electrolyte during cycling thus stopping void formation at the interface between the electrode and electrolyte. This is achieved by producing an anode from a two-phase material consisting of the lithium metal, to be cycled, and a lithium intermetallic that remains as a three-dimensional scaffold to provide both electrical and ionic contact between the current collector and the solid electrolyte throughout cycling. This concept was shown to be viable by Jia et al [3] electrochemically cycling the Li-Ca system with a liquid electrolyte. However, development of these alloys requires a full understanding of the mobility and bonding of lithium through the distinct phases and the microstructures produced by manufacturing and how those change with cycling.

Methods
A combination of plasma focused ion beam milling and scanning electron microscopy energy dispersive x-ray characterisation are used for the identification of the two-phase material on a larger scale and to identify regions of interest for the production of transmission electron microscopy lamella. Given the air sensitive nature of these materials, anaerobic sample preparation and transfer was used between instruments. Transmission electron microscopy and electron diffraction are used confirm the creation of the desired phases. Like most battery materials these lithium containing phases and the lithium metal that surrounds them are highly beam sensitive. Preliminary data shows that the pure lithium metal phase loses crystallinity (as observed by disappearance of electron diffraction spots and directly imaged lattice planes) when illuminated with a dose rate greater than 6 e/A2/s.

Results
Energy dispersive X-ray spectroscopy shows the material as cast has two distinct phases one containing the group two element and one without. Standard EDX spectroscopy cannot detect lithium meaning that the composition of alloys cannot be determined at this step and additional techniques are required for phase characterisation. Transmission electron diffraction from extracted lamella shows that the anode materials formed have two distinct crystallographic phases, in each case containing a lithium metal phase and a lithium intermetallic. The three intermetallic phases studied range in complexity from large face centre cubic (Li23Sr6 F m -3 m) to large hexagonal (CaLi2 and Li4Ba P 63/m m c). The microstructure varies dependent on the alloying element and the local composition during solidification with the width and homogeneity of the scaffold phase reducing with reduced group two element concentration. Preliminary cycling data shows that the material is capable of working as an anode and characterisation shows that the intermetallic scaffold remains after lithium has been stripped from the electrode. The graphic shows that the removal of the lithium metal is observed to be preferential to the conversion of the intermetallic and voids form first in the metal regions closest to the electrolyte. In the graphic the voids are highlighted in red at the top of the anode after a partial strip furthest from the stainless-steel current collector interface denoted in green. In each of these PFIB cross section images the dark regions are lithium metal and the light regions left behind are the LiCa2 intermetallic. Electron diffraction from lamella produced from these cycled anodes shows that the calcium containing regions have not changed phase compared with that observed in the uncycled material.

Conclusion
This work acts as the first step towards producing an anode for all solid-state batteries that is resistant to failure during cycling due to loss of ionic and electronic pathways at the interface. The knowledge gained here will direct future anode development that will be the foundation of successful creation of a commercially viable all-solid-state battery for electric vehicles.

Acknowledgements
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Graphic caption:
i) pristine as cast lithium calcium two-phase anode, ii) partially stripped material, iii) fully stripped anode. The dark phase in each image is lithium metal and the light phases are lithium calcium intermetallic (the material below the green line is the stainless-steel current collector).

Keywords:

lithium alloy anodes, solid-state batteries

Reference:

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Unveiling the Optoelectronic and Thermal Properties of SnSe Polycrystals via EELS

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Poster Group 1

Tin Selenide (SnSe) has become a globally focused research topic in the field of thermoelectricity thanks to its low thermal conductivity and high electrical transport performance, which combined result in remarkable thermoelectric efficiencies. In its crystalline form, SnSe suffers from poor mechanical properties, rigid controlled crystal growth conditions and high production costs [1]. This has led the research to focus instead on SnSe polycrystals which, although less performing with respect to their crystalline counterpart, have great potential for significant efficiency enhancement [2]. A potential strategy to overcome the performance degradation, mainly related to the higher thermal conductivity of Tin oxide (SnOx) which percolates in the material through the grain boundaries, is by selective doping [3]. Here, we prove the effectiveness of Na doping in limiting the formation of SnOx and substituting the oxide at the grain boundaries in form of coherent precipitates through Scanning transmission Electron Microscopy (STEM). The ultralow spatial resolution imaging (~1Å) achieved with the aberration corrected STEM Spectra 300, combined with EDX spectroscopy, allows to map the elemental distribution at the grain boundary with atomic resolution, highlighting the presence of Na confined within the boundary.
The effect of the Na inclusions on the electronic and vibrational properties of SnSe polycrystals is further investigated through Low-Loss EEL Spectroscopy. Monochromated operation on the beam accelerated at 60kV, combined with low beam currents (~70pA) and convergence and collection semi-angles of 30 and 20 mrad, respectively, allow to collect the forward-scattered and higher momentum transfer signals in the first Brillouin Zone. From here, the information on the bandgap together with the plasmonic and phonon signals can be extracted for each probe position. In the specific, bandgap fitting across the doped grain boundary is supported by a customized Python algorithm [4], which highlights the decrease in the pure SnSe bandgap value due to the Na inclusions, in accordance with the DFT calculations. Further investigation on the vibrational signals within the low range of the EELS spectrum is performed to evidence the phonon modes that appear when in proximity of the grain boundaries. The specific vibrational-modes mapping will in the future enable the acquisition of phonon dispersion at grain boundaries, interfaces, edges, and various nature defects unlocking a powerful tool for thermoelectric performance evaluation at the atomic scale.

Keywords:

Thermoelctricity, EELS, Bandgap, Phonon modes

Reference:

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4. Nature Communications 13.1 (2022): 4089.

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Quantitative comparison between the diffuse scattering from three-dimensional electron diffraction and single-crystal X-ray diffraction

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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

Background incl. aims
In contrast to perfectly periodic crystals, materials with short-range order produce diffraction patterns that contain both Bragg reflections and diffuse scattering. In this talk, we will show a quantitative comparison between three-dimensional electron diffraction (3D ED) and synchrotron single-crystal X-ray diffraction, both for the Bragg reflections and the diffuse scattering. The thermoelectric material Nb0.83CoSb was chosen as a reference material.
Methods
3D ED allows the study of nanometre-sized crystals, which are too small to be studied with single-crystal X-ray diffraction. The average structure (occupancies and atomic positions) was refined from the Bragg reflections, whereas the local structure (the vacancy distribution) was refined from the diffuse scattering. A model of the short-range order in Nb0.83CoSb was created by assuming that nearest and next-nearest neighbour vacancies avoid each other. The correlations between the first and next-nearest neighbour vacancies were refined using both a Monte Carlo refinement in DISCUS [1] and a three-dimensional difference pair distribution function (3D-ΔPDF) refinement in Yell [2].
Results
We found that diffuse scattering data used for quantitative analysis are best acquired in selected area electron diffraction (SAED) mode using an energy filter. Both the average and the local structure could successfully be refined from both the 3D ED and synchrotron single-crystal X-ray diffraction data acquired on Nb0.83CoSb. Fig. 1 shows that a good agreement was achieved between the simulated and the experimental intensity distribution of the diffuse scattering. The higher R-value for 3D ED compared to single-crystal X-ray diffraction is likely due to residual multiple scattering. The model of the short-range order in Nb0.83CoSb can easily be applied to determine the short-range order parameters in other materials with similar diffuse scattering, such as the lithium-ion battery cathode material LiNi0.5Sn0.3Co0.2O2.
Conclusion
The diffuse scattering in 3D ED data can be obtained with a quality comparable to that from single-crystal X-ray diffraction. Short-range order parameters could successfully be refined from the diffuse scattering in 3D ED data. As 3D ED requires much smaller crystal sizes than single-crystal X-ray diffraction, this allows to refine short-range order parameters in many technologically relevant materials for which no crystals large enough for single-crystal X-ray diffraction are available.

This research was funded by the Research Foundation Flanders (FWO) (grant No. G035619N).

Keywords:

3D ED, diffuse scattering, thermoelectrics

Reference:

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In situ electron beam irradiation of Ti3C2Tz MXenes. A STEM-EELS study

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Poster Group 2

Background incl. aims
MXenes are a family of 2D transition metal carbide or nitride layers obtained from the exfoliation of nanolaminated ceramics called MAX phases [1]. Their chemical formula is Mn+1XnTz (n = 1, 2 or 3), with M a transition metal, X being C and/or N, and T are surface terminations (e.g. O(H), F, Cl) inherited from the exfoliation process. They present very good electrical conductivity, as well as high hydrophilicity, making them very promising materials for various applications, including transparent conductive electrodes or energy storage devices [2]. Our previous study [3] showed the strong influence of ion irradiation induced defects on the structural and physical properties of Ti3C2Tz MXenes, resulting in a reduced hydration potential, an increase in the charger carrier density as well as a modification of their optical properties. In the meanwhile, a large conductivity is preserved. Electron beams have also been shown to be a valuable tool for the structural engineering of MXenes on the nanometer scale [4]. Here, we present a new experimental protocol, which aims towards localized defect engineering in MXenes, using electron beam irradiation in situ in a transmission electron microscope (TEM), performed in cryogenic conditions.
Methods
This is achieved by adjusting the exposure time, the beam current and the number of frames in STEM-EELS (electron energy-loss spectroscopy) measurements, to reach an electron dose that allows for the introduction of defects into the sample. The study was performed on pristine Ti3C2Tz, exfoliated from its MAX phase precursors following the protocol detailed in [5], the powder was then dispersed in water to delaminate the MXenes sheets and dropped on a TEM lacey carbon grid. The TEM grid was placed on a cryo sample-holder and measurements were performed at liquid nitrogen temperature.
Results
At low temperature, irradiation conditions can be controlled precisely, by the creation of either punctual depressions, roughly the size of the electron beam, or bigger “crater-like” dips spanning over hundreds of nm² (Figure), depending on the irradiation conditions. The evolution of the structure and chemistry of the sample is monitored as a function of the electron dose using core-loss spectra acquired both during and after irradiation.
Conclusion
This study gives a valuable insight into the defect formation mechanisms at play in electron beam irradiation of MXenes, by observing the behavior of the surface functional groups, as well as the core atoms in regards to the incoming high-energy electrons. This innovative approach can potentially be generalized to other MXenes and 2D materials, as well as up scaled to thin films using other electron irradiation techniques such as SEM.

Keywords:

MXenes, STEM-EELS, irradiation, defects, quantification

Reference:

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In situ scanning precession electron diffraction study of chemo-mechanical interactions during Zr oxidation

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Poster Group 2

Background
Zirconium (Zr)-based alloys, serving as cladding materials for nuclear reactor fuel, are susceptible to oxidation under harsh operation conditions. The stress redistribution and strain anisotropy induced by oxidation could elevate the risk of reactor failure and limit the operational economy. Efficient in situ strain mapping techniques are crucial for elucidating the fundamental mechanisms of oxidation processes and advancing the development of more sustainable cladding materials to ensure the safety of nuclear reactor operations. Recent developments of MEMS-based NanoReactors enable in situ TEM studies of reactions at temperatures up to 1000 °C and pressures up to 2 bar. The integration of a vaporizer extends in situ gas phase TEM from dry to humidified gas environments. Specifically, for Zr-based alloys, these innovations facilitate oxidation studies under both dry oxygen and water vapor conditions.
In this work, we pioneer an in situ strain mapping methodology to explore oxidation processes in zirconium by integrating four-dimensional scanning transmission electron microscopy (4D-STEM), MEMS gas-cell holder, precession electron microscopy (PED), and direct electron detector (DED). Central to our successful strain mapping is achieving optimal signal-to-noise ratio within nanobeam electron diffraction patterns (NBEDs).
Methods
A DENSsolutions' in situ gas phase TEM system Climate G+ and a FEI Tecnai 20 with NanoMEGAS ASTAR and Topspin platform were used in this work. The Zr sample was first cut and thinned by the focused ion beam (FIB) and then transferred to the Climate nano-reactor.
Results
Figure 1 depicts the influence of precession on a representative nanobeam electron diffraction (NBED) pattern, an area strain mapping, and an area orientation mapping by toggling precession off and on over the identical region on the Zr sample. The application of precession visibly reduces their sensitivity to the sample thickness and its local bending, increases the number of higher-order reflections, and improves the signal-to-noise ratio. Therefore, it enhances the straining mapping precision and orientation reliability.
A comparison between in situ gas-cell 4D-STEM mapping results using PED at Argon gas pressures of 0 mbar and 500 mbar tells although both PED patterns retain similarity to kinematic ones, the increased pressure lowers the signal-to-noise ratio. Consequently, the reliability of the strain mapping at higher pressure is reduced, as marked by the additional noise present in the strain mapping.
Further details on how we tackle the challenges of FIB sample preparation on a gas cell, the conflict of sample evolution process in a few seconds and SPED data acquisition in a few minutes and so on will be presented on site.

Conclusion
Our approach facilitates precise, nanometer-scale strain mapping across a large field of view during zirconium oxidation. This developed workflow has significant implications for corrosion studies, offering profound insights into the chemo-mechanical evolutions in materials subject to harsh environments.
Figure caption
Figure 1 Comparison of precession's importance in enhancing the quality of diffraction pattern (left), hydrostatic strain map (middle) and ASTAR orientation map + reliability (right).

Keywords:

In situ, SPED, strain mapping

Reference:

No

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Sub 3Å cryoEM structure using a standard LaB6 120kV TEM upgraded with direct electron detector

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Poster Group 2

Background incl. aims
Cryo electron microscopy (Cryo-EM) single particle analysis (SPA) has become a major structural biology technique in recent years. High-resolution cryo-EM structures typically require higher voltage cryo-TEMs with coherent FEG sources, stable columns, autoloader systems and direct electron detectors. These setups are specialised for Cryo-EM work and expensive to establish and maintain. More recently the concept of using 100keV cryo-TEMs has been introduced as a way to make cryoEM more affordable and hence accessible to a larger group of researchers. However, so far, the implementation of these 100keV have relied on specialised cryo-TEMs with FEG sources and better and more stable optics than usually present in common 120keV TEMs.

Methods
We here explored whether a standard 120keV TEM, commonly available at many places worldwide, can be upgraded with a direct electron detector and used for higher resolution cryo-EM. Our setup consisted of a TFS Tecnai Spirit G2 with lanthanum hexaboride (LaB6) thermionic electron emitter and with a Gatan 626 cryo-transfer holder. The microscope was upgraded with a Gatan Alpine direct detection camera optimised for 100 to 200keV. Automated data collection was set up using Serial EM.

Results
Using this imaging configuration, we were successful in achieving a 2.7Å reconstruction for the cryo-EM standard apoferritin. We were also able to resolve a more challenging small 64kDa protein haemoglobin to 4.5Å. Furthermore, we solved an asymmetric membrane protein, 160kDa GPCR (M4 muscarinic acid receptor) to a resolution of 4.5Å. Importantly, all the results were achieved using limited datasets ranging from 700-1500 movies, making it feasible to collect these datasets with a side entry cryo-holder.

Conclusion
These results showcase a potentially widely accessible microscope configuration to obtaining interpretable cryo-EM structures. However, we suggest that the true value of using standard 120keV TEMs is in the potential for many EM facilities and laboratories to setup high quality cryo-EM SPA sample screening without the need to procure specialised Cryo-TEMs. This could hence help to considerably lower the entry barrier for cryo-EM SPA and contribute to the “democratisation” of Cryo-EM.

Keywords:

democratisation, structure, GPCR, screening, optimisation

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Microstructure characterization of CIGS/GaP/Si tandem solar cells

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Poster Group 2

Background incl. aims
The conversion efficiencies of single-junction solar cells have reached values very close to the theoretical Schockley-Queisser limit (~33%). One strategy for exceeding this value is to use tandem solar cells [1] combining a top cell optimized to absorb solar radiation in the UV/visible range with a bottom cell optimized for IR absorption. As part of the ANR EPCIS project [2], we have developed this type of cell using a Cu(In,Ga)S2 (CIGS) layer (with a band gap of 1.65-1.70 eV) deposited at low cost by co-evaporation on a Si substrate (band gap of 1.1 eV). However, the adhesion of a CIGS layer to Si is very poor, so an intermediate layer is needed to improve adhesion. The GaP material was chosen for 3 main reasons : 1) its cubic symmetry with a lattice parameter close to that of Si and also to that of CIGS, for a GGI close to 20% (chalcopyrite material with pseudo-cubic quadratic symmetry with c~2a) can promote epitaxy growth of the CIGS film, 2) such layer can act as a diffusion barrier to prevent diffusion of species between layers during the growth process, and 3) its low absorption coefficient (2.26 eV indirect bandgap) coupled with an electron affinity around 3.8 eV make it an ideal selective contact for holes. Despite the above-mentioned advantages, electrical performances of our synthesized tandem solar cells are still very low. We combined different characterization techniques including S/TEM to try understanding the relation between microstructure and optoelectonic performances.

Methods
Growth parameters including temperature, GGI=[Ga]/([Ga]+[In]) and CGI=[Cu]/([Ga]+[In]) can be adjusted during the co-evaporation process to deposit the CIGS layer on the GaP/Si substrate. When CGI is greater (less) than 1, the film is described as "Cu-rich" ("Cu-poor"). Various S/TEM techniques (ACOM-TEM, probe-corrected S/TEM imaging, spectroscopies) were used to study the 'Cu-rich' and 'Cu-poor' CIGS films: the epitaxial growth of the chalcopyrite CIGS layer, the presence of other crystalline phases and their orientation, and the identification of certain intragranular defects. The interdiffusion of elements and the possible presence of an additional layer between the CIGS and the GaP were characterised by atom probe tomography (APT).

Results
In the Cu-poor sample, the existence of a CuIn5S8 thiospinelle phase (TS) was demonstrated in addition to the CIGS chalcopyrite phase (CH) with this epitaxial relationship: CH[100](001)//TS[100](001)//GaP[100](001)//Si[100](001). In the case of Cu-rich sample, the epitaxial relationship is the same, except that there is no TS phase (Figure). However, the interface between GaP and CIGS layer is much more abrupt in the Cu-poor case than in the Cu-rich case where a few nanometers thick layer made of Cu and P is detected.

Despite the presence of TS phase and interface layers, the desired chalcopyrite phase is the most present. Unexplained contrast in HAADF-STEM images for both kind of samples led us to consider defects such as cation antiphase boundaries. On the basis of these contrasts, three types of CAPB were conceptualized.

Conclusion
Combined with other more macroscopic techniques, S/TEM and APT have enabled us to show that the growth of a CIGS layer on a GaP/Si pseudo-substrate for the application of tandem solar cells is far from ideal. Differences in Cu stoichiometry in the CIGS film were also highlighted.

Acknowledgments :
TEM measurements in Nantes were performed using the IMN's characterisation platform PLASSMAT, CIMEN TEM Microscope having been funded by the French Contrat Plan Etat-Région (CPER), the European Regional Development Fund of Pays de la Loire (FEDER), the Région Pays de la Loire, Nantes Métropole and CNRS.
The authors acknowledge financial support from the CNRS-CEA ''METSA'' French network (FR CNRS 3507)

Keywords:

Tandem-solar-cell, epitaxy, secondary phases, interdiffusion

Reference:

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Phonon dispersion surfaces from the electron microscope compared with calculations including dynamical diffraction

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background
Thermodynamic properties such as heat capacity or thermal conductivity depend critically on phonon dispersion. Determining these properties from nanometer sized regions would enable significant advances in nanoscience. Measuring phonon dispersions in the electron microscope with spatial resolution of a few nm was demonstrated in 2016 [1] and later optimized for parallel acquisition [2-3]. We have now extended this technique to sample two momentum directions, thus obtaining phonon dispersion surfaces in 3D momentum-energy space. To study the influence of Umklapp scattering (scattering into higher Brillouin zones) and dynamical diffraction on the measured dispersion, we compare experimental results to electron-phonon scattering simulations that include dynamical diffraction.

Methods
A FIB lamella from diamond in the [110] zone axis was cut from a single crystal and polished to about 80 nm thickness. We used a Nion HERMES microscope operated at 80 kV with a convergence semi-angle of 1.6 mrad and equipped with a Dectris ELA direct detector to acquire phonon dispersions in parallel acquisition mode utilizing a slot as the EELS entrance aperture to define a line in momentum space. The diffraction pattern was consecutively displaced over the slot aperture by 4.1 mrad per step with a slot width of 6.6 mrad to obtain individual slices of 3D energy-momentum space. The data from each position was post-processed by registration and integration of the 120 frames of 1 s acquisition time (26 min total acquisition time for the 13 positions). The zero-loss lines were straightened and other processing steps applied before stacking the slices in 3D to reconstruct the diffraction plane. The data was then visualized using tomviz [4].
To simulate electron scattering from the diamond sample including phonon scattering and dynamical diffraction, a Bloch wave-based method developed by Rez and Singh was used [5]. This method applies operators for dynamical diffraction, phonon scattering followed by further dynamical diffraction to evaluate the phonon scattered intensity. The experimentally determined sample thickness of 76 nm was used in the calculations.

Results
Fig. 1a shows the phonon dispersion from diamond spanning multiple Brillouin zones. While the diffraction intensities fade quickly with increasing scattering angle, the phonon branches first become more intense and then decrease only slowly. This clearly demonstrates that Umklapp scattering dominates the interaction between the fast beam electrons and phonons in the sample. Fig. 1b shows the experimental phonon dispersion surfaces and the results from the calculation are given in Fig. 1c. We will quantitatively compare the experimental and calculated results. We also

investigate the influence of high-angle phonon scattering, which should be a multi-phonon process known as thermal diffuse scattering, by comparing experiment with calculations for large scattering angles.

Conclusion
We have demonstrated the experimental reconstruction of phonon dispersion surfaces in momentum-momentum-energy space using electron microscopy. The comparison of such rich data sets with Bloch wave-based electron-phonon scattering simulations that include dynamical diffraction makes it possible to understand the fundamental processes of electron-phonon interactions. This paves the way to obtain quantitative phonon densities of state at nanometric spatial resolution, which can be used to determine thermodynamic properties such as heat capacity or thermal conductivity.

FIGURE 1: a) Experimental diamond phonon dispersion covering multiple Brillouin Zones (BZ). b) Experimental phonon dispersion surface volume of diamond. c) Calculated phonon dispersion volume including dynamical diffraction.

Keywords:

Monochromated EELS, phonons, DFT

Reference:

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Influential factors affecting the quantification accuracy of magnetic moments with electron magnetic chiral dichroism technique

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Poster Group 2

Background:
Electron magnetic chiral dichroism (EMCD), a transmission electron microscopy (TEM) technique based on electron energy-loss spectroscopy (EELS), allows quantitative measurements of element-selective spin and orbital moments at a high spatial resolution [1-2]. Since EMCD was first demonstrated in 2006 [1], the investigation of the influencing factors of quantitative determination has always been fundamentally important. The influencing factors of asymmetrical diffraction condition, plural scattering effect and signal-to-noise ratio (SNR) have been mostly discussed in the literature, and some data treatment methods have been correspondingly proposed. However, more questions are open. First, deconvolution has been routinely applied to EMCD spectrum to remove the plural scattering, but how effective the deconvolution of momentum-resolved spectra can be? Second, how the universal problem of electron damage can affect the EMCD quantification? Third, what is relationship between SNR and quantification accuracy? We explored our answers to these questions.

Method:
For the first question, TEM experiments were performed on a wedge-shaped area in a plane-view TEM specimen of Fe/MgO(001) thin film. A series of low-loss EELS, conventional in-axis core-loss EELS with Fe-L2,3, and pairs of q-resolved core-loss EELS with Fe-L2,3 edges at two chiral positions were acquired from areas of different thicknesses, using spectrum imaging in the scanning TEM mode (STEM-SI), as in Figure (a). The in-axis EELS and q-EELS before and after deconvolution with changing thickness were then compared.
For the second question, TEM experiments were performed on a cross-sectional TEM specimen of Co/MgO(001) thin film. The surface oxidation layer and amorphous layer were damaged under continuous electron irradiation, as in Figure (a). The changes in shape and intensity of EELS spectrum Co-L2,3 edges under continuous electron irradiation were followed and its influence on the shape of EMCD spectrum was discussed.
For the third question, a pair of EELS spectra at Co-L2,3 edges were mathematically constructed. The background, L3 edge, L2 edge and continuum background of each spectrum were respectively described by one inverse power exponent function, two gaussian functions and one double arctan step function. Various degrees of white gaussian noise were added to the EELS spectra. EMCD spectra with different SNR were thus obtained, and quantification results were compared.

Results and Conclusions:
(1)The q-resolved spectra after deconvolution still contain residual plural scattering, which is more significant in thicker areas than thinner ones, as shown in Figure (c-d). It is then derived that the existence of such residual plural scattering would bring artifacts to ml/ms value. The thicker the detected area is, the higher the ml/ms value would be obtained even after deconvolution. We suggest that even deconvolution would be performed, EMCD spectra should be acquired from

sufficiently thin samples for minimizing the plural scattering effect in originally detected spectra before any deconvolution [3-4].
(2) Under sustained electron irradiation during spectra acquisition, a gradual removal of the thin surface oxidation layer, rather than a simple continuous thickness reduction that changes the diffraction and plural scattering conditions, can lead to notable residual nonmagnetic components in EMCD spectra and may make the quantified result of the orbital-to-spin moment ratio remarkably higher than the actual value, as in Figure (f-g). It was thus proposed to pay great attentions to the surface oxidation and to minimize the effect of oxidation layer by performing electron irradiation on the target area prior to EMCD experiments, for improving the quantification accuracy [5].
(3) For quantitative measurement of orbital-to-spin moment ratio of Fe, Co, Ni with an error range within about 50% of actual value, the minimum noise degree should be smaller than 0.01.

Keywords:

TEM, EMCD, quantification, magnetic moment

Reference:

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Structure, Chemistry, and Interface of Dispersoids in Powder Metallurgically Processed Ni based 617 ODS Alloy

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background incl. aims:
Ni base Alloy 617 super alloy is well-known to the metallurgists and materials scientists owing to solid solution strengthening aided with precipitation strengthening with the formation Ni3(Al,Ti) type γ' precipitates. Interestingly, this alloy system does not undergo any phase transformation upto its melting point and has impressive high temperature strength and oxidation resistance upto about ~ 1000 °C making this alloy system quite attractive for structural applications at elevated temperatures. However, the Ni3(Al,Ti) type γ' precipitates dissolve in the matrix from about 700 °C following which, carbides provides the strength to this alloy system. In order to retain the strength of Alloy 617 upto 1050 °C while maintaining its oxidation resistance properties, this alloy has been conceptualized and developed by powder metallurgical route using fine yttria as (~ 0.6 wt%) dispersoid [1]. During the high temperature processing of the material, yttria undergoes solid-state reactions with different alloying elements present in the matrix and form different kinds of dispersoids/ precipitates (terminology maybe debated). Detailed electron-microscopy characterizations including High Angle Annular Dark Field (HAADF) analysis in a probe aberration corrected scanning TEM of the solid state reacted dispersoids/precipitates shows different contrast [2]. STEM-HAADF, being a Z^2 dependent technique, the contrast difference of the dispersoids is directly related to the composition of the dispersoids. Most exciting finding of the present work is the formation of a bi-phase precipitates resembling as Janus particle comprising Y-Al-O and Al-O. Detailed correlative microscopy analysis including probe aberration corrected differential phase contrast imaging (STEM-iDPC), STEM based tomography and 3D-Atom Probe Tomography (APT) have brought out details about their chemical composition, structure, and a possible mechanism of formation of these bi-phase Janus particles. The studies have also fascinatingly revealed natural methods of the system to minimize interface strain through formation of core-shell structures in some cases.
Methods:
Alloy 617 ODS alloys have been developed by powder metallurgical route. The Alloy was prepared by ball milling water atomized powders of Alloy 617 and 0.6 wt. % of nanocrystalline bcc-Y2O3 followed by consolidation in SPS. Corelative microscopy including ultrahigh-resolution Cs-aberration-corrected scanning transmission electron microscopy (STEM) based techniques and 3D atom probe tomography have been used to resolve the structure, chemistry, and interfaces of the nano-dispersoids in this alloy.
Results:
STEM-HAADF analysis of the complex nano-oxides shows a variety of contrasts, namely, bright, dark, and bi-phases. STEM-XEDS and STEM-EELS demonstrates the differences in chemical composition of these particles. Most intriguing aspect of the study is the discovery of bi-phase Janus particles. Interestingly, the bi-phase contrast of the Janus particle consists both the heavy and light atoms together. One phase of the Janus particle has been identified as Y-Al-O complex oxide, whereas the

other phase mainly comprises relatively light Al-O. In overcoming the limitation of conventional STEM-HAADF imaging, the integrated differential phase-contrast imaging technique was employed to investigate the oxygen atoms along with other elements in the yttrium rich dispersoids and its interface with the matrix. Detailed atomic resolution information of both phases has been analyzed using STEM-iDPC technique. For the monoclinic Y-Al-O complex oxide structure a few atoms thick Al-O interlayer (shell) around a monoclinic Y–Al–O core in the Ni-matrix. On the other hand, when the dispersoid is a hexagonal type Y–Al–O complex, the interface energy is already low; maintaining a semi-coherent interface and it was devoid of a shell. STEM based tomography analysis has explicitly revealed the shape of the Janus particles, where a 'cup shaped' Al-O surround the 'cone shaped' Y-Al-O complex oxides as shown as inset in Fig. 1. Interestingly, APT analysis confirms the presence of two different types of Janus particles in the system with two different morphologies, (i) cup shaped Al-O phase surround Y-Al-O complex oxide and (ii) cup shaped Y-Al-O surround the Al-O. However, APT analysis shows that for the later type of the Janus particle, core is Al-O rich and the shell is also Al-O rich but contains 3–5 at. % of Y. Direct structure imaging of the interfaces between Y-Al-O/ Al-O and Al-O/ Ni based matrix of the Janus particles have been carried out, where detailed STEM-HAADF and iDPC analysis of the interface between Al-O/ Ni based matrix indicate the periodic disappearance of atom columns from Al-O phase (Fig. 1). Understanding of this phenomenon is quite intriguing and can be explained in terms of vacancy ordering along Al-O/ Ni matrix interface. Quantitative analysis including STEM-HAADF simulation and microstructure modelling towards understanding mechanism of the formation of Janus particle and particularly interface structure is in progress.

Conclusion:

Bi-phase Janus particle is produced as a solid solution product during the processing of alloy 617 ODS alloy during powder metallurgical route. The Janus particles are stabilized in a 'cup-cone' shaped morphology, where both the phases are compositionally stabilised as Y-Al-O complex oxide and Al-O oxide or vice versa. Formation of vacancy ordering has been observed at the interface between Al-O bi-phase and matrix, which is a natural method adopted by the material to minimize the misfit strain across the interface.

Keywords:

Janus particles, STEM-iDPC, 3D-APT, Interface

Reference:

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Mitigating radiation damage in beam sensitive battery materials by adapting scanning parameters

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
Electron-beam sensitivity of battery materials hinders the full potential of high-resolution STEM-EELS insights on battery failure mechanisms. It is therefore important to understand how electron beam induced alterations can be minimized. Beam damage mitigation in STEM can be achieved by adjusting electron probe properties such as beam size, current and acceleration voltage, by changing dwell time, but also by changing the scan pattern and thus, exploiting the spatial component of beam damage (1). For the latter, many different approaches have shown great promise. Alternative scanning techniques, such as leapfrog scanning, which explores the effect of increasing pixel spacing (2), or sparse scanning, which irradiates only a percentage of pixels (3), can reduce damage compared to conventional raster scanning. Since beam damage is a process that is highly dependent on the type of material being irradiated, damage mitigation techniques require material-specific investigations to ensure effective adaption of the scanning parameters. Here we are interested in Li-containing battery materials, where for LiF, for example, radiolysis is more dominant than knock-on damage (4). Methodical experiments are necessary to understand how irradiation conditions like acceleration voltage, electron dose, and scan pattern can be optimized for specific electron beam sensitive battery materials.
Methods
In order to determine the optimum acceleration voltage, damage cross-sections for different acceleration voltages are calculated for various battery materials. To gain a better understanding of the damage mechanisms involved, damage is systematically induced on Li-containing battery materials and monitored with STEM-EELS ((S)TEM Themis Z 3G). The effect on materials damage of conventional scan patterns is compared to different “alternative” scan patterns.
Results
The calculated damage cross-sections show, that avoiding knock-on damage by imaging below a threshold voltage is not applicable for (S)TEM measurements of lithium-containing battery materials. STEM-EELS observations provide insights on the degradation of LiF to metallic Li, being consistent with radiolysis being the dominant damage mechanism. EELS spectra of LiF over radiation time show that the LiF bulk plasmon peak and the Li K-edge for LiF at 61 eV decrease, while the bulk plasmon peak for metallic Li appears at 7 eV. Lastly, scanning variations give insights on damage delocalization in battery materials. Damage imposed by conventional scanning is compared to different alternative scan patterns, by quantifying electron beam induced alterations in image intensity.
Conclusion
With the gained understanding for radiation damage in specific battery materials, optimized acceleration Voltage and scan pattern can be chosen to reduce electron beam induced alterations. Optimized scanning parameters enable higher imaging flexibility and could provide new insights into battery failure mechanisms.(5)

Keywords:

beam-damage, scan pattern, battery materials

Reference:

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(5) Acknowledgements: The authors thank the OPINCHARGE project, which received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101104032. We acknowledge Battery2030+ for their support to the OPINCHARGE project. Measurements were performed using the IMN's characterization platform, PLASSMAT, Nantes, France.

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High-resolution 3D chemical imaging of light elements by time-of-flight mass spectrometry

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Poster Group 2

Background incl. aims
It is a common practice to correlate multiple imaging techniques to achieve a comprehensive understanding of the interplay between material structure and function. Beyond the geometric structure, the chemical composition, particularly at the nanometer scale, plays an essential role. However, there is a shortage of instruments capable of imaging light-mass elements with sufficiently high spatial resolution and sensitivity. This gap is addressed by TOFWERK's fibTOF platform, offering high spatial resolution and 3D chemical imaging of all the elements of the periodic table, including light-mass elements. This capability is demonstrated through the application examples below, which are taken from the battery and metallurgy sectors.
Methods
fibTOF is a Secondary Ion Mass Spectrometry (SIMS) detector designed to be attached to a Focused Ion Beam (FIB) Scanning Electron Microscope (SEM). fibTOF extends microscopic analysis to probe the chemical composition of solids at the nanometer scale with sensitivities down to the ppm range. The properties of a FIB as a primary ion source to erode and ionize material is used. For each pixel probed by the FIB, the released secondary ions are extracted and characterized. The combination of the mass spectra/pixel, results in an intensity map of elements for a mass range of 0-500 Th. As the FIB repeatedly scans the region of interest (ROI), a 3D data set is generated.
Results
The value of the fibTOF is demonstrated by the investigation of a Solid-Electrolyte Interface (SEI) composition at the anode after galvanostatic cycling. We explore how various molecular additives affect its stability.
Lithium ions have been observed at various penetration depths, indicating differences in SEI formation efficacy based on the additives used. The lower SEI formation efficiency explains the diminished battery performance due to ongoing electrolyte degradation. In addition, measurements of Mn and Ni on the anode corroborate irreversible phase changes and metal dissolution of the cathode.
The use of the fibTOF has been shown as well in correlation with hardness tests done by nanoindentation of an M3 high-speed steel alloy. Certain regions of the sample, chemically characterized as hard phases, exhibited soft mechanical properties. These regions also showed the presence of hydrogen, hinting at a potential link to hydrogen embrittlement. Notably, both case studies were carried out using elevated sputtering and detection rate of the fibTOF.
Conclusions
The studies presented demonstrate fibTOF's efficacy in light-mass element imaging, showcasing its versatility and reliability in analyzing diverse materials and complex microstructures. Furthermore, the demonstrated capability of fibTOF to manage enhanced signals bolsters its potential for a broad spectrum of applications.

Keywords:

FIB-SIMS, high-resolution imaging, lithium, battery

Reference:

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In-situ electrical characterization of MOSFET transistors using AFM-in-SEM solution

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Poster Group 2

Background incl. aims
The semiconductor industry is continuously working on improving the performance of modern electronics and new components development. Key physical parameters like dopant concentration levels, carrier types, and crystalline defect densities are fundamental factors that influence the electrical performance of semiconductor devices. To improve device performance, novel methodologies, instrumentation, and workflows are being developed. The key requirement is the ability to investigate samples with nanoscale features to characterize device reliability or failure root cause. One example of a progressive approach for characterizing complex physical and electrical properties of semiconductor materials is correlative in-situ microscopy. A combination of different imaging systems provides a comprehensive understanding of the sample properties without the need to move the sample between multiple instruments.
Methods
The AFM-in-SEM approach, combining Atomic Force Microscopy (AFM)-based techniques with Scanning Electron Microscopy (SEM)-based or Focused Ion Beam (FIB)/SEM-based techniques, provides means to integrated correlative approach for studying semiconductor materials and devices. This solution allows for non-destructive mapping of diverse electrical properties of trenches, measuring gate dimensions, or localizing defects, which could help to understand the device processes. This approach provides the advantages of combining the benefits of capabilities of site-specific sample preparation by FIB, and ultra-high resolution imaging by SEM and AFM techniques. This integration helps in revealing the structures below the sample surface and measuring various properties at the exact location under in-situ conditions. Additionally, it provides quantitative 3D information while avoiding sample or environmental changes such as differential pressure or sample contamination.
Using the AFM module LiteScope, integrated inside the FIB/SEM, it is possible to measure high-resolution topography, and various electrical properties using techniques such as Conductive Atomic Force Microscopy (C-AFM), Scanning Spreading Resistance Microscopy (SSRM) and Kelvin Probe Force Microscopy (KPFM). C-AFM enables electrical conductivity measurement with nanoscale resolution, while SSRM can provide valuable information on dopant concentration profiles in semiconductors. KPFM is a non-destructive technique that measures surface potential, giving insight into electronic properties.
Results
With such an instrumentation, the samples are either in-situ lifted-out, or bulk x-sectioned. First we calibrated the probe measuring SSRM resistance on p- and n-doped silicon substrate, reference samples for dopant concentration measurement in SEM. Then, we analyzed MOSFET high power transistor by SSRM, showing the resistance of different components of the sample, see Fig. 1. The resistance can then be calculated to the dopant concentrations, using the calibration data from reference samples. This workflow proves that the dopant concentration steps of transistors can be

safely measured under in-situ conditions in SEM, thus, enabling quality control and failure analysis of semiconductor components.

Conclusion

In summary, integrated AFM-in-FIB/SEM is a valuable instrumental combination for studying semiconductor materials or devices in order to improve device performance and enhance failure analysis success.

Keywords:

in-situ, semiconductors, SSRM, AFM, SEM

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Correlative imaging of the human anterior cruciate ligament by micro-CT and histology

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Background incl. aims
In case of a damage to the anterior cruciate ligament (ACL), a detailed identification of its morphological structure is essential for the biomechanically valid reconstruction of the ligament [1]. However, so far, most anatomical studies on the ACL were based on macroscopic evaluations [2] or low-resolution clinical imaging technologies (e.g. clinical computed tomography – CT, magnetic resonance imaging – MRI) [3,4]. The objective of this study was to investigate the 3D morphology of ACL in anatomical flexion and extension positions, focusing on the arrangement of its anterior and posterior fiber bundles and its femoral and tibial bone attachments. This was achieved through the correlative imaging by micro-CT, a non-destructive high resolution 3D imaging technique, and histology, leading to the identification of the four layers insertion zone of the ligament.

Methods
Healthy human knee samples (n = 2) were chemically fixed in formalin solution in knee flexion and extension and dehydrated by soaking the specimens in solutions of ascending ethanol concentration and in hexamethyldisilazane. Micro-CT analyses were carried out using an EasyTom XL Ultra 230–160 micro/nano-CT scanner (RX Solutions, Chavanod, France) with a voxel size of 17.5 µm. The generated CT datasets were analysed to create 3D models of both knees in flexion and extension using CTAn and CTVox softwares. After the micro-CT analysis, both knees were rehydrated in solutions of descending ethanol concentration, washed in distilled water, and decalcified with an aqueous solution composed of formic and nitric acid. The micro-CT 3D models were used as references to guide the orientation of the tissue for the histological analysis. Histological 5 µm thick sections were stained with hematoxylin and eosin (H&E) and Safranin O and registered, using Dataviewer software (Bruker micro-CT, Kontich, Belgium), with the corresponding orthogonal micro-CT sections.

Results
The micro-CT imaging analysis permitted the morphological 3D visualization of both soft and hard tissues of the knee in extension and flexion. These anatomical knee components were digitally identified for subsequent 3D modelling analysis, which showed a differential orientation of the ACL bundles when the knee was flexed. In particular, in the extended position the ligament exhibited a parallel course of the bundles, whereas when the knee was flexed a twisting of the bundles disrupted the parallel orientation. Direct comparison with histology permitted to identify the ACL insertion structure, comprised of four layers: ligament, fibrocartilage, mineralized fibrocartilage and bone. Moreover, this versatile method can be applied to define important joint structures, including blood vessels and nerves.

Conclusion

This study underlined the efficacy of micro-CT method to visualize the intricate anatomy of the ACL. Our protocol enabled correlative imaging using micro-CT and histology, allowing the precise assessment of ACL shape and positioning within the knee joint across different extension and flexion states. A detailed 3D description of ACL anatomy can pave the way for new regenerative medicine and tissue engineering approaches with the aim of improving diagnostics and treatment strategies for ACL-related knee pathologies.

Keywords:

Micro-CT, Correlative imaging, ACL, 3D-Imaging

Reference:

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Exploring the 3D architecture of native and stained human intervertebral discs through micro-CT

Raluca-Ana-Maria Barna[1,3,4], Federica Orellana[1,2], Marie-Rosa Fasser[4,5], Daniel Valent[4], Jonas Widmer[4,5], Annapaola Parrilli[1]
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Poster Group 1

Background incl. aims
The disruption of normal intervertebral disk (IVD) structure and its subsequent degeneration significantly contributes to the onset of low back pain [1]. To date, the human IVD 3D microstructure characterization has been confined to imaging the annulus fibrosus (AF) [2] and the subchondral endplate [3]. Our study aimed to propose a step-wise approach for the complete morphological investigation of human IVDs at various tissue processing stages using a non-destructive micro-CT analysis. This approach allowed detailed 3D visualization of distinct IVD anatomical components: nucleus pulposus (NP), AF, and the orientation of collagen fibers. Additionally, the method provided a precise quantification of lesions and calcifications within the disk, which affect the integrity of its microstructure and thus its functional and biomechanical characteristics [4].

Methods
The IVDs (n = 14) were examined through the use of the micro-CT EasyTom XL Ultra 230–160 micro/nano-CT scanner (RX Solutions, Chavanod, France) with a voxel size of 40 µm. Each sample was scanned label-free (both frozen and thawed) and after formalin fixation and staining of the tissue in an iodine-based contrast agent solution. The calcifications and lesions present in the tissue were determined with an automatic segmentation approach using the software Avizo (Thermo Fisher Scientific, MA, USA). For each processing stage, the calcification and lesion volume fractions were compared considering the entire disc and selecting the AF region. In addition, the degree of contrast enhancement has been defined calculating the Hounsfield Units (HU) relative to the acquired CT values.

Results
Thawed samples allowed an accurate quantitative analysis of lesions and calcifications present in the native IVD. Additionally, the analysis of frozen samples provided valuable insight into the alternate laminal pattern of the AF. Iodine staining, while providing a homogeneous increase in contrast and simultaneous visualization of the IVD anatomical components, was time-consuming (2 weeks of staining) and resulted in the extrusion of the NP, leading to an increase in lesion volume fraction. The contrast measured in HU increased between the different preparation steps.

Conclusion
The examination of frozen, thawed, and chemically fixed-stained IVD tissues using micro-CT revealed distinctive microstructural information, presenting different benefits for each stage. The systematic methodology used in this study had the aim to define the trade-off between contrast enhancement and processing artefacts. A label free imaging of the IVD represents a valuable option for a 3D

morphological investigation of the sample, especially when combined with biomechanical testing due to its non-destructiveness thus preserving the original stiffness and geometry.

Keywords:

Micro-CT, IVD, 3D-anatomy, X-ray imaging

Reference:

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Developing a multimodal imaging pipeline for molecular biochemical studies with a 3D approach

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Background incl. aims
It is increasingly important to understand the 3D ultrastructure of cells and tissues particularly in the study of diseases and infection. The study of infection requires a complete understanding of where the virus, parasite or pathogen is located within the cell, how it interferes with the host cell's mechanisms and to follow its development. Several types of imaging are extremely powerful techniques in the study of these processes: XRFM & PC imaging/tomography, XAS & cryoEM/ET. Each of these techniques produces unique information that is crucial in the overall understanding of the problem. However, each technique requires different sample preparation (e.g. ice protective layer thickness) & different supports and holders, which makes it extremely difficult to locate and study the same area of interest in each sample in diverse imaging modalities.

Methods & Results
A complete experimental setup (Fig. 1) accompanied by an efficient workflow, to reach an optimised preparation process allows the easy transfer of the sample from one technique to another. New supports and sample holders are being designed and developed to be compatible with diverse targeted cryo-imaging techniques on near-native state frozen-hydrated samples (Fig. 2). EasyGrid machine [1] is used to automate and validate the sample vitrification. The preliminary results on the dose show that the radiation damage is very limited, suggesting that we can study the same sample by EM after having targeted a region of interest (bio-elemental accumulation/targeted organelles) with XR imaging. The optimization of the sample vitrification with a better amorphous ice quality and a reduction of crystallin ice, ice cracks and a better control of the ice thickness seem to allow a reduction of the flux used for the same image quality. For a multimodal and multiscale sample analysis, a new multimodal imaging pipeline is currently in development with the new design of sample support (collaboration with Silson company) compatible with the above-mentioned imaging and sample preparation instruments. It will allow the study of the same region of the same sample across many scales and tracking down the full process of biochemical mechanisms.

Conclusion
The generation of a new cryo-sample preparation process, suitable for XRFM, PC and Cryo-EM techniques, will make the beamtime (synchrotron/EM) use more efficient, potentially improving the success rate of multimodal imaging projects that will benefit a wide user community. The target community is the historical X-ray imaging users in biological fields who need to overcome difficulties in certain challenging projects, and a rapidly growing new population of users: the non-experts who will benefit directly from a complete multimodal imaging pipeline.

Keywords:

Electron Microscopy/Tomography, X-rayFluorescenceNanoscopie, phase contrast,EasyGrid

Reference:

[1]Gemin et al. EasyGrid. BioRxiv, in review in Nature Methods (2023)

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3D atomic-resolution imaging of nanomaterials based on exit wave reconstruction

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Poster Group 2

Breakthroughs in transmission electron microscopy (TEM) have opened for visualizing nanomaterials with atomic resolution and uncovering the surface terminations that are governing e.g. catalytic properties. In a TEM experiment, the most informative outcome is the recovery of the electron wave function exiting the nanomaterial. While different experimental and mathematical reconstruction schemes exist to recover the underlying exit wave, the transformation of exit waves into a three-dimensional (3D) structural model is still being debated. Recently, our group presented an analytical model to describe the 3D atomic-scale arrangement in an excited 2-dimensional Co-Mo-S nanocrystal [1]. The model was based on channeling theory and made for the first time use of the full shapes of the atomic columns in the exit wave. Extending this model to an arbitrary 3D nanomaterial is challenged by the role of extinction distance in relation to the sample thickness as well as the irregular surface both at entrance and exit of the nanomaterial. Here, we will outline the development of this analytical approach to investigate arbitrary nanomaterials at 3D atomic-resolution.

We examine nanometer-sized metal nanoparticles with a focus on their terminating surface structure. The exit waves are reconstructed from experimental focal series of bright-field TEM images, which carries most signal pr. electron. This is vital for investigating surfaces of nanomaterials since under coordinated atoms at the surface are particularly prone to beam-induced alteration compared to the bulk. Imaging is therefore done with low electron dose rates using direct electron detectors (DED) to establish sufficient SNR for the reconstruction while maintaining the pristine structure of the material.

With the complex exit wave, we use the channeling approaches in [1,2] to determine the 3D atomic position and stoichiometry of atomic columns of a carbon supported Pt nanoparticle with a diameter of 5 nm. We present a strategy to counteract the influence of extinction and show how this drastically reduces the elongation of the 3D tomogram along the beam direction. Furthermore, we also present a strategy for the non-uniform entrance and exit surface of the nanoparticles.

In this work, we present an approach to reconstruct 3D structural information about nanoparticles from electron wave functions exiting the sample. Based on channeling theory, an analytical model is outlined to establish the location and stoichiometry of the atomic columns and approaches to counterbalance the role of extinction and irregular entrance and exit plane surfaces are

demonstrated. The present work therefore build a foundation to recover a 3D atomic model of a nanoparticle and thereby uncover its exposed surface structure. The present approach should be generally applicable to any nanomaterial even under exposure to reactive environments and should thus aid the understanding of gas-surface interaction is diverse fields such as crystal growth, corrosion, and catalysis.

Keywords:

Holography, exit waves, 3D atomic-resolution

Reference:

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[3] The Center for Visualizing Catalytic Processes is sponsored by the Danish National Research Foundation (DNRF146)

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Novel combination of integrated FLM with cryo-FIB allows targeted cryo-ET sample preparation from challenging samples

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

Cryo-electron tomography (cryo-ET) has revolutionized structural biology by enabling the observation and characterization of biological samples at a molecular level in their near-native state [1]. This technique provides an unprecedented level of structural detail, but it necessitates thin specimens (typically below 200nm) for effective electron beam penetration. Presently, the prevailing method for achieving such thinning involves Cryogenic Focused Ion Beam (cryo-FIB) milling [2].

During this milling process, it is easy to miss the regions of interest (ROIs). Fluorescence labeling and fluorescence light microscopy (FLM) can improve targeting [3]. However, the conventional use of a stand-alone cryo-FLM invokes extra transfer steps, increasing the risk of ice contamination, devitrification, and mechanical damage [4]. Moreover, the correlation of the two imaging modalities is cumbersome and often inaccurate, leading to the loss of ROI inside the lamella, rendering it useless, reducing the overall efficiency of the whole workflow.

To address these challenges we present the novel combination of the TESCAN AMBER cryo- FIB/SEM with a fully integrated high quality FLM: Delmic's METEOR system [5]. Thanks to the software integration and position of FLM parallel to the FIB, this novel combination allows smooth and accurate 3D correlation of FLM , FIB and SEM data. Furthermore, the integration of the FLM significantly reduces handling and ice contamination. Additionally, a full collision model allows safe control of the microscope ensuring novel users can adapt the technique quickly. The high-end fluorescent light microscope provides excellent resolution and brings background noise to an absolute minimum allowing researchers to image and mill challenging samples.

In this talk we will delve into the practicalities of this accurate, easy-to-learn and high-throughput correlative cryo-FIB workflow. We will show that the workflow can be used for targeted fabrication of cryo-lamellae from thin specimen (on-grid lamella), larger volumes frozen by waffle method , and cryo lift-out from the most challenging samples. Additionally, we will present the latest results showing EM and FLM data of high-pressure frozen C. elegans and plunge frozen U2OS cells. We will also provide an outlook and highlight the potential of plasma FIB milling, enabling analyses of large biopsies, tissues and small organisms are also discussed.

The novel combination of METEOR with TESCAN AMBER cryo-FIB/SEM allows targeted preparation of cryo-TEM specimen from challenging samples, like high-pressure frozen samples and flat U2OS1 cells.

Acknowledgement
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Keywords:

CLEM, Cryo-FIB, Cryo-ET

Reference:

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Measuring electric fields with 4D-STEM: Demonstration of pitfalls by the example of GaN and SiGe

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Poster Group 2

Background
The development of fast pixel-based detectors in (scanning) transmission electron microscopy ((S)TEM) has resulted in 4D-STEM becoming almost a standard tool [1]. The computationally simplest analysis of 4D-STEM data is the calculation of the center of mass (COM) of the intensity in the diffraction pattern (the so-called COM or first-moment method) [2]. The COM corresponds to the expectation value of the net momentum transferred to a beam electron during the interaction with the sample. The momentum transfer is caused by a magnetic or electric field [3]. However, it is anything but easy to draw conclusions about an electric field in the sample from a measured COM. In this contribution, we use the example of AlN/GaN as well as of SiGe/Si to demonstrate effects that influence the measured COM and which may lead to artifacts in the measured electric field. A knowledge of these effects is important for a correct interpretation of the COM measurement [4,5]. We focus primarily on two aims: (i) Deriving the difference of polarization induced fields in GaN and AlN and (ii) measuring the relative mean inner crystal potentials (MIPs) using the electric field at the interface between different materials.
Methods
The investigations are performed by multislice simulations and by experimental 4D-STEM. In the experimental case, an FEI/Thermo Fisher Titan 80/300 and a Thermo Fisher Spectra 300 were used.
Results
Figure 1a shows the projected electric field obtained from a simulated COM measurement [4] along a line profile of an unrealistic, non-atomistic supercell in which a layer with the MIP of AlN is embedded in a material with a MIP corresponding to that of GaN. The x-axis shows the line-scan position, the y-axis shows the simulated sample thickness.
Apparently, the beam electrons at both interfaces experience a momentum transfer towards GaN, i.e. towards the material with the higher MIP. This is intuitively understandable, with the interpretation that an electric field exists - due to the (mean inner) potential difference - that deflects the electrons.
If one now considers the complex propagation and interaction of the beam electrons inside the specimen using an atom-based supercell, one gets a completely different picture shown in Figure 1b: the strength with which the beam electrons are deflected at the interface between GaN and AlN is not just higher - it points in the opposite direction. It can be shown that this simulation result agrees with experimental measurements.
In addition to this example, we will also address the influence of sample geometry (inclined surfaces) and examine what effect beam convergence has and how strongly nanobeam-electron diffraction (NBED) is influenced by it.
Conclusion

The interaction of the beam electrons in the STEM with the sample is strongly influenced, for example, by dynamic scattering. Thus, there are also effects on the measured COM of the diffracted intensity in 4D-STEM image series. Caution is therefore advised when interpreting these COM results (e.g. for quantifying electric fields). For complex and especially small-scale structures, comparison with simulations is a must.

Keywords:

4D-STEM, COM, electric fields

Reference:

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Electron single pixel imaging enabled by ultrafast optical modulation of the illuminating wavefunction

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background incl. aims

Transmission electron microscopy (TEM) provides sub-angstrom imaging resolution unmatched by alternative modalities, but cumulative radiation damage to soft-matter specimens can pose an obstacle to applying TEM in the life sciences. Electron Single Pixel Imaging (ESPI) is a computational imaging technique with the potential to dramatically reduce the electron dosage needed to produce high-resolution images [1]. The key idea behind ESPI is to carefully imprint patterns onto the illuminating wavefunction and record the dependence of total scattering on pattern choice [2]. Controlling the illumination in this way ensures that every scattering event contributes useful information toward image reconstruction [3]. The outstanding instrumentation challenge when implementing ESPI is to modulate the electron probe. Here we report a novel, versatile method for imprinting arbitrary patterns onto the electron wavefunction that allows new patterns to be selected several times per second. We show proof-of-principle experimental results demonstrating image reconstruction of a MAX-phase nanoparticle.

Methods

Our modified JEOL TEM, shown in Fig. 1. is operated in pulsed mode: we drive electron emission via femtosecond laser pulses at repetition rates up to 600 kHz. Our column includes a secondary sample holder with a view port located directly below the acceleration section and above the primary specimen holder. In this secondary location, an electron-transparent, optically reflective metal film intercepts both the probe electron pulse and a control laser pulse, mediating an interaction between probe electrons and optical near fields. A Spatial Light Modulator (SLM) imprints a pattern onto the control laser, and the near fields at the metallic film transfer the SLM pattern to the electron beam. The patterned electron beam then scatters off the principal specimen, and diffraction-mode electron optics at long camera length enable detection of the total scattering of the modulated beam.

Results

Figure 2.a. shows a specimen of Ti3Al0.4Sn0.6C2 imaged with conventional TEM. Figure 2.b. shows a representative set of near-field modulated probe illumination patterns, while Fig. 2.c. shows the result of the image reconstruction algorithm applied to this basis set of patterns.

Conclusion

We are actively developing the ESPI technique, in particular, improving the coupling efficiency between control laser and electron probe pulses to provide sensitivity on the level of single-photon exchange. Simulation results suggest that by placing the specimen in the fourier plane of the electron-laser interaction point, we can achieve beam patterning with nanometer feature sizes given a sufficiently coherent probe. We anticipate performing experiments on Li-based electrochemical samples and biologically embedded gold nanoparticles to demonstrate this capability.

This work is part of the SMART-electron Project that has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 964591.

Keywords:

Ultrafast electron microscopy, single-pixel imaging

Reference:

[1] A. Konečná, et al. "Single-Pixel Imaging in Space and Time with Optically Modulated Free Electrons," ACS Photonics 10, 1463-1472 (2023)

[2] M. F. Duarte, et al. "Single-pixel imaging via compressive sampling." IEEE Signal Process. Mag. 25, 83-91 (2008).

[3] J. H. Shapiro, "Computational ghost imaging," Phys. Rev. A 78, 061802 (2008).

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Panta rhei - tuning the radiation chemistry of silver nitrate solutions via flow in LP-STEM

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background incl. aims
Operando liquid phase electron microscopy (LP-EM) has revolutionized the insights in chemical reactions. Yet, electron irradiation of liquid phases always comes at the cost of radiolysis. Recent advances in simulation allow to estimate the chemical environment and show that the results are drastically altered for different, diluted aqueous systems[1]. With the new generation of liquid cells however, active flow is feasible experimentally to allow a dynamic exchange of the solution.[2] This demands to systematically access these effects. Recently, flow simulations on irradiation of pure water indicate that the flow reduces the local concentration of some, especially long-lived species. Their lack in turn increases the concentration of short-lived reactive species within the irradiated volume.[3] However, experimental validation is pending - a prerequisite to a mature application of LP-EM to more applied systems.

Recent attention has been drawn to silver nanoparticles for CO2 upconversion. To facilitate such studies in in LP-EM, the radiation chemistry of such systems must be well understood. While the radiation chemistry of AgNO3 was estimated in TEM, STEM, and under heatin[4] , the impact of direct flow on beam-induced Ag nanoparticle evolution remains an unknown parameter.

Methods
To overcome this knowledge gap, we provide systematic experiments using a DENSsolutions Stream system and a Thermo Fisher Scientific Talos F200i at 200 kV in STEM mode operated with a beam current of 22 pA. By using AgNO3 as probe solution, we systematically vary flow velocity and dwell time to characterize growth and etching kinetics.

Results
Our findings show that radiolytically-grown Ag nanoparticle behavior ranges from fast growth to dissolution, suggesting a change between reductive and oxidative regime. This is achieved by a change of either flow rate or dwell time, which allowed to empirically map the parameter space. Preliminary results are shown in Figure 1: Growth rate analysis of silver particles (colorbar) for varying dwell times of the scanning electron beam (y-axis) and flow rates(x-axis).

Supporting to the experimental analysis we provide further advances to the automated radiation chemistry tool AuRaCh[5] including interrupted (scanning) irradiation and flow. These findings will provide further insight into the chemistry observed in flowed liquid-phase experiments.

Keywords:

LP-EM, silver nanoparticles, flow velocity

Reference:

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[4] Lee et al., 2023, ACS Nano 17, 6
[5] Fritsch et al., 2022, Adv. Sci. 9, 2202803

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Glovebox coupled TEM – a new method for versatile investigations of air-sensitive samples

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Poster Group 1

Background incl. aims
Transmission electron microscopy is a versatile and indispensable technique when investigating catalysts. When studying metal containing materials, it is of utmost importance to treat the samples under inert conditions in order to preserve the status of air-sensitive samples and their oxidation states as they get easily destroyed or transformed upon contact with air. Usually this is performed using vacuum-transfer sample holders. However, using such holders poses limitations to the investigations that can be performed (e.g., limited tilting range, shadowing effects, etc.). We therefore developed a unique system that allows for the use of all available sample holders without limitations under inert conditions.

Methods
Our novel setup consists of a glovebox system that is directly connected to the TEM. The interface is designed such that it can keep the inert atmosphere while operating and transferring the sample from the glovebox to the TEM. At the same time its construction avoids the transfer of vibrations to the TEM while investigating the sample. The experimental workflow includes the final preparation steps of the sample into the sample holder being performed in the glovebox after inert transfer of the catalyst material to the box. The sample holder is subsequently transferred to the TEM. For high-resolution (S)TEM investigations the glovebox is put in a special operating mode which includes reduced circulation and shutdown of the vacuum pump.

Results
In order to test the specifications of the system we measured the HR-TEM information limit and STEM resolution after connecting the glovebox and compared the results with the tests performed prior to the modifications. The unique modified system is able to operate within the same specifications as the non-modified TEM, in our case even exceeding the specification limits of the vendor. To test whether the samples can be handled under inert condition from inserting to the sample-holder until insertion to the TEM, we investigated two showcase materials. A reduced Cu-metal based catalyst [1] was transferred via the glovebox-system to the TEM and kept under inert conditions at all times. Additionally, a commercially available metallic Cu sample was investigated without inert treatment. Figure 1 a) and b) show an EELS map and extracted energy-loss spectra from the inertly treated sample. It can be seen that the Cu metal is present in its reduced form in the bulk and on the surface. For comparison, figure 1 c) and d) show the same experiments performed on the sample which was not treated under inert conditions exhibiting surface oxidation which can be seen from the Cu_2O spectrum in figure 1 d).

Conclusion
As it is of utmost importance to investigate metal-containing catalyst materials under inert conditions, we designed a modified TEM-system with a glovebox directly attached. We showed that the system is able to function within specifications of the non-modified TEM and allows the investigation of sample while keeping them under inert atmosphere during preparation and insertion to the TEM. This modified glovebox-TEM is currently successfully employed for investigations of different air sensitive catalyst materials as it allows us to use all available sample holders and perform the investigations without the limitations of a vacuum-transfer sample holder.

Figure 1: a) EELS map of a reduced Cu based catalyst [1] investigated in the described glovebox-TEM under inert conditions. b) Extracted EEL spectra from the regions marked in a). The presence of only metal Cu on the surface and in the bulk of the investigated nanoparticle is visible by inspection of the Cu-$L_{2,3}$ edge. c) EELS map of a metallic Cu sample without inert treatment. d) Extracted EEL spectra of the Cu-$L_{2,3}$ edge from the regions marked in c). The presence of Cu_2O in the surface region is clear from inspection of the spectra.

Keywords:

Instrumentation, inert-transfer, catalysis, oxidation-state, EELS

Reference:

[1] Schumann et al., ChemCatChem 2014, 6, 2889-2897

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Atomic-scale model of the platinum (111)-water interface revealed by angstrom resolution off-axis phase shifting holography

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Poster Group 2

Background
The colossal challenge against climate change highlights the necessity of scaling up renewable energy storage capacity and enhancing green hydrogen production by electrocatalysis. However, developing stable and high-performance electrocatalysts for hydrogen production using abundant materials requires a further fundamental atomic-scale understanding of the charge transfer process at the catalytic interfaces.
Although the metal-water interface is the preeminent part of interest for a basic understanding of electrocatalysis and has become an extensively discussed topic in current catalysis research, the atomic structure of the electrolyte, the active sites of the reactions, and the role of single atoms and step edges on the catalyst surfaces remain elusive.
Environmental TEM research enables atomic-scale assessments of metal-water interfaces. We present an open-cell in-situ study of the interface between catalytically active platinum (111) and water vapor under experimentally feasible pressures. Under a water ambient condition, we measure the projected potential across the interface using the angstrom-resolution electron holography method. The results obtained at various external bias conditions are compared to the atomic structures from ab initio molecular dynamics (AIMD) simulations. We will discuss the metal-water interface structures and their natures from experiment and theory.
Methods
A two-electrode MEMS setup is exposed to 50 microbar water vapor, which forms an ultrathin condensed water layer at the Pt electrode surfaces. An in situ analysis is carried out by employing an image-aberration-corrected Titan environmental TEM. Off-axis phase-shifting electron holography is used in conjunction with an improved drift-correction scheme to reconstruct the exit-wave with a spatial resolution up to the information limit of the microscope (<1.0 Å).
Results
The in-situ HRTEM image series and phase reconstructions identify dynamic platinum adatoms at the metal-water interface. The adatoms are residues of the metal-water interface formation and appear only after the electrode is exposed to the water. The existence of the adatoms may depend on the preparation procedures of the metal electrode. By comparing the quantitative frozen-lattice multi-slice simulations, we retrieve the specimen thickness at the edge and, subsequently, the coverage of the dynamic platinum adatoms. At the metal-water interface, the oscillations of the projected potential up to 5 Å above the platinum adatoms are monitored. We interpret the oscillations as preferential orientations of the water molecules in the first water layer with the help of predicted holography data from multi-slice simulations using the AIMD trajectories of the model interface

configurations. Furthermore, we provide an outlook on the influence of external bias on the potential profiles and expectations based on AIMD-simulation for different surface conditions.
Conclusions
In conclusion, we identify the dynamic platinum adatoms at the platinum- (111) interface to water in an open cell ETEM experiment under in-situ conditions. Applying the angstrom resolution phase shifting off-axis electron holography captures the signatures in the projected potential of the first water layers. Based on the multi-slice simulations derived from AIMD trajectories, we attribute these signatures as the preferential ordering of the interfacial water. First experimental indications of the influence of external an bias on the water layer are discussed with respect to AIMD predictions.

Keywords:

metal-water interface, atomic-scale, holography, environmental-TEM

Reference:

Lindner, J., Ross, U., Meyer, T., Boureau, V., Seibt, M., & Jooss, C. (2024). Reconstruction of Angstrom resolution exit-waves by the application of drift-corrected phase-shifting off-axis electron holography. Ultramicroscopy, 256, 113880.

Huang, J., Zhang, Y., Li, M., Groß, A., & Sakong, S. (2023). Comparing Ab Initio Molecular Dynamics and a Semiclassical Grand Canonical Scheme for the Electric Double Layer of the Pt (111)/Water Interface. The Journal of Physical Chemistry Letters, 14(9), 2354-2363.

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Tracking the host inflammatory response via the MAP kinase pathway

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Background incl. aims

The MAPKs (mitogen-activated protein kinases) are a family of intracellular protein kinases that form signalling cascades, responding to a number of stimuli, that control fundamental mechanisms such as proliferation, differentiation, inflammation and cell death. The MAPK signalling cascade is highly complex, involving multiple signals (infection, cell stress) and hundreds of interacting protein kinases. In order to tightly control this signalling, the interaction between both activating MAPKs, and deactivating phosphatases, is mediated through recruitment via a binding site between the proteins, distal from the active site, called the D-motif. We are interested in studying the MKK6/p38α MAP2K/MAPK pair as it is a crucial step in a cell's inflammatory and innate immune responses. Both kinases have also been the subject of intense study by the pharmaceutical industry, with several compounds targeting p38α in phase II clinical trials [1]. The interaction between p38α and MKK6 is transient and the timing and location of each component during stress is unknown at high resolution. We have generated HEK 293T cell lines expressing MKK6 and p38α, respectively harbouring europium and nickel binding tags. Using XRF microscopy we seek to trace the cellular movement of the two partners in the cytoplasm and nucleus during steady state conditions and under cellular stress. Crucially, the possibility of differential labelling will be demonstrated, allowing us not only to track the proteins in the cell at high resolution but also to see when and where they co-locate. We have recently determined the cryo-EM structure of the MKK6/p38α transient complex [2] (Fig.1) and complementing molecular details with cellular spatio-temporal insights will further help deciphering the inflammatory response.

Methods

This project involves the use of a HEK 293T derived cell line expressing tagged MKK6 and p38. Such cell lines will be targeted in the same experiment for both Ni2+ (p38α tag) and Eu3+ (MKK6 tag).
1. HEK 293T modified cell line will be cultured in complete medium (DMEM+10% FBS) supplemented with 500 ug/ml geneticin & 4 ug/ml puromycin. HEK cells (WT) will be cultured in complete medium.
2. 24h before the Ni/Eu treatment, the double tagged cell lines will be treated with doxocyclin (1ug/mL) inducing expression of tagged p38 and MKK6.
3. HEK 293T modified & WT 293T will be seeded on Si3N4 membrane (Silson, UK) (20000 cells/membrane) [3].
4. Cells will be treated with Ni2+ and Eu3+ (250 uM each) for 24h. A negative control will be performed with HEK WT cells without any Ni/Eu treatment. (concentrations defined based on cytotoxicity assay analyses).
5. After treatment, cells will be washed and subcellular organelles will be labelled with fluorescent markers for 30 min.
6. The cell samples will then be cryofixed in LN2-chilled ethane with Easygrid [4] and stored in LN2 prior to analysis at ID16A.
7. Samples will be placed into the cryo-stage at ID16A for analysis by cryo-correlative X-ray fluorescence microscopy and phase contrast imaging.

Results & Conclusion
We already obtained the cryo-EM structure of the MKK6-p38α complex during activation showing how the MAPK is bound and activated by its MAP2K (Fig.1). However, we would like to go a step further and study the MKK6/p38α transient complex [2] in cellulo and complementing molecular details with cellular spatio-temporal insights will further help deciphering the inflammatory response. Tagging each protein with a different metal atom, we will then be able to select the region of interest showing where and when the two proteins interact. An optimal sample preparation is then required and using the EasyGrid platform [4] which is an automated cryo-EM grid preparation and control system enables high reproducibility and improved in-depth vitrification of cells (compared to traditional plunge-freezing techniques), we might obtain ideal conditions to perform the full study. In order to define the cell regions before XRF, we will use fluorescent markers to identify the location of organelles in order to relate them to the distribution of the tagged proteins. This will also help to preselect the region of interest of our samples before XRF studies. Our expectations that the double tag might be validated very soon with synchrotron X-ray fluorescence nanoimaging beamtime on the ESRF ID16A beamline. Even if the concentration of the Ni/Eu that will be bound to the cells are extremely low, thanks to the high synchrotron flux we are waiting to see a colocalization of Ni-Eu signal at locations within the cells. Obtaining this information on the inflammatory response will be a crucial to decipher and understand the inflammation chain reaction involved in diseases and could lead to the onset of new therapies.

Keywords:

ElectronMicroscopy/Tomography, X-rayFluorescence, Double-tagProteins, Nickel-Europium, InflammatoryChainReaction

Reference:

[1] Patnaik, et al, (2016) A First-in-Human Phase I Study of the Oral p38 MAPK Inhibitor, Ralimetinib (LY2228820 Dimesylate), in Patients with Advanced Cancer. Clin Cancer Res 22, 1095–1102
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[3] C. Bissardon,et al. Cell Culture on Silicon Nitride Membranes and Cryopreparation for Synchrotron X-ray Fluorescence Nano-analysis, Journal of Visualized Experiments (JOVE), (2019) DOI:10.3791/60461)
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Unraveling the composition of monolayer-thick InGaN/GaN quantum wells: A quantitative analysis via probe-corrected HRSTEM

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Poster Group 2

Short-period superlattices comprising ultra-thin InGaN/GaN quantum wells (QWs) with thicknesses of a few (0002) monolayers (MLs) have attracted considerable interest within the field of advanced optoelectronics. These nano-heterostructures offer the potential to manipulate the bandgap through precise control over both the QW and GaN barrier thicknesses [1]. Furthermore, their potential applications in quantum computing and spintronics have been spurred by the possibility of topological insulator behavior [2]. These crucial properties are intricately linked to the indium content within the QWs. Growth efforts strive to achieve high-quality heterostructures with high indium content and QW thickness of less than 5 MLs. This study aimed to determine the impact of growth temperature on indium incorporation and minimum QW thickness down to a single ML, by employing a methodological approach utilizing quantitative analysis of probe-corrected HRSTEM observations.

Methods

Multi-quantum-well (MQW) samples were grown by plasma-assisted molecular beam epitaxy under highly indium-rich conditions. These conditions included In:N flux ratios of 3:1 or 6:1 and InN growth rates of 0.25 or 0.15 ML/s. The objective was to kinetically stabilize QWs with nominal thicknesses of only 1 or 2 MLs. The MQWs were fabricated with either 3 or 5 periods. The thickness of the GaN barriers was maintained at 10 nm. Both the QW and GaN barrier growth temperatures were systematically varied. An integrated characterization approach was implemented to comprehensively analyze these nano-heterostructures. This approach encompassed quantitative analysis of probe-corrected HAADF HRSTEM observations. A peak finding algorithm utilizing 2D Gaussians was implemented to determine the atomic column positions within the experimental HRSTEM images with sub-Angstrom precision. These positions were subsequently used for nanoscale strain mapping and measurement of the atomic column intensities via a 2D Voronoi tessellation applied to each image. For Z-contrast quantification of the atomic column intensities, comparison to frozen-phonon HRSTEM image simulations was employed. The latter were generated from supercells relaxed using either density functional theory or empirical potential calculations with a modified embedded atom method interatomic potential [3-5].

Results

HAADF HRSTEM observations along the <11-20> zone axis of the wurtzite structure were employed for quantitative Z-contrast and strain analysis. Empirical potential calculations were conducted to determine the strain behavior of QWs with thicknesses of 1 and 2 MLs. A significant number of InxGa1-xN/GaN QW supercells spanning the entire compositional range, including both ordered and random alloy configurations, were constructed, and relaxed under biaxial strain conditions. These

calculations yielded composition-dependent strain profiles. Subsequently, the relaxed supercells served as input for the multislice HRSTEM image simulations. Consequently, graphs of InxGa1-xN/GaN column intensity ratios versus TEM foil thickness were generated, facilitating the correlation between QW composition and average column intensity. The analysis explicitly considered the effects of thin foil relaxation, the presence of amorphous surface layers and the cross-scattering phenomenon. The latter was identified as leading to an artificial intensity enhancement of the adjacent GaN MLs of the QWs, particularly at large foil thicknesses. The HAADF HRSTEM observations were then compared to the strain and intensity calculations in order to determine the indium contents within the QW MLs. The evaluation of the results allowed the elucidation of the crucial role of growth temperature in governing the indium incorporation and the QW thickness. A synthesis model explaining the nanoscale processes occurring during epitaxial growth was subsequently proposed.

Conclusion

This work emphasized the importance of an HRSTEM-based methodology for determining the composition of ultra-thin InGaN/GaN QWs. The indium content was determined directly through quantification of Z-contrast and indirectly through measurements of lattice strain. Quantification of Z-contrast utilized thickness-composition-intensity ratio maps obtained from image simulations. Similarly, lattice strain measurements were translated to indium composition by employing the theoretically derived strain-composition coupling of such thin nano-heterostructures. The development and application of this integrated approach were necessitated by the requirement for sub-nanometer accuracy in compositional and strain analysis of low-dimensional materials. This accuracy is crucial for defining their structure-composition-properties relationship.

Acknowledgments

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Keywords:

III-nitrides, Monolayers, Strain, Z-contrast quantification

Reference:

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Nanoparticle formation mechanisms and molecular intermediates revealed by liquid phase EM and reaction pathway analysis

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Poster Group 2

Classical nucleation theory (CNT) is used to describe the growth of metal nanoparticles by seed formation from insoluble metal atoms generated by chemical reduction of precursors containing metal ions. It involves a nucleation step, and subsequent monomer attachment on the seeds which is limited by diffusion of metal atoms.

In contrast, liquid phase transmission electron microscopy (LPTEM) has revealed that nanoparticles do not form by the above-mentioned process but via cluster-cluster aggregation instead.[1] Although this non-classical growth of nanoparticles has been observed frequently, precise knowledge about atomic and cluster species remains concealed. Mechanisms comprising thermodynamically preferred cluster sizes have been suggested in prior studies. However, these magic clusters cannot explain formation mechanisms involving strong reductants at room temperature where kinetics are dominated by the reaction.

In LPTEM, reaction kinetic modeling has been shown to facilitate uncovering reaction conditions in irradiated solutions and how to tailor redox chemistry [2] and acidity [3] in precursor solutions with radiation.

Unraveling ionic precursor species guiding the formation pathways of metallic nanoparticles would enable to specifically design synthesis routes and thus to deliberately adjust their nanostructure properties.

Herein we correlate liquid phase electron microscopy with reaction throughput analysis to demonstrate that nucleation and growth of silver nanoparticles is based on aggregation and attachment of various metal clusters and ions. Therefore, we utilize LPTEM to study nucleation and growth rate of silver nanoparticles induced by radiolysis of aqueous silver nitrate solution. [4] Observations are complemented by a time dependent radiolysis model of the precursor solution (Figure 1a and 1b) illustrating the concentration of most silver species being directly proportional to electron dose rate (Figure 1c).

LPTEM measurements revealed a nearly invariant nanoparticle growth rate with increasing electron dose rate and a decreasing nucleation rate (Figure 1d and 1e). Furthermore, our reaction kinetic model suggests that Ag_4 and Ag- have steady state concentrations orders of magnitude larger than those of the other species which are also almost invariant with dose rate. Experimental dose rate dependent growth and nucleation rates were fitted by power laws yielding growth exponents of $(2.5 \pm 7.5) \cdot 10^{-2}$ and -0.17 ± 0.07, respectively (Figure 1d and e, red lines). The large concentrations of Ag_4

and Ag- in combination with their dose rate dependent power law exponents of $(-7.5 \pm 1.0) \cdot 10^{-3}$ and $(6.5 \pm 2.6) \cdot 10^{-3}$ indicated by our kinetic model and located within the error margin of the experimentally obtained data, suggest a nanoparticle growth by attachment of Ag_4 and Ag- to the silver seeds. However, the nucleation rate exponent did not match any of the simulation results. Furthermore, CNT evaluation yields very high supersaturation values suggesting unrealistically small critical radii of no more than a single atom.

The inconsistency of the dose rate dependency of the observed nucleation rate with that of all silver species and CNT suggests a silver nanoparticle growth via a non-classical pathway. Our results provide evidence, that nucleation rather takes place in a millisecond time scale which is dominated by Ag_4^{2+} cluster-cluster aggregation. Overall, this work showcases the power of quantitative LPTEM combined with transient reaction kinetic modeling for disentangling which chemical processes are involved in nanoparticle formation.

Figure caption
Figure 1. (a) and (b) Radiolysis model for a dose rate of 0.56 MGy/s and 5.6 MGy/s. (c) Steady state concentration of silver species as a function of dose rate. (d) Growth rate multiplied by the squared particle size as a function of dose rate. The blue symbols represent experimental measurements. The red line is a fit assuming diffusion limited growth to the Lifshitz-Slyozov-Wagner (LSW) model. (e) Nucleation rate as a function of the dose rate fitted by a power law. Green symbols are experimental nucleation rates and the red line a power law fit. The green shaded region represents the 3σ confidence interval for the fits.

Keywords:

LPTEM; nucleation; growth; silver; nanoparticles

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Retrieving sub-angstrom resolution from low order dynamical diffraction intensities

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Poster Group 2

Background incl. aims
While dynamical scattering has typically been viewed as a hindrance when attempting to retrieve an unknown crystal structure from electron diffraction data, it can also be of direct benefit since it encodes phase information in the intensity of the electron wave function exiting the crystal [1]. For example, recent advances in 3D electron diffraction have utilized dynamical effects to complement and significantly improve kinematical refinement methods [2]. Building on prior work by Feng et al. [3] we have developed a structure retrieval method which, using experimental large-angle-rocking-beam electron diffraction (LARBED) data, is not only capable of reconstructing complex crystal structures, but also shows that dynamical scattering can be directly used to increase the resolution of a reconstruction beyond what would be achievable under assumption of purely kinematical conditions.

Methods
The approach by Feng et al. [3], which was demonstrated on a rather simple structure (SrTiO3), used a conjugate gradient method to minimize the sum of squared differences between an experimental LARBED pattern and one simulated via the Bloch wave formalism from reconstructed structure factors. We expanded on this method by switching to the adaptive moment estimation (ADAM) optimization algorithm as well as introducing analytical calculation of gradients of the scattering matrix. Significant improvements were achieved by utilizing a series expansion of the scattering matrix [4] to develop a weighting scheme which selectively prioritizes matching those diffraction intensities which have a dominating linear dependence on the structure factors of the crystal. Experimental measurements were performed using a Dectris ELA detector mounted to the end of the IRIS spectrometer of a Nion HERMES microscope, allowing zero-loss filtering with the energy-selecting EELS beam trap. The results presented below were achieved using a roughly 40nm thick lamella of β-Ga2O3 which was prepared with the FIB technique from a bulk crystal grown by the Czochralski method [5].

Results
Figure a) shows the 2D projected potential of β-Ga2O3 in [010] zone axis, generated from reconstructed structure factors, with the theoretical structure overlaid on top. The structure factors were refined from 1129 diffraction patterns of an experimental LARBED tilt series, collected with an accelerating voltage of 200 kV and a maximum tilt angle of 100 mrad. Figure b) shows the Fourier transform of the reconstructed potential with the red circle indicating the range of visible frequency components (image is not to scale with a)). Figure c) shows the mean of all experimental diffraction patterns collected in the LARBED measurement. While many excited beams are visible, only the first 8 (indicated by green circles) were actually used in the reconstruction of the crystal structure. Our

method reaches a sub-angstrom resolution of 1.39 Å-1, which is over 5 times finer than the maximum resolution of 0.27 Å-1 which one obtains when only using the structure factors corresponding to these 8 beams, as in kinematic scattering theory. The reconstructed potential shows good agreement with reference data, further validating this result. In addition to the crystal structure, a specimen thickness of 36.9 nm was also retrieved. The reconstruction was performed ab-initio, i.e. without initializing it with any reasonable starting guess. Besides a small regularization term in the loss function to aid convergence, no further constraints were placed on reconstructed structure factors and no prior information about the crystal, except for its lattice parameters (which can also be extracted from the diffraction data), were used.

Conclusion
In summary, we have directly utilized experimental dynamical diffraction data to perform an ab-initio reconstruction of a crystal at a much higher resolution than would be possible with purely kinematical methods. While we show the best result in this text, our method has successfully been tested on experimental data from further crystals, such as BaTiO3 and Al2O3. An interesting consequence of these findings is that it may be possible to reconstruct a 3D structure from 2D rocking curve data, since there is no fundamental difference in how structure factors corresponding to different reciprocal lattice vectors influence the diffraction pattern produced by the crystal. Preliminary testing on simulated data appears to be promising in this regard and will be further discussed.

Keywords:

dynamical scattering, crystallography, electron diffraction

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4D STEM and EELS Acquired Simultaneously with a Fast Pixelated Direct Detector with Center Hole

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Poster Group 1

In four-dimensional scanning transmission electron microscopy (4D STEM) imaging, a focused beam of electrons scans the sample in a two-dimensional (2D) raster pattern, while a two-dimensional (2D) image is recorded at each scan position. This technique is meanwhile commonly used and is applied to a wide range of materials. We present an ultrafast and direct electron detection system, the pnCCD (S)TEM camera which is equipped with a novel chip with a center hole in the middle of the detector. The annular pnCCD sensor with a central hole (264x264, 48µm2, physical hole 2.5mm diameter) allows the central beam component to pass through the detector, thus becoming available to the EELS instrumentation, without losing the possibility to reconstruct 4D data. The pnCCD (S)TEM camera is compatible with electron energies ranging from 10 keV to 300 keV and can be operated with up to 7500 frames per second. If long dwell times are used for the EELS measurement, online averaging over any number of frames is possible to reduce data while extending the dynamic range by reading out multiple times per scan pixel.

The simultaneous acquisition of electron energy loss spectroscopy (EELS) data and diffraction signal using two hardware synchronized instruments adds attractive information about the sample like elemental composition, phonon or plasmon excitation or core loss phenomena. With formerly available detector systems, a decision needed to be made if either a 4D STEM or an EELS measurement is taken.

In this contribution, we show details of the new sensor and first measurement results recorded at 200keV on a JEM-F200 (JEOL Ltd.) with a cold field emission gun using a prototype camera. The data show that the experimental conditions for EELS and 4DSTEM can be matched and, most importantly, no disturbance in the EELS data is found due to that fact that the electron beam passes through the pnCCD detector.

Keywords:

4D-STEM STEM EELS Phase

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Towards in-situ 4D-STEM observation of texture evolution in nano-crystalline thin films

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background incl. aims
Texture describes the preferred orientation of grains in crystalline materials, which dictates their anisotropic, for example, (opto-)electronic, thermal transport, and mechanical properties. The texture is evaluated conventionally by probing the reciprocal space using X-ray and/or electron diffraction methods, where spatial information, e.g., the orientation relationship of particular grains, is hardly accessible. Probing the local diffraction pattern with a focused electron beam, i.e., nano-beam diffraction (NBD) 4D-STEM, provides local structure information. Recent detector technology opened new possibilities to perform such experiments at ever-higher temporal resolution.
In this contribution, we discuss the optimized conditions towards in-situ 4D-STEM observations of texture evolution in thin films by a combination of state-of-the-art fast direct electron detector (DED), dose-efficient experimental approach, and novel algorithms of data analysis.
Materials and methods
Two material systems, (I) a beam robust poly-crystalline gold thin film and (II) an extremely radiation-sensitive bulk hetero-junction (BHJ) blend used as active layers in organic solar cells serve as model samples in this study.
For poly-crystalline gold, we deposited 15 nm gold with PVD on the SiNx windows of MEMS heating chips (DENSsolutions Wildfire) and annealed the film in the TEM at 150^{o}C for 180 s while observing coarsening of the nano-crystalline structure. 4D-STEM experiments were carried out using a Thermo Fisher Scientific Spectra 200 TEM operated at 200 kV in a regular nano-beam diffraction setup and data was acquired using a DECTRIS ARINA detector. The orientation maps are then analyzed using the ACOM [1] routine within the py4DSTEM package and custom codes to streamline the output orientation matrix to the Orix package [2] for further evaluation of the texture evolution.
The BHJ thin film of nominal thickness of 80 nm comprises small molecule donor DRCN5T blended in fullerene acceptor PC71BM as reported earlier [3,4]. The major challenge is the dose budget (< 5 e-/Å2 at room temperature) that limits the applicable probe current for structural elucidation.
Results and discussions
For gold nano-crystal thin film (Fig. 1a), despite being radiation robust, it remains very challenging to observe evolution at high spatial and temporal resolution with sufficient angular resolution and sampling in diffraction space for orientation determination. This is due to the high dimensions of data required and the limited dose rate at the single pixel level of DED detection. To overcome this limit, we realized that an appropriate convergence angle and camera length are the key factors (using a standard NBD setup) that control the reciprocal space coverage and sampling. It is important to balance DED saturation in the primary beam and sufficient SNR of weak Bragg disks for accurate disk detection and subsequent crystal orientation matching. With a larger convergence angle, a higher probe current at a given high speed of detector readout, thus a higher dose, can be applied. While a small convergence angle seems arguably to favor angular resolution for Bragg disks detection, it

lowers the applicable probe current due to detector saturation, resulting in weaker Bragg disks fading in the diffuse scattering background. After optimizing conditions on our experimental platform, we found that time resolution of 5 – 10s and sub-3 nm sampling resolution (probe size <1 nm) for a statistically relevant field of view is realizable.
We further discuss strategies to improve the dose-effectiveness in terms of Bragg peak detection and eventually, in-situ 4D-STEM experiments supported by experimental results. These include (I) elastic energy filtering, (II) amplitude grating using patterned probe-defining aperture [1], and (III) applying precession-assisted 4D-STEM [5].
Finally, we were able to visualize the grain orientation from optimized, standard NBD 4D-STEM datasets and track the evolution of texture evolution of a defined ROI from the early stage on. We observed the growth of <111> oriented grains at the cost of neighboring high-index oriented grains (Fig. 1a). The 4D-STEM datasets allow grain orientation relationships to be analyzed qualitatively as well as quantitatively, shedding light on the complex interplay between various factors including types of grain boundaries, defects, and even local strain.
In the case of extremely dose-budget limited scenarios like the molecular nano-crystallites in the BHJ, the ultimate Bragg peak detectability is limited by the Poisson noise of the scattered electrons that strike on detector pixels. Therefore, the focused electron diffraction pattern is advantageous.
Inspired by earlier work of ultra-high angular resolution ω-q mapping [P. Midgley, Ultramicroscopy 76(1999) 91], we established a dose-efficient approach, 4D-scanning confocal electron diffraction (4D-SCED), to study the texture structure, i.e., face-on and edge-on domains, of nano-crystallites of the donor in the BHJ (Fig. 1b). 4D-SCED applies defocused pencil beam illumination on the sample and combines a confocal electron optic setup with a pixelated detector to record focused spot-like diffraction patterns. The defocused illumination reduces the dose and generates a homogenous beam-specimen interaction. At the same time, the confocal optics generates spot-like diffraction signals, boosting both the signal-to-noise ratio and signal-to-background ratio even in Poisson noise-limited scenarios [4]. We demonstrated a quasi-in-situ observation of the molecular nanocrystallites' structural evolution during an annealing experiment using a CMOS detector [3] (Fig. 1b). We further discussed new avenues to further reduce dose, improve experimental speed (temporal resolution) and throughput using a fast hybrid pixel detector, and advantage of the frame-based detector over the current generation of event-based detector for these applications [4].
Conclusion
In the study of nano-crystalline structural evolution, the achievable temporal resolution of in-situ 4D-STEM experiments is dose-limited at detection saturation level, independent of sample radiation resistance. Besides calling for further improvements of detectors, we present and discuss several experimental strategies for both beam robust and beam sensitive samples to optimize conditions to spare the meaningful dose to Bragg signal detectability and structural determination.

Keywords:

4D-STEM,
in-situ,
texture,
thin film.

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4D STEM in SEM with a Fast Pixelated Direct Detector

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Poster Group 1

In the TEM community, four-dimensional scanning transmission electron microscopy (4D STEM) imaging is meanwhile a commonly used technique for a wide range of materials. In a scanning electron microscope (SEM), usually the backscattered and emitted electrons from the sample surface are recorded by the available detectors. The used electron energy is typically at 30keV or lower. If a thin sample is to be measured in transmission, single cell or multi-channel STEM diodes are used to collect STEM images at a fixed distance below the sample, as there is no optical lens between the sample and the detector. This allows for basic bright field (BF) or differential phase contrast (DPC) signatures to be measured.

To open the use of SEM instrumentation to a broader range of applications and more complex investigations, we used our pixelated pnCCD (S)TEM camera [1] with 264x264 pixels to perform first 4D STEM measurements in a SEM. The camera can record up to 7500 frames per second, which corresponds to a dwell time of 133 microseconds. We modified the camera design such that a compact detector module is placed below the sample position along the optical axis of the microscope, while the camera electronics is mounted outside the vacuum chamber.

In this contribution, we present first measurements with the pnCCD (S)TEM camera operated at room temperature (see Fig. 1) in a Tescan Mira3 SEM, taken at different electron energies from 9keV to 30keV.

Keywords:

4D-STEM STEM SEM

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Identification of inorganic fibres in workplace air by SEM-EDS

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Poster Group 2

When assessing fibre dusts in the air at workplaces, it is crucial to identify and classify the type of fibre in order to distinguish carcinogenic fibres from others from an occupational health and safety perspective. Up to now, this has been done using an error-prone method of product fibre identification. The aim of the project is to create a database for determining the type of fibre independently of reference materials from the sampled work areas and, based on this, to define identification criteria.
Using available reference materials from the synthetic inorganic fibres used in work areas (synthetic mineral fibres, high temperature wool, micro glass fibres, textile glass fibres, etc.), the range of element composition of the fibre types is determined by means of EDS analysis. In addition, systematic investigations are used to determine the quality of these analyses of fibres with diameters in the micrometre range, identify systematic problems and derive possible differentiation criteria. This database is supplemented by information from publications and manufacturer data, which have been structured for comparison with EDS analyses
A scheme was established that allows fibres in airborne dusts to be classified largely independently of reference materials. It is important not only to look at individual particles separately, but also to classify the fibres found on the basis of previous grouping.
The presented solution to the problem of identifying fibres shows the limits of manual classification. A further step will be the establishment of self-learning neural networks, which have already achieved successful results in initial tests in this area and provide constructive support for identification.

Keywords:

fibres, workplace air, SEM/EDS, identification

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Automated feedback correction of the specimen drift at the atomic scale

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background
Transmission electron microscopy (TEM) has always been an active subject of research, in particular with the advent of new instrumentation like brighter electrons sources, sensitive cameras (direct electron detection), phase plate and vortex beam, aberration corrector, high collection angle EDX detector. Such new advanced capabilities have improved conventional TEM techniques to higher resolution (spatial and in energy) and better signal–to-noise ratios (SNR).
In parallel to these instrumental and methodological developments, automation of the electron microscope has become an essential tool in cryo-microscopy for recording images of many specimen areas and defocus values for single-particle analysis [1], or in the acquisition of tomographic tilt series, diffraction tomography, and holographic tomography [2]. Further refinements include object displacement and focus prediction to accelerate acquisition of tilt series for shorter acquisition and less beam induced damage, online reconstruction of tilt series for preliminary inspection of data at the microscope, alignment of individual particles in images to correct for beam-induced movement. More recently, automation has been developed for 4D-STEM data acquisition, and pipeline acquisition and analysis for routine sample studies.
In these examples of automation, the specific sequence of images is recorded with predetermined experimental conditions. High numbers of images can thus be easily acquired without requiring any operator interaction, leaving human errors and fatigue out of the acquisition process. The computer-controlled execution can thus reduce the time the specimen is exposed to electrons and improves the studies of beam-sensitive materials. In addition, series of images acquired on the same area with a short exposure time to minimize the effect of instabilities, mainly the specimen drift, can be realigned using sophisticated post-processing and summing to improve the SNR. However, the latter approach generates large volumes of data, requires significant calculation time and reduces the field of view of the final image by keeping only the common area of all the images making up the series. The field of view is all the more reduced if the drift is significant. It is therefore interesting to consider whether the specimen drift could be carried out continually and autonomously during image acquisition, or experiments in general, and to investigate the precision with which dynamic drift correction can be achieved [3].

Methods
For several years now, we have been developing software tools to dynamically stabilize experiments. The position of the region-of-interest is determined continuously by analyzing the live flow of images from the camera coupled with feedback control to the specimen stage, or image deflection coils, to stabilize the position mechanically or optically, respectively [4]. This plugin can be installed on any platform (microscope + computer) equipped Digital Micrograph and any type of STEM camera or detector.
The specimen drift measured between two successive images can be corrected on a time scale of less than a second by the use of speed-optimized routines. Whilst the image deflector coils can be used

for optical correction, we have mainly worked on mechanical stabilization of the stage in order to preserve the optical alignment and to maintain identical conditions during observations.

Results
We tested our approach on several different microscopes from different manufacturers. In addition, different modes of observation (Lorentz mode, high-resolution TEM or STEM) have been used, combined with in-situ experiments (biasing, temperature). We will show that stabilization at the atomic scale can be obtained for an unlimited time if the stage is equipped with piezoelectric movement.

The figure shows HAADF high-resolution STEM images acquired on a BaTiO3 thin film epitaxially grown on a SrTiO3 substrate. On the left are presented an image recorded in normal conditions during 30 s with the corresponding phase image extracted using the geometrical phase: scan error and distortions are clearly visible. On the right the same images recorded during 60 s using automated feedback of the piezostage allowing accurate correction at the atomic scale.
We will thus discuss the precision of the correction and the perspectives for HR-STEM studies for which scan errors and distortions are eliminated, or for studies of change at the atomic scale as a function of temperature.

Keywords:

Specimen drift, automated feedback, control

Reference:

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Quantification of Dynamic Scattering Effects in Molecular Crystals using Large Angle Rocking Beam Electron Diffraction

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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

Background
Electron crystallography provides a pathway to solve structure of small crystals (< 1um) in size, and thus overcomes difficult synthesis constraints involved in growing large crystals. Generally, electron diffraction data is collected in the form of integrated intensity using continuous rotation or by precession. The measured intensities in 3D are utilized for structure solution. Using this approach, structure solution of difficult crystals, such as small crystals of zeolites, metal-organic frameworks, molecular crystals, and proteins , can be solved by electron diffraction.
However, electron structure solutions are regularly reported with higher R-values than x-ray or neutron diffraction. While similar structures are found despite the high R-values, the differences in the measure intensity and theory calculated intensity limit information that can be extracted by electron diffraction. Previous work demonstrated that including multiple-scattering effects significantly reduce the R-values . Thus, it is critical to be able to quantify the dynamical diffraction effects in molecular crystals.
Aims
Here, we introduce the large-angle rocking beam electron diffraction (LARBED) technique implemented with hardware synchronization for the quantifying dynamical scattering in large unit cell and dose sensitive crystals, such as zeolites. We report LARBED measurement from zeolite-Y crystal. The resulting patterns show the effects of dynamical interactions when scattering through an imperfect crystal. Our aim is to provide an example of the type of interactions which are limiting the statistical accuracy of methods like microED. Furthermore, we suggest that LARBED can be utilized as a complementary method, where solving the LARBED pattern will provide enhanced statistical accuracy.
Methods
The LARBED technique was implemented on an FEI Themis-Z microscope operating in precession mode with a TEM nanobeam diffraction probe. Here we establish a dark field pivot point aligned to the bottom of the sample with the range of the LARBED pattern set as the precession angle, here, 60mrad. This setup allows for a scan generator to drive the scan coils to tilt the beam rather than rastering the beam in real-space as in standard 4D-STEM techniques. The signal then incrementally tilts the beam with a step size corresponding to the precession angle divided by the integer size of supplied signal grid. The signal was provided using the synchronized scan generator of an electron direct detector. This allows for high-throughput data collection, where 128x128 scans are taken in 17seconds providing high resolution rocking beam curves while maintaining a lower dose. Additionally, dose was minimized using the monochromator slit to 2pA and ~ 0.2mrad convergence angle as a 4.5nm FWHM probe. The Zeolite-Y sample was prepared as a dried powder on an ultrathin lacey carbon grid.
Results
Figure 1 shows the LARBED pattern of a 60 mrad [100] obtained from a Zeolite-Y crystal. The collected datasets are a 4-dimensional stack of diffraction patterns of shape. Here, data are individual

point-like diffraction patterns as a 2D array, with one pattern taken at each point on a 2D array grid of tilts. Figure 1a is the average electron diffraction pattern over all tilts, where the intensities are quasi-kinematic due to the averaging of the intensities. Figure 1b shows the expected structure of the Zeolite at the [100] orientation. Figure 1c is the recovered LARBED Patterns for individual selected disks from the incident beam to the (0,2,6) family of reflections. The bright lines perpendicular to the g-vector direction are generally where the Bragg excitation is satisfied. Note the exact zone axis displays the most complex interactions with highly varying intensity in nearly all beams. This is indicative of multiple scattering effects between many beams as they are all in or near excited conditions. This represents, to our knowledge, the first example of this type of pattern on a large unit-cell, dose-sensitive crystal.

The details within the disk excitation lines also show the effects of small crystalline differences. For a perfect Ia-3d cubic crystal we expect each family of reflections to maintain expected mirror and rotational symmetries(4mm1R). In this case, there are several excitation lines with variations in the families of reflections. These deviations will also be present when performing microED and will subsequently affect the intensity integration. These types of deviations are not accounted for by multiple scattering alone.

Conclusions

Our results show unexpected intensity variation relevant to electron diffraction intensity integration. The splitting of excitation lines is the result of dynamic effects from small defects, orientation, or thickness differences in the sampled region of the crystal. These effects are apparent in LARBED patterns and can potentially be utilized to supplement microED data in the future. Careful selection of the intensities from LARBED patterns have the potential to aid statistical analysis of solved crystal structures.

Keywords:

Electron Diffraction, Crystallography, Dynamic Scattering

Reference:

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Imaging Toxoplasma gondii tachyzoite infection: tracking a single protein effector by X-ray fluorescence microscopy

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Background incl. aims

Toxoplasma gondii is an intracellular parasite that infects over half the world's population and is the causative agent of toxoplasmosis – a condition extremely dangerous for developing foetuses and those with a weakened immune system. However, most people are unaware of infection as the organism tightly controls its host's immune response using a range of secreted effector proteins. A common strategy for intracellular pathogens is to take control of host signalling networks in order to re-wire the response to its own requirements (parasitism). There is increasing evidence that T. gondii uses a repertoire of products stored in a large heterogeneous family of secretory dense granules (DG) as effectors to subvert key functions of the host cell, primarily by taking over host cell gene transcription during infection [1,2]. However, the molecular mechanisms, and crucially, transport of these proteins, both out of the parasite vacuole and from host cytoplasm to nucleus, are unknown. We study the mechanisms, dynamics and transport of proteins delivered by T. gondii in human cells following invasion using a new metal-tag for XRF combined with phase contrast imaging and structural biology.

Methods

1. HFF primary cells (HFF-1 (ATCC® SCRC-1041TM)) cultured on Si3N4 membrane (Silson) in DMEM medium supplemented with 10% heat inactivated FBS (Invitrogen) [3]
2. Cells infected with Toxoplasma: Type I RH strain endogenously tagged at the GRA16 locus (RH ku80 GRA16-His12-HA(Hyaluronan)-Flag)
3. Samples cryofixed with EG at 24h exposure. Pressure wave generation spread the liquid and remove the excess, ethane jet vitrification on both support sides [5] and stored in LN2
4. Samples analysed (PC/XRFM)

Results

T. gondii uses a repertoire of products stored in a large heterogeneous family of secretory dense granules (DG) as effectors to subvert key functions of the host cell [1,2]. GRA16 was the first DG stored protein (GRA protein) shown to be exported to the host nucleus4 and binds USP7 (HAUSP) and PP2A-B55 via unknown mechanisms [4]. Once in the nucleus it controls p53 levels to determine cell fate by somehow modulating the activity of USP7 to deubiquitinate p53. Exported DG proteins are intrinsically disordered and cannot be exported if they contain a folded region. This has limited the study of these proteins by fluorescence labelling. We have labelled GRA16 with a short metal binding motif (12 histidines collating Ni2+) that will not interfere with export and will be labelled in vivo. A proof-of-concept experiment demonstrated that the tagged protein can be visualised by XRF (Fig.1).

Conclusion

By directly mapping these proteins in their native environment at high resolution, we hope to gain insight into how, when and where these proteins emerge from the parasite and enter the host cell for the first time. We reveal the location and timing of GRA16 and is also relevant to other parasite diseases such as malaria, Chagas disease and sleeping sickness. The concept of labelling these proteins with metals and imaging at high resolution has been successful and opens a large range of applications in the study of these and other proteins. We plan to complement these experiments with correlative techniques including sub-cellular mapping of the samples by light microscopy (staining using MitoTracker, CytoPainter…) before X-ray fluorescence as well as X-ray tomography. The knowledge gained would provide much needed information on how these parasites adapt the host cell to become a supportive environment that could be used for potential therapeutic applications.

Keywords:

X-rayFluorescenceMicroscopy, ToxoplasmaGondii, Ni-tag, ElectronMicroscopy, EasyGrid

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Fully Integrated Pixelated 4D-STEM Detector for Scanning Electron Microscopes

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Poster Group 2

Four dimensional scanning transmission electron microscopy (4D STEM) refers to a technique where the electron beam scans across a 2 dimensional array on the sample while a detector positioned below the sample records a 2 dimensional pattern for each point in the array, thus creating a 4 dimensional dataset. 4D STEM is a common technique in TEM and is used for virtual imaging, orientation and strain mapping, and differential phase contrast. This work focuses on developments in detector design, centred on miniaturization and low energy sensitivity, which enable a 4D STEM detector to be used in scanning electron microscopy(SEM).
Presented is a 4D STEM solution based on a Timepix 3 hybrid pixelated detector. This detector consists of a matrix of 256 x 256 smart digital pixels (pixel pitch 55 µm), each containing advanced electronics for signal processing including digital registers. The digitisation is performed immediately for each detected electron recording complex information (position, energy and time) which suppresses unwanted signals and therefore selects only relevant events. This principle significantly improves image quality, reduces noise and increases the resolution and contrast in the acquired images. For 4D STEM applications the main advantages of this approach is the so-called data-driven readout, where every single detected event is streamed directly out of the chip, thus allowing dwell times of hundreds of nanoseconds, effectively removing the traditional bottleneck arising from the requirement to store a full pattern for each point in the array.
Figure 1 shows a map of 1536 x 1024 pixels of a stainless steel specimen, acquired in only 80 s. The full dataset with individual diffractograms along with the subsequent virtual diffraction image is shown. In this example, the high contrast arises from the noisless operation of the detector paired with the low energy sensitivity and the virtually unlimited dynamic range.
With a full integration of the detector into a microscope vacuum chamber the complete workflow from lamella preparation to 4D STEM imaging can now be realised. This approach significantly improve the performance capabilities achievable within SEM.

Keywords:

4D-STEM, Timepix, diffraction imaging

Reference:

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3. "Four-dimensional scanning transmission electron microscopy in a FIB-SEM instrument", ID: FW06010396, national TAČR grant

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Advanced electron microscopy of trimetallic core-shell Pt/Au_xCu_{1-x} nanoparticles

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Poster Group 2

Background:
Noble metal catalysts are widely used in heterogeneous catalysis due to their superior catalytic performance. By coating an inexpensive nanoparticle core with a noble metal, a core-shell catalyst is obtained, which can exhibit an increased catalytic activity[1], whilst limiting the use of expensive noble metals[2]. The increased activity of the core-shell catalyst compared to their monometallic counterpart can be caused by two distinct phenomena; i) lattice strain in the shell material induced by the lattice mismatch between the core and shell metal, and ii) electronic interactions of the core with the shell material[3]. In many cases, these effects are difficult to disentangle and a suitable model system to solely investigate the influence of shell lattice strain on catalytic activity is missing. In this work, we achieve such a system through the synthesis of trimetallic Pt-shell Au_xCu_{1-x}-core nanoparticles and investigate its structure using electron diffraction and high-resolution scanning transmission electron microscopy, yielding insight into the composition and crystal structure of this trimetallic system.

Methods:
Three transmission electron microscopy (TEM) techniques were applied to investigate the trimetallic Pt-shell Au_xCu_{1-x}-core system: selected area electron diffraction (SAED), high resolution STEM (HR-STEM) and elemental mapping (STEM-EDX). Pt/Au_xCu_{1-x} nanoparticles were dropcast onto Carbon Type-B, 200 mesh Hex, Molybdenum grids from Ted Pella, and then cleaned to limit carbon contamination using a literature procedure[4]. STEM-EDX maps and SAED patterns were acquired with a Talos200X microscope operated at 200 kV. I) SAED patterns were acquired for 1000-2000 Pt/Au_xCu_{1-x}nanoparticles with a selected area aperture size of 200 µm, a camera length of 520 mm and a screen current of 0.045 nA. II) STEM in combination with energy dispersive X-ray (EDX) mapping was used in a 512x512 px region with a pixel size of 0.512 nm/px, a dwell time of 5.00 µs/px and a screen current of 0.750 nA. III) HR-STEM images were acquired with an aberration-corrected Spectra300 microscope operated at 300 kV at a camera length of 145 mm and a screen current of 0.150 nA for a 2048x2048 px region and a pixel size of 27.29 pm/px.

Results:
In this work, we show the synthesis and characterization of Pt/Au_xCu_{1-x} nanoparticles (Figure 1a). Our nanoparticles were monodisperse in size with an average diameter of 12-15 ± 2.0-3.0 nm and a composition that could be varied from x = 0.81 to x = 0.23. Using SAED, STEM-EDX and HR-STEM, we show that it was possible to control the structural and compositional properties of these nanoparticles, without compromising size and shape control. A typical SAED pattern of many Pt/$Au_{0.59}Cu_{0.41}$ nanoparticles is shown in Figure 1b and allowed for the determination of the FCC lattice parameter, which was varied as a function of copper content. Combining this with HR-STEM imaging (Figure 1c), and STEM-EDX maps and linescans (as shown in figure 1d & 1e), the epitaxial overgrowth of the Pt-shell over the Au_xCu_{1-x}-core was directly related to the core composition and structure.

Conclusions:
In this study, the structure, composition and morphology of trimetallic Pt/Au_xCu_{1-x} nanoparticles, with x varying from x = 0.81 to x = 0.23, was elucidated. Advanced electron microscopy techniques revealed monodisperse core-shell nanoparticles with an epitaxially grown Pt shell and an average FCC lattice parameter that could be controllably varied as a function of copper content.

Keywords:

Colloids, HRSTEM, SAED, core-shell, trimetallic

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Growth of Au-seeded GaAs-GaSb nanowires explored in environmental TEM

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Group III-Sb semiconductor nanowires are an important material system with potential uses in applications such as quantum electronics, optoelectronics, and sensing. This is due to their excellent electrical properties, including high carrier mobility and narrow band gap. However, current device development is slowed down by the difficulty to control the growth of Sb-based nanowires, as compared to the more common III-As materials. To a large extent this is caused by the complex growth conditions of antimonides, as elemental antimony exhibits low vapour pressure and surfactant effect.[1,2] In order to understand the limits and possibilities of the III-Sb nanowire system, a thorough investigation of the growth behaviour is therefore needed. Since atomic scale dynamics and liquid catalyst composition are parameters that are not readily accessible during conventional nanowire growth, in-situ investigations are crucial to develop a comprehensive understanding of the nanowire growth.
Methods
Herein we utilize a Hitachi HF-3300S environmental transmission electron microscope (ETEM) to grow and analyze Au-seeded GaAs and GaSb containing nanowire heterostructures in-situ as demonstrated in Figure 1 (a). This allows us to acquire high frame rate videos of nanowires using a Gatan OneView IS camera in addition to acquiring X-ray energy dispersive spectroscopy (XEDS) compositional data using an Oxford Instruments SDD X-MaxN 80T system. In order to conduct the in-situ growth studies Norcada MEMS chips with ex-situ aerosol deposited Au particles were used.[3] To perform the growth of GaSb nanowires trimethylgallium (TMGa) and trimethlyantimony (TMSb) were used as precursors.
Results
When conducting in-situ XEDS measurements during the growth of Au-seeded GaSb segments we have shown that the Ga concentration in the particle is in the range of 66 – 94 at.%, while the Sb concentration is in the range of 3-4 at.% depending on the used V/III ratio between precursors. The particle composition had a significant impact on the particle volume, which in turn affected the nanowire diameter as shown in Figure 1 (b-c). We observed that steady-state axial growth of GaSb nanowires with diameters in the range of 50-100 nm can be achieved (for the lowest and highest Ga concentration, respectively).[4] Varying the vapor phase composition and particle composition had a further effect on the growth dynamics. We examined the growth process by extracting incubation and step-flow process times from the nanowire growth videos at different V/III ratios. These processes describe the time it takes for a new layer to nucleate and subsequently the time it takes the layer to cover the liquid-solid interface, respectively. Our experimental observations and Monte-Carlo modelling of the growth revealed both processes to be predominantly affected by Sb atom abundance (in vapor and liquid phase). Lastly, we examined GaSb-GaAs heterostructure formation dynamics. Here we were able to show qualitative analysis of the kinking mechanism often found in these and similar high lattice mismatch heterostructures. Furthermore, we were able to demonstrate

that the growth of these highly lattice mismatched heterostructures can be attained via ternary GaAsxSb1-x segments.

Conclusions

In this study we offer a thorough in-situ analysis of the Au-seeded GaSb nanowires. Firstly, we demonstrate how the precursor flows affect the seed particle composition, which in turn affects the seed particle volume subsequently leading to dramatic changes in nanowire morphology. Furthermore, we examine in close detail the layer-by-layer growth dynamics revealing how precursor flows affect the step-flow and incubation processes revealing the growth to be mainly Sb limited. Lastly, we demonstrate how GaSb-GaAs heterostructures can be attained without nanowire kinking which offer insight into high lattice mismatch nanowire heterostructure growth strategies.

Keywords:

In-situ, MOCVD, nanowires, GaSb, VLS

Reference:

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Investigating the ultrafast phase transition of vanadium dioxide using time-resolved three-dimensional electron diffraction

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

Background incl. aims
Vanadium dioxide (VO_2) exhibits a remarkable phase transition at around 68°C, accompanied by significant changes in its electronic and structural properties. This transition holds great promise for the development of advanced electronic switches, optical detectors, logic and memory storage and nonlinear circuit components [1,2]. However, the ultrafast dynamics of this transition are not fully understood, limiting its practical application. The temporal evolution of the structural phase and the question whether thermal or nonthermal effects dominate remains a controversial, debated topic to date [3], in particular, whether VO_2 transitions directly from the monoclinic phase (M1 - $P2_1/c$) to the rutile (R - $P4_2/mnm$) phase, or whether an intermediate monoclinic phase (M2 - C2/m) exists under certain conditions in a transient state. This potential intermediate phase which typically occurs under applied strain was proposed by X-ray spectroscopy and later disproved by ultrafast electron diffraction (UED). Our experimental approach which allows for a complete determination of the phase will shine light on this most recent debate.

Methods
Here, to investigate the spatio-temporal evolution of the phase transition we use an ultrafast transmission electron microscope (UTEM) which combines the nanometer spatial resolution of the TEM with the temporal resolution of femtosecond pulsed lasers. Our approach relies on the combination of an optical pump pulse for exciting the sample and a photoemitted electron probe pulse to detect structural dynamics in our sample. The UTEM allows us to probe the atomic structure on a sub-micron area with femtosecond resolution, thus extending the capabilities offered by UED [4] and X-ray techniques. Using this pump-probe technique, a change of the delay of the laser excitation with respect to the electron probe allows us to capture time-resolved diffraction movies in a stroboscopic approach. Given the similar diffraction patterns of the different structural phases of VO_2, the phases taken along well-defined zone axes can easily be confused. This is especially true with the conditions relevant for ultrafast electron microscopy, where one is further limited by low electron count rates (SNR), low spatial coherence and resolution. We overcome these difficulties by extending ultrafast electron diffraction, for the very first time, to ultrafast three-dimensional electron diffraction (3D ED [5]), where at each time delay the diffraction pattern of the sample is collected at multiple angles oriented off zone axes. This tilt series of diffraction patterns can then be combined and analyzed to build a complete 3D model of the atomic crystal structure without prior knowledge of the material. This approach allows us to clearly distinguish the different structural phases in VO_2 at moderate SNR and without the need of atomic structure simulations for comparison.

Results
We characterized the ground state of a single-crystalline VO_2 lamella made via focused ion-beam milling in a conventional TEM and confirmed its excellent crystalline quality by electron diffraction. Defocused imaging revealed bending and stress contrast in the lamella (see Graphic). Static 3D ED of VO_2 as a function of temperature (between room temperature and 100° C) showed the structural transition from the M1 to the R phase, without any evidence of a stress-induced M2 phase (see Graphic). These results were consistent, regardless of the electron probe size and position on the lamella.
The ultrafast 3D ED results are distinctly different from the static results. We unambiguously observe a transition pathway between the two monoclinic phases (see Graphic), from M1 to M1+M2, on a timescale of 500 fs. This can be indicative of several scenarios: (i) either the nature of the ultrafast photo-induced phase transition is different compared to the thermal phase transition, or (ii) the M2 phase is a metastable, transient state (and thus cannot be resolved in a static measurement), or (iii) the photo-excitation leads to excitation of intense acoustic modes which induce notable stress in the material, placing VO_2 on a transition trajectory to a different structure. Our results were reproducible, and the sample did not show any sign of photoinduced damage. Importantly, the laser repetition rate of 12 kHz was kept such that the system could return to its ground state between sequential pump laser pulses.

Conclusions
In summary, we have for the first time demonstrated the feasibility of 3D ED in the ultrafast regime. The results allowed us to unequivocally determine the crystal structure of VO_2 in the time-resolved regime. Surprisingly, and in contradiction to existing literature, we unequivocally detect the presence of a monoclinic M2 transition state in our well-defined, single-crystalline sample. The M2 phase persists over several picoseconds. We plan to extend our study by collecting more data sets at longer temporal delays and with the intention to gain further insights into the underlying mechanisms governing the transition. Of special interest is how photoexcited acoustic modes or different lamella geometries affect the transient stress and ultimately the transition pathway. We believe that ultrafast 3D ED will become indispensable for transient structural determination in the near future. In contrast to three-dimensional X-ray or neutron diffraction, our approach is more readily available, does not require large-scale facilities, and offers better spatial resolution, thus facilitating the exploration of micro/nano-crystals and individual domains.

Keywords:

Ultrafast, three-dimensional electron diffraction

Reference:

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Effect of preparation and oxygen evolution catalysis on CoNiFe oxide electrocatalysts revealed by STEM-EELS

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Poster Group 1

Background incl. aims
Hydrogen is considered as a competent and green energy carrier that can be obtained by water electrolysis [1]. However, this process is mainly limited by the water oxidation half reaction, the OER (oxygen evolution reaction). Therefore, substantial efforts for this reaction focus on the development of catalysts that are comprised of abundant metals, and which simultaneously fulfil the requirements of high current densities, low overpotential, high mass activity and excellent stability. Here, such a system is presented using CoNiFe oxide in alkaline oxygen evolution. The effect of the synthesis procedure as well as the influence of the catalytic conditions on the material are investigated by STEM coupled with EELS and EDX.

Methods
The catalysts were investigated by a probe corrected JEOL JEM-ARM200F (Schottky emitter) equipped with a Gatan Enfinium ER EELS and a JEOL JED-2300 EDX spectrometer having a silicon drift detector (dry SD60GV). A rather high beam current was applied to keep the acquisition time low. The FWHM of the zero-loss peak (ZLP) was about 1.2 eV and the DualEELS mode was applied. The spectra were recalibrated at each pixel by the position of the ZLP.

Results
CoNiFe (2:2:1)/M, synthesized under an oxygen-deficient atmosphere (1 vol% air in Ar), shows the best catalytic performance. Synthesis of CoNiFe (2:2:1) under a reductive atmosphere (CoNiFe (2:2:1)/H; 5% H2 in Ar) or oxygen-rich conditions (CoNiFe (2:2:1)/A; air) leads to less active catalysts. While CoNiFe (2:2:1)/M and CoNiFe (2:2:1)/A show a quite uniform distribution of the incorporated metals, CoNiFe (2:2:1)/H shows enrichment of metallic Ni containing nanoparticles on the bulk. More pronounced differences can be observed in the EEL spectra, in particular at the O K edge. Whilst CoNiFe (2:2:1)/A shows a pronounced pre-edge feature at about 528 eV, it is only barely noticeable and slightly shifted to higher energy losses in CoNiFe (2:2:1)/H and CoNiFe (2:2:1)/M. Additionally, CoNiFe (2:2:1)/M exhibits a feature at about 545 eV, which was not present in the other materials. This behaviour is also observed in the spectral regions (L edges) of Co, Ni, and Fe. All M L edge features of CoNiFe (2:2:1)/A ressemble that of oxidized metals M [2-4]. In contrast, CoNiFe (2:2:1)/H and CoNiFe (2:2:1)/M show both, reduced and oxidized states of the contained metals. In CoNiFe (2:2:1)/H, metallic Ni was observed, with Co and Fe remaining oxidic. In addition to metallic Ni, CoNiFe (2:2:1)/M exhibits also partially reduced Co. The incomplete reduction of the metal centers in CoNiFe (2:2:1)/M and CoNiFe (2:2:1)/H is also reflected by the presence of an oxygen signal.
The most active catalyst CoNiFe (2:2:1)/M was also investigated after electrolysis in the absence of nafion. The observed decrease of the ECSA (electrochemically active surface area) was also reflected by a denser appearance of the particles and a decreased size of the crystallites constituting these particles (see graphic). The composition of the material has also changed considerably with a non-uniform distribution of the constituting elements. Some regions lack almost completely Ni and

exhibit a reduced contents of iron, leading to a cobalt enriched material. The EEL spectra at the O K edge ressemble those of the fresh catalyst, while the L edges of Co and Fe point towards the presence of these metals in their oxidized forms.

Conclusion
STEM-EELS is a powerful method to investigate changes of electrocatalysts at the nm scale. The different preparation procedures of CoNiFe (2:2:1) yield materials with differently oxidized metals which can be observed by EELS. While CoNiFe (2:2:1)/A exhibits completely oxidized metals, metallic Ni is present in CoNiFe (2:2:1)/H and non-oxidic Ni and Co are present in CoNiFe (2:2:1)/M. After electrolysis in the presence of CoNiFe (2:2:1)/M the signal shape at the O K edge is retained, however, the material is lacking some Ni. STEM-EELS is thus able to detect changes at a very low concentration of the analyte that may escape the eye of bulk analysis methods.

Keywords:

STEM, EELS, OER, oxide, catalysis

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L-TEM characterization of controlled skyrmion nucleation in synthetic antiferromagnetic multilayers

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Poster Group 1

Background incl. aims
Skyrmions are circular magnetic textures whose topologically protected nature makes them great candidates for the development of novel information storage devices which are both power efficient and non-volatile [1]. Effective control of the skyrmion nucleation process is required for the development of such devices. Previous research has demonstrated how a focused ion beam (FIB) microscope can be used to engineer skyrmion nucleation sites in ferromagnetic multilayers by creating artificial point-life defects [2]. Such defects induce intermixing at the interfaces between the layers, which results in local modifications of the magnetic properties in this kind of samples where the strength of exchange interactions is highly correlated with the sharpness of the interfaces.
The present work builds on this research by applying this technique to a synthetic antiferromagnetic (SAF) multilayer sample. SAF multilayers are characterized by the presence of the RKKY interaction, which creates antiferromagnetic coupling between individual ferromagnetic layers. SAF skyrmions, which have been found to arise in such systems under specific field regimes, are characterised by minimal dipolar fields and are not susceptible to the skyrmion Hall effect. These properties make them more stable and better suited for spintronic applications than ferromagnetic skyrmions [3].
Methods
In this project, an array of point-like defects was irradiated on a SAF multilayer using the 10 nm probe (FWHM) of a Ga+ FIB. Defects were induced by a range of Ga+ ion doses, spanning from 10^{13} ions/cm² to 10^{18} ions/cm². The magnetic behaviour of the sample within the irradiated region was observed under Lorentz transmission electron microscopy (L-TEM) in the Fresnel mode, which allows for the direct visualization of magnetic textures. This led to the in-situ observation of the skyrmion nucleation and pinning behaviour in the presence of an external magnetic field. The magnetic behaviour of the sample outside of the skyrmion phase was also recorded.
Results
Nucleation and pinning of skyrmions were observed at the location of the defects. This behaviour was observed in the ferromagnetic regime of both sides of the hysteresis loop (Fig.1). We note that, in the non-irradiated state, skyrmion nucleation is only observed when starting from a saturated state, and only after the field had passed through zero.
Conclusion
The behaviour recorded in the irradiated areas of this SAF system differs from the behaviour recorded prior to irradiation. The field regime at which we observe skyrmion nucleation and pinning at the location of the artificial defects indicates that these skyrmions are likely to be ferromagnetic in nature in this initial study. The effects of FIB irradiation on the SAF phase of the sample are currently under investigation.

Keywords:

Magnetism, Skyrmions, L-TEM, Synthetic Antiferromagnets

Reference:

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Quantum phase modulation and retrieval in an ultrafast transmission electron microscope

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Poster Group 2

Background incl. aims

Coherent phase modulation of the free-space electron wavefunction has the potential to dramatically extend the applications of electron microscopy by enabling, e.g., enhanced image contrast in Cryo-Electron Microscopy [1], Single Pixel Imaging [2] and quantum information processing [3]. Measuring the phase degree of freedom is challenging because conventional approaches to electron detection are sensitive to deposited energy. Pump-probe ultrafast electron microscopy allows for the implementation of phase retrieval algorithms by comparing pre and post-excitation images, using the pre-excitation image to define a reference phase. Here we demonstrate phase retrieval performed on a probe beam passing through a fast moving, dilute free electron gas.

Methods

Our specimen in pump probe experiments is shown in Fig. 1 a. and consists of a nanometer thin gold film suspended on a standard 3 mm gold TEM grid. We illuminate the specimen with point projection optics (i.e., Lorentz microscopy) and collect the scattered beam at long diffraction camera length, so that the electron intensity on the detector mixes momentum and real-space information, a critical experimental detail for enabling phase reconstruction. We pump the specimen with 1030 nm, 290 fs laser pulses, emitting a low energy cloud of electrons in the path of the probe beam. We record changes in the detected probe caused by the electron cloud as a function of pump-probe delay. The specimen geometry is invariant in one of the two directions transverse to the probe beam trajectory and we improve signal to noise by averaging along the invariant direction, as indicated in Fig. 1 a.

Results

Figure 1. b. shows the modulation of the transmitted beam intensity as a function of position on the sample and delay time. Relying on charge conservation, a deflection angle is computed from the local change in beam intensity as a function of delay as shown in Fig. 2, and from this deflection angle the phase-change of the electron beam is calculated. Figure 3. shows the phase shift as a function of delay time and position using a 2π periodic colormap.

Conclusion

We have demonstrated how the combination of pump-probe specimen modulation with point projection electron optics (Lorentz microscopy) enables phase retrieval. In subsequent experiments,

phase retrieval has allowed us to go further and extract key experimental observables from our data, e.g., the charge density of the specimen gas.

This work is part of the SMART-electron Project that has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 964591.

Keywords:

Pump-probe electron phase retrieval

Reference:

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Hard X-ray nano-imaging of Toxoplasma gondii tachyzoites & merozoites

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Background incl. aims

High resolution imaging of biological samples is currently undergoing several revolutions. Light microscopy can obtain details at subcellular scales in organs and whole organisms and cryo-electron tomography is approaching atomic resolution of macromolecules in situ. Hard X-ray nano-imaging is an emerging technique allowing high resolution phase contrast imaging combined with X-ray fluorescence microscopy of biological samples. Phase contrast tomographic imaging of biological samples using X-rays fills several spatial and temporal gaps in the imaging tool box and when correlated with other techniques it will provide breakthrough results in understanding cellular processes. Tomograms can be collected providing 3D reconstructions, at resolutions of 50 nm, that can be combined with the distribution of elements within the sample. We have been applying this technique to the study of toxoplasma tachyzoites and pre-sexual merozoites in human cells. This study is already providing important information on the physical interaction between these developmental stages (Figure 1C) and their effects on the host cell but also on the effects on metabolism, for example iron distribution in the cell.

Methods

1. HFF primary cells (HFF-1 (ATCC® SCRC-1041TM)) cultured on Si3N4 membrane (Silson#) in DMEM medium supplemented with 10% heat inactivated FBS (Invitrogen) [1]
2. Cells infected with Toxoplasma: Type I RH strain endogenously tagged at SEC14 or GRA1 locus.
3. Samples cryofixed with EasyGrid at 24h exposure. Pressure wave generation spread the liquid and remove the excess, ethane jet vitrification on both support sides and stored in LN2[2].
4. Samples analysed (PC/XRFM)

Results

Several types of imaging are extremely powerful techniques in the study of these processes: one of them is XRF & PC imaging. Phase contrast tomographic imaging of biological samples using X-rays fills several spatial and temporal gaps in the imaging tool box and when correlated with other techniques it will provide breakthrough results in understanding cellular processes. Tomograms can be collected providing 3D reconstructions, at resolutions of 50 nm, that can be combined with the distribution of elements within the sample. With this technique at cryogenic temperature, we can obtain crucial information on the morphology and the divalent cations distributions within the samples in near-native conditions. It is important to provide an overview of the different elements and then proteins that can be implicated in the metabolism and development of the disease. We then managed to obtain information that is providing new insights in parasite research. This study is already providing important information on the physical interaction between Toxoplasma tachyzoites and pre-sexual merozoites [3] and their effects on the host cell but also on the effects on metabolism, for example iron distribution in the cell. We also studied mutants revealing interesting features regarding the

calcium and other ion distributions within the cells and the parasite itself (Fig 1). In order to find some insights of the bio-metabolic mechanisms of the different stages of TG infection [4], we studied the ionic distribution of all the elements present intrinsically within the infected cells and parasite itself. While potassium K expressed the well-preservation of the samples – parasite, other elements – iron Fe & calcium Ca – present some very interesting & intriguing distributions. They seemed to be concentrated in the dense granules, and this phenomenon appeared to be overexpressed in the case of TG mutants.

Conclusion
Thanks to reproducible and automated sample preparation, the study of the cells infected by TG parasites through diverse techniques especially the XRFM. EG offers the in-depth vitrification of the samples with a very good ice quality (no crystalline ice present in the sample) and a vitrification that is homogenous on the whole cell height which allows a high-quality data collection. The preliminary results are extremely promising. Fe- & Ca-ionic distributions with the parasites and the cells reveal a change in the metabolic processes in TG mutants.

Keywords:

X-rayFluorescenceMicroscopy, PhaseContrastImaging, ToxoplasmaGondii, Ni-tag, electronmicroscopy

Reference:

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[2] Gemin et al. EasyGrid. BioRxiv, in review in Nature Method (2023)
[3] Antunes et al. 2023 Nature

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Physico-chemical and biological characterization of Ag- and Cu-doped ZnO thin films coated with calcium phosphates

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Poster Group 2

Background incl. aims
Bacterial contamination of biomedical surfaces is a serious threat that can lead to nosocomial infections, with a prevalence reported up to 10 %.1 Treatment of such infections is complicated by emerging antimicrobial resistance.2 Potential solution lies in the development of novel antibacterial surfaces that prevent the initial bacterial adhesion. In this sense, magnetron sputtered ZnO thin films are attracting attention as the properties, including biological, of the thin films can be regulated by changing the sputtering conditions. In addition, doping ZnO with elements from group I and IB (e.g. Ag and Cu) can influence the structural and morphological properties of the thin films. However, a major disadvantage for the biomedical application of this material is the lack of porosity.3 The aim of this research was to evaluate the differences between Ag and Cu doping of ZnO and to determine whether changes in surface morphology due to the coating with calcium phosphate can influence the biological responses of the material.
Methods
Ag- and Cu-doped ZnO thin films were prepared by magnetron sputtering, followed by biomimetic deposition of calcium phosphate to potentially improve bioactivity. Characterization focused on microscopy techniques including scanning electron microscopy (SEM), helium ion microscopy (HIM) and atomic force microscopy (AFM) to investigate microstructural changes. Additional characterization of structure and composition included grazing incidence small-angle X-ray scattering (GISAXS), energy dispersive spectroscopy (SEM/EDX), and X-ray diffraction (XRD). Wettability and surface free energy were assessed, and ion release was measured using inductively coupled plasma mass spectrometry (ICP-MS). Biological characterization included cell viability assays with MG-63 cells and biofilm formation assays with Staphylococcus aureus and Pseudomonas aeruginosa.
Results
Microscopic analysis revealed that the thin films have irregular granular structures in all cases, with Ag or Cu doping influencing the size and shape of grains. Increased Ag or Cu content had opposite effects on the grain size of the nanostructured thin film. Further SEM/EDX and XRD analyzes indicated that Ag or Cu were incorporated into the ZnO crystal structure. Calcium phosphates were successfully deposited as individual crystals or aggregates. The deposition of calcium phosphates slightly improved cell viability, with the effect being greater for Ag-doped ZnO. The addition of calcium phosphates also showed better prevention of biofilm formation.
Conclusion

Ag- and Cu-doped ZnO thin films with calcium phosphates were investigated as antibacterial surfaces for biomedical applications. The doping allowed control over the morphology of the nanoparticles, while the deposition of calcium phosphates showed the potential to improve cell viability and inhibit biofilm formation. These results are promising for the development of effective coatings to prevent nosocomial infections and for a variety of other antimicrobial applications.

Keywords:

Magnetron sputtering, calcium phosphates, biofilms

Reference:

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Influence of titanium surface modifications on formation of composite calcium phosphates / silver nanoparticles coatings

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Poster Group 2

Background incl. aims
Due to their chemical and mechanical stability, as well as biocompatibility titanium and its alloys are among most frequently used materials for hard tissue implant materials [1]. However, they are biologically inert, a problem which is usually surpassed by coating them with bioactive calcium phosphates, which results in improved adhesion of coating material and cells [2]. As major problem in orthopaedic surgery are implant associated infections, the coatings should in addition have antimicrobial properties. One way of achieving this is incorporation of antimicrobial components in calcium phosphate coatings. In this sense, silver nanoparticles attract attention due to the fact that silver exhibits a broad spectrum of antimicrobial activity and is unlikely to cause bacterial resistance [3].
Methods
The titanium surface was chemically modified using two different methods using strong acids, namely hydrochloric acid previously described by Lu et.al. [4] and using a mixture of hydrochloric and sulfuric acid previously described by Wang et.al. [5]. After modification of the titanium surface, composites of silver nanoparticles and calcium phosphates were coated by wet chemistry method. The influence of the different surface modifications on the formation of the composites was investigated by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), Raman spectroscopy and atomic force microscopy (AFM).
Results
The modification of titanium surfaces changes surface properties such as roughness and wettability, as shown by AFM and contact angle measurements. Composites of calcium phosphates and silver nanoparticles formed on treated, but also on untreated titanium surfaces. SEM and EDS measurements showed that the morphology and uniformity of the coatings change depending on the surface modification.
Conclusion
Surface modification of titanium is an important step in a process for coating titanium with functional materials such as composites of calcium phosphates and silver nanoparticles, as it modifies surface properties and thus influences the deposition of coatings. The results obtained can contribute to the development of an ideal procedure for the preparation of antimicrobial coatings on titanium surfaces.

Keywords:

composites, titanium implants, surface modification

Reference:

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Low-voltage Secondary Electron Emission Spectromicroscopy using a Scanning Auger Microscope

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background
Having been utilized in scanning electron microscopes, secondary electron emission is considered a well-established nano-scale probe for mapping the surface morphology of materials. It has also been demonstrated that secondary electrons (SE) emitted from materials can provide additional information on the local work function, bulk density of state (DOS), surface potential, charging/discharging characteristics, and elemental/chemical properties of bulk materials. The nano-scale lateral resolution and surface sensitivity of low-voltage scanning microscopes give them a unique advantage for the investigation of surfaces. However, the surface contamination caused by exposure to electron beams has always been a limiting factor for this purpose. Since the yield of SE emission is higher than that of Auger emission, the secondary electron emission spectromicroscopy (SEES) performed in an ultra-high vacuum chamber using a scanning Auger microscope (SAM) can be a very powerful tool for surface characterization, especially in the case of ultra-thin materials. Moreover, the concurrent SEES and Auger spectromicroscopy can also provide useful information on the analysis of light elements and hold promise for enabling nanoscale hyperspectral electron spectromicroscopy studies of 2D materials.
Method
We adapt our scanning auger microscope (SAM), equipped with a cylindrical mirror analyzer (CMA) and operated in an ultra-high vacuum, to SEES by tilting the sample holder and applying a negative bias to the sample. We also measured DOS/SEES signals on different materials at low voltages of 500 and 1000 eV.
Results
The SEES results showed that the SAM can perform SEES on different materials when the sample is tilted and negatively biased. The experimental SEES results on metals are in agreement with the Chung and Everhart theory. Moreover, quantum-state contrast on different materials, including ultra-thin films, can be mapped using the SEES acquisition approach. However, the DOS information can be extracted partially, due to a degree of distortion in the signal.
Conclusion
From the results, one can conclude that the SAM can be a proper platform for SEES. However, a lens should be commissioned to pre-accelerate the SEs properly before the entrance to the CMA, mitigating the field distortion around the sample holder. Moreover, the technique can promise the quantum-state mapping of 2D materials due to the limited degree of beam-induced surface contamination.

Keywords:

Auger microscope, Secondary electron, spectroscopy

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Impact of Electron Beam Irradiation on Carbon Black Oxidation

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Poster Group 2

Background
The use of environmental transmission electron microscopy (ETEM) in oxidation studies of nanocarbons, such as carbon black (CB), has revealed new, and confirmed previously suggested, oxidation mechanisms [1]. However, connecting ETEM studies to technical processes (occurring at very different conditions) is not necessarily straightforward. Primarily, the influence of the electron beam can present a large issue that is not easily resolved. For nanocarbon oxidation, there is a clear increase in oxidation rate depending on the dose rate, however, there are few studies reporting any quantification of this effect [2].
To better understand the origin of the beam-enhanced oxidation rate, we note that CB reacts with oxygen gas only at active sites, imperfections in the graphitic shell. These active sites are inherent to CB but can also be generated through interactions with the electron beam through 3 distinct mechanisms. Firstly, ionized oxygen species can react directly with the graphitic shell of CB, these species can be produced through ionization interactions with either primary electrons (PE) or secondary electrons (SE). Additionally, active sites can be generated through atomic displacements in CB by PE. These three mechanisms; ionization of O2 by PE [3], ionization of O2 by SE [4], and atomic displacements by PE; have different underlying mechanisms and respond differently to variation of reaction and imaging conditions.
Methods
In our study, we have observed the oxidation of CB at varying electron dose rates and electron energies in two ETEM setups. Data were collected using electron energy loss (EEL) spectroscopy time-series for determination of an average oxidation rate over agglomerates, as calculated from the time-dependent decrease in the C-K signal. This data were complemented with data high-resolution time resolved image series to investigate the local effect on individual particles and to validate the EEL spectroscopy results.
Results
Our experiments show that in situ beam-enhanced CB oxidation is localized to the irradiated area, that the oxidation rate is not significantly affected by changes to the electron energy, and that the in situ beam enhancement is non-linear in nature. Additionally, the reaction only occurs in the presence of an oxidizing gas, i.e. not in inert gases like N2. These findings contradict that ionization by PE is the driving force behind the increase in oxidation rate in TEM, as the ionized species are expected to spread out over an area larger than the illuminated, and the rate at which ionized oxygen species is expected to approximately double as the electron energy is reduced from 300 keV to 100 keV. Similarly, oxygen ionization by SE, is also expected to increase when the electron energy is decreased, however, this mechanism may have a minor impact if the CB sample is located on a support, in which case an excess of SEs is generated. Rather, the results are consistent with atomic displacement as the main driving force; given that the electron energy required to knock an atom out of its lattice position is exceeded, the probability of displacement does not change to a large extent over the relevant electron energy scale. Additionally, atomic displacements can only occur where the electron beam has been located, and no damage is observed outside of the irradiated area.

Conclusion
We have shown that CB oxidation is mainly affected by elastic high-energy electron-sample interactions, with a smaller contribution from inelastic electron-gas processes from secondary electrons emitted from the sample and sample support. While this process suggests that there are no safe conditions, the beam effect can be made vanishingly small compared to the intrinsic oxidation. We show how EELS can link processes seen at individual particles to whole agglomerates. This further illustrates the importance of comparing results obtained in the ETEM with results obtained via other methods such as TGA or its like.
Graphic
Figure: Summary of methods and primary results of study. a) EEL spectroscopy time-series (blue) of the C-K signal and linear fit (orange) to the linear region of the time-series. The inset illustrates selected average spectra from times s. b) The linear oxidation rate as calculated from the linear fit in time-series as in a) illustrating no significant difference between 80 keV and 300 keV. c) Results from an image time-series with insets showing a CB particle at various stages of oxidation. d) Aggregate results of image time-series, the green region represents an “inherent” oxidation rate with intermittent imaging.

Keywords:

Electron beam nanocarbon oxidation ETEM

Reference:

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Atomically sharp domain walls in antiferromagnetic thin films

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Poster Group 1

High-quality thin films of antiferromagnetic tetragonal CuMnAs, grown by molecular beam epitaxy, have recently found application in spintronic devices. These devices leverage various intriguing magnetic phenomena, such as Néel vector reorientation via spin-orbit coupling, or thermal quenching into high-resistivity states, to achieve novel functionalities.
In this study, we conducted scanning transmission electron microscopy (STEM) measurements to investigate the magnetic structure of epitaxial CuMnAs thin films on the atomic scale. Our aim was to explore the origin of sharp transitions observed by DPC-STEM technique and relate them to the presence of an abrupt change in the antiferromagnetic order .
Complementary results obtained by 4D-STEM strongly suggest the existence of atomically sharp antiferromagnetic domain walls in epitaxial CuMnAs thin films, at least on the qualitative level. The existence of such features not only contributes to a deeper understanding of the physical mechanisms underlying the intriguing functionalities of CuMnAs devices but also sheds light on the intricate interplay between crystalline and magnetic structures in antiferromagnets.
Our more recent findings suggest that some of the intriguing phenomena previously observed exclusively in CuMnAs may have a more universal origin and can be observed in other materials. We will highlight a family of materials which, while grown in the form of thin films, have nearly the same crystallographic structure as CuMnAs but exhibit distinct ferromagnetic and antiferromagnetic magnetic orders. This naturally epitaxial material platform holds great promise for the design of high-quality magnetic heterostructures, but can also serve for further development of advanced STEM techniques.

Keywords:

spintronics, 4D-STEM, magnetism, domain walls

Reference:

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STEM-EELS studies of interfaces between perovskite oxide membranes and single-crystal carrier substrates

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Perovskite-structure transition metal oxide epitaxial thin films exhibit a variety of functionalities that make them highly promising for the development of novel electronic devices, including non-volatile memories, sensors, and flexible electronics. In recent years, interest has arisen in transforming these thin films into perovskite membranes, that can be detached, transferred and layered similarly to architectures established for 2D materials. Compared to epitaxial heterostructures, this offers potential benefits of, for instance, no structural mismatch strain effects, possibilities to create texture (wrinkles), versatile stacking and, ultimately, scaling down to the 2D limit [1]. Recently, such membrane structures have been studied with Cs-corrected scanning transmission electron microscopy (STEM) using a plan-view geometry [2]. In contrast, here we focus on cross-section studies, where atomic structural quality and flatness/roughness can be precisely assessed. By combining with electron energy-loss spectroscopy (EELS), we further apply this approach with a goal of probing the bonding of membranes transferred to single-crystal substrates, and how this evolves with thermal annealing.
For the measurements, 30 nm-thick $SrTiO_3$ membranes are grown on a 16 nm-thick $Sr_3Al_2O_6$ sacrificial layer on a (001) $SrTiO_3$ substrate using pulsed laser deposition. After dissolving the $Sr_3Al_2O_6$ layer in deionized water, the resulting $SrTiO_3$ membrane is transferred onto a Nb-doped $SrTiO_3$ (001) carrier substrate. This membrane heterostructure is either left “as is”, or is subjected to a thermal annealing at one of a series of temperatures. Focused ion beam milling is then used to prepare a cross-section lamella. These samples are studied in STEM mode using a double-corrected FEI/Thermo Fisher Scientific Titan Themis 60-300, operated at 200 or 300 kV, with STEM-EELS maps recorded in counting mode using a Gatan GIF Continuum ERS equipped with a K3 detector.
By imaging membranes in cross-section from both before and after the steps of $Sr_3Al_2O_6$ dissolution and transfer, it is found that the quality of the $Sr_3Al_2O_6$ sacrificial layer is crucial for final membrane quality, with a flat and uniform $Sr_3Al_2O_6$ layer being necessary for obtaining a flat $SrTiO_3$ film having a high quality of epitaxial growth. The figure shows an example cross-section high angle annular dark-field (HAADF) STEM image of a $SrTiO_3$ membrane transferred to the Nb:$SrTiO_3$ carrier substrate. Because of the twist of the membrane to the carrier substrate, while the cation columns are resolved in the substrate, the membrane depicts only atomic planes. This is, however, still sufficient to study the atomic nature plane-by-plane using Cs-corrected STEM-EELS mapping. Membrane and substrate are separated by a ~2 nm interface gap.
From a STEM-EELS map, the left-hand panels of the figure show EEL spectra of the Ti L-edge and O K-edge, integrated from three different regions: the carrier substrate, the membrane, and also the 2 nm interface gap. It can be seen that there are no significant differences between the membrane and carrier substrate, where the Ti L_3, L_2 peaks show the signature t2g and eg splitting for Ti^{4+}, and the O K-edges share the same fine structure [3]. To the right of the HAADF image, a line-profile is shown of

the Ti L-edge signal extracted across the heterostructure. Surprisingly, the intensity does not decay to zero within the interface gap. Looking at the corresponding EEL spectrum, the lack of splitting in the Ti L_3, L_2 peaks structure indicates that, in the interface gap, Ti exists in a reduced form [4]. This may be due to the free surface of the membrane and substrate having dangling Ti bonds that are observed in projection.
In summary, we are applying atomic-resolution STEM in a cross-section geometry to study the chemical bonding of transition metal oxide membranes transferred to carrier substrates. By thermally annealing these heterostructures, we expect to create different bonding states between the membrane and the substrate, that will be measured using STEM-EELS.

Keywords:

perovskite membrane, EELS, chemical bonding

Reference:

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Breaking the atomic resolution depth of field limit using multi-slice electron ptychography and tomography

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Poster Group 1

Background incl. aims

Aberration-corrected scanning transmission electron microscopy (AC-STEM) has evolved into a powerful method for atomic-level visualization and chemical analysis of materials, thanks to equipment improvements and the incorporation of electron-matter interactions into reconstruction algorithms. Particularly, 4D-STEM has become an impressive technique, combining 2D position- and 2D momentum-resolved measurements, capable of high-precision imaging and extensive materials characterization, with the aid of fast direct electron detectors and computational imaging methods. One notable method is Multi-slice Ptychography, which allows for obtaining not only a highly resolved transverse image but also an axial one; however, the axial resolution is currently limited by a few nanometers, preventing its use for de novo atomic structure determination.
To overcome this limit, tilt-series 4D-STEM data collection and tomography reconstruction have been used for 3D atomic resolution phase contrast imaging [1], however the reconstructed volume has so far been limited by the projection approximation.
Here we present and experimentally demonstrate an approach to discern individual atoms in a substantial volume, three times larger than the traditional depth of field, while retaining atomic resolution in three dimensions.

Methods

We applied a two-step multi-slice electron ptychographic tomography method, where 4D-STEM scans are conducted with overlapping probes at various tilt angles using a fast-framing direct electron detector in an AC-STEM. Thirty-six 4D-STEM datasets were captured from a Co3O4 nanocube. The diffraction images were obtained using a the 4Dcamera, capturing full diffraction patterns at each position. The reconstruction used multi-slice ptychography on the captured images, which accounted for the wave propagation through the sample and onto the detector. Five slices along the depth were reconstructed, slightly oversampling the 5nm depth of field due to the 13nm size of the cube (see Fig. 1).
The initial ptychography reconstructions underwent denoising and contrast adjustments to highlight the nanoparticle over the substrate, followed by an alignment process for accurate 3D reconstruction. A developed two-step optimization algorithm refined the alignment through iterations of reconstruction and alignment adjustments of down-sampled volume. The final reconstruction at full resolution utilized the obtained alignment and Gaussian blurring to maintain fidelity within the Nyquist limit, resulting in clear visualization of the nanocube's inner structure and lattice.

Results

Due to its strong contrast, the amorphous carbon substrate introduces more noise into the reconstructed volume compared to prior ADF-STEM tomography reconstructions of metal nanoparticles. This substrate scattering challenges direct tracing of Co atomic peaks. Instead, the experimental Co lattice is aligned with the Co_3O_4 crystal model by fitting Bragg peak positions in Fourier space and optimizing volume translations in real space, revealing the expected positions of Co and O atoms. This method is preferable to previous peak-by-peak tracing due to contrast variations from the substrate. The results display good agreement between the fitted atomic model and the reconstructed volume.
Higher-symmetry zone axes allow for clearer differentiation of Co atoms, while lower-symmetry axes don't resolve Co atoms less than 2 Å apart due to substrate contrast. Three-dimensional resolution is measured using the 3D power spectrum; the resolution along the axes was estimated, showing sufficient clarity to distinguish neighboring Co atoms in the structure. However, to resolve Co and O atoms effectively, our simulations indicated that a 3D resolution of 1 Å is necessary owing to the weaker signal from O atoms.

Conclusion
We have achieved unprecedented 3D phase-contrast imaging in electron microscopy, attaining an axial resolution of 2.04 Å and transverse resolution of 0.7 Å within a volume surpassing the depth of field limit by a factor >3x. Our method, sequential multi-slice ptychographic tomography, removes the depth-of-field limits of existing techniques and suggests the possibility of reconstructing crystalline oxide samples up to 70 nm thick. Although the resolution was not sufficient to discern oxygen atoms within the Co_3O_4 lattice or to cope with the high background from amorphous substrates, we anticipate that future improvements in algorithms, experimental design, and detector efficiency will address these challenges. Adopting crystalline substrates for the interim may aid in resolving weakly scattering atoms at atomic resolution. Moreover, incorporating explicit atomistic models in ptychographic tomography can better account for effects like thermal diffuse scattering, further enhancing resolution. Importantly, ptychographic electron tomography shows promise due to its amenability to experimental automation at atomic resolution by trading in experimental complexity of ADF-STEM tomography against data size and compute, positioning it as a forerunner for large-scale, high-resolution, 3D imaging within the realm of 4D-STEM.

Keywords:

4D-STEM, multi-slice ptychography, tomography, reconstruction

Reference:

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Strain analysis comparison in complementary and nanosheet field-effect transistor devices: nanobeam vs Bessel electron diffraction

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Poster Group 2

Complementary Field-Effect Transistors (CFETs) represent a significant innovation in semiconductor technology currently drawing a lot of interest as they can be a potential solution for extending Moore's law by allowing considerably higher density compared to conventional CMOS. The novelty of these devices lies in the possibility to integrate different polarity transistors within a single structure, stacking nmos on top of pmos or vice-versa. That allows substantial density scaling benefits with enhanced performance and reduced power consumption. [1] CFETs represent an evolutionary step from the nanosheet transistors (NSFETs) which consist of several vertically stacked lateral nanosheets per device and where pmos and nmos devices are positioned next to each other on the same horizontal plane in the wafer instead of vertically atop one another as in CFETs. In both architectures, unlike finFET-based CMOS, in order to reduce their parasitics, inner spacers are introduced in-between the vertically stacked NS and prior to the source/drain (S/D) epitaxial growth, a flow addition which will also impact the S/D epi process. [2]

Strain engineering is a crucial technique for enhancing the mobility of conventional semiconductor devices. In general, channel strain, induced by the S/D in both conventional and novel CMOS, depends on the epitaxial layer composition used for the S/D (with SiGe typically used for pmos), its geometry and defectivity. Understanding the strain distribution within these structures is important for optimizing device characteristics. Previous studies on NSFET devices [2] demonstrated that the presence of inner spacers impacts S/D growth and, by consequence, the strain distribution induced in the channels. In this work, we evaluate and compare the channel strain distribution in aggressively scaled (CFET-NS) relative to more relaxed (NSFET) gate pitches, employing nanobeam electron diffraction (NBD) and Bessel diffraction [4] to discern the most apt technique for studying strain in these novel architectures.

TEM samples are prepared by focused ion beam lift-out technique. Strain analysis is performed by NBD and Bessel in a double corrected Titan3 G2 60-300 transmission electron microscope operating at 200kV. CFET and NSFET samples consist of fin-shaped structures of Si/SiGe layers. The sacrificial SiGe layers, destined later for etching to release the NS, are also recessed post-S/D recess to accommodate inner spacer deposition. CFET samples feature a single pmos Si channel sandwiched between SiGe50 S/D at the bottom and one nmos Si channel at the top, while the NSFET sample consists of a dual Si-channel pmos. Notable, the main difference between NSFET and CFET devices in this study lies in the gate pitch dimensions, with NSFET at 100nm and CFET at 50nm.

Strain profiles along the Si channels (indicated by blue arrows in Figure 1a and b) in double Si-channel NSFET (Figure 1a) and single Si-channel CFET (Figure 1b) devices, processed until after epitaxial SiGe S/D growth, are depicted in Fig1c and Fig.1d, respectively. Both single-level NSFETs and bottom NS of CFET exhibit tensile channel strains, a significant departure from traditional finFETs characterized by compressive strain. [3] This tensile strain originates from the lattice mismatch of the SiGe/Si

multilayer stack in the fins, which persists after fin patterning. The introduction of inner spacers further amplifies tensile strain in the channels. Inner spacers lead to separate SiGe epitaxial growth from the bottom and lateral fronts, resulting in defects or air gaps at the interface where the three growing fronts converge. In addition, CFET exhibits lower strain compared to NSFET likely due to their smaller S/D and gate pitch than in NSFETs. TCAD simulations could offer additional insight into local strain behavior in these devices. Furthermore, we perform a comparison between the NBD and Bessel techniques for assessing strain in CFET devices. As shown in Figure 1d, while the NBD technique reveals minimal or no strain, the Bessel method (dotted curves) enables measurable strain detection. As previously observed [5], the Bessel technique demonstrates superior sensitivity compared to NBD, becoming the preferred technique for strain evaluation in such innovative architecture devices.

Keywords:

Strain, CFET, nanosheet-FET, NBD, Bessel

Reference:

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Off-Axis Electron Holography of In-Situ-Biased Highly-Doped p-AlGaAs/n-GaInP Junctions for Solar Cell Applications

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Introduction and motivation
Multi-junction solar cells that are based on MOVPE-grown III-V compound semiconductor layers achieve the highest solar cell efficiencies, as they absorb light from a large part of the solar spectrum. Tunnel junctions provide electrical interconnections to the device sub cells, while yielding high current capabilities and high optical transparency. As the tunnelling probability decreases exponentially with increasing depletion width, high dopant concentrations are required to achieve the narrowest junctions. Structural and electrical analysis with high spatial resolution is essential to provide feedback to optimize the growth process. Electrostatic potentials at p-n junctions can be measured quantitatively with nm spatial resolution using off-axis electron holography (EH).
Methods
Here, we combine EH with in situ electrical biasing and optimized TEM sample preparation to study electrically-contacted p-AlGaAs/n-GaInP tunnel hetero-junctions grown in upright and inverted configuration. First electron-transparent specimens of different thicknesses were studied to assess the influence on the measured electrostatic potentials of the presence of electrically-inactive specimen surface layers introduced during focused ion beam (FIB) milling. The crystalline thickness of each specimen was measured using convergent beam electron diffraction (CBED) in a scanning TEM (STEM) (Fig. 1B). The TEM specimens were prepared using dedicated chips for an electrical biasing TEM holder (Protochips Aduro 500). Reliable contacting was achieved by developing two different FIB milling procedures. First, conventional FIB milling was used to extract a region of interest, while preserving a pre-deposited Ohmic top contact (Fig. 1A). Tungsten was then deposited in the FIB workstation onto the bottom of the sample to provide a second electrical contact. The second approach involved the use of plasma FIB milling to extract slices of the wafer that contained the original top and bottom metal contacts. Electron hologram stacks were recorded from both junction configurations to improve the sensitivity and spatial resolution of the results. Measurements of depletion width were recorded as a function of both specimen thickness and applied external bias.
Results
Phase images (proportional to projected electrostatic potential) were reconstructed from the holograms as a function of applied electrical bias. Figure 1C shows a phase image recorded at zero bias and a corresponding line profile of the phase across the junction.
The inverted and upright junction configuration exhibit similar depletion widths measured at zero bias. Analysis under external bias is necessary to reveal differences in tunnelling performances occurring in the current regimes of operation under light. Our results demonstrate the importance of optimizing specimen preparation when performing in situ electrical biasing experiments. In particular, to create Ohmic electrical contacts, as opposed to the Schottky contacts that are normally formed using FIB metal deposition. By using an optimized specimen preparation approach, we could

compare the electrostatic potential distribution across upright and inverted p-AlGaAs/n-GaInP junctions reliably.

Conclusions

This study demonstrates the progress that has been achieved in realizing successful in situ characterization of dopant potential distributions in III-V tunnel hetero-junctions in real solar cell devices.

Keywords:

Electron Holography, III-V Multijunction PV

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Multimodal and correlative imaging approaches to study early stages of SARS-CoV 2 infection

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Background
In this work we aim to investigate SARS-CoV-2 entry mechanisms using cutting edge cryo-methodologies to obtain structural insights in the mechanism of infection. SARS-CoV-2 is the causative agent for the recent Covid pandemic and as such has been the centre of both scientific and public attention over the past few years. While this has resulted in numerous publications over a very short amount of time, much is still unknown, also about the entry pathway. For the virus to enter it needs 2 steps, first the viral Spike protein needs to bind to a host cell surface receptor, the most common being ACE-2, although others have been reported to facilitate SARS-CoV-2 entry. Two possible pathways have been identified: direct viral fusion at the cell membrane or viral endocytosis and fusion once internalised. This depends on the presence of host-cell proteases (most commonly TMPRSS2) at the cell surface, as the viral spike protein needs to be cleaved before fusion can commence.
Precisely identifying sites of binding and/or fusion is difficult when doing an infection experiment. Here we aim to use correlative and multimodal imaging in cryogenic conditions to study the viral entry within the very first hour, at a time that the cell-population is basically unaware of an ongoing infection and not releasing stress-effectors. For this we are using cells that can only be infected through the endocytic pathway and a fluorescently labelled virus, which we want to track in a correlative manner. High-resolution fluorescence data is essential to localize the areas of interest and the recent purchase of the cryo-stellaris 8 confocal microscope (Leica) allows us to collect this type of data. After initial mapping and localization of areas of interest, we aim to study the samples using either cryo soft X-ray tomography (cryo-SXT), and/or cryo electron tomography (cryo-ET). Both methods have the advantage of providing 3D information of biological samples preserved as close as possible to their native state. Cryo-SXT is a transmission based, medium-resolution (30 nm half pitch) but high throughput synchrotron technique that relies on the absorption of the X-ray light by the sample. It is a powerful technique to study the cellular ultrastructure of thick samples (<10 micron) and can even be used for quantification by comparing the absorption coefficients of different structures. Cryo-ET is a transmission-based technique that does require very thin samples (<300nm) but can provide nanometre resolution data in return. Using this technique, molecular interactions can be visualized and using sub-tomogram averaging, protein structures can be resolved in situ.
Methods
Mammalian cells are cultured on classical TEM Quantifoil grids and infected with SARS-CoV-2 at an MOI of 10. To synchronize the start of the infection, the virus and cells are kept at 4 degrees Celsius, as at this temperature no fusion takes place, and after 30 minutes the cells are moved back to the incubator at 37 °C. The grids are then fixed by plunge-freezing at various timepoints during the first hour of infection. Next, cryo-fluorescence is used to localize sites of interest. The same sample can then be used either for cryo-SXT acquisitions, or for cryo-ET of cell peripheries and of cryo-FIB milled

lamellae. Collected tilt series have to be aligned and reconstructed to obtain the final 3D tomograms which can be used for the correlation, segmentation and analysis.
Results
By registering the fluorescent map obtained at the confocal microscope with the low magnification TEM map of the grid, we were able to locate areas of interest. Lamellae preparation was required only for the later timepoints as in this timeframe the internalised virus is located in thicker areas of the cells (>300 nm). We visualized the virus at the cell surface and inside vesicles, bound to surface proteins or in the process of fusion. Using cryo-SXT we were able to confirm some of the phenotypes that we observed by cryo-ET, and it allowed us to put it into perspective of a larger area of the cell. It was possible to compare cellular morphologies, and the changes thereof over the course of the first hour of infection.
Conclusion
Visualizing different stages of infection within their native environment can greatly improve the understanding of their underlying cellular processes. Here we use 3 complementary modalities that allow us to look at the SARS-CoV-2 infection process with complementary imaging methods at low (cryo-confocal), medium (cryo-SXT) and high (cryo-ET) resolution. While this approach is very time-consuming, it gives a more complete multiscale overview of the complex mechanism of virus-host interaction.

Keywords:

cryo-CLEM; CLXM; SARS-CoV-2; Host-Pathogen-Interaction

Reference:

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Impact of physical exercise in bone healing around bio-resorbable implants imaged by synchrotron techniques

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background incl. aims
Bio-resorbable magnesium implants are promising candidates for the treatment of bone fractures due to their mechanical properties, their immunological response during healing, and the reduced need to perform a second surgery for implant removal. Some studies show how the placement of bio-resorbable implants alters the bone nanostructure, which changes from the preferred orientation along the bone shaft to a wrapping around the implant surface [1].
There are, however, no accounts in the literature on how physical exercise applied immediately after implantation might influence the bone nanostructure and the healing process around magnesium implants. This question is of great importance for understanding the impact of mechanical stimuli during healing and could be of relevance for post-surgical recovery treatments.
Methods
We designed an animal study on two groups of rats, one group underwent physical exercise immediately after implantation and the other one was sedentary. The bone with the implant was explanted after 2 and 6 weeks and the explants were studied.
For the aims of the study, we used two complementary synchrotron 3D imaging techniques, i.e. small-angle scattering tensor tomography (SASTT) and phase-contrast high-resolution computed tomography (PCµCT). Each one of the samples was measured with both techniques and the results were correlated with each other.
Results
We have investigated the role physical exercise plays in bone healing around Mg implants in a short-term study. With SASTT we studied the orientations of the bone nanostructure in 3D [2]. We have observed how the degree of orientation of hydroxyapatite, the thickness of the mineral particles, and the nanostructural orientations in bone change when physical exercise is applied. With PCµCT we characterized the osteocytes' network (considered responsible for mechanotransduction) by imaging the lacunae in 3D. We performed a shape and orientation analysis of the osteocytes to study the effect that physical exercise has on this network, and we correlated the results of both techniques.
Conclusion
Our study proved that physical exercise favors remodeling and bone healing around bio-degradable implants and alters the orientation of the bone structures at different length scales. These results are highly relevant for understanding degradable implants' behavior and are expected to be of clinical significance in the treatment of bone fractures.

Keywords:

Bone, implants, physical exercise, X-ray

Reference:

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Grain evolution during annealing of a semisolid Al-Cu alloy studied with lab-based diffraction contrast tomography

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
3D experimental data of simultaneously high temporal and spatial resolution is key to validation of computational modelling of materials phenomena. In this study, we exploit lab-based diffraction contrast tomography (DCT) [1], to capture the evolution of grain structure over a series of interrupted annealing treatments of a semisolid Al-Cu alloy [2,3]. The time resolved response measured on the present Al-Cu model system provides insights into the rearrangement, densification and coarsening of powder compacts at late-stage sintering. The wealth of the experimental data lends itself particularly well to investigations of both grain size and orientation (rotation) evolution.

Methods
The semisolid Al-Cu alloy specimen was subjected to ten sequential isothermal heat treatments, each 15 min at 630°C, and we used lab-based DCT to non-destructively track the microstructural evolution of individual grains through the corresponding eleven temporal states of interrupted annealing. The lab environment setting allows us to reconstruct the results and analyze changes to the microstructure before subsequent treatment of the sample. Furthermore, we take advantage of the recently developed DCT advanced acquisition schemes [4] to cover a large sample volume of 10 mm^3.

Results
During the experiment, we observe both grain coarsening (from 1934 grains with a mean grain size of 194 µm at the initial state to 934 grains with a mean grain size of 247 µm after ten annealing steps) and grain rotations. A statistical study of the evolving grain structure reveals that the disappearing grains are generally among the smaller ones at the beginning of the experiment. In addition, the rotations of individual grains are typically small fluctuations irrespective of grain size, but when an abruptly large rotation is observed, it is more likely to occur for a smaller grain at the last annealing step(s) before the grain vanishes. Finally, the experimental data indicates that Σ3 twin boundaries are especially stable, and that grains sharing such a boundary will rotate together in their local environment to keep the boundary intact.

Conclusion
Our investigations show that the crystallography of grain contacts undoubtably plays a pivotal role in the microstructural evolution of semisolid systems during heat treatment and should be included in predictive simulations of coarsening. To help incorporate our experimental observations into the next generation of models, the eleven grain maps have been made publicly available to the scientific community via the Materials Data Facility [5].

Keywords:

Lab-based DCT, Coarsening, 4D structure

Reference:

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Detection of weak ELNES signals using dose-fractionated spectrum imaging combined with direct detection

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Poster Group 2

Background:
Recent studies showed that mixed Fe-Co oxide nanoparticles could replace more expensive Ir oxide and Ru oxide for the oxygen evolution reaction for water electrolysis [1]. Acquiring spectra at a high resolution is especially advantageous for understanding how the valence state of Fe and Co changes across the nanoparticles and potentially influences their catalytic behavior. EELS combined with STEM has proven effective at studying chemical changes at the sub-nm level [2]. Previous studies systematically characterized how the Fe L2,3 edge changes with changes in local symmetry based on the phase of Fe oxide [3]. The L2,3 edges of the first-row transition metals probe the unoccupied 3d states, which are sensitive to spin-orbit coupling, linking local symmetry changes to oxidation state. However, the transition metal K-edges probe unoccupied 4p states and are more sensitive to nearest neighbor distances, bonding angles, and the onset of the K edge undergoes measurable shifts with changes in oxidation state. Characterizing the Fe K edge at the sub-nm scale could add more profound insight into changes in structure and chemistry.
The cross-section of the transition metal K edges is ~3 orders of magnitude smaller than the corresponding L2,3 edge. This requires increased acquisition times and the likelihood that radiolysis and sample drift will compromise the measurement. It was shown in polymer blends that the performance of the microscope does not limit the resolution of an EELS spectrum image. Instead, the resolution is limited by a critical dose above which the polymer structure changes [4]. Gatan recently implemented new software and hardware tools that acquire and analyze weaker signals without compromising the sample. Using direct detection cameras for EELS on the GIF Continuum increases the detection efficiency needed to collect weaker, higher-energy K-edges, mitigating radiolysis by reducing acquisition times [5]. Upgrades to DigiScan 3 enable continuous in-line drift correction, where features in the simultaneously acquired ADF image are used to monitor drift, reducing latency during the drift measurement. Coupled with the fast, sub-millisecond frame rate of these direct detection cameras, accurate and frequent drift correction is critical in ensuring no spatial blurring of the spectrum image during long acquisition times. Finally, the in-situ spectrum imaging in DigitalMicrograph allows tracking and monitoring of the experiment over time and the total dose to be fractionated over multiple, individually saved passes. This enables integration or removal of passes after acquisition, enabling the enhancement of the SNR or removal of passes that may be compromised by sample damage or contamination.
In this study, we demonstrate why it is critical to combine the sensitivity and speed of direct detection with the ability to fractionate the dose over several passes using in-situ spectrum imaging when acquiring weaker ionization edges. We show how these tools can be utilized to study the spatial resolution limits based on the dose threshold of Fe oxide and mixed Fe-Co oxide nanoparticles. Ultimately, we explore how the material's interaction with the electron beam limits the resolution of the measurement and how this compares to analogous techniques such as scanning XAS.
Methods:

A JEOL F200 with a cold-FEG with a post-column Gatan Continuum Spectrometer was used to acquire EELS spectrum images (SI) at 200 kV. The spectrometer is fitted with single electron counting (K3, Gatan) and hybrid pixel thresholding (Stela, Gatan/Dectris) cameras, and were used to collect SI datasets targeting the Fe K edge (7112 eV). DigitalMicrograph's Elemental Quantification tool and concurrent standards were used to study the reduction of the Fe oxide particles as a function of applied dose. The linear-least squares tool in DigitalMicrograph was used to measure the shift in the Fe K-edge across the nanoparticles by fitting Gaussian functions to the pre-peak and main peak after the edge onset.

Results :

An EELS spectrum image acquired from a cluster of Fe¬2O3 nanoparticles is shown in the ADF image in Figure 1a. The spectrum image was acquired using multi-pass spectrum imaging. A step size of 5 nm and a pixel dwell time of 330 µs was used to minimize the total dose per pass while being able to resolve individual particles in the SI. The short pixel dwell time combined with inline drift correction enabled frequent and accurate drift correction to minimize drift artifacts during the measurement. In Figure 1b, an elemental map was created from the integrated intensity of the Fe K-edge, and the smaller particles can be resolved in the cluster. The Fe K-edge ELNES extracted from the Fe¬2O3 particles is plotted in Figure 1c, along with the fitted Gaussian functions of the pre-peak and the main peak of the K-edge. Shifts in the pre-peak and the main peak were measured by mapping the position of the fitted Gaussian in Figure 1e and 1f, respectively. The pre-peak originates from a weak 1s to 3d quadrupole transition. As a result, the SNR is lower than the main peak, producing a larger variability in the fit in Figure 1e. The main peaks in the Fe K ELNES originate from a stronger 1s to 4p dipole transition, resulting in a signal that is an order of magnitude larger than the pre-peak. A shift in the main peak is observed in Figure 1f, from 7130 eV in the smaller particles to 7132 eV in the larger particles. To confirm this trend, further measurements are needed from well-separated particles.

Conclusion:

Detailed analysis of the weaker Fe K-edge is possible across individual particles. However, further experiments are needed to determine why the main peak of the Fe K edge shifts with changes in particle size. In-situ experiments fractionating the dose over several individually acquired passes are needed to confirm that the shift in the main peak is caused by radiolysis. We will apply this workflow to a mixed Fe-Co oxide system to study how the oxidation state changes with the concentration of Fe and Co, by examining changes to the ELNES of the Fe and Co K-edges. Finally, we aim to study how radiolysis limits the spatial resolution of weaker ionization edges.

Keywords:

Dose Fractionated EELS, ELNES, Catalyst

Reference:

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Arctis WebUI redefines lamella preparation for cryo-electron tomography workflow

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Poster Group 2

Background
Cryo-electron tomography has emerged as a valuable technique for visualizing proteins and protein complexes in their natural cellular environment. To achieve high-resolution imaging, the preparation of thin cryo-lamellae containing the region of interest is a critical step. However, the usual methods for lamella preparation are often time-consuming and require the use of several applications, presenting challenges for inexperienced users in the cryo-tomo workflow.

Methods
The Arctis cryo-Plasma Focused Ion Beam system plays a pivotal role in the automated production of cryo-lamellae from cells. The system is supported by the Arctis WebUI, with a web-based user interface that streamlines the creation of cryo-lamellae with minimal user inputs. WebUI is a unified platform where users can map and navigate through the sample using electron, ion, or optical tilesets. Additionally, the integration of a fluorescent light microscope enables in-situ targeting of specific cellular structures. Prepared lamellae are transferred to a Transmission Electron Microscope for cryo-electron tomography. The Thermo Fisher Scientific Selectris Imaging Filter is utilized to significantly enhance contrast during data acquisition through zero-loss filtering. Data collection is facilitated by Tomography 5 software, enabling unattended data acquisition, seamlessly connected with Tomo Live software, allowing automatic on-the-fly data reconstruction.

Results
We will provide a comprehensive overview of the application workflows for lamella preparation facilitated by the Arctis WebUI. The key advancement is the use of the fully automated optical tileset, which allows the selection of cells directly based on their fluorescently labelled features that are not visible through standard electron or ion imaging. With the combination of the 3D targeting solution, users gain valuable insights into the cellular content and can precisely identify target areas for lamella preparation. The automatic determination of ROI depth in the optical stack significantly accelerates and simplifies the 3D targeting process, eliminating the requirement for manual browsing through the entire optical stack. Once the lamellae are milled, they are transferred to a cryo-Transmission Electron Microscope through the common sample loading interface and utilized for cryo-electron tomography data collection.

Conclusion
With the capability to seamlessly process multiple (up to 12) grids with cells, Arctis WebUI streamlines the preparation of cryo-lamellae for tomography, enhancing efficiency and productivity in the field of cryo-electron microscopy. The integration of optical tileset and 3D targeting enables more precise lamella placement, guarantees data collection from specific sites in cryo-TEM, and ensures the presence of accurate structures in the resulting tomography data. These workflows significantly reduce the need for extensive user input, allowing researchers to concentrate on solving

biological problems while the WebUI automates the sample preparation process for cryo-tomography.

Keywords:

Arctis WebUI, cryo-electron tomography, cryo-lamellae

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Ptychographic imaging of nanoscale 3D objects with soft x-ray synchrotron and EUV table-top sources

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

X-ray/Extreme ultraviolet (EUV) coherent diffraction imaging (CDI) is an efficient technique for the characterization of nano-structures. Unlike conventional microscopy, CDI does not rely on complex high-quality X-ray/EUV optics. However, in order to obtain a reconstructed image of the object, generally, a large amount of diffraction data is required. To reach the necessary level of oversampling, ptychography is often utilized. It is a form of CDI in which a focused light beam is scanned over the sample, obtaining a unique diffraction pattern at each scan position. This allows to reach the necessary amount of over-sampled data. The technique enables reconstruction of both amplitude and phase distributions, much like holography, via advanced iterative phase retrieval algorithms. Utilizing information about both absorption and phase shift in every pixel of the object's image yields chemical sensitivity. In addition, the relative transparency of many materials in the EUV/soft x-ray range allows buried structures and interfaces to be imaged that would otherwise be opaque in electron or probe-based microscopies.
Given the high spatial resolution that can be achieved with ptychographic CDI, we recently proposed [1] its exploitation to image atom probe samples. Atom probe tomography (APT) is highly complementary to transmission electron microscopy (TEM), as it enables nano-scale compositional analysis on 3D objects, which has triggered lots of attention in the semiconductor industry [4]. For APT analysis, the sample is shaped into a nano-sized object that resembles a tomography pin [2] but with much tighter aspect ratio and dimensions, i.e. the tip's endpoint has a radius of less than 100 nm, the length can be several hundreds to thousands of nanometer while the thickness of the sample changes along its z-axis with typical tapper angles around 5˚ to 10˚.
While APT is capable of atom-level resolution, the fidelity, and the resolution in APT is sample dependent. The reason is, in an atom probe microscope, the sample itself acts as the ion optics and thereby its shape and properties define the magnification during analysis.
Knowledge of the tip's actual shape, and the ability to monitor it during APT measurement is expected to provide substantial improvement in resolution and fidelity of APT reconstructions.
So far, most high-resolution EUV/X-ray ptychographic images were taken from thin film samples with imprints or holes, showing spatial resolution below 10 nm [3]. In this work we show the results of ptychographic imaging of APT tips with two distinct sources: soft x-ray produced by a synchrotron radiation at 800 eV (1.5 nm), and tabletop EUV light at 92 eV (13.5 nm). The shape of a typical APT tip significantly differs from both a typical thin planar sample for which the ptychography resolution is studied, and the more arbitrary 3D samples like grains or cells. The APT tip represents a conical needle with radius from 150 nm down to ~50 nm, where both the internal and surface structure of the top few hundred nm is of interest [4]. This leads to several challenges, on the one hand, the typically deployed planar approximation of the object is no longer valid, which leads to a decrease of resolution in the lower, and thus thicker part of the tip. On the other hand, the apex of the tip is very small both in lateral and axial dimensions. This leads to a miniscule change in the absorption and the

phase shift resulting in low contrast and, consequently, also lower resolution, and higher requirements for the detector's dynamic range.
We demonstrate capabilities for quantitative analysis of chemical composition, shape determination and resolving of embedded structures. The imaging test was performed with two types of tips: Si fin and Mo/Si Multilayer, as the first one represents a 3D low-contrast structure and the second – a high-contrast layered structure. The fin-type tips analysis allows to study and demonstrate 3D imaging while the characterization of high-contrast layer structure allows analysis of the dependency of ptychography resolution on the thickness of the sample.
Using 800 eV light produced by a synchrotron together with a CDI setup with a 0.07 detection NA, on the example of the first needle we here demonstrate the ability to image buried interfaces with sub-30 nm resolution, while simultaneously determine the exterior geometry with 11 nm resolution as shown in Fig. 1 a,b.
Using the tabletop EUV source with 0.5 detection NA, we determine the exterior geometry of the second APT needle with 15 nm resolution, see Fig.1d. For comparison scanning electron microscope images of the two needles are presented in Fig. 1c, e, respectively. Please note that since the angle between the impinging SEM beam and the tip axis is 52 degrees, the SEM images are vertically elongated by 27% The EUV imaging clearly suffers from rather low signal-to-noise ratio due to large focal spot size (~20 µm) and sub-optimal EUV flux at our current setup compared to the synchrotron source. Nevertheless, we were able to resolve the needle exterior geometry in the colour-phase brightness-amplitude image (Fig 1.d) which perfectly matches SEM image (Fig 1.e).
Our first experimental results prove the feasibility of ptychographic imaging of APT tips using both synchrotron soft x-ray and tabletop EUV light sources. Ultimately, this might be a potential path forward to establish relevant information to feed into APT data reconstruction schemes to improve the spatial accuracy of reconstructed atom probe data [5].

Keywords:

CDI, ptychography, EUV, x-ray, APT

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Unveiling Strain Fields and Plastic Relaxation in Narrow-Core GaAs/In(Al,Ga)As Nanowires with High-Resolution Electron Microscopy

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Background incl. aims
Narrow GaAs nanowires (radius in the range of 10 nm) can undergo extreme elastic stretching when overgrown by much thicker, highly mismatched In(Al,Ga)As shells. Subsequently, GaAs in such core/shell heterostructures has demonstrated up to 40% bandgap reduction [1] and 30-50% boost in electron mobility [2]. Compared to planar epilayers, such nanowires offer superior flexibility by facilitating elastic relief along the side facets and strain partitioning between the core and the shell. This effectively reduces the strain energy and expands the coherency limits. However, plastic relaxation ensues once the lattice mismatch surpasses a critical threshold. This critical limit is determined by the intricate 3D strain distribution within the nanowires and the plastic relaxation mechanisms involved. In this contribution, we determine experimentally the critical misfit of such core/shell GaAs/In(Al,Ga)As nanowires featuring narrow cores and thick shells, using (scanning) transmission electron microscopy ((S)TEM) methods. Additionally, we utilize high-resolution (S)TEM (HR(S)TEM) combined with theoretical calculations, to obtain the 3D strain fields. Finally, we employ topological analysis with HR(S)TEM to identify the operating plastic relaxation mechanisms.
Methods
The [111]-oriented GaAs/Inx(Al,Ga)1-xAs core/shell nanowires were epitaxially grown on Si(111) substrates in the self-catalysed vapour-liquid-solid mode by molecular beam epitaxy. The shell composition was varied systematically from x = 0.2 to 1, while the core radius and shell thickness were fixed at 10 nm and 80 nm, respectively. (S)TEM and HR(S)TEM observations, as well as energy dispersive X-ray spectroscopy, were performed in a 300 kV probe-corrected ThermoFisher Scientific Titan Themis 60/300 microscope and a 200 kV JEOL JEM F200 CFEG microscope. Samples were prepared along <1-10> and <11-2> zone axes by ion-thinning, as well as along [111] with cross-sections prepared by ultramicrotomy. The 3D strain fields were directly determined from the HR(S)TEM observations, using geometrical phase analysis (GPA). The experimentally-extracted strain fields were compared to theoretical ones obtained by finite element analysis, performed using thermal expansivity to model the lattice mismatch, and with atomistic energetical calculations, employing the Tersoff interatomic potential [3]. Plastic relaxation mechanisms were investigated by employing diffraction contrast TEM and HR(S)TEM observations.
Results
Experimental observations showed the axial strains to be uniformly distributed. In contrast, the elastic relaxation along the free facets, combined with the hexagonal geometry of the core, resulted in opposite radial and tangential strain gradients in the core and shell as determined by GPA on (111) cross-sections of the nanowires. Even the plastically-relaxed nanowires retained radial and tangential elastic relaxation. These results were consistent with the theoretical continuum and atomistic

calculations. The critical indium content for the initiation of plastic relaxation was determined through the onset of defect introduction. In order to determine the residual elastic strain along the nanowire axis, analysis of Moiré fringe patterns was employed. Shells in plastically relaxed nanowires exhibited extended defects relieving the misfit, such as lattice and partial dislocations whose density varied with the lattice mismatch. Nanowire bending was found to influence the plastic relaxation process. Circuit mapping [4] around interfacial defects unveiled their Burgers vectors and, subsequently, the plastic relaxation nano-mechanisms, which involved both dislocation glide and stacking fault formation.

Conclusions

An integrated methodology, combining atomic resolution electron microscopy with continuum and atomistic calculations, has been employed to achieve a comprehensive understanding of the intricate strain relaxation in GaAs/Inx(Al,Ga)1-xAs core/shell nanowires featuring narrow cores. The 3D strain field distribution in such nanowires has been determined experimentally for the first time along with the maximum sustainable elastic strain in the core, the critical lattice mismatch for plastic relaxation, and the associated mechanisms of defect introduction. These insights can be employed for further straining the core while mitigating the formation of defects that impede electron mobility and internal quantum efficiency.

Keywords:

nanowires, III-arsenides, strain, dislocations, HR(S)TEM

Reference:

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Quantification and denoising of atomic-resolution EDX spectrum images using a Multiscale Bayesian Approach

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Poster Group 1

While latest generation aberration-corrected microscopes have enabled atomic-resolution scanning transmission electron microscopy (STEM) imaging to be relatively straightforward and accessible, atomic resolution spectroscopy, and in particular energy dispersive X-ray (EDX) spectroscopy, remains a challenge. Aside from having a suitable aberration-corrected probe, the relatively long of acquisition times necessary for achieving reasonable signal-to-noise ratios (SNR) impose tougher constraints on stage stability, optics and beam stability, and the resiliency of the sample to beam damage and contamination. To tackle this challenge, microscopists will often apply a simple directive: “acquire faster”. This is the motivation behind the development of EDX detectors with an increasingly large solid angle. However, there is a second way to approach the problem: using more sophisticated data analysis to be able to reduce acquisition times.

In this work, we propose the use of a multiscale Bayesian estimation strategy to deal with the extreme noise and signal sparsity observed in atomic-resolution EDX data. The method, originally developed for noisy LIDAR data[1], relies on two factors. First, it assumes pre-known contributions to the EDX spectrum from each chemical element. In the present work, the elemental contributions to the spectra are obtained from the espm library[2]. It should be noted that, since the espm-generated priors are themselves scaled by the corresponding k-factors, their corresponding abundance maps enable proper atomic percentage quantification. Secondly, it adopts a multiscale model that accounts for the Poisson statistics of the data and exploits spatial correlation between pixels to provide robust abundance estimates of each element in each pixel, even in the presence of high noise. The outputs of the algorithm are maps of the X-ray signal quantified in atomic percentage, which are considerably denoised, and corresponding Bayesian uncertainty maps.

The method has first been tested using (non-atomic) simulated EDX datasets generated using espm, which allow comparison to a ground truth (Figure 1 left). The plot shows the deviation from the ground truth abundance maps as a factor of SNR or, equivalently, virtual acquisition time. These outputs are compared to those made using a commercial analysis software: Velox from Thermo Fisher Scientific. As can be observed, the Bayesian multiscale (BMS) analysis demonstrates better accuracy, especially in low SNR situations, where it is shown to converge more quickly to the ground truth.
Next, the analysis method is tested on a wide range of experimental EDX spectrum images, acquired from several types of samples. Figure 1 right shows an example, where both Velox spatial filtering and BMS are applied to an atomic-resolution EDX spectrum image acquired from a film of $LaVO_3$ grown epitaxially on a $DyScO_3$ substrate (see reference 3 for details). Raw integrated counts maps are also included for comparison. This case is especially interesting, since it demonstrates the conservation of spatial resolution through the multiscale approach. The results shown below show that the obtained quantified maps of X-ray yield keep atomic resolution even when reducing the

acquisition time by a factor 3. As can be observed in Figure 1 a–f, the spots in the Fourier transform of the calculated maps are still clearly visible for the BMS analysis at the lowest acquisition time, while they are lost for the spatial filtering of the Velox analysis, proving that our method enhances spatial resolution even under the most challenging conditions. (We note that these are not absolute maps of atomic columns, owing to scattering processes such as channeling and dechanneling.)

In summary, we present a novel strategy for the denoising of atomic-resolution EDX spectrum images, that incorporates both knowledge of spectral shape and Bayesian multiscale analysis. Testing of this algorithm on experimental data sets acquired from a range of samples shows a consistently better performance compared to quantification and denoising using state-of-the-art commercial software.

Figure 1 Left) Simulated EDX maps results: abundance maps mean error as a function of simulated acquisition time. Right) Overlaid elemental maps of Dy (green). Sc (red), La (orange) and V (Blue) from an $LaVO_3$ thin film on $DyScO_3$ substrate. Rows represent different acquisition times (obtained by reducing the number of frames used), while columns represent the different analysis methods tested: raw integrated peak counts, Velox quantification with 10 pixel Gaussian smoothing, and Bayesian multiscale approach. Fourier transform panels a, c, e correspond to the BMS analysis at the corresponding acquisition times, while b, d, f correspond to the Velox-filtered maps.

Keywords:

EDX, Bayesian analysis, atomic-resolution STEM-HAADF

Reference:

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Observation of novel ferroelectric domain configurations in $PbTiO_3/DyScO_3$ epitaxial films

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Poster Group 1

$PbTiO_3$ is a ferroelectric material with the perovskite structure that exhibits polarization below a Tc of 765 K. $PbTiO_3$ thin films are known to have complex domain configuration as a function of deposition temperature, epitaxial strain, film thickness and electrostatic boundary conditions. In this work, atomic resolution scanning transmission electron microscopy (STEM) characterization is used to investigate the ferroelectric polarization configuration in $PbTiO_3$ films grown under 0.2% biaxial compressive strain on a (110)o -oriented $DyScO_3$ substrate, separated by a $SrRuO_3$ bottom electrode.

Ferroelectric polarization in $PbTiO_3$ is commonly mapped by carefully monitoring small displacements of the Ti atomic columns relative to neighboring Pb columns in aberration-corrected high angle annular dark-field (HAADF) STEM images. However, the thickness of our film of interest is around 240nm, with domains of similar size. Following this approach therefore implies tracking the position of tens of thousands of atomic columns over many tens of nanometers with picometer precision, over large images having high enough pixel density that each atomic column is separated by ≥ 40 pixels. To this end, we acquire 8k x 8k pixel images at relatively low magnifications, while aiming to keep the associated scan distortions to a minimum and maintaining a probe with atomic resolution.

Even when the acquisition of these large experimental images is successful, the challenge of analyzing them remains. In this work, we innovate an approach where each large-scale image is split into sub-images, each containing just one perovskite unit cell. Compared to whole-image analysis, we find that tracking the positions of atomic columns in these sub-images is greatly facilitated. It further yields a dataset that is suitable for multivariate analysis (figure 1a-d); an approach analogous to that shown by Ziatdinov et al.[1,2].

The results are large-scale polarization maps that reveal novel a/c domain configurations in the form of needles, stacked a-domains (figure 1 g-h), as well as strongly and weakly charged 180° domain walls (figure 1 e-f). Interestingly, a potentially new type of line defect is observed, that may play a role in stabilizing the observed domain walls that we hypothesize to be of high energy.

In conclusion, we present ongoing analyses of novel domain configurations in strained films of ferroelectric $PbTiO_3$, where we combine large-scale atomic resolution STEM imaging with machine-learning analyses to identify and understand domain boundary and structural defects.

Figure 1 . a-d) Non-negative matrix factorization results on unit-cell subimages revelaing the complex structure of a-c configuration with both 90° and 180° domain walls. Scale bars correspond to 2 Å for panels a,c and 15 nm for panels b,d. e-f) Polarization angle of maps of different observed 180° domain walls across a and c domains. Polarization up, down, right and left directions correspond to

blue, red, black and white colors respectively. Scale bars correspond to 20 nm. g-h) Stacked needles configuration of a-c domains revealed by geometrical phase analysis.

Keywords:

Ferroelectric, PTO, STEM-HAADF, atomic resolution

Reference:

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Evaluation of diabetes-associated testicular morphology and the effects of MSC secretome as a therapeutic intervention

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Poster Group 1

Background incl. aims
Diabetes is a metabolic disease characterized by prolonged hyperglycemia, which causes various types of complications, including impaired reproductive function. Diabetes-related microvascular damages, oxidative stress and insulin resistance could bring about a wide range of endocrine organ damages associated with the secretion of reproductive hormones and lead to hormonal imbalance, seminiferous tubule injury, including disrupted spermatogenesis and dysfunction of Sertoli cells. Ultimately, diabetes impacts male sexual function by leading to issues like erectile dysfunction, reduced libido and sperm damage. Mesenchymal stem cells (MSCs) could be a potential therapeutic intervention for the treatment of diabetes and accordingly, associated disorders like dysfunction of the male reproductive system1. Conditioned media (CM) obtained from MSCs contain soluble and non-soluble factors, all of which are collectively considered as one of the best ways of conveying MSCs' therapeutic effects, including angiogenesis, anti-inflammatory and -apoptotic effects, immunomodulation, and promotion of tissue repair and regeneration. Preconditioning of MSCs with different strategies, like incubation in hypoxic or 3-dimensional (3D) cell culture conditions, could improve their therapeutic potential2. In our previous study, the application of CM collected from MSCs cultured in 3D microfabricated scaffold to diabetic rats improved beta-cell regeneration and immunomodulation in comparison to the one obtained in conventional 2D culture conditions3. In this research, it was aimed to investigate the effects of prolonged hyperglycemia on serum reproductive hormone levels and testicular morphology of Sprague Dawley rats with diabetes, and the possible therapeutic effects of systemic application of CM derived from MSCs.
Methods
MSCs isolated from the human umbilical cord by tissue explant method were used for the collection of 2D-CM and 3D-CM. 22 rats were intraperitoneally treated with multiple low doses of streptozotocin (STZ; 5 days, 20 mg/kg) to induce diabetes. By the injection of last dose of STZ, it was confirmed that all the rats were in the range of diabetes (blood glucose level >250 mg/ml). Following the 2nd week of the first dose STZ injection, equal volumes (1 mL) of 2D-CM and 3D-CM were intraperitoneally applied to the diabetic rats (D+2D-CM, n=8; D+3D-CM, n=8) for 4 weeks as 3 doses a week. After 1 week of injection of the last dose, blood samples were collected by cardiac puncture for serum analysis of the hormones. The rats were sacrificed, and testis were obtained for light and transmission electron microscopic (TEM) evaluations. Serum concentrations of testosterone, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined with enzyme-linked immunosorbent assay (ELISA). Light microscopic evaluation was performed with hematoxylin + eosin (H+E) staining. Semiquantitative Johnsen's tubular biopsy score (JTBS) analysis was used to histopathologically evaluate spermatogenesis out of an average 40 seminiferous tubules (STs). In this respect, STs were scored from 1 (no cell in the tubule section) to 10 (complete spermatogenesis and perfect tubules) in accordance with the level of epithelial maturation. Histomorphometric analyses were executed with the Fiji ImageJ software program by measuring the shortest diameter of ST and length of seminiferous epithelium (SE) (the

distance between the basal lamina of SE and the closest spermatozoa to the lumen) out 10 STs for each specimen (n=3 for each group) at 40X magnification. Statistical analyses were performed using SPSS version 20.0 software. P<0.05 was accepted as statistically significant.

Results

ELISA analysis showed that serum LH and testosterone levels of experimental groups significantly decreased compared to the C group ($p < 0,05$). Serum GnRH and FSH levels were found to be significantly reduced in the D and D+2D-CM groups ($p<0,01$ and $p<0,05$ respectively), while there was no significant difference noted between the C and D+3D-CM groups. Our preliminary light microscopic analysis indicated that the JTBS values of experimental groups were significantly lower than the one of the C group while there was mild amelioration in D+3D-CM group in comparison to D group. On the other hand, our preliminary histomorphometric analysis of ST diameter and SE length did not show notable differences among the groups. TEM evaluation of SE revealed that the C group had normal ultrastructural morphology while spermatogonia with dispersed heterochromatin, primary spermatocytes having damaged mitochondria with loss of lamellar properties, cytoplasmic vacuolization in spermatogenic cell series and large intercellular spaces were noted in the D group. Additionally, assessments of spermatids at different stages of spermiogenesis demonstrated the presence of spermatids with disrupted chromatin condensation in the D group. In both treatment groups, such impairments were relatively fewer than the ones in the diabetic group and considerable restoration of ultrastructures of Sertoli and spermatogenic cells in seminiferous epithelium was observed.

Conclusion

In this diabetes model induced with multiple low doses of streptozotocin (5 days, 20 mg/kg), our preliminary light microscopic and TEM evaluations indicate that diabetes-associated damage in testis, specifically STs, was at a distinctive degree, and application of CM led to improvement in spermatogenesis and ultrastructural morphology of SE. On the other hand, when the differential changes in concentrations of serum reproductive hormones, including testosterone, GnRH, LH, and FSH, were considered, hyperglycemia seemed to affect the hypothalamic-pituitary-gonadal (HPG) axis by interrupting the feedback mechanism. The treatments with the CM, especially 3D-CM brought about considerable restoration of GnRH and FSH serum levels, but this was not enough to improve serum testosterone level, which is both a product and regulator of the HPG axis in males. The discrepancy between the morphological and hormonal analysis also suggests considering unrevealed mechanism of therapeutic action for MSCs derived CMs.

Keywords:

Mesenchymal-stem-cells, conditioned-medium, diabetes, testicular-damage, male-reproductive-hormones

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The effect of mesenchymal stem cell's secretome on hyperglycemia-related complications: focus on reproductive system disorders

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Poster Group 1

Background incl. aims
Type 1 diabetes (T1D) is characterized by insulin deficiency due to the autoimmune destruction of β-cells in the pancreas, which causes hyperglycemia. Although exogenous insulin is very effective in increasing the survival of patients, inadequately controlled hyperglycemia leads to secondary complications. One of the complications associated with T1D is reproductive disorders. In males with long-standing T1D, this can appear as sexual dysfunction or reproductive issues, primarily stemming from impaired spermatogenesis, reduced testosterone production and release, and changes in glucose metabolism within Sertoli cells [1]. Mesenchymal stem cells (MSCs) hold promise for handling the therapeutic requirements of T1D due to their involvement in the immunomodulation process and cytoprotective, antiapoptotic, and antioxidant properties. It is established that MSCs exert their therapeutic effects via paracrine mechanisms, releasing a diverse array of bioactive molecules termed the secretome, which includes growth factors, cytokines, chemokines, exosomes, and other bioactive factors. The composition of the secretome can be modified through various inductions, presenting significant potential for targeted therapy development. Among these induction types are a hypoxic cell culture environment, cultivating the cells on a three-dimensional scaffold, and using various chemicals. Conditioned media (CM) represents a tool to use the therapeutic benefits of the secretome exclusively, obtained by collecting the medium in which MSCs are cultured, free of cellular components [2]. The CM is important in the treatment of T1D due to the factors it contains [3]. Furthermore, the systemic application of CM could be crucial in preventing and repairing tissue damage caused by hyperglycemia in the body, despite this not being the primary treatment goal. This comprehensive approach is valuable as it addresses the entirety of T1D, which affects the entire organism. So, this study aims to investigate the effects of human umbilical cord MSC-derived CM, used for therapeutic purposes in the rat experimental T1D model, on testicular damage and the disrupted hormone mechanism, caused by hyperglycemia.
Methods
MSCs were isolated from human umbilical cord tissue by the tissue explant method, followed by characterization experiments. Two distinct CM types were obtained: CM obtained from MSCs cultured in normal culture (N-CM) conditions and CM obtained from MSCs preconditioned with a hypoxia mimetic agent, deferoxamine (150 µM) (DFX-CM). Sprague-Dawley rats were used for in vivo experiments, and the T1D model was induced using a single high-dose of streptozotocin (55 mg/kg). Experimental groups included control (C), diabetes (D), diabetes with N-CM treatment (D+N-CM), and diabetes with DFX-CM treatment (D+DFX-CM), with 6 rats in each group. Following the 4th week of the STZ injection, diabetic rats received intraperitoneal injections of equal amounts of N-CM and DFX-CM (each containing at least 15 µg of protein) four times a week for three weeks. Blood samples for hormone analysis were collected via cardiac puncture one week after the final CM dose injection, and the rats were sacrificed. After sacrification, serum levels of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were quantified via enzyme-linked immunosorbent assay (ELISA) for hormonal profiling. Testicular tissues stained with hematoxylin and eosin were used to perform Johnsen's scoring via light microscopy to

assess spermatogenesis. For this analysis, an average of 10 seminiferous tubule sections were analyzed in each rat (n=3). Seminiferous tubule diameter and epithelial thickness were also measured using the Image J analysis program. Finally, cellular ultrastructure was examined using transmission electron microscopy (TEM). All findings are presented as preliminary outcomes. Statistical analysis was conducted using the Kruskal-Wallis test, with p-values <0.05 considered significant.

Results

Upon examination of reproductive hormone levels in serum, statistical significance was identified among the groups for GnRH, FSH, and LH ($p<0.05$, $p<0.001$, and $p<0.05$, respectively). All hormone levels decreased in the D group. It was noteworthy that GnRH, FSH, and testosterone levels tended to increase in the D-DFX-CM group compared to the D group. Johnsen's scoring indicated impairment in spermatogenesis in the diabetes groups. On the other hand, the measurements of seminiferous tubule diameter and epithelial thickness did not reveal statistically significant differences. TEM analysis of seminiferous tubules revealed that the C group exhibited normal morphology. In contrast, the D group exhibited elevated lysosome levels in Sertoli cells, spermatogonia with dispersed heterochromatin, and primary spermatocytes with disintegrated nuclear membranes, alongside increased intercellular spaces. In both treatment groups, such defects were relatively less than in the diabetic group, and a substantial improvement in the ultrastructure of Sertoli and spermatogenic cells within the seminiferous epithelium was observed. Examination of spermatids showed that some in the diabetes group had impaired chromatin condensation. Axoneme structures were generally preserved (Fig.1).

Conclusion

Ultrastructural examinations revealed that the secretome of MSCs improved the seminiferous tubule epithelium by exerting a cytoprotective effect. Despite no significant impact on the hypothalamic-pituitary-gonadal axis, which is impaired by hyperglycemia, it is noteworthy that DFX-CM increased GnRH and testosterone release from the hypothalamus. However, this effect was insufficient, leading to no observable improvement in spermatogenesis. These preliminary findings underscore the necessity for further investigation into the observed ultrastructural improvement in the seminiferous epithelium and the need to uncover new cellular-level data elucidating the mechanisms of action of the secretome of MSCs.

Keywords:

Mesenchymal stem cells-Testicular damage-Conditioned medium

Reference:

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Microstructure and Phase Analysis of an Al-Mg-Si Alloy Produced by Laser Power-Bed Fusion

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Poster Group 2

Background incl. aims
Aluminum (Al) alloy additive manufacturing (AM) offers a groundbreaking approach to metal fabrication, providing unmatched design flexibility and material efficiency. However, achieving optimal performance hinges upon understanding and controlling microstructures. Microstructure analysis is critical for evaluating properties like grain morphology, size distribution, and phase constitution, which directly influence mechanical properties. By tailoring AM parameters and post-processing techniques based on microstructural insights, engineers can elevate the integrity and performance of Al alloy components. Careful microstructure analysis ensures AM-produced parts meet the stringent quality standards and reliability demanded by diverse industries, from aerospace, automotive, and beyond. Nevertheless, the rapid solidification and intricate thermal histories inherent in AM-produced Al alloys result in microstructures significantly different from conventional processes (e.g., casting), posing substantial challenges in accounting for microstructural elements across various length scales [1]. In this study, a scale-bridging approach [2] combining multiple techniques in scanning electron microscopy (SEM) and transmission electron microscopy (TEM) was employed for obtaining a clear microscopical picture of an Al-Si-Mg alloy [3] produced by Laser Power-Bed Fusion (LPBF).
Methods
Samples are prepared using electropolishing in combination with ion milling from the AM Al alloy. Conventional imaging and diffraction techniques as well as energy-dispersive X-ray spectroscopy (EDS) in both SEM and TEM are used to characterize the as-built microstructure (Figure 1). To reveal the underlying effect of additive manufacturing on the grain structure and phase distribution, a correlative approach for orientation imaging and phase mapping is then employed by using EBSD in SEM and ASTAR in TEM.
Results
Detailed microstructural features such as melting pools, grain structure and distribution/morphology of eutectic phases in the investigated AM alloy will be revealed and analyzed (to be continued).
Conclusion
This comprehensive approach will provide valuable insights into the microstructural evolution of AM-produced Al alloys, facilitating further advancements in material design and process optimization.

The authors acknowledge Dr. Daniel Knoop from Leibniz-IWT in Bremen for providing the specimens and extend the gratitude to the support and resources provided by ZGH at Ruhr-University Bochum. Part of this work was performed at the DFG-funded Micro- and Nanoanalytics Facility (MNaF) of the University of Siegen (INST 221/131-1).

Keywords:

Additive-manufacturing; LPBF; AlMgSi; EBSD; ASTAR

Reference:

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Towards 3D quantitative imaging in FIB-SEM for applications in battery materials

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Poster Group 1

Background incl. aims
The development of more efficient and safer battery materials stems from the commitment to green energy initiatives. To fully understand the failure mechanisms of newly developed battery materials at the micrometer level and below, scanning electron microscopy is a technique of choice. Focused ion beam (FIB) combined with SEM broadens the characterization capabilities of electron microscopy by revealing sub-surface microstructure and phase distributions. The technique permits quantitative analyses from the reconstruction of volumes from collected 2D SEM datasets. Moreover, the collection of Energy-dispersive X-ray spectroscopy (EDS) and Electron Backscatter Diffraction (EBSD) maps from the generated x-rays and EBS patterns can help explain further the chemical and structural stability of the materials in 3D. Degradation over time during cycling can originate from alterations of the microstructure of particles, such as the apparition of cracks due to volume expansion, changes in grain morphology or activity at grain boundaries. This work aims to obtain quantitative information on the distributions of the different phases of lithium-based cathodes from field-emission (FE)-SEM datasets of 2D images, including porosity. Porosity is a critical parameter in battery design, since it directly affects electronic and ionic diffusion processes [1,2] and therefore battery performance. An interest will be given towards the changes in the phase distributions as a result of cycling, therefore pristine (uncycled) and cycled specimens will be compared including EDS and EBSD data.
Methods
Hitachi Ethos NX5000 FIB-SEM microscope located at the Facility for Electron Microscopy Research at McGill University will be used to prepare volumes using the slice and view technique of pristine and cycled cathodes. SE and BSE images will be acquired simultaneously at low voltage (2kV) during the slice and view routine to ensure the characterization of the fine pore structure from the small probe interaction volume. Ultim Max 100 SDD detector and Symmetry S2 EBSD (up to 4500 patterns per second collection) from Oxford Instruments will be used with 20 keV at given slices to collect chemical and crystalline information on the cathodes. Dragonfly software from Comet [3] is used to segment the 2D image datasets into 3 semantic phases (porosity, active material (particles) and binder (carbon)) using Deep Learning methods. Watershed algorithms are used to differentiate the labeled voxels in the phases and separate nearby voxels into individual elements for pore and active material to obtain statistical data on each phase.
Results
A preliminary dataset was obtained from a pristine lithium based cathode using Ga FIB milling with 3.5nA current with a slice thickness of 40nm. A total of 441 frames were collected with in-lens BSE(U) and BSE(L) detectors using simultaneous acquisition and a pixel size of 50nm. A volume of 19,6µmx20,5µmx17,5µm of the sample was reconstructed after registration of the slices and cropping. A U-net neural network was trained on a subset of the total stack manually segmented using thresholding on the mixed BSE U+L images, with 20% of the data retained for validation. It was found that 51,9% of the volume constitutes of carbon binder, 37,2% Li-based active material and 10,9% porosity. Histograms of the pore and active material populations were constructed after single

particle separation with the watershed algorithm. Following fractal geometry theory from Mandelbrot[4], the probability distribution of particles P is linked to the fractal dimension D with P(r>R)=AR^(-D). Deriving the equation to get the probability density ρ(R), the fractal dimensions D of both subsets can be extracted using the equation D=1/(m-1), knowing the radius R is expressed proportional to the volume V^(1/D) and m is the slope of the fitted straight line obtained from taking the logarithm of both sides of the derived equation. The fractal dimension of the pores yields D=0.60 and the active material is found to have D=0.99.

Conclusion

Preliminary image analysis using a FIB-SEM dataset on a pristine cathode showed the distributions of active material, carbon binder and pores. A neural network was trained to segment the cathode's phases and object analysis was used to study the pristine cathode. It was determined that the fractal dimension D of the pore medium in the cathode is 0.60, while the active material particles, containing Li, is set at 0.99. In both cases, the fractal dimension value is lower than one and therefore indicate that objects composing the phases are disconnected. Analysis of cycled specimens will enable us to observe the behavior of percolation pathways over time. The newly installed Ultim Max 100 SDD detector and Oxford Instrument's Symmetry S2 EBSD will be used to generate 3D volume reconstructions on pristine and cycled Li-based cathodes in addition to the image segmentation and quantitative results obtained from EM datasets. The addition of 3D chemical and crystalline information will give new insights into the effect of charge-discharge cycles on the distribution of phases and permit the observation of defects in 3D that can help explain battery failure mechanisms.

Keywords:

FIB-SEM, 3D-EDS,3D-EBSD, quantitative imaging, battery

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In-situ TEM study of ice nucleation and growth

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PS-07 (1), Plenary, august 29, 2024, 15:00 - 16:00

Ice is one of the most omnipresent solids on Earth, yet its crystallization is not fully understood. In particular, a long-standing debate is whether natural ice can form in a metastable pure-cubic phase. A mainstream view attributes the divergence to the stacking disordered ice (Isd) with a mixture of cubic and hexagonal sequences that preferentially nucleate and grow at low temperatures typically under 200 K, where complex kinetics in ice crystallization has also defied understanding thus far. This is due to that the direct and reliable determination of the structure of metastable ice requires ultra-high spatiotemporal resolution in an in-situ freezing environment, which remains a major outstanding challenge in the community. Here, through developing the in-situ cryogenic TEM, we directly tracked and unveiled the heterogeneous polymorphic ice nucleation and crystallization with molecular resolution during the water vapor deposition process at 102 K. We further pinpointed the atomic structure of polymorphic ice crystallites and identified distorted ice tetrahedral configurations as crystal defects with molecular resolution, which were not accessible before. Employing the electron beam as both the imaging probe and activation source, we were able to trigger and consequently monitor the kinetic transition process of stacking disordered ice (Isd) to ice Ic, demonstrating the unexpected meta-stability of ice Isd with respect to ice Ic. Our measurements shed new light on the structure and dynamics process determined heterogeneous nucleation of polymorphic ice. While a deep understanding of ice crystallization requires microscopic details, our realization of direct real-space imaging of growing ice crystallites at the molecular level is a key step towards reconciling experimental and theoretical investigations into the structure, nucleation, and crystallization based complex phenomena in ice family and polymorphic crystals.

Keywords:

Ice, In-situ TEM, polymorphism, Crystallization

Reference:

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QCBED Measurements of Vacancy Concentrations, Lattice Contraction, and Bonding Electron Densities Surrounding Aluminium Nanovoids

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background incl. aims
We set out to make the first position-resolved measurements of bonding electron density around a nanostructure in an inhomogeneous crystalline material.

All bonding electron density and potential studies to date have only involved homogeneous, single phased materials; however, most materials that serve us have hybridised properties because of the nanostructures that they contain, often by design. We also note that materials defects are ubiquitous and unavoidable, so the assumption that we can derive materials properties from notionally perfect regions of single, homogeneous crystal is limited in scope and "real" applications. This work aims to provide a new capability for interrogating bonding electron densities around nanostructures in nanostructured and inhomogeneous materials. Our first attempt involves nanovoids in nominally pure (99.9999+%) aluminium.

On the way to realising this aim, we have made several discoveries as a result of having to accurately map vacancy concentrations and determine the associated lattice contraction due to vacancies in order to be able to measure the Fourier coefficients (structure factors) of the crystal potential and electron density precisely and accurately (to <0.1% uncertainty).

Methods
Over the last 15 years, we have developed a multislice [1,2] approach to quantitative convergent-beam, electron diffraction (QCBED) refinements. We call this method QCBEDMS. This has facilitated the necessary analyses to generate the results summarised below. For more details regarding the method, please come to the talk – they will not be discussed here.

Results
From 45 CBED patterns collected through many different voids and the surrounding matrix, in different orientations and with different electron energies:

- We measured the volume of a vacancy with 5-fold less uncertainty than previous work.
- Observed that nanovoids in aluminium can "heal" even after significant beam damage.
- We mapped vacancy concentrations around aluminium nanovoids in 3 dimensions.
- We have shown that the bonding electron density around aluminium nanovoids is diluted by the elevated vacancy concentration surrounding these voids.

Conclusion
Our work and the results from it are a precursor to measuring and mapping bonding electron densities around many other types of nanostructures (eg. nano-precipitates) in other nanostructured, inhomogeneous materials. Furthermore, we are confident that our technique can be extended to mapping solute atom concentrations around such structures and that this will go together with accurate bonding electron density measurements within and surrounding these features in other nanostructured materials.

Keywords:

QCBED, multislice, bonding electron densities

Reference:

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Transformation of Al2O3 to Cr-rich spinel during spark plasma sintering of alumina dispersed 316L steel

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Poster Group 1

Background incl. aims
Dispersion of oxide particles in sintered steels is generally applied to improve the mechanical properties (e.g. fracture toughness, creep resistance) of steels. Spark plasma sintering (SPS) is a relatively new method [1] when a sequence of electric pulses are applied at high temperature and pressure. As an advantage, SPS offers rapid heating, limitation of grain growth, and efficient densification kinetics, which may provide benefits over hot isostatic pressing in certain applications. Manufacturing by SPS can also provide significant savings by increasing production volume and reducing energy costs.
Methods
Oxide dispersion strengthened (ODS) 316L stainless steel alloys with dispersed alumina were prepared by attrition milling and spark plasma sintering process at 900°C. The location and crystal structure of the oxide phase was investigated by a Cs corrected Themis (Thermo Fisher) transmission electron microscope including HRTEM, SAED [2] and EDS elemental mapping. TEM specimens were prepared by Ar ion beam milling at incidence angle of 4° at 10kV using a Technoorg Linda equipment [3]. The thinning procedure was finished at low-energy of 300 eV in order to minimize the possible ion beam damage.
Results
During sintering, the dispersed alumina transformed to spinel phase, dominantly with Cr2AlO4 composition. The spinel crystallites were observed between the grains of stainless steel in the sintered composites. The spinel crystallites were surrounded by an amorphous silica phase. The necessary Cr and Si may segregate from the stainless steel alloy at SPS conditions. The lattice parameter of the formed spinel phase is 8.36Å independent of the local composition variation as indicated by FFT of high resolution images.
Conclusions
The lattice parameter of the spinel phase is close to the high end among chromite related spinels which implies that octahedral sites of spinel structure are mainly occupied by Cr3+ or possibly Fe3+ cations [4]. These findings are consistent with literature describing geological formation of spinels in mafic or ultramafic rocks with low oxygen fugacity and assistance of silica phase in cation exchange [5].

Keywords:

Keywords:
spinel, cation exchange, sintering

Reference:

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EasyGrid: a new automated cryo-multimodal sample preparation

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

Background incl. aims
In structural biology, Cryo-EM is one of the major imaging technologies. Even if the limits of the EM towards higher resolutions and faster data-collection have been really improved, the sample preparation step still remains poorly automated and difficult to manage for untrained and trained users (reproducibility issues). Many developments (e.g., Vitrojet1, Chameleon2) exist to make cryo-EM sample grid preparation easier, faster, and more reliable, and some of them also offer time-resolved module than remains a less user-friendly solutions (TrEM3, Joachim Franck's mixing/spraying method4). However, no machine currently offers a universal and easy solution. Several relative issues also exist in the field of X-ray imaging where there is no unique, reproducible sample preparation. This is the main reason why the instrumentation team of EMBL-Grenoble began the development of the EasyGrid5 device in 2017 that aims to provide a versatile tool that addresses most of the current difficulties encountered in the cryogenic preparation of diverse sample supports.

Methods & Results
This fully automated procedure (Fig.1a,b) uses in-line plasma treatment, picolitre drop dispensers, blotless liquid removal, ethane jet vitrification, automated cryo-storage and sample quality control at cryogenic temperature. The in-depth vitrification step is crucial to study native sample conditions for cells. Here we report initial results in two main domains; thin film sample vitrification for SPA experiments (cryo-EM), and whole-cell vitrification for cryo-ET/-XRF deep in large cells (Fig 1c,d,e).

Conclusion
EasyGrid proposes a versatile and robust multimodal sample preparation framework that can be easily adapted to a large variety of sample preparation protocols: cell freezing, classical SPA sample grid preparation, light triggered time resolved freezing, on-grid mixing, and this for diverse imaging modalities requiring different supports and conditions. The adherent cell vitrification protocol has been adapted for XRF using Si3N4 membranes (Silson), however, other supports can also be envisaged. The blotless liquid removal process allows for control of the ice layer thickness, a crucial parameter to optimise, to limit radiation damage on the sample during data-collection and maximise signal and contrast. The fully automated sample preparation procedure, leveraging precise robotics and an axis control system with sub-millisecond time-base, is highly reproducible. Hence, process optimization for different samples becomes accessible, and the screening of each process parameter can be reliably executed.

Keywords:

ElectronMicroscopy, X-rayFluorescence (XRF), Single-particleAnalysis, Cryo-samplePreparation

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A cryo workflow combining light, electron and soft x-ray microscopy provides targeting of unlabeled features

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Background incl. aims
While Electron Microscopy (EM) reveals extensive subcellular information in exquisite detail the technique has some limitations; lack of 3D cellular context, limited field of view and demanding sample preparation protocols compared to other techniques such as light or x-ray microscopy. To overcome these limitations correlative workflows have been developed that combine light with volume EM techniques such as focused ion beam scanning electron microscopy (FIB-SEM), serial block face scanning electron microscopy or array tomography, thus facilitating the localization of specific regions of interest within an extended sample volume. Nonetheless, these workflows remain largely confined to chemically fixed samples requiring labor intensive workflows, as the contrast from frozen-hydrated samples can be limited, making direct SEM imaging of native samples challenging. Soft x-ray tomography (SXT), on the other hand, is a unique x-ray imaging modality which enables imaging of frozen-hydrated specimens like entire mammalian cells or thick tissue sections with a few tens of nanometers spatial resolution and minimal sample preparation. The recent development of a laboratory scale SXT microscope opens the possibility of integrating this novel technique into light and electron imaging workflows. The SXT microscope features an integrated light microscope for overview imaging and fluorescence targeting, allows for swift acquisition of 2D and 3D images covering extensive areas on the specimen, and enables efficient and rapid identification of cells of interest. The (x,y,z) feature location can be recorded and the specimen passed to a cryo FIB-SEM for lamella extraction and subsequent cryo-ET imaging at ultra-high resolution.
Methods
Cryo SXT was used to identify target regions of interest within whole, frozen hydrated cells. Soft x-rays from 284 to 543 eV (2.34 to 4.4 nm) allow SXT to retrieve quantitative x-ray absorption information of protein content in biological cells with high native contrast. The resulting 3D datasets were imported to the correlative module in the TESCAN AMBER cryo FIB-SEM and used as a reference for targeted extraction of cryo lamella from the specimen.
Results
Resulting cryo-ET tomograms provide proof of concept for presented workflow.
Conclusion.
This workflow of correlative light, electron and soft x-ray microscopy (CLEXM) combines the strengths of both SXT and EM while also avoids any adverse effect of chemicals used for fixation in traditional EM methods, and therefore is particularly suited for studying rare events or features which cannot be labelled with fluorescent tags. We will discuss recent progress in this novel workflow development.
Acknowledgement
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Keywords:

CLEM, Cryo-FIB, Cryo-ET, SXT, CLEXM

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Electron-beam-induced surface diffusion of contaminants and growth of carbon contamination

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

Background incl. aims
Carbonaceous contamination deposited on samples illuminated by a beam degrade the quality of images and aggravate structural and compositional analysis of the material. Contamination results from irradiation-induced polymerization of contaminants, which can be hydrocarbon molecules already present on the sample surface or molecules adsorbed from the residual gas of the instrument chamber [1]. Several authors concluded that the contribution of surface contaminants is dominant [2,3], but already earlier models consider an additional source from the residual gas in the instrument [4].
The goal of this work is to describe the flow of contaminants and their polymerization in the irradiated area with a number of parameters reduced to a minimum necessary. To analyze possible sources of contaminants, both, a surface diffusion process and the supply of contaminants from the residual gas are considered. It is hypothesized that the diffusion process is driven by the gradient of the surface density of contaminants, generated by the impact of the electron beam probe. The contribution of the residual gas atmosphere in the instrument is described by the tendency to re-establish an equilibrium surface density of contaminants.
A time-dependent reaction-diffusion model is elaborated to describe contamination growth on surfaces illuminated by an electron beam. The unknown parameters of the model are determined by comparing the theoretical predictions with experimental results. The experiments are designed such that the influence of each parameter can be unequivocally separated. Successive contamination measurements, performed at distinct time intervals allow to follow the dynamic of the process. Examination of the individual contribution of contaminants from the sample surface and from the residual gas of the instrument is facilitated by experiments with appropriate variations of these parameters.
Methods
Circular contamination patterns were grown in a FEI DualBeam Strata 400S on commercial thin (~10 nm) amorphous carbon films by illuminating the sample homogeneously with a defocused electron beam. The radius of the irradiation disk was chosen to be sufficiently large (~ 700 nm) to separate the influence of the distinct parameters on the contamination growth. Thus, the inflow of the surface contaminants by diffusion was noticed at the margin of the disk, while the residual gas contributed on the entire sample surface. The dynamical evolution of the process was followed by successive contamination measurements performed up to 20 minutes at time intervals of 5 minutes.
The local height and shape of the contamination rings were quantified by taking high-angle-annular-dark-filed (HAADF) images in scanning-transmission-electron-microscopy (STEM) mode between the irradiation cycles, and comparing their intensities with corresponding Monte-Carlo simulations. Both, irradiating and imaging, were conducted at electron beam energy of 20 keV and with a current of 120 pA.

To elucidate the supply of contaminants from the residual gas, the chamber pressure of the microscope was varied by different pumping periods and the usage of a cooling vessel trap. The initial density of the surface contaminants was altered by subjecting the sample to variable duration of in-chamber plasma cleaning.
Consideration of the main parameters of the process lead to a reaction-surface-diffusion model with an additional source term. It was supposed that the relative density of contaminants $n = N/N_0$, normalized to an initial density $N_0 = N(t = 0)$, changed locally due to polymerization to amorphous carbon contamination by the illuminating beam. The occurring gradient of n generated a surface diffusion process of contaminants, characterized by the diffusion coefficient D. The reaction of the irradiating electrons with the contaminants was described by the reaction-frequency σ. An adsorption frequency η of molecules from the residual gas considered for a source term with the tendency to re-establish the initial density of the surface contaminants. With these terms, the reaction-diffusion equation was elaborated as follows:
$\partial n/\partial t = D \cdot \Delta n - \sigma \cdot n + \eta \cdot (1 - n)$,
with Δ representing the Laplace operator in cylindrical coordinates for radial symmetry.
Comparing the predictions of this model with the time evolution of the experimentally grown contamination, the parameters of the process could be determined. All results were considered with respect to reference measurements, performed prior to the experiments with parameter variation.
Results
A surface-plot in Fig.1 a) shows the HAADF-STEM image intensities of contamination grown during 20 minutes of local irradiation with a defocused electron-beam of 20 keV. The modeled thickness profiles (continuous lines) in comparison with the measured radial profiles (dashed lines) of the contamination ring are depicted for distinct time intervals in Fig.1 b). Comparing the predictions of the reaction-diffusion model with experimentally grown contaminations, the diffusion of the surface contaminants was described by $D \sim 10^3$ nm²/s and the polymerization of the contaminants by a reaction frequency $\sigma \sim 1$ Hz. Small variations of D and σ for distinct measurements could be explained by local differences of the contaminants type of the corresponding experiments.
The contribution of contaminants from the sample surface and from the residual gas were separately determined by varying accordingly the experimental conditions by plasma cleaning, respective pumping and cooling of the instrument chamber. Already short plasma cleaning durations significantly reduced contamination growth and the exponential decay in time of surface contaminant density N_0 showed an effective removal of surface contaminants.
With higher chamber pressures, the residual gas supply of contaminants increased, as indicated by the raise of the adsorption frequency of contaminants. However, its value $\eta \sim 0.002$ Hz remained very low in comparison with the other parameters and contributed only with a small amount to the total contamination growth. Its direct influence in the illuminated area was relatively reduced, but became noticeable for contaminant adsorption outside the illuminated area and their subsequent diffusion into it.
Conclusion
In conclusion, it can be asserted that contaminants stem from the sample surface and diffuse into the illuminated area. This underlines the effectiveness of in situ plasma cleaning. The residual gas contributes less to direct contamination growth but should be considered for experiments of longer duration.

Keywords:

contamination, surface diffusion, electron microscopy

Reference:

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CL-EBSD-TUNA Correlative Multi-microscopy Study of Grain Boundaries in Pseudo-symmetric Cu(In,Ga)S2 Solar Cell Absorber

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Poster Group 1

Introduction
High quality and defect free absorbers materials are usually essential for high performance solar cells. Different defects, such as point defects and grain boundaries (GBs), may strongly limit the performance of solar cells via enhancing the non-radiative recombination and impeding the charge carrier movement.1 However, Cu(In,Ga)S_2 (CIGS) solar cells exhibit a distinct scenario, in which polycrystalline absorbers with a high density of GBs can achieve high performance, exceeding 15% conversion efficiency.2 The fundamental physics underlying the high efficiency of polycrystalline CIGS solar cells, particularly the influence of different GBs, is still a significant question. In this research, we study the interplay between structural, electrical, and opto-electronic properties of GBs in CIGS solar cells absorbers by employing a multi-microscopy approach, integrating hyperspectral cathodoluminescence (CL), electron backscatter diffraction (EBSD), and tunnelling current atomic force microscopy (TUNA).
Materials and Methods
The CIGS samples were fabricated using a 3-stage co-evaporation process. All investigated samples were polished using a low energy broad Ar ion beam to achieve the low surface roughness essential for EBSD and TUNA measurements. We started the multi-microscopy investigation with a TUNA measurement conducted using a Bruker Dimension Icon AFM equipped with a Pt/Ir-coated SCM-PIC tip to minimise the impact of possible carbon contaminations in the SEMs. These measurements were then followed by EBSD measurements which were performed using a Zeiss GeminiSEM equipped with an Oxford Instrument Symmetry S3 detector. The initial EBSD datasets were indexed using Hough-based indexing, having a high density of mis-indexed pixels due to the pseudo-symmetry of the CIGS tetragonal lattice. The pseudo-symmetry arises from the subtle lattice parameter difference between c and 2a, which will significantly affect subsequent phase orientation analysis and grain boundary (GB) calculations. In order to mitigate the impact of pseudo-symmetry, we re-indexed the EBSD datasets with a tetragonal lattice system using a dictionary indexing approach. The open-sourced software, EMsoft, is used to create the master Kikuchi pattern using relevant lattice parameters of the investigated material and to carry out dictionary indexing on experimental EBSD patterns. Hyperspectral CL data was acquired using an Attolight Allalin 4027 Chronos dedicated CL-SEM. We employed Gaussian fitting to extract the CL emission intensity, energy, and full-width-half-maximum of each peak at each pixel from hyperspectral CL dataset. Direct correlation between the three techniques was achieved through sample markers and AFM images. AFM and TUNA data were processed and analysed by using Bruker Nanoscope Analysis software. Hyperspectral CL datasets were analysed by using open-sourced python packages Hyperspy and Lumispy. EBSD results were processed and analysed by open-sourced MATLAB package, MTEX.
Results
Analysing the EBSD maps we obtained structural information about the CIGS samples, including grain structure, phase orientation, and GB misorientation. We divided the GBs in CIGS into two categories,

twin boundaries (TBs) and random high angle grain boundaries (RHAGBs). TBs had a high relative frequency of more than 40%, and were observed with two distinct misorientation angles, 60° and 70°, corresponding to {112} and {110} planes, respectively. The CL spectrum shows two peaks, a strong emission peak at 1.6 eV corresponding to near band edge (NBE) emission and a weak and broad peak at 1.3 eV associated with defect-related emissions. Grain structure-like patterns were found from CL maps, wherein grains with strong NBE emission were separated by low emission intensity boundaries. Similar patterns, displaying strong current flows within grains and weak current flows at boundary positions, were observed in TUNA maps.
By using our multi-microscopy approach and correlating the CL and TUNA results with the EBSD maps for the same region, we were able to directly associate changes in the opto-electronic and electric properties to variations in the local sample microstructure. We found the strongest impact on the TUNA current and CL emission intensity and emission energy along RHAGBs, with distinct variations of the optical properties at some TBs. Inter grain variation was generally found to be weak and no correlation of either optical or electrical properties with grain orientation was found. Strong reductions in both TUNA current and CL intensity compared to the surrounding area were observed at all RHAGBs, suggesting that RHAGBs strongly inhibit the local charge carrier movement and locally enhance non-radiative recombination. The influence of TBs on local material properties is more complicated. Approximately 50% of the TBs appeared indistinguishable from the surrounding GI in both TUNA and CL maps, while around 25% of TBs behaved similar to RHAGBs with remarkably reduced TUNA current and CL emission intensity. An increase in emission intensity with weak redshift and narrower emission peak was observed from the remaining 25% of the TBs. No linkage between TB behaviour and misorientation angle has been found. The variation in both the electrical and opto-electronic properties likely links to the atomic scale structure of the different GBs. We attribute the reduction in both the TUNA and CL signal intensity at RHAGBs to a disruption of crystallinity and an agglomeration of point defects at the boundaries. The behaviour at TBs was found to be more complicated and we suggest a combination of imperfect TBs and local type-II quantum well like features to explain the varied optical response to TBs.
Conclusions
In conclusion, we carried out a directly correlated CL-EBSD-TUNA multi-microscopy study to comprehensively examine the electrical and opto-electronic behaviour of different GBs in CIGS on the nanoscale. Our findings demonstrate that all RHAGBs strongly inhibit the local conductivity and radiative recombination, while TBs were found with three different types of scenarios: behaving similar to surrounding GIs, strong reduction in CL intensity and TUNA currents, and showing enhanced radiative emission.

Keywords:

Cathodoluminescence, EBSD, TUNA, $Cu(In,Ga)S_2$, Solar-cells

Reference:

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Blockwise Processing of Hyperspectral Analytical Scanning Transmission Electron Microscopy Data for Enhanced Analysis

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Processing the vast amounts of data generated by STEM spectroscopy techniques, particularly hyperspectral data obtained through energy dispersive X-ray spectroscopy (EDXS), poses significant computational challenges. Current approaches based on machine learning algorithms of the family of multivariate statistical analysis often struggle with the monolithic analysis of very large datasets that exceed available computer memory. A second challenge with very large datasets is that minority phases might be discarded with the noise if the dataset is treated in one block. Our research addresses these challenges by extending a monolithic, in-house, non-negative matrix factorisation (NMF) method specifically tailored for model-based treatment of EDXS data[1], called ESpM-NMF – that enables meaningful interpretation of the resulting spectral and spatial components – into a new tool where the dataset can be treated blockwise before a global reconstruction is performed.
First, we split the hyperspectral data into spatial blocks, the optimal size of which is based on the spatial structure of the initial dataset. Next, we perform principal component analysis (PCA) utilising singular value decomposition (SVD) to identify the number of relevant components within each block, by visual inspection of the SVD output. Subsequently, an initial NMF decomposition is performed with our ESpM-NMF algorithm on a blockwise basis, to estimate spectral components. In the following post-processing stage, we integrate a priori information concerning elemental composition, phase details, and chemical compound identification. The resultant spectral components are correlated using a custom spectral clustering algorithm to identify shared features across the different data blocks. With this step, we are able to reduce the number of spectral components to the exact total number of components to be expected for the whole dataset. Finally, we use the definitive spectral components relevant to the entire dataset to derive the corresponding spatial components capable of monolithically representing the input dataset by solving the least squares problem in a blockwise manner and concatenating the resulting blocks.
For the purpose of testing our algorithm, we generated a synthetic dataset with 5 spatially-separated phases using a previously-developed EDXS dataset simulation algorithm[2]. We use four spherical particles sitting on a silicon substrate. The substrate corresponds to a thickness of 10 nm, the sphere sizes are 10, 10, 20 and 50 nm for W, Pd, $ScVO_4$ and $SrTiO_3$, respectively. As shown by the comparison of monolithic to blockwise dataset decomposition in the figure below, our methodology shows clear improvements for processing large hyperspectral EDXS datasets. Through the integration of prior knowledge and the utilisation of blockwise NMF-based decomposition, we attain enhanced accuracy in separating spectral and spatial components, while also enabling the identification of minority phases that are not adequately detected in the monolithic approach. Leveraging spectral clustering aids in identifying shared spectral features across blocks with remarkable noise resilience, thereby improving results consistency, reliability and interpretability. Crucially, our approach enables efficient out-of-core processing, surmounting memory constraints and enabling the analysis of datasets previously deemed impractical without access to high-performance computing clusters.
In summary, we introduce a new tool for out-of-core hyperspectral data processing. Our NMF-based methodology not only overcomes memory constraints but also enables the detection of minority

phases that are not successfully retrieved using monolithic processing. This advancement opens avenues for more comprehensive and detailed analyses in STEM spectroscopy, paving the way for deeper insights into material structures and compositions. Progress is under way to publish the tool in an open-source format.

Keywords:

Physics-guided NMF, Out-of-core Processing

Reference:

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Generation of AFM cellular datasets and classification by ML

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Poster Group 1

Background incl. aims
Mechano-biological measurements have the potential to distinguish healthy cells from pathological cells. Since 2007, a number of articles have shown that metastatic cancer cells have a Young's modulus (or elastic modulus) measured by atomic force microscopy (AFM) on living cells that is significantly lower than that of benign cells. However, cells are not inert and purely elastic materials, but dynamic and viscoelastic systems.
Methods
In order to measure these viscoelastic properties in living cells, we carried out dynamic mechanical analysis (DMA) measurements using AFM. After indenting a cell, we apply a strain and record the stress. The strain is proportional to the pulsation and therefore to the 6 frequencies we chose (from 1 to 200 Hz), and the stress is proportional to the phase shift of this pulsation. Knowing the strain and stress, we can then calculate G' (shear storage) in phase with the strain and G'' (shear loss) in phase with the strain rate. In our study, we sought to classify a population of cancer cells from a population of non-cancer cells. On each cell, 16 force curves (FC) and 19 characteristics/FC, corresponding to the viscoelastic properties and constituting a part of the mechanome, were calculated.
Results
All the FCs were then classified using machine learning (ML) tools with a statistical approach based on a fuzzy logic algorithm, trained to discriminate between non-malignant and cancerous cells (training basis, up to 51 cells/cell line). The proof of concept was carried out on non-malignant (RWPE-1) and cancerous (PC3-GFP) prostate cell lines.
Conclusion
After developing an algorithm to automate AFM measurements, we were able to measure 141 cells in dynamic mechanical analysis and demonstrated the ability of our method to correctly classify between 68 and 74 % of cells (31 cells in the test base/cell line).

Keywords:

AFM, DMA, viscoelasticity, ML, classification

Reference:

(Cross et al. 2007), (Wang et al. 2021), (Proa-Coronado et al. 2019), (Hedjazi et al. 2015)

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Chitin in the cuticular capsule of tardigrade cysts

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Poster Group 1

Background incl. aims
Chitin is a biomolecule classified under carbohydrates, wherein N-acetyl-D-glucose-2-amino units are connected through β-1,4-glycosidic linkages, forming a polysaccharide biopolymer. It is a prevalent structural element in the body wall of various invertebrate groups, including nematodes, annelids, molluscs, onychophorans, and arthropods. This polysaccharide has also been observed in tardigrades, microinvertebrates commonly found in diverse habitats. Tardigrades are characterised by their ability to withstand unfavourable environmental conditions through cryptobiosis and diapause. Encystment, a form of diapause, is a natural process observed in some tardigrades, leading to the formation of a specific cyst form. Thulinius ruffoi (Tardigrada, Isohypsibioidea: Doryphoribiidae) is one of the species capable of undergoing encystment. During this process, the animal undergoes visible changes and produces a cuticular capsule composed of three layers – an outer, middle, and inner sheath. Additionally, the individual enclosed within the capsule possesses its own cuticle covering its body. This work aims to analyse the presence and localisation of chitin within the cuticular capsule of the cyst using selected methods for chitin detection on semi- and ultrathin sections.
Methods
Analyses were conducted using both light and electron microscopy. This study represents the first analysis of the composition of the cuticular capsule in tardigrade cysts. We adapted the 'chitosan-iodine' test to detect chitin indirectly on semi-thin sections. This method involved the deacetylation of chitin into chitosan, protonation of amine groups, and formation of the complex with iodine. Another method included the use of Calcofluor White, which specifically binds to β-1,4-linked polysaccharides. Control reactions in both cases involved specific enzymatic pretreatment using chitinase. Analyses were also conducted at the ultrastructural level. The first method involved a comparative analysis of material treated and untreated with chitinase. The second method utilised Wheat Germ Agglutinin (WGA) conjugated with gold particles to recognise and bind to chitin's N-acetylglucosamine (GlcNAc). The control reaction involved applying the method after prior chitinase treatment.
Results
The results revealed the presence of chitin in the cuticle of encysted animal. Also, the cuticular capsule inside which the animal was enclosed showed chitin localised within the middle and innermost sheaths of the cuticular capsule.
Conclusion
The methods used allow for an analysis of the presence and localisation of chitin on semi- and ultrathin sections, and those dedicated to transmission electron microscopy enable a more precise localisation of chitin. However, implementing the control reactions, such as treatment with chitinase, is necessary for a more specific verification of the obtained results. The analyses demonstrated the presence and precise location of chitin in the cuticle of the examined T. ruffoi. The research also allowed for the detection and analysis of the location of chitin in the cuticular capsule of tardigrade

cysts on the example of the studied T. ruffoi, which is the first analysis of the chemical components of the cuticular capsule in tardigrades.

The study was supported by the Polish National Science Centre (NCN Preludium 2020/37/N/NZ8/00170).

Keywords:

chitin, cuticular capsule, encystment, tardigrades

Reference:

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Janelt K, Poprawa I., 2020. Analysis of encystment, excystment, and cyst structure in freshwater eutardigrade Thulinius ruffoi (Tardigrada, Isohypsibioidea: Doryphoribiidae). Diversity, 12:62. https://doi.org/10.3390/d12020062

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Mapping of interstitial atoms using super-resolution and optimized machine-learning techniques

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Poster Group 2

Abstract- Dedicated machine-learning techniques applied to superresolved HRSTEM images are used to map interstitial atoms with decananometric spatial resolution and picometric precision. This methodology is illustrated with the technologically-relevant case of etched GaN, used for power devices. In general, an average chemical concentration evolution close to a typical erf implantation profile is obtained, in agreement with atomistic simulations.

Keywords: imaging, super-resolution, superresolution, machine-learning, mapping, interstitials, TEM, STEM, HRSTEM

I. BACKGROUND AND GOALS

The interstitial atoms have a potential impact on the performance and reliability of microelectronic or electro-optic or quantum devices, but the precise mapping of their distribution is a very difficult task. In the field of MOSc-HEMT power devices, the etching steps may induce a detrimental ion implantation at critical interfaces in the case of recessed hybrid processes. These defects might potentially induce parasitic leakages currents and a detrimental reduction of breakdown voltages. The local defectivity increase due to such technological steps usually occurs in an ultrashallow range (nanometric or below), which is very difficult to precisely estimate. To tackle this major characterization challenge, novel methodologies have been developed, patented [1], and will be detailed here.

To map the chemical concentration profiles, many state-of the art techniques are currently used, such as secondary ion mass spectrometry (SIMS), electron energy loss spectrometry (EELS) or Energy dispersive X-ray analysis (EDX) among others, but all the techniques have their intrinsic limitations. For instance, it is usually very difficult to distinguish between substitutional and interstitial atoms with these characterization methods. Interstitial atoms are so small that they are generally very difficult to detect by conventional transmission electron microscopy [2]. Hence, it is often preferable to use aberration-corrected scanning transmission electron microscopy (HRSTEM or 4D-STEM) in HAADF mode (Z-contrast imaging) for heavy atoms and annular bright filed (ABF) or customized imaging modes for lighter elements [3],[1]. Light atoms such as H, Li or O play an important role in energy devices, and their mapping at the picometer level is useful to assess their potential electrical activity. In this presentation, we use our patented tools to extract the precise positions of interstitial atoms from superresolved HRSTEM images and optimized machine-learning algorithms.

II. METHODOLOGY

The original HRSTEM-HAADF images are obtained by aberration-corrected electron microscopy using the FEI Titan Themis microscope operated at 200 keV.

All the details of the methods are provided in the following patents. The patent EP4020378B1 is used to denoise the image by convolution.

The patent EP 4020379A1 provides the superresolved image, with a resolution typically increased by a factor of 5 compared to the original image.

Then the patent EP3671190B1 is used to obtain the positions of the interstitials using machine-learning based methodology with 3 binary classes: interstitial atoms, substitutional atoms and elsewhere. Only 3 areas are sufficient for an efficient supervised training with the Fast Random Forest algorithm [4], based on tree bagging and feature sampling.

III. RESULTS AND DISCUSSION

A typical result of atomic mapping is displayed on Fig. 1, in the case of etched GaN. The substitutional atoms are represented in grey, whereas the interstitial atoms are colorized. The spatial Abbe resolution is estimated from the superresolved diffractogram. The pic (-6,12,0) provides an horizontal resolution of 27 pm while the presence of the (0,0,13) reflection implies a vertical resolution of 38 pm, which is close to the record obtained by ptychography [5].

The vertically-averaged profiles are typical of implantation profiles, in agreement with atomistic simulations (Fig. 1 bottom). These results are consistent with the energetics of the plasma etch, when ion bombardment is expected. The possible vertical alignment of interstitials is attributed to possible channeling effects inside the implantation tracks.

This approach, validated by simulations and atomistic modeling, provides a universal methodology to map the interstitial atoms at the atomic scale, with a spatial resolution around 30 pm or even better in the case of ultrathin (about 20 nm thick) TEM lamellae with sample preparations close to perfection.

IV. CONCLUSIONS

Using super-resolution and machine-learning, it is possible to obtain accurate maps of interstitial atoms from high-resolution electron microscopy images, to analyze and optimize the technological processes involving ion implantation or surface damage. This method is universal, and the spatial resolution achieved by these combined techniques is close to the best currently achievable.

Keywords:

super-resolution, superresolution, machine-learning, TEM, interstitial

Reference:

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Reducing user-interaction in the processing of electron energy-loss spectra

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Poster Group 1

Background
Conventional processing of electron energy-loss spectra (EELS) relies heavily on user-determined parameters, like for instance location and width of a background window, or choice of fine structure region. This makes results operator-dependent, more subjective and harder to reproduce.
Here we introduce a model-based processing of EEL spectra that relies on a linear model for which physical constraints are derived and subsequently imposed with the aid of quadratic programming. The model's linearity ensures fast convergence and the absence of local optima. Since the model includes all relevant EELS-features---background, atomic cross sections, fine structure, and multiple scattering---minimal user-interaction is required.
Methods
The background is described as a sum of 4 to 5 power-laws with fixed exponents ranging from -1 to -5. During the fit the coefficients are subjected to constraints that ensure non-negativity, monotonous descent and convexity. This background was proven to hold for energy ranges up to 1500 eV and to be superior to the conventional power-law background model [1].
The atomic cross sections are taken from a data base [2] that contains all edges of all elements up to atomic number 108. They were obtained from numerical relativistic solutions to the Dirac equations.
The fine structure is modeled as a quadratic spline with either linearly or quadratically spaced sample points. Working out the Bethe sum rule, assuming independent atomic shells and the dipole approximation, shows that the fine structure must have a weighted sum of zero. This constraint, too, is enforced through quadratic programming.
Results
In the figure the elemental maps of a TbScO3 sample are shown, in order to compare our approach to three alternative methods:
1. Unconstrained fit, with fine structure, no energy ranges excluded from fit.
2. Unconstrained fit, without fine structure, fine structure region excluded from fit.
3. Conventional approach: background region in front of each edge, background extrapolated and subtracted.
These 3 methods require a substantial amount of user choices, which we made to the best of our abilities. In comparison, our unsupervised approach yields the highest-contrast elemental maps, while offering a better precision, as demonstrated in the concentration histograms in the figure.
Conclusions
Imposing physical constraints on the EELS model through quadratic programming---in this case non-negativity, monotonous descent and convexity for the background, and the Bethe sum rule for the fine structure---greatly improves the quality of the result and removes most of the user-dependent input from the fitting process.

Keywords:

EELS; Constrained optimization; Background; Fine-structure

Reference:

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Atomic Lensing Model for Atomic Scale Multi-Elemental Quantification in STEM

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Background incl. aims
To understand the structure-property relationship in nanostructured materials, we need to probe their crystal structures and compositions at the atomic scale. Advanced materials typically consist of multiple elements in a complicated structure. Significant challenges remain to disentangle the contributions of composition and thickness in STEM due to dynamic scattering, necessitating detailed simulations. However, the combination of the computational cost of the multislice calculation and the enormous ordering possibilities for a given composition makes the quantification of mixed columns almost impossible. To address these challenges, we here report the development of the atomic lensing model for the fast prediction of ADF-EDX scattering cross-sections of mixed columns [1].

Methods
The channelling effect originates from the fact that a fast negatively charged electron will be attracted by the positively charged atomic nuclei. As a consequence, an atomic column with periodically spaced atoms along the beam direction acts as a waveguide dynamically focusing the electrons. This leads to a non-linear signal as a function of depth for atomic resolution ADF and EDX, which significantly complicates elemental quantification. Based on the channelling theory of incoherent imaging, the so-called atomic lensing model was therefore developed to take the ordering of multiple elements into account [2]. In the atomic lensing model, dynamical scattering is treated as a superposition of individual atoms focusing the incident electrons. This model predicts the ADF scattering cross-section of a mixed column from the libraries of pure elements. Based on the incoherent imaging of ADF and EDX signals, this model has been shown to work for EDX as well and allows us to predict mixed column cross-sections with any atomic ordering efficiently. The validity of the atomic lensing model was verified numerically with frozen phonon multislice simulations. The speed and accuracy of the atomic lensing model were compared against multisclice and PRISM algorithms. For instance, the atomic lensing model is the only feasible approach that can explore all the ordering possibilities for a 20-atom-thick binary alloy column, taking 30 s to loop over 1 million orderings with an accuracy of ± 1 atom.

Results
We explained the linear dependence between ADF and EDX cross-sections from scattering theory and examined it numerically with multislice simulations, which is the basis for the extension of the atomic lensing model to both signals under the incoherent imaging mode. As shown in Fig.1 for a Au@Pt nanorod, one cannot distinguish the elements with adjacent atomic numbers from an ADF image but can fingerprint them with EDX. The predictions, based on the atomic lensing model, are in good agreement with multislice simulations and significantly deviate from the linear model in which a

simple scaling with the number of atoms is assumed. Based on the atomic lensing model and the correlation between ADF-EDX due to signal incoherence, we are able to count the number of atoms from an experimental ADF-EDX dataset of a Au@Ag core-shell nanoparticle [2]. We also demonstrated that this model can reliably predict EDX values for an Al-Ag-Pt-Au high entropy alloy up to 10 nm. Beyond this thickness, the contribution of neighbouring columns becomes significant.

Conclusion
The atomic lensing model significantly accelerates the prediction of ADF-EDX scattering cross-sections. It enables exploring atomic arrangements in multi-elemental materials with high throughput and opens new avenues for quantitative analysis in materials science.

Figure 1. (a) Atomic model of a Au@Pt core–shell nanoparticle. (b) Comparison of the scattering cross-sections (SCS) quantified from multislice simulations, the atomic lensing model (ALM), and a linear incoherent model.

Keywords:

Quantitative STEM-EDX, Scattering cross-section

Reference:

[1]Zhang, Z., Lobato, I., De Backer, A., Van Aert, S., & Nellist, P.D. (2023) Ultramicroscopy, 246, 113671.
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Relativisitic EELS cross-sections for all elements

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Poster Group 1

Background incl. aims
The rich information obtained from EELS originates from the complex inelastic scattering process, wherein fast electrons transfer energy and momentum to atoms, exciting bound electrons to higher unoccupied states. To quantify compositions using EELS, the common practice is to fit the observed spectrum with the scattering cross-sections calculated from experimental parameters and the general oscillation strength (GOS) database [1]. A common commercial solution uses GOS tables obtained from the Hartree-Fock solution of atomic orbitals [2]. Here, almost 40 years after, we attempt to go beyond some of the limitations of this database by including the full relativistic effects and making use of dramatically improved computation capabilities in calculating a tabulated GOS solving the Dirac equation (Dirac-Hartree-Slater method within the local density approximation).
Methods
We calculated the transition density matrix using Fermi's golden rule, with the core orbital's bound state as the initial state and the continuum state as the excited state.
We assume a single independent atom, ignoring the solid state effects on the density of states and therefore the rich fine structure that is present in experimental EELS cross sections. We use the frozen core approximation, which assumes the potential remains unchanged after ionizing the core-shell electron to the continuum state, to ensure the orthogonality of the initial and final states. Both large and small components of the Dirac solution are needed to compute the transition matrix element. The convergence is checked by including the final states until the contribution of GOS of the last three states falls below 0.1%.

Results
A complete GOS database was constructed for all elements with atomic number Z=1-118 and all shells, which resulted in 2143 entries for which we store a tabulation of the GOS as a function of energy and momentum with fine sampling. We chose an energy range sampling from 0.01 - 4000 eV with 128 sampling points and a momentum range of 0.05-50 with 256 sampling points, both using a logarithmic sampling scheme. The database was saved in HDF5 file format [3] and will be supported in various software (e.g. Digital Micrograph, Hyperspy and pyEELSModel) so that users could switch easily between different flavours of GOS. As shown in Fig.1(a), the newly developed database has a much larger coverage of energy-momentum space with fine sampling for EELS cross-section quantification. It includes the effects of spin-orbital coupling, e.g. for the Ti L shell [Fig. 1(b)] and shows a good agreement with experimentally measured ionization energies across the periodic table [Fig. 1(c)]. We evaluated the computed spectrum based on different atomic calculations for the experimental spectrum fitting and elemental mapping [Fig. 1(d-f)]. We also considered the relativistic electrodynamics of the fast incoming electron on the orbital transition when computing the EELS cross-sections.

Conclusion
The accuracy of EELS quantification relies on the accuracy of the cross-section calculations represented in the GOS database as a function of energy loss and momentum transfer. In this study, we developed an open-source Dirac-based GOS database for all elements and shells for large energy and momentum space with fine sampling. The open-source Dirac-based GOS database [4] is expected to bring benefits to the EELS community for both academic use and industry integration.

Fig.1 (a) The sampling of Dirac-based GOS compared to Hartree-Fock-based commercial database for K-edge ionization of a C atom, with white grids indicating the sampling; (b) Example of the Ti L2/3 ionization energies comparison; (c)Plots of the experimental (from EELS Atlas) and calculated ionization energies for different elements and orbitals; (d-f) Spectrum fitting and elemental mapping with GOS computed by different atomic calculation methods.

Keywords:

Quantitative EELS, General oscillation strength

Reference:

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Atomic transitions analogue in phased-shaped electron energy-loss spectroscopy

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background incl. aims
Electron energy-loss spectroscopy (EELS) has proven its efficiency in probing nano-optical excitations down to the atomic scale e.g. plasmonic resonances, excitons or plexcitons. However, EELS being directly proportional to the magnitude of the electric field, it is unsensitive to a plethora of important properties of nano-optical excitations - such as e.g. their phase, chirality, field lines symmetry.
Over the last decade, several routes have been explored to enhance the EELS scheme and enable the detections of these so-far hidden properties. One solution – first proposed in 2014 [1] – consists in shaping the amplitude and phase of the electron beam before its interaction with the sample and sorting the final electron states after. This EELS scheme based on state preparation-selection is generally called phase-shaped EELS (PSEELS) and has attracted both a strong theoretical [2,3] and experimental interests [1,2]. Most of these efforts have been focused on the measurement of the chirality of matter through dichroic PSEELS measurements [1,2] and several other properties of matter remain to be explored through the prism of this technique.
In this conference, we will present our latest work on PSEELS aiming at giving a different physical perspective on this technique as well as exploring its application to the probing of multipolar resonances.
Methods
In a first part, we will show that most of the theoretical results found in the literature [1,2,3] can be demonstrated using a different approach treating the shaped and post-selected electron beam in an analogue way to an electron bounded to an atom. More precisely, we will demonstrate that PSEELS is physically equivalent to a spontaneous transition between two bounded electron states - known as Purcell effect – where the initial and final state are in our case shaped and post-selected electron states. This reformulation of the problem provides an elegant and intuitive derivation of the standard result of PSEELS [1,2,3], without the need of complex derivations.
Results
In a second part, we will focus on PSEELS transition between Hermite-Laguerre-Gauss states, which have drawn a strong experimental interest over the last years [4,5]. For this family of states, our formalism enables an exact formal analogy between PSEELS and transitions in a two-dimensional harmonic oscillator – a standard textbook problem. This enables us to express the PSEELS problem in a 2nd quantization language (using ladder operators) and derive closed-form expressions for the PSEELS transition probabilities. This approach has the benefit of directly highlighting the conservation rules of physical quantities such as the linear and angular momentum, thus giving an intuitive interpretation of PSEELS.
Conclusions
In a last part, we inject a multipolar development of the electromagnetic field into the previous formalism. Thus, we demonstrate that, by carefully selecting the in and out free electron states, one can directly measure the different multipolar moment of the electromagnetic field in PSEELS. We numerically illustrate our finding by simulating a PSEELS experiment on a plasmonic quadrupolar field using a boundary element method – as shown on figure 1. In particular, we show that simply by using

1st and 2nd order Hermite-Laguerre-Gauss beams (demonstrated experimentally [4]) one can selectively measure the in-plane quadrupolar field component, as well as the twist of the electric field lines, thus revealing the 3D field topology in EELS.

Keywords:

PINEM, Angular momentum, Numerical, nano-optics

Reference:

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[3] Bourgeois et al., Science Advances 9 (51) (2023)
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Electrochemistry in LP-EM & Effects Induced by Irradiation of Metal Electrodes

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Poster Group 1

Background incl. aims

Electrocatalytic processes are a prerequisite for efficient decarbonization, i.e. by providing green energy via green H_2 synthesis or chemical precursors via CO_2 upconversion. However, this requires a profound understanding of these processes and a complete characterization of the materials employed in these technologies to reveal and prevent potential degradation mechanisms. Nowadays, in situ characterization techniques that use irradiation sources such as electrons or X-rays gained interest since mechanisms can be temporally and spatially resolved giving insights into the atomic scale of such complicated electrochemical reactions. [1]

The challenge that remains when using these techniques is the inevitable interaction of the irradiation source with the specimens in study. An effect widely studied is the generation of radiolytic species that alter the chemical environment, whether evolving beam-induced redox chemistry, or altering the solution/electrolyte acidity. [2] However, a so far overseen aspect when performing liquid-phase electron microscopy (LP-EM) experiments is the design of the three-electrode setup used for these experiments.

A common setup consists of utilizing noble metals like platinum (Pt) as working, counter, and reference electrodes. By having this configuration, effects like increased inelastic scattering, galvanic corrosion effects [3,4], changes in electrochemical response by variation of electrolyte flowrate, or interferences on the reference electrode signal [4] have been suggested. These effects need to be understood and considered as unrelated to the electrochemical reactions to avoid misinterpretations as degradation mechanisms.

Methods

To investigate into these proximity effects, 10 mM silver nitrate ($AgNO_3$) solution flowed (0.8 µL/min) through a pattered Pt three-electrode microchip in a DENSsolution Stream system. The specimen was constantly irradiated with a Thermo Fisher Scientific Talos F200i at 200 kV acceleration voltage in STEM mode (dwell time of 2 µs, and beam current of 533 pA). The Pt working electrode was biased until electrochemically Ag dendrite growth was observed and then stopped to observe the evolution of the process.

Results

Our work reveals that electrochemically grown Ag dendrites etch radiolytically as function of electrode distance. Particularly, the structures closest to the working electrode showed an accelerated dissolution (Figure 1: (a) Electrochemically grown Ag dendrites at time = 0 s, (b) Radiolytically etched Ag particles at time = 71 s, green area marked showing accelerated Ag dissolution when closer to the Pt working electrode.). This is supported by DSSIM analysis [5].

We discuss several possible mechanisms, including secondary electron-based local dose rate increases, Pt-catalyzed generation of oxidative species, as well as galvanic corrosion. Additionally, we report irradiation-driven potential overshoots when irradiating in the proximity of the reference electrode during electrochemical experiments.

Conclusion

In summary, we explore effects associated with the proximity and irradiation of the electrodes to further increase the control over and understanding of in situ analysis of electrochemical processes.

Keywords:

LP-EM, electrode-irradiation effects, galvanic corrosion

Reference:

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A twist to superlubric sliding in bilayer graphene uncovered by in situ TEM

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims

Twist in two-dimensional (2D) Van der Waals (vdW) bilayers, which results in the formation of moiré superlattice, has garnered attractive interest and can lead to unprecedented exotic physical properties including superlubricity.1 The twist angle (θt) offers not only a new and unique degree of freedom for modulating the properties, but also small and local variations in θt can result in exceptional differences in these properties.2 Indeed, it was discovered that the superlubricity in graphite is intimately dependent on the interlayer twist angle (thus incommensurability), interfacial cleanliness, and thickness. Fruitful experimental exploration on the superlubric features in artificially stacked graphite surfaces at a wide range of twist angles has been achieved and the underlying mechanism has been readily explained as the twist-related incommensurate stack between the sliding layers using a rigid-body model. 3,4 However, the scenario of the transition to superlubricity becomes more complex due to atomic scale structure relaxation and reconstruction at a small twist angle regime ($\theta t < 4^{o}$), thus normal rigid-body model fail to predict. Meanwhile, the existing experimental approaches fail to capture the details of superlubric sliding, and direct insight with high precision measurement into how the interface between two atomic layers undergoes transitions from structurally reconstructed bilayers to incommensuration to approach superlubricity is still lacking.

In this work, we report the observation of the superlubric sliding between two monolayer graphene in free-standing bilayer graphene (BLG) by the implementation of an in situ transmission electron microscopy (TEM) tensile straining setup combined with 4D scanning transmission electron microscopy (4D STEM) to probe local twist angle and strain.

Methods

We transfer bilayer graphene samples (ACS materials, 1cm×1cm) to a homemade Cu support with a small window, and then clean the Poly (methyl methacrylate) coating by acetone. Tensile straining was imposed by using a single-tilte Gatan straining holder 654 and in situ TEM experiments were carried out using a ThermoFisher Scientific Spectra 200 microscope operated at 80 kV. Dark-field TEM (DF-TEM) images, selected area electron diffraction (SAED) patterns, and ($1\bar{1}\bar{1}20$) DF-TEM image series were recorded throughout the straining process to visualize the dynamics of basal plane dislocations during straining. The mechanical loading was paused in between to carefully characterize the basal plane dislocations and to acquire 4D-STEM datasets (Fig. A). The 4D STEM experiments was carried out using micro-probe mode with a nominal convergence half-angle of 0.47 mrad and the data acquired using a Ceta-S camera running at ~300 fps. These datasets were analysed using py4DSTEM package and custom codes. A similar straining experiment and data acquisition process was succeeded for a second BLG sample. Both straining experiments were terminated until fully detachment of the monolayers.

Results

We realized the strain experiment on graphene samples with a monolayer-bilayer-monolayer (MLG-BLG-MLG) configuration (Fig. B). Before straining, basal plane dislocation with line direction parallel to the straining direction was observed (Fig. B), which is likely resulted from film buckling. After an

incubation period during straining, where the width of the bilayer region decreased, we observed nucleation of new basal plane dislocation with line direction perpendicular to the straining direction, indicating initiation of twist between the monolayers. Further straining resulted in continuous nucleation of dislocations and a dramatic increase in the density of these dislocations, thus the increase in twist angle (Fig. C and D). Using 4D STEM analysis, we quantified the twist angle within the bilayer region by measuring the angular difference of Bragg disks in the local nano-beam diffraction (NBD) patterns, which are extracted from the left-side and right-side monolayer regions. Similarly, the local elastic strain in the monolayer regions was determined by analysing the displacement of Bragg peaks throughout all datasets, referencing a free region near the edge of MLG as zero strain. The established relationship between twist angle and strain, derived from extensive measurement points in both samples, clearly disclosed a consistent tendency of decreasing in strain with increasing twist angle. Leveraging the strain-twist angle correlation, and employing the quasi-kinetic equilibrium force model alongside the elastic properties of monolayer graphene (Fig. A), we investigated frictional properties associated with twist angle in bilayer graphene. Notably, a smooth reduction in the coefficient of friction (CoF) was observed as a function of twist angle, with the CoF dropping below 0.01 for twist angle exceeding 1.5^{o}, implying the approach of superlubricity in bilayer graphene.

Conclusions

Our study delved into the phenomenon of twist-modulated superlubric sliding between monolayer graphene by directly visualizing the dynamic transition from commensurability to incommensurability in marginally twisted bilayer graphene. These findings provide the direct experimental observation of superlubric feature between atomic graphene layers and open a new avenue to build up twist angle gradients in 2D materials for investigating their twist-related exotic properties.

Keywords:

Graphene, twist, in-situ straining, superlubricity

Reference:

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(5) X. Zhou, et al. in preparation.

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Combined 3D electron diffraction and nanotexture analysis for the study of planetary materials

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Poster Group 2

Background incl. aims
We propose a method which combines 3D electron diffraction (3D ED) [1] and nanotexture analysis in a TEM to characterize small impact ejecta (usually spherules of few hundred microns) with the aim to identify new mineralogical phases and to understand their formation mechanism/conditions. The method of analysis consists of: a FIB thin section preparation on an area of interests determined by SEM imaging and EDS analysis; identification of the crystal structures of the main phases through 3D ED, a single crystal diffraction technique on nanometric grains; texture and phase analysis using a scanning diffraction method known as PACOM (Precession Assisted Crystal Orientation Mapping) [2].
Methods
The FIB section is usually inspected both by Z-contrast STEM imaging and EDS analysis to identify areas and crystal grains of interest. For identifying the phases present in the sample, fast 3D ED data collections, covering just 30°-40° of reciprocal space, are collected on selected crystal grains with the aim of unit cell determination and consequent phase identification. If the phase determination is uncertain a wider angular range (<80°) 3D ED data collection can be employed for its structure solution. The relative phase relations and possible coherent growth are then determined by recording a sequence of ED patterns while the electron beam is scanning the area of interest with a spatial resolution of a few nanometers. Those patterns are then indexed with a cross correlation algorithm employing banks of ED patterns (using the software of the ASTAR Nanomegas system) created on the bases of the determined crystal phases present in the sample.
We applied this methodology to two sets of samples: impactite ejecta belonging to the Australasian tektite/microtektite strewn field and Fe-Ni oxide spherules generated by the impactor which created the Kamil crater (Egypt).
Results
In the case of the Australasian ejecta 3D ED allowed the identification and structure solution of the TIO2-II high pressure polymorph, which resulted to be a Zr-free srilankite endmember. The texture analysis indicates that TiO2-II is usually surrounded by well-ordered rutile grains which retains a topotactic relation with the embedded TiO2-II suggesting a partial transformation due to P-T waves during the impact.
In the case of the spherulites from Kamil crater it has been possible to depict a complex scenario in which a matrix of Fe-Ni oxides grains showing fine exsolution of two different phases (STEM HAADF image in the right picture) is permeated in the interstitial volume by a Si rich fluid which, in most of the cases, is amorphous. PACOM and HREM imaging revealed that the two exsolved Fe-Ni oxide phases have a trevorite (the Fe-rich component) and a bunsenite (the Ni-rich component) structure and that inside each micrometric grain they are coherent. The amorphous interstitial part usually separates into two components: SiOx amorphous droplets and a second silicic component rich in Fe

and P. This second component has been found both in an amorphous and in a crystalline form. When crystalline, 3D ED identified it as olivine with a P content as high as 3%-4%at. The full crystallochemical characterization at the nanoscale depicts a formation model in which the Ni-Fe-P melt from the meteorite oxidizes and mixes with the melted quartzite from the target. The oxidized Fe and Ni crystallizes in Fe-Ni oxides micrometric grains which, upon cooling, exsolve the two abovementioned phases (bunsenite in green and trevorite in light blue in the left picture). The remaining Fe with all the P enriches the silicic melt which form a glass that fills the interstitial void. This glass further separates into two components: one depleted in Fe and P which stays amorphous and a second which, depending on its thermal history, can or cannot crystallize forming P-rich fayalite.

Conclusions

The combination of 3D ED with PACOM allows at the same time the discovery of new crystal phases present in micrometric planetary materials and obtaining a full crystallochemical charaterization which is essential for understanding the thermal history and for building a formation model of the retrieved sample. We envisage a crucial application of this methodology to any kind of planetary material coming from sample return missions.

Keywords:

electron diffraction, meteorites, texture analysis

Reference:

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Probing the chiral behavior of nano-optical near-fields through angular momentum resolved PINEM

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

Background incl. aims
Usually performed in a ultrafast transmission electron microscope (UTEM), photon induced near-field electron microscopy (PINEM) consists in mapping the magnitude of an optical near-field pumped by a laser with a fast electron beam [1]. Benefiting from the sub-nm spatial resolution of TEMs as well as the high fluence and energy resolution of ultrashort lasers, this technique can probe deep-subwavelength structural details of the optical near-field in resonant and non-resonant structures.
Methods
In addition, and contrary to electron energy-loss spectroscopy (EELS), PINEM displays a high degree of selectivity thanks to the laser illumination. For example, by properly tailoring e.g. the wavelength and polarization of the laser, one can selectively excite individual optical modes [2]. This idea can be extended to probe the chirality of nano-optical near-fields. Indeed, as first demonstrated by Harvey and collaborators in 2020 [3], one can perform a so-called “chiral PINEM” experiment – where a right- and left-handed polarized laser beam is sequentially used to excite the structure and measure the differential PINEM signal. In that configuration, spin angular momentum (SAM) of the incoming beam is tuned so that this technique is denoted as SAM-dichroic PINEM (see figure 1).
Nevertheless, by shaping its phase profile, a laser beam can also carry orbital angular momentum (OAM), and a long-standing question remains on the difference between OAM- and SAM-based dichroic experiment [4]. A natural candidate to tackle this issue is thus a combined SAM-dichroic and OAM-dichroic PINEM experiment – an experimental scheme that we will refer to as angular momentum resolved PINEM (AM-PINEM).
Results
In this conference, we will present our latest work on angular momentum resolved PINEM, showing its application to the probing of the coupling between chiral light and optical near-fields. In a first part we will show that the theoretical framework based on the local density of states (LDOS) used to explain EELS and CL experiments [5] can be extended to PINEM, combining in an elegant way these three main TEM spectroscopies. In particular, we will demonstrate that AM-PINEM can be connected to the concept of chiral radiative LDOS.
In a second part we will focus on the numerical simulation of AM-PINEM experiments using pyGDM - an electrodynamics simulation toolbox based on the green dyadic method that we enhanced to simulate polarized electron spectroscopies. The large library of different illuminations allows for simulation of both SAM- and OAM-resolved PINEM experiments. We will focus on practically realizable structures such as the gold trimer (see figure 1) in which OAM- and SAM-dichroism present clear differences.
Conclusions
In a last part, we will show our first experimental results on SAM-dichroic PINEM realized with a new cold field emission UTEM on simple achiral structures, revealing dichroic behaviors at the deep sub-wavelength scale. Finally, we will present our preliminary work towards the realization of the OAM-dichroic PINEM experiments.

Keywords:

PINEM, Angular momentum, Numerical, nano-optics

Reference:

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Impact of oxidation state on copper nanoparticle stability in industrial Cu/ZnO/Al2O3 hydrogenation catalyst

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Poster Group 2

Background
The escalating concentrations of atmospheric CO2 represent an urgent challenge for the global climate system, driving intensive research efforts aimed at mitigating this trend through innovative strategies for CO2 reduction, conversion, and sequestration. The catalytic conversion of CO2 into liquid fuels and high-value chemicals emerges as a pivotal area of focus within the realm of environmental stewardship and energy efficiency. Among these strategies, the hydrogenation of CO2 to methanol stands out as a particularly promising avenue for the effective large-scale deployment of CO2 utilization technologies. The production of methanol through the conversion of CO2 using green hydrogen obtained from sustainable energy sources like wind, solar, and biomass, serves the dual purpose of CO2 utilization and the storage of renewable energy into chemical compounds.
The standard methanol catalyst Cu/ZnO/Al2O3 has been industrially applied for more than 50 years. Still, its efficacy is marred by exceeding 50% decline in activity within the initial weeks of operation, primarily due to thermal sintering and structural reconfigurations. It underscores the critical need for an in-depth exploration into the mechanisms underlying catalyst deactivation, with the ultimate goal of engineering more resilient catalyst systems. Previous studies attribute this deactivation to the sintering of copper, a process intimately linked to the dynamics and stability of Cu nanoparticles (NPs).
In the quest to elucidate the dynamic behaviors and migration patterns of NPs, electron microscopy stands as the method of choice. Specifically, in situ Transmission Electron Microscopy (TEM) offers unparalleled insights into catalyst structures under near-industrial operational conditions, including the intricate dynamics of nanoparticulate entities. This study, therefore, harnesses the power of in situ TEM to meticulously examine the behaviors of Cu NPs on industrial Cu/ZnO/Al2O3 catalysts throughout the entire CO2 hydrogenation lifecycle, spanning activation, steady-state operation, and eventual deactivation. By focusing on the oxidation state of copper under varying operational conditions, this investigation seeks to unravel the complex interplay between the chemical state and the mobility of Cu NPs, with the overarching aim of fostering advancements in catalyst design and CO2 conversion technologies.
Methods
In situ TEM experiments were carried out using a windowed gas cell nanoreactor (Climate, DENS solutions) comprising of two electron-transparent Si3N4 windowed chips. Commercial Cu/ZnO/Al2O3 catalyst (Alfa Aesar, 45776) was dispersed in ethanol and deposited on the bottom climate chip before constructing the climate holder and sealing the nanoreactor. The nanoreactor consists of a top and bottom chip of 50 nm and 30 nm thickness respectively. The internal temperature of the microreactor was accurately controlled by a 4-point probe method.
Microscopy measurements were conducted using a probe aberration-corrected FEI Titan Themis microscope operating at 300 kV. The beam convergence angle was set to approximately 26 mrad. EEL spectra were recorded with an ELA hybrid pixel direct electron detector from Dectris that was retrofitted with a CEFID energy filter from CEOS. The energy dispersion of the ELA detector was 0.747 eV per channel, and the dwell time was set to 1 s.

Results
Upon careful reduction following standard industrial procedures, the catalyst was activated, demonstrating significant changes in the behavior of Cu NPs across various stages of treatment, as monitored by in situ STEM and complemented by EDS and EELS analyses.
Initially, under high vacuum at room temperature, Cu NPs exhibited stability with no discernible movement; the only observable change was the slight contraction of the overall catalyst structure. EELS indicated a pronounced white line, suggesting an oxidized state of copper.
Heating to 180°C under a 1 bar N2 atmosphere triggered dynamics within Cu NPs, extending them towards the sample's edge to form larger entities. EDS data revealed Cu's segregation from Zn, highlighting Cu's higher mobility. This segregation could contribute to deactivation, as the Cu-Zn synergy is deemed crucial for catalytic activity. Notably, areas not subjected to electron beam exposure did not exhibit similar changes, suggesting that temperature or gas composition, in conjunction with beam interaction, might facilitate these dynamics. Post-sintering, EELS data suggested a reduction in Cu, with a diminished white line intensity and reduced mobility.
At 260°C in a 1 bar H2 atmosphere, Cu NPs demonstrated sintering with no alteration in the white line intensity of Cu EELS before and after observation, indicating a stable oxidation state that was reduced compared to the initial state but more oxidized than beam-induced sintered NPs. Some level of dynamic behavior was still observed, albeit less than in N2. Significant movements, such as approaching beyond the edge of the specimen, are indiscernible, suggesting that the beam-demage is minimized in H2 atmosphere.
Exposure to a 1 bar CO2+H2 atmosphere at 260°C resulted in immediate stabilization of Cu NPs, with EELS revealing a lower white line intensity, approaching that of beam induced sintered NPs.
Finally, under a 1 bar O2 atmosphere at 260°C, Cu NPs were re-oxidized, regaining high mobility. The presence of O2 also facilitates the redispersion of Cu NPs.
Conclusions
The comprehensive investigation delineates the nuanced mobility hierarchy within the catalytic system, conclusively establishing that the mobility of copper is positively related to its oxidatino state. The discovery holds significant importance within the realm of Cu/ZnO/Al2O3 deactivation, as corroborated empirically that deactivated Cu/ZnO/Al2O3 catalysts exhibit the presence of partially oxidized copper. The study further corroborates the role of oxygen in facilitating the reoxidation of Cu to CuO, a process that significantly contributes to the sintering, underscoring the detrimental impact of oxidation processes on catalyst longevity and performance. The mobility difference between copper and zinc could potentially cause their segregation and contribute to deactivation, as the Cu-Zn synergy is deemed crucial for catalytic activity. Moreover, the addition of CO2 to the hydrogen flow enhances the reduction power, likely through the formation of CO by Reverse water gas shift. Collectively, these insights not only advance our understanding of the dynamic interplay between catalytic components under varying environmental conditions but also pave the way for the development of more resilient and robust hydrogenation catalyst systems.

Keywords:

Cu/ZnO/Al2O3, In-situ-TEM, Gas-Cell, Sintering, Stability

Reference:

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Damage reduction in amorphous ice by a non-conventional scan strategy in cryo-STEM

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

Background
For many important classes of materials, electron beam damage in transmission electron microscopy (TEM) has a detrimental effect that limits the capability of the instrument to obtain information at different scales, atomic or structural scale, for instance. Organic and biological materials are among the most beam sensitive materials. Biological samples are commonly studied using cryo-TEM, where the samples are preserved in amorphous ice and illuminated by a parallel beam. Unfortunately, the ice matrix is also sensitive to the electron beam. For these reasons, electron doses in the order of only 10's of e-/Å² are usually employed in cryo-TEM. Potentially, scanning transmission electron microscopy (STEM) offers some benefits over TEM such as the compositional contrast, through the high angle annular dark field (HAADF) signal, and the capability to observe thicker specimens [1]. In STEM, it is well known that beam damage can extend over an area larger than the size of the scanning probe so areas that come later in the scanning sequence can be damaged prior to observation with the probe. This effect can have many physical sources, which can depend heavily on the material itself: diffusion of heat, diffusion of radicals, delocalized inelastic scattering, electrostatic charge, etc. [2]. Some of these effects are dynamic in nature and have both a spatial and a temporal scaling parameter. It has been shown that this behavior can be modeled as a diffusion process and that beam damage can be mitigated by changing the temporal distribution of the electron dose [3]. In this work, since evidence of a diffusion type damage behavior was found in vitreous ice, we compare beam damage when using an interleaved scan, see Figure 1, and the conventional raster scan with the aim to mitigate damage effects and increase the applicability of cryo-STEM on biological samples.
Methods
To make the experiments reproducible, vitreous ice served as a test sample. A JEOL ARM300F2 microscope operated at 300 kV and equipped with an external scan generator was employed to scan two adjacent areas using raster and interleaved scan. In an effort to perform the experiments in areas of relatively uniform thickness, the experiments were carried out close to the center of the holes of quantifoil grids filled with ice, see Figure 1. An electron dose budget approximately 100x higher than in conventional cryo-TEM was used in order to make the effect of damage more evident and easier to quantify. The quantification was done from images acquired using the HAADF analog detector where darker areas indicate more damage after mass removal when scanning. The electron dose was fractionated by sequential acquisitions for both scan methods and also compared to single acquisitions, maintaining a constant total dose.
Results
A significant reduction of beam damage of more than 20% is observed when using interleaved over raster scan when scanning with long dwell times (more than 250 µs) and only a few passes (two or less); as shown by the use of line profiles in Figure 1. However similar amounts of damage occurred

when using a short dwell time (20 µs) and several passes (around 25). Our results also show that the damage rate increases as the vitreous ice thickness decreases.

Conclusion

Our observations support the diffusion like process, at least for this specific material. It shows that beam damage is not only proportional to the total electron dose, but also the distribution in time and space plays an important role. Further work is required to identify the physical nature of the diffusion process and its link to beam damage.

Keywords:

Cryo-STEM, diffusion process, beam damage.

Reference:

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Atomic-scale Defects Critical to the Performance of Perovskite Solar Cells

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Poster Group 2

Background
Metal halide perovskite semiconductors have emerged as a focal point of research due to their potential applications in solar cells, characterized by exceptional power conversion efficiencies, extended carrier lifetimes, reduced recombination rates, and cost-effective manufacturing techniques. Despite their considerable potential, challenges such as structural instability, ion migration, hysteresis, and defect intolerance are impeding their practical application. There is a critical need for a detailed atomic-scale understanding of the processes that control these properties and performance, however, atomic-scale electron microscopy studies have been relatively limited due to the extreme sensitivity of these materials to the electron beam. Here we develop and apply low dose electron microscopy methods to provide new insights into the structure-property relationships of these materials, including the role of nanoscale heterostructures, octahedral tilts, and point and planar defects, that are critical to their distinctive properties.

Methods
Transmission Electron Microscopy (TEM) offers valuable insights into the local atomic structure of materials. The application of TEM in the study of perovskite solar cells is extremely challenging due to their extreme sensitivity to electron irradiation, which can lead to rapid changes to their pristine structures with doses as little as a few electrons per Å2, far below conventional techniques, including STEM, EDX and EELS. To circumvent this limitation, we have developed and applied a suite of specialized TEM protocols encompassing specimen preparation, inert atmosphere transfer, diffraction pattern and image acquisition, and data interpretation.

Results
Our study reveals the sequence of subtle, atomic-level structural transformations leading to the degradation of mixed-cation Cs1-xFAxPbI3, beginning with the formation and ordering of ion vacancies and followed by a composition-dependent octahedral tilt. Additionally, we elucidate the atomic configurations of planar defects, including twins and Ruddlesden-Popper phases, and establish a correlation between these defects and solar cell performance. Our findings indicate that certain types of twin defects adversely affect solar cell efficiency, and their removal markedly enhances device performance.

Conclusion
The insights gained from our study into ion loss, ion migration, octahedral tilt modes, and the influence of A-cations shed light on the atomic-scale structural mechanisms responsible for the

instability of perovskite solar cells. By correlating specific atomic defect structures, including planar defects, with optoelectronic properties, we outline strategies for the amelioration of perovskite solar cells through defect engineering, paving the way for their improved performance and stability.

Keywords:

Low-dose TEM, perovskite solar cells

Reference:

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Field emission properties of a single-crystal diamond needle under ultrafast laser illumination

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Poster Group 1

Stable sources of ultra-short electron pulses are an important issue in the development of time-resolved electron microscopy. Here we report on experimental investigation of static and pulsed electron emission from single crystal diamond needles and LaB6 nano-tips, fabricated by focused ion beam milling [1-2]. Diamond attracted a lot of attention due to its unique properties: chemical inertness, mechanical stability and high thermal conductivity [3]. LaB6 has been traditionally used as a thermionic electron source for electron microscopes, but has been hardly put to practical use as cold field emitter (CFE) [4]. In the present work we study the emission properties of diamond and LaB6 nano-tip under static electric field (static emission) and under femtosecond laser illumination using infrared laser (at 1.04 □m and 2.25□m, wavelength) and high repetition rate ranging from 40 kHz to 13 MHz.

Field emission measurements were performed in an ultrahigh vacuum system at a pressure of 4 × 10−10 mbar at room temperature. A negative bias voltage Vtip is applied to the sample. The electron kinetic energy spectrum is measured with a retarding field energy analyzer using concentric hemispherical grids with a resolution of about 0.2% of Vtip. The detector is composed of a stack of two micro channel plates (MCP) coupled with a phosphorescent screen each of 8 cm in diameter. A CCD camera is used to recorded the impacts on the phosphorescent screen. This setup enables the measurement of the 2D map of the emission pattern. The MCPs have a hole in the center of 0.5 cm in diameter. The laser beam is focused on the tip emitter through a spherical mirror of numerical aperture NA = 0.3 and a working distance WD = 25 mm.

Conductive diamond and LaB6 emitters show significant increase of electron emission current under laser illumination. Electron spectroscopy measurements suggest multi-photon nature of the electron emission processes. The increasing of the laser intensity strongly modifies the emission pattern, leading to the emergence of a new emission region at high peak power [2-5].
In particular, the field emission pattern, when compared with the field ion microscopy image of the LaB6 surface, suggests that electrons are emitted from the [001] pole and [011] poles under strong laser illumination. By further increasing the laser power, a transition from multiphoton to optical field emission occurs, emitting electrons of energy higher than the Fermi level.

Figure. Ultra-fast diamond-based photofield emission source. (a) Schematic view of the emission dynamics. (b) Electron emission microscopy (EEM) pattern and (c) energy spectra of the sites 1 (black dots), 2 (red dots) and 3 (green dots) defined in (b), at Vtip = 300 V under laser illumination at Ipeak =

13 GW cm-2. The (X, Y) coordinates indicate the position on the CCD camera in number of pixels. The colors map corresponds to the normalized density of impacts.

Keywords:

Ultra-fast electron emission, ultra-fast laser

Reference:

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Electron beam imaging of extremely confined optical modes in topology-optimized dielectric photonic cavities

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Poster Group 2

Background incl. aims
Dielectric photonic cavities can store light efficiently and have proven capable of spatially confining light far below the diffraction limit, making them promising building blocks for integrating photonics and electronics.[1] The small mode volumes realized in these structures reveal the limitations in spatially resolving the optical modes by conventional optical characterization techniques. In comparison, transmission electron microscopy (TEM) can reach sub-nanometer spatial resolutions, making this technique attractive for characterizing nanophotonic structures.[2] In particular, the swift electrons in an electron microscope can couple to the optical modes of nanostructures, leading to electron energy losses.[3] The electron's energy loss can be measured by electron energy loss spectroscopy (EELS) with high spatial resolution when combined with scanning transmission electron microscopy (STEM). This contribution aims to visualize the fundamental mode at 1550 nm wavelength of a topology-optimized photonic cavity with extreme dielectric confinement (EDC).
Methods
Si-based dielectric photonic cavities are designed by an inverse design process using topology optimization and are subsequently fabricated by dry etching techniques in the cleanroom.[1, 4] The dielectric photonic structures are lifted out individually from the substrate using a plasma focused ion beam (PFIB)/scanning electron microscope (SEM) and attached to a TEM-compatible grid. The lifted out cavities are examined using a Thermofisher TEM instrument equipped with a monochromator and an aberration corrector, giving energy and spatial resolutions better than 0.1 eV and 0.2 nm, respectively. EELS spectrum images are acquired at various sample tilt angles, revealing information about the optical modes' evolution in space. The EEL spectra are analyzed using different fitting approaches after a Richardson-Lucy deconvolution of the zero loss peak to produce the electron energy loss maps for the mode of interest at approximately 0.80 eV. EEL spectra are simulated using a quasinormal mode expansion of the electromagnetic Green tensor to facilitate a detailed analysis of the experimental data.[5]
Results
STEM images acquired in the tilt range 0°-90° show the structure of the dielectric photonic cavity used for the present study (Figure, panel a). EEL spectrum images acquired for each tilt angle reveal the excitation of different cavity modes, with the fundamental mode at 0.80 eV being most strongly excited at the highest tilt angle, which is visualized by EELS intensity maps (Figure, panel b) and sum spectra from the bowtie region of the photonic cavity (Figure, panel c). This observation agrees well with the simulations and is caused by the in-plane polarization of this mode tailored by the inverse design process. EELS intensity maps of this mode confirm its tight confinement in the center bridge of

the bowtie of the cavity structure. Besides the fundamental mode, additional optical modes with different energies, polarizations, and spatial confinements are observed. The simulated EEL spectra for different sample positions agree very well with the experimental results.

Conclusions

The results of this contribution showcase the application of EELS in nanoscale imaging of optical modes in dielectric photonic cavities and its potential for gaining deeper insights into light's interaction with matter and confinement at deep sub-wavelength dimensions.

Keywords:

STEM, EELS, nanophotonics, photonic cavities

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Unveiling Atomic-Scale Defects and Optical Properties within Quasi_2D Perovskite single crystals

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Poster Group 2

Perovskite materials have emerged as a transformative force in the field of optoelectronics, particularly in the development of solar cells, light-emitting diodes, and photodetectors. While three-dimensional (3D) perovskite thin films exhibit exceptional optoelectronic properties, their performance can be hampered by issues such as grain boundaries, surface roughness, and stability concerns. In this context, the advent of perovskite 2D single crystals offers a promising avenue to address these challenges and optimize the morphologies of 3D perovskite thin films. Unlike their 3D counterparts, 2D perovskite crystals are characterized by their ligand divided atomically thin-layers nature, a chemical formula of L2An-1BnX3n+1,1 which often leads to large exciton binding energy with enhancing radiative recombination rate and hinder ion migration to improve stability. Understanding the growth dynamics, nucleation processes, and crystallographic orientations of these 2D structures is critical for harnessing their full potential in optoelectronic applications. Despite their potential, the atomic-scale defects influencing their optical behaviour remain poorly understood. While previous studies have individually explored either the structural or optical aspects, a comprehensive correlation between the two is lacking. This study aims to bridge this gap by combining cutting-edge microscopy techniques to unravel the nanoscale origins of optical properties in 2D perovskite single crystals.

Keywords:

2D Perovskite Single Crystals,4D-STEM, HRSTEM

Reference:

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Modification of topological phenomena at hybrid Bi2Se3/organic interfaces

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Poster Group 1

Background
Topological insulators such as Bi_2Se_3 have interesting transport properties, including undamped transport via topologically-protected surface electronic states[1]. Manipulation of surface plasmons has been previously demonstrated using magnetic dopants, but these cannot be altered after growth. In contrast, the use of organic molecular overlayers such as C_{60} could be advantageous because it is possible to 'gate' the C_{60} interaction through electrical biasing and thereby develop tunable devices[2]. Here, we investigate plasmonic interactions at the interface of an as-deposited thin film sample of Bi_2Se_3/C_{60} using electron energy loss spectroscopy (EELS). We show changes in the characteristic plasmonic behaviour of the Bi_2Se_3 surface in the presence of C_{60}, providing greater understanding of the topological insulator-organic interface.
Methods
Bi_2Se_3 thin films were grown on a c-plane sapphire substrate by molecular beam epitaxy (MBE) with immediate subsequent deposition of C_{60} overlayers to avoid interfacial contamination. Cross-sections were extracted from the thin films by focused ion beam techniques and thinned to an electron transparent thickness of 35 nm for imaging. STEM and EELS were carried out on a mono-chromated instrument at 60 kV (the Nion UltraSTEMTM 100MC 'HERMES' instrument at the UK SuperSTEM facility).
Momentum-resolved EELS was used to map the plasmon dispersion with a convergence and collection semi-angles of 2 mrad and 1.1 mrad respectively. Spectra were collected containing only electrons scattered with specific momentum transfer from the lattice at momenta 0.0±0.3, 0.7±0.3, 1.0±0.3 and 1.4±0.3 1/Å along the ΓM direction of the first Brilliouin zone of Bi_2Se_3 to map the dispersion.
Results
EELS data and plasmon dispersions were obtained across two interfaces Al_2O_3/Bi_2Se_3 and Bi_2Se_3/C_{60} and are presented in figure 1. Panel is a HAADF STEM image of the stack, showing excellent film quality that includes clear crystallinity in the C_{60} layers. EELS spectra of bulk Bi_2Se_3 from the centre of the thin film (annotated blue in figure 1a) revealed two volume plasmons, at 7.2 and 17.3 eV, as well as two Bi core-loss edges between 25-28 eV (not shown). The dispersion of the 17.3 eV volume plasmon is shown in figure 1d and follows a parabolic trendline as expected from literature implying the classical nature of this plasmon.
A surface plasmon from Bi_2Se_3 was observed at 5 eV, localised to the Al_2O_3/Bi_2Se_3 interface, as shown in figure 1b. Its plasmon dispersion was observed to follow either a linear or root trend, plotted in figure 1e, which is similar to that predicted for π-electrons in graphene[3] and suggests the presence of a strongly confining interfacial potential. Comparison with simulations suggests that the nature of this surface plasmon could be a result of Bi_2Se_3 π-electrons confined in 2D to the surface[3]. The

carrier density obtained from a fit of the surface plasmon dispersion concurs with the predicted number of carriers arising from Bi_2Se_3 π-electrons by DFT simulations.
At the other side of the thin film, additional features could be isolated as originating from interaction with C_{60} due to their absence at the Al_2O_3/Bi_2Se_3 interface. Across the Bi_2Se_3/C_{60} interface, the surface plasmon energy was shifted higher in energy to 5.9 eV, shown in figure 1c. This interface contained features from bulk Bi_2Se_3 and C_{60} along with the additional surface plasmon. In bulk C_{60}, three interband transitions were observed, at 3.6, 4.7 and 5.8 eV, consistent with literature[4]; these exhibited very little dispersion with increasing momentum. At the Bi_2Se_3/C_{60} interface, some contribution of these non-dispersive interband transitions remained present. In momentum-resolved EELS spectra of this interface, dispersion of the observed surface plasmon was more difficult to map due to the additional spectral features present, however, the spectra could be decomposed into a linear combination of distinct contributions, revealing the presence of a dispersing feature, localised at the interface and that we identify as the interfacial plasmon.
Unusual plasmon dispersion of the Bi_2Se_3 surface plasmon was observed by momentum-resolved EELS similar to dispersion of 2D π-electrons in graphene. Upon fitting, an agreement in carrier concentration suggests the surface plasmon origin could be from the 2D confinement of Bi_2Se_3 π-electrons to the surface. Through the introduction of organic molecules such as C_{60}, the surface plasmon of Bi_2Se_3 was altered. Further work will characterise the interface between Bi_2Se_3 and other organic molecules such as H_2Pc.

Keywords:

EELS, topological insulator, plasmons

Reference:

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AI Automation for Transmission Electron Microscope Alignment

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims
Transmission electron microscopes, like other scientific instruments, are becoming increasingly complicated. Consider the I2TEM in Toulouse, a dedicated TEM for electron holography and in-situ research (HF-3300 C from Hitachi) which has a cold-field emission gun, 9 lenses, 4 apertures, 3 biprisms, 18 pivot points to align, and nearly as many elements in the corrector. The operation involves more than one hundred configurable parameters, but with approximately 10^300 theoretically possible configurations, one wonders if the instrument is used to its full potential. Furthermore, appropriate microscope alignment takes between 20 minutes and an hour each day, depending on the experiment and the microscopist's effectiveness. We propose to explore the use of artificial intelligence to automate the alignment, therefore tackling the complexity and reducing the time taken by the task.
Methods
To address this complexity, we first developed full computer control of the microscope. Hitachi provided access to every single element (including aperture positions, deflector currents and alignment) as well as details about the communication protocol. This enabled us to develop dynamic automation of the microscope to stabilize the specimen and hologram alignment through traditional control and feedback loops in real-time [1]. But to go further, we wondered if the computer could take complete control of the microscope according to the user's needs using artificial intelligence (AI).
Machine Learning, such as Convolutional Neural Networks (CNN) [2] is gradually replacing older forms of automation in various industries. We developed an Application Programming Interface (API) to automatically change the microscope parameters while acquiring images (Figure 1). This allowed us to create training datasets for matching the configuration to the images produced by the microscope. Because most configurations would not create an image on the screen, we first aligned the TEM before randomly shifting the parameters around their respective values. This ensures that we stay close to a configuration producing an image and by knowing the difference between the aligned value and the shift we can form a dataset linking the image to the shift. This allowed us to predict image characteristics based on the microscope configuration, as well as configurations that met specific image characteristics. In parallel, we have developed a realistic simulation of the I2TEM to produce a dataset of virtual experiments [3] which allows us to test models on various scenarios without the associated cost of directly using the microscope.
Results
This allowed us to train models for each step of the alignment process on the I2TEM (aperture alignment, condenser astigmatism correction, pivot points correction, eucentric position correction and focus correction). The only remaining step is to correct the objective lens astigmatism, which is handled by the corrector using a different API than the rest of the microscope. Each step of the correction, while not as good as human performance yet, takes up to twenty seconds at most, reducing the correction time to less than two minutes. Figure 1 depicts the first step in the alignment: aperture correction.

Conclusion
The results show that AI allows for TEM alignment automation, and while precision is still not on par with human levels, the complex task of finding a good alignment is met, allowing for much faster alignment overall. The method could be extended to other microscopes if they can be controlled via an API and the captured images can be processed in real-time. Additionally, the method used could be modified to address issues like object stabilization, or the calibration of new components.
The aim is to integrate the entire solution into an application that allows microscopists to use the automation functions seamlessly while also allowing them to easily generate or strengthen new automation routines. We are also considering developing a reinforcement learning [4] model that can achieve or maintain a set of meta-parameters according to the user's needs. The meta-parameters are the microscope's parameters that users are most interested in, such as beam size, beam position, focus and magnification, rather than the microscope configuration itself. We intend to encode the image in the meta-parameter space using a constrained variational auto-encoder [5], with limitations to ensure that they either coincide with a previously identified parameter, such as the focus or are at least intelligible to humans. The user can then manipulate the encoded image, and the model will change the configuration accordingly for the microscope to produce an image meeting the user's needs.
Acknowledgements
The authors acknowledge funding from the European Union under grant agreement no. 101094299 (IMPRESS). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

Keywords:

TEM, AI, CNN, Automation

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Correlating absorption and diffraction contrast tomography on earth and space materials

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Poster Group 2

Background incl. aims
Minerals in polycrystalline rocks on Earth have formed over durations spanning solar system formation up to our present geosphere and cryosphere. The conditions of rock and ice formation are recorded in the relative crystallographic orientations, sizes, shapes, defects, and distributions of their crystals or grains that, together with chemistry, chronicle planetary dynamics over timescales of seconds to billions of years. The three-dimensional (3D) mineral architectures are therefore fundamental to understanding celestial bodies and the solid earth, while providing crucial context within which we explore the origins of life on our planet and the interactions between its cryosphere, biosphere, and atmosphere. Observations, however, have long been restricted by 2D analyses and the destructive sample preparation required for 2D analytical techniques, as well as an inability to directly observe textural or mineralogical evolution. Maintaining 3D and time-dependent contexts of microstructures is vital to fully understand the dynamic formation and efficient exploitation of earth materials, both as recorders of earth processes and to support environmentally sustainable innovation.

Methods
Minerals are defined by both composition and crystallographic structure. X-ray absorption contrast tomography (ACT) is ideally suited to derive information on porosity and to characterize boundaries between higher- and lower-density phases. However, many minerals have similar X-ray attenuation, and some mineral systems have little or no variation in mineral type (e.g., ice), leading to minimal image contrast. Complimentary to ACT, lab-based diffraction contrast tomography (DCT) produces 3D maps of the grain morphologies and crystallographic orientations for the different constituent phases. ACT and DCT, both non-destructive in nature, can be performed on the same micro-CT system, and combining the two can provide earth and planetary scientists with unprecedented new knowledge – in 3D – while keeping the often rare or precious samples intact.

Results
We present different examples of combined ACT and DCT studies on earth and space materials to illustrate some of the current imaging capabilities. Results from an olivine chondrule inside a carbonaceous chondrite, a type of meteorite that can host organic compounds thought to contain the ingredients for life, reveal previously unobserved relationships between grain shape, size, composition, and chemical zoning that inform models of melting during short heating events in the proto-planetary disc. Ice is also explored with the goal of characterizing the 3D microstructural and porosity evolution of glacial ice, which contextualizes chemical and isotopic measurements used to extract climate records from ice cores.

Conclusion
Together, quantitative ACT and DCT can be combined to create 3D petrography: a non-destructive method of identifying and characterizing multiphase materials (rocks, alloys, etc.) without a priori knowledge. As a step towards that goal, this work is developing the utilization of joint ACT and DCT in addressing different challenges in geoscience.

Keywords:

Lab-based DCT, absorption contrast tomography

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Effect of electron microscopy sample preparation protocol on the preservation of liposomes in cell culture

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Poster Group 1

Background: Liposomes are powerful vehicles for the delivery of a broad range of drugs and other compounds to specific locations within the human body. This has made them a versatile, and often used, nanoformulation within the pharmaceutical industry. Accurately localizing these liposomes within cells and tissue is vital to understanding the uptake mechanisms and ultimately the efficacy of the liposomes. Electron microscopy is the ideal tool to localize liposomes within cells and tissue, however there are numerous challenges that must be addressed. 1st, Liposomes exhibit a pronounced sensitivity to their surroundings, and the harsh conditions often employed to prepare cells for electron microscopy may result in the dissolution of the liposome of interest. 2nd, Liposomes are composed of carbonaceous material much like the surrounding cellular matrix, making it difficult to distinguish. In this work we aimed to characterize the effect that sample preparation has on the preservation of liposomes within a cell culture sample.

Methods: 100 million CT26 colon carcinoma cells were incubated unloaded stealth liposomes for 3 hours to allow for uptake. The cells were then divided into 4 groups for further processing. Group 1 was processed using a standard chemical fixation, staining and dehydration protocol. The cells were placed in 4% paraformaldehyde, 2% glutaraldehyde in 0.1 M Na Cacodylate Buffer for 1 hour at room temperature. The cells were then pelleted, suspended in 10% gelatin, and stained with 1% osmium tetroxide in water at 4 C for 1 hour. The cells were then stained with 1% uranyl acetate in water overnight before being progressively dehydrated in ethanol and propylene oxide. After dehydration, the cells were embedded in EMBED 812 epoxy resin.

Groups 2-4 were all high pressure frozen using a Leica EM-ICE and underwent 3 different freeze substitution protocols using the Leica AFS2. Group 2 underwent a standard freeze substitution protocol in a 1% osmium tetroxide in acetone solution over the course of 3 days. This was followed by room temperature staining in 1% uranyl acetate for 1 hour before embedding in EMBED 812 Epoxy resin. Group 3 underwent a quick freeze substitution protocol over the course of 4 hours in a 1% Osmium tetroxide, 1% uranyl acetate in acetone solution before being embedded in EMBED 812 Epoxy resin. Group 4 underwent freeze substitution with a 1% uranyl acetate solution and low temperature embedding in Lowicryl HM20 at -50 C.

Ultrathin sections from each sample were prepared and imaged using a Tecnai T12 at DTU Nanolab.

Results: All 4 samples were successfully prepared into 100 nm sections for imaging. Liposomal structures were observed for all 4 samples with the samples undergoing freeze substitution exhibiting qualitatively the best-preserved liposomes. Imaging is ongoing to obtain enough data to try and definitively state which freeze substitution method will result in the best preservation.

Conclusions: Freeze substitution appears to provide better preservation of liposomes in cell culture. Additional characterization is ongoing and necessary to determine which freeze substitution method provides the best results for preserving liposomes. Even with improved preservation of the liposomes, it is still very often difficult to ensure that the object being imaged is a liposome and not some other vesicular body within the cell.

Keywords:

sample preparation, TEM, Liposomes

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Thermal vibrations in inverse dynamical electron scattering

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IM-03 (4), Plenary, august 29, 2024, 14:00 - 15:00

Background incl. aims
Inverse multislice (IM) schemes have enabled structure retrieval in the presence of dynamical scattering, extending the applicability of electron ptychography assuming single scattering. This allows structure determination for thick specimens with super-resolution, to date only limited by the thermal atomic vibrations [1]. These thermal vibrations cause thermal diffuse scattering (TDS) which becomes partly dominant at elevated specimen thickness. Since the TDS governs the darkfield in diffraction patterns (DP) which contains crucial information about the atomic structure and chemical composition, its correct consideration in IM is a prerequisite for accurate specimen retrieval. Because various IM approaches evolved, differing by their implicit or explicit consideration of TDS, studying the impact on specimen structure reconstructions suggests itself. For this purpose different slicing concepts are studied as to the effect of TDS on the reconstruction and on the capabilities to yield atomic types and thermal mean square displacements, thus local temperature, quantitatively [2].
Methods
The multislice approach calculates momentum-resolved STEM data by propagating an electron wave through a set of slice transmission functions (STF), mathematically performed by iteratively multiplying the wave function with one STF and then propagating it to the next slice, e.g., formulated as an artificial neural network [3]. The inverse model then applies a loss function to the calculated DPs and those of the reference data set and minimizes the loss by optimizing the STF to reconstruct the specimen structure. To this end the home-built, gradient-based TorchSlice program utilizing the PyTorch framework was used [4]. We discuss the influence of TDS on three different optimization models performing reconstructions of simulated momentum-resolved STEM data for an SrTiO3 crystal as the ground truth. To incorporate TDS in the simulation we used the frozen phonon (FP) model which incoherently averages multiple diffraction patterns calculated from different phonon ensembles. First we optimize one slice transmission function pixel-wise which is reused to describe the interaction in each slice, precluding any ensemble averages thus being incapable to incorporate TDS correctly. Second, 50 slices are optimized pixel-wise and individually, providing the opportunity of incorporating TDS by allowing displacements of atomic potential maxima from their equilibrium position from slice to slice [5]. Third, a parameterized approach [4] is used which constrains the STF to be a sum of atomic potential functions, and a set of specimen parameters characterizing the STF is optimized every iteration. This model incorporates a full FP simulation ensuring physically correct inclusion of TDS within the FP approximation using the Einstein model.
Results
Optimizing wrapped slices pixel-wise the STF's phase grating (PG) shows stripe-like artefacts superimposed to the atomic structure (Fig. 1a), not being physically reasonable but reproducing the diffuse intensity in the corresponding DPs (Fig. 1b). It is shown that the strength of the artefacts and the cutoff angle up to which the loss is calculated are directly correlated. Restricting the solid angle such that TDS is excluded from the loss calculation, or using different regularization methods was found to successfully suppress the artefacts in the PG, however, suppressing the diffuse intensity in the DPs, too (Fig. 2). Optimizing each STF individually, the diffuse intensity was reconstructed very

accurately (Fig. 3a). The phase maxima at the atomic sites are displaced from their equilibrium position, following a slightly narrowed Gaussian function (Fig. 3b) having, however, a standard deviation of only 28% and 12% of the theoretical values for the Sr and the O columns, respectively. Still showing artefacts in the PG, the diffuse scattering within this model is produced via both, physically reasonable but too small thermal displacements and artificial phase noise to achieve numerical consistency with the ground truth. The parameterized optimization approach achieved the smallest DP error compared to the other optimization methods, and could retrieve the atomic phases very accurately which has been observed case-dependent for pixel-wise approaches. Also the mean thermal displacement could be received with an accuracy of at least 89%. The accuracy of both parameters is shown to increase with increasing number of phonon ensembles underlining the importance of incorporating TDS correctly. A final simulation study examining the sensitivity of the loss to the atom type and the mean thermal displacements clarifies the observed accuracy of these parameters and is used to outline the capability of IM to map the local chemistry by including TDS-affected Z-contrast in the inverse FP multislice model.

Conclusions

Different IM slice optimization concepts show a varying accuracy of reconstructed parameters among the models i.e. maximum atomic phases and mean thermal displacement and, when explicitly precluding thermal displacements from the model, the occurrence of physical unreasonable phase artefacts. We show various ways for suppressing these artefacts to achieve a smooth PG. In particular, we also demonstrate that for retrieving quantitative information on temperature and chemical composition, including TDS in IM within the FP approach is essential.

Keywords:

STEM, Inverse Multislice, Thermal Vibrations

Reference:

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Characterization of Solid Electrolyte-free Si anodes for all-solid-state batteries (ASSBs) using Cryo-STEM

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Poster Group 1

Background:

Solid-state lithium-ion batteries (ASSBs) hold great promise for delivering higher energy density and safer performance than conventional lithium-ion batteries. Lithium metal is a highly studied choice for the anode due to its high energy potential. However, its use in ASSBs is hindered by the issue of dendrite formation and growth. Conversely, silicon offers a capacity that is tenfold greater than that of graphite, presenting itself as a cost-effective and promising substitute for lithium metal.[1] Unfortunately, the potential of Si as an active anode material hasn't been fully tapped in ASSBs. Recently, solid electrolyte (SE) free Si anodes have been found to have improved performance compared to their SE-containing counterparts;[2] however, there is still considerable room for improvement. One of the ways to enhance these electrodes is to have detailed insights into the microstructure concerning various electrochemical treatments.

Methods:

Si particles (mixed with 0.5 wt % of polyvinylidene fluoride binder) are first pressed to form a pellet. This electrode is cycled against an In/InLi counter electrode using LPSCl as an SE. We utilize cryo aberration-corrected scanning transmission electron microscopy (AC-STEM) to characterize the microstructure of SE-free Si anodes after the 1st cycle. Since ASSB samples are air and moisture-sensitive, the cells are dissembled in an Ar-filled glove box, and the Si electrodes are retrieved. The sample is transferred from the glove box to a plasma FIB (PFIB) in an argon atmosphere. It is prepared at approximately -190 °C using an Ar-ion beam from the Helios 5 Hydra CX PFIB to thin and polish the specimen. Afterward, the samples are moved back into the argon atmosphere of the glovebox. The sample is then mounted onto a double-tilt Atmos Defend Holder from Melbuild within the glove box. This holder is capable of transporting the sample from the glovebox to the S/TEM under an argon atmosphere, reaching temperatures of about -170 °C during measurements. At the S/TEM, the samples are measured at roughly -170 °C employing multiple techniques, such as HR-S/TEM, energy dispersive X-rays spectroscopy (EDXS), electron energy-loss spectroscopy (EELS), four-dimensional STEM nano-beam diffraction and precession electron diffraction (4D STEM NBD/PED), etc., under cryogenic conditions.

Results:

Figure 1(a) is a STEM high-angle annular dark field (HAADF) image of pristine Si particles. The cloudy contrast on the edges of the particles shows SiOx particles (confirmed vis EDXS, not shown here).

Figure 1(b) is a cryo-STEM HAADF image of the bulk electrode after the 1st cycle. A massive microstructure transformation is observed with Si particles showing an amorphous structure (confirmed by PED, not shown here). Figure 1(c) is a cryo-STEM HAADF image of the region marked in Figure 1(b), showing a unique texture of the Si amorphous structure, commonly found after 1st cycling. A cryo-STEM HAADF tomography has also been performed in this region, showing a sheet-like structure (not shown here). Figure 2 shows Li and Si cryo-STEM EELS maps of a selected region after the 1st cycle. SiOx consumes a considerable amount of irreversible Li at the surface of the Si particles.

Conclusion:

Though the detailed analysis of the samples after subsequent electrochemical treatments is still underway, the drastic evolution of the Si electrode structure after 1st cycle is critical to finding the lithiation behavior of novel SE-free Si electrodes for ASSBs.

Keywords:

Si-anode, STEM, Cryo, EELS, HAADF

Reference:

References:

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Direct observation of the interplay between stacking polytypes and self-intercalation in epitaxial Nb1+xSe2 films

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Poster Group 2

Background
Stacking and intercalation are crucial for modifying and engineering the properties of two-dimensional (2D) van der Waals (vdW) materials [1, 2]. Stacking, as a control parameter, involves varying the lateral registry or twist angle between adjacent layers to realize, for example, exotic electronic phases in a moiré superlattice [3]. Intercalation refers to the insertion of atoms or molecules into the vdW gap between two layers [4]. This can be achieved by synthetic routes or electrochemistry, potentially leading to emergent superconducting or magnetic states. While the individual potentials of stacking and intercalation have yet to be fully explored, a natural progression is to investigate the synergistic combinations of these two tuning parameters. Understanding the interplay between stacking and self-intercalation is therefore important. However, current knowledge of the interplay between stacking polytypes and intercalation is often based on macroscopically averaged probes, which fail to accurately identify the exact atomic position and chemical state of the intercalants in real space. Atomic-scale observation of the cross section of vdW materials is essential for further clarification.

Methods
In this work, utilizing atomic-resolution electron energy-loss spectroscopy (EELS) in a scanning transmission electron microscope, we systematically investigate the atomic and electronic structures and local chemistry in epitaxial transition-metal dichalcogenide Nb1+xSe2 films grown by "hybrid" pulsed laser deposition and Nb1+xSe2 crystals grown via chemical vapor transport techniques. Density functional theory calculations were performed to evaluate the roles of thermodynamics and kinetics in these observations.

Results
Using high spatial resolution scanning transmission electron microscopy (STEM), we observe that thin films with an average x ~ 0.29 comprise a nanoscale phase mixture of NbSe2 layers stacked with both 180° and 0° in-plane rotations. The 180°-stacked layers exhibit significant self-intercalation with Nb at the octahedral interstitial sites, likely reaching several tens of percent occupancy, whereas the 0°-stacked layers contain few detectable intercalants at their octahedral interstitial sites. Our findings extend beyond merely imaging intercalants or different stacking structures to establishing a correlative relationship between the two. Density functional theory (DFT) confirms that the energetically favored stacking orientation transitions from 0° to 180° when the self-intercalation exceeds a threshold of approximately x ~ 0.25. However, achieving this threshold appears to necessitate kinetic pathways distinct from those in the thin films. Efforts to replicate these

observations with Nb1+xSe2 crystals grown via chemical vapor transport (CVT) yield a homogeneous phase of intercalated, 0°-stacked layers with an average x of at most ~0.20. The presence of Nb intercalants reduces the size of the Fermi hole pockets and suppresses superconductivity in NbSe2, and these altered properties may provide a foundation for fabricating junctions and nanostructures with more precise control over stacking and self-intercalation.

Conclusion
Using STEM and EELS, we have directly visualized a stacking-selective self-intercalation phenomenon in epitaxial Nb1+xSe2 films. Our results offer not only renewed mechanistic insights into stacking and intercalation, but also open up prospects for engineering the functionality of TMDCs via stacking-selective self-intercalation. [5]

Fig. 1 stacking-selective self-intercalation phenomenon in epitaxial Nb1+xSe2 films

Keywords:

STEM, NbSe2, staking polytypes, self-intercalation

Reference:

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Atomic Imaging of Lattice and Electron Ordering in Tensile-Strained LaCoO3 Films

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Background
Strong multiple interactions between degrees of freedom (spin, orbital, charge, and lattice) in complex oxides give rise to rich electronic phase diagrams and thus to intriguing macroscopic functionalities, such as ferromagnetism, superconductivity, and multiferroicity [1, 2]. A famous example is LaCoO3 (LCO), in which unexpected ferromagnetism occurs in tensile-strained epitaxial LCO thin films, in contrast to paramagnetism in bulk LCO [3]. However, the underlying mechanism of emergent ferromagnetism and spin-state transitions remains controversial, with the tensile strain-induced ferroelastic deformation and oxygen vacancy ordering are known as two possible driving forces. Previous density functional theory (DFT) calculations on the ferromagnetism of tensile-strained LCO have shown that the tensile strain-induced changes in lattice constants are insufficient to stabilize the long-range ferromagnetic order [4]. Suppression of CoO6 octahedral rotations should be considered to modify the eg orbital order configuration and induce a spin-state transition to a ferromagnetic state. To date, no direct experimental evidence has been reported to support this theoretical prediction.

Methods
In this work, we systematically investigate the atomic and electronic structures, and their correlation with the spin-state transition and the ferromagnetic insulating state of high-quality LCO epitaxial films grown by pulsed laser deposition.

Results
Scanning transmission electron microscopy (STEM) and electron energy-loss spectroscopy (EELS) were used to examine the 6 uc and 25 uc thick LCO films for comparison. High-angle annular dark-field STEM results support the appearance of dark stripes only in the 25-uc thick LCO film, showing well-ordered dark stripes with a period of ~ 3 uc in the interior part of the film (Fig. 1). EELS maps of the interface region show a sharp interface between LCO and STO without the formation of any misfit dislocations. Energy-loss near-edge structure analysis was performed to investigate the electronic structures of the LCO films at the atomic scale. In the dark stripes, the pre-peak of the O-K edge almost disappears, and the intensity of the O-K edge peaks is significantly reduced. Meanwhile, the peak positions of the Co-L2,3 edges shift to lower energies, and the Co-L3/L2 ratio is larger in the dark stripes than in the bright regions. These features fingerprint a reduction of the Co valence state in dark stripes due to the formation of oxygen vacancies. Quantitative analysis of annular bright-field STEM results reveals previously unreported long-range suppression of CoO6 octahedral rotations throughout 25-uc thick LCO epitaxial films, inducing an increase in the angle βCo-O-Co from bulk 163.5o to 172.5o. DFT calculations further unravel the underlying physical mechanism of the spin-state transition to induce an emergent and robust ferromagnetic insulating state.

Conclusion

This work provides new insights into how the interplay between degrees of freedom drives the ferromagnetic insulating state in tensile-strained ferroelastic LCO epitaxial films [5].

Fig. 1 STEM images of the LCO films with thicknesses of 6 uc and 25 uc, respectively.

Keywords:

EELS, STEM, LaCoO3, octahedral rotation

Reference:

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Insights into the Structural Dynamics of Cu@Ag Core-Shell Nanoparticles during CO2 Reduction

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Poster Group 2

Electrochemical CO2 reduction reaction (eCO2RR) is one of the most promising and sustainable approaches to CO2 conversion to reduce atmospheric CO2 concentrations and mitigate the impacts of climate change [1]. For this purpose, the use of metallic nanoparticles (NPs) as electrocatalysis is on the rise, with Cu/Ag bimetallic interfaces being particularly popular for enhancing the eCO2RR towards CO and C2 products [2]. However, the electrochemical performance and product distribution of Cu@Ag core-shell configurations can change drastically under reaction conditions, particularly during the first few minutes of the reaction. In this study, our goal is to reveal the underlying structural changes these NPs experience at the critical first steps of the eCO2RR to understand their structure-properties relationship.

In this study, we utilized EDS analysis and high-resolution electron tomography to characterize the evolution of the Cu@Ag core-shell NP structure. The electrochemical measurements were performed in a homemade two-compartment, three-electrode cell, where the TEM grid was used as the working electrode, allowing for direct observation of the NPs before and immediately after the crucial first minutes of the reaction.

The Cu@Ag core-shell NPs used were found to be oblate and not homogeneously covered by Ag, with atomic-sized pinholes that expose the Cu core. Based on 3D characterization of various nanostructures found after current application, we hypothesized a transformation pathway. We observed an enlargement of these pinholes, most likely caused by the catalytic activity of the Ag shell, resulting in Cu leaching and, subsequently, a complete structural transformation of the NPs. Remarkably, the transformed Cu-Ag core-shell structure almost doubles the production of CO, presumably due to the combined effect of the different structures at various stages of transformation, as these transformations do not occur simultaneously [3].

Through the combined use of electrochemistry and advanced electron microscopy techniques, we have gained a better understanding of the relationship between the structure and electrochemical properties of Cu@Ag core-shell NPs, and how this affects their electrocatalytic performance. This research enhances our knowledge of Cu@Ag core-shell configurations and provides insights into other largely immiscible metal combinations. The study emphasizes the significance of the crystallinity of the central metal nanoparticle in deciding the formation of a complete and uniform second metal shell, which will be the key parameter towards nanoparticle stability.

Keywords:

Cu@Ag core-shell, CO2 reduction, Tomography

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A Robust Toolkit to Correlate High Dimensional Multimodal Microscopy

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Background
Spatially correlated multimodal microscopy allows an experimentalist to gain a holistic understanding of their sample by compiling complementary information from separate imaging modalities. This however relies on the robust registration of images between techniques- a difficult task with differences in the contrast mechanism, resolution and presence of artefacts all posing unique challenges. Furthermore, these problems are exacerbated by state-of-the-art instruments which produce high dimensional datasets (often upwards of hundreds of gigabytes), making the nuanced evaluation of scientific hypotheses challenging. Herein we present a general approach to coregister and interpret spatially correlated scanning electron diffraction (SED) and hyperspectral photoluminescence (PL) measurements performed on hybrid perovskites, an emerging class of semiconductor used as active layers in solar cells, LEDs and radiation detectors. SED, a variant of 4D-STEM, allows for information on crystallographic phase, orientation, and defects to be uncovered, whereas hyperspectral PL provides information on optoelectronic characteristics and performance. Given the fact that hybrid perovskites show striking spatial heterogeneity in both structure and performance, the spatial correlation of crystallographic information and PL is motivated by the prospect of unequivocally linking structure-property relationships in these materials.

Methods and Results
To find common areas between separate microscopes, gold fiducial markers are synthesized and deposited onto a thin film similarly to the work of Jones, Osherov and Alsari et al. [1]. Due to the resolution differences between SED (typical probe size ~5 nm) and hyperspectral PL (resolution diffraction limited to ~100s of nm) we record multiple contiguous SED scans before they are stitched together during post processing. To stitch the SED scans together virtual bright or dark field images are formed which act as a proxy for the 4D dataset. A keypoint detection and matching algorithm is then applied between the images; classical choices for this include the scale invariant feature transform (SIFT) or binary robust invariant scalable keypoints (BRISK) algorithms, commonly implemented using OpenCV [2]. Although these are undoubtably valuable tools, it has been empirically found that pretrained neural network-based approaches, such as Key-Net-AdaLAM implemented using Kornia, provide improved mappings [3]. Once the keypoints are detected, the random sample consensus algorithm (RANSAC), or variants thereof, is used to define an affine transform which stitches one image onto the other while capturing differences in rotation, shear, and translation [2,3]. To make these tools accessible a Python based GUI has been developed.

The registration of hyperspectral PL and stitched SED datasets is now considered. Due to the differences in pixel size between the two techniques the hyperspectral PL is upsampled via linear

interpolation to match the SED pixel size. To coregister the resultant datasets several options are available. 1) An image transform can be defined by finding where the normalized cross correlation is maximal between the two images. Due to the differences in contrast mechanism a mask can be applied such that only the Au fiducial marker is considered during this process. 2) The Python package AntsPy, primarily built for the registration of medical images can be utilized [4]. AntsPy uses 'multi-resolution gradient descent' and metrics such as cross correlation or mutual information to define the mapping. Importantly, as all image transforms are known we can then calculate where each 'pixel' from a SED scan corresponds to in the hyperspectral PL data.

Once the two datasets are coregistered the interpretation of any interrelationships between them poses a further separate challenge. Merely considering the SED data the scale of the problem becomes apparent— if 50 SED scans are acquired, each of which contains 512 x 512 diffraction patterns (typical during one experimental session), this amounts to a total of 13,107,200 individual patterns. To rapidly reduce data dimensionality, we have adapted the simple linear iterative clustering (SLIC) algorithm prevalent in the field of remote sensing and applied this to the SED data [5]. This approach allows us to reduce hundreds of thousands of individual diffraction patterns to ~100 single crystal patterns obtained by averaging diffraction patterns over individual grains of the polycrystalline halide perovskite film. SLIC can be thought of as a variant to K-means clustering and provides a general, intuitive, robust, and computationally inexpensive methodology to cluster SED data. Key principles underlying the algorithm are to remove points on your detector which have a low dynamic range or variance prior to clustering such that burdensome computation is only performed on a reduced subsection of the data. Additionally, cluster centroids only consider data in their close vicinity meaning unnecessary computation is further reduced. The spatial and channel Euclidean distances are combined into a single distance measure, with a weighting value being used to define the importance between them. This encodes intuition that 'pixels' close together likely belong to the same cluster. Finally, clusters which are highly correlated are combined before being used to calculate the mean diffraction patterns from the original data. Remarkably this approach proves exceptionally computationally efficient with typical compute times taking approximately a minute using a standard desktop machine (32Gb RAM, 11th Gen Intel(R) Core(TM) i5-11400 CPU). An automated indexing procedure of the clustered SED patterns can then be employed to obtain phase and orientation maps at low computational cost.

Conclusion

The toolkit introduced herein can elucidate structure-property relationships in state-of-the-art optoelectronic materials and provides a robust framework to perform correlative microscopy in related fields.

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Keywords:

Correlative Microscopy, 4D-STEM, Energy Materials

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Molecular Microscopy by Thermo-Fisher-Scientific: FTIR-Imaging of a Tintoretto-Fresco and Raman-Imaging of the Strain-Distribution in Semiconductors

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Poster Group 1

Background incl. aims
Molecular spectroscopy tools are employed to investigate the vibrational properties of a wide variety of samples. The chemical composition and physical properties of materials are stored in the molecular bonds and can be read from the vibrational spectrum. Fourier transform infrared (FTIR) spectroscopy has long been utilized to investigate materials present in artwork. This study of Tintoretto's work at the Scuola Grande di San Rocco (Venice, Italy) aims to understand the composition of its pictorial layers. The second part of this talk is dedicated to the influence of a buried stressor on the strain-dependent site control of InGaAs quantum dots. Raman spectroscopy is highly sensitive to subtle changes in the crystal lattice structure. Under strain, its molecular bonds shift slightly, altering the vibrational frequencies of the lattice. The resulting shifts in Raman peaks contain information about the amount and type of strain present.

Methods
The cross-section of the Tintoretto painting sample was embedded in resin to assess the five layers. The sample was analyzed in several regions using a Thermo Scientific Nicolet RaptIR FTIR microscope in corroboration with scanning electron microscopy with energy-dispersive x-ray spectroscopy. All mappings were performed in ATR mode with a Germanium micro-ATR. The quantum dot sample was analyzed with a Thermo Fisher Scientific DXR3xi Raman imaging microscope and a 532 nm laser. The results are correlated with atomic force microscopy assessments of the sample height profile.

Results
The cross-section of the painting revealed the existence of a lipidic preparation layer and finishing layer. The preparation layer contains traces of proteins, pointing to the use of animal glue. The sandwiched layers contain different pigments and filler materials. The study confirmed assumptions and knowledge on the use of materials and techniques in the 16th century. In contrast, semiconductor nanostructures are state-of-the-art technology of the 21st century. InGaAs quantum dots grow at sites exhibiting a considerably larger strain than their surrounding. Tuning of the buried stressor size results in the precise control of the distribution, emission, and density of the quantum dots. The overlay of Raman strain mappings with atomic force microscopy height profiles allows for a deeper understanding of the physical properties. In combination with computational simulations, the results forecast the design of new devices with distinct emission patterns.

Conclusion
All materials that were expected to be found in the paint sample were successfully identified using the Nicolet RaptIR FTIR Microscope, combined with the multicomponent search function of OMNIC Paradigm Software. Besides the chemical composition, Raman spectroscopy is powerful in assessing the physical properties such as the strain in semiconductors. The power and beauty of these

spectroscopic techniques are pivotal in the creation, conservation, consolidation, and understanding of materials across all scientific fields.

Graphics
(left) Cross section of a Tintoretto painting, highlighting the five layers.
(center) Tintoretto fresco at the Scuola Grande die San Rocco in Venice, Italy.
(right) 3D recreation of a quantum dot sample from Raman imaging. False color image of the vibrational frequency translating into the amount of strain in the material.

Keywords:

FTIR, Raman, Semiconductor, Material-Identification, Composition

Reference:

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Assessing the Precision of Local Temperature Measurement by Plasmon Energy in In-Situ Heating Electron Microscopy

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background incl. aims
In-situ transmission electron microscopy (TEM) has become increasingly important in the characterization of microstructures in functional and structural materials when real-time observation under application conditions is required to reveal underlying structure-property relationships [1]. With the advancement of microelectromechanical systems (MEMS) technology for the application of stimuli on the materials, the high sample stability and precise control of the stimuli, especially in elevating temperature with a local accuracy within 4% [2], enable atomic-scale resolution in such in situ TEM experiments. However, uncertainty remains regarding the exact temperature profile across a TEM sample itself, which limits the interpretation of observed structural phenomena in in-situ TEM heating experiments.
Methods
Here, we exploited plasmon energy expansion thermometry (PEET), detecting temperature-dependence of the bulk plasmon peak in an energy electron loss spectrum (EELS) [3] and testing its validity and accuracy using tungsten (W). The reasons to assign W as the model material come from the high melting temperature which is above 3000 °C and the sharp plasmon resonance in EELS, as shown in figure(a). These advantages benefit PEET through improved precise EELS peak determination over a wide implementation temperature range.
For sample preparation, W lamellas with varying sample thickness of 30 to 70 nm were prepared using a ThermoFischer Hydra Plasma FIB (Xe plasma at 30 kV) and were mounted on a DENS Wildfire heating chip [2] using W-gas assisted deposition. Two types of FIB-cut W lamellas were prepared: One is placed at the center of the spiral heater where we expect a homogeneous temperature distribution, as shown in figure(b). The other one is placed at an off-central position where we expect a thermal gradient [4].
Ex-situ temperature measurements were conducted using Raman spectroscopy with a 532 nm laser of beam size less than 1 µm. Si particles of 45 nm diameter were drop-casted on the heating chips. Exploiting the temperature dependence of the bonding vibration, the local temperature can be obtained by the Raman frequency of the Si-Si bond, which shifts with temperature at approximately 0.0232 cm-1 per °C.
For the in-situ heating experiments, both a ThermoFischer Themis (at EMAT) and Spectra (at DTU) S/TEM equipped with X-FEG mono have been operated at an accelerating voltage of 300 kV. For our PEET experiments, the monochromator was excited to achieve an energy resolution of 0.12eV. The effect of the STEM convergence semi-angle (3.6 to 18 mrad) as well as of the EELS collection semi-angle (6 to 70 mrad) was investigated in the PEET mapping experiments. The bulk plasmon peak was collected using Quantum 966 Gatan Image Filter with a dispersion of 0.01 eV per pixel and dwell time of 0.1 second per pixel.
Results
PEET mapping on the W lamella at the center of the spiral heater (figure(a)) reveals a temperature-dependent plasmon energy shift of 1.8 eV as the temperature progressively increases from room

temperature (figure(c)) to 1100 °C (figure(d)). Correspondingly, the standard deviation when measuring at multiple positions also considerably increases, from 0.027 eV at room temperature (figure(c)) to 0.471 eV at 1100 °C (figure(d)), respectively. To understand this variation in plasmon peak energy, we attempt to correlate the PEET mapping and the local estimated sample thickness (using the log-ratio method in EELS) and find a noticeable correlation as one factor responsible for this large deviation.
Leveraging these findings as calibration data, we further extended the application of PEET for detecting the temperature difference over space. The profound temperature gradient of the off-central W lamella was predicted by COMSOL simulation and verified by ex-situ Raman measurements. The results show that the thermal gradient can reach approximately 106 °C /m (from 667 to 772 °C within a 10 µm length) at a set temperature of 1000 °C. Interestingly, on the PEET map of the off-central W lamella at the same set temperature of 1000 °C, the plasmon energy shifts to higher values as the position moves farther away from the spiral center. By using the calibration data for correlating the plasmon energy to the local temperature, the data from the PEET map closely matched the thermal gradient observed in the ex-situ Raman measurement and simulation results.
Conclusion
Our study demonstrates that PEET is capable of obtaining quantitative temperature measurement results with the consideration of the sample thickness. Moreover, it effectively identifies pronounced thermal gradients within the sample. The improvement of the temperature determination in PEET provides a more reliable analysis of structure-properties correlation under various thermal conditions.

Keywords:

plasmon energy expansion thermometry

Reference:

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Enabling discovery by in-cell structural biology

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

Most structural biology focuses on the structure and function of individual macromolecular complexes, but falls short of revealing how they come together to give rise to cellular functions. Here, cryo-electron tomography (cryo-ET) provides a unique opportunity for obtaining structural information across a wide range of spatial scales - from intact cells, 3D cultures, tissues and model organizms frozen in their close-to-native state, to individual macromolecular assemblies embedded in their native functional environments. We develop and employ advanced sample preparation techniques for in-cell cryo-ET, including cryo-focused ion beam thinning guided by 3D correlative fluorescence microscopy. Preparations of such site-specific ‘electron-transparent windows’ in appropriate cellular model systems visualizes molecular structures directly from three-dimensional stills of intact cells and can reveal their molecular sociology. Using the genome-reduced human pathogen Mycoplasma pneumoniae as a minimal cell model, we further demonstrated the synergistic application of whole-cell crosslinking mass spectrometry and cryo-ET to determine an in-cell integrative structural model of actively transcribing RNA polymerases coupled to translating ribosomes. Recent computational breakthroughs now allow resolving these molecular machines to near-atomic resolution directly inside the cell, reveal small molecule antibiotics bound to their active site in ribosomes within the intact pathogen, and provide snapshots of their structural dynamics along reaction cycles. We will discuss novel technologies that extend these applications and depth of information to mammalian cells and tissues. These cutting-edge methodologies unlock an enormous potential for system-spanning discovery enabled by label-free in-cell structural biology.

Keywords:

cryo-FIB, cryo-CLEM, cryo-ET, integrative modeling

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Low-Dose 4D-STEM Investigations of the Octahedral Network Structure in Formamidinium Lead Bromide Nanocrystals

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

Background incl. aims
Hybrid organic-inorganic metal halide perovskites have shown great potential as semiconductors for new optoelectronic devices, such as photovoltaics, light-emitting diodes, and X-ray detectors. Their outstanding optoelectronic properties, including a tunable bandgap, high charge carrier mobility, high photoluminescence quantum yield, and low recombination rate of photogenerated carriers, have contributed to their rapid development.(Dey et al. 2021) The structure of metal halide perovskites (MHP) is described by the chemical formula ABX3, where A is a monovalent organic cation (e.g. formamidinium (FA+) [CH(NH2)2+] or methylammonium (MA+) [CH3NH3+]) or a metal cation (e.g. Cs+), B is a divalent metal cation (typically Pb2+or Sn2+), and X is a halide anion (X = Cl-, Br-, I-). The perovskite structure consists of [BX6]4-octahedra that are connected through the corner halides. The octahedral network structure is closely linked with the polymorphic nature of MHPs, which is of great importance since phase instability is still one of the major roadblocks for long-term applications of MHPs. Only the “black” phase demonstrates photoactive properties, which dramatically decrease once the MHPs convert to the “yellow” phase. From the high-symmetry cubic phase, the transition to the lower symmetry phases can be described by rotation or shearing of the octahedra. It is crucial to understand the role of the octahedral network to unravel the structure-property connection and correlate it to transformations during degradation. Transmission electron microscopy (TEM) is an excellent method for investigating the local structure of nanocrystals (NCs), even down to the atomic level. Nevertheless, when applied to MHPs, their sensitivity to the electron beam is highly challenging. Irradiation with the electron beam easily causes degradation of the MHP NCs with PbX2 and Pb as resulting products.
Methods
In this study we utilize four dimensional scanning transmission electron microscopy (4D-STEM) to obtain phase contrast image reconstructions to investigate the local structure of colloidal FAPbBr3 NCs, which were synthesized following a hot injection method. Phase contrast imaging is beneficial for materials in which both heavy and light elements are simultaneously present, such as organic cations and lead in perovskites. Moreover, 4D-STEM is superior with respect to information-richness and dose-efficiency as compared to high-angle annular dark-field (HAADF) STEM, where only electrons scattered to relatively high angles are used. The 4D-STEM datasets were acquired with a custom-made Timepix3 detector, which is an event driven hybrid pixelated direct electron detector. To retrieve the phase information from the recorded 4D datacube, we applied a recently developed convolutional neural network (CNN).(Friedrich et al. 2023) The CNN was trained based on a large

synthetic dataset, using atomic structures extracted from the materials project database. Moreover, to analyze the local structure we fitted the shape of the projected atomic columns. For this purpose, a parametric model was used which consists of a sum of two-dimensional elliptical Gaussians, each centered on the atomic column positions. Additionally, the experimental STEM results were compared with a series of molecular dynamics (MD) simulations at constant temperature and pressure, which were performed using a machine learning potential trained on underlying Density Functional Theory energies and forces.

Results

The high dose efficiency of the CNN reconstruction enables to study these beam-sensitive NCs with an electron dose that is sufficiently low to avoid the formation of Pb-rich clusters due to electron beam irradiation while maintaining a high signal-to-noise ratio. It is noteworthy that at a total electron dose below 50 e-/A2, all atomic columns, including the light formamidinium cations and Br anions can be clearly detected in the CNN reconstructions (see panel (a) in Figure 1). Moreover, a close inspection of the projected Br columns revealed deviations from perfect round projections. A possible reason are alternations in the position of the Br atoms along the viewing direction. The ellipticity ratio for the projected Br atomic columns was obtained by fitting elliptical Gaussians. In panel (b) the arrows illustrate the ellipticity ratio of the projected Br atomic columns and the overlay represents the different atomic columns ((Br = purple, Pb-Br = blue, FA = orange). This indicates that the Br atoms have a small flexibility in the octahedral network structure and can be displaced perpendicular to the Pb-Br-Pb bond, which is schematically illustrated by the red arrows in panel (c) in Figure 1. Furthermore, the performed MD simulations confirmed the displacement of the Br atomic columns. This observation is also in good agreement with previous synchrotron XRD measurements of FAPbBr3 NC, where the measured Pb-Br-Pb bond angles deviate up to 15 ° from the ideal 180 ° angle due to local disorder caused by displacement of Br anions.(Yazdani et al. 2023)

Conclusion

Thus, our approach via low-dose phase image reconstructions enables to study the local octahedral network structure of perovskites and could be transferred to in situ experiments in the future to investigate degradation mechanisms under environmental triggers.

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Keywords:

perovskite, 4D-STEM, phase contrast imaging

Reference:

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In-situ beam driven experiments by Electron time-correlation microscopy of amorphous structures

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Poster Group 2

Background & Aims
Amorphous materials are widely present and not straight-forward to study. However, similar to X-ray photon correlation spectroscopy (XPCS) the method of electron correlation microscopy (ECM) was introduced [1]. This approach using the time correlation of diffracted or dark-field intensities was applied at elevated temperatures. However, it was shown that this way of data analysis gives also proper materials parameters at room temperature (RT) [2]. Besides that, also pure materials made amorphous by ion implantation were recently studied [3]. To understand the influence of the beam and the measured signals, results from different materials are compared and discussed. In this work, the imaging and analysis conditions are examined to understand the arising quantitative signals, and to be able to compare ECM data obtained at elevated temperatures to that of electron time-correlation microscopy (EtCM) obtained at any temperature, even at RT where the thermal activation is small.

Methods
The analyzed materials are on the one hand metallic glasses as FeNiP or PdNiP, and on the other hand pure elements as Si or Ge. The metallic glasses were processed by rapid quenching techniques, and the pure elements were synthesized by ion implantation. EtCM is used to probe the dynamics and relaxation times from RT up to the glass transition temperature. The samples for the heating experiments were prepared using a focused-ion beam (FIB) and the lamellae were mounted onto a in-situ heating chip. Additionally, all samples were also structurally characterized using diffraction including fluctuation electron microscopy (FEM). Besides a basically disordered structure, also a preferred length-scale for ordering within the amorphous phase can be given. This number should typically not change during the EtCM experiments.

Results
The analysis of time series of dark-field images enables to measure the intensity variation over time, related to a structural relaxation time. The function of the intensity correlation can be given for every image pixel, thus also a relaxation time constant and a stretching exponent can be deduced. The measurement needs a certain amount of time and dose to achieve reliable numerical values. Different materials show different numbers, however, all materials show that the electron beam and its dose-rate or cumulative-dose are relevant for the achieved numerical values. The dose-rate can be even too high, to induce crystallization at RT. Thus, the dose-rate is measured and given for all experiments.

Conclusion
The imaging conditions during EtCM matter for the numerical values and further treatment of the result. Best results are obtained using low-dose settings and long measurement times. However, it must be noted that amorphous structures show relaxation times on basically all time scales, from

atomic jumps on the pico-second scale up to bulk relaxation in terms of (millions of) years. Experimentally, the maximum measurable time scale is less than half of the overall experimental time, and the minimal measurable time scale depends on the frame time for two frames. It can be concluded, that EtCM experiments at RT are beam driven relaxation studies, helpful to understand the intrinsic time scale for relaxation in any amorphous matter.

Keywords:

amorphous, metallic glass, in-situ, time-correlations

Reference:

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Magnetic behavior of steel studied by in-situ Lorentz microscopy, magnetic force microscopy and micromagnetic simulations

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Poster Group 1

Many industrially relevant steels are ferromagnetic such as ferritic-pearlitic steel characterized in this study. Ferritic-pearlitic steels are commonly used, for example, in automotive components. Microstructural features of ferromagnetic materials influence their mechanical and micromagnetic properties. Traditionally, the microstructure, for example, cementite (Fe_3C) content, has been studied with destructive methods. To save time and money, destructive methods should be replaced with non-destructive testing (NDT) techniques. One of the potential NDT methods is magnetic Barkhausen noise (MBN) testing. It is used in industry to detect for example localized microstructure and stress variations. MBN testing is based on the motion of magnetic domain walls in ferromagnetic materials exposed to a time-varying external magnetic field. The motion of domain walls is hindered by the microstructural pinning sites such as carbides, grain boundaries, and dislocations. In the varying magnetic field, domain walls stop and finally jump over the pinning features, causing discontinuous and abrupt changes in the magnetization of the workpiece. These changes result in electromagnetic burst-like signal, i.e., Barkhausen noise, measured with an inductive coil. However, the applicability of the Barkhausen noise method is currently limited due to the stochastic nature of the phenomenon itself. Thus, more scientific knowledge is needed. Recently, we mimicked and visualized the Barkhausen noise measurement by in-situ Lorentz microscopy. This study gives general information about the behavior of domain walls in ferromagnetic steel. In addition, we have utilized magnetic force microscopy and micromagnetic simulations to deepen our knowledge on the magnetic behavior of steels.

In this study, we used multi-instrumental and computational approach. Traditional microstructural characterization of ferritic-pearlitic steel was carried out by SEM-EBSD-TKD and (S)TEM-EDS. The magnetic structure of the thin sample was studied by Lorentz microscopy (Fresnel mode), while the bulk sample was studied by magnetic force microscopy (MFM). The dynamics of domain walls were studied by in-situ Lorentz microscopy. A varying, external magnetic field was generated by a normal objective lens of TEM, and the images were collected in LOW MAG mode using objective mini lens. The recorded frames of each sample were jointly post-processed as a single video using video denoising and frame alignment procedures. To measure a single point magnetic flux density generated with different excitation values of the normal objective lens inside the TEM, we used a custom-made holder equipped with a Hall-effect sensor. We also run micromagnetic simulations to verify domain wall dynamics in certain magnitudes of magnetic fields.

Our multi-instrumental characterization, dynamical in-situ Lorentz microscopy studies, and micromagnetic simulations with the complex ferritic-pearlitic structure revealed the interaction of different domain walls and pinning sites. Thus, we could visualize and verify hypotheses related to

the origin of Barkhausen noise signal. Comparing Lorentz microscopy and MFM results, we indicated that thin and bulk samples studied have similar magnetic structure. So, TEM studies are also relevant from the industrial point of view, although usually bulk samples are used in industrial applications. To measure the magnetic field strength generated by the normal objective lens of TEM in dynamical in-situ studies, we built a custom-made Hall-effect sensor holder. It measures the flux density at the same location as the TEM sample. Based on the measurements, the objective lens of our TEM has almost linear response to the magnetic field strength, and when the objective lens is switched off, the magnetic field in the sample area is close to 0 mT.

Based on our studies, the carbides are very strong pinning sites for domain walls. In addition, larger globular and thicker lamellar carbides can have their own magnetic structure. In the increasing magnetic field, domain walls in the ferritic matrix perpendicular to the lamellar cementite carbides begin to move first. Then, the domain walls inside the carbides start to disappear. Finally, domain walls parallel to the lamellar carbides move. However, some of them are very strongly pinned by carbides. When the magnetic field is decreased back to 0 mT, the domain walls appear in the opposite order. We simulated the magnetization dynamics where microstructural information is extracted from the SEM-TKD and (S)TEM results. To explain the domain wall behavior in certain magnitudes of the magnetic field as observed using in-situ Lorentz microscopy, we ran dynamical micromagnetic simulations to reproduce the domain wall disappearance in the globular carbide. In general, the simulations supported very well the interpretation of the experimental findings, although the re-appearance of the domain walls with decreasing field could not be reproduced. In the next step, our multi-instrumental and computational approach will be extended by transport of intensity equation (TIE) method and off-axis electron holography. These results will be reported in the near future.

Imaging methods and micromagnetic simulations have their own limitations. Despite using multi-imaging techniques, it is probable that not all magnetic features can be visualized by microscopes and on the other hand, simulations cannot always reproduce all events observed experimentally. However, our combined multi-instrumental and computational approach gives novel knowledge on how iron-based carbides affect magnetic domain wall dynamics in ferromagnetic steel.

Keywords:

Ferromagnetism, Lorentz microscopy, Micromagnetic simulations

Reference:

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Thapsigargin induces non-apoptotic programmed cell death in RBL-1 cells

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Poster Group 2

Background and aims:
Thapsigargin (TG) is a potent sarco/endoplasmic reticulum (ER) Ca2+-ATPase (SERCA) inhibitor and, accordingly, elevates intracellular Ca2+ levels. Recent studies demonstrate that TG induces cell death in a variety of cancer cells. While it is assumed that TG triggers cell death by apoptosis, our cytological studies on rat basophilic leukemia cells (RBL-1) indicate a non-apoptotic mode of cell death.
Methods:
Since the ultrastructural hallmarks of TG-induced cell death are only sparsely described, we studied the morphological consequences of TG exposure in RBL-1 cells using 2D transmission electron microscopy (TEM) and 3D TEM tomography in correlation with laser scanning microscopy (LSM). To visualize and quantify the effects on different organelles, live-cell fluorescence markers such as ER-tracker, HOECHST, endocytosis-tracker and MitoTracker were used.
Results:
TG-exposed RBL-1 cells showed prominent ballooning of the perinuclear space, vacuolization, increased vesicle formation, mitochondrial enlargement and degradation as well as ER- swelling and anomalies. In particular, the TEM data failed to show apoptotic hallmarks such as nuclear fragmentation or the formation of apoptotic bodies in TG-exposed RBL-1 cells.
Conclusion:
Contrary to the prevailing theory that TG triggers apoptosis, our results suggest a non-apoptotic programmed cell death. Moreover, numerous non-apoptotic morphological hallmarks were found, which are reminiscent of autosis and paraptosis. Thus, while TG represents a potential tumor therapeutic agent, the underlying mechanisms of cell death are still ambiguous and might differ in distinct cell types.

Keywords:

apoptosis, autosis, paraptosis, thapsigargin, cancer

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Multimodal control of the magneto-structural phase transition in FeRh studied by TEM

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Background incl. aims
The onset and properties of long-range ordering in solids come from a fine interplay between different order parameters. Materials featuring magneto-structural phase transitions constitute optimal systems for investigating the interplay of structural, electronic, and magnetic order parameters. The equiatomic FeRh alloy is a prototypical system undergoing a first-order magneto-structural phase transition from antiferromagnetic (AF) to ferromagnetic (FM) order upon heating (350 – 380 K), that is accompanied by a lattice parameter expansion [1]. Broad options for the tunability of the transition temperature via doping, strain, magnetic field, or controlled disorder make the system attractive for micro- and nanoscale spintronics [2], magnetocalorics, and sensors. The fullfilment of the application potential however requires the understanding of the phase coexistence, phase boundary properties, and transition dynamics at nanoscale. Here we demonstrate broad possibilities of controlling and sensing different ordering parameters of the FeRh phase transition using TEM.

Methods
TEM investigations are performed in free-standing FeRh thin fims which were epitaxially grown on MgO substrates via magnetron sputtering and subsequently released by chemically dissolving the MgO substrate. Different external stimuli are applied in-situ in TEM to drive the FeRh transition, namely, magnetic field, stage-induced static heating, or fs-laser-induced heating [3]. Other stimuli are applied ex-situ to locally tune the transition properties, such as ion irradiation. The induced structural and magnetic order modifications in FeRh are then explored using different TEM-based techniques, such as Lorentz TEM (LTEM), Transfer of Intensity (TIE) reconstruction of LTEM [4], Differential Phase Contrast (DPC), Electron Magnetic Circular Dichroism (EMCD) [5], or Selected Area Electron Diffraction (SAED).

Results
The studied free-standing FeRh film exhibits a well-defined phase transition as evidenced by the thermal evolution of the magnetic and structural ordering parameters that vary between AF and FM phases, as shown in Fig 1(a). TEM enables unprecedented nanoscale visualization of the evolution of the phase transition upon following magnetization via LTEM or lattice parameter from SAED. For instance, Fig 1(b) shows the temperature dependence of the FM phase domain content as measured via LTEM. Apart from stage-driven static heating, the AF-FM transition in FeRh can be driven by fs-laser light or locally by ion irradiation as can be seen in Fig 1(c) and 1(d), respectively. High-resolution vectorial magnetic reconstruction can be done either using TIE-LTEM or DPC as shown in Fig 1(e) and (f), respectively.

Conclusion
Overall, we demonstrate that multimodal TEM represents a unique experimental platform to tackle state-of-the-art challenges in probing the interplay between different order parameters in magnetic systems at the nanoscale. In particular, we show that in combination with different stimuli, nanoscale phase coexistence of structural and magnetic orders in FeRh can be uniquely probed and disentangled. Furthermore, high-resolution magnetic imaging using STEM-DPC represents a unique tool for investigating magnetic phases and their boundaries at the nanoscale.

Figure 1: (a) Schematics of the phase transition in FeRh and the sample geometry for TEM. (b) Temperature-dependent FM phase fraction in FeRh as obtained from LTEM. (c-d) LTEM imaging of the FM phase induced by fs-laser induced heating, and by localized ion irradiation using gallium FIB, respectively. (e) High-resolution image of the laser-induced FM phase using TIE reconstruction. (f) High-resolution image of the ion irradiation-induced FM phase using STEM DPC.

Keywords:

FeRh, TEM, magnetic imaging

Reference:

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Defects and alloy ordering in Pt-Cu nanoparticulate electrocatalysts

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Poster Group 1

Background incl. aims
Proton exchange membrane fuel cells are an alternative to fossil fuel-powered engines in the mobility and energy sector. They use hydrogen as fuel and do not directly generate greenhouse gas emissions. However, the cost of the electrocatalyst for the oxygen reduction reaction (ORR) still poses an issue, as it commonly contains scarce platinum. Currently, supported Pt-alloy nanoparticles are commonly used to lower the amount of Pt but retain good ORR performance. Despite numerous studies on structure-property relationships of such electrocatalysts, the effects of selected structural features on the catalytic activity and stability remain unexplored. Ensembles of supported metallic alloy nanoparticles typically vary in size, shape, composition, crystal structure, presence of defects, and other structural subtleties, all of which impact catalytic performance. Therefore, advanced characterization methods are needed to obtain average and local information about the catalyst structure to correlate it to its properties [1]. The study aims to provide an accurate description of the presence and type of defects and crystal ordering in carbon-supported platinum-copper nanoparticles and an insight into how advanced microscopy complements electrochemical methods in terms of answering questions about the structure-property relationship.
Methods
Various analogs of carbon-supported platinum-copper nanoparticles were synthesized using a wet impregnation method and subsequent thermal annealing in a tube furnace using specified temperature-time profiles and atmospheres. X-ray diffraction, scanning transmission electron microscopy (STEM), 4D-STEM, and electrochemical measurements were used to characterize the samples. Simulated X-ray and electron diffraction patterns and images were used to interpret experimental results. 4D-STEM datasets were collected for individual nanoparticles of a selected sample. Virtual detectors were used to construct real-space images from the 4D dataset, and clustering and matrix factorization were used to segment the dataset and determine commonly occurring patterns that can be associated with specific structural features within the catalyst.
Results
The platinum-copper alloy with a composition close to $PtCu_3$ crystallizes as an ordered or disordered alloy, depending on the annealing temperature. For analogs that were annealed in a reductive atmosphere at a temperature at a transition between the two well-known phases, diffraction patterns revealed the presence of periodic anti-phase boundaries, separating ordered alloy crystallites. The modulated unit cell was previously reported for a bulk material, but not in the context of nanomaterials. Based on differences in column intensities, an anti-phase boundary was observed in real space on an atomically resolved STEM micrograph, where the expected column intensities were confirmed with STEM image simulations, as seen in the Figure. The presence of anti-phase boundaries has not proven itself critical in determining the catalytic activity of the investigated material. For an analog, exhibiting a mix of the ordered and disordered alloy phases, a 4D-STEM investigation of individual nanoparticles provided diffraction patterns containing information on the

local crystal structure. The data analysis enabled the discovery of notable features within the data without explicitly specifying what diffraction signal they should cause. In the case of platinum-copper nanoparticles, it revealed a spatial distribution of the signal, associated with the ordered alloy phase, which is one of the factors previously shown to govern the catalytic activity towards the ORR.

Conclusion

The investigation resulted in a quantitative description of periodic anti-phase boundaries in platinum-copper nanoparticles following a specified thermal annealing protocol [2]. Simulated data for both powder diffraction patterns, as well as STEM images, helped explain experimental results. 4D-STEM proved itself useful in providing local crystal structure information, which can be inferred from the dataset in an unsupervised way. This imaging modality can bring added value to studies, probing the complex structure-property relationships of nanoparticulate electrocatalysts.

Keywords:

electrocatalysis, nanoparticles, crystal structure, 4D-STEM

Reference:

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[2] A. R. Kamšek, A. Meden, I. Arčon, P. Jovanovič, M. Šala, F. Ruiz-Zepeda, G. Dražić, M. Gaberšček, M. Bele, N. Hodnik. Mater. Today Nano. 23 (2023) 100377.

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Whole-brain 3D quantification of alpha-synuclein spreading and toxicity in a mouse model of Parkinson's disease

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LS-05, Lecture Theater 5, august 26, 2024, 14:00 - 15:00

Background & Aim: Progressive spreading of alpha-synuclein (aSyn) aggregates in the brain plays a key role in the prodromal phase of Parkinson's disease (PD). While several preclinical models of synucleinopathies have been developed for studying the neurotoxicity of prion-like aSyn aggregate spreading, they remain to be systematically explored with regards to early pathological events that could potentially be targeted to slow down or prevent progression of PD. Using whole-brain light sheet fluorescence microscopy (LSFM), the present study aimed to provide a detailed 3D map of progressive pathological aSyn spreading and tyrosine hydroxylase (TH) expressing neurons and projections in the aSyn pre-formed fibril (PFF) mouse model of PD.

Methods: 8-weeks old C57BL/6 male mice received two unilateral, intrastriatal injections of murine aSyn PFFs (5 µg per injection). Mice were terminated at 1, 4, 8-, 12-, 16-, or 26-weeks post-injection (wpi), whole-brains were dual immunolabelled for aSyn phosphorylated at serine-129 (pS129-aSyn, marker of aSyn aggregation) and TH, optically cleared (iDISCO+) and scanned using LSFM at cellular resolution. AI-based computational analysis enabled automated whole-brain mapping and quantification of pS129-aSyn and TH fluorescence intensity across 840 individual brain regions using a custom mouse brain atlas.

Results: Distinct spatiotemporal phases of endogenous aSyn aggregate spreading observed in aSyn PFF mice over time. The phases included progressive spread of aSyn aggregates to primary seeding regions (amygdala, substantia nigra, and several cortical areas) and secondary seeding regions (entorhinal area and hippocampal formation) based on their interconnectivity to the injection site (striatum), followed by redistribution of aSyn aggregates in specific brain regions. In parallel, TH expression was progressively downregulated in the nigrostriatal pathway, suggesting axonal damage in terminal areas preceding dopaminergic neuronal loss in the aSyn PFF mouse model.

Conclusion: We here report a complete whole-brain map of aSyn aggregate spreading in an industry-standard aSyn PFF mouse model of PD. The anatomical complexity of aSyn aggregate spreading in the model underscores the unique applicability of whole-brain 3D LSFM imaging to fully capture spatiotemporal dynamics in aSyn and TH expression, making the model highly instrumental for the evaluation of therapeutic modalities that may prevent aSyn aggregate spreading and dopaminergic neuronal loss.

Keywords:

3D microscopy, tissue clearing, synucleinopathies

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NP-SAM: Implementing the Segment Anything Model for Easy Nanoparticle Segmentation and Analysis in Electron Microscopy

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background incl. aims
Nanoparticles (NPs) have important use cases in catalysis, nanomedicine, photonics and plasmonics, and their properties are very dependent on elemental composition, structure, size, and shape. Reliable characterization methods are, therefore, very important in developing these technological areas. With X-ray-based scattering methods, large volumes of NPs can be probed, which gives volume-averaged ensemble characteristics. (Scanning) transmission electron microscopy ((S)TEM) on the other hand can give detailed information about the individual particles, but thousands of NPs must be analyzed to get statistically significant information about the sample. This calls for automatic NP characterization. Although numerous approaches exist, many require choosing the right combination of segmentation parameters or training machine learning algorithms. Here, we present NP-SAM [1], an easy-to-use segmentation and analysis software for NP characterization with advanced filtering based on NP characteristics and with the ability to run with varying degrees of human intervention.
Methods
NP-SAM is based on the Segment Anything Model (SAM) developed by Meta AI Research [2]. We have implemented SAM into NP-SAM and added extra functionalities such as a user-friendly mask filtering mechanism, overlap handling, core-shell analysis, and automatic generation of different types of useful output. The figure shows the general workflow of NP-SAM. Electron microscopy image(s) are first segmented with SAM, resulting in a mask for every segmented particle in different colors. Unlike most other segmentation methods, masks can overlap the same regions, making it possible to analyze neighboring NPs that overlap to a certain degree. An optional graphical user interface (GUI) allows undesired masks to be easily filtered based on the most relevant characteristics such as area, intensity, overlap, etc. For advanced users, even more advanced filtering is possible. Finally, NP-SAM produces different kinds of output: 1) Histograms and statistics of user-chosen characteristics, 2) an overview .pdf file for quick sharing that contains the histograms and summarizes the segmentation and filtering parameters (for reproducibility), 3) a .csv file with the particle characteristics, e.g. area, perimeter, orientation, etc., 4) a flattened binary mask image of all the particles found, and 5) a mask for every individual particle enabling further advanced workflows of i.e. core-shell particles or hyperspectral data.
Results
We have tested NP-SAM on various electron microscopy images including high-angle annular dark-field (HAADF) and bright-field TEM images. 24 HAADF images of PdCu NPs were analyzed, and 2352 NPs were analyzed in about 11 minutes. The size distribution found with NP-SAM agrees with manually measured sizes. NP-SAM's segmentation can be adjusted depending on the accuracy needed and the computer power available. Four segmentation model weights are available: Huge,

large, base, and fast, with huge being the most accurate but also computationally heaviest and fast being lightweight and less accurate [3]. Segmenting the 24 HAADF images with the fast model weights took 3 minutes, and 1925 NPs were found to give a size distribution that again resembles the manually measured one.
The segmentation is based on a grid of points determining where the segmentation model searches for objects. For a HAADF image of very polydisperse Ag NPs and with many small NPs, a finer grid helped find more NPs. We have also implemented a feature called crop and enlarge that can help detect small NPs by dividing the image into four enlarged and segmented sections individually.
One of the exciting features of NP-SAM is its ability to handle overlapping NPs. The overlap between masks is calculated since masks are allowed to occupy the same pixels. This overlap is an important filter parameter, and the user can freely choose the amount of overlap tolerated. This is shown with a HAADF image of AgCuIrPdPt NPs with complicated HAADF contrast.
NP-SAMs sensitivity and ability to handle overlapping masks also enable advanced analysis of core-shell NPs. With core-shell NPs being segmented as two masks, one being the entire particle and the other being the core within the particle, the core and shell can be analyzed individually, and their characteristics can be linked. For example, we can plot shell thickness as a function of core diameter.
NP-SAM is available at our GitLab at https://gitlab.au.dk/disorder/np-sam with installation instructions, source code and user-friendly example notebooks. It is written in Python and can easily be installed using pip after installing PyTorch. A CUDA-compatible GPU speeds up computations significantly, and 8 GB of VRAM is recommended for the huge model weights. NP-SAM can also be run in a cloud-based Google Colab notebook where GPUs are often available. The segmented masks can then be downloaded and filtered locally using the GUI. Finally, we have also developed NP-SAM as a .exe file that runs NP-SAM through a user-friendly interface, not requiring any Python knowledge.
Conclusion
NP-SAM is a powerful tool for quick, user-friendly, and semi-automatic NP segmentation and analysis. Several images can be segmented in less than a minute per image, facilitating quick and reproducible analysis of thousands of NPs. In general, the size distributions found with NP-SAM resemble those found manually. Because NP-SAM can handle overlap, this allows for useful filtering and advanced analysis workflows such as the analysis of core-shell particles.

Keywords:

segmentation, automatization, machine learning, nanoparticles

Reference:

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Towards advanced polymer membranes for hydrogen technologies through dose-optimised Focused Ion Beams

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Poster Group 2

Background & Aims
Understanding the properties of membranes applied in the CCMs of electrolyser and fuel cell technologies is crucial to advancing the commercialisation and deployment of these technologies to the market. The cross-sections of these membranes are typically studied using a Focused Ion Beam (FIB). A FIB is a technique in which accelerated (Ga) ions spatter material away upon interaction.[1] This technique is used mainly for electron-transparent transmission electron microscopy sample preparation, FIB tomography, or cross-sectional analysis of materials such as semiconductors, ceramics, metals, polymer thin films, and biological specimens. Unlike conductive materials such as ceramics and metals, polymer membranes are non-conductive and thus susceptible to beam-induced heating during ion beam milling.[2] Ion beam-induced heating in polymer membranes creates 'melt-like' artefacts that smear the pristine structural details of the membrane along the cross-section. In addition, it is also shown that accelerated Ga ions can result in the chemical alteration of the membrane's chemical structure, which affects the properties of the membrane. [3] This ion-beam-induced damage is typically circumvented by dose-controlled milling or lowering the specimen temperature via cryogenics. Here, we aim to optimise the milling parameters for polymer membrane cross-section milling by determining the optimum current, accelerating voltage, and dwell time required to minimise beam-induced heating in polymer membranes.
Methods
Polymer membranes were mounted on SEM-FIB stubs and sputter-coated with a conductive layer of carbon. A series of cross-sections were milled using a Zeiss Crossbeam 550 dual-beam at a varying current, accelerating voltage, dwell time, and ion dose. The actual values will be presented with the results.
Results and conclusions
Preliminary results show that reducing the dwell time at low milling currents and high voltage reduces the beam-induced artefacts in the membrane cross-sections. The results of this study will help understand the pristine microstructure of polymer membranes in a standardised and reproducible routine cross-sectional analysis, and ultimately lead to the development of novel membranes for applications in hydrogen technologies.

Keywords:

FIB; Polymer membranes; ion damage

Reference:

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2. S. Kim et al., Ultramicroscopy 111 (2011) 191–199
3. R.J. Bailey et al., Micron 50 (2013) 51–56

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NBED investigations of coaxial (Al,In,Ga)As nanowires

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Poster Group 2

Background incl. aims
GaAs-based semiconductor nanowire (NW) lasers exhibit many advantageous properties for telecom-band data communications and sensing applications, like e.g. their ultra-compact size and their compatibility to Si-based photonics. In comparison to other III-V semiconductors, GaAs allows a fairly mature Au-free NW growth on Si. However, within the coaxially grown multiple quantum wells (MQW) the strain resulting from lattice mismatch accumulates during the growth. This problem can be mitigated by the introduction of an InAlGaAs buffer layer in between the GaAs core and the active region of the MQW-stack [1].
For process control and further engineering of NW laser structures, the strain in the resulting devices must be monitored. We report on strain investigation by means nanobeam electron diffraction (NBED) for such structures.
Methods
GaAs NW cores were grown via a vapor-liquid-solid growth mechanism on pre-patterned Si substrates along the [111] direction. Subsequently, an In0.3Al0.3Ga0.4As buffer layer, followed by an In0.3Ga0.7As QW, an In0.23Al0.23Ga0.54As barrier layer, and a GaAs cap layer were grown coaxially onto the side walls of the GaAs core. A cross-sectional lamella was prepared from the NW using focused ion beams.
HAADF, EDX and eventually NBED measurements were performed using a JEOL GrandArmF2 microscope, operated at 300kV. The NBED data was recorded using the Quantum Detector's Merlin detector.
Results
We applied a custom algorithm to determine the base vectors for the reciprocal lattice visible in the individual diffraction patterns for each scan point. Starting with an initial guess for the base vectors and the origin of the pattern, the algorithm consists of several steps: (i) for each lattice point spawned by the base vectors, a sub-region of the diffraction pattern is extracted; (ii) within each subregion the position of the reflection is determined with sub-pixel projection; (iii) a new set of base vectors is calculated by weighted linear regression, using the inverse precision from step (ii) as weights; (iv) unless the resulting set of base vectors has converged to a predefined precision, the algorithm is repeated from step (i) again. From the resulting base vectors of the reciprocal lattice observable in the diffraction patterns, the strain components can be calculated in reference to the GaAs core.
In comparison of the resulting strain data with finite element calculations short-scaled variations of the determined strain components are observed in areas where smooth strain components are expected. We attribute these variations to dynamical diffraction effects stemming from small specimen inhomogeneities created during preparation, for example from thickness variations. We discuss the influence of experimental and evaluation parameters, like the convergence angle or the method used to determine the reflection position, on the precision of the obtained strain components.
Conclusion

NBED generally allows the evaluation of strain data with nanometer precision. However, the resulting strain maps suffer from dynamical diffraction effects, which can be further mitigated by optimized experimental parameters and optimized evaluation procedures.

Keywords:

4D-STEM, NBED, Strain

Reference:

[1] P. Schmiedeke, A. Thurn, S. Matich, M. Döblinger, J. J. Finley, G. Koblmüller; Appl. Phys. Lett. 118, 221103 (2021)

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Surface Polarity Dynamics and Strong Reconstruction in Partially Reduced Nickelate Films

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims
Surface polarity dynamics profoundly influence the electronic and structural properties of oxide thin films. This study aims to investigate the atomic structure and electrostatic characteristics of partially reduced nickelate films to elucidate the mechanism behind strong surface reconstruction.

Methods
Advanced STEM techniques, including annular bright field (ABF) imaging, four-dimensional scanning transmission electron microscopy (4D-STEM), and electron energy-loss spectroscopy (STEM-EELS), were employed to probe the atomic and electronic structures, along with the near-surface electrostatic charge distribution.

Results
Surface analysis revealed a polar distortion coupled with octahedral rotations in both fully oxidized and partially reduced nickelate films. A stronger polar distortion was observed in the reduced sample, indicating a relationship with oxygen vacancies generated during topochemical reduction (Fig. 1a,b). Direct evidence of homogenous Sr doping and distinct valence variations of Ni atoms near the surface were provided by 4D-STEM imaging. EELS analysis confirmed changes in Ni valence and oxygen concentration, consistent with the observed surface reconstruction.

Conclusions
This study bridges the gap in experimental examinations of surface effects in oxide thin films, providing crucial insights into the nuanced interplay between surface polarity, electronic structure, and structural transformations. The observed surface reconstructions, influenced by oxygen vacancies, impact electronic transport properties, contributing to the understanding and engineering of surface polarity at the atomic scale in functional materials [1].

Figure 1: (a) ABF-STEM image of a $Pr_{0.8}Sr_{0.2}NiO_{2+x}$ film on a (110)-oriented $NdGaO_3$ substrate. (b) Enlarged ABF images of the regions marked in (a) and corresponding structure model.

Keywords:

Surface polarity, polar distortion, nickelates

Reference:

[1] C. Yang et al., Nat. Commun. 15 (2024) 378, https://doi.org/10.1038/s41467-023-44616-x

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Characterization of structure and mixing in nanoparticle hetero-aggregates using convolutional neural networks: 3D-reconstruction versus 2D-projection

Dr. Christoph Mahr[1], Dr. Florian Krause[1], Jakob Stahl[2], Beeke Gerken[1], Dr. Marco Schowalter[1], Dr. Tim Grieb[1], Prof. Dr.-Ing. Lutz Mädler, Prof. Dr. Andreas Rosenauer
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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background
Functional properties of nanomaterials depend to a large extent on the underlying structure and chemical composition. An example are hetero-aggregates in which nanoparticles of two different materials are mixed. The mixture of titanium-dioxide (TiO2) and tungsten-trioxide (WO3) nanoparticles shows enhanced functionality if applied as a photo-catalyst compared to pure TiO2[1]. The performance of the material depends on the mixing. Best photo-catalytic activity is achieved in completely mixed hetero-aggregates[1].
Improvement of functional properties requires a characterization of structure and mixing. In conventional STEM, two-dimensional (2D) projection images of the samples are acquired, information about the third dimension is lost. This drawback can be overcome by STEM tomography, where the three-dimensional (3D) structure is reconstructed from a series of projection images acquired using various projection directions. However, 3D measurements are expensive with respect to acquisition and evaluation time. Hence, the measurement of 3D-reconstruction can only be done for a limited number of hetero-aggregates.

Methods
TiO2-WO3 hetero-aggregates are generated in a double-flame spray pyrolysis setup. Precursors of the two materials are sprayed and combusted in the two separate flames. Nanoparticles form by nucleation and coagulation and aggregate to clusters of the same material. After a certain distance both flames intersect and clusters of both materials form hetero-contacts. The length of the intersection distance has an influence on the mixing.
To obtain statistically relevant results, information on many hetero-aggregates has to be gathered. Positions of many nanoparticles have to be determined in 2D and 3D data. This can be challenging especially in regions where many particles overlap in crowded regions in 2D-projection data. In recent years, it was demonstrated that the application of artificial intelligence (i.e. convolutional neural networks, CNNs) outperforms a manual measurement or classical object detection algorithms[2,3]. The application of CNNs requires a training of the network first. To this end, many training images in which particle positions and material types are known are required. In the present contribution, we simulate realistic 2D-projection and 3D-reconstruction data of computer-generated virtual hetero-aggregates, in which particle positions and material types are known. We evaluate the trained CNNs using simulated data that has not been used during the training process and apply the networks to experimental 2D-projection and 3D-reconstruction data. For evaluations of 2D-projection data we train a Mask R-CNN[4], for evaluation of 3D-reconstructions we train a StarDist-3D network[5].

Results

An example evaluation is shown in the figure. Part (a) shows a STEM image of a TiO2-WO3 hetero-aggregate. Part (b) shows particles detected by the Mask R-CNN, where TiO2 and WO3 are represented in red and green, respectively. The material discrimination shows a good agreement with the energy dispersive X-ray spectroscopy (EDXS) map in (c). The nanoparticles as detected by the StarDist-3D network in the STEM tomography reconstruction is shown in (d). The material discrimination is in good agreement with the EDXS map in (c) again.
To correlate structure and chemical composition with functional properties it is required to obtain quantitative information from 2D-projections and/or 3D-reconstructions. In the present contribution, we evaluate the number of particles, the WO3 mass fraction, particle size distributions and the fractal dimension, which is a measure for the hetero-aggregate structure. To quantify the mixing, we measure the heterogeneous coordination number, i.e. the average number of neighbour particles of a different material. A higher heterogeneous coordination number indicates better mixing. We show that for a measurement of mass fractions and for the characterization of mixing, the evaluation of less expensive 2D-projection data is sufficient, whereas for a measurement of the fractal dimension a 3D-reconstruction is required.

Conclusion
Results of the present contribution will help for future characterization of nanoparticle hetero-aggregates, if only the less expensive 2D-projection data is available. In these cases, results of this contribution will provide the possibility to relate 2D-projection evaluations with the 3D structure of the samples.

Keywords:

nanoparticle-mixing
object-detection
neural networks
tomography

Reference:

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Investigation of metal-to-metal hydride phase transformations in magnesium thin films using STEM techniques

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Background incl. aims
In the pursuit of a decarbonized society and economy, hydrogen (H2) stands out for its zero-emission potential as a fuel, energy storage medium, and chemical feedstock. However, achieving compact hydrogen storage, especially in safe, solid forms such as metal hydrides, remains a significant challenge.
Enhancing storage efficiency requires a deep understanding of the nano/atomic-scale dynamics of, e.g., the metal-to-metal hydride transformation, including nucleation, growth, and the effects of stress, strain, and defects on hydrogen storage properties. Therefore, to pave the way for improved storage solutions, we must develop a deeper understanding of hydrogen sorption and desorption dynamics through the real-time observation of the transformation process in scanning/transmission electron microscopy (S/TEM).
Methods
We used magnesium (Mg) thin films as a model system to study metal-to-metal hydride phase transformation. The Mg films were grown using a DC magnetron sputtering to a thickness of 500 nm with an 8 nm adhesion layer of Titanium (Ti) and a catalyst layer of Palladium (Pd) of 100 nm. A ThermoFisher Hydra plasma-focused ion beam (PFIB, Xe, 30kV) was used to prepare the TEM lamellas. Structural investigations were performed using a ThermoFisher Spectra Ultra equipped with an X-FEG mono and a Gatan Image Filter (GIF) Continuum HR. These phase transformations were studied under both in-situ and ex-situ conditions using STEM, low-loss plasmon electron energy loss spectroscopy (EELS), and electron diffraction (ED).
Results
The hydrogen absorption/desorption process in Mg is observed using the bulk plasmon shift in EELS due to a structural change from a hexagonal closed-packed (HCP) to a tetragonal crystal structure. During absorption, TEM dark-field imaging confirms that the Mg thin film loses its initial morphology and transforms into nanocrystalline particles, which is attributed to volume expansion. Low-loss EELS further confirms that the hydride phase transformation occurs through nucleation and growth, distinguishing it from the desorption process. The desorption process starts with the formation of pores/voids at the interface between Mg and Ti, followed by crack formation that initiates Mg formation. The Complete H2 desorption in thin films results in a fully porous structure, with subsequent coalescence leading to void formation in Mg. The thin film's increasing porosity and void formation with subsequent cycling indicate a deterioration of its durability. However, initial porosity at the Mg/Ti interface promotes Mg nucleation during desorption. In order to validate our findings, we combine both in-situ and ex-situ studies at high spatial resolution in STEM, enabling us to compare our results and interpret the effect of the e-beam dose rate on the analysis.
Conclusion
We demonstrate that STEM imaging, low-loss EELS, and ED allow us to track the phase transformation from metal-to-metal hydride in Mg thin films along with its structural and

morphological changes. The nucleation and growth of Mg are influenced by the stress/strain generated during the hydride transformation, distinct from the desorption process.

Keywords:

STEMEELS
Phasetransformation
Hydrogen storage
MgH2.

Reference:

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Optimizing Backscattered Electron Detection in SEM: Diode Layout and Collection Efficiency

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Poster Group 1

Scanning electron microscopes (SEMs) with their variety of attachments designed for surface observation and analysis stand as a key instrument in research institutes and quality testing facilities worldwide. The essence of the SEM is to induce the emission of various electron products from the specimen, among which backscattered electrons (BSEs) play a pivotal role. These BSEs emerging from the specimen carry information of composition and structure and play an important role in material and life science. Therefore, while most SEMs are equipped with a standard BSE detector, optimizing the measurement process of BSEs becomes crucial when considering the wide range of measurements into account and pushing the limits for aiming the maximum performance. In addition to the detection efficiency of the detector, the geometry of the set-up also plays a decisive role. We therefore address the question of how the layout of the detector affects the specific geometries present in SEM setups [1].
In SEM in general, ensuring clear visualization of specimen features relies on several factors, including beam current, pixel dwell time, working distance, and detector efficiency. Below a certain minimum contrast threshold, specimen details may become indistinguishable. However, for certain specimens or setups it may not always be feasible to increase beam currents, adjust beam energy, or change the working distance to enhance contrast. In such cases, maximizing the collection of electrons (here BSEs), becomes essential for obtaining satisfactory results. Achieving this involves optimizing the diode layout, thus emphasizing the importance of selecting suitable detectors aligned with specific measurement requirements for effectively optimizing SEM imaging parameters [2]. But despite the important role of BSE collection efficiency across scientific and industrial domains, the literature addressing this aspect remains relatively sparse, posing a significant challenge for comprehensive research in this field.
We conduct experiments measuring the backscattered electron collection at various working distances to determine the optimal conditions for each type of diode. To provide a quantitative assessment, we introduce the Geometric Collection Efficiency (GCE), defined as the ratio of BSE impinging on the active area of the diode to the total number of BSE leaving the sample. We present GCE values for different diode layouts across varying working distances. These results are supported by simulations of GCE based on the cosine distribution of BSE and the setup geometry.
Our study aims to illuminate a crucial aspect influencing the performance and accuracy of Back-scattered electron Detector (BSD). We seek to quantitatively understand the collection efficiency of backscattered electrons across a diverse range of BSD. Employing BSD with varying active areas ranging from 40 to 420 mm^2 and hole diameters ranging from 1 to 5.6 mm. Moreover, these findings enable the identification of the most suitable diode layout for specific measuring geometries.
Typically, the diode aperture should be sufficiently small to minimize backscattered electron (BSE) loss at shorter working distances, while the outer diameter of the diode must be large enough to capture a greater proportion of BSE at larger working distances. However, this general approach must be adapted to fit within an existing setup, where the hole size additionally impacts the field of view, and the outer diameter may obscure or interfere with other detectors within the SEM. Therefore, it is imperative to determine the optimal diode layout considering both geometric

collection efficiency and mechanical constraints of the setup. Subsequently, we will showcase images from various applications, spanning from low working distance (WD) and low keV scenarios to high-current applications accompanied by parallel energy-dispersive X-ray spectroscopy (EDX) measurements at higher WDs.

In conclusion, our investigation utilizes various BSD with distinct geometries to assess their suitability for diverse applications in SEM. Through systematic evaluation of detector performance under varying experimental parameters, we aim to offer insights into selecting the most appropriate detector for specific imaging tasks or analytical objectives.

Fig. 1. Comparing simulated and experimental Geometric Collection Efficiency (GCE) values for diverse Backscattered Electron (BSE) chips with different geometrical configurations. The analysis reveals a close agreement, providing insights into the performance and accuracy of each chip.

Keywords:

Scanning Electron Microscope,Backscattered Electrons,Detector Efficiency

Reference:

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Empowering STEM in SEM: Integrative Approaches for Enhanced Detection

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Poster Group 1

In parallel to finding new records in resolution in high energy Transmission Electron Microscopes (TEM) applications - somehow a little behind the scenes - the Scanning Electron Microscopes (SEMs) and TEM machines reach out for each other. Scanning Transmission Electron Microscopy (STEM) in SEM becomes a more and more versatile tool for economical STEM examinations. This may be as a pre-study for further STEM measurements in high end machines or as the final inspection tool. Even though this is an ongoing trend, there is still a gap between the detection capabilities of transmitted electrons in SEM and TEM machines. In SEM, STEM detectors mostly range from passive detection - via generated secondary electrons - to diode arrays and simple segmented diodes. In contrast, in TEM machines the varieties span from single cell detectors over multi segmented ring diodes to fully pixelated electron cameras. Nevertheless, there is a strong demand of expanding the detection possibilities in the SEM closer to TEM machines in order to increase the overlap in detection variability. Here, we introduce a versatile multi-channel STEM setup which can be used in SEM as well as TEM sharing the same platform but addressing the specialties of both worlds.

To perform STEM in SEM with the versatility of the TEM system one needs to address the differences in SEM in relation to TEM :

1. In SEMs the electron energies with <30keV are vastly lower than in TEMs. Therefore, the detector diodes are optimized to support electron energies from below 2 keV to 300keV.
2. SEMs in general offer higher scanning rates than TEM machines. Therefore, the pixel dwell times of down to few tens ns are supported.
3. While all diodes can be used in TEM and SEM, new diode geometries are added to address the different setup geometry in SEM. As seen in figure 1. the range starts with 4 channel segmented diodes and goes up to multi-segmented ring structures with and without BF cell.
4. Two mechanical concepts are chosen to fit most microscope chambers as well as addressing the radiation safety in SEM and TEM. Figure 2(a) shows the manual insertion mechanism for SEM systems. It is optimized for x-y-z positioning in SEM chambers. Figure 2(b) shows the pneumatic insertion mechanism mainly for TEM systems with y-z positioning. While the SEM mechanics cannot be adapted to TEM systems, the pneumatic mechanics can also be used for SEM and is therefore mentioned here.

Besides these differences the system shares the same platform. This includes the amplification chain, the acquisition system, and the ability to support up to 13 individual channels. The platform is divided into two configurations. For the use of the image acquisition system of the microscope there is the possibility to use a 4-channel amplification system. This outputs a user specified combination of the 4 signals (4 to 1) to be digitized by an auxiliary input of the SEM. This system also offers minimal pixel dwell times of few tens of ns but is limited in displaying information in parallel.
To utilize the capability of acquiring up to 13 channels in parallel the second configuration comes with a separate imaging acquisition system. Each of the signals is amplified and digitized separately

and the signals can then be digitally combined if needed. Here the minimum pixel dwell time is below 200 ns and there is no limitation in parallel acquisition, displaying or mixing of the individual signals.

In our contribution we will show the results of both configurations with different diode layouts. These examples show how these systems enlarge the STEM in SEM capabilities by sharing more information from the specimen and thus help to close the gap between transmitted electron detection in SEM and TEM.
Fig. 1. Examples of diode layouts for STEM application in SEM and TEM

Fig. 2. Mechanical insertion mechanism for positioning the detectors in the microscope. (a) Manual mechanism for use in SEM. (b) Pneumatic mechanism for use in SEM and TEM

Keywords:

Scanning Transmission Electron Microscopy,Diode geometries

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Seeking peak precision in atomic EELS mapping with counting mode direct electron detection

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Since the advent of aberration-corrected scanning transmission electron microscopy (STEM) with electron energy-loss spectroscopy (EELS) spectrum imaging capabilities, atomic resolution STEM-EELS has become a vital technique for probing both elemental compositions and bonding states across epitaxial thin films.[1] Nevertheless, data quality are often limited in terms of spatial and spectral precision. In large part, these limitations have derived from the charge-coupled devices (CCD) that, until recently, have been the primary sensors used for EELS, which are associated with a host of deficits: poor detective quantum efficiency (DQE) and point spread function (PSF); slow read out time and poor duty cycle; and correlated channel noise. Typical consequences for atomic resolution mapping include: spatial distortions from scan drift; strong trade-offs regarding the measurement of sharp spectral features versus large spectral range for measuring many elements; high beam currents and/or long dwell times for obtaining sufficient signal-to-noise; and a noise structure ill-suited to denoising using machine learning algorithms. In comparison, direct electron detection promises to address all of these deficiencies, at the same time. Here, we explore the benefits brought by direct electron detection using a large array monolithic active pixel sensor having ~3.4 k energy channels, coupled to a state-of-the-art EEL spectrometer, with the goal of seeking maximum precision for both spectral peaks (EELS fine structures) and spatial peaks (atomic columns).
As test objects, we take superlattices of perovskite rare-earth nickelates, which constitute both pure and alloyed layers.[2] Not only is it paramount to characterize their chemical nature for correctly interpreting their physical properties, these samples also provide interesting challenges for testing atomic-resolution STEM-EELS, such as the overlap of the rare-earth M edges with each other and with the Ni L edge. The STEM-EELS measurements are made using a Gatan Continuum ERS spectrometer equipped with a K3 detector operated in counting mode, installed on a double-aberration corrected and monochromated FEI Titan Themis 60-300, run at 200 (or 300) kV. Different acquisition strategies are tested: single-EELS, dual-EELS, single frame, multi-frame with on-line drift correction, multi-frame with off-line rigid and non-rigid registration.
The figure shows example results from a superlattice of layers with target compositions of alloy $(0.7Nd,0.3La)NiO_3$ and pure $SmNiO_3$, grown on $LaAlO_3$. The spectrum image was acquired using the detector's maximum speed of 0.34 ms per pixel, with 0.25 Å pixel size, a beam current of 100 pA, 20 and 47 mrad convergence and collection semi-angles, for a total of 5 integrated frames, with frame-by-frame drift correction. The right-hand side of the figure shows background-subtracted raw spectra integrated from regions of $SmNiO_3$ (top), $(Nd,La)NiO_3$ (middle) and $LaAlO_3$ (bottom). Despite the broad spectral range covering all major elemental edges of O K (532 eV), Al K (1560 eV), Ni L (855 eV), La M (832 eV), Nd M (978 eV) and Sm M (1080 eV) from the 0.45 eV/channel dispersion, because of the good PSF, the L and M edge white lines are remarkably sharp. This can be seen in the right-hand insets showing zooms of the La M–Ni L overlap region, where the shapes of both edges can be seen in the $(Nd,La)NiO_3$ alloy. The left-hand insets show the O K-edge onset. Both nickelate layers show a

prepeak at ~528 eV, that derives from hybridization of the O 2p with the Ni 3d, rare earth 5d, and Ni 4sp electronic states. The narrow prepeak is distinctly visible. Further, it acts a sensitive marker of electron flux-induced damage.[3] Its undiminished magnitude in the data show that, because of the detector speed, sensitivity and 100% duty cycle, we were able to stay below the flux damage threshold even with the small 0.25 Å pixel size; an order of magnitude improvement compared to the previous results taken with a CCD-based spectrometer. After denoising the spectrum image using principal component analysis (PCA), a standards-based approach was used to generate the displayed elemental maps, where the O K edge is fitted as either nickelate (O_nick) or $LaAlO_3$ (O_LAO). A clear atomic resolution is attained in all maps; even for Al whose K edge shows very small signal intensity. Fourier transform analysis indicates that the summed denoised spectrum image signal attains a spatial resolution of 1.2 Å. In the single-element Sm map of an $SmNiO_3$ layer, displayed at the bottom right, a resolution of 1.7 Å is achieved. More impressively, the spatial precision is such that this map clearly shows the characteristic ± 20 pm up-down antipolar displacements of the Sm cations in this orthorhombic phase.

In conclusion, a latest-generation counting mode detector shows a step-change improvement for atomic resolution mapping of perovskite oxide heterostructures. While ideal acquisition strategies are still being refined, current results already demonstrate the potential for high precision mapping, both spectrally and spatially. Although PCA-denoised maps are superficially correct, we note that this processing systematically generates spectral artefacts, as shown in the pixel-by-pixel sequence of 3 reconstructed spectra across a Sm column in the bottom right of the figure. Potentially this can be understood as the decomposition identifying the spectral structure of a phase, which however does not properly represent the sharp spatial modulations of the chemically-different atomic columns. Work is in progress to identify other denoising approaches that leverage the Poisson noise structure of the counting mode EELS data while generating a physically-correct output.

Keywords:

direct electron detection; EELS; atomic-mapping

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WhatEELS upgrade: the software tool based in Python for EELS analysis

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Poster Group 1

BACKGROUND INCLUDING AIMS
Electron Energy Loss Spectroscopy (EELS) employing a Scanning Transmission Electron Microscope (STEM) offers precise elemental characterization with exceptional spatial resolution. Multiple Linear Least Squares fits are one of the approaches with short convergence times that are typically favoured for spectral unmixing in the quantitative analysis due to the complexity and sizes of the EELS spectrum pictures datasets collected by the state-of-the-art instruments. However, Non-Linear Least Squares (NLLS) fitting could be a better option for analysis in some circumstances, since it does not need calibrated reference spectra and yields information for each of the constituent parts of the fitted model. For efficient analysis of complex datasets, combining clustering segmentation with Non-Linear Least Squares fitting enhances parameter control and accommodates mixed-composition samples. To facilitate this approach, integrating clustering, and NLLS, a modular software solution implemented in Python by J. Blanco-Portals was developed and open for the public in 2022, WhatEELS [1]. In the present work, we update the WhatEELS software with elemental quantification tools [2] using full relativistic cross-sections [3].
METHODS
The interactive shell of WhatEELS is based on Python and, in particular, in the following modules: Panel and Holoviews, and the graphical backend on Bokeh. Below the surface, the NLLS fitting, and background removal are based on a library called lmfit, which expands the SciPy fitting capabilities. The machine learning analysis is based on Hierarchical Density-Based Spatial Clustering of Applications with Noise, Uniform Manifold Approximation and Projection and Support Vector Machines, which the first and the last one are packages from the Scikit-learn library. The full relativistic cross-sections are calculated from Quantum Mechanics following Salvat [3].
RESULTS
We successfully implemented the relativistic elemental quantification in WhatEELS, This testing was applied to iron oxide core-shell nanocubes and iron and manganese oxide core-shell nanoparticles, obtaining the expected results for the quantification.
CONCLUSIONS
WhatEELS is a software tool designed to analyze multiple-pixel EELS datasets by combining clustering algorithms for image segmentation and NLLS fitting routines for improved workflow efficiency. WhatEELS is advantageous for scientists and students who are just starting in EELS data analysis for materials science because of its open-source nature and low programming requirements.

Keywords:

STEM, EELS, software

Reference:

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Use of prior knowlege and physics-guided NMF for improved phase segmentation of EDX datasets.

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

While modern analytical transmission electron microscopes fitted with an energy-dispersive X-ray (EDX) detector can acquire a huge amount of data at an ever-increasing speed, there is still a need to improve processing tools. Machine learning algorithms based on multivariate statistical analysis are widely used to reduce the dimensionality of datasets and perform denoising. On the other hand, to perform quantification, one needs to fit the EDX spectrum with Gaussians for the X-ray lines and a mathematical model taking into account the Bremsstrahlung background. Usually, those two steps are performed independently: first the statistical decomposition and denoising; and then the fitting and quantification.

With a new Python package named espm, that provides a new algorithm entitled ESpM-NMF, we propose to perform the two steps jointly, where the machine learning algorithm directly learns the parameter characterizing the model of the spectrum. We have, in addition, implemented an advanced regularization procedure to take into account the relatively smooth nature of the spatial distribution of components.
Our method has many advantages:
- It is much more resilient to noise and enables analysis and quantification of datasets with much lower signal than conventional methods.
- It allows prior knowledge to be easily taken into account, such as the fact that some regions of the image may contain only one phase or that some of the phases do/do not contain certain chemical elements. With this, we drastically improve the chances that the results of the decomposition have an actual physical meaning.
- The results of the decomposition naturally contain the information needed for quantification, as we directly learn the height of the Gaussian peaks modelling the X-ray lines. There is therefore no need for a further step of fitting the spectra to obtain relative abundances of elements.

In the following paper, we will demonstrate how we can easily use reasonable a priori knowledge on a sample to guide a decomposition. The sample is a synthetic mineral assemblage used to study Earth's mantle. It has a homogenous starting composition with major elements Si and Mg (besides O), minor elements Fe, Ca & Al, and some dopants. The sample is synthesized in a diamond anvil cell at pressure of 55 GPa, and heated with a laser to around 3200-3500 K, resulting in a complex microstructure of a matrix phase (bridgemanite) containing inclusions (ferropericlase and calcium perovkites). The overall composition of the main phases has, in addition, a gradient towards the center of the heated spot, that is of particular scientific interest. Small regions of the datasets are excluded from the analysis via a mask because they contain an iron-rich core which is not of interest (top right) and a hole due to the FIB preparation (bottom).

An unguided decomposition with two components leads to a nearly realistic result with two phases: a matrix (a), with inclusions (b). However, a close analysis shows that the intensity of the image of the component #2 never goes to 0, indicating an imperfect unmixing. Moreover, the Si content obtained in the matrix phase (panel (a)) is 52 at. %, which is above the expected maximum of 50%. Using a Mask (c), it is possible to force the inclusion component to be 0 in the yellow areas. This is a very reasonable constraint based on evidence from the general structure of the specimen. By doing so, we obtain a more realistic composition of the matrix phase with 48 at. % Si. This finding further supports the supposition that the mask selects a pure phase region containing only the matrix.

A further investigation can be performed using a decomposition with 4 components, of which 2 are forced to represent the matrix by setting the same mask on the other 2 components. This will allow for variations inside each of the 2 main phases. Indeed, as seen in the lower 4 panels of the figure, with this 4-component decomposition, one sees a compositional gradient from top right (close to the central laser spot) to bottom left. In general, both the matrix phase and the inclusions contain more iron as they approach the iron-enriched core.

While not shown in the figure, with our algorithm, it is also possible to constrain the chemical composition of some of the components. The use of well-chosen constraints can be both a way to reach a more realistic segmentation in the actual phases composing the specimen, but is also a powerful method to explore and better understand a complex specimen. As such, ESpM-NMF both redefines the standard pipeline for EDX dataset processing, and offers strong versatility for tailoring the analysis to the specific nature and characteristics of a sample.

Keywords:

EDX NMF pysics guided ML

Reference:

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3D ED for accurate structure analysis of nanoparticles

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Background incl. aims
When it comes to characterizing nanomaterials, electron microscopy offers an unrivalled range of tools. This contribution focuses on the accurate structure analysis of individual nanoparticles (NP) through electron diffraction, particularly on 3D electron diffraction (3D ED) approaches1. In recent years, 3D ED has emerged as a valuable method for accurate structure analysis, specifically in cases where sample size limitations prevented structure analysis by X-ray diffraction. Nevertheless, very few experimental studies have investigated the applicability of 3D ED to small particles (10 nm and less), in particular by examining whether this approach can provide relevant quantitative information concerning the presence of elements in the structure and their localization.

Methods
In 3D ED, diffraction patterns of individual particles are captured at different goniometer tilt positions, using continuous or stepwise acquisition methods. Then, a 3D reconstruction of the reciprocal space of the NPs can be obtained and used for subsequent crystallographic analyses, including determination of cell parameters, space group and overall structure (atomic positions and occupancies). We tested several data acquisition protocols, including continuous rotation 3D ED, precession-assisted 3D ED and serial ED, using different instruments and parallel beam sizes. For small NPs (10 nm or less), the capability to generate parallel beams matching the size of the particles enables the acquisition of high-quality data. With a diffraction volume considerably reduced, lower diffracted intensities are expected, and the use of hybrid pixel detectors is an asset to investigate the size limit of NPs for 3D ED by improving the diffraction signal-to-noise ratio. To meet these requirements, we used a JEOL F200 electron microscope equipped with an ASI Cheetah M3 hybrid pixel detector as our main experimental setup. Beam precession motion, when applied, was achieved using a Nanomegas DigiStar unit. We also found advantages in employing a Gatan Elsa cryogenic and tomographic holder.

Results
The model materials used in this methodological study were nanoparticles of TiO2 (brookite), In2-xSnxO3 (ITO), and MgTi2O5. To assess the capability to localize light elements, lithium was incorporated into the brookite TiO2 structure, resulting in the compound LixTiO2. The feasibility and efficiency of various data acquisition protocols are first established for different particle sizes. For larger NPs, around 100 nm in size, rapid automated data acquisition is possible for different beam sizes. However, for NPs around 10 nm in size, only precession-assisted 3D ED with a small parallel beam has proven effective for subsequent accurate structure refinement (see Graphic rectangle 1). In this case, it becomes possible to achieve atomic position precision on the order of 0.01 Å or 0.03 Å depending on whether we consider dynamical effects in refinement2 or not.
Another approach, similar to serial ED, was also successfully tested in cases where the NPs are subjected to uncontrolled movement induced by an external stimulus. In our example, where NPs are

set in motion by beam energy that modifies the amorphous support (see Graphic rectangle 1), the induced rotation of NPs can be used advantageously to collect hundreds of diffraction patterns from different NPs. Here again, an atomic position accuracy of about 0.03 Å was achieved, considering only kinematical approximation refinement.
In the final part of this contribution, we successfully applied the established 3D ED data acquisition protocol and analysis (i.e. precession-assisted 3D ED and 'dynamical refinement') to two case studies. Firstly, we explored the feasibility of localizing lithium atoms in LixTiO2 (x=0.4) brookite nanorods, which have a maximum width of approximately 100 nm (see Graphic rectangle 2). Second, we addressed the challenge of determining the ratio of magnesium to titanium atoms sharing the same atomic positions within a MgTi2O5 NP of about 30 nm in size (see Graphic rectangle 3).

Conclusion
Our research highlights the ability of 3D ED to perform accurate structural analysis of NPs as small as 10 nm. Although still not widely used, 3D ED is an advanced EM technique offering complementary information to that obtained through imaging or spectroscopy. As examples, we demonstrate the localization of light elements, such as lithium, and the determination of element ratios in atomic positions with a mixed occupancy (in this case Mg/Ti). We are confident that these findings will capture the interest of the community and enhance the understanding of crystallographic structures of new nanoparticles, which are vital for applications in energy materials and catalysis.

These results were obtained as part of the European project NanED (Electron Nanocrystallography – H2020-MSCA-ITN GA956099).

Keywords:

3D ED, nanoparticles, battery materials

Reference:

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Unsupervised Quantification of large EELS datasets

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims
Electron energy loss spectroscopy (EELS) has experienced substantial steps forward over the last decades in terms of energy resolution from monochromators, signal to noise and speed from improved detectors, vastly improved optics and stability in spectrometers and advances in the statistical treatment of the recorded data.
Yet, despite these efforts, EELS relies heavily on the interpretation skills of expert operators. This limits the widespread adoption of EELS and leaves room for experimenters' bias posing a significant reproducibility risk.
In this talk, I will give an overview of recent efforts towards the goal of an entirely autonomous data processing workflow, which could significantly improve the quantification of EELS spectra in terms of ease of use, precision and accuracy.
Methods
The method relies on model-based quantification as was e.g. implemented in EELSMODEL [1] but improves on several important aspects:

- The physical model is made entirely linear. This results in a single solution without the need for user provided initial parameter estimates.
- The background modelling process is significantly improved through a combination of a linearized model and constrained quadratic programming methods [2].
- The fine structure is described with a piecewise linear function with a nonlinear energy sampling to express that most significant ELNES features occur near the edge onset
- A complete Dirac-based database of open generalized oscillator strengths significantly improves the fit with experiments and provides a way to improve accuracy and precision. [3]

We investigate automatic identification of all EELS edges present in a dataset making use of neural network approach [4]. This allows building a complete model without any user input thereby completely removing the experimenter's preferences and opinions from the process. We discuss achieved precision approaching the statistical limit and show attempts to evaluate the accuracy with reference samples.

Results
The result provides a reliable quantification with highest possible precision and excellent accuracy and seamlessly provides background subtracted and deconvolved ELNES features without any user intervention. This is particularly interesting for high energy K-edges as they provide a direct lab-based alternative for XAS measurements even for edges that are traditionally considered to be far outside the achievable EELS energy range.

Conclusion
In summary, we believe that the days of manual processing of EELS data are over and fast and fully unsupervised data interpretation is available. Together with advances in EELS spectrometers, this widens the scope of EELS significantly and confirms its position as an indispensable analytical tool, also for non-expert users. It also satisfies the need for high reproducibility, essential in particular for industry applications.

Keywords:

EELS, model based fitting, signalprocessing

Reference:

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doi:10.1016/j.ultramic.2004.06.004
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https://doi.org/10.1016/j.ultramic.2023.113830
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[5] This project has received funding from the ECSEL Joint Undertaking (JU) under grant agreement No 875999. The JU receives support from the European Union's Horizon 2020 research and innovation programme and Netherlands, Belgium, Germany, France, Austria, Hungary, United Kingdom, Romania, Israel.

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MAX phase-based nanocomposites for LIBs negative electrodes investigated by multi-approach TEM analysis

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PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

Background incl. aims
In a landscape where the global demand for clean and reliable energy sources, and the need to mitigate the environmental impacts of fossil fuels, are constantly growing, lithium-ion batteries (LIBs) represent the most widespread energy storage technology, due to their reliability and excellent electrochemical performance. Nonetheless, the drawbacks of graphite, the most used negative electrode in LIBs, pose the need to study materials that present better outputs. In the limited landscape of alternative anodic materials, one of the most interesting is the family of MXenes: 2D materials with Mn+1XnTx stoichiometry (M represents a transition metal, X carbon or nitrogen, T the functionalization of the layers). However, these materials are obtained by etching the corresponding MAX phase precursor with HF, a seriously hazardous operation. To overcome the issue, we changed the paradigm and thermally treated Sn-doped MAX phases with different levels of Sn doping, obtaining peculiar MAX nanocomposites, and employing them as anodic materials in LIBs. [1] Since the key to understanding their electrochemical behavior lies in the morphology, crystal structure, and formation mechanism of the nanostructures, a multi-approach Transmission Electron Microscopy (TEM) analysis has been the main character in the investigation of the materials, consisting of static High-Resolution TEM (HRTEM), Operando TEM upon electrochemical materials lithiation [2] and pump-probe dynamic Ultrafast TEM (UTEM). [3]
Methods
The Ti3Al(1-x)SnxC2 MAX phase samples with nominal x = 0, 0.4, and 0.7 were synthesized through Spark Plasma Sintering (SPS). They were heated in air at 600°C and studied as anodic materials in LIBs by galvanostatic cycling with potential limitation (GCPL) in two-electrode coin cells. The sample were characterized by neutron and X-ray diffraction, CHNS, thermal gravimetric analysis, Scanning Electron Microscopy and Raman spectroscopy. Static HRTEM was executed with a JEOL JEM 2100 Plus operated at 200 kV at UNIMIB; Operando electrochemical TEM measurements were obtained with a JEOL JEM 2100 Plus at 200 kV at UNIST coupled to a Nanofactory Instruments in-situ Dual-Probe sample-holder used to apply bias and execute the lithiation. The pump-probe UTEM measurements are going to be acquired with a JEOL JEM 2100 coupled with a fs PHAROS laser in stroboscopic mode: the 1030 nm IR laser is both used as the pump and upconverted into UV light used to generate the photoelectron pulses used as the probe.
Results
The electrochemical performances show a very large improvement for the oxidized samples; moreover, the cells show a remarkably increasing capacity for a higher Sn content: it reaches 200 mAh g-1 for Sn0.4_Ox and 250 mAh g-1 for Sn0.7_Ox at 1 C, more than double to the non-Sn-doped material. Moreover, the samples showed an excellent value of Coulombic Efficiency (99.6%) and good stability upon cycling. The structural analysis evidenced the appearance of rutile and SnyTi(1-

y)O2 mixed oxides in the thermally treated samples, and the HRTEM imaging highlighted the morphology of such nanometric crystallites on the MAX phase surface as shown in Figure 1. Since the MAX phase is inert, the potential profiles from the GCPL suggest that for the LIBs the measured capacity is due to mixed mechanisms of intercalation, conversion, and alloying in the nanostructured oxide composite. The main contribution of SnO2 conversion and alloying has been confirmed thanks to the operando electrochemical TEM analysis, which shows the enlargement of the surface structures (clearly depicted in Figure 2), typical of the mentioned mechanisms.
According to our analysis, the presence of Sn lowers the MAX phase resistance to oxidation, giving a lower oxidation temperature and a higher percentage of oxides, which is confirmed also by CHNS; for a complete understanding of the process, as sketched in Figure 3, pump-probe dynamic UTEM measurements will be crucial to unveil the onset of the transient structural modification upon laser heating which leads to oxidation in the presence of oxygen.
Conclusions
Our study has highlighted that the unaltered conductive MAX phase cores show a beneficial role to the surrounding oxide nanoparticles since they can ensure sufficient electrical contact. In addition, as testified by the good stability of the samples, the SnyTi(1-y)O2 solid solution prevents the huge volume changes that pure SnO2 particles typically show, which cause an important loss of capacity after a few cycles due to active material shattering. Our investigation underscores the indispensable role of a diverse TEM analysis in elucidating the electrochemical behavior and structural transformations of the Ti/Sn oxides and Sn-doped MAX phase composite, paving the way for the rational design and optimization of such nanocomposites for next-generation LIBs.

Keywords:

Energy-Storage, lithium-metal-batteries, Ultrafast-TEM, pump-probe, operando-TEM

Reference:

[1] Ostroman, I., Ferrara, C., Marchionna, S., Gentile, A., Vallana, N., Sheptyakov, D., Lorenzi, R. & Ruffo, R. (2023). Highly Reversible Ti/Sn Oxide Nanocomposite Electrodes for Lithium Ion Batteries Obtained by Oxidation of Ti3Al (1-x) SnxC2 Phases. Small Methods, 7(10), 2300503.

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Many-body effects in the electron-atom ionization cross section in Quantum Electrodynamics

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Poster Group 1

The elemental composition of materials in the Transmission Electron Microscope is probed by inner shell ionization of atoms from highly energetic impact electrons. The ionization process is localized and can be well described by the electron-atom ionization cross section for the purpose of elemental quantification of TEM samples. The inelastic differential cross section is calculated in the context of Quantum Electrodynamics (QED) for a target of a neutral free atom, which is coupled to the QED Lagrangian as an external source. The beam-atom interaction factorizes and is treated perturbatively in QED, where the contributions of the transverse photons along with the Coulomb interaction are explicitly included. The multi-electron atomic transition currents are calculated in the context of the self-consistent Dirac-Hartree-Fock method. Hence, the relativistic effects on the atomic structure are explicitly included in this derivation. Higher order relativistic corrections of the electron-electron interaction within the atom are also assessed. The nuclear charge density which is used in the Dirac-Hartree-Fock calculation is modeled as a Fermi distribution. The many body effects are known to be particularly important for excitation processes in atoms, and they are shown to have significant impact on the shape of the cross section near the ionization threshold in Electron Energy Loss Spectra. The ionization energies are also calculated in this context and are shown to agree with experimental results.

Keywords:

EELS, Inner Shell Spectroscopy

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Determination of Magnetic Symmetries by Electron Diffraction

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Poster Group 2

Background incl. aims
Neumann's principle states that symmetries of physical properties (e.g., dielectric / piezoelectric / elastic tensor) derive from structural point (space) symmetry groups of the crystal lattice. The same can be applied for magnetism: the symmetry of magnetic properties (e.g., anomalous Hall effect, multiferroicity, altermagnetism) is determined by the magnetic point (space) group symmetry of the magnetic crystal. Consequently, the ability to determine such symmetries is crucial for the prediction of physical properties of new materials. Currently, the only probe for magnetic point and space group symmetries was neutron diffraction, requiring rather large samples and scarce beam time to be carried out.
It is well known, on the other hand, that convergent beam electron-beam diffraction methods can be used to determine spatial symmetries of crystalline samples, due to the direct relationship between the diffraction groups (symmetry group of convergent electron diffraction patterns) and the point (space) group of the sample [1]. In this work we employ group theory and electron scattering simulations to demonstrate an extension of CBED toward capable to determine magnetic point groups. The method would be applicable in Transmission Electron Microscopes with spatial resolution in the range of 10 nanometers.
Methods
Magnetic electron diffraction groups are composed of standard electron diffraction groups [1] plus time (magnetization) reversal symmetry. We used group theory to obtain and classify all 125 possible magnetic electron diffraction groups obtained by this procedure. The cornerstones for such a theory are the commutation of time-reversal with all other symmetry transformations and its antiunitary character. In a second step we establish the relations between the 122 crystallographic magnetic point groups and the electron diffraction groups for different zone axis orientations of the sample in the electron diffraction experiment.
The verify the theory, dynamic electron scattering simulations for samples with different magnetic point group symmetries and crystal orientations were conducted to obtain convergent beam electron diffraction patterns with magnetic Bragg discs.
Results
We provide the group theory for convergent beam electron diffraction of magnetic crystals and give a complete mapping of magnetic point groups to corresponding diffraction groups for all possible crystal orientations. We conduct electron scattering simulations for selected antiferromagnetic samples (NiO, etc.) in order to verify the group theoretical considerations. It is shown that obtained symmetries from simulated electron diffraction images correspond to the expected magnetic point groups of the samples.
Conclusion
This work proposes convergent beam electron diffraction as an alternative of neutron diffraction for unambiguous determination of magnetic point group symmetry. With a given map of magnetic point

groups to corresponding electron diffraction groups one can determine the latter from a finite series of diffraction experiments at different crystal orientations. Simplicity, accuracy and relatively small spatial resolution of a proposed method makes it an intriguing candidate for magnetic symmetry detection and investigation.

Keywords:

magnetic symmetry groups, diffraction groups

Reference:

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Optical and acoustic plasmons in the layered material Sr2RuO4

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PS-10, Lecture Theater 5, august 26, 2024, 15:00 - 16:00

Background incl. aims
In conventional metals, the conduction electrons show Fermi-liquid behavior with a characteristic quadratic dependence of the resistance on temperature. In contrast, in so-called “strange” metals the Fermi-liquid behavior breaks down due to strong correlation of the charge carriers, which has various consequences, e.g. a linear dependence of the resistance on the temperature. On the other hand, “strange” metals show unconventional and partially also high temperature superconductivity. Since the latter is supposed to be related to the non-Fermi-liquid behavior, the electronic structures of “strange” metals are of great interest in the field of solid state physics. Here we study the charge density-density response connected with plasmon excitations in the layered material Sr_2RuO_4, a representative of the class of “strange” metals [1]. We also wanted to test a theoretical prediction based on holography calculations [2] that plasmons in “strange” metals are overdamped.
Methods
Focused ion beam milling was used to prepare a lamella in which the layers are oriented perpendicular to the surface. This lamella was investigated using momentum resolved electron energy-loss spectroscopy (M-EELS) in a monochromized transmission electron microscope. Plasmon excitation in dependence on the momentum parallel and perpendicular to the layers was measured almost over the whole Brillouin zone [see Fig. 1 (a)] with a spectral and momentum resolution of 120 meV and 0.04 1/Å, respectively. The experimental data was compared to theoretical predictions of the Fetter model [3] and with the susceptibility χ_0 calculated from a tight binding band structure, all derived in terms of mean-field random-phase approximation.
Results
The M-EELS data show dispersing plasmon excitations below 1.8 eV with a continuous transition between the optical plasmon at qc=0 [purple squares in Fig. 1 (b)] and the acoustic plasmon at qc=0.4 1/Å [red squares in Fig. 1 (b)] close to the edge of the Brillouin zone at qc=0.49 1/Å. All curves are generally in good agreement with the theoretical calculations within the Fetter model taking into account a coupled system of 2D plasmons in the layered crystal structure. However, for qa ≥ qcrit there are differences between the theoretical curves and the experimental data, which can be explained by Landau damping [see open symbols in Fig. 1 (b)]. Here, qcrit is the momentum at which the dispersion curves merge into the single-particle-particle continuum χ_0 [see gray shaded regions in Fig. 1 (b)].
As predicted by the Fetter model, the measured dispersion at qc=0.4 1/Å extrapolates approximately to zero energy, which is characteristic for an acoustical plasmon. A fit of the experimental plasmon data to the Fetter model reveals that the dispersion is determined by the spacing of the layers and is only weakly influenced by many-body interactions.
Conclusion
The long wavelength qa dispersion of the qc dependent plasmons, including the optical and the acoustic collective excitations, resembles the data of the Fetter model, i.e., that of coupled 2D

plasmons. Moreover, the critical momentum qcrit, at which the experimental data starts to deviate from the theoretical curves, agrees well with the calculated susceptibility χ0. In summary, all data can be well explained within the framework of a mean-field random-phase approximation model. On the other hand, we found no evidence supporting theories predicting an overdamped plasmon, i.e., a transition of the plasmon into a featureless, momentum-independent continuum already at very small momentum [2].

Keywords:

EELS, Plasmonics, Layered Materials

Reference:

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Single-crystal and pentatwinned nanorods reverse the handedness of chiral plasmonic nanocrystals: an electron tomography study

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background and aims.
Nanomaterials with chiroptical properties absorb differently left- and right-handed light, an asymmetry that is reinforced by the local surface plasmon resonance of metallic nanoparticles. The synthesis of such chiral particles with controlled shapes and optical properties is of major interest for a host of potential applications including bio-sensing, therapeutics or catalysis, and can be realized by transferring chirality from an organic inducer to the inorganic material in seed-mediated colloidal synthesis [1,2]. Usually, the handedness of the inducer is thought to be transferred to the product [1]. However, recent studies showed that the geometry of the achiral seeds can also influence handedness, even if the same inducer is used [3], and it remains unclear if such effect is related to morphological or structural (surface facets, twin boundaries, defects, …) properties.
Here, we aimed to understand the effect of the seeds on the growth of chiral features. Specifically, we investigated the growth starting on well-defined single-crystal (SC) or penta-twinned (PT) gold nanorod (NR) seeds. To uncover the complex morphological changes at the nanoscale, we used electron tomography (ET), a method that enables three-dimensional (3D) reconstructions down to the atomic scale. In two different synthesis protocols, micelle-templated or chemically induced, 3D reconstructions and nanoscale morphological analysis showed that the final products had reverse handedness and strong morphological differences when using PT or SC seeds [4].
Methods.
We studied the growth of chiral features on well-controlled achiral gold seeds synthesized by wet chemistry. The two systems investigated here were PT NRs featuring five {100} lateral facets and caped by {111} facets at the tips, and SC NRs with an octagonal cross-section, {520} lateral facets and {110} and {100} tips. Chiral features were grown by two protocols. The chemically induced pathway used the chiral L-cystine (L-cys) amino-acid, which was previously reported to yield highly twisted structures on SC seeds [2]. The micelle-templated protocol used chiral, worm-like micelles formed by (S)-1,1’-binaphthyl-2,2-diamine (S-BINAMINE) and cetyltrimethylammonium chloride (CTAC), that are known to yield wrinkled, helical, NRs [1]. The optical properties of the seeds and of the products were characterized by extinction spectroscopy and circular dichroism (CD) measurements.
To characterize the morphology and structure of the final products, we used ET, whereby projection images acquired at incremental tilt-angles are used to compute a 3D reconstruction of the object. Tilt-series were acquired in 2-3° increments and in scanning transmission electron microscopy (STEM) high angle annular dark field (HAADF) mode to provide mass-thickness contrast and meet the requirements for accurate reconstructions. The reconstructions were computed using an expected maximization (EM) algorithm or a constraint SIRT approach developed in-house and implemented in

Matlab with the ASTRA toolbox. Morphological analyses included helicity measurements and orientation tracking. Helicity uses a surface mesh extracted from reconstructions to compute a pseudoscalar metric indicating how close to a perfect helix the shape is and its handedness (positive helicity means right-handed, negative means left-handed) [5]. Orientation tracking reveals the dominant orientation of features around a NR to study the local variations of chirality and was implemented using a combination of the ImageJ OrientationJ plugin and in-house code.
Results.
Regardless of the synthetic pathway, starting from PT or SC seeds resulted in products with opposite signs in CD spectra, indicating a reverse handedness. Strong discrepancies were further seen in the morphology of the particles, as reconstructed in ET. With the L-Cys protocol, SC seeds resulted in strongly twisted NRs with clear right-handed morphology (Figure, a). This observation was supported by helicity measurements, showing strongly positive values. On the other hand, PT seeds resulted in poorly defined, highly complex shapes with no obvious handedness (Figure, b). Helicity measurements were typically weak, with a complex combination of right and left-handed features in each particle leading to some NRs exhibiting a dominantly left handedness and others with right handedness (Figure, b). This observation suggests that optical handedness is not limited to helical features. Overall, a strong influence from the seed crystal structure was expected in this growth protocol, as chirality emerges through the preferential growth of chiral facets stabilized by the chiral inducer. More surprisingly, micelle-templated NRs grown from PT seeds also had an opposite sign in CD spectra as compared to their SC counterpart. ET reconstructions showed closely resembling wrinkled morphologies in both cases, with a major difference in the orientation of the wrinkles (Figure, c, d). PT NRs showed left-handed wrinkles, coherently with positive g-factor plots, while SC NRs showed right-handed features. Helicity measurements confirmed this observation by showing close absolute values but opposite signs.
To further probe how the morphology of the seeds was impacting the final micelle-templated products, we assessed if the presence of corners and lateral facets influenced the wrinkle morphology beyond the global particle handedness. Notably, the tips featured better aligned wrinkles on SC NRs, and wrinkles on the lateral facets grew at different angles. Tracking the dominant orientation of wrinkles around the particles also revealed that PT NRs retained a 5-fold symmetry with alternating areas of flat and oriented wrinkles (Figure, c, d). In contrast, SC NRs had a 4-fold symmetry with similarly alternating flat and oriented wrinkles, coherent with an intermediate with square cross-section we previously identified. These observations confirm that the way micelles adsorb on the seeds' surface is influenced by geometrical (angles, number of facets) or crystallographic (facet index) consideration, in turn impacting the orientation of wrinkle growth and, likely, the handedness of the particle.
Conclusion.
We used ET and detailed nanoscale morphological analyses to show that the nature of the achiral seed in chiral seeded growth has a major influence on the handedness of the products and on their chiroptical properties. Furthermore, this observation in micelle-templated growth shows that wrinkle growth is not random but influenced by geometrical or crystallographic factors. This work enhances the toolbox for controlling chirality at the nanoscale and demonstrates the benefits of ET in linking morphology and optical properties, supporting the development of novel optical nanomaterials.

Keywords:

TEM, tomography, chirality, plasmonics, nanorods

Reference:

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Characterization of Aberration-Corrected Lorentz TEM Applying a Magnetic Field with Objective Lens

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Poster Group 1

Lorentz microscopy is one of the powerful observation methods without the influence of magnetic field from the objective lens (OL) of a transmission electron microscope (TEM). Normally, the magnetic field around the TEM specimen is about 2T. This field could affect the observation of magnetic material. To avoid the influence, OL is turned off and the lens under the OL is used as focus lens, where it is called an objective mini-lens (OM) in JEOL instrument. We can use the OL to apply some amount of magnetic field to samples in Lorentz microscopy [1]. Recently, the spherical aberration correction was applied to Lorentz microscopy, the resolution was improved [2]. In addition, the applied magnetic field with OL is possible with spherical aberration correction. In this research, we characterized the detailed capabilities of spherical aberration-corrected Lorentz TEM while applying magnetic field by the OL.

In this time, JEM-ARM300F2 with TEM spherical aberration corrector was used. The pole-piece was wide gap pole-piece. The TEM resolutions with / without spherical aberration correction were investigated in 300kV. The varied resolution with applying the magnetic field and the field of view were also measured at that time. The aberration measurement was done by diffractogram tableau method. The correction area was about 4mrad and the third order spherical aberration coefficient of OM was under 10mm.

The Young's fringe test was performed to check the TEM resolution. The resolution without spherical aberration correction was 1.83nm without applying the magnetic field, 1.75nm with 0.5T and 1.61nm with 1T. On the other hand, they were improved with spherical aberration correction. 0.76nm, 0.67nm and 0.58nm with 0T, 0.5T and 1T were achieved. The effective field of view (FOV) was changed as applying magnetic field with objective lens. FOV was decreased as a function of the applied magnetic field. They were measured to be 200µm with 0.3T, and 20µm with 1T. This limitation was occurred by replicating of electron beam. The replicating was caused by the aberration of imaging system. On the other hand, the ratios of magnification were increased 6% with 0.5T and 45% with 1T.

The principal plane of complex lens was getting closer to the sample plane as applying magnetic field. The complex lens was formed by OM and OL. This lens improved the spatial resolution, and this result showed the resolution was limited by the chromatic aberration.

We have addressed making a setting of aberration-corrected Lorentz TEM imaging mode. The TEM resolution was improved by spherical aberration correction of OM. Additionally, applying the magnetic field by the OL, the resolution was also slightly improved. The magnification and FOV were changed as a function of applied magnetic field. In the presentation, which condition could be the better for imaging a magnetic sample will be discussed.

Keywords:

Lorentz, Aberration correction, Magnetic field

Reference:

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Quantification of chirality from electron tomography data

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Poster Group 1

Background and aims.
Some optical nanomaterials exhibit the intriguing property of absorbing differently left- and right-handed polarized light. This optical handedness often arises from morphological chirality, the fact that mirrored shapes do not superimpose, but the quantitative relationships between morphology and optical properties are poorly understood. Establishing this knowledge requires accurate characterization the nanomaterials' morphology, as well as the calculation of chirality descriptors relevant to the optical properties of interest [1]. A prime example of such combination is the quantification of helicity from electron tomography (ET) three-dimensional (3D) reconstructions, which we previously presented [2]. Applied to wrinkled or twisted geometries such as those of chiral Au nanorods (NRs), this method informs about the helical character of the NRs and their handedness and is an excellent predictor of the sign of their circular dichroism (CD) spectra. However, helicity is a radially averaged measure, which limits its local interpretation, and entails strong geometrical assumptions, which limits its value for non-helical shapes even as they may still be chiral and exhibit clear optical handedness.
Here, we explored how different descriptors of chirality obtained from ET reconstructions can complement each other to link nanoscale morphology and optical handedness. To understand how local chiral features were varying, we first implemented a measure of their orientation, providing insights into both global and local handedness [3]. To overcome geometrical assumption, we then studied methods quantifying asymmetry on the basis of the difference between a geometry and its mirror image, including the Hausdorff chirality measure [1,4]. We finally tested if, beyond individual metrics, chiroptical performance could be statistically linked with a learned combination of morphological and chirality descriptors.
Methods.
As a prerequisite to study and establish chiral descriptors, a large dataset of ET reconstructions of chiral Au NRs was assembled. The particles in this dataset were typically 50-150 nm-long and were synthesized by seed-mediated growth. Two synthesis pathways were included: micelle-templating, whereby chiral micelles coil around achiral Au NR seeds, resulting in the growth of wrinkled particles whose helical morphology is characterized by a narrow pitch and a small helical orientation; chemical-inducing whereby chiral molecules such as amino-acids induce the preferential growth of high order, chiral surface facets. This latter approach yields highly helical, twisted structures with long pitches and high helical orientations when starting from single-crystalline seeds, but also strongly deformed, asymmetrical structures without clear geometrical handedness nor helical

character but a handed optical signal when starting from pentatwinned seeds. In addition, the dataset includes the optical properties of all batches from which the particles are sampled, including absorption and CD spectra.
ET tilt-series were obtained in high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) and typically span 130-140° in 2 or 3° increments. 3D reconstructions were performed using the SIRT, expected maximization (EM) or constrained SIRT algorithms with in-house code or the ASTRA toolbox.
Surface orientation was computed using the ImageJ plugin OrientationJ while other computations and the statistical analyses were performed in python or Matlab with in-house code.
Results.
We first investigated how to obtain local analysis of the orientation of chiral features. Typically, chirality measures provide summary data, with a single metric indicating the degree of chirality and/or the handedness. Here, the orientation of chiral features on Au NRs was tracked and analyzed with spatial accuracy around the NR, evidencing local variations that were not visible when analyzing helicity (Figure, a-d).
To further quantify the chirality of non-helical structures, we investigated methods based on the calculation of the difference between a reconstruction and its mirror image. Such a descriptor effectively quantifies asymmetry, without considerations of handedness, and the difference is typically evaluated on the basis the Hausdorff distance, yielding the Hausdorff chirality measure. We found that optimizing for the minimum Hausdorff distance as required to calculate the measure requires long computation times if the full surface is used, limiting the applicability to simple shapes. As an alternative, we used a shape overlap measure (Figure, e-g), which can be calculated at a fraction of the cost. This method provided results similar to the Hausdorff chirality measure for classifying particles on the basis of their asymmetry and demonstrated that the morphology of non-helical structures could still be highly chiral (Figure, g-h), thus complementing helicity and orientation-based analyses.
Although powerful because of their simple interpretation, orientation, helicity and chiral distances are descriptors that provide part of the information only. We finally investigated if a combination, rather than a single descriptor, can predict optical properties from morphological data. Given the vast amount of data accumulated by our lab in the recent years, we will present how such combination could be learned statistically from ET reconstructions and the corresponding optical spectra.
Conclusion.
Beyond helicity, orientation tracking and asymmetry quantification provide insights into different aspects of morphological chirality. The key towards fully characterizing chirality might be in their adequate combination. Focusing on interpretable descriptors, this work expands the toolbox for microscopists to quantify chirality from ET reconstructions and paves the way towards establishing quantitative relationships between the morphology of chiral nanomaterials and their optical properties.

Keywords:

ET, tomography, plasmonics, chirality, descriptors

Reference:

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Femtosecond electron beam probe of ultrafast electronics

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

Background and Experiment

The need for ever-faster information processing requires exceptionally small devices that operate at frequencies approaching the terahertz and petahertz regimes. However, electronics itself is too slow to characterize a record-breaking device. Here we show how ultrafast electron beam probe with terahertz-compressed electron pulses can directly sense local electro-magnetic fields in electronic devices with femtosecond, micrometre and millivolt resolution under normal operation conditions [1].

Figure 1 shows the concept of our experiment. We use a femtosecond laser (red) that creates femtosecond electron pulses (grey) with only one electron per pulse. This electron pulse is then further shortened in time to less than 100 fs by using all optical terahertz compression [2]. These ultrashort electron pulses probe the local electromagnetic fields in our device under test (DUT), which is shown in Figure 1. We create a short voltage pulse by exciting a photoconductive switch. The generated femtosecond voltage pulses (magenta) travel through our device under test and the device response deflects the electron beam as a function of space and time. From the resulting electron beam deflections, we then obtain the strength and direction of the local electromagnetic fields [1]. The electron beam does not distort the dynamics under investigation and the measurement is therefore contact-less and impedance-free.

Results and outlook

In a proof of principle experiment, we investigate a coplanar waveguide which is the standard transmission line in high-frequency electronics. We fabricate the structure by lithography and drill two holes into the substrate for investigation. We observe the impulse response, signal reflections, attenuation and waveguide dispersion directly in the time domain. We find that the experiment has a sensitivity to electric potentials down to tens of millivolt or -20 dBm. With our 100-fs electron pulses, the bandwidth exceeds 10 THz which is well sufficient for almost any modern or near-future circuitry. In order to eventually obtain a full movie in space and time, we also report the design of an ultrafast transmission electron microscope with terahertz compression [3]. The use of an optical cavity with magnetic or electric nodes at the point of electron-terahertz interaction provides an almost aberration-free control. In this way, we can reach nanometer spatial resolution and reach even shorter electron pulse lengths down to 19 fs [3].

Our femtosecond time resolution and the capability to directly integrate our technique into existing electron-beam inspection devices in semiconductor industry make our femtosecond electron beam probe a promising tool for research and development of next-generation electronics at unprecedented speed and size.

Fig. 1. A laser pump pulse (red) creates a voltage pulse (magenta) by closing a photoconductive switch. This pulse then travels into the device under test and triggers its operation. Femtosecond electron pulses (grey) probe the local electric and magnetic field (magenta) [1].

Keywords:

Ultrafast electron microscopy, THz electronics

Reference:

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BACKSCATTERED ELECTRON AND X-RAY IMAGING FOR ARRAY TOMOGRAPHY PROVIDES RAPID SPECIMEN CHARACTERISATION AND ROI TARGETING

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Poster Group 1

Background: Array tomography is a flexible volume electron microscopy (vEM) workflow due to its non-destructive nature, multi-scale imaging (mm to nm sized imaging regions) and well-established correlative light and electron microscopy (CLEM) compatibility. Sections are collected on a solid, conductive support, and can be imaged multiple times at different resolutions.
There is a constant evolution and improvement of protocols/techniques for array tomography. Acquisition bottlenecks have pushed the development of workflows to tackle imaging efficiency. However, establishing standards for reproducible specimen preparation remains unsolved. There are a wide variety of preparation methods, contrasting techniques, and individual heterogeneity that affect uptake of stains and the visualisation of ultrastructure. This makes it difficult to directly compare experiments and different types of specimens, which can have repercussions on the reconstruction of vEM data. Quantitative compositional analysis, such as energy dispersive x-ray spectrometry (EDS), can help to identify common baselines and standards, positively impacting comparative data analysis and potentially facilitating automated segmentation of volume data.
We present Backscattered Electron and X-ray (BEX) imaging for the simultaneous and combined acquisition of backscattered electron (BSE) and x-ray data in a scanning electron microscope. BEX acquires ultrastructural and composition data simultaneously, providing fast and automated mapping across a large area of sample.

Methods: We used Unity (a BEX detector, Oxford Instruments, UK) combined with an Ultim 100 (Oxford Instruments, UK) to image array tomography slices of mouse brain tissue prepared with an adapted ROTO protocol. Large areas were collected automatically, using cartography mode, in a Zeiss 460 (Carl Zeiss Microscopy GmbH, Germany) operated at 8kV, 1-2nA probe current, at 7.5mm WD, 10µs dwell time.

Results: BEX cartography data achieved high resolution and fast mapping of array tomography brain slices. Large areas were imaged with a relatively short beam dwell time (10µs), which reduces beam damage, drift, and resin charging (Figure 1). Elemental information was acquired simultaneously and provided chemical differentiation that can be used to further distinguish between sample features, opening the possibility of improving subsequent segmentation of data. BEX also provided information about strain distribution, which EDS quantified. Being able to measure the amount of stain taken up by the sample improves our ability to make direct comparisons between samples and also enables us to optimise our sample preparation techniques.

Figure 1. Large area 7x8 tiles acquired using cartography mode. Top inset displayed at 50% zoom allows to easily distinguish different cell borders. Bottom inset at 100% zoom with a 10nm pixel size with detailed membranes in mitochondria and small vesicles (15-30nm range).
Conclusions: Creating reference specimens for the vEM community has been previously proposed, as benchmarks to compare microscopes, imaging conditions and image segmentation tools. As a high speed and sensitive imaging technique, BEX opens the way towards controlled reproducibility by complementing array tomography ultrastructural images with chemical information.

Conclusions: Creating reference specimens for the vEM community has been previously proposed, as benchmarks to compare microscopes, imaging conditions and image segmentation tools. As a high speed and sensitive imaging technique, BEX opens the way towards controlled reproducibility by complementing array tomography ultrastructural images with chemical information.

Keywords:

Array tomography, Backscattered Electron X-ray

Reference:

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Fabrication of 2-dimensional disordered assemblies of gold nanoparticles and investigation of localized surface plasmon resonances

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Poster Group 2

Background incl. aims
Interaction of electromagnetic waves, such as electron beams or light, with conductive material can lead to localized surface plasmon resonances (LSPRs) where the incoming energy can be deposited in a collective excitation of electrons of the conduction band, which in turn can result in coherent localized plasmon oscillations. LSPR in metallic nanostructures, such as nanoparticles (NPs), which are sensitive to geometry, material composition and environment, are currently utilized in a wide range of applications, such as surface-enhanced Raman spectroscopy, plasmonic wave guides, improved solar cells, on-chip particle accelerators and nanoantennas. A host of studies that focus on plasmonic NPs ranging from single NPs with several shapes (cubic, spherical, tetrahedral) over 1D assemblies of NPs such as chains [1], to ordered 2D assemblies of NPs [2] show an increase of the complexity regarding the hybridization behavior of LSPRs eventually lead to delocalized Surface Plasmons. Furthermore, Anderson predicted in 1977 [3] the absence of diffusion or delocalization of waves in disordered systems, which has been discussed as the underlying mechanism for LSPRs localization in disordered metallic thin films and ultrathin 2D networks [4]. Our aim is to further develop these studies on the surface plasmon localization in disordered structures by (1) developing a novel NPs assembly fabrication method that allows fabricating disordered assemblies of NPs of a wide range of NPs sizes, and (2) probing the LSPR with high-resolution electron energy-loss spectroscopy (EELS). Moreover, the dominant dipolar interaction between the NPs, also facilitates an efficient numerical modeling of these systems, which in comparison with the experiments allows for an in-depth study of the impact of various geometric parameters as well as retardation and life-time damping on the observed localization behavior.

Methods
To synthesize 2D disordered assemblies of gold NPs on a TEM transparent silicon oxide substrate, a new synthesis routine was developed. This procedure is based on sublimation and redeposition of a gold microparticle precursor induced by an electron beam in a scanning electron microscope (SEM) operated at 30 kV. To characterize the assembly of synthesized NPs in terms of size, shape and spreading over the substrate, TEM measurements were conducted subsequently. To study LSPRs experimentally, EELS in scanning transmission electron microscopy (STEM) mode was carried out. The numerical modelling of LSPRs was performed using a self-consistent dipole model.

Results
The synthesized 2D disordered gold NPs assemblies (see Fig. 1a) exhibit a gradient in the NPs mean size, which ranges from 100 nm close to the precursor location down to 2 nm at a distance of more than 20 µm from the precursor location. Additionally, the interparticle distance between the gold NPs increases with increasing distance to the precursor location.

The experimental investigation (see Fig. 1b) as well as the numerical simulation (see Fig. 1c) of the LSPRs demonstrate a localization behavior that decreases toward larger energies, which is driven by the disorder of the NPs assembly (mainly the random particle distance). That localization behavior stays in contrast to what was found in ultrathin 2D gold networks showing increasing of localization towards higher LSPRs energies. By varying the geometric parameters of the NPs assembly in the simulation, we could identify the NPs thickness as the parameter, that determines the energy-dependence of the localization. Specifically, a critical thickness of approx. 10 nm separates the two localization regimes, which correlates to the energy of the dipole mode resonance crucially depending on the thickness of the NPs.

Conclusion

2D disordered assemblies of gold NPs of a wide range of NPs sizes and distances can be synthesized directly on thin substrates facilitating structural characterization and EELS measurements in a TEM. It could be shown that such assemblies exhibit LSPRs with a localization behavior that may be tuned by the NPs sizes (including thickness) and interparticle distances. The proposed synthesis of random NPs assemblies opens new avenues for fundamental studies on Anderson localization in disordered plasmonic structures as well as its applications such as surface-enhanced Raman spectroscopy where localization behavior must be tuned to specific wave lengths.

Keywords:

SEM, TEM, LSPR, Au-NPs-Synthesis, Disordered-Assemblies

Reference:

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Exploring the infection cycle of Vaccinia virus using 3D EM with the FIB-SEM

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LS-07 (1), Lecture Theater 4, august 28, 2024, 11:30 - 12:30

Background incl. aims
Since its successful use as a live vaccine to eradicate Variola virus, the etiologic agent of smallpox, Vaccinia virus (VACV) has become a model to study the mechanisms underlying key processes of infection by poxviruses. At early stages of infection, viral genome replication takes place in the replication compartments, also known as viral factories. Following early viral genome replication, these viral factories subsequently seem to dissociate concomitant with VACV assembly that rely on a unique membrane acquisition mechanism. In brief, assembly of new virions involves the recruitment of vesicles from the endoplasmic reticulum, which are then ruptured-open and associate as crescents with open membrane ends. After packaging of the viral genome, the crescents are closed, forming the spherical immature virions. We aim to decipher the remodeling of VACV-infected cells during the stages of virion assembly by 3D reconstruction using the FIB-SEM technology.

Methods
Cells are cultured and infected with VACV Western Reserve strain on glass coverslips, followed by fixation using aldehyde. Subsequent steps involve post-fixation and staining with osmium tetroxide and uranyl acetate. After dehydration with an ethanol gradient, the cells are embedded in araldite resin and polymerized for 48 hours at 60°C. The resin blocks are then mounted on the FIB-SEM stub using argent lacquer, with cells protected by a platinum layer. To acquire a 3D volume, the slice-and-view approach is employed, starting with acquisition of an image stack. Subsequently, the raw images are pre-aligned using the SIFT algorithm through translation to create a template. Then, the raw data is aligned with the template created using affine transformations. Once the volume is obtained, segmentation can be performed using various software tools for subsequent data analysis.

Results
Spatial reorganization of VACV-infected HeLa cells was investigated through the acquisition of slices from a 3D volume at 8 hours post-infection, employing a FIB-SEM Cross-beam 550 system. The images, captured using electrons backscattered by the EsB detector, exhibited a pixel resolution and slice thickness of 15 nm each. Within the acquired volume, discernible changes in the organization of the endoplasmic reticulum (ER) were observed, indicative of the VACV viral replication compartment. Additionally, crescent-shaped membranes were evident in the assembly compartment, suggesting ongoing viral assembly processes. . These findings are presented in 3D format following segmentation of the various compartments and VACV virions.

Conclusion
We successfully optimized the sample preparation protocols for 3D EM analysis by FIB-SEM of VACV-infected HeLa cells. Additionally, we established an imaging workflow and implemented data acquisition of large volumes using the FIB-SEM slice and view approach. Through these efforts, we elucidated the virus-induced cellular reorganization, revealing the presence of the viral replication compartment, along with assembly involving both immature and mature virions.

Keywords:

FIB-SEM, Vaccinia Virus, ER Segmentation

Reference:

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The geometry of STEM tomography

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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Electron tomography (ET) by scanning transmission electron microscopy (STEM) [1], is a mature technique which has become part of the standard repository of tools available to electron microscopists in the physical sciences [2]. The acquisition of datasets is often carried out using commercially purchased automated acquisition tools, available from a number of microscope [3], accessory manufacturers or freeware solutions [4]. These automated tools save time, minimise dose, reduce the potential for error and prevent the operator dying from boredom. Whichever package is used for the acquisition they tend to follow a set approach, codified during the development of STEM tomography in the early 2000's [1]. This, while it works, has a number of problematic characteristics that have never been addressed in subsequent studies. One of these problems is the mismatch between the relative geometries of the STEM acquisition and that which is ideal for tomographic reconstruction. This is a consequence of choosing the scan rotation to place the fast scan direction parallel to the tilt axis; usually to allow the use of dynamic focussing. This choice just happens to maximise the degradation of the quality of the data due to specimen drift. The nature of this mismatch will be explored and demonstrated using both theory and experiment. The theory will be demonstrated with phantoms and the experiments will apply "forced" drift to accentuate the issues. A number of different solutions will be suggested and demonstrated. The potential for improved quality in reconstructions will be explored and discussed.

Keywords:

Tomography, STEM

Reference:

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4D-STEM/PNBD: Fast and easy powder electron diffraction in SEM

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Poster Group 2

Background: We have developed a new method, named 4D-STEM/PNBD (i.e., four-dimensional scanning transmission electron microscopy/powder nanobeam diffraction), which can convert a modern SEM microscope to a simple, fast, and user-friendly powder electron diffractometer [1, 2]. The only hardware requirement is that the SEM microscope must be equipped with a 2D-array detector of transmitted electrons (also referred to as 2D-array STEM detector or pixelated STEM). In 4D-STEM/PNBD, we reduce a huge and complex 4D-STEM-in-SEM dataset to a single 2D powder diffraction pattern (as shown in the attached figure). The final powder diffraction patterns are equivalent to those from TEM/SAED as documented in our previous studies [1-3]. They can be compared to theoretically calculated powder X-ray diffraction patterns (PXRD) in order to identify the investigated nanocrystals [2, 3]. This contribution deals with the recent improvements of our method, which should make it even more user-friendly and robust [3, 4].

Materials and methods: We performed TEM/SAED (selected area electron diffraction) and 4D-STEM/PNBD measurements on three types of nanocrystalline samples on an electron-transparent carbon film. The samples differed by signal-to-noise ratio (SNR), where signal and noise are represented by the intensity of diffraction peaks and the amorphous background, respectively. The samples could be grouped as follows: (i) Au nanoislands with high SNR, (ii) GdF3 and TbF3 nanocrystals with good SNR, and (iii) magnetic iron oxide nanoclusters with or without amorphous silica shell with intermediate SNR. The 4D-STEM/PNBD calculations were performed with recent version of our open-source Python libraries STEMDIFF (conversion of 4D datasets to 2D powder diffraction patterns; https://pypi.org/project/stemdiff) and EDIFF (conversion of 2D-diffraction patterns to 1D radially averaged diffraction profiles and their comparison with theoretically calculated PXRD; https://pypi.org/project/ediff).

Results and discussion: Our initial studies [1-3] showed that the 4D-STEM/PNBD works very well for reasonably small, highly diffracting crystals with low absorption. If the crystals are thicker and/or surrounded by amorphous matrix, the extraction of 2D powder diffractogram can become difficult or impossible. This results from the lower-energy electrons in SEM (E < 30 keV), which suffer from higher absorption and inelastic scattering than the higher-energy electrons in TEM (E > 100 keV). The better 4D-STEM-in-SEM datasets and, subsequently, the higher-quality 2D powder diffractograms can be obtained with better hardware (i.e. better SEM microscope and/or pixelated STEM detector), better experimental parameters (optimized scanning speed, dwell time etc.), and better software (i.e. data processing). For given hardware and optimized experimental conditions, the decisive factor is the data processing. Consequently, we made several improvements of our STEMDIFF software. At first, we introduced better, multi-criteria filtering of the raw 4D-STEM-in-SEM dataset. The better filtering allows us to select the highly-diffracting locations and ignore the rest, which contain high noise. Moreover, it enables us to get the better estimate of the point-spread-function (PSF) of the

primary beam, which can improve the quality of the individual diffractograms by means of 2D-PSF deconvolution. At second, we increased the speed of the above-mentioned time-consuming 2D-PSF deconvolution step ca 5x by introducing multicore processing. Last but not the least, we improved the user interface and extended the original software package STEMDIFF (the conversion of 4D-datasets to 2D diffractograms) with a sister package EDIFF (the conversion of 2D diffractograms to 1D profiles and their comparison with theoretically calculated PXRD patterns). The recent versions of STEMDIFF and EDIFF employ the well-established Jupyter notebooks as interactive templates for complete data processing (4D → 2D → 1D) without any third-party software. The notebooks are written and documented in such a way that the data could be processed in step-by-step way by any SEM user without detailed knowledge of diffraction theory. The above-listed improvements enabled us to process not only the 4D-STEM-in-SEM datasets of strongly diffracting samples with high SNR (such as Au nanoislands), but also samples with intermediate SNR (such as iron oxide nanoclusters enveloped with amorphous silica). The samples with low and poor SNR remain as a challenge for our ongoing work, which comprises advanced 2D-PSF deconvolution methods and automated, machine learning-based noise reduction.

Conclusion: The 4D-STEM/PNBD method brings a simple, fast, and easy-to-use electron diffraction technique to SEM users. The classical SEM microscopes offer imaging modes (such as SE or BSE) and spectroscopy modes (such as EDX). The modern SEM microscopes equipped with pixelated STEM detectors add also the third mode – the electron diffraction. The pixelated detectors can be installed in a common SEM port like any other detector. The 4D-STEM datasets are easy-to-collect, but difficult-to-process – at least for non-crystallographers. Our STEMDIFF package reduces a complex 4D-STEM dataset to a simple 2D diffractogram. The sister EDIFF package enables a fast conversion of 2D diffractogram to radially averaged 1D diffraction profile and its comparison with theoretically calculated PXRD diffraction pattern. Both packages aim to be as user-friendly as possible, so that the new 4D-STEM/PNBD method could be employed by all users of SEM microscopes.

Acknowledgement: Project TN02000020 (TA CR) and Thermo Fisher Scientific company for a high-resolution SEM with pixelated detector installed at ISI CAS.

Keywords:

4D-STEM-in-SEM, powder electron diffraction, nanocrystals

Reference:

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In situ size dynamics and manipulation of nanoparticle interaction under electron beam irradiation

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Poster Group 1

Background
The size-dependent properties of gold (Au) nanoparticles (NPs) have unique applications in various fields including science and technology ranging from environmental sensing and catalysis to drug delivery and biomedical imaging due to their excellent catalytic, electronic and optical properties. The practical applications of AuNPs in all fields usually require external stimuli, such as electron beam irradiation, temperature variations and mechanical compressions, which provide a further understanding of dynamics including the influence of kinetics of nanoparticle transformations and thermodynamic stability. However, such external stimuli induce structural transformations in AuNPs depending upon their intensity. In recent years, several studies have been conducted to understand the dynamic behavior of AuNPs under the control conditions to exploit their full potential and broaden their applicability in diverse fields. For this purpose, the comprehensive use of transmission electron microscopy (TEM) has already been implemented to study real-time in situ dynamic changes in NPs. The most common coalescence phenomenon is well documented in the literature, nonetheless, the nanoparticle size dynamic and repulsion phenomenon are considered here as a background for the knowledge gap.
The use of high-intensity electron beam irradiation in TEM outcomes in a beam-induced phase transition, surface charging, ionization and defects, providing insights to observe structural changes in individual metal NPs and interactions between a pair of coupled metal NPs at the nanoscale [1,2]. This study aims to investigate the dynamic behavior of AuNPs under the influence of electron beam irradiation including the interaction between closely placed a pair of AuNPs resulting in coalescence or repulsive behavior between them. The study made use of different surface substrates, such as carbon and silicon nitride (SiN) to additionally explore the effect of substrate-nanoparticle interactions. Furthermore, we target our focus on the interaction between different coupled NPs induced by surface charges, electrostatic forces, interparticle distance and size dynamics. By accomplishing these objectives, we aim to contribute to a deeper understanding of NPs manipulation for their applications in advanced photonic and plasmonic nanodevices.

Methods
Different-sized AuNPs on carbon and SiN substrates were irradiated under a controlled dose of an electron beam using TEM. In situ imaging was acquired using the OneView Gatan camera system to monitor dynamic changes in AuNPs.

Results
Several intriguing phenomena were observed upon the irradiation of the electron beam. Firstly, the individual spherical NPs showed remarkable structural transition, involving the structure transformation, for example, from face-centered cube (fcc) to a decahedral facet structure. The structural changes observed with atomic diffusion and structure reconstruction across the substrate are due to the transferred energy from the electron beam, during the electron-matter interactions.

Furthermore, two more phenomena were observed in the case of coupled AuNPs, such as coalescence and repulsion between a pair of coupled AuNPs under an electron beam depending on the electron dose, interparticle distance and size dynamics. Coalescence is a favorable phenomenon which is initiated with the formation of a neck-like structure with the lattice reorientation between two NPs in order to transform to a more stable structure by minimizing the overall surface energy and generating more localized plasmon at the particle-particle interface [3,4]. On the other hand, the repulsion phenomenon is also affirmative under the electron beam interactions governed by surface charge dynamics and electrostatic forces on a carbon substrate, which play a pivotal role in stability and dispersion under electron beam irradiation.

Conclusions

This study provides valuable insights into nanodynamic behavior of AuNPs under the influence of electron beam interaction to explore different nanoscale phenomena on carbon and SiN substrates. The dynamic behavior of AuNPs showed the structural transformation, coalescence and repulsion phenomena on carbon substrate under the electron beam irradiation. Contrarily, strong adhesive interaction between AuNPs and SiN substrate restricted the NPs diffusion across the surface leading to the melting of NPs under electron beam irradiation due to the transferred energy, consequently confining the coalescence and repulsion phenomena. Whereas heating treatment favored the coalescence phenomenon on SiN substrate. Furthermore, the dynamic behavior of AuNPs was recorded as perturbation and rotation of NPs on both substrates under electron beam effects. These outcomes contribute to a deeper understanding of AuNPs size and surface dynamics to manipulate for various applications, such as in material science including nanodevices, and nanotechnology. Future research directions may consider the substrate-nanoparticle interaction in depth using controlled experimental and computational methods.

Keywords:

Structure transformation, Coalescence, Repulsion, Size-dynamics

Reference:

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4. Phys. Rev. B 2010, 82, 235429

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Molecular mechanism of a bacterial Retron

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background incl. aims:
Retrons are reverse transcriptase-carrying prokaryotic immune systems that protect bacteria against phages. Retrons are formed of three components: A reverse transcriptase (RT), a non-coding RNA (ncRNA), and one or several effector proteins with diverse enzymatic activities. Typically, the RT synthesizes a multi-copy single-stranded DNA (msDNA) from the ncRNA template, a feature that has been exploited for gene editing and synthetic biology applications. Upon phage infection, the effectors induce cell dormancy or death, preventing the phage from spreading throughout the population – a phenomenon known as abortive infection (Abi). To avoid cellular toxicity in the absence of infection, Retron effectors are frequently kept in low-activity states, and RT and ncRNA/msDNA are believed to play a neutralizing role in this process. This work aims to elucidate the molecular events underlying the immune response by a Retron.
Methods:
We combine structural biology (CryoEM), biochemistry, mass spectrometry, and bacterial genetics to address the research question.
Results:
Here, we reveal the molecular mechanism of a Retron. First, we characterized the activity of the effector in vitro and during phage infection. CryoEM structures of the Retron complex illustrate that the msDNA stabilizes the effector in a low-activity state. Interestingly, msDNA's mutations induce the release of the effector from the complex and cause toxicity, underscoring the msDNA role in immunity. In addition, we identified and characterized a phage-encoded Retron inhibitor that suffices to offset the immune response.
Conclusion:
Collectively, our work outlines the structural basis of the Retron defense system and highlights the intricate interplay between bacterial defense systems and phages.

Keywords:

Bacterial Immune System, Retron, CryoEM

Reference:

Carabias et al. (2024) Manuscript under review.

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Data Augmentation and Innovative Machine Learning Approaches for Classifying EEL Spectra of Transition Metals Oxides

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Background including Aims:
The field of Scanning Transmission Electron Microscopy (STEM), especially through the application of Electron Energy Loss Spectroscopy (EELS), has experienced significant advancements due to technical innovations such as aberration correctors, direct detectors, and increased computing power. These advancements have facilitated the collection of large and complex datasets, highlighting the need for effective data management and analysis solutions in the STEM community. Machine Learning (ML), with its two main categories of supervised and unsupervised learning, has emerged as a crucial tool for addressing challenges in EELS, including classification, clustering of spectrum images, and denoising tasks [1,2].
However, the effective application of supervised ML is often hindered by the requirement for large, labeled datasets, which are difficult to acquire due to the susceptibility of samples to electron beam damage. Addressing this drawback, this study aims to compare the effectiveness of two supervised ML techniques: soft-margin Support Vector Machines (SVM) and Artificial Neural Networks (ANN) in classifying EEL spectra for the determination of oxidation states in transition metal oxides, particularly focusing on iron and manganese oxides. Additionally, we present a novel unsupervised learning approach that employs Generative Adversarial Networks (GANs) for data augmentation to address the problem of labeled data scarcity and improve the precision and effectiveness of oxidation state detection using EELS.
Methods:
This research presents a comparative analysis of soft-margin SVMs and ANNs, adapted to the specific challenges in EELS data classification [3]. We evaluate these classifiers based on their ability to accurately identify features indicative of oxidation states, such as white lines and the oxygen K edge, and the effect of energy shifts or noisy spectra. To enhance the classifiers' robustness and adaptability, we investigate the impact of incorporating energy-shifted spectra into the training process and explore various normalization methods, including maximum and L2 norms. The latter is analyzed by dimensionality reduction techniques, particularly Uniform Manifold Approximation and Projection. Additionally, we undertake a systematic exploration of ANN architectures through Random Search and Tree-structured Parzen Estimator (TPE) algorithms, aiming to pinpoint the most effective combinations of architecture and parameters for EELS data classification. Finally, we include the innovative use of GANs for data augmentation, which enables the generation of synthetic EEL spectra from a reduced set of experimental data. This approach not only amplifies the diversity and volume of available training data but also reduces the dependency on extensive, experimentally acquired datasets.
Results:

Based on white lines as particularly reliable indicators for oxidation state classification, SVMs are very robust against energy shifts for the EEL features under investigation, and they perform even better when trained on energy-shifted spectra. In comparison, ANNs, especially those employing convolutional layers, demonstrate a superior ability to adapt to the complexities of EEL spectra, achieving a level of precision comparable to the best SVMs. The analysis of normalization techniques and the strategic use of the cosine kernel in SVMs emerge as effective strategies for avoiding normalization while keeping classification accuracy. Finally, the use of GANs for data augmentation marks a pivotal advancement, since this approach generates synthetic data that closely mirrors the variability and complexity of large experimental data collections, facilitating the training of these classifiers to be both more accurate and more generalized, capable of adapting to the diverse spectra encountered in EELS analysis.

Conclusions:

This work not only elucidates the comparative advantages of SVMs and ANNs in the classification of EEL spectra but also introduces a groundbreaking strategy for overcoming the challenges imposed by the limited availability of labeled datasets. SVMs are particularly recommended for simpler classification tasks where data volume is limited, offering an efficient solution that does not compromise performance. On the other hand, ANNs are more suited to tackling complex classification problems that involve larger datasets, benefiting from their enhanced capacity for learning and adaptation. The successful integration of GANs for data augmentation represents a significant advance, substantially reducing the reliance on extensive labeled datasets and paving the way for more efficient and effective classifier training.

Acknowledgments:

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Keywords:

ML, EELS, GAN, SVM, ANN

Reference:

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Three-dimensional imaging to study early bone mineralization

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
Two-dimensional imaging represents a serious limitation when studying the structure of complex hierarchical materials such as bone. Three-dimensional visualization is essential for understanding the interactions that occur between the cellular network, collagen fibrils, and mineral precursors during the formation process. Over the last decade, FIB-SEM has revolutionized the study of mineralized tissues, providing not only three dimensional information in the nanometer range, but also the possibility to nanofabricate mineralized lamellae avoiding the mechanical stress caused by standard sectioning techniques.
These technical advances helped elucidate the mineral deposition in collagen-based materials, driven by a spherulitic-like crystal growth [1]. Initially, disordered mineral aggregates form in the interfibrillar spaces, and subsequently the mineral infiltrates adjacent collagen fibrils, which provide the structural framework for the formation of layered spherulites. These spherulites (also called mineral ellipsoids) imbricate forming a new hierarchical level of organization in bone termed tessellation [2]. Although the mechanism has been described in several systems [1, 2, 3], detailed data on the interaction of the organic and the mineral phases remain insufficient.
Methods
The present study combines electron tomography (FIB-SEM serial surface imaging) which provides 3D information, with the fabrication of lamellae for scanning/transmission electron microscopy (S/TEM), selected area electron diffraction (SAED) and energy dispersive spectroscopy (EDS) chemical mapping to elucidate crystal distribution and orientation throughout the collagen matrix.
Results
The study reveals the internal structure of the forming fibrolamellar bone at nanometer resolution. A connective tissue with dispersed and non-preferentially oriented collagen fibrils seems to be deposited first, serving as a scaffold for the deposition of more aligned collagen. During embryonic development, these osteocytes initiate the mineralization process and become buried in the mineral matrix, which expands both vertically and laterally to form the nascent fibrolamellar units. At the collagen-mineral interface, a multitude of mineral spherulites proliferate and grow to confluence. Their profiles are still recognizable in the consolidated mineral layer.
Conclusions
Our study confirms that the formation of mineral spherulites also drives the mineral deposition in embryonic fibrolamellar bone. This fact demonstrates that this protein-mediated crystal growth mechanism occurs in different types of bone tissue and in different species, indicating that it is a common and homologous mineralization mechanism in type I collagen-based materials [4].

Keywords:

S/TEM, FIB-SEM tomography, bone mineralization

Reference:

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Unraveling the original dissolution mechanism of LiFePO4 when treated for recycling

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
This presentation aims at demonstrating the original mechanism involved during the acid leaching treatment of the very common material used in Li-ion batteries, LiFePO4. Such treatment is the basis of the hydrometallurgical process used for recycling the material and the understanding of the chemical reactions involved is critical to optimize the recycling process. To date and to our knowledge, such a mechanism established thanks to advanced TEM experiments among which operando liquid TEM and STEM EELS for phase mappings has never been highlighted.

Methods
We first carried out operando liquid TEM experiments to follow the evolution of the shape of the particules and to see if the dissolution process was monotonous or composed of severals growth and dissolution steps. Ex situ characterisations on materials residues recovered from partial dissolutions, including HRTEM imaging and STEM-EELS experiments, were then performed. They enabled the identification of the phases and their localisation. All the results are finally correlated and confronted to global chemical and structural analyses by the means of ICP-MS and XRD experiments.

Results
Operando liquid TEM experiments are demonstrating a monotonous dissolution process without any reprecipitation or growth of particles. Moreover as already reported by us [1], it was possible to show the kinetic observed operando was comparable with the one deduced from ex-situ dissolution experiments. Ex-situ XRD analyses are evidencing a rather quick formation of crystallites composed by the FePO4 phase with still the presence of the pristine LiFePO4 one. Discriminating both phases is a challenge but STEM-EELS and HRTEM analyses enabled to prove the coexistence of both phases into the same particles and they demonstrate that a core-shell type or shricking-core mechanism does not occur as it is usually observed during such reactions.

Conclusion
During this talk we are presenting the original dissolution mechanism that is based on the tranformation from monocristalline LiFePO4 particles toward polycrstalline FePO4 ones separated by LiFePO4 residues. We demonstrate the evolution occurs into the same particules without any nucleation of new particules. Then, this is the level of pH that is governing the continuous dissolution process or its interruption. This study is of importance since it allows process optimization. It is also of great interest on a fundamental aspect since we demonstrate the generation of particles where the LiFePO4 and FePO4 phases coexist. Such phenomenon is not really expected when we know the bahavior of the material that is always presenting monophased particles when used in a li-ion battery [2,3].

Keywords:

Operando-liquid-TEM, HRTEM, EELS, LiFePO4.

Reference:

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Scanning electron diffraction reveals the nanoscale ordering of cellulose in a hierarchically structured hybrid material

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Poster Group 2

Background
Biopolymers are electron beam-sensitive materials with low scattering power, which poses a significant challenge for nanoscale characterization. One promising technique to overcome these issues is scanning electron diffraction (SED), utilizing the strong interaction between electrons and matter. In SED, the sample is raster scanned using a near-parallel electron probe while simultaneously capturing a diffraction pattern at each beam position. These patterns provide highly localized crystalline information from the sample, including strain, phase, and orientation, later characterized through post-acquisition data analysis.
Cellulose, with its unique properties and crystalline structure, has emerged as a promising resource for sustainable composite materials. Controlling its hierarchical organization is an essential consideration in cellulose utilization. The aligned orientation of the highly anisotropic cellulose nanofibers is vital to macroscale mechanical properties. Fundamental insights into the intrinsic arrangement of cellulose enhance its exploitation in composites with new and improved properties.

Methods
The wood-based composite material was sectioned using ultramicrotomy in two orthogonal directions, longitudinally and transversely, relative to the elongated wood cell structure. The microscope was configured with a convergence angle of 0.1 mrad, resulting in a probe diameter of less than 10 nm. SED data was acquired using a beam current of 2 pA with a dwell time of 5 ms to minimize beam damage. Cellulose orientation and degree of alignment in each beam position were determined through post-acquisition data processing. 360 virtual detectors were arranged in an annular pattern around the unscattered beam, with a radius corresponding to the scattering angle of the most prominent 200-reflection of cellulose to optimize the signal-to-noise ratio.

Results
In this study, we employed SED to unveil the hierarchical assembly of cellulose nanofibers in transparent wood, a composite material prepared by infiltrating wood with polymerized methyl methacrylate (PMMA)[1]. Our results reveal a well-ordered hierarchical arrangement of nanofibers in the secondary cell wall with a spatial resolution of 15 nm[2]. In the inner regions, the nanofibers are aligned parallel to the cell elongation, including the innermost part closest to the lumen. In the outer part, the nanofibers transition to a tangential orientation. Based on the quantitative SED data, we can conclude that this reorientation occurs smoothly over 1.5 µm. Despite the change in direction, cellulose nanofibers stay well-aligned. Uniform cellulose orientation in the inner part and a plateau in the outermost part support a layered cell wall structure.
Furthermore, wood cells sectioned longitudinally exhibit clockwise or counterclockwise nanofiber rotation towards the outer part. This rotation difference is caused by sectioning the cells at different depths and is consistent with a helical arrangement of nanofibers with a gradually changing pitch.

Peak width analysis of transversely sectioned transparent wood discloses a higher degree of nanofiber alignment in the outer part of the cell wall than in the inner part.
Preserving the anisotropic organization of cellulose in this new biocomposite retains the mechanical properties of wood.

Conclusions
This work demonstrates the potential of Scanning Electron Diffraction in characterizing biobased composite materials. Combining sensitive detectors and innovative data analysis enables the detection of very subtle signals from the sample. SED provides quantitative crystal information with exceptionally high spatial resolution from a relatively large field of view (20x20 µm).

Keywords:

scanning electron diffraction, hierarchical structures

Reference:

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Picoseconds and nanometers; time and spatially resolved cathodoluminescence spectroscopy to characterise nonradiative defects in semiconductors.

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

The drive for miniaturization in the ever evolving field of semiconductor technology has pushed the ability to provide in depth material and device characterization to its limit. This is particularly strongly felt in the characterization of the opto-electronic properties, as the established techniques (such as photoluminescence) are inherently limited in their achievable spatial resolution. Nevertheless, being able to characterize not only the steady state optical properties on the nanoscale but also time dependent properties is paramount for further scientific and engineering progress. Especially as nanoscale features such as point and extended defects can significantly influence material and device properties.
While cathodoluminescence spectroscopy for characterization of steady state properties on the nanoscale has been available for quite some time, reports on (hyperspectral) time-resolved characterization with picosecond resolution have been much more limited (1), (2), (3). This work demonstrates hyperspectral time-resolved cathodoluminescence (TR-CL) spectroscopy for the analysis of the interplay between extended defect and point defects in UV-C light emitting AlGaN quantum wells (QWs), single nonradiative point defects in an InGaN/GaN QW and extended defects in InAs QDs on Si substrates.

We are using an Attolight Chronos dedicated TR-CL scanning electron microscope with laser pulsing system. We have achieved time-resolved measurements with 7 ps resolution in point and shoot mode, and 100 ps time resolution when recording hyperspectral TR-CL maps. All investigated samples were grown by MOVPE. Sample analysis has been performed using the open source Python packages Hyperspy and Lumispy.

We have successfully measured the carrier lifetime in AlGaN-based, UV-C light emitting, QWs with emission wavelengths ranging from 260 nm to 220 nm (with QW composition varying from 56% to 82%) at temperatures from 80 K to 300 K. We found, that the carrier lifetime decreases strongly with increasing QW Al mole fraction, indicating a significant increase in the point defect density. Further, spatially resolved, investigation of the interplay between threading dislocations and point defects show no evidence for a significant influence of the local threading dislocation density on the carrier lifetime, indicating that the optoelectronic properties of these materials are being controlled by their point defect density.
Observation of decay dynamics in InAs QD systems using hyperspectral TR-CL reveals changes in the carrier lifetime in and around misfit dislocations. In contrast to our observations in AlGaN we can observe a significant decrease in carrier lifetime, when compared to the surrounding material, at the extended defect. This does highlight both the strong effect extended defects can have on carrier

dynamics and that differences in the point defect density and carrier localization can significantly impact local recombination channels. We also observed a lack of impact of the misfit dislocation on the lifetime on the material immediately surrounding the dislocation which we attributed to a short carrier diffusion length, preventing carriers from reaching the non-radiative recombination centre. This in turn causes the observed high tolerance of the internal quantum efficiency for defects in this system (4).
Lastly, hyperspectral TR-CL has been carried out on a carefully designed InGaN/GaN QW system for the investigation of isolated point defects. The system contains an ultrathin QW (3 ~bilayers thick) on a low dislocation density template (~106 cm-2) with a point defect filtering layer, resulting in a point defect density of about 109 cm-2 . The low density allowed us to first find isolated point defects and then measure hyperspectral TR-CL maps containing these isolated point defects. Extracting spatially resolved carrier lifetimes from the hyperspectral dataset then allowed us to not only compare and analyse decay times across the mapped area but also to visualize carrier diffusion within the system. Our findings in this system reveal the direct influence of isolated nonradiative point defects on carrier recombination dynamics and open the pathway for indepth studies of the optoelectronic properties of point defects.

We have demonstrated the unparalleled ability of TR-CL to characterize the opto-electronic properties of defects in a wide range of materials systems on the nanoscale. Further development of TR-CL capabilities will only strengthen this research capability and provide future research on nanoscale devices and objects with an invaluable tool for the characterization of spatially resolved carrier dynamics.

Keywords:

Cathodoluminescence, Time-resolved, Hyperspectral, Nitrides, Arsenides

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STEM-XEDS spectrokinetic analysis of oxygen: a tool to understand redox processes in nanostructured oxide catalysts

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Poster Group 2

Background
Understanding the details of oxygen exchange processes becomes key to understand the performance of catalysts used in redox processes as well as to fix the most convenient pretreatment conditions to activate the catalyst prior to reaction. At macroscopic level, hydrogen temperature programmed experiments (H_2-TPR) are the most commonly used tool to propose the different phases and processes resulting from this activation step. Nevertheless, the interpretation of these TPRs becomes cumbersome and most of the times rather speculative.
A reliable determination of the actual phases appearing during the different oxygen evolution processes involved in these TPRs requires a precise quantification of the oxygen content of the materials at different steps of the reduction process. Such information, combined with structural analysis at local level provides the necessary information to interpret the H_2-TPR trace at quantitative level on solid backgrounds.
Regarding the compositional aspects. STEM-XEDS stems as a possible technique to face the quantification of the oxygen content at nanoscale in oxides with interest in redox catalysis. However, such approach requires to overcome two major difficulties: (a) dealing with absorption effects in the quantification of the oxygen signal; (b) disentangling the influence of beam reduction effects, which are particularly relevant in the case of high surface area, beam sensitive oxide nanostructures.
This contribution illustrates how combining time-resolved STEM XEDS experiments recorded during the in-situ reduction under the electron-beam performed on an aberration-corrected microscope equipped with large area detectors; an O-quantification scheme based on the use of the so-called ζ-factors method [1] and modeling of the kinetics of oxygen evolution, it is possible to determine with high accuracy the composition of the intermediate phases involved in the oxygen exchange processes of oxide nanostructures. In particular, the methodology is applied to the quite challenging case of nanorods of a complex, extremely beam sensitive, multicationic K-modified manganese oxide with hollandite type structure highly active in CO oxidation.

Methods
The starting K0.11MnO2-δ nanoxide was prepared from Mn(SO4)2, HNO3 and KMnO4 solutions under reflux over 24 h. This oxide was characterized at microscopic and macroscopic levels by Electron Probe Microanalysis (EPMA) and Neutron Diffraction (ND). The implementation of the ζ-factors method was done starting from Electron Tomography experiments performed in a Titan Themis 60-300 microscope operated at 80 kV. For reconstruction, a methodology based on TVM3D algorithms was employed to ensure high accuracy in the determination of local thickness values the oxide nanostructures [2]. XEDS maps were acquired with a Super-X G2 detector, using a beam current ranging between 135 and 145 pA. Maps were recorded in both single and multiple frames

modes setting a pixel dwell time between 0.128 and 5.000 ms respectively. These conditions result in a frame acquisition time of approximately 3-100s, after which the drift was corrected using cross correlation. XEDS line profiles were extracted using Velox 3.1 and the quantification was carried out using home-made scripts coded in Matlab.
Hydrogen temperature-programmed reduction (H_2-TPR) experiments were performed in an AutoChem II 2920 automated characterization system, equipped with a calibrated thermal conductivity detector (TCD).

Results
The quantitative analysis of XEDS spectra recorded at different irradiation times under the electron beam allowed us tracking the evolution of the O/Mn molar ratio of different oxide nanostructures, Figure 1 (left). Extrapolation to t=0 of the O/Mn ratio vs t plot provided an estimate of K0.13MnO1.98 stoichiometry for the pristine material. Full agreement with the K/Mn ratio determined by EPMA (K/Mn=0.125) and with the composition obtained by ND (K0.11MnO1.96) and H_2-TPR (K0.13MnO1.99) validated the oxygen quantification procedure proposed by us.
Formation of K0.13MnO1.61 as steady-state product of the electron-beam reduction process, which could be perfectly fitted to a contracting-volume kinetics, suggested that oxygen evolution proceeds in steps of 1/3 of the oxygen located at the O1 or O2 positions of the unit cell.
Taking this experimental observation into account and analysing in quantitative terms the deconvolution of the H_2-TPR experiments, Figure 1 (middle), all the steps involved in the reduction under hydrogen of this type of oxides could be perfectly interpreted and the corresponding intermediate phases proposed (Figure 1, right).
Combination of these results with those coming from DFT calculations allowed us confirming that the usual pre-reduction procedures used to activate these oxides lead to the elimination of all the oxygen residing at the O2 sites of the hollandite structure, rather than just all the oxygen sites (O1 and O2) at the surface of the oxide nanorods, as it has been proposed on speculative basis in the existing literature.
Likewise, it was possible to determine that the formation of MnO concludes at 553°C after the so-called δ step, rather than after the γ one, just at 423°, as it has also been proposed in literature.
Moreover, it has been possible to clarify that the intensity of this δ step is totally determined by the initial K content of the hollandite, this suggesting that oxygen evolution in this peak is related to O at the interface between Mn and K.

Conclusions
A novel methodology to implement the ζ-factors scheme to quantify with high accuracy the oxygen content in multi-cationic, beam sensitive, materials has been developed [3]. This methodology has been fruitfully applied to analyse in full quantitative terms time-resolved STEM-XEDS experiments recorded during the in-situ e-beam irradiation of nanorods of a potassium-modified manganese oxide with hollandite-type structure.
The quantitative results obtained at nanoscopic level fully agree with reference data obtained at macroscopic level by EPMA, ND and H_2-TPR, which supports the reliability of the developed approach. Moreover, from the nanoscopic results and the deconvolution of the TPR diagram on the basis of α step processes, it has been possible to determine the composition of the different intermediate phases involved in the reduction of the oxide under hydrogen, avoiding erroneous assignments in the literature.

Acknowledgements

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Keywords:

Time-resolved-STEM-XEDS, oxygen-stoichiometry, quantitative-XEDS-at-nanoscale, beam-sensitive-materials, ζ-factors

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Deep orientation estimation of macromolecules in cryo-electron tomography

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Background incl. aims

The standard method for detecting macromolecules in cryo-electron tomography (cryo-ET) images is template matching (TM), which suffers from high computational complexity and difficulties in identifying particles with similar structures. In TM, one uses template density maps of a specific macromolecular particle and computes the cross-correlation score at every voxel across the whole tomogram. The highest-ranked cross-correlation scores correspond to possible particle locations. In addition to the particle location, one also obtains an estimation of the particle orientation. As a final step, sub-volumes are extracted at those locations which can be used in turn for other tasks like classification and segmentation.

Recently, the investigation of crowded cell environments using cryo-ET has been attempted with deep learning (DL) methods. Models like DeepFinder [1] improve particle picking by being much faster while at the same time providing a reasonable accuracy. Prediction of the particle orientation using DL methods, however, has, to the best of our knowledge, not yet been achieved. This, we believe, is mainly because learning based on representations like Euler angles or quaternions fails due to discontinuities of the representation space [2].

Here, we investigate DL-based particle orientation estimation using a continuous representation with six degrees of freedom (6DoF) that empowers neural networks for the optimal estimation. Input to the neural network is a 3D image patch containing the particle. Since for experimental data, usually no ground truth is available, we generate test data using the PolNet software that was recently published [3]. We evaluate the accuracy of the orientation estimation using an end-to-end model on this test data. Our promising results suggest that particle orientation using DL methods is indeed feasible.

Methods

Different rotation representations may be used to train a machine learning model for orientation estimation. Inspired by the work of Zhou et al. [2] , we address the problem of orientation estimation as a regression problem. We use M =[a1, …, an] and SO(n) to denote a rotation matrix and the space of n-dimensional rotations, respectively, and ai represents a column vector. In consequence, having a set of 3D rotations, one can define the original space X=SO(3). Zhou et al. [2] showed that representations for 3D rotations are discontinuous in four or lower dimensions; hence, representation spaces for rotations based on Euler angles and quaternions are discontinuous.

Let R and X be the representation space and the original space of rotations; then the neural network should predict an intermediary representation in R that can be mapped into the original space X. In other words, we are looking for a representation (f, g) such that f:R→X maps from the representation space to the original space and g:X→R maps back to R, preserving the continuity. Figure 1 illustrates these definitions. However, the problem is that the limit of g is undefined for zero rotation, i.e. limit of g in one direction gives 0 and in the other 2π.

One possible solution is to employ identity mapping. Although this guarantees that the network output is back in SO(3), it results in matrices of size 3 × 3 which can be computationally exhaustive due to orthogonalization. As a result, we perform the orthogonalization in the representation space. Having the original space X=SO(3), a representation space $R=R^{3\times2}\backslash D$, and a rotation matrix M, the mapping g is simply defined as dropping the last column of the rotation matrix, resulting in [a1, a2], as suggested by Zhou et al. Here, the set D represents that part of the space that f cannot map to SO(3) [2].

In addition to these challenges, designing a network structure that can reduce the computational cost while accurately estimating the orientation is a profound task. One such structure is a multi-layer perceptron (MLP). We use a four-layer fully connected network with 32 nodes per layer and a regression layer with tanh activation function. The number of output nodes in the regression layer equals the dimension of the orientation representation, i.e. 3, 4, and 6 for Euler angles, quaternions, and 6DoF, respectively.

Results

We performed experiments on a synthetic dataset generated by PolNet [3] that for uniform random sampling of the rotations uses the Algorithm S2 in [4]. We generated 150 tomograms of resolution 10A° containing the ribosomal complex (4v4r) and Thermoplasma acidophilum 20S proteasome (3j9i). We extracted centred patches of size 40^3 for all 4v4r particles, leading to 26703 samples. 130 tomograms (23128 samples) were used for training and validation, and 20 tomograms (3575 samples) for testing. Note that the synthesized tomograms contain the missing-wedge artifact and noise.

We trained our network for 50 epochs using Adam optimization, batch size 32, learning rate 0.0001, and patch size of 40^3. We used Huber loss to calculate differences between ground truth and predicted orientation representation. Our experimental results suggest that the continuous representation performs much better in practice. Figure 2, left, shows the ground truth and predicted orientations on a test tomogram for the 6DoF representation. Green and red colors represent ground truth and prediction, respectively. Our model predicted 81% of the test samples correctly using the Crowther criterion [5] with an angle difference threshold of 20 degrees. Visual analysis shows that most incorrect predictions occur in regions where the particles form a cluster. While training time was about 1 hour, inference time was only approximately 25 seconds on a single GPU. We achieved an R2 score of 0.96 on training data and 0.87 on test data for the 6DoF representation. These values downgrade to 0.76, -0.07 on training and -0.47, -0.07 on test data for the quaternion and Euler representations, respectively. Figure 2, right, shows a histogram chart of the angle differences for all three representations.

Conclusion

We studied the use of MLP to estimate macromolecule orientation using various representation spaces, namely Euler angles, quaternions, and a 6DoF-continuous. The continuous representation space shows a huge advantage over the others. Our future work includes developing more complex models to perform multiple downstream tasks along rotation estimation.

Keywords:

machine learning, cryo-electron tomography, orientation

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Direct observation of Ni nanoparticle growth in carbon supported nickel under carbon dioxide hydrogenation atmosphere

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background:
Understanding nanoparticle growth is crucial to increase the lifetime of supported metal catalysts. The recent advances in in situ transmission electron microscopy (TEM) using closed cell nanoreactors, in which samples can be exposed to gas or liquid phase environments, allow the visualization of nanoparticle catalysts under 1 bar gas. This enables the analysis of changes in nanoparticle morphology, size, composition and shape of metal nanoparticles under reaction conditions, which is highly relevant to the field of catalysis.[1]
Nickel-based catalysts are of interest for the conversion of CO2 and renewable H2 into synthetic natural gas (Power-to-Gas process). The catalytic performance in terms of activity is controlled by the available surface area of the metal nanoparticles. The exposure of nanoparticles to reaction atmospheres and elevated temperatures can result in nickel particle growth and activity loss. In this study,[2] in situ gas phase TEM is employed to visualize the movement and growth of ensembles of tens of nickel nanoparticles supported on carbon for CO2 hydrogenation at atmospheric pressure in real time.

Methods:
Carbon supported nickel catalysts were prepared via incipient wetness impregnation using an aqueous Ni(NO3)2 precursor solution, followed by a heat treatment under H2 atmosphere. In situ gas phase TEM was performed at 450 °C under 1 bar H2:CO2 (4:1) atmosphere using a Protochips Atmosphere holder. The results were compared to ex situ experiments, amongst others in a catalytic setup, to study the influence of beam dose, which was kept at a maximum of 20 e- A-2 s-1.

Results:
Various movement and growth mechanisms of a supported Ni catalyst were directly observed in real time under CO2 hydrogenation conditions, using in situ gas cell TEM at 1 bar and 450 °C. During 50 min under reaction conditions, the average Ni particle diameter grew from d = 5.7 ± 1.4 nm to d = 7.7 ± 1.9 nm, while the number of particles decreased from 72 at t = 2 min to 34 at t = 50 min. This correlated to a decrease in specific Ni active surface area from 105 to 78 m2 gNi-1. Comparison of the averaged particle growth within a nickel on carbon catalyst in situ and ex situ validated the relevance of the in situ obtained results.
Furthermore, the in situ TEM experiments enabled the analysis of individual nanoparticles in real time. We observed two modes of particle movement with an order of magnitude difference in velocity: fast, intermittent movement (vmax = 0.7 nm s-1) and slow, gradual movement (vaverage = 0.05 nm s-1). Two distinct particle growth mechanisms were visualized: diffusion and coalescence, and Ostwald ripening. Figure 1A shows the electron microscopy images of particles at selected times with the projected areas of the four particles highlighted in Figure 1B. Figures 1C-F show the evolution of the projected areas of the four particles over time. Particle 2 and particle 3 disappeared due to coalescence with particle 1, highlighted in the purple regions. Interestingly, particle 4 showed

different growth behavior. After the first 20 minutes in which particle 1 had grown to ~60 nm2, particle 4 started shrinking, until it completely disappeared after 50 minutes. Hence a striking interplay between the two mechanisms was observed: first coalescence took place, followed by Ostwald ripening, caused by the increased difference in particle size.

Conclusions:
Our direct visualization of the complex nanoparticle growth mechanisms highlights the relevance of studying nanoparticle growth in supported nanoparticle ensembles under reaction conditions, and contributes to the fundamental understanding of the stability in supported metal catalysts.

Figure 1 - Direct observation of particle growth via coalescence (purple) and Ostwald ripening (green). A) In situ transmission electron microscopy images of carbon supported nickel nanoparticles acquired at t = 2, 20, 22, 24 and 70 min. B) Analysis of the projected areas of individual particles overlayed on the original EM images. The bottom row shows the evolution of the projected area versus time of C) particle 1, where the arrows indicate coalescence with particles 2 and 3, D) particle 2, E) particle 3 and F) particle 4.

Keywords:

in situ TEM, catalysis, nanoparticle-growth

Reference:

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Development of simple image processing for in-situ TEM toward live processing

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Poster Group 1

Background incl. aims
In-situ transmission electron microscopy (in-situ TEM) has become an essential tool for studying dynamic phenomena at the nanoscale [1]. Observing nanoscale phenomena with in-situ TEM allows us to understand the mechanism in more detail, which leads to improving material properties or increasing the process efficiency. However, most of the information is available only after a long time of analysis, which ends up as a passive approach. To actively investigate the phenomena, more information should be extracted during the in-situ experiment to manage the stimuli in real time. Live processing during the in-situ TEM experiment can allow to overcome this limitation by actively controlling the stimulus conditions according to the observed features, thus allowing dynamic adjustments during the experiments. Achieving live processing requires two critical components: 1) establishing real-time communication channels between the TEM, the in-situ stimuli, and the processing server, and 2) developing fast and efficient image processing algorithms capable of handling the high throughput of data generated during in-situ TEM experiments. Until now, artificial intelligence (AI) has not been able to help with live processing due to the large amount of computing power and time required. While the establishment of real-time communication channels requires cooperation with TEM manufacturers, this paper focused on the development of image processing algorithms suitable for live processing.
In this work, we present the development of simple but effective image processing techniques suitable for live processing in in-situ TEM. Three cases of segmentation from electrochemical in-situ TEM experiments were shown as: (Case 1) plating and stripping of Zinc (Zn), (Case 2) dendritic growth of Zn, and (Case 3) particle-like plating and stripping of Copper (Cu) [2, 3]. Through these case studies, we demonstrate the ability of simple image processing to understand electrochemical phenomena and its potential for applications.

Methods
a) Performing in-situ electrochemical TEM experiments
In-situ Liquid TEM holder (Stream, DENSsolution) assembled with 3-electrode Micro-Electro-Mechanical System (MEMS) chips was used for experiments. In case 1, Pt electrode MEMS chip and 0.1 M ZnSO4 solution (flowing) were used and cyclic voltammetry (CV, -1.5 V ~ +0.8 V for 0.01 V/s scan rate) was applied. In case 2, Pt electrode MEMS chip and 0.1 M ZnSO4 solution (non-flowing) were used and chronopotentiommetry (CP, 5 µA for 10 seconds) was applied. In case 3, TiNx electrode MEMS chip and 0.02M CuSO4/0.01M KH2PO4 solution (flowing) were used and chronoamperometry (CA) with 4 cycles (one cycle: +1.5 V for 15 seconds and –1.5 V for 15 seconds) was applied. Case 1 and 2 were monitored at STEM mode and case 3 was monitored at TEM mode.

b) Developing simple image processing methods dedicated to in-situ TEM
All the image processing methods are developed as python codes. To reduce the noise level, a gaussian filter was applied as pre-processing. Key strategies are 1) subtraction of the reference image (pure electrode) from target image (during experiment) to extract the information changes and 2) thresholding the subtracted image to binary-segmenting as feature and background. Especially case 2, the subtraction was performed between images with 1 second difference, in order to emphasize the fast growth of dendrite within 1 second. After the segmentations, the deposition information such as deposited area was numerically extracted and plotted.

Results
In case 1 (Figure 1a), Zn plating and stripping phenomena were recorded with CV stimuli. On the recorded STEM images, the plated Zn is well visible, but the brightness of the plated Zn looks similar to the rectangular Pt electrode on the right side. After the simple image processing as described in methods, the plated Zn is clearly visible without Pt electrode and the area of plated Zn can be measured easily. After measuring the area throughout the whole CV process, the plated Zn area can be synchronized with the CV stimulus data, which leads to a quantitative understanding of the phenomena.
In case 2 (Figure 1b), Zn dendritic growth was recorded with CP stimuli. From the video, a dendritic growth took only a few seconds, so the information related to the growth rate is more important than the amount of growth. By subtracting the 1-second difference between frames, the grown dendrite is visualized in 1 second, which can directly show not only how fast the dendritic growth is, but also which direction is preferred. In this case, Zn dendrite growth occurred to the left where the counter electrode is located.
In case 3 (Figure 1c), Cu particle-like plating and stripping was recorded with cyclic CA stimuli. On the recorded TEM images, plated Cu particles are well visible on the top of the electrode (round shape with hole), but small dots are also visible at the outside of electrode. Large Cu particles on the top of the electrode are formed based on electrochemical deposition and small Cu particles outside of electrode are electron beam induced deposition. To focus on electrochemical deposition, the only electrode area was selected to further particle segmentation. After the image processing, each individual Cu particle was segmented and could be tracked during the plating and stripping process. The area and number of particles were plotted with a time scale to study the plating and stripping phenomena.

Conclusions
In-situ TEM has been enabled to observe countless phenomena in nanoscale, but quantitative information was very limited due to huge amount of dataset which cannot be handled by human. This paper showed that the bunch of quantitative information can be extracted from recorded in-situ TEM video by simple image processing. We believe that simple image processing will allow to actively investigate the phenomena at nanoscale by correlating the observed feature and applied stimuli in real time.

Figure 1. Examples of image processing from in-situ TEM dataset. (a) STEM image and segmented image of metal deposit. (b) STEM image and reconstructed image of metal dendrite growth within 1 second. (c) STEM image, segmented image of particle shape deposits and graphs about deposit area and number of particles vs. time. [2, 3]

Keywords:

in-situ TEM, Live, Image processing

Reference:

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In-situ TEM reduction of a solid oxide cell with NiO/YSZ and NiO/BZCY fuel electrode materials

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background
Increasing the durability of solid oxide cells is one of the main goals for achieving wider industrial application. The quality of the electrode plays a major role in the performance and durability of a fuel and electrolysis cells. Ni/YSZ or Ni/BZCY (BZCY stays for the state-of-the-art proton conducting ceramic material BaZr1-x(Ce,Y)xO3-d) electrodes of solid oxide cells are commonly reduced from NiO/YSZ or NiO/BZCY under hydrogen atmosphere at high temperatures, prior to operation. It is known from commissioning that e.g. reduction temperature and H2 pressure influence the initial performance of the fuel electrode by governing Ni particle size and shape. The reduction results in a significant change in microstructure. As specific microstructural properties are crucial to achieve high performance and durability of the cell, a comprehensive understanding of the reduction process is required.
Methods
With in-situ TEM we are able to observe the reduction of NiO in real time while exposing the sample to hydrogen gas and heat [1]. Using in-situ TEM atmosphere system from Protochips we studied the electrode reduction at the H2 pressures up to 1 atmosphere and temperatures up to 850 °C which fit the real working condition of a conventional solid oxide cell based on oxygen ion conductor as an electrolyte.
Results and conclusions
With this set-up we can observe the initial steps of NiO reduction, local change in oxidation state of Ni and formation of defects in Ni grains. Grain boundaries and triple junctions between NiO and YSZ or BZCY are determined as the starting points of the reduction process at lower temperatures. Closely reproducing the reduction conditions of the real cell, we demonstrate that NiO reduction mechanism is different from the previous findings. We also showed that the initial temperature of the reduction is crucial to achieve a high number of electrochemically active triple phase boundaries between e.g. Ni/YSZ and gas. In-situ results go in good agreement with ex-situ results obtained from a bulk cell reduced in a test bench described in Ref. [2].
Figure 1. Step-by-step reduction of NiO/YSZ material for fuel electrode. Ni becomes porous and nanocrystalline after the reduction. The grain size grows with temperature and pores disappear due to the Ni coarsening.

Keywords:

In-situ gas TEM, SOC, hydrogen

Reference:

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Assessing Ptychographic Methods for Maximum Low Dose Performance

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Poster Group 2

For beam sensitive materials, the dose that can be applied and the dose efficiency of the imaging method often sets the achievable resolution, rather than just the capabilities of the optical system. This is crucial in biology but also for many materials science samples such as metal organic frameworks (MOFs), zeolites, and organic perovskites. While scanning transmission electron microscopy (STEM) has come to dominate high resolution materials science, for the most beam sensitive materials TEM [1] has remained predominant due to the dose efficiency of its phase contrast imaging. Recent technological advances have, however, started to challenge this status quo for low dose imaging, through the combination of fast 4D STEM and ptychography. Ptychography can exceed the dose efficiency of TEM but has been limited until very recently by the relatively slow speed of cameras, which makes 4D STEM exceedingly slow for high probe position count datasets. This bottleneck has recently been overcome using event driven camera technology [2], and conventional framing cameras are also increasingly closing the gap between 4D STEM scan speeds and high-speed conventional STEM, greatly facilitating drift-free and low dose 4D data acquisition.

However, there are many forms of ptychography and so far, sufficient broad comparison of the low dose performance of the various ptychographic methods has been lacking. Ptychographic methods can be grouped into iterative and direct algorithms. Iterative methods are most often performed with a defocused probe, as the higher signal per diffraction pattern facilitates convergence, but with a careful choice of reconstruction parameters they can also produce useful phase images in a low dose focused probe configuration. Here, we present a comparison of a broad spectrum of these algorithms, both iterative and direct, for reconstructing low dose data sets.

Figure 1 compares ptychographic reconstructions of 2.5 nm thick Methylammonium lead iodide (MAPbI3) at a dose of 50 e-/A², using a convergence angle of 13 mrad, with a focused probe. The iterative gradient descent (GD) algorithm converges, but to a relatively poor quality image showing very little atomic structure. The rPIE, ePIE [3], ER and WASP [4] iterative and direct SSB algorithms, all perform better under these conditions, revealing relatively similar atomic structures from which the iodide and the Pb sites can be located. On the heavy Pb atomic sites, contrast reversals are present. These reversals can be easily corrected using our new phase offset method [5], which can greatly improve the interpretability of ptychographic data.

Switching to a defocus of 20 nm, as shown in Figure 2, improves the GD result significantly, however it remains dominated by low frequency features of limited utility, with high resolution information still less clear than the other iterative methods. Although the SSB becomes blurred when the probe is defocused, as expected due to the optical sectioning effect, this can be corrected post collection as we show in the figure.

In addition to the choice of algorithm, the parameters used are critical to obtaining the best solutions. For iterative schemes, probe step size and defocus are linked in their importance to the amount of overlap of adjacent illuminated regions. The convergence angle determines which frequencies will be transferred most strongly and sets the upper limit on conventional image resolution. Although ptychography can provide super-resolution and exceed this limit, at very low doses there is insufficient scattering to higher angles for super-resolution to provide significant benefits; rather, at low doses, correction of residual aberrations is expected to play a greater role.

Keywords:

Ptychography, Low-dose, 4D STEM

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Electron microscopic investigation of photothermal laser printed ZnO nanoarchitectures

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Poster Group 2

Background incl. aims
Multi-photon 3D laser printing of polymers can be used to create complex structures on the micro- and nanoscale [1,2]. This technique involves focusing a laser into a liquid ink, initiating a reaction that solidifies the material at the laser focus. While this technology has primarily been employed with polymers, recent developments aim to extend its capabilities to inorganic materials. However, these materials often need post-processing at elevated temperatures, facing challenges for multi-material architectures due to the potential impact on polymer structure [3]. Recent advances in the direct printing of semiconducting ZnO through hydrothermal synthesis offer a promising alternative since no post-processing step is needed [3]. This enables the direct laser printing of functional microelectronic devices, possibly combined with polymers. Using electron microscopy, we demonstrate that single crystalline ZnO can be printed on amorphous substrates, opening the possibility of new applications, e.g., non-linear properties for devices in optics. However, a deeper understanding of the growth of the ZnO crystals and the crystal rotation is necessary for further applications in microelectronic devices.

Methods
The ZnO structures are photothermally printed from a liquid ink (zinc formate and sodium citrate in dimethyl sulfoxide) onto a glass substrate using a focused continuous-wave laser (532 nm wavelength).
An FEI Helios G4 FX combined focused ion beam (FIB) and scanning electron microscopy (SEM) dual-beam system was used to characterize the crystallinity and crystal orientation of the printed ZnO with electron backscatter diffraction (EBSD) with a Bruker eFlash detector. EBSD requires a polished sample surface, for which we used FIB milling since the ZnO structure sizes are in the µm range. First, a Pt protection layer is deposited using the FIB. Then, the sample is tilted so that the sample surface is aligned at an angle of 10° relative to the Ga+-ion incidence. After polishing, the sample is tilted to a 70° effective sample tilt relative to the electron beam incidence for EBSD data acquisition. The Bruker Esprit software was used for the collection of the EBSD patterns, data processing, and indexing using the Hough transformation of EBSD patterns to detect the Kikuchi lines . Subsequent orientation analysis was performed using the MTEX toolbox for MATLAB [4], providing, e.g., inverse pole figure (IPF) maps.

Cross-section samples for scanning transmission electron microscopy (STEM) were prepared using the in-situ lift-out technique in an FEI Strata 400S Ga+-ion FIB/SEM instrument. A Pt protection layer was deposited by FIB to protect the underlying material during FIB milling. High-resolution high-angle annular dark-field (HAADF-) STEM images were acquired on an FEI Titan³ 80-300 at 300 keV. An FEI Tecnai Osiris operated at 200 keV and equipped with ChemiSTEM technology was used for chemical analyses with energy-dispersive x-ray spectroscopy (EDXS). The EDXS data was processed using Bruker Esprit.

Results

The continuous laser is focused at the interface of ink and substrate, resulting in the deposition of crystalline ZnO. Residual ink is removed by a solvent and the ZnO line is left (Fig. 1g).
SEM -EBSD was applied to analyze the crystallinity of ZnO (Fig. 1a). Single crystalline ZnO with the wurtzite crystal structure (P63mc) grows on the amorphous substrate for a 1 µm/s printing speed and a laser power of approximately 0.8 mW. The initial ZnO orientation is likely random since different lines show different initial orientations (not shown). In the IPF maps, one large ZnO single-crystal can be identified due to one single general color in each IPF map (Fig. 1b-d). However, a gradient of the color in all IPF maps indicates that the orientation of the crystal varies along the printed line, which means the crystal rotates along the printing direction. The corresponding color legend is presented in a triangle color legend (Fig. 1e). The orientation of the hexagonal lattice of ZnO at the start and the end of the printing is schematically shown (Fig. 1f). Earlier works have reported rotating lattice crystals in Sb-S-I glass system, by using different materials and laser printing parameters [5]. A total misorientation of 20° over the entire printed line (7.9 µm) is observed, corresponding to a linearly changing rotation rate of 2.6°/µm.
STEM-EDXS is used for chemical analysis of a cross-section TEM sample, which shows porous ZnO formed on silica (Fig. 1h). Nanometer-sized pores in the ZnO layer are visible as dark regions in the HAADF-STEM image result from the printing process (marked with arrows) . The Pt layer on the ZnO is deposited by FIB and acts as a protection layer during TEM lamella preparation. Also chemical analysis shows that the substrate layer below ZnO consists of 20 nm SiO2 and the layer below is a 250 nm Si layer. The silica glass under the Si contains Ti. The atomic resolution image shows a small region of the single crystalline laser-printed ZnO, here viewed along the ZnO<2-1-10> zone axis orientation (Fig. 1i). The Zn atomic columns appear bright and the O columns are not visible due to the HAADF-STEM Z-contrast.

Conclusion

To conclude, we provide an extensive electron microscopic examination of ZnO single crystals produced by photothermal laser printing at relatively low printing speed and laser power on an amorphous substrate. EBSD confirms the single crystallinity of ZnO with a rotating lattice over a distance of 7.9 µm. We will explore different printed geometries for a better understanding of the rotating lattice ZnO single crystals and the growth process regarding laser printing parameters. The aim will be to use such laser-printed semiconductor devices in microelectronics applications [3].

Keywords:

Electron microscopy
EBSD
laser-printing
ZnO

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Towards atom-counting from first-moment STEM images

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Poster Group 2

Background incl. aims
Over the past few years, a model-based approach has been established to extract quantitative measurements from atomic resolution HAADF STEM images. In this approach, the image is modelled as a superposition of 2D Gaussian peaks from which unknown structure parameters, such as atomic column positions, peak intensities and scattering cross-sections, can be estimated. This approach has been used to achieve accurate and precise determination of the chemical composition of materials and to count the number of atoms with single atom sensitivity. Atomic column intensities in HAADF STEM scale with the square of the atomic number and so light columns are easily hidden by the stronger intensity from heavier columns, making simultaneously estimating structural parameters for both light and heavy atomic columns challenging. However, this limitation might be overcome by quantifying other types of STEM image via a model-based method.
Methods
Recent advances in pixelated detectors, along with the capability to collect 4D STEM datasets, offers significant flexibility in generating diverse STEM imaging modes. One example is first-moment STEM images, in which the center of mass (COM) of convergent beam electron diffraction (CBED) patterns is recorded at each scan position. Typically, the COM is determined in two perpendicular directions, generating COMX and COMY images. Within the phase object approximation, the COM is proportional to the gradient of the projected potential, which is expected to scale linearly with the number of atoms in atomic columns as well as atomic number. Therefore, COMX(Y) images are expected to narrow down the signal difference between light and heavy atomic columns. By means of simulations, our study has explored the possibilities to estimate structure parameters and to perform atom counting based on COMX(Y) images. The precision of counting atoms with COMX(Y) images is also evaluated and compared to that using the traditional HAADF regime.
Results
We have performed model-based quantification on simulated COMX(Y) images of aluminum crystals. The integrated-intensities of atomic columns have been determined as a function of thickness by fitting a parametric model of a superposition of X(Y) derivatives of 2D Lorentzian-functions. For aluminum, a monotonic increase is observed up to a thickness of 30 atoms (~12nm), suggesting these intensities can be used as a quantitative measure for atom counting. Based on repetitive noise realizations of both COMX(Y) and HAADF images, it is possible to estimate the precision with which intensities can be measured. Furthermore, examining the overlap of the integrated-intensity distribution between atomic columns with consecutive thickness offers a means to evaluate the atom counting precision. This overlap can be quantified in terms of its relative width. The reduction of the relative width suggests the possibility to attain more precise atom counts when using COMX(Y) images as compared to the use of HAADF STEM images. Finally, the integrated-intensities from an aluminum wedge, whose thickness ranges from 5 to 15 atoms, is compared to those values from the aluminum crystals. The excellent agreement demonstrates the robustness to use bulk crystal simulations as a library, where the atom counting for samples with different shapes can be achieved by comparing to those library values.

Conclusions
In this study, a model-based quantification approach for COMX(Y) images has been developed. Unknown structure parameters, especially the integrated intensities of atomic columns, can be estimated, which enables us to perform atom-counting. As a comparison to traditional HAADF STEM imaging, atom-counting based on COMX(Y) images shows better precision and has great potential for further use in materials where both light and heavy elements are present.

Keywords:

Quantitative 4D-STEM, atom counting,

Reference:

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Multi-scale cements and concrete characterization using X-ray Microscopy, automated phase classification, and machine learning

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Poster Group 2

Background incl. aims
There is a growing need to quantify and characterize the varying components within cements and concretes in multiple dimensions and at the multi-scale. This is particularly true for cements and concretes developed for the nuclear industry, whether as vessels for nuclear reactors or for nuclear waste containment, and to understand their constituents more holistically in three dimensions. However, cements and concretes are generally challenging to image in 3D, particularly at high resolution; this is because of typically large core sizes and consequential limited X-ray penetration, as well as varying chemistries of the components, and differing grain sizes. Further, because most of the components are calcium based, it can be difficult to differentiate them using density-based greyscale data alone when using traditional reconstruction (FDK) techniques. Because of these difficulties, scans usually need to contain many projections and long exposure times, therefore leading to long scan times and limited throughput. Here, we apply a variety of machine learning and AI approaches in a new XRM > machine learning reconstruction > AI quantified phase classification workflow. This workflow vastly improves the resulting scan data and enhances the component quantification process.

Methods
Here, we use non-destructive 3D imaging via X-ray Microscopy (XRM), combined with a novel 3D automated quantitative phase classification technique to spatially characterize the mineralogical phases in a variety of cements and concretes. We collected multi-scale 3D scans on a ZEISS Xradia Versa 620 X-ray Microscope (XRM), the data being subsequently run through numerous reconstruction options belonging to the Advanced Reconstruction Toolbox (ART): this includes DeepRecon Pro, a machine learning based approach for advanced denoising, enhanced contrast, and faster scans, and DeepScout, which allows users to upscale higher resolution interior tomographies to larger fields of view without sacrificing resolution. Finally, we use Mineralogic 3D to spatially characterize and quantify the mineralogy/phases within the concretes and cements in 3D (Mitchell et al., 2024).

Results
We find that DeepRecon Pro is effective at reducing noise in the resulting scan data (Figure 1). We also find that by using DeepRecon Pro, we can collect fewer projections (801 rather than 2401), resulting in better quality data, and we are able to collect scans three times quicker (29 minutes instead of 1 hour 35), leading to greater throughput of samples. We also find that we can improve the size of the field of view for high resolution scans; we are able to upscale 5.8 um voxel size scans from 5.9 mm3 field of view to 23 mm3, resulting in a field of view increase, and resolution recovery, of roughly 4x. This consequently leads to more representative segmentations over larger sample areas and better-quality data. In the final step we have applied Mineralogic 3D to achieve

quantitative phase classification of the concrete components; we are able to distinguish components of similar chemical composition and contrast (in particular, those that are Ca-rich), segment, and quantify them, which is an improvement on standard thresholding which does not take into account the scan conditions and relative density of the material. The results presented here show how new software options (DeepRecon Pro, DeepScout, Mineralogic 3D) are valuable to improve the image quality, characterization, and quantification of cements and concretes in 3D.

Keywords
Cements/concretes; automated phase classification; Tomography; Image processing

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Mitchell, R. L., Holwell, A., Torelli, G., Provis, J., Selvaranjan, K., Geddes, D., Yorkshire, A., & Kearney, S. (2024). Cements and concretes materials characterisation using machine-learning-based reconstruction and 3D quantitative mineralogy via X-ray microscopy. Journal of Microscopy, 1–9. https://doi.org/10.1111/jmi.13278

Keywords:

concrete; phase classification; tomography

Reference:

Mitchell, R. L., Holwell, A., Torelli, G., Provis, J., Selvaranjan, K., Geddes, D., Yorkshire, A., & Kearney, S. (2024). Cements and concretes materials characterisation using machine-learning-based reconstruction and 3D quantitative mineralogy via X-ray microscopy. Journal of Microscopy, 1–9. https://doi.org/10.1111/jmi.13278

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Microstructure and thermal stability of ultrafine-grained CuZn5 processed by HPT

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Poster Group 2

Background and aims:
High-pressure torsion (HPT) is widely known as an effective method for severe plastic deformation (SPD). This intense shear strain, combined with high pressure, not only results in notable grain refinement but also introduces a substantial density of dislocations, thereby enhancing mechanical strength [1].
Concurrently, ensuring the stability of ultrafine-grained (UFG) microstructures is crucial for their reliable performance in practical applications. Grain growth can compromise the enhanced strength of UFG materials, highlighting the importance of investigating microstructural stability for their commercial feasibility [2].
This concern is particularly notable in certain pure metals with low stacking fault energy such as fcc metals. In order to avoid grain growth, the grain boundary (GB) migration has to be limited. This can be done through the segregation of solute elements, which depends on the GB character. In this work, we use copper (Cu) as bulk material and add different amounts of Zn to examine the impact of varying Zn content on microstructural changes and thermal stability of GBs after HPT processing at room temperature.

Methods:
CuZn5 solid solutions were processed using HPT with a pressure of 4.5 GPa. The thermal behaviors of these materials were studied using differential scanning calorimetry (DSC). Scanning transmission electron microscopy (TEM) in combination with energy dispersive X-ray spectroscopy (EDX) was used to investigate the microstructure and composition of Cu-5at.%Zn (CuZn5), with a focus on GB segregation before and after annealing at different temperatures. Additionally, 4D-STEM (ACOM) was utilized to extract information on grain size distribution, grain orientation, and grain boundary types.

Results:
Annealing of CuZn5 up to 300°C by DSC shows a single exothermic peak at about 275°C. it should be mentioned that no grain growth or changes in the grain orientations occurred up to 200°C. High-angle annular dark-field (HAADF)-STEM imaging helps to understand the effect of Zn on the microstructure and GBs. From the 4D-STEM orientation mapping, the GBs can be classified into low-angle GBs, special coincidence site lattice (CSL) boundaries as well as high-angle general GBs. Together with STEM-EDX, it was found out that low energy boundaries such as Σ3 twin boundary is not enriched by Zn. On the other hand, Zn general GBs are segregated with Zn. This observation agrees well with the theory that solute segregation depends strongly on the GB energy. While special Σ-boundaries have low energy, they are less affected by solute segregation.
Conclusion:

Both CuZn5 and CuZn30 maintain a single-phase structure even after annealing, suggesting that the improved thermal stability of the alloys compared to pure Cu is attributed to the presence of solute content. This is due to the solute-drag effect [3], hindering the diffusion of GBs and contributing to the enhanced thermal stability of the alloys

Keywords:

HPT; Cu alloy; Thermalstability; DSC

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Evaluation of Conventional Adherent Cell Enumeration Methodologies alongside Image-Enhanced Flow Cytometry

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Poster Group 2

Background incl. aims
Enumeration and characterisation of cell populations are vital processes in both clinical and industrial laboratories. Accurate, reproducible counts of viable and apoptotic cells are crucial to many biological assays and require the development of robust experimental protocols. Currently, interlaboratory comparability between cell measurements is limited due to the lack of alignment in cell analysis techniques and methodologies. In general, the enumeration of adherent cells poses a greater metrological challenge than suspension cells, due to morphological complexity and diversity these cells can present. The impact of cellular morphology and growth characteristics on cell counting is yet to be fully investigated for many conventional enumeration techniques. The use of DNA and membrane stains allowed for quantification of cell confluency as a function of cell count using microscopy, demonstrating the impact of cell type on limits of quantification and linearity. Overall, this study evaluated the efficacy of three common enumeration techniques: haemocytometry, microscopy and flow cytometry using five distinct adherent cell types. Finally, a novel counting technique, which uses combined brightfield imaging flow cytometry, was investigated as a means of overcoming the current limitations associated with conventional flow cytometry.

Methods
Five adherent cell types; MCF-7, HeLa, MRC-5, HUVEC, and CHO were selected to cover a range of morphologies and disease states. Cells were seeded at commonly used concentrations, recommended by the ATCC. Cells were analysed forty-eight hours after seeding, using six separate instruments across three different counting techniques: haemocytometry (automated Countess and manual), microscopy (confocal and widefield), and flow cytometry (Attune CytPix and Beckmann Coulter CytoFlex). Cells for haemocytometry and flow cytometry were detached for counting using TrypLE. Cells for microscopy were fixed using 4 % paraformaldehyde, then DNA and membranes stained using Hoechst 33342 (2 µg/ml) and Wheat Germ Agglutin 594 (2.5 µg/ml) respectively. Imaging was performed using a Zeiss LSM 880 confocal or a EVOS FL 2 Auto widefield microscope. Automated image analysis of nuclei counts, and membrane area was performed using CellProfiler 4.2.1.

Results
Cell counts measured using hemocytometry demonstrated that this technique is least impacted by cellular morphology and growth characteristics. Low sampling volumes and user bias are the primary sources of counting variations which result in a relatively high limit of detection when compared to other techniques. The efficacy of microscopy-based counting was the most influenced by cellular morphology. Cells which displayed densely populated growth characteristics, such as CHO and HeLa, proved challenging in the identification and segmentation of single cell nuclei. These limitations resulted in a loss of linearity in cell count v confluency plots, which showed cell counts continuing to rise despite having a 100 % confluency. Both conventional flow cytometers consistently underestimated cell counts when compared to the other four techniques. This was primarily due to

the presence of cell aggregates in the sample which were incorrectly counted as single cell events and was not resolved following single gating approach of scatter height against width. In particular, cells displaying clustered growth morphologies, such as MCF-7, were found to produce the lowest cell counts in flow cytometry. Using the Attune CytPix brightfield imaging and analysis capabilities, cell aggregates could be identified and the number of cells in each aggregate determined. Image enhanced cell counts (CytPix En.) displayed values significantly more in-line with alternative techniques, demonstrating the efficacy of this novel counting methodology (graphic).

Conclusion

Results throughout this cell counting study demonstrated the impact of cell morphology and growth characteristics on countability. Several key factors have been highlighted which must be considered when aiming to develop robust cell counting methodologies and standards. Microscopy has been shown to be a useful tool for cell counting, however, this study notes that its limit of linearity and quantification is dictated by cell type. The combined use of flow cytometry and imaging for cell counting presents a quantitative method of determining cell count without the use of reference materials. This method overcomes the limitations of both microscopy and flow cytometry, in particular, removing the uncertainties associated with user-based single-cell gating strategies.

Keywords:

Cell-counting, Imaging Flow Cytometry, Microscopy

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Structure and Function of Fructose 6-phosphate aldolase

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Poster Group 1

Background
Fructose 6-phosphate aldolase (FSA) catalyzes formation of fructose 6-phosphate from dihydroxyacetone and D-glyceraldehyde 3-phosphate via an aldolization reaction (1).
Methods
Cryo-EM structures of FSA from E. coli were obtained of the wild type enzyme and a mutant, L107C/A129G/R134V/L163C/S166G (FSAm), that had been identified as having substantially improved activity(2).
Results
Both proteins form D5 symmetrical decamers and X-ray structures (pdb: 7qxf, 1l6w) could be used for model building(2,3). FSAm samples contained the substrate 2-hydroxyacetophenone and the map had density corresponding to the crucial iminium reaction intermediate captured in the active site. The refined model showed a modification of a lysine residue (Lys85), 2-imino-2-phenylethanol-lysine not previously described in the Chemical Component Dictionary of the pdb database. Moreover, the side chain of the critical residue Y131 was rotated 100 degrees shifting the position of the phenolic group 3 Å. A water molecule participating in a hydrogen bonding network at the active site and thought to be involved in proton relay was shifted similarly.
Conclusion
The new structure can guide identification of additional FSA variants that display improved carboligation activities with 2-hydroxyacetophenone and phenylacetaldehyde.
Keywords
Catalysis, Fructose 6-phosphate aldolase, cryo-EM

Keywords:

Catalysis, Fructose 6-phosphate aldolase, cryo-EM

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Surface microstructural evolution during Rolling Contact Fatigue of Rolling Element Bearing steels

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PS-02 (1), Lecture Theater 5, august 28, 2024, 10:30 - 12:30

Background incl. aims
To reduce CO2 emission and comply with European legislation, automotive market implements new electrical powertrain. It implies changes in the operating conditions of its bearings requiring new material development (grades and/or thermochemical treatments) for bearing manufacturers. A typical mode of failure for bearings operating in contaminated lubricant is surface-initiated spalling on a dent[1,2], such as in gearboxes. The denting process creates micrometer-scale shoulders, intensifying localized stress [3] and leading to plastic deformation over the operational lifespan (Figure 1,a). Therefore, cracks are often observed to be initiated at the vicinity of the shoulder. However, the life cycle of the bearing was observed to be dependent of its initial microstructure[4]. To guide the development of new materials, a better understanding of the link between microstructure of the material and its performance in contaminated conditions is needed. Understanding the influence of each microstructural constituent on microstructural evolution, fracture development, and propagation is paramount for the development of more durable materials.

Methods
This study presents a multi-scale characterization of martensitic 100Cr6 bearings before and after RCF under standard test conditions. Samples were collected from bearings before and after the tests. Observations were realized at different locations (outside the dent area, under the dent, under the shoulder) using techniques such as SEM, SEM/FIB, EBSD, TKD and TEM-ASTAR.

Results
Prior to RCF, finishing operations leads to the formation of a 0.5 µm thick surface layer consisting in refined martensite and spread primary carbides. After RCF, away from the dent, no further grain refinement was observed along the raceway; the refined surface layer being resistant enough to plastic deformation during fatigue. Surprisingly, at the bottom of the dent, were large plastic deformation occurred during the indentation phase, no significant additional alteration of the microstructure was observed.
The major microstructural evolutions were observed beneath the dent shoulder where plastic flow occurred during the indentation and running-in, and where the stress is localized during the rolling process. First a fibered region is observed below surface, with a thickness of around 1µm (Figure1b). In this region, martensite grains are refined so that nano-grains are observed, and primary carbides are sheared. Below and down to 3 µm deep, far from finished surface, an ultra-fine grain layer is observed, mixing coarse and fine martensite. The martensite morphology, size and disorientations maps suggest that martensite undergoes refinement through twinning and continuous Dynamic Recrystallisation (Figure 1,b).

Crack initiation was preferentially observed in the nano-grain layer, at carbides/matrix interfaces. Above the crack, another refined grain region is detected that was interpreted to be due to high deformation and stresses through crack propagation.
In all characterized areas, nanosized austenite islands were observed. The initial retained austenite does not completely transform during fatigue in this case while it is known to transform under surface-RCF [5]. The major microstructural evolution observed is related to the refinement/reorientation of martensite grains that occurs through twinning and/ cDRX depending on the initial orientation of the grains.

Conclusion
The multiscale characterization of such localized deformed area, in non homogenous pieces (indentation being a random process) is a complex and tedious task to achieve. However, it gave crucial information about the microstructural deformation mechanisms. We infer that a material producing dents with smaller shoulder and less primary carbides at its surface would improve its lifetime.

Fig. 1: (a) Schematic representation of a dent after RCF, (b) SEM-BSE image of a crack initiated under the dent shoulder, and c) ASTAR-ACOM map of the white square area.

Keywords:

Steel, Rolling Cycle Fatigue, Microstructure,

Reference:

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[2] F. Ville et D. Nélias, Tribology Transactions, vol. 42, pp. 231-240, 1999.
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Strain mapping of a Σ5(310) grain boundary in Cu bi-crystal using scanning transmission electron microscopy

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Poster Group 2

Background incl. aims
Grain boundaries (GBs) are pivotal in determining the physical characteristics of materials. The presence of extended strain fields localized at GBs can significantly impact atomic transport within these boundaries. Our research focuses on understanding the atomic structure of GBs and its correlation with the strain state along these boundaries. To delve deeper into the influence of strain on GB diffusion, we are investigating the evolution of strain along GBs under mechanical treatment using two distinct methods across varying length scales to establish a connection between microstructure and atomic transport properties.
Methods
A Cu bi-crystal containing a Σ5(310) GB was fabricated using a modified Bridgman technique, followed by annealing at 800 °C. An electron-transparent sample was prepared using FIB lamella target preparation to observe the GB in a cross-sectional view. The elastic strain along and across the GB was measured at the nanometer scale using a stack of nano-beam diffraction patterns (NBDPs) acquired in STEM mode with a 1 nm probe size, while ensuring grains were oriented in zone axis conditions. A custom-written code was utilized to extract the strain maps [1]. On an atomic scale, strain was characterized using the geometrical phase analysis (GPA) applied to high-resolution STEM images, based on measuring small displacements of lattice fringes relative to a reference lattice in the HR(S)TEM images [2].
Results
In the reference state of the pure Cu GB structure, shear strain, dilation, and rotation were measured along and across the GB using the NBDP method. The strain in the vicinity of the GB was found to be negligible (<0.1%). The results were consistent with the GPA method, showing almost constant small values of strain (<0.5%) in both halves of the crystal. These measurements were compared with strain mapping on cobalt-deposited samples.
Conclusion
Strain analysis of the GB in the Cu bi-crystal was conducted using two methods, GPA and NBDP, both of which yielded consistent results for strain across and along the Gb. GPA provided localized information at the nanometer scale, while NBDP offered strain information over a larger area. The strain analysis using GPA was correlated with the high-resolution structure of the GB. The high-resolution image of the GB provided insights into the variation of motifs within the boundary, potentially influencing strain distribution across both crystal halves.

Keywords:

Grain boundary, Strain, GPA, NBDP.

Reference:

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[2] Hÿtch, M. J.; Putaux, J.-L.; Thibault, J. (2006). Stress and strain around grain-boundary dislocations measured by high-resolution electron microscopy. Philosophical Magazine, 86(29-31), 4641–4656. doi:10.1080/14786430600743876

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CEFID: A flexible platform for spectroscopic experiments

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

TEM-related instrumentation has been developing rapidly in the last few years and a state-of-the-art experiment often involves a variety of third-party components ranging from advanced in-situ specimen holders and new generation detectors to pulsed lasers and programmable phase plates. The CEOS Energy Filtering and Imaging Device (CEFID) is an energy filter and spectrometer offering state-of-the-art specifications and the flexibility to implement ambitious and unprecedented experiments.
Under a concept of separation of function that mirrors the design of a TEM itself, the filter design comprises highly optimised and stable optics up to the energy-selecting slit, and a flexible and minimalist projective stage [1]. This gives high performance and stability, while allowing to hop between modes (imaging, spectroscopic dispersions) with little to no re-tuning. The Python/Qt-based graphical software used for the filter operation, implements interactive and automated procedures for alignments, common workflows ranging from EFTEM to 4D-STEM, and tools for on-the-fly analysis such as live DFT, EELS maps computation/quantification, Center-of-Mass, etc.
The software is highly extendable and offers a scripting and plug-in API in python and a remote control interface for the integration into third-party software.
A wide range of detectors and scan generators from different manufacturers has already been integrated and can be used for both the tuning and data acquisition, and the filter can be installed on TEM columns from the three major manufacturers. The capability and versatility of the system make it well suited for rapidly acquiring and evaluating spectroscopic information on samples of interest [2], while its flexibility and compatibility make it a platform for complex experiments where different tools need to work in unison, such as the synchronization of acquisition with in-situ stimuli, or photon-induced near-field electron microscopy [3].
Recent developments on the CEFID will also be shown. This includes the recently introduced Dual Range EELS, a solution to rapidly switch energy range so that two different losses can be recorded concurrently, as well as methodological developments in energy-momentum mapping, and in the measurement of high-loss edges.

Keywords:

EELS, Spectrometer, Energy Filter

Reference:

[1] F. Kahl, et. al, Characterization of a New Post-Column Imaging Energy Filter.
Advances in Imaging and Electron Physics, 212, 35-70, (2019).
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Nature 600, 653–658 (2021).

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Improving Transmission Kikuchi Diffraction workflows

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Poster Group 2

Transmission Kikuchi diffraction (TKD) is an increasingly popular technique in the scanning electron microscope (SEM) for the characterization of nanoscale structures in wide range of materials [1-3]. The convenience of being able to utilize a conventional commercial electron backscatter diffraction (EBSD) system in a field emission SEM or focused ion beam (FIB) SEM has made TKD a particularly attractive alternative to orientation mapping techniques in the transmission electron microscope (TEM).

Improvements to focused ion beam instruments and sample preparation methods [4], have added analysis capabilities but not significantly improved the overall workflow or data quality. Methods such as the large area preparation method have made it possible to apply many standard approaches used for EBSD, however there is scope for TKD specific improvements. related to data processing during data acquisition, offline reprocessing and the geometry in which data is being acquired.

We will discuss the latest improvements to TKD workflows, improving the acquisition of high-resolution data, making the end-to-end workflow easier, and improving data processing to obtain better data quality in relation to TKD analysis.

Keywords:

TKD, EBSD, FIB, FIB-SEM

Reference:

[1] R.R. Keller and R.H. Geiss, J. Microscopy, 245 (2012), p. 245-251.
[2] P.W. Trimby, Ultramicroscopy, 120 (2012), p. 16-24
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[4] P.W. Trimby et al., Microscopy and Microanalysis, 27(S1) (2021), p. 1596-1598.

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Panta Rhei: A software platform for acquisition and processing of image and spectral data

Angelika Leibscher[1], Dominique Lörks[1], Michael Krieger[1], Dr. Giulio Guzzinati[1], Heiko Müller[1], Martin Linck[1], Pirmin Kükelhan[1], Ingo Maßmann[1]
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Poster Group 1

Recent developments of add-on equipment in transmission electron microscopy cause a demand for modular and flexible data acquisition software. Fully integrated commercial solutions exist, but these are typically tailored towards a certain combination of proprietary hardware components from one manufacturer. For off-line data analysis and very specific high-throughput workflows open source software packages became available during the last years. Nevertheless, a lack of highly interactive software, directly usable during the operation of the instrument with its different components, is obvious.
To close this gap, CEOS Panta Rhei is designed as an interactive platform for data acquisition, processing and visualization in electron microscopy written in Python using the Qt, ZMQ and numpy libraries.
The primary purpose of Panta Rhei is to enable simple steering and supervision of workflows combining the control of the microscope and its accessories with data acquisition, online data analysis, and direct visual feedback. The capabilities for online data evaluation are progressing. We currently concentrate on functionality for quick and meaningful assessment of data quality and online session planning like image filters, data statistics, diffractograms, live 4D-STEM evaluation, and elemental mapping and quantification for EELS as well as EFTEM. Recently, the fitting of EELS spectra (and maps) using a library of precomputed generalised oscillator strengths (GOS) was also added [1]. Data can be interchanged with other software using common file formats like npz, hspy, mrc, msa, and tiff.
Interactive dialogs are provided to control the supported hardware, and perform interactive data acquisition from the separate device (e.g. camera images or STEM images), or combining multiple hardware components (e.g. STEM-EELS mapping).
The acquisition (via cameras or scan detectors) can generate a high volume of data which has to be transferred and processed efficiently. Therefore, a central component of Panta Rhei is a separate server process called Repository providing access to a managed shared memory. As soon as an acquisition device stores data in the Repository other clients are notified and can directly access the data with minimal CPU load and memory consumption. Clients themselves may also use the Repository to store processed data.
The Panta Rhei GUI is an application to acquire, display and process data and control hardware components which connects as client to the Repository. It displays Views of data from the Repository via a multiple document interface (MDI). Views are used to live display certain aspects of the data and the numerous available DataTools continuously calculate dependent data from updated input. The name Panta Rhei (πάντα ῥεῖ -- everything flows) is motivated by these chains of transformations that may even run in separate processes.
For custom extensions, a scripting interface provides control of data processing and display tools as well as hardware devices. For easy external access to hardware control, an RPC-interface is available.

Currently, Panta Rhei has interfaces for the TEMs of 3 manufacturers, 5 families of detectors, 4 different scan generators, and the CEOS Imaging Energy Filter (CEFID [2]). We expect that the number of compatible devices will continuously grow over time.

Keywords:

Software, data evaluation, image processing

Reference:

[1] Generalised Oscillator Strengths for the simulation of EELS spectra, with a broader coverage of high energy and minor edges, https://zenodo.org/records/7645765
[2] F. Kahl et al. (2019) AIEP 212 including Proceedings CPO-10.

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MÖNCH: A 25um hybrid pixel detector with sub-pixel resolution using deep learning

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background incl. aims
The adoption of direct electron detectors (DEDs) has led to the "resolution revolution" in cryogenic electron microscopy (cryo-EM) [1]. In Cryo-EM the state-of-the-art is back-thinned Monolithic Active Pixel Sensors (MAPS) due to their small pixel size (< 15 µm) and high-quality imaging capabilities. However, back-thinned MAPS have several drawbacks in terms of radiation hardness, frame rate, and non-optimal performance for low-energy electrons (≤ 120 keV), which are of increasing interest for better image contrast for a given electron dose and lower operational costs [2].
An alternative type of direct electron detector, Hybrid Pixel Detectors (HPDs), have been widely adopted for diffraction-based modalities in electron microscopy on the account of their high frame rates (> 1 kHz) and large dynamic range. However, currently available HPDs are limited in their suitability for imaging by their large pixels (≥ 55 µm) and multiple scattering of electrons in their thick (≥ 300 µm) sensors, such that the signal produced by incident electrons is recorded by many pixels, despite the large pixel pitch. MÖNCH [3] is a general-purpose, charge integrating HPD under development which is notable for its small, 25 µm pixels. Therefore, to fully realize the potential benefits of fast, radiation hard HPDs across the widest range of electron energies and experimental modalities of electron microscopy, we are developing deep learning methods to reconstruct the impact points of incident electrons from their complex track in the sensor with sub-pixel resolution.
Methods
We generated training samples including detector responses and impact points of individual incident electrons from both simulation and measurements. The simulation configuration closely mirrors the design parameters of the MÖNCH. Whereas previous studies [4] have been entirely based on simulations, we have, uniquely, prepared training samples with precise labels from experimental measurements by developing two novel data acquisition approaches, which will be presented in detail. For this purpose, as well as for the measurement of the detector's performance as quantified by its modulation transfer function (MTF) and detective quantum efficiency (DQE), a MÖNCH 03 prototype was mounted on a JEOL JEM-ARM200F NEOARM at the Paul Scherrer Institute for data collection.
Based on the simulated and experimental datasets, deep learning models were developed for impact point reconstruction. We will show details of the deep learning model design, training scheme, and evaluation results.
Results
Sub-pixel resolution was attained at all tested energy levels (200, 120, 80, 60 keV). For example, at energy levels of 200 keV and 60 keV, the resolutions were 1.8 pixels and 0.4 pixels for the conventional charge centroid method, while via the deep learning approaches the resolutions were 0.6 pixels and 0.3 pixels, respectively. Images of a test sample consisting of continuous carbon with gold nanoparticles at 200 keV are shown in Figure 1, processed using the conventional charge

centroid method (left) and the deep learning approach (right). With the deep learning method, we were able to image the spacing of the {1 1 1} gold atomic planes, with a spacing of 2.35 Å. The MTF and DQE results at all energy levels, with and without the deep learning algorithms applied, will also be presented as a benchmark of the system's capabilities. Lastly, the data processing pipeline for the MÖNCH detector for its use in electron microscopy will be discussed.

Conclusion

In this work, we have successfully developed deep learning approaches for localizing electron impacts on MÖNCH. Using measurement-based training enabled by the novel acquisition setup we have developed, sub-pixel spatial resolution was achieved across all electron energies tested. The improvement relative to the results obtained with the charge centroid method increased with electron energy. The spatial resolution obtained for 200 keV electrons (0.6 pixels) represents a threefold enhancement compared to the charge centroid method. Our results show that MÖNCH, with its spatial resolution enhanced using deep learning, is a highly promising detector for electron microscopy studies using electrons up to 200 keV.

Keywords:

Hybrid Pixel Detector, Deep-Learning, Cryo-EM

Reference:

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Nanoscale Chemical Segregation to Twin Interfaces in τ-MnAl-C and Resulting Effects on the Magnetic Properties

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Poster Group 1

Background incl. aims
Among various candidates for the rare-earth free permanent magnets, the ferromagnetic τ-MnAl-C (L10, P4/mmm) shows great potential to replace ferrites and bonded Nd-Fe-B magnets for its attractive magnetic properties and the non-critical nature of the raw elements [1, 2]. Further magnetic properties enhancement will depend on understanding of the effect of various crystallographic defects in the magnet, e.g. twin boundaries, and on developing novel processing routes for microstructure optimization.
Twin boundaries are frequently observed in both the as-transformed and hot deformed τ-MnAl-C magnets [3]. Three different types of twin boundaries have been discovered in the τ-MnAl-C magnet and they are described as true twins, order twins and pseudo twins [4]. Considering the tetragonal structure of the chemically ordered τ-MnAl-C magnet, it is reasonable to assume that a different atomistic structure exists at these three types of twins, as well as its local magnetic properties.
Methods
In this study, aberration-corrected scanning transmission electron microscopy coupled with electron energy-loss spectroscopy (STEM-EELS) was used to investigate the atomistic structure and chemical composition at various twin boundaries in τ-MnAl-C [5].
Results
The results show differing levels of structural disorder at the various types of twin boundaries and EELS data reveal the presence of a Mn-enriched layer at the twin boundary surrounded by Al-enriched layers. The thickness of these layers and the magnitude of the chemical segregation vary with the level of structural disorder. Micromagnetic simulations based closely on the experimental results showed that the coercivity tends to increase with increasing structural and chemical disorder at the twin interface.
Conclusions
These results suggest that targeted doping of interfaces in τ-MnAl may be a promising strategy to increase the coercivity of the material for applications.

Keywords:

Twin boundaries
STEM-EELS
Elemental segregation

Reference:

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Overcoming the aberration-limit of a non-corrected Transmission Electron Microscope with computational ghost imaging

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Poster Group 2

Background incl. aims

The invention of aberration correctors at the end of the 20th century has made modern Transmission Electron Microscopes (TEMs) one of the most sought-after scientific instruments in modern material science and life science laboratories since they now allow to image and characterize samples with the highest lateral (spatial) resolution. [1,2] The most important technological advancement which further increased the interest in TEMs was the invention of spherical aberration correctors. [3,4] Unfortunately, aberration correctors are quite costly and are reaching performance limitations: this has prompted some research groups to work on computational imaging methods to increase the lateral resolution in TEMs.
Here we present the results from a simulated computational ghost imaging (CGI) scheme for TEMs where we demonstrate numerically that it's possible to overcome the resolution limit imposed by aberrations using well-characterized structured probes and a bucket detector.

Methods

In computational ghost imaging the image of the sample (and its spatial information) is computationally recovered by illuminating the sample with a series of known structured beams and collecting the integrated transmitted intensity via a single-pixel bucket detector positioned after the sample. In fact, if the measurement is linear we can see that it can be described in matrix notation with the equation $I = P \hat{} T$ where I is the array containing the N signals recorded by the single pixel detector, P is the array containing the intensity pattern of the N probes and T is the sample transfer function. By knowing both P and I it is possible to recover T and many algorithms exist to this end [5]. We have developed a custom python-based algorithm that allows us to simulate the generation of structured patterns, provides us with the value of the signal measured by a virtual bucket detector (even in presence of noise and realistic coherence effects) and recovers T with three different reconstruction algorithms: the traditional one, the alternated projection algorithm and the conjugate gradient descent algorithm.

Results

For the generation of the simulated structured patterns we assumed a 300kV microscope with Cs=2.7mm, a 15.4 mrad probe convergence angle and as defocus four times the Scherzer STEM defocus. All other aberrations were neglected since we had such a large value of Cs. The electron modulator is assumed to be in the last condenser aperture, while the sample of choice (a twisted

bilayer of MoS2 – Figure1a) is conventionally positioned in the sample plane. As single pixel detector we used the annular dark field detector, but in principle we could use other single pixel detectors such the bright field detector or those used for energy dispersive X-Ray imaging, electron energy loss spectroscopy or cathodoluminescence.
As it is possible to appreciate from figure 1, our CGI scheme is able to overcome the aberration limit imposed by the instrument (figure 1e) with a two-fold increase in spatial resolution compared to an aberration-limited STEM image (figure 1c) as confirmed by the analysis of their FTTs (figure 1f and d, respectively).

Conclusion

Here we have demonstrated that, if the optical system is well characterized and the aberrations that act on the illumination are known and quantified, it is possible via a computational imaging technique to overcome the aberration-limit.
We are already working on an experimental implementation where the phase plate for structuring the beam has been realized using MEMS technology. As a next step for further improvements, we are working on the possibility to use machine learning (ML) to further optimize our electron modulator and the pattern that it can generate, with should enhance both our simulated results and more importantly the future experimental ones. Moreover, we are also working on a mixed scheme where we also control the scan coil of the TEM to optimize the illumination, increases reliability and acquisition speed, and should allow for the reconstruction of the probe.

Figure 1 Caption: a) schematic of the electron-optical setup, (b,d,f) comparison between the sample atomic potential, the aberration-limited STEM image, and CGI reconstruction and their corresponding FFT (c,e,g). For the CGI reconstruction reported here no noise and no realistic coherence effects (temporal or spatia) were considered, but we have also performed simulations in those scenarios. In the FFT images the yellow inner circle corresponds to 2α, while the red outer circle corresponds 4α, where α=7.3 mrad. Scalebar in b,d and f is 0.5nm. In h) is shown an example of a structured pattern utilized for the CGI reconstruction and in i) is shown complex image of its FFT.

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Keywords:

Phase-plates, electron-beam-shaping, single-pixel-imaging, high-resolution, MEMS

Reference:

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Characterization of the crystal structure of In-Ga-Zn-O materials via Precession Electron Diffraction

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Poster Group 2

Indium-Gallium-Zinc-Oxide (IGZO) compounds have been regarded as attractive material for the channel in transparent thin-film transistors (TFTs), with low leakage in the off-state and high mobility. IGZO-based materials belong to the class of high band-gap semiconductors and their crystallographic properties are the key characteristic determining the final performances of the electronic devices. Amorphous IGZO, for example, is easy to grow and has good electron mobility, in contrast to other semiconductors such as Si and Ge in the amorphous state. However, in the perspective of the fabrication of electronic devices, amorphous IGZO TFTs suffer from electrical instability. Crystalline IGZO mostly exists in two polytypes having spaces group R3̄m (160) and P63/mmc (194), and has showed improved electrical performances. There is also an intermediate crystal phase where the periodicity is kept only in the c-axis, while being structureless in the a and b-axis. This phase is called CAAC (c-axis aligned crystalline) IGZO and it is less prone to defect formation. More sophisticated configurations have been employed in TFTs: for example, a double layer design, constituted of amorphous and CAAC-IGZO, has shown good device stability [1]. To further enrich the family of IGZO compounds, a new a spinel polytype has been recently demonstrated, which can be grown via PVD using intermediate conditions derived from the growth of amorphous and CAAC-IGZO, and exploiting a Ga2ZnO4 (GZO) layer as a seed [2].
In such a complex panorama, there is a need for an appropriate technique to characterize the huge diversity of crystal phases in IGZO-based TFTs. To this end, we referred to Precession Electron Diffraction (PED) [3] executed via a Transmission Electron Microscope (TEM) in scanning (STEM) mode. In fact, standard electron diffraction, due to sample thickness, is significantly affected by multiple/dynamic scattering, which leads to diffracted intensities not easy to be interpreted. PED was introduced to solve this issue and analyze crystal structures with better sensitivity. In PED, the electron beam is rocked at a fixed angle to the optic axis above the sample, forming a hollow cone, and then it is de-rocked below the sample. It can be demonstrated that the final diffraction pattern has many more reflections than in the case of an unprecessed beam, having intensities unaffected by dynamic scattering. We applied scanning PED to the study of IGZO thin (about 20 nm) films having spinel structure and grown on GZO layers having different thickness (from 2 to 10 nm). We prove the possibility to correctly identify materials with similar electron diffraction patterns, such are IGZO and GZO, using PED and Phase & Orientation mapping of the scanned area (ASTAR, NanoMEGAS) [4]. We demonstrate that high sensitivity can be achieved to properly resolve even narrow GZO layers, as thin as 2 nm, from the above IGZO. This can be inferred from Figure 1, which shows the combination of phase map and indexation for two different IGZO samples, grown on GZO having thickness of 10 nm (a) and 2 nm (b), respectively. Moreover, phase and orientation mapping allowed us to study the grains structural properties in IGZO grown on GZO layer with different thickness. We show that, as expected, IGZO grows epitaxially on the GZO seed layer. Moreover, IGZO grain growth is related to the thickness of the underlying GZO layers: size of the IGZO grains is coarse when GZO has higher thickness, while columnar growth of narrower and IGZO grains is reached when thin GZO is used. For GZO having 2 nm thickness, IGZO and GZO present texture, with growth of IGZO/GZO along the

<111> direction. Finally, we study the case where thermal annealing in a hydrogen atmosphere reduces the IGZO film and induces the formation of In-rich grains dispersed in the IGZO matrix. In this context, we study the crystal structure of these grains and their matrix.
This study is preliminary to the characterization of TFTs devices employing complex design and based on IGZO compounds as channel material. In general, these results can open the way to the study of different devices, where the common feature relies in the close link between the crystallographic structure and the final performances.

Keywords:

In−Ga−Zn oxide, Precession Electron Microscopy

Reference:

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Investigating stability of ZIF-8 metal organic framework in operational environments: potential candidate for host-guest chemistry

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PS-07 (1), Plenary, august 29, 2024, 15:00 - 16:00

Background incl. aims
Ultra-small atom precise metal nanoclusters (NCs) have emerged as (electro)catalysts due to their large surface area and abundant unsaturated active sites. However, surface ligands and strong agglomeration tendency limit NCs' catalytic activity [1]. Zeolitic imidazolate frameworks (ZIFs), a sub-set of metal-organic-frameworks (MOFs) emerge as excellent support to host NCs due to their well-controllable pore size of 11.6 Å, pore aperture 3.4 Å and high internal surface area. Understanding the state and the stability of these NCs inside the ZIF pores under operating condition is crucial for tuning the catalytic properties.
Generally, in high-resolution TEM (HRTEM) experiments few atom NCs appear to be more stable than ZIF when exposed to the electron beam. The atomicity of Pt NCs is measured using spectroscopy techniques as mentioned [2]. Therefore, HRTEM experiments to understand the structure, e.g. of Pt27@ZIF-8 nanocomposites (shown in fig. 1(a)), are very challenging because ZIF-8 tends to lose crystallinity already at a cumulative electron dose of ~ 25 e-Å-2, which is far below the required dose for standard high-resolution images [3]. So far, no quantitative measurement of a critical electron dose limit for real-time observations of ZIF-8 (and NCs@ZIF-8) under operating conditions (elevated temperature and various gaseous environments) has been reported. Hence, the structural and morphological integrity in such experiments as well as the mechanism of possible loss of crystallinity under the exposure of electron beam are still largely unexplored. However, we consider this to be a prerequisite before conducting an (in-situ) study of such a beam sensitive material.
Methods
To establish the critical electron dose for ZIF-8, we systematically investigated here the effect of the accumulated electron dose on the ZIF-8 crystallinity using selected area diffraction (SAD). Thereby we quantitatively measured 1. the fading of intensity of specific Bragg planes as well as 2. the relative displacement of Bragg planes, both as a function of accumulated total electron dose. We have varied the TEM sample support (graphene versus amorphous holey carbon) and we have applied different TEM imaging parameters such as accelerating voltage (200 vs 300 keV) as well as electron dose rates (0.33, 0.5, 1, and 2 e-Å-2s-1). The bright field (BF) image and the corresponding SAD pattern of single crystalline ZIF-8 particles are shown in fig. 1(b, c). The fading of the relative intensity of 431 Bragg ring in the SAD pattern as function of cumulative dose is shown in fig. 1(d).
Further, we investigated the stability of ZIF-8 at different sample temperatures, varying the temperature from -176°C to 550°C. Both the TEM cryo and heating experiments were performed in a ThermoFisher Titan microscope using a Gatan 626 and Inconel holder, respectively. In the heating experiment the temperature was varied from 200 to 550°C with a step of 50°C.
Under all experimental conditions, the critical accumulated dose was determined by measuring the fading of the respected Brag spots. In order to understand the mechanism of loss in crystallinity we further measure any crystal lattice expansion or shrinkage indicated by changes in the radius of the SAD rings (= Bragg planes).

At last, we investigated the stability of ZIF-8 under oxygen atmosphere and elevated temperatures, using a ThermoFisher Titan ETEM (300 keV) and a Gatan Inconel heating holder. The temperature was varied from 350°C to 550°C under constant O2 pressure of 2.7 mbar. Again, the transformation of ZIF-8 to ZnO nanoparticles was observed in BF images and SAD pattern, and the change in the crystal structure and morphology of the particles were studied.

Results

Based on our systematic study, we report the critical limit of cumulative dose of ~10 e-Å-2 to enable non-invasive structural characterization using HRTEM imaging. We thereby provide insights into the fundamental mechanisms of electron beam-induced damage in ZIF-8.

The influence of different imaging conditions, substrate materials and temperature on the critical dose will be discussed. Finally, the challenges and development of in-situ gas experiments on beam-sensitive materials will be discussed, taking ZIF-8 MOF to ZnO transformation as a reference.

Conclusion

This work provides a deeper understanding of the interaction of electrons with MOF (here: ZIF-8) under different HRTEM imaging conditions, an understanding of the underlying damage mechanism and the identification of optimal conditions for (in-situ) characterization experiments.

These findings have significant implications for the further development of NC@MOF nanocomposites in a wide range of applications, such as catalysis.

Keywords:

Nanoclusters, metal-organic-framework, HRTEM, ETEM, In-situ-TEM

Reference:

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Denoising of 4D-STEM Dataset using Pix2Pix GAN Algorithm and Artifact Reduction Strategy

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Background and Aims
4D Scanning Transmission Electron Microscopy (4D-STEM) has revolutionized the study of nanoscale materials by offering localized structural imaging capabilities through electron diffraction patterns [1]. However, the inherent noise within these patterns often impedes critical structural details, posing challenges to accurate analysis, particularly in orientation-based clustering [2]. In response, this paper presents a comprehensive approach to denoising 4D-STEM datasets, focusing on leveraging Pix2Pix Generative Adversarial Networks (GANs) to reduce noise and the influence of the artefacts [3].
Methods
The methodology is to focus on training a Pix2Pix GAN architecture using paired noisy-clean 4D-STEM image data. Adjusting the conditional GAN framework, the generator network learns to map noise from input images to their corresponding clean counterparts, guided by the discriminator network, which distinguishes between real-clean images and generated ones [3]. This approach effectively captures the intricate relationships between noisy and clean data, facilitating precise denoising. To address artifacts commonly encountered in GAN-generated images [4], we integrate additional regularization techniques and architectural modifications into the generator. Furthermore, architectural adjustments such as skip connections and multi-scale discriminators are implemented to enhance image fidelity and minimize artifact occurrence.
Results
Extensive experimentation was conducted on both synthetic and real-world 4D-STEM datasets to evaluate the effectiveness of our approach. Quantitative metrics, including peak signal-to-noise ratio (PSNR) and structural similarity index (SSIM), were employed to assess denoising performance, complemented by visual comparisons to highlight the clarity and fidelity of denoised images. Results demonstrate significant noise reduction and artifact suppression, enabling clearer visualization of nanoscale structures and more precise analysis. Importantly, our approach offers a substantial time-saving advantage compared to traditional methods, reducing processing time from 15 hours (using e-Pattern processing [5]) to just 0.2 hours.
Conclusion
In conclusion, our methodology provides a robust solution for denoising 4D-STEM datasets, leveraging Pix2Pix GANs while effectively addressing the challenge of artifact reduction. Significantly, this work contributes to advancing the field of materials science by enhancing the utility of 4D-STEM imaging techniques and emphasising the potential of GAN-based approaches in complex image-processing tasks.

Keywords:

Deep Learning, Pix2PixGan, Denoising, 4D-STEM

Reference:

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Analytical electron tomography, fine structure EELS and nanoscale X-ray CT of nanoporous Copper materials

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Background incl. aims
For systems with a complex morphology, 2D information can be insufficient, which requires tomographic approaches. Together, electron tomography and nanoscale X-ray CT cover the range from 3D sub-nanometer resolution for small sample volumes, up to samples sized several tens of micrometers, and in the electron microscope this information can be coupled with analytical information from associated spectroscopy techniques.
Among advanced modern materials, nanoporous metals (usually represented by nanoporous gold or other noble metals) can form the backbone for various applications, such as catalysis or supercapacitors. Copper is a promising alternative to rare and expensive noble metals due to its cost, redundancy and scalability. Nanoporous Cu materials can be made by electrochemical dealloying[1] where the size of resulting pores is tailored to fit the intended application. Due to its less noble nature, Cu is more prone to oxidation, and oxide chemistry on the pore surfaces plays a significant role for intended applications. It is therefore imperative to know the morphology and inner surface chemistry of the nanoporous material as well as to be able to assess pore parameters, such as size distribution or local curvatures for scalable pore sizes ranging from 20 to 500 nm, which requires adapting available characterization methods.
Methods
Nanoporous Cu structures, dealloyed from either Cu-Mn or Cu-Al alloys with different dealloying conditions and optional subsequent coarsening, were investigated with following methods: high-angle annular dark field scanning transmission electron tomography (electron tomography), energy-dispersive x-ray spectroscopy (EDXS), electron energy loss spectroscopy (EELS), Zernike phase contrast laboratory nanoscale x-ray computed tomography (nano-CT). Samples for electron tomography were prepared as 100-300 nm thin pillars via focused ion beam milling (FIB). Samples for nano-CT (60 µm thick) were formed by laser ablation with a finishing touch of FIB.
Results
We use electron tomography to capture in detail the nanoporous Cu network morphology from HAADF data, while also reconstructing 3D elemental distributions from EDXS. Implementing multimodal joint reconstruction of all channels (EDXS and HAADF) with total generalized variation regularization (TGV)[2] algorithm we can capture the surface oxidation of Cu in 3D, as well as local residuals of Mn after dealloying. We use EELS fine structure to self-reference multiple linear least squares fitting (MLLS) segmentation to classify and map present Cu and Mn oxide species. We further implement Zernike phase contrast laboratory nano-CT for statistical pore parameter analysis of coarsened samples.
Conclusion

In nanoporous copper samples dealloyed from Cu-Mn alloy we were able to identify and map Cu and Mn oxide species. Based on Mn oxides present we suppose a different reaction path on the dealloying stage to the one suggested in literature.[1] We have reconstructed and segmented 3D distributions of present elements and overall nanoporous Cu morphology to quantify porosity as well as pore size, distribution and connectivity. With statistically-rich nano-CT segmentation we show that at the testing stage coarsening procedure works differently on np-Cu dealloyed from Cu-Mn or Cu-Al alloys likely because of the complexity of phase diagram of Cu-Al. We report that pore coarsening on Cu-Al has good scalability but suffers from residual presence of unreacted mother alloy bubbles. [3]

Keywords:

nanoporous metal, electron tomography, EELS

Reference:

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M-SIS Software: Automatic tilted series acquisition for environmental (gas, liquid and temperature) multi-scale electron tomography

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Poster Group 1

Background incl. aims

Electron tomography is a technique that allows 3D data analysis at the nanometer scale. It has always been considered a difficult and time-consuming technique, from tilt series acquisition to volume reconstruction and data analysis. In the beginning, the tilt series acquisition was performed in several tens of minutes in bright field mode to several hours in scanning transmission mode (STEM). Huge technical developments have been made to make tilt series acquisition faster and faster, and to enable fast electron tomography compatible with environmental electron microscopy. [1,2] Environmental electron tomography in gas, and even more in liquid state, is very sensitive to the electron beam, therefore recording fast tilt series in STEM mode with a very low electron dose received by the sample is a real challenge even for the most experienced users. One solution to ensure the recording of fast tilt series with low electron dose at a constant rate is to automate the recording process.
This presentation will introduce the M-SIS software. Its purpose is to take advantage of the power and robustness of the computer code to assist the operator and improve his/her skills while acquiring a series of tilts in electron microscopy. The automation minimizes the electron dose received by the sample by reducing its exposure time to the electron beam.

Methods

The M-SIS code (Figure1) is written in Python and is compatible with different environments and machines. It can be installed on an Environmental Scanning Electron Microscope (ESEM), which is compatible with Thermo Scientific™ Autoscript, as well as on an Environmental Transmission Electron Microscope (ETEM), as a plug-in of DigitalMicrograph™. In addition, built-in libraries enable hardware control, such as the Smaract MCS-3D piezo-inertial stages used to control the stage and detector in the ESEM.

Results

The M-SIS software is compatible with ESEM and ETEM, enabling multi-scale electron tomography in environmental mode at very low electron dose in STEM (scanning transmission) imaging mode. It is able to automatically set the sample at the eucentric position. The brightness and contrast of the images are automatically adjusted, and then fast tilt series are acquired. One of the problems of fast tilting is that the sample drifts out of the field of view due to mechanical imperfections while tilting. The M-SIS software automatically corrects the drift of the specimen during image acquisition and keeps it in the field of view during the tilt. The code allows the acquisition of multiple imaging modes

simultaneously, i.e. SE, BF, DF and HAADF, all while protecting the sample from beam damage. During validation tests, the total electron dose received by the sample ranged from 1,042 e-/nm2 in ESEM at 30 keV to 16,000 e-/nm2 in ETEM at 300 keV both recorded in liquid mode, for the acquisition of more than 100 images per imaging mode, in about 10 min. Volumes have been reconstructed on very sensitive samples from materials science and biology, at different hydration states, and quantitative data could be reliably extracted.

Conclusion
We have developed a software based on Python language, which is compatible with ESEM and ETEM electron microscopes, allowing the recording of low electron dose tilt series in STEM mode in environmental mode at multi-scale. The software is user independent, automatically sets the sample to the eucentric position, corrects brightness and contrast, and records fast tilt series with controlled electron dose, while tracking the sample as it rotates. [3]

Figure 1: M-SIS software, a Python based software for electron tomography in environmental conditions i.e. gas, liquid and temperature, conceived for beam sensitive samples investigation.

Keywords:

tomography automation in-situ

Reference:

[1] S. Koneti et al, Fast electron tomography: Applications to beam sensitive samples and in situ TEM or operando environmental TEM studies. Materials Characterization 151, 2019.
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Capturing the diffusion of individual atoms in 3D: heat-induced alloying in Au@Ag nanoparticles

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
In recent decades, bimetallic core-shell nanoparticles (NPs) have attracted great attention in materials science, primarily due to their unique optical properties based on surface plasmon resonances. However, the practical application of these materials, particularly under elevated temperature conditions, often leads to particle reshaping and redistribution of metals between the core and shell of the particle, gradually altering nanoplasmonic properties. Consequently, advancing nanoplasmonic-based technologies necessitates a comprehensive understanding of heat-induced transformations, considering factors like nanoparticle size, shape, and defect presence. Conventional two-dimensional Scanning Transmission Electron Microscopy (2D STEM) imaging falls short in reliably analysing diffusion, especially in asymmetric NPs. Thus, employing three-dimensional (3D) characterization techniques becomes paramount. Herein, electron tomography (ET) emerges as a pivotal tool. Moreover, achieving atomic resolution in the 3D investigation of elemental redistribution is crucial. This enables drawing conclusions regarding the structural parameters influencing diffusion kinetics in bimetallic NPs, such as core-shell Au@Ag NPs.
Methods
To conduct a thorough 3D investigation of heat-induced alloying in individual Au@Ag NPs, ET based on High-Angle Annular Dark Field (HAADF) STEM was combined with in situ heating holders.1 To reach ET reconstructions with atomic resolution, an aberration-corrected Themis Z transmission electron microscope offering a spatial resolution of 60 pm was used. The 3D reconstruction of each studied NP was performed after several heating steps using advanced reconstruction algorithms2 supported by convolutional neural network, allowing for compensation of scanning distortions during the acquisition of HAADF-STEM projections and missing wedge artifacts.3 Mass-thickness contrast (Z-contrast) of HAADF-STEM ET enabled us to distinguish between individual Au and Ag atoms, facilitating the determination of atom positions. Additionally, a set of selected slices through the obtained reconstructions were used as an input for StatSTEM analysis,4 to correlate the kinetics of atomic redistribution with the presence of lattice distortions.
Results
As reported in our previous work, we observed significantly faster alloying kinetics in pentatwinned (PT) Au@Ag NPs compared to their single-crystalline (SC) counterparts, which we attributed to lattice distortions near the twin boundaries, facilitating faster atomic transport.5 To support this hypothesis, heat-induced alloying in SC and PT Au@Ag nanorods (NRs) with similar sizes and compositions was studied using atomic resolution ET. To induce alloying, all particles were heated to 450°C using the specialized heating tomography holder until complete and uniform alloying was achieved. To investigate intermediate states of alloying, the heating process was interrupted, and atomic resolution ET series were acquired after several heating time intervals. The obtained tomography datasets were further utilized as input for advanced ET reconstruction algorithms, where prior knowledge is typically used to enhance the outcome of the reconstructed 3D volumes. Specifically,

the Sparse Spheres Reconstruction (SSR) algorithm2 was implemented, where parameters such as the sizes and intensity values of atoms within an NP can be parameterized, leading to the improved distinction between individual Au and Ag atoms. In this manner, we were able to study the atomic transport in different NPs in 3D and assess the diffusion kinetics in the SC regions of the studied NPs or in the vicinity of twin boundaries, where lattice distortions can be carefully determined from the positions of all atoms obtained from atomic resolution ET reconstructions.

Conclusion

In this work, we demonstrate the capability of advanced ET techniques to conduct in situ investigations and quantitative analyses of heat-induced processes in complex NPs, such as core-shell Au@Ag NRs, with atomic resolution in 3D. By employing these techniques, which involve the combination of state-of-the-art TEM instruments with dedicated heating tomography holders and specialized reconstruction algorithms, we show that diffusion kinetics are faster in regions where twin boundaries cause higher interatomic distances compared to the "perfect" crystal lattice of Au and Ag.

The project has received funding from European Research Council (ERC Consolidator Grant 815128, REALNANO) and European Commission (grant 731019, EUSMI).

Keywords:

Electron tomography, in-situ, bimetallic nanoparticles

Reference:

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Unveiling the three-dimensional ultrastructure of Poly Lactic Acid (PLA) spherulites by means of electron microscopy

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PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Background incl. aims
Among biobased polymers, Poly Lactic Acid (PLA) is widely used in a variety of daily-use products from packaging to biomedicine, thanks to its properties (high strength, lightweight, toughness, corrosion resistance, transparency) and its ability to be easily and economically processed from renewable resources via traditional manufacturing techniques, such as injection-moulding. It is therefore a good candidate for replacing conventional petrochemical-derived plastics [1]. To improve the fabrication of this promising material, and optimize its properties (mechanical, thermal, etc.), a deeper comprehension of PLA morphology is mandatory. During the injection-moulding fabrication process, molten PLA solidifies into semicrystalline structures called “spherulites” (figure A), which are constituted by fibrillar lamellae (with thickness ranging from 10 to 30 nm), radially growing from a nucleation center (figure B). Depending on the manufacturing process, spherulites vary their morphology leading to a great impact on the macroscopic (e.g. mechanical) properties of this material [2]. To date, few studies extensively describe PLA spherulites’ morphology [3], but generally their characterization is limited to two-dimensional observations. Here we report our work in the three-dimensional (3D) investigation of PLA spherulites, by means of electron microscopy, toward a better comprehension of the relation between micro- and macroscopic properties of this material.
Methods
Dog-bone semicrystalline PLA samples were obtained by injection-moulding technique. PLA blocks (approx. 5 mm each side) were retrieved from the central portion of the dog-bone specimens, while keeping track of the orientation of the material within the hot mould. Then, using an ultramicrotome, we sectioned PLA blocks for transmission electron microscopy (TEM) imaging, by carrying out two different sectioning approaches: 1) directional sectioning; 2) serial sectioning. The first requires that the sample was cut into three mutually orthogonal directions, to investigate the PLA spherulites respective 3D arrangements in the block. The latter was instead chosen for 3D reconstruction of a single spherulite, by collecting sequential PLA sections, and imaging the same structure across the entire series of sections. We also performed scanning electron microscopy (SEM) imaging of the PLA sections collected on silicon wafers and tested the capabilities of focused ion beam scanning electron microscopy (FIB-SEM) for the spherulite volume imaging. Finally, ultramicrotome sectioning and FIB-SEM were used for fabricating PLA blocks suitable for X-ray ptycho-tomography.
Results
TEM analysis of directionally sectioned PLA blocks revealed the 3D organization of spherulites within the samples (figure A), which appeared to have an elliptical shape, with lower ellipticity extent at the faces perpendicular to the hot polymer flow direction during the injection-moulding processing. This would be directly related to the shear flow generated at the hot mould walls, which favored the elongation of the spherulites in the direction of the hot polymer flow.

Concerning the 3D characterization of a single spherulite, TEM imaging of the same structure across the entire series of sections collected with the serial sectioning approach allowed to reconstruct the lamellar arrangement within the spherulite, for a total volume of about 1.1 um (figure C).
In order to reconstruct larger volumes of PLA spherulites, SEM analysis as well as FIB-SEM tomography were tested too, but spherulite's lamellae were not visible with the same resolution achieved with TEM.
For this reason, we are now approaching a new imaging technique (figure D): X-ray ptycho-tomography, pushed to the limit of spatial resolution (voxel side) down to tens of nanometers. This would give for the first time information along the third dimension about spherulites morphology and lamellar arrangements at the nanoscale.
Conclusion
PLA morphology and its mechanical/thermal characteristics are strictly related. Thus, the knowledge of PLA ultrastructure is particularly important for optimizing the fabrication process and finally obtaining a material with tailored macroscopic properties, such as thermal behavior, flexibility, strength. Our observations demonstrate the importance of microscopy in polymer science, through the application of cutting-edge (electron) microscopy techniques. Here we bring new insights of the PLA morphology, paving the route towards optimized industrial processing of this versatile and environmental friendly material.

The authors gratefully acknowledge support from the European Union's Horizon 2020 research and innovation program under the FET Open grant agreement 5DNanoPrinting - no. 899349.

Keywords:

Polymer, electron microscopy, three-dimension, ptychotomography

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High resolution characterization of Y(Mn,In) Blue Chromophoric Oxides

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Background
Throughout history, bright colours have contributed to the advancement of art and science through inspiring creativity, improving mental wellbeing, and enhancing the transfer of information. However, since many common pigments are produced using toxic materials, there has been a recent burst of interest in producing non-toxic, colourfast, long-lasting pigments for various colour treatment applications [1], led by the discovery of a durable, environmentally friendly inorganic blue pigment Y(Mn_{1-x}In_x)O_3 [2].
The two end compounds of this mixture, $YInO_3$ and $YMnO_3$, are white and black, respectively. Both compounds crystallize in a layered hexagonal lattice, while the intermediate compounds form a solid solution in which In and Mn occupy the same lattice sites. Mixing a small amount of Mn into the $YInO_3$ lattice results in a startling blue colour originating from absorption of other colours by electronic transitions from the Mn 3d states, including nominally forbidden d-d transitions. These transitions are made possible by the non-centrosymmetric trigonal biypramidal (TBP) environment of O around the Mn ions [2,3].
In this study, we present a thorough characterization of the structural and spectral characteristics of Y(Mn_{1-x}In_x)O_3 across the full compositional range. We discuss the origin of the strong blue colour and its correlation with the crystallographic properties of this oxide material.
Methods
We use a Nion HERMES100 STEM operated at 60 keV, with an average energy resolution of 5 meV, to acquire high resolution EEL spectra across a broad range of the electromagnetic spectrum: from the mid-IR to the UV and core-loss regions. We complement our EELS results with high resolution imaging of the crystal structure using STEM, EDS, and iDPC on a double-corrected ThermoFisher Spectra Ultra STEM operated at 200 keV. JEMS enables us to visualize the crystal structure and simulate HAADF images of the various orientations we find in the STEM.
Results
In our EEL spectra, we observe several trends in the mid-IR to UV regions of the spectrum. In the mid-IR, the phonon modes redshift with more In content due to the larger relative atomic mass and size of In compared to Mn. In the visible region of the spectrum, $YMnO_3$ has strong extinction peaks arising from Mn 3d transitions (Fig. 1A), resulting in broadband absorption across the visible spectrum and a black colour in the bulk material. $YInO_3$, on the other hand, has no peaks in this spectral regime until the bandgap onset in the near-UV, resulting in a material which reflects all colours of light and appears white. In the intermediate compounds, such as Y(Mn_{0.1}In_{0.9})O_3, we observe a small peak around 2.2 eV and a change in

the higher energy bandgap onset relative to the pure In compound. Further increasing the Mn fraction results in stronger intensity on the 2.2 eV peak, and a new peak at 1.9 eV which redshifts to 1.7 eV and dominates the lower energy part of this spectrum. Additional extinction signal also arises near the bandgap onset, which overall shifts to lower energy and results in absorption of purple and UV light. This combination of transitions leaves only blue light to be reflected.
Through HR-STEM-EDS, we are able to identify the lattice sites occupied by Mn even in samples with low In concentration, such as Y(Mn_{0.9}In_{0.1})O_3 (Fig. 1B). From our EDS results, we observe that the Mn and In occupy the same lattice sites, substituting for each other in a disordered fashion, for the most part. One notable exception that we have observed is in the Y(Mn_{0.5}In_{0.5})O_3 oxide, wherein the Mn ions are distinctly noted to occupy specific lattice sites in a unit cell larger than former studies suggest (Fig. 1C), whereas the In ions do not show the same behaviour in this lattice. The origins of this mysterious ordering remain to be understood.
Through further analysis of our HR-STEM images, we observe an anisotropic expansion of the crystal lattice as Mn ions are replaced with the larger In ions. The lattice exhibits more expansion along the apical than the basal directions, with the c lattice parameter increasing by 1 Å in $YInO_3$ compared to $YMnO_3$. We observe through selected area diffraction that in several of the intermediate compounds, the crystal lattice has a twist, likely due to the mismatch in size of the substitutional Mn and In ions. Further, using iDPC imaging, we are able to observe distinct structural features in alternating lattice planes, with a repetition matching the size of the system's unit cell (Fig. 1D).
Conclusion
In conclusion, we present a thorough characterization of the blue chromophoric oxide, hexagonal Y(Mn_{1-x}In_x)O_3. We examine the crystallographic and spectral characteristics of the material across the range of compositions to understand the origin of the optical properties that make this such an attractive pigment. We directly confirm the trends observed in x-ray based studies of this material and uncover evidence of Mn ions preferentially occupying unexpected sites in a larger supercell in Y(Mn_{0.5}In_{0.5})O_3.

Keywords:

Oxide, optical, HR-STEM, HR-EELS

Reference:

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Twinning in HfO2 nanocrystals

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Poster Group 1

Background
Twinning, a phenomenon observed in a variety of crystalline solids, often arises during growth, deformation, and phase transformations [1]. The symmetry connecting adjacent crystal twins differs from the point group symmetries of the parent crystal. These additional symmetry operations, known as twin operations, typically include rotation, reflection, and inversion [1].
Materials with polar point group symmetries exhibit spontaneous polarization. An emergent polarization from twinning is anticipated when the additional symmetry coincides with a polar point group's symmetry. Nonetheless, the complex interplay between twinning and polarization has been scantily addressed in the literature.
The twinning associated with the tetragonal to monoclinic phase transformation in ZrO_2 and HfO_2 has undergone extensive study. Twin planes are commonly observed on the (100) plane and potentially on others, such as (001) and {110} [2]. A thorough understanding of twinning necessitates resolving both the heavier metal and lighter oxygen atoms.
The negative spherical aberration (Cs) imaging (NCSI) technique provides optimal negative phase contrast under conditions of negative Cs and overfocus in conventional transmission electron microscopy (TEM) mode [3]. Comparative studies have demonstrated the superiority of NCSI over positive Cs imaging in terms of image contrast, signal intensity, and noise robustness. Notably, NCSI excels in visualizing all atoms, including oxygen, in metal oxides.
This work aims to elucidate the atomic structure of twin boundaries in HfO_2nanocrystals using the NCSI technique [3].
Methods
In this study, NCSI TEM images were captured at 200 kV with a Gatan OneView phosphor-CMOS camera on an FEI Titan 50-300 PICO electron microscope, featuring a Schottky field emission electron gun and a CEOS Cc/Cs corrector. Additional NCSI images were obtained at 300 kV using a Thermo Fisher Scientific Spectra 300 microscope equipped with a high-brightness X-FEG source, a piezo-enhanced CompuStage, a CEOS CETCOR Cs corrector for the S-TWIN objective lens, and a fast Ceta CMOS camera. The monoclinic HfO_2 nanocrystals under study were synthesized via a sol-gel method [5].
Results
Using the NCSI TEM technique, we resolved both hafnium and oxygen atoms within individual HfO_2 nanocrystals (Fig. 1). The NCSI findings disclosed both individual and unit-cell-wise multiple consecutive twinning on the (200) plane of colloidal HfO2 nanocrystals. Each twinning event involves two-fold screw symmetry, leading to a unit-cell scale polar orthorhombic phase with a $Pbc2_1$ space group at the twin boundary. The unit-cell-wise multiple consecutive twinning gives rise to a novel antipolar phase with the Pbca space group. These interpretations were corroborated through an iterative two-step optimization process for image matching between experimental and simulated images.
Figure 1 Top: Atomic resolution TEM image of an HfO_2 nanocrystals recorded using the negative spherical aberration (Cs) imaging (NCSI) technique. Middle: Map of polarization vectors and structure

model (blue: Hf, orange: O, grey: bond) superimposed on the NCSI image of the region marked in the top. Bottom: Structural illustration of polarization resulting from twinning.

Conclusions

We have revealed the correlation between twinning and polarization in HfO_2 colloidal nanocrystals via the NCSI technique [3]. The polarization induced by twinning correlates with sub-nanometer ferroelectric and antiferroelectric phases. The discovery of twinning-induced polarization may offer a new avenue for identifying novel ferroelectric phases in ionic compounds beyond oxides.

Keywords:

NCSI, Twinning, Polarization, HfO_2

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Microscopy techniques show the assembly of nanovesicles around lipid droplets via the tumor protein TPD54

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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

Background : TPD54 belongs to the TPD52-like family of tumor proteins which are of great interest because they are overexpressed in aggressive metastatic cancers with poor prognosis. Recently an article showed the membrane association of TPD54 with small vesicles of around 30 nm, half the size of conventional transport vesicles, and its involvement in cell migration (1). To determine the underlying mechanism, our collaborators showed that this protein has an ALPS motif (Amphipathic lipid packing sensor), which recognizes curved membranes, and binds nanovesicles via ALPS-dependent and -independent mechanisms (2). However, its functions remain unclear. To further understand the function of TPD54 in intracellular trafficking, we worked on cultures of epithelial cells (RPE1) overexpressing various forms of GFP-TPD54, including phospho-mimetic mutants.
Methods : By confocal microscopy, the localisation of the two mutants GFP-TPD54 S166E and S166A was analyzed. A cell ultrastructural analysis was realized by different transmission electron microscopy (TEM) methods and correlative light and electron microscopy (CLEM) techniques: a) classical chemical fixation and epon embedding, and tomography b) Tokuyasu immunogold TPD54-GFP localization, c)CLEM on HM20 resin sections on samples prepared with high pressure freezing, and freeze substitution in uranyl acetate 0,2%. Sections were observed with a JEOL 1400 TEM.
Results : Confocal microscopy shows that GFP-TPD54 phosphomimetic S166E is localized to Golgi apparatus, whereas GFP-TPD54 S166A is also found at big spherical structures at the periphery of the nucleus and colocalized with Rab11, a small G protein associated to recycling endosomes. The GFP fluorescence is also found around lipid droplets. When overexpressed in RPE1 cells, we observed by TEM that TPD54 induces the formation of huge assemblies of very small vesicles and tubular structures in close contact but still at a defined distance (10-20 nm) from lipid droplets. The diameter of the vesicles (38 +/- 8 nm) is about half the size of classical transport vesicles (e.g. COPI and COPII vesicles). These assemblies contain selective membrane markers including the autophagy transmembrane protein ATG9 and RAB11. The immunogold staining and CLEM confirm the localization of TPD54 to the vesicular aggregates.
Conclusion: Our EM observations validate the existence of the TPD54 nanovesicles without the artificial trick of mitochondrial attachment published previously by Larocque et al. Furthermore, they suggest an intimate link with lipid droplets. This process seems controlled by phophorylation.

Keywords:

Electron Microscopy, nanovesicles, CLEM, TPD54

Reference:

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Additive effects in phase transformation of calcium phosphate to hydroxyapatite using high-throughput transmission electron microscopy

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background:
Calcium phosphate (CaP) is a crucial biomineral essential for human health, primarily forming the foundation of bones and teeth [1]. The nucleation of CaP begins from amorphous calcium phosphate (ACP) phases and transitions to hydroxyapatite (HAP), a key process in the mineralization of bones and teeth [2]. The mechanisms behind this phase transformation are complex and, despite decades of research, remain a topic of ongoing debate, particularly regarding the influence of additives such as Mg2+ [3]. This study aims to delve into the effects of Mg2+ on the transformation of ACP to HAP, employing high-throughput transmission electron microscopy (TEM) [4]for detailed analysis.

Methods:
To assess the impact of additives on phase transformation, we designed an automated sample preparation platform. This system uses a standard 24-well plate, complemented by a picoliter-volume liquid dispenser and a motorized stage under precise control. Custom Python scripts automate the liquid dispensing and stage movements, facilitating the accurate placement of 30 samples on a single TEM grid for comprehensive TEM analysis. Chemical reactions were initiated with three base solutions: 20 mM calcium chloride (CaCl2), 10 mM magnesium chloride (MgCl2), and 12 mM sodium phosphate (NaH2PO4). CaCl2 and MgCl2 solutions were mixed in various Mg2+/Ca2+ ratios (0, 0.01, 0.06, 0.3, and 0.5), as shown in Figure 1a. The addition of sodium phosphate solution and extra water marked the start of the reaction. Samples were extracted 3 minutes after the reaction's initiation, with a 300 pL aliquot deposited onto a TEM grid. This sampling continued at 45, 90, 135, 180, and 225-minute intervals on the same grid. Each 300 pL sample dried in about 2 seconds, creating distinct areas roughly 130 µm in diameter as in Figure 1a. The dispenser was cleaned after each sampling. Results were analyzed using bright-field TEM and electron diffraction, revealing insights into the samples' morphological and structure as illustrated in Figure 1.

Results:
Samples 1, 2, and 3 showed similar morphological evolutions: spheroidal nanoparticles formed within 3 minutes, transitioning into sheet-like nanostructures characteristic of HAP, as evidenced in Figures 1b and c. Diffraction patterns at 45 minutes confirmed the crystalline structure of HAP, indicating the shift from ACP to HAP. Mg2+ addition was found to expedite this crystallization, as seen in sample 3's radial average diffraction pattern, where HAP's distinctive peaks were more pronounced compared to samples 1 and 2. However, excessive Mg2+ concentrations delayed or prevented the ACP to HAP conversion, as demonstrated in Figures 1c and d, where sample 4 showed a delayed HAP transformation at 225 minutes, and notably, sample 5 did not transition to HAP even after 225 minutes.

Conclusion:
The innovative automated sample preparation platform significantly enhances our capacity to deposit multiple samples on a single TEM grid, enabling thorough investigation of the ACP to HAP

phase transition with and without additives across various reaction times. This study highlights the advantages of applying high-throughput TEM for deep dives into chemical and biochemical reaction mechanisms. Our findings suggest that while Mg2+ does not affect the initial ACP formation, it does modify the crystallization rate. Crystallization accelerates up to a Mg2+/Ca2+ ratio of 0.06, after which it slows down or stops the ACP to HAP conversion. Future work will aim to refine TEM techniques, such as optimizing sampling rates and integrating data acquisition processes, including in-situ methods like liquid-phase TEM [5], to further elucidate the mechanisms in-situ.

Keywords:

hydroxyapatite, additive, high-throughput TEM

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In situ TEM thermal study of MBE and CVD GeSn layers: cross-section and plan-view geometries

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

Introduction
Direct band gap GeSn epilayers have great potential in high-performance Si-based electronics and optoelectronics. A transition to a direct band gap can be achieved in GeSn layers when the Sn concentration exceeds 6 at% [1], a value well above the solubility limit. Therefore, these non-equilibrium GeSn alloys with the desired materials concentrations are unstable at high temperatures, resulting in phase separation and decomposition processes [2]. Two main epitaxial methods used for the material synthesis are molecular beam epitaxy (MBE) and chemical vapor deposition (CVD). Depending on the method, the properties of the GeSn can vary strongly. For example, the CVD GeSn films exhibit better optical properties and, to the best of our knowledge, all GeSn-based lasers were grown via this technique. Thermal stability is another property that can vary. The excess of Sn and its possible segregation that, can occur during material growth, has a major influence on thermal stability. Understanding this is important for predicting the thermal budget that a Ge1-xSnx layer can be exposed to during device fabrication.
Methods
We use the powerful technique of in situ transmission electron microscopy (TEM) to study the dynamic process during thermal annealing experiments. We analyze how the Sn concentration and the presence of dislocations affect the thermal stability. In this regard, samples grown by CVD and MBE, 50 nm thick epilayers with 6-14 at% Sn on Ge substrate, were analyzed in cross-section and plan-view geometries. The cross-sectional lamellae were cut and installed on micro-electro-mechanical system (MEMS) heating chips with a Ga+ focused ion beam (FIB). In the case of the plan-view, first, the samples were prepared by wedge polishing technique, and then with FIB transferred to the MEMS chip and finally thinned [3]. The experiments were performed by heating-cooling-cycles with an increasing maximum temperature up to 750°C, investigating the sample via complementary HRTEM and STEM EDXS. In cross-section geometry, we can trace precipitation in relation to present interfaces and surfaces, while in plan-view geometry we can gain information about the morphological changes and particle size distribution on the surface of interest.
Results
For the MBE samples, two different concentrations of 10 and 14 at% Sn were investigated. The cross-section specimen with 10 at% Sn is stable up to 500°C. At 500°C, Sn-based precipitates are formed in the GeSn epilayer. HRTEM Fast Fourier transform (FFT) analysis of these inhomogeneities reveals a pattern well-fitted with the β-Sn crystal structure [4]. At the same temperature, we monitored the formation of Sn drops in the plan-view sample. In the case of a cross-section sample with 14 at% Sn, the start of the precipitation process is found at a lower temperature of 350°C. For a CVD GeSn layer with 11.5 at% Sn, the specimen shows stability up to 450°C where also β-Sn precipitates appeared. However, to achieve these results the cross-section specimen had to be prepared by the wedge

polishing technique, reducing the FIB processing and Ga contamination. Without this measure, the CVD-grown sample decomposes already at 200°C under the influence of Ga impurities implanted during FIB-assisted specimen preparation. To better understand the present Ga contamination effects and the relation with defects, we analyzed CVD samples with different growth and buffer qualities, which influence the density of threading dislocations. However, all samples were highly susceptible to Ga contamination during the standard lamella preparation. Applying our optimized wedge polishing technique, we analyzed additionally a CVD sample with 6 at% Sn, which demonstrated stability up to 650°C during the in situ TEM experiment.

Conclusion

The measured Sn segregation temperature for cross-sectional and plan-view geometries correlate well, however, there are differences in the precipitate densities which are attributed to the free surface diffusion of Sn for the plan-view specimen compared to the suppressed one for the capped cross-section. Regarding the two MBE specimens, since no formation of liquid phases was observed, a solid-state diffusion and precipitation mechanism is believed to occur. Ga contamination during preparation can lead to the formation of a GaSn liquid phase during annealing [4], leading to a fast decomposition of the GeSn layer. CVD GeSn layers are more susceptible to this effect, which we believe is due to the presence of point defects induced by the CVD method. Nevertheless, using the wedge polishing approach it was possible to overcome this challenge. The obtained results show the highest stability for the sample with 6 at% Sn and the lowest for the sample with 14 at% Sn. The gained results indicate a strong dependence of the decomposition temperature on the Sn content, which is summarized in the figure presented.

Keywords:

In-situ TEM, GeSn, MBE, CVD

Reference:

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Illuminating the Microscopic Realm: Application of Resin R221 for CLEM in microbes and plant tissues

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Poster Group 1

Background incl. aims
Correlative light and electron microscopy (CLEM) is a powerful imaging tool that combines the advantages of light/fluorescence (LM/FM) and electron microscopy(EM). In CLEM , FM images can be used to observe the localization of one or more molecules of interest indicating where the region of interest (ROI) the needle in the haystack is located whereas EM provides the ultrastructural information. Samples used for investigation of ultrastructure by EM are subjected to chemical fixation and are treated with heavy metal salts and contrasting agents such as osmium tetroxide and uranyl acetate to improve imaging contrast and later embedded in resin as it stabilizes the samples so that it withstands the vacuum condition of the EM and enables long storage. However, chemical fixation and resin embedding process which is essential for good quality of the ultrastructure is impaired by the contrasting agents or the florescence protein tag in the samples lose their ability to fluoresce due to protein denaturation which possess a major challenge for CLEM. The discovery that fluorescence can be retained in resin-embedded specimens following moderate heavy metal staining revolutionised CLEM. R221 (CryoCapCell) is a methacrylate based acrylic resin with promising results allowing florescence preservation facilitating CLEM approaches to identify the ROI and obtain high resolution ultrastructural images of the targeted ROI. We aim to use Resin R221 to identify the interface of plant microbe interaction which is ROI under florescence microscope with the help of florescent tags before thin sectioning for EM ultrastructural analysis. This immensely reduces the time required to locate the ROI in plant samples. For instance, symbiotic bacteria present in root cells can be easily identified if the bacteria are florescent tagged allowing efficient identification of the bacteria infected cells for EM analysis.
Method:
In the initial phase cyanobacterial and algal strains are subjected to high pressure freezing followed by freeze substitution(FS) in a cocktail containing uranyl acetate, glutaraldehyde, H2O and acetone(at – 90 to -30°C) followed by R221 resin infiltration and polymerization in low oxygen level and exposure to UV light to ensure proper polymerization. After polymerization the specimen blocks are stored at room temperature protected from light to preserve florescence. Thin sections of 0.7 µm70 nm are placed on copper grids coated with collodium which are then mounted on glass slides with cover slips sealed with paraffin wax for CLEM analysis.
Results :
Three different fluorescent tags could be detected in sections of samples prepared in the resin R221 so far. This facilitates the application of CLEM analysis to identify the cyanobacterial and algal strains as well as plants associated with microbes under the TEM. The R221 resin paves a way for multimodal analysis without having to follow different sample preparation for florescence and electron microscopy.
Conclusion:

While resin already gave us very promising results, there's still work to do in fine tuning the workflow for plant materials to facilitate the identification of ROI and target rare events. Additionally, it is also essential to explore chemical fixation methods as it is a more convenient method especially for plant materials. Subsequent steps will involve application of this optimized workflow to various This will be followed by applications of different plant material, plant-microbe associations and implementation of other plant material and with other fluorescent tags.

Keywords:

R221, Fluorescence microscopy, CLEM, TEM

Reference:

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4D-STEM and EELS Analysis of Complex C-based Sensor Architectures

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Poster Group 2

Background
The transformation of precursors with high carbon content into open-porous carbon foams and coatings via thermal processes is gaining traction as a promising and eco-friendly method for a range of applications including catalysis, energy utilization, and sensing [1]. In this investigation, we employ a one-step laser patterning technique to locally pyrolyze doctor-bladed ink coatings on flexible PET substrates. This process yields highly porous and intricate CO2 sensor architectures with a thickness of around 50 µm. The ink composition includes glucose as a pore-forming agent and adenine as a nitrogen source, contributing to the sensing capabilities. Laser treatment in an oxygen-rich environment results in flexible and highly porous sensor structures with distinct nitrogen and oxygen functionalities within different regions. The laser intensity varies with depth, leading to the creation of a well-defined highly porous graphitic surface layer (acting as the electric transducer layer) and a less porous nitrogen-rich lower sensor layer, interconnected by a thin transition zone [2].
Methods
To understand the formation of these structures and their relationship with functionality, we conducted a thorough TEM investigation on cross-sections (0.25 - 0.5 t/λ) of the entire device prepared by microtomic cross-sectioning.
Results & Conclusion
STEM-EELS elemental distribution maps reveal a clear distinction in chemical composition between the upper and lower layers of the sensor. Principal component analyses were utilized to unravel the complex sensor structures, comprising various crystalline and amorphous phases containing carbon and nitrogen. 4D-STEM analysis unveils the distribution of the crystalline graphitic phase and the alignment of graphite basal planes relative to the pore walls of the open-porous sensor. Understanding these parameters is crucial for deciphering the electrical performance of the sensor (refer to Figure 1) [3].

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Keywords:

4D-STEM, open-porous carbon, laser pyrolysis

Reference:

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In-situ/ Opernado (S)TEM on Mass Selected Pt Clusters Deposited on CeO2 for CO Oxidation Catalysis

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

The study of model catalyst systems with mass selected Pt, Pd and Pt-Pd clusters on nanostructured metal oxide supports like CeO2, Al2O3 in exhaust gas environments can be helpful to understand the fundamental processes in catalytic reactions of exhaust gases, to unravel the complex relationship between the structure and the catalytic properties. During a catalytic reaction, the active metal clusters/ particles dynamically change their structure due to the interaction with the gas environment at elevated temperatures. In-situ TEM can be of aid to track the dynamic transient stages involved such as structural or morphological changes of clusters under catalytic reaction conditions. In this work the behavior of size selected Pt clusters (50-200 atoms) deposited on CeO2 support are studied using advanced scanning transmission electron microscopy (STEM) techniques, under pure gas environments (H2, Ar, O2, CO, CO2) and during CO oxidation (with CO and O2 as input gases).

Size selected Pt clusters are deposited using an ultra high vacuum (UHV) cluster ion beam deposition (CIBD) system on top CeO2 thin films, which is deposited on in-situ TEM nanoreactors[1]. Pt clusters are characterized calculating the pair distribution function from 4D-STEM datasets. The morphology changes in the clusters, cluster-support interaction, cluster-gas interaction etc. are studied realtime in pretreatment and exhaust gas environments using in-situ STEM imaging & spectroscopy. The CO2 generation and the level of conversion at different temperatures can be qualitatively studied using high sensitivity residual gas analyzer (RGA).

Figure 1(a) shows the 4D-STEM image of Pt55 deposited directly onto Si3N4. In order to calculate the PDF, it is necessary to substract the background signal of Si3N4 in the reciprocal space. The PDF is calculated by averaging the signal from the whole particle and compared with one another. The integrated PDF of all Pt55 clusters is comapred to the neighbouring distances expected for Pt FCC (Fig. 1). Additionally PDFs are obtained from multislice simulations of Pt55 icosahedra for comparison with experimental PDFs from individual clusters[2].

In-situ (S)TEM on Pt200 supported on CeO2 at pretreatment conditions (oxidation and reduction cycles at 300-500°C) are done and the clusters are found stable at both reducing and oxidative atmospheres. The clusters dynamics under catalytic reaction conditions (in CO & O2 atmospheres) are tracked in-situ at different temperatures from 200-700°C. The RGA showed a constant CO2 partial pressure at all the reaction temperatures, over a conversion of 15%. With continuous imaging, the clusters are found hopping through CeO2, sintering only at 200-300°C. The movement is not identified at higher temperatures when tracked with the electron beam. Clusters in the areas which are not tracked with e- beam are found coalesced, moved to other locations at higher temperatures.

This shows that the effect of the electron beam on the reaction sites are inevitable and has to be taken into account during acquisition.

To summarize, the study concentrates on the structural characterization of Pt clusters using 4D-STEM PDF. The results show clear differences from bulk FCC-Pt but resemble towards isomeric shapes. Comparison of PDFs of Pt55 icosahedron at various orientations confirms shift of pair distances and intensity variations according to the changes in diffraction intensities with different orientation. Additionally, in-situ evaluation of the model catalyst system shows changes in the morphology as well as movement and sintering of the clusters on CeO2 under catalytic reaction conditions.

Keywords:

In-situ, Catalysis, 4D-STEM, CO oxidation

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Electron microscopy and X-ray techniques correlative in situ studies in microfluidic conditions on hybrid perovskites

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Poster Group 1

Hybrid organic-inorganic perovskites (HOIP), a class of materials with a cubic unit cell (ABX3) possess remarkable physical and chemical properties and have aroused growing interest in the scientific community in the last decade. The A-cation, at eight corners of the cube, is surrounded by twelve X-anions (mainly halides, Cl− , Br− , I−), while the B-cation (typically divalent metal ions such as Pb2+, Sn2+ or Cu2+) is located at the body center surrounded by six X-anions in an octahedral [BX6]4- cluster. When the A cation is replaced by methylammonium cations (MA=CH3NH3+), three-dimensional hybrid organic–inorganic structures are obtained, known as 3D hybrid perovskites, with potential applications in solar cell technologies. Their high power conversion efficiency (PCE), together with their low-cost and scalable, solution-based fabrication processes lead to intense research efforts towards their industrialization. Despite these advantages, 3D perovskite solar cells also face significant challenges. The long-term stability of these materials remains a major concern, as they can be sensitive to moisture and other environmental factors, leading to a premature degradation. In this context, there has been growing interest in 2D HOIPs. These materials include larger organic cations as hydrophobic spacers, isolating the inorganic metal halide octahedra and leading to improved stability compared to their 3D counterparts. A wide variety of structures can be obtained for these materials, depending on the organic or inorganic cations and the synthesis conditions. Indeed, their synthesis in solution implies complex chemistry, with pre-organization, nucleation, reorganization and precipitation. Controlling the properties of these materials need a precise control over their structure, hence over their synthesis pathways.
For a better understanding of the synthesis of HOIPs by ligand assisted re precipitation (LARP) and to control their structures and properties, we developed a new approach for observing the structural evolution of 3D/2D lead halide hybrid perovskites based on a time-resolved acquisition protocol of the structural data. By combining in situ liquid transmission electron microscopy (TEM) and X-ray absorption techniques, we can monitor in real time the birth and the structural and chemical evolution of the different phases in well-controlled and realistic conditions and have a better understanding of the associated mechanisms. Specific microfluidic devices were developed for this work, which were used to perform in situ X-ray absorption studies and can be applied to other X-ray techniques as well. This new device reproduces the precipitation of 3D/2D lead halide hybrid perovskites under X-ray in particular for X-ray absorption near edge structure (XANES), for Extended X-ray Absorption Fine Structure (EXAFS) and for Small-angle X-ray scattering (SAXS) analyses. This micro fluidic chip has been designed to be versatile and adaptable to the specific needs of synthesis. The internal microfluidic pathway can be easily changed in design, from a T to observe the interface between two fluids, or a mixer to reproduce the conditions required for precipitation. By combining this information with those obtained from in situ liquid TEM with the Protochips technologies Poseidon AX and X-ray diffraction, we propose mechanisms for the formation and structural transition of different phases of this family of perovskites. This method highlighted the importance of studying the precursor solution to the LARP method, at which stage an atomic arrangement was found. This indicates that the synthesis of HOIPs begins before precipitation with the formation of a

forerunner atomic structure of the intermediate phase. We will also show how the method presented could be applied to the study of a variety of nanomaterials under in situ liquid conditions.

Keywords:

perovskite, insitu TEM, microfluidic device

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Atomically Sharp Domain Walls in an Antiferromagnet

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Poster Group 1

Introduction
Efficient manipulation of antiferromagnetic (AF) domains and domain walls has opened up new avenues of research towards ultrafast, high-density spintronic devices [1,2]. AF domain structures are known to be sensitive to magnetoelastic effects, but the microscopic interplay of crystalline defects, strain and magnetic ordering remained largely unknown. Recently, we have explored antiferromagnetic CuMnAs thin films in which imaging by x-ray photoemission electron microscopy (XPEEM) revealed that its AF domain structure is dominated by nanoscale crystalline defects [3]. However, even smaller magnetic objects were indirectly observed in the material, but they remained below the detection limit of the used established XPEEM methods.
Materials and Methods
Scanning transmission electron microscopy (STEM), differential phase-contrast (DPC) and 4D-STEM techniques are utilized CuMnAs epilayers grown by molecular beam epytaxy.
Results
Here, we achieve atomic resolution imaging of abrupt AF magnetic domain walls in CuMnAs epilayers [4]. The identification of the magnetic domain DPC signal is based on the specific symmetry of the CuMnAs crystal, where the opposite magnetic Mn sublattices occupy crystallographically distinct noncentrosymmetric sites, Fig. 1A. With focus on small field-of-view high-resolution imaging, we could associate the DPC-STEM signals with two types of abrupt Néel vector reversals, schematically illustrated in Fig. 1C and D: The first type occurs at a crystallographic antiphase boundary defect (Fig. 1C), while the second type forms in a part of the epilayer with no crystallographic perturbation detectable by STEM (Fig. 1D).
Conclusions
The results emphasized the crucial role of these defects in determining the AF domains and domain walls, and provided a route to optimizing device performance in term of scaling limits for the data density in the bulk of the antiferromagnet.

Keywords:

DPC, 4D-STEM, Antiferromagnets

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Visualizing microbial interactions and CRISPR-Cas interference using FISH applied to environmental archaeal biofilms

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Poster Group 2

Background incl. aims
Identifying microbial virus-host interactions is essential to understand viral dynamics in ecosystems and their impact on nutrient cycling. In environmental samples, their identification relies mostly on bioinformatic approaches, i.e., detecting matches between spacers encoded in clustered regularly interspaced short palindromic repeats (CRISPR) systems and their corresponding protospacers present in viral genomes. To complement and confirm these predictions, we established sophisticated microscopy techniques. First, by employing virus-targeted direct-gene fluorescence in situ hybridization (virusFISH), we demonstrated the lytic life cycle of an in silico predicted virus to infect Candidatus Altiarchaeum hamiconexum, an uncultivated free-living, biofilm-forming archaeon, via fluorescence microscopy. Secondly, we characterized the morphological changes of altiarchaeal virocells, i.e. virus-infected prokaryotic cells, using correlative fluorescence and scanning electron microscopy. This effort culminated in a protocol to link a viral genome to the ultrastructure of its virus-like particle and to co-localize these with their host. However, the CRISPR-Cas immune response in single environmental virocells remains unexplored due to current technical constraints.
Methods
As a next step in expanding our microscopy toolkit for the study of virus-host interactions in environmental samples, we utilize various FISH-based methods to visualize the presence and activity of CRISPR-Cas systems within a biofilm of Ca. Altiarchaeum hamiconexum. In the first step, we developed HRP-probes to target the host crRNA of 11 abundant spacer that target a host-specific lytic virus. As the CRISPR spacer diversity in altiarchaeal biofilm is complex, the CARD-FISH based amplification method enhances the signal intensity of low-abundant crRNA in environmental samples.
Furthermore, we developed a distinct set of probes to traget the mRNA of the cas3 protein. Amplification of the signal was achieved by employing 50 different oligonucleotide probes, each fluorescently labeled with so called FLAP tails, targeting the same mRNA segment.
The established protocol can also be combined with virusFISH, allowing a nuanced perspective on the dynamic interplay between the CRISPR system and actively infecting viruses in a natural ecosystem.
Results
Our findings uncover the spatial expression of specific CRISPR spacer sets targeting one virus population within an environmental archaeal biofilm, with a confluence of the most abundant spacers targeting the same virus covering nearly the entire biofilm. Additionally, we are able to detect mRNA of the Cas3 protein within our environmental sample. Our two independent fluorescence microscopy methods enabled us detecting active CRISPR-Cas systems in environmental samples along with the targeted viral genomes.
Conclusions

Spacers targeting the same virus within a biofilm can be heterogeneously expressed across the individual cells, resulting in an active defense of the population. Nevertheless, active infections are observed in these biofilms, suggesting an ongoing competition between the host defense system and the virus. In sum, our results and newly developed techniques open novel avenues to understand the intricate relationships between viruses and their microbial hosts.

Keywords:

Microbiology, virology, FlSH, CRISPR, archaea

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Observing Magnetic Skyrmions in Pt/Co/Cu Multilayers using in-situ Lorentz Transmission Microscopy

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Poster Group 1

Magnetic multilayer systems hosting skyrmions at room temperature have attracted considerable interest for their potential application in the forthcoming generation of magnetic data storage devices. Pt/Co/X multilayer systems, where X represents a metallic element, stand out as a prospective candidate due to their versatile adjustability of magnetic properties. Recent theoretical predictions indicate that the size of skyrmions within this multilayer architecture can be finely tuned by varying the repetition of stack layers, the thickness of individual metallic layers and the element X [1]. A pivotal milestone towards employing these multilayer systems in data storage devices lies in the capacity to both stabilize skyrmions and manipulate their movement across the material. In this work, in situ in Lorentz transmission electron microscopy (LTEM) is used to investigate nucleation, stability, and mobility of magnetic phases in [Pt/Co/Cu]x5 multilayer specimens.

[Pt/Co/Cu]x5 multilayer specimens were grown on Pt buffer layer grown on an insulator substrate (Al2O3 [111]) using molecular beam epitaxy (MBE) [2]. Cu was chosen as a X mainly for two reasons: 1) its close lattice parameter to Co and Pt favors an epitaxial grown of the layers, and 2) there isn't a magnetic dead layer in this system [3,4]. Cross-section and plan-view specimens were prepared using dual focused ion beam (FIB) instrument. High angle annular dark field (HAADF) STEM imaging and energy-dispersive X-ray (EDX) mapping analysis in cross-section specimens was performed to characterize the layered structure of specimens. L-TEM in Fresnel mode with variable applied magnetic field was used to investigate magnetic domain structure and skyrmions in plan-view specimens. As a further step, biasing experiments applying current (DC and pulses) using an in-situ TEM holder (DENS Wildfire-Lightning) connected to a pulse generator were performed to explore the stability of magnetic phases and its dynamics.

Room temperature magnetic phases as a function of applied magnetic field (from 0mT to 300mT) are identified by LTEM imaging, showing "labyrinth" texture at 0mT that evolves into magnetic stripes domains followed by a transition to isolated bubbles-like skyrmions around 135mT [2,5]. The contrast in the LTEM images, suggest coexistence of multitype bubbles-like skyrmions, Bloch-type and Néel-type. This observation is quite surprising since a Néel-type configuration is expected for such interfacial Dzyalloshinskii−Moriya interactions (DMI) system. As a result of our biasing experiments, it was observed that skyrmion's type, stability and density can be modulated by the thermal effect caused by current pulses or DC current. Ultimately, the correlation between current density and applied magnetic field for this system is summarized in a magnetic phase diagram.

Our findings reveal a broad range of magnetic conditions enabling the manipulation and control of skyrmions by varying biasing current parameters and could be considered within similar systems.

Keywords:

Magnetic multilayer, LTEM, Skyrmions

Reference:

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Nanoscale T-cell membrane protein imaging across complex topography using accelerated large depth-of-field localisation microscopy

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LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
The nanoscale topography of cells plays a key role in a variety of biological processes, particularly for T cells, where finger-like structures scan the environment to sense pathogens. Conventional imaging often considers individual protein distributions, which can result in perceived clustering due to the membrane curvature. Without considering topography, it becomes impossible to decipher protein distributions and therefore its role in biological processes. Imaging cell topography (Fig. 1a) with localisation microscopy in 3D is thus important, but challenging, due to the relatively large scales involved and the requirement to achieve sufficient localisation densities. Point accumulation for imaging in nanoscale topography (PAINT) can be used overcome these challenges, but is typically hampered by high background, particularly for large depth-of-field 3D imaging.

Methods
Here we use actively controlled probes to greatly increase the effective concentration in PAINT, without influencing the background. By building up a concentrated and reversible reservoir of photoactivatable or spontaneously blinking probes the localisation rate of PAINT can be greatly accelerated. We combine this method with double-helix point spread function engineering that enables large depth-of-field localisation microscopy, which we apply to image the cell membrane of fixed T cells. Finally, the membrane imaging is combined with conventional membrane protein localisation microscopy to characterise nanoscale T-cell protein distributions across the complex topography of the cell membrane.

Results
We demonstrate that our approach (resPAINT) can increase the localisation rate of PAINT up to 50-fold without increasing background (Fig. 1b), which is essential for achieving suitable localisation densities to study membrane topography. We then show that our approach is suitable for imaging topography as we apply it to volumetric super-resolution imaging of entire T-cell membranes (Fig. 1c). Finally, we achieve correlative membrane and protein imaging (Fig 1d) to resolve the distribution of key T-cell membrane proteins: the T-cell receptor, the phosphatase CD45 and the microvilli marker CD62. By considering the local distribution of neighbouring proteins and membrane topography, we demonstrate how eigenvector analysis can be used to decipher preferential organisation and clustering of membrane proteins.

Conclusion
We have developed a technique for performing correlative membrane topography and membrane protein imaging, which we show is key for understanding how proteins distribute at the nanoscale. This represents a new approach to understanding how proteins organise and redistribute to achieve

important biological functions, such as the recognition of pathogens by the adaptive immune response.

Keywords:

Super-resolution, Single-molecule, Membrane proteins, Biophysics

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Active focus stabilisation using astigmatism with universal objective lens compatibility and sub-10 nm precision

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Poster Group 1

Background incl. aims
Super-resolution microscopy techniques can resolve biological samples with nanoscale resolution that surpasses the diffraction limit. This, however, requires focus stabilisation to correct for axial drift, which is particularly important for high-throughput automated imaging. Many solutions have been developed to tackle this problem. Marker-based methods are highly accurate, but the introduction of fiducials during sample preparation can give rise to additional challenges. With a beam-based approach, it is common to monitor the reflection of an infrared laser from the coverslip-sample interface. Nevertheless, there are limitations of current beam-based approaches that can be summarised as: (i) relatively low sampling rates; (ii): only being compatible with high numerical aperture (NA); (iii) electronic components requiring soldering and custom circuit boards and (iv): the need to put a focus stabilisation module close to the objective lens as the performance is limited by laser pointing stability.

Methods
Here, we present a standalone, cost-effective, and fiducial-free focus stabilisation system that operates over a long axial range with nanoscale precision and can be implemented using off-the-shelf components. In our focus stabilisation system, an infrared laser beam is focused by the objective lens, back-reflected from the coverslip-sample interface, and then imaged onto a camera (Fig. 1a). Introducing a cylindrical lens in front of the camera creates an astigmatic point-spread-function (Fig. 1b). Real-time monitoring of variations in the shape (Fig. 1c) of this astigmatic intensity profile as a response to focal drift allows for the transmission of a control signal to a piezo z-stage, consequently facilitating the stabilisation of the sample.

Results
We characterise the performance of our astigmatism-based drift correction system and find that it is much less sensitive to optical component stability, unlike TIR-based systems that need to be close to the coverslip. We achieve sub-10 nm axial drift estimation and correction. We then show the astigmatism leads to one of the advantages of our system – its ability to operate within a large axial range, extended over 20 µm by employing cylindrical lenses with different focal length. This trade-off between precision and axial range makes it possible to achieve 20 nm precision with low numerical aperture 10x objective lenses. We have implemented our solution on a Raspberry Pi platform that can perform stabilisation at 100 Hz (Fig. 1d), which is suitable for drift correction on most super-resolution methods.

Conclusion
With compatibility across different objective lenses, we have created a straightforward and low-cost optical device that can seamlessly integrate into most microscopy setups, offering advantages such as ease of implementation, universality, and robustness.

Keywords:

Autofocus, Focus stabilisation, Drift correction

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Largely improved momentum resolution in STEM-DPC imaging of Si(110) with a segmented detector

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Poster Group 2

Differential phase contrast (DPC) imaging in scanning transmission electron microscopy (STEM) is one of the promising methods to precisely characterise the electric field and the charge density distribution in solids at atomic resolution. DPC measurements are based on the detection of the lateral shift in the centre of mass (CoM) of intensity distribution in the detector plane. This shift of the CoM is detected by a position-sensitive detector such as a segmented or pixelated detector and can be related to the transferred momentum, which is imposed on the electron beam by the electrostatic potential of the specimen via the Coulomb force [1]. Besides the sensitivity to electric fields, DPC measurements are influenced by many factors such as residual lens aberrations, specimen thickness and tilt [2], as well as the detector response function. For segmented detectors the latter is determined by the polar and azimuthal detection angle intervals of the detector segments. Due to the small number of segments and their comparably large detection angle intervals, segmented detectors have a much lower momentum space resolution than pixelated detectors. This significantly influences the measurable DPC signal [3]. The detection intervals of a segmented detector can intentionally be modified: The polar detection intervals can be varied via the camera length; the azimuthal detection angle intervals can be set via the detector rotation. It is mandatory to know how and to what extend the detector response function of a segmented detector influences the features in a DPC image. For quantitative DPC imaging, it is therefore in the best interest to give methods for identification of artefacts and optimisation of the detector response function. This is presented in this contribution, including a comparison of DPC measurements of Si(110) acquired with segmented and pixelated detectors.
Experimental DPC images of a 30 nm thick Si(110) specimen are acquired with two probe-side corrected microscopes, a JEOL JEM-ARM200F and a JEOL JEM-ARM200F NEOARM, both operated at 200 kV. For DPC imaging, the former is equipped with a rotatable eight-fold segmented detector, the latter with a fast GATAN OneView camera for 4D-STEM imaging. To evaluate the DPC signal of the pixelated detector, the CoM determined from the complete intensity distribution and from intensities of virtual detector segments, which are projected onto the pixelated detector in post-processing, are utilised. While a thickness of 30 nm is ill-suited for quantitative DPC, it is appropriate to reveal and study the influence of the detector response function. Analogous image simulations are obtained using the software Dr. Probe [4], which is based on a multislice algorithm.
Comparative DPC investigations of Si(110) acquired with the segmented and the pixelated detector (Fig. 1 a-d) reveal that measured electric field and charge density distributions match in the order of magnitude. However, a strong influence of the segmented detector can be seen from significantly different image features, e.g. resulting in unexpected positive charge densities at interatomic pixels (Fig. 1b) which are not detected with a pixelated detector (Fig. 1d). To study the influence of the segmented detector's response function, series of measured and simulated DPC images are compared for different detector rotations. This is facilitated with the so-called scattergram, which is a 2D histogram of the transferred momentum distribution of the DPC image. The scattergrams exhibit characteristic intensity distributions depending on the detector rotation. A good agreement between experimental and simulated scattergrams is observed. In addition, a method is presented to find the

optimum polar and azimuthal collection angles of a segmented detector to obtain a DPC image with high similarity to the one of a pixelated detector. This is achieved by comparing the DPC images extracted from the full intensity distribution of the measured 4D-STEM data set with the DPC image acquired by projecting virtual detector segments on the same 4D-STEM data set. In case of a 30 nm thick Si(110) specimen the optimum detector response function is achieved when the direct beam illuminates the complete detector at a detector rotation of 45° to the Si dumbbell axis (inset in Fig. 1b). Ultimately, a novel method to increase the azimuthal momentum space resolution of segmented detectors is presented which uses a series of DPC images of equidistant detector rotations. Using this method, artefacts such as the positive charge density at interatomic pixels are minimised even for such large specimen thicknesses (Fig 1.f).
The results show a strong influence of the detector response function of segmented detectors and emphasise the importance of minimising this influence on DPC images for correct image interpretation. In particular, the here presented method for increasing the azimuthal momentum space resolution of segmented detectors enables to obtain equally good DPC measurements with a segmented detector as with a pixelated detector.

Keywords:

DPC, detector response function

Reference:

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Interaction of transduction enhancing peptide nanofibrils with cells and virions assessed by complementary EM techniques

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Poster Group 2

Background and aims:
Retroviral gene therapies are applied to treat cancers. In chimeric antigen receptor (CAR) T-cell therapy the gene for the fusion protein CAR is inserted into the patients' T-cell genome by retroviral transduction in vitro. After reintroduction into the patient, the reprogrammed T-cells expressing CAR are able to recognize and destroy cancer cells (Cappell and Kochenderfer, 2023). For cost efficient application of CAR T-cell therapy, researchers aim at enhancing transduction efficiencies. Transduction efficiencies are often low due to the low retroviral titers. Another reason is the electrostatic repulsion between the negatively charged virions and the negatively charged plasma membrane. To overcome this repulsion, positively charged peptide nanofibrils (PNF) were developed. One type of PNF, enhancing factor C (EF-C), is actively engaged by cellular protrusions and leads to a 9.3-fold enhanced transduction rate in HeLa cells (Schütz et al., 2021). However, EF-C fibrils formed large aggregates, which could clog the bloodstream when introduced into the patient and are, thus, not suitable for clinical application. A novel peptide, Derivative-4 (D4), was identified by in-silico screening (Rauch-Wirth et al., 2023). To better understand the morphology of D4 PNF and to characterize their interaction with virions and cells, electron microscopy was performed.
Methods:
The morphology of D4 PNF was characterized by negative staining and transmission electron microscopy (TEM). The binding of D4 to virions and the cell surface was then studied by scanning electron microscopy (SEM) of cells after critical point drying. To obtain a more complete view of binding of D4 to cells, samples were analyzed by TEM and STEM tomography. For both, high-pressure frozen and freeze-substituted cells were used (Bergner et al., 2022).
Results:
TEM showed that D4 PNF are shorter than EF-C. In cell culture, most of the D4 aggregates were formed by structures measuring up to a few hundred nanometers in length ("D4 network"). SEM analysis revealed that D4 networks bind to cells via cellular protrusions. When the networks were considerably smaller than the cell, the plasma membrane formed indentations close to the network, indicated starting cellular uptake. TEM analysis of cell cultures treated with PNF and virions revealed a second D4 morphology; besides D4 networks also aggregates of µm-long, thick D4 nanofibrils were found. We then analyzed virion binding patterns to these different D4 morphologies by TEM and STEM tomography. Irrespective of the D4 morphology, virions bound to D4 aggregates. However, the D4 morphology appeared to influence virion binding. Virions bound to the surface of the D4 networks and were also found within the network. In contrast, at aggregates formed by the long and thick D4 fibrils, virions were only found on the surface of the aggregate.
Conclusion:

Using 4 complementary EM techniques, we obtained a better understanding of the transduction enhancing function of D4. This understanding is important for the further development of D4 PNF and their clinical applications.

Keywords:

STEM tomography, SEM, gene therapy

Reference:

Bergner et al., 2022, Near-Native Visualization of SARS-CoV-2 Induced Membrane Remodeling and Virion Morphogenesis. Viruses. doi: 10.3390/v14122786
Cappell and Kochenderfer, 2023, Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol. doi: 10.1038/s41571-023-00754-1.
Rauch-Wirth et al., 2023, Optimized peptide nanofibrils as efficient transduction enhancers for in vitro and ex vivo gene transfer. Front. Immunol. doi: 10.3389/fimmu.2023.1270243
Schütz et al., 2021, Viral Transduction Enhancing Effect of EF-C Peptide Nanofibrils Is Mediated by Cellular Protrusions. Advanced Functional Materials. doi: 10.3389/fimmu.2023.1270243.

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Structure retrieval by parameterised inverse multislice accounting for partial coherence and thermal effects

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background
Inverse multislice uses experimental momentum-resolved scanning transmission electron microscopy (4D-STEM) data to reconstruct the individual slice transmission functions (phase gratings) of a specimen. It solves the problem of inverse dynamical scattering, and takes partial coherence effects into account to obtain spatial resolutions in the range of thermal vibrations [1]. Both the incident probes and phase gratings are usually optimized on a pixel-wise grid, involving the determination of 10^6 to 10^7 unknowns. In this work, we present an inverse multislice concept which uses a consequently physical parameterisation of both probes and specimen. A few aberration coefficients, focal spread and source size describe the illumination comprehensively, and the phase gratings are parameterised via atomic types, positions, occupancies and thermal vibrational amplitudes. Consequently, the number of unknowns is reduced by at least four orders of magnitude. This leads to both a more efficient and stable inversion strategy, and the capability of performing inverse frozen phonon (FP) multislice. The latter incorporates thermal diffuse scattering (TDS) and exhibits enhanced sensitivity to temperature and chemistry. We present both the methodological concept and its application to measuring ferroelectric displacements experimentally in $Pb(ZrTi)O_3$ (PZT) with picometre precision [2].

Methods
Inverse multislice starts with an initial guess for the probe and the phase gratings, performs forward multislice so as to calculate a loss with respect to the experimental 4D-STEM data. The update for the subsequent epoch is derived from the gradient of the loss as to the parameters of interest. We developed an efficient implementation of this scheme by (i) expressing the multislice as an artificial neural network following van den Broek [3] within PyTorch, being able to (ii) seamlessly allocate all gradients in a single forward run. We start with conventional, pixel-wise inverse multislice reconstructions, followed by the detection of atomic structure, types and probe parameters to initialise a parametric model. Instead of complex-valued pixels, gradients decisively update real physical parameters, especially atom positions. We then firstly employ the Debye-Waller approach with absorptive potentials for the reconstruction, and secondly switch to a full inverse FP multislice which also inverts TDS using several thermal snapshots while still considering more than 10 probes to account for partial coherence. Our method is verified using FP ground truth 4D-STEM simulations first, before experimental 4D-STEM data of PZT (EMPAD recording on probe-corrected FEI Titan, 200kV) is analysed inversely using 52 slices, corresponding to a thickness of 22nm.

Results
Concerning forward scattering the FP concept involves averaging over multiple configurations of thermal atomic displacements $\vec{u}$ from the equilibrium positions as illustrated in Fig. 1a, being responsible for TDS. Via the mean squared displacement $\langle u^2 \rangle$, TDS is explicitly accounted for in our inverse multislice as forward scattering theory demands. At the same time, our model is also

differentiable with respect to the atom positions, as shown in Fig. 1b. Here, we simulated 4D-STEM data of 20nm thick PZT using FP multislice including ferroelectric displacements. For the reconstruction, no polarisation was used as prior by initialising with a centrosymmetric model and taking projected potentials from literature [4]. For 101 epochs, Fig. 1b shows the optimisation trajectories of the Zr(0.2)Ti(0.8) site treated as a virtual atom obtained by linear interpolation, and one oxygen site. Even though the forward simulation used the discrete random mixture of the Zr(0.2)Ti(0.8) site, the reconstruction with a virtual atom yields a final position between the ground truths (dashed cross) for Zr and Ti, shifted proportionally according to the chemistry of the atomic column. Moreover, the lower graph indicates an exact reproduction of the oxygen site after 101 epochs.
Applying the methodology to experimental 4D-STEM data of PZT results in an atomic structure depicted by its projected potential in Fig. 1c for the reconstructed region, whereas the initial model started with a nonpolar crystal. The polarisation of the final state is visible by eye and free of artefacts such as mistilt which we took into account by a modified Fresnel propagation that was also optimized. The displacements from the symmetry positions are mapped in Fig. 1d, with different markers for the different atom columns and the ferroelectric displacements colour-coded. The high-precision homogeneous polarisation in the scan region becomes obvious, and is confirmed by the statistical evaluation in Fig. 1c (table). The displacements are accurate to a few picometres, can distinguish oxygen from the (ZrTi) site in the same column and fit excellently with literature for the same alloy. We discuss our findings via a quantitative comparison with atom positions retrieved a posteriori from phase gratings retrieved pixel-wise assuming a unimodal specimen. In this case, several unit cells exhibit the wrong polarisation direction.

Conclusion
Inverse multislice taking multimodal states of both illumination and specimen into account is capable of exploiting TDS in the reconstructions. Since TDS is a major contribution in thick specimen for which (inverse) multislice is designed, this opens several possibilities to accurately measure atom positions, atom types and temperature or Debye parameters, whereas sensitivity to the latter parameters will be discussed in our contribution based on simulations.

Keywords:

Inverse multislice, Ptychography, 4D-STEM

Reference:

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[5] Funding from the Bavarian HighTech Agenda (EQAP project), DFG (EXC 2089/1 – 390776260) and Helmholtz (VH-NG1317) is kindly acknowledged.

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Temperature-dependence of beam-driven dynamics in graphene-fullerene sandwiches

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Poster Group 2

Aberration-corrected high-resolution transmission electron microscopy was used to investigate C60 fullerenes encapsulated between graphene sheets at different temperatures (ca. 93K, 293K, and 773 K), along with molecular dynamics simulations. Cryogenic and heated conditions were reached using a custom in situ MEMS heating system. The study focused on the beam-induced dynamics of the C60 fullerenes and the encapsulating graphene, measuring the critical doses for the initial damage to the fullerenes and following the beam-induced polymerization. We observe that the doses for the initial damage are not significantly affected by temperature. However, the clusters formed by subsequent polymerization exhibit more tubular shapes at lower temperatures, while sheet-like structures are generated at higher temperatures.
These experimental findings are supported by the results of first-principles and analytical potential molecular dynamics simulations. The merging of curved carbon sheets is promoted at higher temperatures and occurs rapidly over segments of only a few nanometers.

Keywords:

C60 Graphene 2D HRTEM MEMS

Reference:

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Viral replication organelles revisited by cryo-electron microscopy

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Coronaviruses and their distantly related arteriviruses belong to the order Nidovirales. Similar to other positive-strand RNA viruses infecting eukaryotes, nidoviruses hijack host membranes converting them into specialized structures often termed viral replication organelles. These replication organelles exhibit diverse morphologies depending on the virus group but serve a common purpose: to establish specialized micro-environments conducive to viral RNA synthesis, potentially shielding viral replication intermediates from cellular immune surveillance. A hallmark of nidovirus infection is the emergence of double-membrane vesicles (DMVs) originating from endoplasmic reticulum membranes, which serve as sites of viral RNA synthesis. Typically, nidovirus genomes encode 3 nonstructural proteins (nsps) that contain multi-spanning transmembrane (TM) domains (TM1, TM2 and TM3) and that are the main drivers of DMV formation (1).

Traditionally, viral replication organelles have been studied by conventional electron microscopy (EM) methods on resin-embedded infected cells. However, recent advancements in cryo-EM, particularly the introduction of focused ion beam (FIB) milling technology that makes internal regions of cells accessible for cryotomography, have revolutionized our ability to investigate these virus-induced structures. This cutting-edge approach allowed us to investigate viral replication organelles in cells infected with coronaviruses with exquisite detail (2). Through this methodology, we uncovered a molecular pore spanning the membranes of the DMVs, potentially serving as a gateway for viral RNA synthesized within the DMVs to access the cytosol for translation or encapsidation into progeny virions. Using subtomogram averaging, this crown-shaped pore complex of around 3 MDa was solved to 3 nm resolution and revealed to have an overall 6-fold symmetry. Additionally, we identified that the largest viral nonstructural protein, which contains the TM1 domain, as a component of this complex. Furthermore, in situ cryo-tomograms provided compelling evidence of dynamic interactions between the pore complex and other macromolecules on both sides of the pore. Based on these observations, we postulated that the viral replication machinery may interact with the pore side facing the DMV interior, while the nucleocapsid protein, responsible for encapsulating new viral RNA, would associate with it on the cytosolic side.

Our investigations have extended to the distantly related arteriviruses, revealing the presence of similar DMV-spanning pore complexes. Notably, viral nucleocapsids and putative ribonucleoprotein assemblies were often observed in close association with the arteriviral DMV pores, providing further support to the notion of a functional linkage between RNA export from the DMVs and encapsidation, mediated by interactions of the pore complex with the nucleocapsid protein. Interestingly, DMV pore complexes were also formed in non-infected cells that expressed the 3 arterivirus TM-containing nsps. This indicated that the formation of a DMV-spanning pore structure is independent of the presence of viral RNA, the replication machinery, or the structural proteins. Moreover, expression of only the nsps containing TM1 and TM2 was sufficient to form DMV-like structures in which pore complexes were present. This observation is consistent with recent reports of the formation of DMV-

spanning pore complexes in cells expressing coronavirus TM1- and TM2-containing nsps (3). Taken together, these results suggest that DMV-spanning pore complexes may be a shared characteristic among nidoviruses. These pore complexes would have a similar mechanism of formation driven by TM1- and TM2-nsps, which appear to be core components of the complex, and would fulfill a critical role in coordinating viral RNA synthesis, export and encapsidation.
Interestingly, recent studies focusing on other +RNA viruses that induce a morphologically different type of viral replication organelle consisting of an invaginated spherule, have unveiled crown-shaped multimeric complexes located in the neck of the spherules (4,5). These complexes bear intriguing similarities to the DMV-spanning complexes observed in nidoviruses. Collectively, these studies define an emerging class of viral complex that seem to underscore common strategies employed in the replication of evolutionarily distant positive-strand RNA viruses. Further elucidation of their structural and functional details will not only deepen our understanding of viral replication but may pave the way for the development of novel antiviral strategies.

Keywords:

coronaviruses, arteriviruses, cellular cryotomography

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Influence of dynamical diffraction on DPC measurements of 2D materials

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Poster Group 2

Dynamical diffraction effects in (scanning) transmission electron microscopy often hamper direct image interpretation. While dynamical diffraction effects are commonly neglected in conventional STEM investigations, they are crucial in quantitative phase retrieval techniques as multiple coherent scattering events alter the intensity distribution during the interaction of the electron wave with the specimen. One of these phase retrieval techniques is differential phase contrast (DPC) imaging, which is based on the detection of the center of mass in the post specimen intensity distribution and is promising for the precise quantification of atomic electric field and charge density distributions in solids. It is commonly accepted that qualitative DPC imaging is only feasible for specimen thicknesses below 20 nm [1] due to the influence of dynamical effects resulting in beam broadening and intensity redistribution within the specimen. For quantitative DPC imaging, the maximum specimen thickness is estimated to be about 2 nm, depending on the atomic species [2].
Due to their inherently low thickness, 2D materials are therefore of great interest for DPC measurements and are intensively investigated also by other phase retrieval methods [3,4]. Among the 2D materials there is the class of transition metal dichalcogenides (TMD) which consist of molecular layers with the stoichiometric form MX2 (M: transition metal, X: chalcogenide). The molecular layers are bound to each other only by weak van-der-Waals interactions. This allows for a comparatively simple preparation of specimens that are only one or a few molecular layers thick and are therefore suitable for quantitative DPC imaging. However, the possibility that dynamical effects may influence the measurable electric field distribution even in 2D materials, has only been rudimentarily investigated.
Since slight changes in the intensity distribution along the propagation of the electron wave through the specimen alter the measurable electric field and charge density distributions measured by DPC, it is important to understand the influence of dynamical effects on DPC measurements in a few-layer 2D TMDs.
In this contribution, we present investigations on the influence of dynamical diffraction effects on the DPC images of mono- and multilayers of tungsten diselenide, which is a 2D TMD. This is achieved using multislice simulations performed with the software Dr. Probe [5] and a comparison with experimental DPC images. For the acquisition of the latter, an eight-fold segmented bright-field detector installed at a probe-side Cs-corrected microscope is used and the same configuration is assumed in the simulations. In addition to simulations for 2D WSe_2, image simulations are also conducted for isolated atomic columns to investigate the obtainable DPC images in dependence of the atomic species, the atomic spacing and the acceleration voltage. By comparison with the DPC image of a single atom, the influence of dynamic effects is revealed. Furthermore, the propagation of the electron beam in the vicinity of different atomic columns is investigated by analyzing the real space probe intensity as a function of the specimen thickness.
The DPC measurements and simulations of WSe_2 [0001] are in good qualitative agreement. However, they show an unexpected ratio of electric field magnitudes and charge densities at W and Se atomic columns, even for a monolayer: despite their lower projected atomic number, Se atomic columns exhibit an increased electric field and positive charge density magnitudes compared to W. This can be explained by dynamic diffraction effects superimposed with other influences such as lens

aberrations. These findings are supported by simulations for the isolated atomic columns which reveal that the measurable mean DPC signal of W and Se atomic columns is always smaller than the mean DPC signal of a single atom multiplied by the number of atoms in the column. In addition, the DPC image is significantly affected by the atomic spacings in beam direction and number of atoms in an atomic column. The behavior of the real space probe intensity for the interaction with different atomic columns is presented and related to the measurable DPC signal, again supporting the finding that the atomic species of the atomic columns strongly affect the influence of dynamical diffraction on the DPC image. The results highlight that for quantitative DPC measurements even on 2D materials a careful consideration of dynamic influences must be made.

Keywords:

2D materials, differential phase contrast

Reference:

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Sensing the Invisible: Ultrathin (UT) Membrane Chip for In-Situ Microscopy

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background incl. aims
Operando or in-situ S/TEM methods utilizing amorphous silicon nitride (SiNx) membrane encapsulated chips to confine fluids for electron microscopy have become popular in recent decade, A great number of prior innovators have shown this to be an effective approach for probing fluid-surface/nanostructure interactions and related phenomena. Such “closed-cell” platform has many practical and technological advantages over the differential pumping environmental TEM (ETEM). Unfortunately, however, conventional fluid-cells suffer from additional and significant electron scattering from the top and the bottom membranes, which are typically 30-50nm thick to maintain integrity/stability during the operation. Thus, the total thickness of >60-100 nm of the encapsulating membranes imposes many adverse effects on the post electron optics, such as increased chromatic aberrations. This naturally results in significant degeneration of signal quality and loss of spatiotemporal resolution, diffuse interference in the electron diffraction, and plasmon-dominated electron energy loss spectra (EELS).
Methods
We have recently reported development of a robust, functional and scalable backing support strategy to enable the thinnest possible (<10 nm) SiNx gas encapsulation material [1, 2]. Inspired by the natural honeycomb geometry, our novel design provides for hexagonal backbone that can neatly anchor ultrathin (~<10 nm) SiNx membrane with excellent stability and consistent performance. Unlike graphene-based encapsulations, stability under the electron beam is comparable to a 50 nm SiNx membrane, which is sufficient for most high-resolution S/TEM applications on non-electron sensitive materials.
Results
We show that our UT chip increases contrast of typical nanoparticles at 1 atm Ar gas by ~70 % and the accessible information limit is enhanced by >130 % compared to the conventional encapsulation. More importantly, the t/λ_i is reduced from nominally ~1.0 to 0.3 using a 1 Atm gas cell. This greatly enhances spectral visibility and significantly improved S/N for EELS excitations. Thus, spatiotemporal detection of gas species, down to ~nanometer scale is now being routinely achieved.
Conclusions
The presentation will cover the design and implementation of UT membrane fluid-cell for in-situ gas-solid interactions. It will also argue that combining monochromatic source with UT membrane may open new opportunities for molecular-scale understanding of dynamic fluid-surface phenomena. [3]

Keywords:

Ultrathin (UT) membrane, in-situ microscopy

Reference:

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Impact of Clostridium botulinum C2 Toxin on the ultrastructure of cells

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Poster Group 1

Background: The C2 toxin of Clostridium botulinum is a prototypical representative of the binary actin ADP-ribosylating toxin family. It belongs to the AB toxin class, which comprises two components: the enzymatic unit A and the binding and transport unit B. In C2 toxin, C2I represents the enzymatic unit A, and C2II represents the binding component B. To gain its biological activity, C2II must be activated by proteolytic cleavage, converting it to the active component C2IIa. Upon activation, C2IIa binds to the cell surface as a homoheptameric structure and is subsequently endocytosed together with C2I into the cell. In the cytosol, the enzymatically active component, C2I, ADP-ribosylates G-actin. By this, G-actin acts as a capping protein that blocks the polymerisation of actin filaments. This alteration of the actin cytoskeleton eventually leads to cell rounding and ultimately, to cell death[1].
The effect of the C2 toxin on cells was investigated with various assays including fluorescence microscopy of labelled actin[2]. Nevertheless, to date, the effect of C2 toxin on the cellular ultrastructure was not studied. Therefore, we performed transmission electron microscopy (TEM), scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) tomography of C2 intoxicated cells. Furthermore, to better understand the mechanism of C2 intoxication, we also investigated the effect of the individual subunits on the cell architecture.
Methods: Analysis of the ultrastructure of C2 intoxicated cells was performed using TEM and STEM tomography. For this, cells were high-pressure frozen, freeze-substituted, and embedded in Epon[3]. The impact of the C2 toxin on the cell surface was investigated using SEM of critical point-dried cells. To test whether the observed effects are mediated by actin, Cytochalasin, an actin polymerisation inhibitor, was used as a control[4].
Results: TEM analysis demonstrated that cells exposed to either C2I or C2IIa did not exhibit any ultrastructural changes compared to non-intoxicated cells. However, cells intoxicated with complete C2 toxin displayed many intracellular vesicles and a reduction in cell size. Additionally, blebs were observed along the cell membrane. Control cells, treated with Cytochalasin B, exhibited similar effects as the C2-intoxicated cells, albeit to a lesser degree.
SEM analysis revealed that C2 treated cells rounded up and confirmed plasma membrane blebbing. Furthermore, a lack of filopodia on the cell surface was observed. The combination of SEM and TEM analysis revealed that the filopodia were endocytosed by the cell. Cytochalasin B control cells seemed to be close to rounding up, with fewer filopodia on the surface compared to non-treated cells. However, the uptake of filopodia, as seen upon C2 intoxication, was not observed.
STEM tomography of C2-intoxicated cells revealed large vesicles containing cellular debris, as well as stress granules in mitochondria and swollen endoplasmic reticula. In contrast, STEM tomography of untreated cells did not exhibit any of these observations.
Conclusion: The three electron microscopic methods provided a deeper understanding of the impact of C2 toxin on cellular architecture. It was found that only the complete C2 toxin acts as a stressor

and affects filopodia, causing them to be engulfed into the cell. Further examination is necessary to gain a better insight into the mechanism behind filopodia engulfment.

Keywords:

AB-Toxin, TEM, SEM, STEM tomography

Reference:

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Nondestructive structural characterization of 2D materials by hybrid pixel direct detector

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Poster Group 2

The remarkable properties of 2D materials, including their high carrier mobility, conductivity, and mechanical strength, have generated significant interest among researchers. However, characterizing these materials in their self-standing form or when deposited on substrates is a challenging task. As a result, there is a growing demand for SEM and FIB/SEM-based techniques. Currently, available techniques lack site-specificity and fail to accurately represent 2D materials on substrates, often requiring destructive cross-sectioning or TEM lamellae preparation [1]. Therefore, there is a need for a suitable SEM/SDB technique.
One newly developed technique that shows promise is Backscattered Electron imaging and Reflected Kikuchi Diffraction (RKD) facilitated by a hybrid pixel direct detector [2]. The detector mounted on Apreo SEM on a retractable arm consists of four Timepix1 chips configured around the optical axis on a retractable arm. This configuration allows capturing the electron backscatter patterns while also counting the amount of backscattered electrons from each analyzed point. The WSe2 flakes were deposited on the TEM supporting grid. Based on the calibration, evaluation of patterns, and intensity of measured backscattered electrons, we can elucidate layer thickness changes under the assumption that interaction volume extends to the substrate.
Figure a) shows a median electron count map of a multilayer specimen of WSe2 measured in Reflected Kikuchi Diffraction (left map) and in standard (70° tilt) EBSD geometry. Both maps were acquired at 10 keV acceleration voltage, 5 keV electron energy threshold, and approximately 3.2 nA probe current. We have taken and plotted the average values along the red area shown on both maps to demonstrate the differences in both methods. It can already be seen that the RKD method is more sensitive to the layer thickness compared to the standard EBSD. Further comparison can be seen on the graph on the right showing the averaged values along the red arrows. One can easily distinguish the three separate layer thicknesses along the profile in the case of the RKD method. No such profile is evident on the dataset acquired on a standard EBSD detector. Figure b) shows an example pattern from the RKD detector from the area under the red rectangle, whereas Figure c) shows a dynamic simulation of the pattern in Fig. b).
For the first time, it was demonstrated that the possibility of characterizing 2D materials, in this instance on WSe2 flakes, using RKD. By using the median electron counting method, it is possible to distinguish small deviations in specimen thickness. In contrast, a conventual geometry EBSD detector of an equivalent kind was unable to provide layer thickness distinction. We anticipate that this method could be developed to provide characterization down to single atomic layers.

Keywords:

EBSD, WSe2, Diffraction, thin layers

Reference:

[1] Londoño-Calderon, A., Dhall, R., Ophus, C., Schneider, M., Wang, Y., Dervishi, E., ... & Pettes, M. T. (2022). Visualizing grain statistics in MOCVD WSe2 through four-dimensional scanning transmission electron microscopy. Nano Letters, 22(6), 2578-2585.
[2] Marshall, A. L., Holzer, J., Stejskal, P., Stephens, C. J., Vystavěl, T., & Whiting, M. J. (2021). The EBSD spatial resolution of a Timepix-based detector in a tilt-free geometry. Ultramicroscopy, 226, 113294.

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Elucidating functionalities of N-doped carbonaceous materials by means of in-situ TEM

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Poster Group 2

Background incl. aims
Nitrogen-doped carbonaceous materials gained broad interdisciplinary attention as low-cost, metal-free materials for photocatalysis, carbon capture, energy storage and water remediation. Their performance in real devices relies on the precise control of their nanostructure. Usually, nanostructural characterizations are done after completion of either the synthesis or the device operational cycle. Here we present two scientific questions related to functionalities of N-doped carbons, for which in-situ/in-operando TEM studies were crucial: (1) How N-doped carbons condensate from molecular precursors, and how can we thus guide their design as materials for CO_2-sorption[1] and (2) What is the nanoscale mechanism of Na storage in N-doped carbons when they are used as anodes in Na-batteries[2]. The Methods and Results sections below are structured according to these two questions. Generalized conclusions on the application of the in-situ/in-operando methods to polymeric materials exemplified by N-doped carbons are given.
Methods
In this study we combined ex-situ high-resolution STEM, energy-filtered electron radial distribution function analysis, energy dispersive X-ray analysis and electron energy loss spectroscopy (at (1) different stages of condensation and (2) at different states of charge during electrochemical cycling) with in-situ STEM investigations: (1) in-situ heating was performed using a heating/biasing Protochips Fusion Select holder; (2) an in-operando Na half-battery was constructed using a Protochips Poseidon holder. 1 M solution of $NaPF_6$ in ethylene carbonate and diethyl carbonate was used as electrolyte.
Results
(1) By combining in situ condensation inside a STEM and ex situ analysis of the products of condensation at different temperatures and atmospheres, we were able to follow the structural, morphological, and chemical evolution of the uric acid and guanine precursors on the nanoscale upon heating and correlate it with the sorption properties of the obtained materials. We showed how one can control and tune the formation of pores in nitrogen-containing carbonaceous materials by varying pressures and reaction rates. We found that these two parameters change how the porosity of the surface develops, forming particles with mesopores (in vacuum) or microporous (in nitrogen) surfaces. Since this process co-occurs with cross-linking, the porous structure of the surface governs the subsequent release of volatiles and the development of the hierarchical pore structure. These findings allow us to synthesize N-doped carbons with a 2-times higher CO_2 uptake, keeping the same selectivity.
(2) N-doped porous hollow carbon spheres (N-PHCSs), which due to their size and shape, serve as an ideal model system to investigate Na-storage at the nanoscale, have been synthesized. By combining the ex-situ characterization at different states of charge with in-operando TEM experiments we found that at the beginning of sodiation a solvated ionic layer forms on the surface of N-PHCSs, followed by irreversible shell expansion due to the solid electrolyte interface formation and subsequent storage of Na(0) within the porous carbon shell. We showed that binding between Na(0) and C creates a Schottky junction making Na deposition inside the spheres more energetically

favorable at low current densities. During sodiation, the solid electrolyte interface fills the gap between N-PHCSs, binding spheres together and facilitating the Na ions' transport in the direction of the current collector and subsequent plating underneath the electrode. The N-PHCSs layer acts as a protective layer between the electrolyte and the current collector, suppressing possible growth of dendrites at the anode.

Conclusion

In situ/in-operando characterization, setups have been successfully applied to the characterization of N-doped carbonaceous materials. However, special attention is required for separating the electron-beam-induced effects and the processes we are interested in. The synergetic combination between ex-situ and in-situ experiments is crucial for obtaining realistic models of the studied processes.

We gratefully acknowledge financial support by the Max Planck Society. We are grateful to Dr. J. Kossmann, Dr. M. Odziomek and Prof. N. Lopez-Salas for help with synthesis, CO_2 sorption characterization and valuable discussions.

Keywords:

N-doped carbons, in-situ microscopy, batteries

Reference:

[1] D. Piankova, J. Kossmann. H. Zschiesche, M. Antonietti, N. L´opez-Salas, N. Tarakina, J. Mater. Chem. A, 2022, 10, 25220-229;
[2] J. Hou, Z. Song, M. Odziomek, N. Tarakina, Small, 2023, 2301415.

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Accessible STEM-DPC Imaging Using ADF Detector Allowing in-situ Magnetic Reversal Studies

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Scanning transmission electron microscopy (STEM) is a powerful tool for both structural and functional characterisation of materials. Phase contrast techniques such as differential phase contrast (DPC) are commonly used to observe functional properties, for example magnetic fields inside samples. However, phase contrast imaging typically requires specialised equipment such as segmented or pixelated detectors[1]. In this work we have imaged nanomagnet arrays to demonstrate the feasibility of a simplified and accessible method for DPC using a monolithic annular dark field (ADF) detector. Using the post-specimen deflector lenses, the electron beam is shifted onto an edge of the ADF detector to translate magnetic deflection into intensity variations in the image[2]. By acquiring such images at four opposing edges of the ADF detector we were able to qualitatively image the magnetic landscape of our nanomagnet structures. The images were corrected for de-scan background intensity variation and then processed like data from an annular four-segmented detector. The resulting DPC image is very well in agreement with that acquired from a center-of-mass calculation from a pixelated detector. Additionally, we show that the increased speed and lowered data set size of this accessible STEM-DPC method, when compared with 4D-STEM, allows for characterisation of the magnetic hysteresis of nanomagnet arrays with different geometries. DPC images of a nanomagnet array in three different magnetic configurations can be seen in Fig. 1. In conclusion, this method makes qualitative DPC available in most STEM-capable TEMs, enables fast and easy in-situ magnetic experiments, and paves an accessible route to correlation of observed function with structural properties.

Keywords:

DPC, in-situ, magnetic hysteresis

Reference:

1. Yang, H., Pennycook, T. J. & Nellist, P. D. Efficient phase contrast imaging in STEM using a pixelated detector. Part II: Optimisation of imaging conditions. Ultramicroscopy 151, 232–239 (2015).
2. Kraut, S. & Cowley, J. M. A simplified mode of differential phase contrast lorentz microscopy. Microsc. Res. Tech. 25, 341–345 (1993).

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Label-free imaging of virus-cell interactions using 200 Hz ROCS microscopy

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background incl. aims
The emergence of SARS-CoV-2 has shown the profound impact that viruses can have on human health and society. Due to their small size and remarkable speed it is very challenging to observe cell-virus interactions in life-cell imaging. Techniques like fluorescence microscopy, often struggle to visualize these interactions, especially due to their susceptibility to bleaching and the difficulty to label different structures without altering their function. This underscores the ongoing need for innovative approaches to unravel the complexities of virus-cell interactions and advance our understanding of infectious diseases.
Methods
In our research we use Rotating Coherent Scattering (ROCS) microscopy in order to visualize the diffusion of 100 nm sized virus-mimicking particles and their interactions with macrophages or epithelial cells. Rotating Coherent Scattering (ROCS) microscopy, a label-free imaging technique, harnesses the coherent backscattering of a rotating laser beam for super-resolution (150nm). The method involves oblique illumination of the sample from all azimuthal directions, producing nearly artifact-free images by integrating all scattered light during a single laser rotation. Currently operating at a frame rate of 200 Hz, ROCS minimizes motion blur, without any need for extensive post-processing. With its capability to adjust the illumination angle up to 70° and to utilize 4 distinct illumination wavelengths in the range of 405 nm to 561 nm, ROCS proves highly versatile in brightfield or darkfield mode, transitioning between total internal reflection (TIR) and non-TIR.
Results
By tracking particle trajectories and analyzing their fluctuations we are able to analyze the speed of our diffusing virus-mimicking particles and are able to follow binding events of single particles to cells. After binding of particles to filopodia or lamellipodia the observed fluctuations decrease over time and Additionally, by using multiple illumination angles and wavelengths in ROCS microscopy we observe different appearances of differently absorbing structures, enabling the analysis of subtle z-motions of particles and the visualization of distinct cellular structures.
Conclusion
Our research highlights Rotating Coherent Scattering (ROCS) microscopy, as a powerful tool to observe virus-cell interactions. Through ROCS, we successfully image the diffusion of virus-mimicking particles and visualize discrete binding events to cells, enabling detailed analysis of particle trajectories and binding properties. Furthermore by capturing scattered light from multiple wavelengths at once, we extend the capability of ROCS towards obtaining specificity for different structures.

Keywords:

Rotating-Coherent-Scattering-Microscopy (ROCS), single-virus tracking, label-free

Reference:

Jünger, F., D. Ruh, D. Strobel, R. Michiels, D. Huber, … A. Rohrbach (2022). "100 Hz ROCS microscopy correlated with fluorescence reveals cellular dynamics on different spatiotemporal scales." Nature Communications 13(1): 1758.

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The ultrastructural analysis of plant-microbe interfaces

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
In natural environments, plants share their habitat with various microbes and are colonized by a rich diversity of bacteria and fungi. Over time, different types of plant-microbe interfaces (PMIs) developed to enable interactions between a specific microbe and its host. The most famous PMI are e.g. the symbiosomes in root nodules of legumes, containing nitrogen-fixing bacteria, the arbuscules of mycorrhiza fungi, which are used for the nutrient exchange with the plant and the so called haustoria of some parasitic fungi or oomycetes. Knowing the ultrastructural details of PMIs is central for the biological and functional understanding of plant microbe interactions. This project aims to generate sample preparation methods for TEM and FIB/SEM techniques, visualize PMIs in 2D electron microscopy images and identify their 3D structure.
Methods
Mesorhizobium loti infected plant tissue of Lotus japonicus root nodules was prepared for TEM and FIB-SEM by chemical fixation and embedding in epoxy resin blocks. After ultra-thin sectioning, 2D images were generated with TEM. Furthermore, image stacks were generated by FIB/SEM to enable 3D modeling of the PMI region of interest by structure segmentation.
Results
TEM images of infected root nodule cells confirmed the presence of symbiosomes, which have a plant derived membrane that encloses a peribacteroid space with rod shaped bacteria of Mesorhizobium loti. The 3D model of a symbiosome visualized the arrangement of bacterial cells in the peribacteroid space tightly sourrounded by the symbiosome membrane. With regard to the TEM images, the model revealed further, that the number of bacteria in one symbiosome can be much higher than it was previously assumed by 2D image data of this symbiosis.
Conclusion
Ultrastructural details of plant-microbe interfaces visible in 2D TEM images were verified and analysed by 3D structure construction using FIB/SEM, which gives insight in the complex arrangement of microbes within infected plant cells.

Keywords:

Plant-microbe interaction, TEM, FIB/SEM

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Event-responsive Beam-modulated STEM with Multi-frame and Sparse Scanning

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Poster Group 2

Recent advances in high-speed signal processing electronics allow any analog STEM detector to operate in an event-counting mode, measuring individual electron detection events in real time. This technology delivers images with zero dark-noise, improved linearity, and SNR limited only by ideal Poisson counting statistics [1]. By combining such event-counting hardware with fast electrostatic dose-modulators (EDM) [2], a real-time 'event responsive' imaging mode can be realised [3]. We call this Trigger Event Modulated Probability Observation STEM, or TempoSTEM for short.
TempoSTEM operates in a fundamentally different way from classical STEM imaging. In classical STEM, we observe fixed periods (dwell times) and record the varying numbers of transmitted/scattered events that arrive at a detector. In TempoSTEM, we specify a fixed number of events and measure the varying time needed for the image signal to reach that number of events in each pixel location. In both pulse counted STEM and TempoSTEM the operator sees images expressed in quantitative units of events-per-microsecond. However, there is one key difference; in TempoSTEM the beam is blanked for the remainder of the pixel once the threshold is met, significantly reducing dose and sample damage. The beam is un-blanked at the start of the next pixel and a fresh measurement begins.
Information theory predicts that for every successive electron detected within each pixel time, there is a diminishing return on information content; this has also been confirmed experimentally [3]. This creates a balance where a lower Tempo trigger exit condition ensures the most efficient detection per-electron, but it does not ensure the most readily analysable SNR overall. Here we present a mode to iteratively update noisy high-variance pixels to achieve improved resolution and SNR.
Another common approach to increase the SNR of an image is through the stacking of multiple frames. Where a hardware EDM is present [2], a further option is to only revisit a subset of pixels in subsequent frames in the series. After the first noisy scan-frame of a TempoSTEM acquisition, such as with a trigger of n=1 electron, we can calculate the local variance of image pixels. On the second scan frame, we can revisit only pixels that are outliers by, say, more than one standard deviation of their neighbours. This might be around 25-30% of the pixels for example. For these pixels, an additional TempoSTEM observation can be made and the beam blanked elsewhere to minimise dose. The rescanned pixels have now received the dose equivalent to an n=2 TempoSTEM exit condition, and the scattering rate estimate is updated. On a third scan for example, the number of outlier pixels reduces and even fewer are rescanned to an effective n=3 exit condition. After some number of rescans the variance is converged, and the image acquisition can be considered complete. For an equivalent target resolution a dose saving of around 2x is achieved even relative to the already low-dose TempoSTEM approach. This method pushes below one event per-pixel-per-frame, maximising the information from each electron, increasing the best achievable combination of resolution and beam damage.

Keywords:

Low-dose, event-responsive, STEM, intelligent-scanning

Reference:

1. JJP Peters et al., Nature Communications 14 (2023) 5184
2. BW Reed et al., Mic. & Microanalysis 28 (2022) 2230.
3. JJP Peters et al., Mic.& Microanalysis 29 (2023) 1754.

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Identification of the phases resulting from the thermal crystallization of Ge-rich GeSbTe alloys using EELS

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Poster Group 2

Background incl. aims

Among many phase change materials, Ge-rich GeSbTe (GGST) alloys are of considerable interest due to their high thermal stability, a specification required for the next generation of embedded digital memories. This stability results from the fact that these alloys do not crystallize congruently but experience phase separation and finally form a polycrystalline material in which small grains of Ge and of several GeSbTe (GST) cubic phases coexist [1-3]. As all these phases, which have different chemical compositions, have almost the same lattice parameters, they cannot be differentiated by X-ray diffraction. On the other hand, as the grains are much smaller than the thickness of the lamella, the compositions measured by energy-dispersive X-ray spectroscopy (EDX) or electron energy loss spectroscopy (EELS) in the transmission electron microscope result from the overlapping of many small grains and do not give access to their individual stoichiometry (Figure 1). Herein, we report an efficient method to deduce the stoichiometry of the different phases of the grains which overlap by exploiting “pixel by pixel” EELS data and by extracting their characteristic Sb/Te ratio. Using this method, we show that the crystallization of the GST phase proceeds through the initial formation of cubic GeTe and its progressive enrichment in Sb during thermal annealing, ultimately leading to the formation of the well-known stable GST-225 phase.

Methods

A 100 nm-thick GGST layer was deposited by physical vapor deposition (PVD) onto a naturally oxidized 300 mm Si(100) wafer and covered by a 20 nm-thick TiN cap layer. The samples were annealed in a conventional furnace under nitrogen flux. Cross-sectional specimens were prepared by focus ion beam (FIB) technique operating with a 30 keV Ga ion beam and finally polished and cleaned at 2 keV–3 pA. High-angle annular dark-field (HAADF)-STEM and dual-EELS data were acquired on a probe corrected ARM JEM JEOL 200F microscope, operated at 200 kV. The convergence and collection angles for EELS were 29.6 and 75.9 mrad, respectively. The quantification process was carried out through the following steps: 1) the zero-loss spectrum is used to subtract the background and plural scattering signals caused by the specimen thickness, 2) high-loss elemental peaks are fitted using the Hartree-Slater model, after exclusion of the ELNES edges (about 50eV width) and 3), the Ge, Sb and Te ratios are normalized and the result obtained at each pixel is plotted in a ternary diagram for visualization.

Results

Figure 2 shows HAADF STEM images and corresponding plotted Sb/Te ratio for GGST samples, annealed at different temperatures and time durations. After 320°C/8h annealing, when the first GeSbTe cubic phase forms, the compositional data points spread along an almost horizontal line (Sb/Te = 0.05). The alignment of the pixels on such a horizontal line indicates that the compositions

which are measured result from the superposition of pure Ge and almost pure GeTe grains in varying proportions, depending on the overall composition of the layer and statistical fluctuations. The vertical shift of this horizontal line upwards reflects the homogeneous distribution of Sb in the material and should be interpreted as being due to the presence of a third highly dispersed "phase" containing all the Sb. When annealing for longer times or at higher temperatures (320°C/16h, 400°C/30min and 400°C/3h), the Sb/Te ratio characteristic of the GST phase which is formed slowly and progressively increases, from 0.05 (horizontal line) to 0.4, the signature of the well-known stable GST-225 phase. In the meantime, the horizontal shift due to dispersed Sb decreases. This shows that Sb, which was initially diluted in the matrix, progressively incorporates into the GeTe crystals during annealing (by substitution, necessarily on Ge sites). Moreover, some pixels point toward Sb-rich GST phases. Complementary TEM analysis shows these phases have the hexagonal structure, the most common being Sb2Te3. Pure Sb2 lamellas can also be identified by HAADF STEM imaging and EELS mapping, as shown in Figure 1.

Conclusions

We have thoroughly investigated the phases resulting from the thermal crystallization a GGST alloy using advanced transmission electron microscopy based techniques. While X-ray diffraction cannot differentiate the various phases found in the polycrystalline material due to their “close” lattice parameters, the careful analysis of the Sb/Te ratio obtained from pixelated EELS data gives access to the stoichiometry of the GST phases in the samples. Based on such analysis, we demonstrate that the thermal crystallization of GGST alloys proceeds through the initial formation of GeTe and Sb-poor GST grains which get progressively enriched in Sb during annealing until forming GST-326 (Sb/Te=0.33) and GST-225 (Sb/Te=0.4) grains. Moreover, excess of Sb and Te can be accommodated within Sb2 lamellas and Sb2Te3 hexagonal grains.

Keywords:

PCMs, GeSbTe, crystallization, EELS, TEM

Reference:

(1) Rahier et al. ACS Appl. Electron. Mater. 2022, 4 (6), 2682–2688.
(2) Rahier et al. Physica Rapid Research Ltrs 2023, 17 (8), 2200450.
(3) Luong et al. Physica Rapid Research Ltrs 2024, 2300421.

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Multi-scale electron tomography in liquid state and its application to the study of beam-sensitive nanomaterials

Dr. Louis Marie Lebas[1], Dr Lucian Roiban[1], Dr. Victor Trillaud[1], Dr. Mimoun Aouine[2], Professor Karine Masenelli-Varlot[1]
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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Background incl. aims

Electron tomography is a technique that allows 3D data analysis at the nanometer scale. In its early days, it was performed in high vacuum and only beam-resistant samples were analyzed due to its time-consuming nature. In the last decade, tremendous technical developments have been made to make tilt series acquisition faster and faster, and to make fast electron tomography compatible with environmental electron microscopy. [1,2]
Environmental electron tomography in gas, and even more so in liquid state, is very sensitive to the electron beam, so acquiring fast tilt series in STEM mode with a very low electron dose received by the sample is a real challenge even for the most experienced users. In this presentation the application of the M-SIS software, developed in our group, in the analysis of aluminum hydrogel suspended in liquid will be shown.

Methods

Al(OH)3 is an extremely beam sensitive sample, so care must be taken to avoid beam damage, especially when the sample is in a liquid state. An aqueous suspension of aluminum hydrogel was inserted into the ESEM in the liquid state, then dried and rehydrated in situ. At each hydrated state, a fast tilt series in STEM mode from -70° to +70° was recorded on the region of interest at low electron dose in order to preserve the sample. In addition, ETEM experiments were carried out without encapsulating the sample. A careful control of the sample temperature and the water vapor pressure enabled the 3D study of the hydrated material at nanometer scale and the pore size distribution was measured. The tilt series were recorded using the custom code M-SIS build in Python that is an automatic tool for electron tomography and can be installed on an ESEM and on an ETEM.[1-3]

Results

Three hydration states of Al(OH)3 were investigated in 3D by ESEM. It was found that the global volume of the sample does not change its structure during the dehydration-rehydration cycle. Its change in volume was measured to be about - 3.4 ± 1.3% during dehydration, followed by a + 1.2 ± 1.0% expansion during rehydration. The spatial resolution of the tomograms was about 10 nm and the total electron dose of each tomogram was between 1050 and 1250 e-nm-1.
In ETEM, tilt series could be acquired with 260 frames in 12 min, with a pixel size of 0.6 nm and a total electron dose of 16,000 e-nm-1. No significant change of the sample structure could be detected during acquisition. The pore size distribution in the hydrated state was found to vary from 5 to 30 nm (Figure 1).

Conclusion

Thanks to the development of the M-SIS software, we are now able to study beam-sensitive materials in liquid in 3D. Al(OH)3, an extremely beam-sensitive material, was analyzed in ESEM in 3D in different hydration states. The morphological changes were quantified with a spatial resolution of 10 nm. The pore size distribution in the hydrated sample was quantified with higher resolution in liquid using electron tomography in ETEM.

Figure 1: 3D model of Al(HO)3 in the initial state in liquid, then after dehydration and finally after rehydration. The yellow particles are Au particles used as fiducial model.

Keywords:

In-situ, liquide, beam sensitive, tomography

Reference:

[1] S. Koneti et al, Fast electron tomography: Applications to beam sensitive samples and in situ TEM or operando environmental TEM studies. Materials Characterization 151, 2019.
[2] X. Jiao et al, Electron tomography on latex particles suspended in water using environmental scanning electron microscopy, Micron, 2019, 117.
[3] the Consortium Lyon Saint-Etienne de Microscopie (CLYM) is acknowledged for microscope access, and ANR for funding (project ANR-20-CE92-0014-01).

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In-situ nucleation of silicon particles using environmental transmission electron microscopy

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Background incl. aims

Silicon (Si) particles are excellent candidates for the development of optical metamaterials with high scattering efficiency for applications in anti-counterfeiting, nonlinear nanophotonics and enhanced Raman scattering. An optimized synthesis method that allows the fabrication of Si nanoparticles with resonant properties that depend on their size, crystallinity and density would open the way to the large-scale production of such so-called metamaterials. Today, one of the most promising synthesis methods is the thermal disproportionation of hydrogen silsesquioxane (HSQ), which produces resonant silicon particles with the desired criteria. However, the disproportionation of HSQ occurs at very high temperatures and the particles produced are polydisperse in size, requiring a separation process to obtain a monodisperse particle.
In this work, the nucleation and growth of Si particles from the thermal disproportionation of HSQ and the formation of silicon nanoparticles with diameters up to 70 nm were investigated. Advanced in situ environmental transmission electron microscopy (ETEM) was used to directly observe the formation of Si particles from HSQ to resonant particles at high temperature under 20 mbar of gas pressure.

Methods

In situ experiments were performed using a FEI ETEM Titan 80-300 environmental microscope operating at 300 kV equipped with a OneView camera from Gatan. HSQ grains were crushed in a mortar and then dispersed in 2-propanol, a drop of the highly dilute dispersion was placed on a Si3N4 chip compatible with DENSsolutions Wildfire sample holder then dried. The experiments were performed under a gas pressure of around 20 mbar. The gas employed was argon or a mixture of N2 - H2 (95%-5%). The temperature was raised from RT to 800 °C at a rate of 10 °C/sec with about 2 min of plateau every 100 °C. From 850 °C to 1300 °C, the temperature was increased by step of 50 °C, at a rate of 10 °C/sec with plateaus of about 20 min – 1h at 900 °C, 1000 °C, 1100 °C, 1200 °C and 1300 °C in order to record images and videos of the nucleation and growth processes. Cooling of the sample to RT was carried out by step of 100 °C at a rate of -10 °C/sec.

Results

As expected, the thermal decomposition of HSQ was found to begin at temperatures above 1000°C. Once the Si crystalline nuclei are formed, two main growth processes are observed, including particle-particle coalescence and, more surprisingly, particle displacement through the matrix, leaving traces through the HSQ matrix due to interfacial chemical reactions. At 1000°C, small particle

nuclei of about 1 nm are visible, then with increasing temperature to 1200°C, particles of 7-11 nm are observed. Then these particles begin to coalesce, indicating high mobility within the system, and the particles formed have a larger projected diameter (Figure 1a).
The second growth mechanism observed shows that the particles move through the sample by changing shape and growing up to 70-80 nm (Figure 1b). They show a liquid-like behavior, are very mobile and change shape rapidly. This liquid-like behavior below the typical melting point of silicon is attributed to a beam effect lowering the melting point during in-situ observation.
The in-situ experiments were complemented by in-situ XRD measurements up to 1200 °C, demonstrating a solid-state growth mode below the melting point of Si. [1,2]

Conclusion

This study was conducted under extreme conditions using an ETEM under 20 mbars of gas and at 1300°C and helped to illuminate the growth mechanism of Si nanoparticles from a silicon rich oxide precursor. It was found that crystalline Si domains grow easily in the solid state starting from 1000°C. For the first time, a highly malleable and mobile phase was observed in real time, suggesting an intermediate liquid-like behavior that facilitates particle growth. Growth processes by coalescing and moving within the matrix after the crystallization were identified. This study was complemented by an ex-situ investigation of the role of the reducing atmosphere in the disproportionation reaction.

Figure 1: Si particles formed after thermal decomposition of HSQ grew by two different mechanisms: a) Particles coalescence, the Si particles have high mobility at 1200°C and during the encounter they form larger nanoparticles. b) At 1300°C the particles have liquid like behavior with high mobility moving through the matrix while growing.

Keywords:

In-situ, HSQ hdisproportionation, Si synthesis

Reference:

[1] Cibaka et al. Chem. Mater. 2023, 35, 20, 8551–8560
[2] the Consortium Lyon Saint-Etienne de Microscopie (CLYM) is acknowledged for microscope access, the European Research Council (ERC) under the European Union’sHorizon 2020 research, innovation program (Scatter, Grant agreement no. 948319) and METSA network are acknowledged for the financial support

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Heating effects in Bi-doped Cu nanowires for spintronics: atomic resolution in-situ insights

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Poster Group 2

Background incl. aims
CuBi alloys are predicted to exhibit a giant spin Hall effect (SHE), making them promising materials for developing spintronic devices. This prediction was supported by the direct observation of SHE in Cu95Bi5 films by X-ray spectroscopy. However, the material composition and structure, e.g. crystallinity, crystallite size, effective Bi insertion into the Cu lattice or formation of metallic clusters of Bi, can affect the spin Hall angle, which is related to the efficiency of the spin-to-charge current conversion. This efficiency becomes more and more critical in the case of reduced dimensionality systems, where some dimensions may become smaller than the spin diffusion length. Changes taking place during device operation, such as those related to Joule heating, may also affect the system performance.

Methods
Here, we present a detailed structural characterization of Bi-doped Cu nanowires (NWs) with a high Bi doping level (up to 7% Bi) and different degrees of crystallinity, grown by template-assisted electrochemical deposition. The combination of in-situ atomic resolution scanning transmission electron microscopy (STEM), diffraction, electron energy-loss spectroscopy (EELS) and high-resolution synchrotron powder X-ray diffraction (PXRD), allows studying in detail the atomic structure of the NWs, which is critical for optimization of the spin transport related properties.

Results
Nanodiffraction (4DSTEM) measurements were used to analyze the crystallite size and orientation of the nanowires (see Figure 1(a,b)), allowing to harness the growth procedures in order to achieve a controlled distribution of grain boundaries, with average crystal sizes ranging from tens of nm to the micron scale. In-situ heating experiments, using both STEM and PXRD techniques, were carried out to reproduce operando conditions subject to Joule heating effects. After heating for an interval of several minutes at 400°C, in-situ PXRD measurements show that a temperature induced structural transition takes place. For an initial Bi doping of 6%, diffraction peaks typical of pure Bi appear after heating, pointing to the presence of significant Bi diffusion (Figure 1(c)). EELS chemical quantification reveals that the NWs average doping decreases to 1 at. % of Bi after heating. A decrease in the unit cell lattice parameter can be detected both by PXRD analysis and STEM images, suggesting that Bi is coming out of solution in a thermally activated process. In fact, a pure Bi phase is detected after the annealing, associated with Bi nucleation on external surfaces and also at grain boundaries. Indeed, high angle annular dark field (HAADF) images show the early stages of Bi nucleation, coming out of

solution from the Cu cubic matrix along the edges of the NWs, indicating preferred orientations for Bi segregation during heating (Figure 1(d)). Density-functional theory will be used to explain these processes, which must be taken into account when trying to understand the behavior of these nanosystems under working conditions in future devices exploiting SHE.

Conclusions
Our study explores Bi-doped Cu nanowires grown via electrochemical deposition. Synchrotron and Advanced microscopy techniques reveal structural details critical for spin transport optimization. In-situ heating experiments expose a temperature-induced Bi diffusion, leading to nucleation of a pure Bi phase. This suggests a thermally activated process affecting NW doping and lattice parameters. Understanding these phenomena is crucial for developing efficient spintronic devices driven by the spin Hall effect.

Keywords:

HR-STEM, EELS, in-situ, nanowires, spintronics

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Transfer of Nanomaterials for in-situ TEM with electrical Currents

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Poster Group 1

Background incl. aims
In-situ transmission electron microscopy (TEM) has evolved to be a crucial tool for studies of dynamic processes in a large variety of specimens. While numerous examples exist that use gaseous/liquid environments, heating, biasing or the application of a mechanical force, studies in which the electrical current (and not bias) has been used as external stimulus are scarce. [1,2] A possible reason is the necessary controlled contacting of the specimen, which is challenging, especially for nanomaterials that need to be kept free from any source of damage and contamination. Here we present a preparation technique that allows a clean and almost damage-free preparation to focus on the materials during the application of electrical currents inside the electron microscope. [3]
Methods
The key element of the process is a holey silicon nitride membrane, which supports the nanomaterial during the transfer with the focused ion beam (FIB) technique using Ga+ ions. In brief, the specimen is first prepared conventionally on a TEM grid made of such a holey silicon nitride membrane (PELCO). In a TEM analysis, a suitable individual nanomaterial can be selected, as exemplarily shown in Figure A for a nanotube (NT) and a flake made of the misfit-layered compound LaS-TaS_2. [4] The grid is then loaded into a dual-beam (SEM-FIB) instrument and the nanomaterial with the supporting membrane is transferred to a microchip using a microneedle and FIB induced deposition (FIBID) of Pt/C (Figures B and C). The properties of the silicon nitride facilitate easy contacting of the membrane to the contact pads of the chip by FIBID. Finally, the membrane is removed in the central part to leave the nanomaterial as only conductive bridge.
Results
Using the above-described transfer technique, we have prepared a range of different devices based on nanomaterials and evaluated in detail the transfer quality. Figure D shows a HRTEM image of the transferred LaS-TaS_2 (NT and flake) sample revealing no contamination or charging. The limits of the methods were tested with a monolayer of WS_2, which was successfully transferred and revealed that Pt/C contamination during FIBID is kept well below a monolayer. For 1D nanomaterials, Ga implantation can be kept to zero, while for 2D materials or thin films, a small amount of Ga is implanted at the edge of the specimen in the final step of removing the membrane in the central part. We will present exemplary experiments conducted on different prepared devices.
Conclusion
The development of a support-based transfer and contacting procedure of individual nanomaterials to microchips, e.g. used for in-situ TEM investigations, allows an almost artifact-free preparation of nanomaterial specimens for electrical in-situ TEM. Suitable nanomaterials can be selected by previous TEM analysis and correlative microscopy techniques. The resulting devices can be used to electrically characterize the nanomaterials and to dynamically study the effect of an electrical current on the sample. This includes Joule heating, which can be used to heat samples to extremely high temperatures, not reachable by standard heating in-situ TEM studies. Further possible investigations

concern electromigration, electrical failure analysis or EBIC. In addition to in-situ TEM, the preparation method can equally be used to prepare devices on related microchips.

Acknowledgments
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Figure caption:
(A) SEM image of LaS-TaS_2 nanotube and flake. Inset shows a low-magnification TEM image of identical sample position. (B) SEM image of micro-needle holding membrane and nanomaterials after contacting and cutting free. (C) SEM image after milling of membrane in hole area of the chip with nanomaterials forming the only bridge between contacts. (D) HRTEM image of a flake and a nanotube (shown in (C)) after transfer. Scale bars are (A) 2 µm, (B,C) 5 µm, and (D) 5 nm.

Keywords:

in-situ-TEM, nanomaterials, sample preparation, focused-ion-beam

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Zernike Phase Plates for aberration-corrected TEM in Material Science

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Poster Group 2

Background incl. aims
Aberration correctors are tools to manipulate the phase of the electron wave, which have strongly increased the resolution and the impact of transmission electron microscopy (TEM) studies in multiple scientific fields. However, the phase cannot be adjusted in a completely random way by such correctors. Specifically, although the aberrations can be corrected for a large range of spatial frequencies allowing to resolve almost all crystal lattices, the overall phase contrast in acquired images is low. In fact, for perfect aberration correction, phase-contrast transfer and thus phase contrast in the image would be zero. Physical phase plates (PPs), developed for life science specimens [1], potentially allow to obtain maximum phase contrast if combined with aberration correction [2]. The aim of this investigation [3] was to demonstrate the promising application of PPs in material science.
Methods
For this study, we fabricated a Zernike PP, consisting of a thin amorphous carbon film with a central hole milled by a focused ion beam. Figure (a) shows a sketch of the objective aperture stripe with mounted PP consisting in a conventional Cu TEM grid (SEM image in (a)), which was implemented in an aberration-corrected (image) transmission electron microscope (Titan3, Thermo Fisher Scientific). Figure (b) shows a SEM image of one of the grid frames showing the carbon film with central hole. The PP was applied to different nanomaterial samples, for example to Fe_3O_4 nanoparticles [4].
Results
Figure (c) shows an example TEM image of Fe_3O_4 nanoparticles at the edge of an amorphous carbon film acquired with PP. While the lattice-fringe contrast of the nanoparticles is clearly resolved, the PP causes a strong phase-contrast transfer also for intermediate spatial frequencies making the carbon film and the nanoparticle morphology visible. The power spectrum of the TEM image (d) shows that the phase contrast is increased for spatial frequencies larger than the cut-on frequency corresponding to the radius of the hole. The power spectrum also reveals the reflections linked to the crystalline nanoparticles. The application of the unheated Zernike PP is limited to thin samples with small crystalline structures as the PPs charges if hit by a diffracted beam with increased intensity [3].
Conclusion
The experimental results obtained with an unheated Zernike PP clearly prove the benefits of applying a PP for high-resolution TEM studies in material sciences allowing to image features at the atomic and nanometric scale at the same time [3]. The combination of aberration correction TEM with PPs is especially promising as it potentially allows to perform a wave-function reconstruction with only two images, containing pure amplitude and phase contrast, respectively. Further technological improvement, such as the implementation of a PP heating device to avoid charging, or the use of electrostatic PPs could overcome the limitations of the unheated Zernike PP used for the presented results.
Acknowledgments

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Figure caption

(a) Sketch of objective aperture stripe with objective apertures and a Zernike PP. (b) SEM image of a frame of the PP grid. (c) Aberration-corrected TEM image of Fe_3O_4 nanoparticles acquired with PP and (d) corresponding power spectrum. Scale bars are (c) 6 nm and (d) 2 1/nm.

Keywords:

TEM, phase-plate, aberration-correction, phase-contrast, nanoparticles

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Enhanced time resolution with a room-temperature energy dispersive X-ray PIN photodiode detector

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Energy dispersive X-ray spectroscopy (EDX) has demonstrated its utility across diverse fields, ranging from materials science to biology, serving as a user-friendly analytical technique in electron microscopy for elemental analysis and mapping of specimens. Detector technology has evolved from liquid nitrogen-cooled lithium-drifted silicon detectors (Si(Li)) to configurations featuring multiple large, tear-drop-shaped thermoelectric-cooled silicon drift detectors (SDDs). The former type allows for high count rates by having a large active area while keeping the anode capacitance low. Incident X-rays are absorbed in the bulk Si region and generate electron-hole pairs. The electrons then drift towards the anode under guidance of an electric field which is established and controlled by several increasingly reverse-biased ring electrodes covering one surface of the detector.
While this method is effective for elemental mapping and offers reasonable acquisition times, the precision of X-ray arrival time is limited by the relatively slow drift speeds and large active area size. X-rays absorbed near the anode are read out significantly faster than those absorbed farther away. This forms a major bottleneck for another promising electron microscopy application, namely EDX and energy electron loss (EELS) coincidence detection. Here the aim is to correlate transmitted electrons and X-rays in time. The coincidence information can greatly enhance the sensitivity for detecting trace elements in a matrix as compared to conventional EELS and EDX. Furthermore, the method allows the determination of the collection efficiencies without the use of a reference sample and can subtract the background signal for EELS and EDX without any prior knowledge of the background shape and without pre-edge fitting region. Another advantage is that the correlation data is revealed while preserving the full EELS and EDX signal without compromise in speed or acquisition time.
To tackle this issue, we are working on a proof-of-concept detector design consisting of a small reverse-biased Si PIN photodiode with a low-noise charge amplifier circuit. Si PIN photodiodes are reported to be suited for X-ray detection and are commercially available at low prices and in small sizes. What truly sets our approach apart is that contrary to the ongoing trend of ever-increasing size of active areas and number of detectors to increase solid collection angle and thus acquisition rates, we ensure sufficient collection by bringing our sensitive area and specimen very close together. The sensitive area can thus remain small, keeping the photodiode's capacitance low enough to allow for high acquisition rates and low noise levels. Moreover, the small size minimizes the temporal broadening as initially aimed for. Regardless of where the X-ray is absorbed, the readout speed in a PIN photodiode will have little spread as the anode stretches over the entire back side of the PIN junction. Besides the fundamental improvement in time resolution, our concept offers numerous practical advantages. It provides a cost-effective method for integrating EDX capabilities into an electron microscope. Additionally, the use of a small PIN photodiode minimizes the necessity for cumbersome cooling, as the limited active area (and thus cross-section of the depletion layer) ensures that dark current (noise) remains limited even at room temperature.
So far several prototypes have been made and tested in a scanning electron microscope (SEM) as this allows for convenient piloting. Over the different iterations the leakage current, signal-to-noise ratio

(SNR) and form factor have been improved. With Cu X-rays the current SNR of the single X-ray pulse signals we are measuring is above 5, with pulses exhibiting sub-50ns rise times (see left side of figure). The entire system consumes less then 750mW of power. We are on the brink of obtaining our first energy spectrum and determining the energy resolution using the XG-lab digital DANTE pulse processor. Currently different photodiodes are being tested and compared as well as ways of preventing the X-rays from landing outside of the depletion layer of the photodiode where they lead to a slow and distorted signal.
In conclusion, we present the development of an in-house build room-temperature PIN photodiode X-ray detector to improve time resolution and allow for advancements in EDX and EELS coincidence experiments that so far have been hampered by the slow drift mechanism in SDD setups.
This work received funding from the Horizon 2020 research and innovation programme (European Union), under grant agreement No 101017720 (FET-Proactive EBEAM)

Keywords:

EDX, coincidence, PIN, charge-sensitive preamplifier

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Development and application of In-situ atomic-scale straining&heating&biasing platform for TEM

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background incl.aims
Due to increasing demands on better performance of materials of various fields working under harsh and complicated conditions and the constant minimization of devices, it is essential to reveal structure-composition-property correlation of materials from atomic-scale under working conditions in real-time. In-situ TEM technology is one of the most promising solutions to the above needs and has advancing rapidly in recent decades. However, it is greatly difficult to simulate working environment involving harsh thermal, mechanical, electrical stimuli, while performing real-time atomic-resolution observation and analysis of materials. For this reason, our group has dedicated to the development of an in-situ atomic-scale straining, heating and biasing system for TEM and has successfully applied the platform to study the atomic-scale response of various materials and revealing the mechanisms underneath.
methods
The multi-functional in situ TEM testing platform was developed mainly based on micro piezoelectric ceramic actuator and dedicated designs of MEMS chips with various heating and/or biasing functionalities. With a unique truss-like design, the heating chip can heat the sample from room temperature to 1200℃ with an accuracy of ±1℃ while allowing straining of the sample at any given temperature. The miniature straining actuator can apply a GPa-level stress, over 4 microns actuation displacement and an actuation resolution of 0.1 nm. The in-situ biasing chip can achieve precise application of electrical signals and pA and μV level measurement. The MEMS chips for heating and biasing and the miniature actuator for straining are fixated onto a rigid frame. A flexible printed circuit (FPC) connects the electrodes on the MEMS chips and the actuator to an outside control unit for the application and measurement of subtle signals. The rigid frame and the FPC comprises a functional cartridge, which can be conveniently connected to a specially designed double-tilt TEM holder.Various kinds of thermal/electrical/mechanical loading of samples can be carried out inside a TEM column. Throughout the entire loading process, the sample can be tilted freely around two orthogonal axes for ±15°. This capability is essential for achieving atomic-scale resolution, which requires precise alignment of the electron beam to a low-index zone axis of the sample during in-situ testing.
Results
With this in-situ platform, we carried out an in situ atomic-resolution study on the sliding-dominant deformation at general tilt grain boundary (GB) in platinum bicrystals. Both atomic-scale sliding along the GB and sliding with atom transfer across the boundary plane were observed directly in real-time.1 For the first time, we uncovered that tungsten fractures at 700℃ in a ductile manner via a strain-induced multi-step body-centered cubic (BCC)-to-face-centered cubic (FCC) transformation and dislocation activities within the strain-induced FCC phase.2 Using this technique, researchers has also

revealed the atomic-scale process of GB dislocation climb in nanostructured Au during in situ straining.3

Conclusion

Based on highly integrated MEMS chips, miniaturized piezo actuator and unique double-tilt system, we have developed an in-situ TEM testing platform that allows real-time observation of micro-structure evolution at atomic scale under flexible coupling of mechanical, thermal and electrical fields. Researches on revealing the atomic scale mechanisms of GB plasticity and brittle-to-ductile transition of various metallic materials were carried out.

Keywords:

In-situ TEM; Atomic-scale; straining; Heating;

Reference:

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Sn Alloying Impact on Structural and Electronic Properties of Core-Shell Ge-GeSn Nanowires: A TEM Study

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PS-03 (2), Lecture Theater 2, august 30, 2024, 10:30 - 12:30

Background incl. aims
Alloying group IV elements in out-of-equilibrium conditions when there is a significantly different atomic size between the atoms of the host lattice (Ge) and the alloying element (Sn) results in peculiar modifications of the structure and electronic properties of the parent phases. Growing alloys in the form of core-shell Ge-GeSn heterostructures adds an additional degree of freedom for strain accommodation. Moreover, such core-shell configuration induces changes in their morphological, chemical, electronic, and optical properties. Investigating these heterostructures, where Ge acts as the core and GeSn as the shell in hexagonally faceted nanowires with a diameter in a range of 80-100 nm and length exceeding tens of µm, poses significant challenges. While there are challenges, these heterostructures offer numerous opportunities for developing silicon-compatible devices with enhanced capabilities in the mid-infrared wavelength range (5-20µm). These capabilities include, among others, CMOS and MOSFET devices, night-vision devices, and lasers [1], owing to their advanced band-to-band transitions and enhanced carrier mobility. Consequently, a thorough investigation and precise characterization, at the atomic level, are crucial for these heterostructures, requiring a high degree of sensitivity and high-spatial resolution. The introduction of Sn to Ge, a high carrier mobility element, will undoubtedly have a profound impact on its bandgap characteristics, influencing transitions within the bandgap [2]. We observe and extract information locally and detect features using the Scanning Transmission Electron Microscopy (STEM) technique, employing an extremely narrow electron probe directed at the sample to obtain detailed insights into the structure and chemical composition of these intriguing core-shell structures. The initial Sn content ranges from below 8 at. % to above 14 at. % representing a substantial amount of Sn. It has been predicted that the transition from an indirect to a direct bandgap will occur at approximately 8 at. % Sn [3], a hypothesis we aim to experimentally confirm and deepen further.

Methods
To study such core-shell heterostructures, we utilize a High-Resolution (HR) STEM with the High-Angle Annular Dark Field (HAADF) detector and Energy-Dispersive X-Ray Spectrometer (EDS). For such purpose we employ the ThermoFisher Scientific Spectra Ultra double-corrected TEM operated at 300 kV with a segmented Ultra-X EDS detector enabling atomic-resolved EDS maps. Additionally, we implement electron energy-loss spectroscopy (EELS) in STEM mode using the Nion HERMES 100 instrument operated at 60 kV, with optimal energy resolution of 4.8 meV for detailed examination of electronic and optical properties.

Results

The results of this investigation unveil a substantial impact of increased Sn content in the core-shell Ge-GeSn nanowires on their structural, electronic, and optical characteristics. The performed EDS analysis validates the distinctive core-shell configuration, highlighting a region depleted of Sn in the core, alongside a discernible degree of its uniformity in the shell (Figure 1a). Additionally, findings show that the notable segregation of Sn adatoms along the hexagonal facets promotes the generation of complex defects and that their complexity is more prominent with the elevated Sn content and incorporated strain. The presence of twinning may contribute to the growth of supplementary structures such as nanobelts (NB) with unique non-hexagonal cross-sectional shapes. Moreover, the coexistence of both diamond cubic (DC) and hexagonal-wurtzite (WZ) structures (Figure 1b) or polytypism, results in notable changes in the polarity of these initially presumed non-polar elements (i.e. we alloy within the same group IV elements), enhancing the complexity and interest in the ordered nature of these structures. The investigation of electronic transitions at a high level of locality with high-energy resolution EELS confirms that the increase of the Sn content from 8 at.% up to 18 at.% leads to a transition from an indirect bandgap of Ge (measured 0.67 eV) to a direct bandgap of GeSn, and a shift from 0.56 eV for 8 at.% Sn to approximately 0.3 eV for 18 at.% Sn (Figure 1c).

Conclusion

In summary, our findings demonstrate that core-shell Ge-GeSn nanowires exhibit a unique morphology and crystalline structure ascribed to the Sn content within the GeSn shell. Furthermore, an in-depth investigation of high-spatial resolution HAADF data unveils that the presence of Sn segregation, occurring with higher Sn concentrations in the shell, significantly impacts the occurrence of intricate defects like twinning. As evidenced by the EELS data, the transition of the bandgap from indirect to direct concomitant with a reduced value (measuring below 0.67 eV for Ge), was confirmed with higher Sn concentrations surpassing approximately 8 at.%.

Keywords:

GeSn core-shell nanowires, bandgap, STEM/EELS

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Cryo-ET Structures of Huntingtin-Actin Complexes Reveal that Huntingtin Organizes Actin Cytoskeleton in Neurons

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Poster Group 1

Huntingtin (HTT) is a causative protein for Huntington's disease (HD) and facilitates intracellular dynamics along the cytoskeleton network. Here, we identify that HTT directly binds to actin filament, inducing a bundling. Furthermore, we determined the sub-atomic resolution structures of Q23 HTT complexes with actin filament via cryo-electron tomography (cryo-ET) followed by subtomogram averaging (STA), revealing that N-HEAT and Bridge domains in HTT wrap around actin-filament, concomitantly, C-HEAT domain being displaced away. In addition, we show that HTT depletion leads to a defect in the axonal growth cone with abnormal cytoskeletal organization. Our data demonstrates that HTT directly interacts with actin-filament and is a dynamic modular scaffold protein organizing cytoskeleton.

Keywords:

cryo-ET, Huntington's disease, actin, complex

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Ultrastructural investigation of human cytomegalovirus tegument protein UL71 and its role in secondary envelopment

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background
The molecular mechanisms underlying the assembly of infectious virions of human cytomegalovirus (HCMV), an important human pathogen associated with complications in immunocompromised patients and the leading cause of birth defects after congenital infection, are still not well understood (Griffith et al., 2015). An important step of HCMV morphogenesis en route to infectious virions is a cytoplasmic envelopment process, referred to as secondary envelopment. Tegument protein pUL71, which is highly conserved among herpesviruses, is known to play an important role in the envelopment process (Schauflinger et al., 2011). Several functional motifs, mainly located in the conserved N-terminus of pUL71, have been characterized in detail (Read et al., 2019; Metzner, 2022), whereas the role of the non-conserved C terminus of pUL71 is mainly unclear. However, a tetralysine motif in the C terminal region of pUL71 has revealed a mechanistic involvement of this region in HCMV morphogenesis (Read et al., 2019). Dissecting the functions of pUL71 during secondary envelopment brings us further to unravel the molecular mechanism of secondary envelopment and could lead to the identification of new antiviral targets.
Methods
The role of the C-terminus of pUL71 was investigated by generation and characterization of a mutant virus (TB71insHA-299) expressing a truncated version of pUL71 fused to an HA epitope lacking the last 61 amino acids with the tetralysine motif and the MIM2 domain. A first phenotypic characterization of TB71insHA-299 after infection of human fibroblasts was performed by immunofluorescence microscopy (IF). For direct visualization of secondary envelopment, transmission electron microscopy (TEM) was carried out on human fibroblasts infected with TB71insHA 299 that were fixated by high-pressure freezing and freeze substitution at five days post infection and embedded in epoxy resin. Stages of secondary envelopment were analysed on ultrathin sections by TEM and compared with cells that were infected with wild-type virus or a previously published virus mutant carrying a mutation in the C-terminal tetralysine motif of pUL71 (Read et al., 2019). In addition, 3D analysis was performed on these samples by focused ion beam scanning electron microscopy (FIB-SEM) and scanning transmission electron microscopy (STEM) tomography (Villinger et al., 2014).
Results
In IF experiments, TB71insHA-299 infected cells showed an accumulation of viral particles at the periphery of the cytoplasmic viral assembly complex (cVAC) at large circular structures which is similar to a mutant lacking pUL71. Quantification of secondary envelopment stages by EM analysis of TB71insHA-299 infected cells revealed an impaired secondary envelopment compared to the wild-type virus but no further defect compared to the well characterized virus with a mutation in the

tetralysine motif at the C terminus of pUL71 (Read et al., 2019). Furthermore, it was shown that the accumulation of viral particles at the periphery of the cVAC at large circular structures in TB71insHA 299 infected cells were multivesicular bodies (MVB) with viral capsids attached to the whole cytoplasmic face of the MVB membrane. Additionally, EM revealed abnormal budding of capsids at multiple budding sites (MBS). These ultrastructural differences and the impaired secondary envelopment are similar to a virus that is unable to express pUL71 (Schauflinger et al., 2011). In the virus with the mutated tetralysine motif, these ultrastructural differences could not be observed (Read et al., 2019).
Conclusion
In conclusion, the last 61 C terminal amino acids of pUL71, containing the tetralysine motif and especially the MIM2 domain of pUL71, seem to be of great importance for HCMV morphogenesis. This work demonstrates that additionally to the already characterized secondary envelopment defect caused by the mutation of the tetralysine motif, the other amino acids, most likely the MIM2 domain, carry additional functions for the formation of an intact cVAC and efficient secondary envelopment. This data argues for the controversially discussed involvement of the ESCRT machinery in HCMV morphogenesis and membrane remodelling.

Keywords:

HCMV, High-pressure freezing, virus morphogenesis

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Strain mapping and simulation of transistor structures in a 22nm FDSOI technology

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Poster Group 2

Background & aims
With the advent of the AI/ML and 5G/Satellite internet era, modern electronic devices need to be operated with low energy consumption, high performance, radiation hardness and manufactured at low cost. One of the technologies is 22FDX offered by GlobalFoundries® which utilizes a fully depleted silicon-on-insulator (FDSOI) technique. Speed and power consumption of devices are improved accordingly. Introducing mechanical strain to transistor channels e.g. by epitaxial growth or process-induced pre-stressed overlayers or periphery layouts alters the crystal lattice and thus also the band structure of the semiconducting channel. As different requirements of strain for N- or P-type transistors e.g., along the direction of the channel, the characterization of strain in electronic devices is necessary, both for monitoring the intended engineered strain but also the unintended strain states. However, for verification as well as enhancing strain, FEM Simulation of Strain is crucial. The second part of this study focuses on the simulation of transistors. A detailed model is developed that includes mechanical and structural parameters. [1, 3]
Methods
Precession Electron Diffraction (PED) is a specialized technique in Transmission Electron Microscopy (TEM) employed to gather electron diffraction patterns while scanning across the designated region of interest. During the scanning process, the electron beam rotates around the central axis, while the beam itself is tilted at a specific angle. This results in a quasi-kinematical diffraction pattern, which facilitates the use of a more sophisticated algorithm for crystal structure determination. The strain simulation, based on the finite element method, started with a basic structural model and mechanical parameters. After validating this fundamental model with corresponding structures at the transistor size level, increasingly complex models were developed and subsequently verified. [2, 4]
Results
In this study, strain maps and graphs will be presented obtained from both experimental data using PED and simulated data from various transistor devices. These strain maps exhibit excellent agreement with each other as well as with electrical measurements. Furthermore, they enable the verification and identification of new stress elements, highlighting potential areas for improvement by adjusting structural dimensions and addressing distinct stress elements.
Conclusion
This holistic approach—combining simulation and measurement—enables a comprehensive understanding and optimization of strain effects in electronic components. It also promotes the progression of 22FDSOI technologies and beyond. Finally highlighting the Significance of Advanced TEM Techniques in the Semiconductor industry.

Keywords:

Strain, FDSOI, Transistor, Simulation, PED

Reference:

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Revolutionizing Electron Microscopy Through Intuitive Language-Driven Interfaces: The Emergence of the EM CoPilot

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

The field of electron microscopy (EM) has long been constrained by the complexity of its operating systems, which require extensive training and expertise to navigate. Traditional graphical user interfaces (GUIs) provide a layer of abstraction that, while useful, can distance the operator from the most efficient and intuitive interaction with the instrument. This study introduces the EM CoPilot, an innovative application of Large Language Models (LLMs) [1-4]. designed to transform the operation of electron microscopes by enabling control through intuitive language commands. This paradigm shift signifies a new era in microscopy, where accessibility, efficiency, and user-friendliness are paramount.
Methodology involved the development of an LLM capable of understanding and translating natural language commands into specific function calls for electron microscopes. The system supports a wide range of commands. The code was integrated into an EM control system, equipped with voice and text recognition capabilities, supporting multiple languages to cater to a global user base.
Key findings demonstrate that the EM CoPilot significantly reduces the learning curve for new users, while enhancing the operational efficiency and flexibility for seasoned experts. Users can execute complex sequences of operations with simple commands, automate routine tasks, and receive operational guidance, thereby reducing operational errors and increasing throughput. Furthermore, the system's ability to handle complex sequence function calls and integrate basic classical image processing tasks directly through language commands opens new avenues for advanced microscopy techniques.
The implications of this study are profound, signaling a move towards more user-centric approaches in the design and operation of scientific instruments. By bridging the gap between advanced technology and user interface design, the EM CoPilot not only democratizes access to high-level electron microscopy but also sets a precedent for the application of LLMs in scientific instrumentation. The integration of LLMs into EM operation paves the way for future innovations in instrument control, potentially revolutionizing how scientific research is conducted across multiple disciplines.
This abstract encapsulates the essence of the EM CoPilot study, highlighting its innovative approach, methodology, key findings, and the broader implications for the field of electron microscopy and beyond.

Keywords:

EM Automation, Large Language Models

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Structural basis of rotavirus spike proteolytic activation

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Poster Group 2

Rotavirus (RV) is the leading cause of severe gastroenteritis with dehydration in children under 5 years of age and causes ~590 million infections per year in all age groups. Rotavirus infectivity depends on the activation of viral particles by spike proteolysis by trypsin-like proteases. Although it has been described how the entry into the cell is mediated by a conformational change in the spike, the molecular mechanisms underlying this proteolytic activation process are unknown. Using cryoEM we have resolved the structure of the infective particle of group A RV before and after proteolytic activation. Despite the low occupancy and high flexibility of the spike, we have built an atomic model of both spikes by combining various computational methods. The resolved structures show that the conformation of the non-proteolyzed spike is conditioned and limited by the position of the loops that surround its structure and that join the lectin domains that form the head of the spike with its body. The proteolysis of these loops breaks this structural limitation allowing the transformation of the spike to a state competent to make the necessary conformational changes to penetrate the cell membrane.

Keywords:

Rotavirus, cryo-EM, dsRNA-virus, viral entry

Reference:

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Cryo electron tomography of impregnated mesoporous catalyst supports

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Poster Group 2

Background

Mesoporous materials are important for industry and society given their wide-ranging application areas including sorbents, separation, energy storage, biomedicine, or heterogeneous catalysis. Particularly heterogeneous catalysts have played a major role in our fossil-based society but they are also facilitating the transition towards a sustainable chemical industry based on renewable feedstocks and energy. One of the most commonly used methods to prepare a supported heterogeneous catalyst is incipient wetness impregnation (IWI). In IWI, the mesopores of the support material (e.g. silica, titania and alumina) are filled with a metal-salt solution that matches the volume of available pore space. Subsequently, the impregnated material is dried, calcined, and reduced yielding supported metallic catalyst particles within the mesopore network of the support. However, fundamental aspects of IWI such as pore filling, solute-support interactions, solid precipitation and/or (re-)deposition are still not fully understood. Furthermore, the corrugated surface and typically disordered mesopore structure of supports, both before, during, and after liquid impregnation have remained unexplored so far.

Methods

We employed cryogenic electron tomography (cryo-ET) to quantitatively assess the 3D structure of wet impregnated and freeze-dried disordered mesoporous supports. To this end, alumina extrudates of a few mm in diameter and length were crushed into small particles and IWI was performed with either water or aqueous solutions containing different molarities of Mo and Ni. Impregnated support particles were deposited on TEM grids and vitrified in liquid nitrogen. In addition, to study the solid deposition upon drying, impregnated support particles were freeze-dried by equilibrating the TEM to room temperature overnight. To capture the liquid-filled and freeze-dried state of a particle in 3D, tomographic tilt series were acquired under cryogenic conditions. A low-dose acquisition scheme combined with a direct electron detector (Falcon 4i) were utilized to minimize electron beam induced damage to the samples. Tilt series alignment and reconstructions were performed in IMOD or Inspect3D. Image processing, segmentation and quantitative analysis were carried out using a combination of Ilastik, MATLAB and AMIRA3D. We quantitatively evaluated the pore size distribution (PSD), specific surface area (SSA), pore volume (PV), pore tortuosity, surface shape index, and surface curvature to explore the change in pore structure and support surface induced by impregnation and solid deposition after drying.

Results

The degree of mesopore filling by a liquid was assessed from tilt series acquired with a cumulative electron dose ranging from 200 to 300 e-/Å2 which prevents beam-induced damage such as liquid bubbling. Numerical slices extracted from the 3D reconstructed volume show good contrast between water and the alumina support structure. However, the contrast decreases with increasing concentration of metal components in the solution (i.e., 1.3 M, 3.0 M, 5.1 M, 8.1 M of Ni andMo). The PSD, SSA, and PV derived from these cryo-ET measurements were close to the bulk values, as determined by N2 physisorption. The additional morphological information uniquely provided by cryo-ET are pore tortuosity, surface shape index, and surface curvature, which were comparable for all samples. However, the sample with the highest molality was an outlier, which we attribute to the much-reduced contrast between alumina and the impregnation solution, hampering precise segmentation.

To study solid deposition during the drying step, an impregnated support particle (5.1 M precursor solution) was 3D imaged in the wet state and after freeze drying. The sufficient contrast between the solution and the support in the wet state allows observing the removal of water after freeze drying. The surface of the dried impregnated particle is covered with precipitated solids, substantially impacting the morphology of some of the pores, while other pores remain unaffected. Due to the low contrast between the precipitated solids and the support, it is not possible to measure the thickness of the deposited layer. Nevertheless, the darker regions at the outer rim of the freeze-dried support particle suggest preferential solid deposition in the outer regions of the impregnated and dried particle. In this state, cryo-ET-derived PSD, SSA, and PV values were lower than those of the bare support. The less tortuous pore structure together with the decrease of the average solid surface shape index, corresponding to an average surface change from ridge-like to saddle-like, suggests preferential deposition in pockets with initially a high negative curvature.

Conclusion

We employed a cryo-ET workflow to study the impregnation and drying steps of IWI performed on disordered catalyst support particles. To this end, wet impregnated support particles were successfully vitrified and imaged in 3D under cryogenic conditions. Good contrast was obtained between liquids containing up to 5.1 M metal (Ni and Mo) precursor and the alumina support, demonstrating the potential of this cryo-ET approach to investigate different liquid-solid systems. Furthermore, solid deposition was evaluated by quantification of the pore network and particle surface before and after freeze drying. Although it was not possible to directly distinguish the deposited solids from the support particles after drying, the deposited solids affected the pore size and particle surface as probed by quantitative analysis of cryo-ET reconstructions. In conclusion, this study shows the potential of cryo-ET to explore the liquid impregnation and/or drying processes across a diverse range of mesoporous materials applications.

Keywords:

Cryo-ET
Quantitative analysis
Catalysts
Mesopores

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Structure-activity relationship of Pt nanoparticles during the CO oxidation reaction

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Poster Group 2

Background incl. aims
The coupling between catalytic activity and the structure of supported nanoparticles under reaction conditions has been a significant driver for the development of in situ and operando characterization techniques in catalytic research. Traditional photon-techniques provide spatially averaged structural information of nanoparticle ensembles in miniaturized reactors, with conversion of reactants measured at the reactor exit by use of e.g. Mass Spectrometry (MS). To improve the spatial resolution of local structure and catalytic function of nanoparticle ensembles it is, however, beneficial using miniaturized reactors in conjunction with Transmission Electron Microscopy (TEM) [1,2,3,4].

Methods
Herein we focus on operando electron microscopy based on a nanoreactor. The nanoreactor is a Micro Electro-Mechanical System (MEMS) device equipped with a unidirectional and micrometer-sized gas channel, including a heating element, which facilitates MS of the exhaust gas from the nanoreactor as well as reaction calorimetry. With an array of electron transparent windows, the nanoreactor permits atomic-resolution TEM imaging of the nanoparticles under ambient pressure levels at different positions along the heated reactor zone [1,2,3]. Previously, we demonstrated how this system offered unprecedented insight into the oscillatory CO oxidation reaction catalyzed by an ensemble of Pt nanoparticles at ambient pressure levels by correlating high-resolution TEM of individual nanoparticles with global MS and calorimetry data. Moreover, a time-dependent first-principal reactor model was established that suggested marked gradients in the reactants along the gas flow channel under conditions of finite conversion levels.

Results
Herein, we will examine experimentally such gradients in the reaction environments by means of Electron Energy-Loss Spectroscopy (EELS). EELS offers sensitive detection of lighter elements in the gas phase with a special resolution defined by the area of illumination with the electron beam. EELS has therefore previously been used investigate gradients in temperature of heating devices [3] and gas composition[4]. Here we employ EELS for the first time to a gas flow reactor. By recording EELS at the Carbon K ionization edge, we examine the CO conversion profile along the nanoreactor and compare with mass-flow calculations through the nanoreactor and we relate the local structure and conversion to resolve spatially dependent structure-activity relationship during the Pt-catalyzed CO oxidation reaction.

Conclusion
In perspective, this operando electron microscopy and spectroscopy approach sets apart from the vast number of operando photon-techniques by the ability to relate nanoparticle structures with

their catalytic activity, selectivity, and stability. However, such analyses take the reactor and mass-flow conditions into account conditions in order to develop chemical meaningful insight.

Keywords:

TEM, EELS, in-situ, Operando, Nanoreactor

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Advanced imaging reveals new lipid droplets dynamics in the malaria parasite Plasmodium falciparum

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Introduction
Plasmodium falciparum, the deadliest species of malaria parasites infecting humans, has a complex life cycle which requires transition between two intrinsically different hosts (human and mosquito). Hence, the parasite faces the challenges of having to navigate growth, proliferation, transmission, and sexual reproduction in vastly different host environments. Each of these cellular processes rely on coordinated lipid access and metabolism mechanisms, with the parasite either synthesising essential lipids de novo or acquiring them from its host environment in order to propagate. Lipid droplets (LDs) are organelles central to lipid and energy homeostasis across all eukaryotes but their roles and importance in P. falciparum are poorly understood.
We therefore set out to characterise the size, composition and dynamics of LDs across the disease causing intraerythrocytic stages of the parasite life cycle.

Methods
We applied a combination of advanced light microscopy techniques (split fluorescence emission analysis) and volume imaging using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) in combination with inhibitors of essential enzymes in neutral lipid metabolism to characterise the dynamics and importance of LDs in the parasite's asexual life cycle stages.

Results
We observed significant LD changes in late schizont stages, suggesting a switch from lipid accumulation to lipid utilisation that precedes parasite egress from the host erythrocyte. We will furthermore report on observed connections between LDs and several other organelles, pointing to potential functional interactions. Employing inhibitors that specifically interfere with the synthesis or break-down of triacylglyerols (TAG), we found that LD function is essential for schizogony and counteracts lipid toxicity.

Conclusions
Our study of LDs across the asexual life cycle grants new insights into the dynamics of lipid synthesis, storage and utilisation in P. falciparum, which might provide a novel avenue into new intervention strategies to combat this devastating disease.

Keywords:

malaria, lipid droplets, 3D FIB-SEM

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Atom-counting for heterogeneous nanostructures using multimodal STEM

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Poster Group 1

Background incl. aims

To understand the structure-property relationship of nanostructures, reliably quantifying parameters, such as the number of atoms along the projection direction, is important. Advanced statistical methodologies made it possible to count the number of atoms for monoatomic crystalline nanoparticles from a single annular dark field scanning transmission electron microscopy (ADF STEM) image [1]. For this purpose, the so-called ADF STEM scattering cross-sections (SCSs), which correspond to the total intensity of electrons scattered by a single column, are a useful measure. Those SCSs scale with the number of atoms and the atomic number Z. Because of the presence of this Z-contrast, for mixed columns, different elements will contribute differently to the SCSs thus significantly complicating a quantitative interpretation. Progress was made to extend atom-counting from homogeneous to heterogeneous nanostructures by combining ADF STEM with energy dispersive X-ray (EDX) spectroscopy [2]. Although this method can be used to unscramble elements even when the difference in atomic number is only one, EDX measurements may not always be the most viable option for beam-sensitive materials due to the substantial electron dose needed to achieve a sufficiently high signal-to-noise ratio in the EDX elemental maps. As an alternative, we investigated multimode atomic resolution ADF STEM [3]. In this technique, distinct scattering behaviours in different annular detector collection regions will help to unscramble the composition and thickness. Both approaches will be discussed and in addition, the feasibility of novel dose-efficient 4D STEM techniques will be explored for atom-counting, since the simultaneous acquisition of both light and heavy elements remains challenging based on ADF STEM images [4].

Methods

As a first step to count the number of atoms, the atomic columns in the ADF STEM image are modelled by a superposition of Gaussian functions. The volume under each Gaussian corresponds to the scattering cross-section. When counting the number of atoms in heterogeneous nanostructures by combining EDX and ADF STEM, EDX STEM SCSs are also derived by integrating the intensity over the atomic column positions from the noisy elemental maps. Since both ADF STEM and EDX imaging are incoherent techniques, a linear relationship between the EDX and ADF STEM SCSs exists. By exploiting this linear relationship, the experimental SCSs are matched to the simulated SCSs by estimating normalisation constants for the EDX SCSs, using an iterative weighted least squares minimisation. Similarly, with multimode atomic resolution ADF STEM, the nanoparticle SCSs are matched with simulated SCSs by minimising the uniformly weighted sum of squared differences in the SCSs from multiple ADF STEM images. The latter method only considers ADF STEM images, i.e.

images obtained by integrating from regions in the convergent beam electron diffraction (CBED) pattern with angles larger than the convergence angle of the probe. When using the flexibility of the 4D STEM datasets, the electrons scattered at low angles can also be employed, facilitating the quantification of light elements. To this end, first-moment STEM images, which measure the centre of mass (COM) position from the CBED pattern are quantified by modelling the COMx(y) image as a superposition of 2D Lorentzian derivatives.

Results

The combination of EDX and ADF STEM images to count atoms is applied to experimental EDX and ADF STEM images of an Au@Ag core-shell nanorod. For this purpose, the EDX mapping was done for 60 minutes, with the results stored every 5 minutes followed by the acquisition of a calibrated ADF STEM image. This resulted in a times series of 12 EDX Ag and Au elemental maps and 12 ADF STEM images. The counting results for both types of elements agree well with the expected results for a symmetrical Au@Ag core-shell nanorod. The average error on the number of atoms in a column equals ±5.6, ±2.5, and ±3.2 atoms for respectively the number of Ag, Au and total number of atoms. With this methodology we also explored the possibility of characterising a simulated Au@Pt nanorod, with adjacent atomic numbers. Those analyses quantitatively demonstrate the opportunities to count the number of atoms corresponding to each specific element, even when the difference in atomic number is only one.
As a more dose-efficient method to reveal the thickness and composition information, we counted the number of atoms of a simulated Au@Ag core-shell nanoparticle based on multiple STEM images originating from non-overlapping detector regions retrieved from a 4D STEM dataset. When using a set of SCSs obtained from 3 independent ADF STEM images, the average root mean squared error for the atom-counting results is ±5.4, ±2.2, and ±3.5 atoms for respectively the number of Ag, Au, and total number of atoms. This is the same order of magnitude as for the EDX analysis, although the incident electron dose was much lower, i.e. 10^5 e/Å² in a single frame, whereas for the EDX experiment the incident electron dose per frame was approximately $4x10^4$ e/Å² and $4x10^6$ e/Å² for ADF and EDX respectively, clearly highlighting the dose-efficiency of multimode ADF STEM.
For light elements and COMx(y) imaging, the possibilities for atom-counting are explored using a simulation of an aluminium crystal. By fitting the 2D Lorentzian derivatives, the integrated intensities of the atomic columns as a function of thickness are calculated. A monotonic increase up to a thickness of 30 atoms is observed, suggesting that the COMx(y) images can be used for atom-counting.

Conclusions

In conclusion, different multimodal strategies are presented to count the number of atoms in heterogeneous nanocrystals. When combining EDX and ADF STEM imaging the number of atoms can be counted even for elements with adjacent atomic numbers, albeit at the expense of high incident electron doses. The analysis of images obtained from processed 4D STEM datasets will be more dose efficient. Given the promising results, it is a viable alternative for atom-counting of beam-sensitive materials.

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Keywords:

atom-counting, multimodal STEM, heterogeneous materials

Reference:

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Microfabricated Sample Carrier for Cryogenic Electron Microscopy

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

Cryo-electron microscopy (CryoEM) has been proven as invaluable tool for small biomolecule structure determination ranging from proteins to viruses and DNA. Nevertheless. since the introduction of TEM and cryoEM the sample carrier (grid) has remained largely unchanged. Meanwhile microfabrication has made leaps due to the semiconductor industry. In this abstract we present a silicon-based sample carrier made with microfabrication techniques.

Current sample carriers consist of grid made by soft metals typically copper or gold and covered by a holey carbon film or lately higher end carriers are covered by a gold film[1] . Despite their desirable high thermal conductivity, soft metals carriers are very easy to bend, and require gentle handling . Clipping the grid (i.e. to create AutoGrids) is required to be compatible with autoloaders. In addition, due to their low robustness, their surface is typically wavy (not flat), making sample preparation far from ideal, especially for applications such as cryo-tomography. The carbon film transfer typically happens on an individual carrier level, resulting in surface inconsistencies between carriers even from the same fabrication batch. Furthermore, any process in the individual carrier level lengthens the fabrication process and hence the lead times.

Our proposed carriers use nanotechnology materials and, via the introduction of microfabrication techniques, we aim to improve all the aforementioned limitations of the current carriers. Our microfabricated devices consist of a silicon frame, holey silicon nitride membrane and a monolayer Graphene layer (Figure 1). Silicon offers a rigid and robust frame, making the handling and sample preparation much more forgiving. Our carriers can be clipped in the standard AutoGrid following the same process and tools as the current grids, but our microfabrication process enables the possibility to produce carriers with the geometry of an assembled AutoGrid, which would help to skip the tedious process of clipping. By meticulously controlling the deposition of silicon nitride on the silicon substrate, we produce a highly reproducible, atomically flat surface. In addition, microfabrication allows precise control material properties such as internal stress that can improve robustness. A large amount of literature reports the advantages of graphene for cryoEM [2-5], ranging from eliminating one air water interface to enabling affinity grids and controlling ice thickness via graphene hydrophilicity treatment. In order to guarantee the scalability of our production, we have developed the processes to introduce graphene on the wafer level, ensuring consistent quality and surface properties for carriers from the same batch.

Initial characterization of the carriers has been performed, with more extensive experimental results expected in the coming months. The first results are very promising. The Graphene coverage, which depends on the hole size, showed a coverage of 66% for 6µm holes, 85% for 3.5µm holes and 96% for 2µm holes. We have confirmed the compatibility of our chips with different vitrification methods (i.e. plunge freezing and jetting). The acquired ice quality results, obtained with an unoptimized protocol using a Vitrobot, indicate amorphous ice of a uniform thickness of approximately 40nm (Figure 2). Several methods for the hydrophilization of graphene have been investigated depending on the users equipment, ranging from O2 & H2 plasma and glow discharge, to annealing and chemical modification of graphene.

We believe that silicon-based sample carriers are the logical next step to enhance and advance the capabilities of cryoEM. Leveraging microfabrication technologies provides us with

unprecedented possibilities to customize the layout/geometry of the chip, allowing us to meet the experiment´s specific requirements precisely.

Keywords:

CryoEM, graphene, Silicon based carriers

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Understanding phase and chemical transitions in Ge-rich GeSbTe based phase change memory: a (S)TEM tribute

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Phase change memory (PCM) has demonstrated potentials to become the mainstream of the non-volatile memory technique at 28 nm node and below and is promising for applications in future computing hierarchy [1]. The data storage of PCM relies on the reversible phase transition of its active material, which exhibits distinct electrical resistances between crystalline (high conductivity, logic “1”) and amorphous (low conductivity, logic “0”) states.
To meet high-temperature data retention requirements, compositional optimized Ge-rich GeSbTe (GGST) alloys are used by the industry for fabricating PCM for embedded applications [2]. While being the key for the improved thermal stability, GGST alloys undergo chemical phase separation during thermal annealing [3]. The crystallized GGST has been found to be a “composite” material, resulting from the formation of multiple crystalline phases, such as GeTe, Ge, GST-225, and Sb2, at different crystallization stages [4].
In PCM cells, GGST alloys experience different kinds of thermal cycles after deposition. In particular, to allow for the switching between 2 logic states, the GGST layer is locally heated by Joule heating generated by applied current pulses, which provide adequate temperatures either to melt-quench or to crystallize it. Owning to the off-stoichiometry nature of the as-deposited GGST, these heating processes may result in different microstructures and elemental distributions within the cell.
While understanding the evolution of GGST materials is critical for building up the physics behind the functionality of such devices, experimental observations are challenging due to the confinement of the active material to the nanometer scale in integrated PCM. We have thus worked at developing a methodology combining various (scanning) transmission electron microscopy ((S)TEM) techniques to identify grains of different phases that define the cells’ microstructure and the structural and chemical transitions that affect them during electrical programming.
Methods
The studied devices were integrated PCM with a “wall architecture”. The cell consists of a GGST active layer of a few tens of nanometers thick, which is deposited on top of the heater and encapsulated by a metallic top electrode. Extremely thin (<30 nm) TEM lamellas were prepared using focused-ion beam (FIB) from cells after electrical programming. The active region of PCM is a dome which can be amorphous (RESET state, see Fig. a) or crystalline.
TEM samples were characterized by various (S)TEM-based techniques: dark-field (DF) and high-resolution (HR) imaging to access crystallographic information, and high-angle annular dark-field (HAADF) imaging and electron-energy loss spectroscopy (EELS) for chemical mapping. In-situ TEM heating was conducted on cells in RESET state under DF imaging conditions using a furnace-type holder. Results were cross-compared with samples after ex-situ baking.
Results
Fig. b shows a typical (S)TEM analysis of the GGST cell for a region near the heater. The cell was programmed by a forming pulse, which is used to activate the material by melting it followed by slow cooling during which the melt recrystallizes [5]. The STEM HAADF image and associated EELS maps

show chemical segregation near the heater: the material is separated into an outer Ge-rich region surrounding a core made of a Sb-rich (left) and a Te-rich (right) regions. TEM HR images of 2 regions in the core show quite similar crystalline lattices. However, when compared using geometric phase analysis (GPA), the corresponding "strain" map indicates the lattice spacing in the Sb-rich region is ~4% larger than found in the Te-rich region. A clear interface is seen which matches with the boundary evidenced in chemical maps. In the SAED pattern, diffraction spots from 2 sets of slightly different lattices are found and show a good match with rhombohedral Sb (ZA 44$\bar{1}$) and face-center-cubic GST (ZA 111), respectively. By selecting the spot arising from one or the other, DF images of Sb and GST grains can be obtained separately. This demonstrates our ability to identify and distinguish main crystalline phases (Ge, GST, and Sb2) in GGST cells using combined chemical and crystallographic analysis.

Fig. c shows a GGST cell after being programmed by a melt-quench pulse (RESET). An amorphous dome can be evidenced in the BF and DF images. Beyond that, composite "BF & DF" images can be generated by superimposing the BF and several different DF images of either the Ge or the GST phase. A very peculiar characteristic of the polycrystalline region surrounding the dome is evidenced by DF images and the EELS map: Ge crystals preferentially accumulate at the lateral sides of the dome while GST grains are mainly located above it.

The thermal stability of PCM, notably of their RESET state, is a key factor for device reliability. Fig. d shows a GGST cell programmed to the RESET state and subjected to annealing in-situ in the TEM. DF imaging shows a GST crystal located above the amorphous dome. When heating to temperatures at which RESET resistance decreases (250 to 300 ˚C), DF images show that this grain progressively regrows by epitaxy into the amorphous region until it occupies almost the entire dome. EELS maps of the RESET state and after partial-crystallization (by ex-situ annealing) show that while the amorphous dome is initially chemically homogeneous, phase separation is observed after recrystallization (Fig. c and d): Sb segregates inside the crystallized region while the remaining amorphous material becomes more enriched in Ge.

Conclusion

In this work, we have set up a (S)TEM-based methodology to study complicated phase and chemical transitions that occur when programming GGST-based PCM cells. By combining chemical and crystallographic analyses, grain distributions of crystalline phases formed during programming can be revealed. A specific polycrystalline environment, involving two Ge "walls" and a "roof" of GST grains, has been identified. Using in-situ and ex-situ TEM, the thermal recrystallization behavior of the amorphous dome has been revealed: the growth of GST crystals from top of the dome is the mechanism dominating the crystallization. Further chemical analysis has evidenced the phase separation inside the dome, which limits the crystallization process.

Such complete (S)TEM-based analysis provides important experimental results for setting up physical models describing electrical conduction in PCM cells and to guide the process optimization.

Keywords:

phase-change-memory, chalcogenide, in-situ TEM, crystallization

Reference:

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Mechanism of WS2 nanotube formation revealed by in-situ/ex-situ imaging and cross-sectional sequences

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Poster Group 2

Multiwall WS_2 nanotubes have been synthesized from $W_{18}O_{49}$ nanowhiskers in substantial amounts for over a decade [1]. The established growth model is based on the “surface-inwards” mechanism, whereby the high-temperature reaction with H_2S starts on the nanowhisker surface, and the oxide-to-sulfide conversion progresses inwards until hollow-core multiwall WS_2 nanotubes are obtained [2]. In the present work, an upgraded in-situ SEM µReactor [3] with an H_2S source has been conceived to study the growth mechanism in detail. Ex-situ TEM technique was developed to gain structural insight to the reaction at selected times. Further inspection was done by cross-sectional ex-situ TEM sequences where preselected long $W_{18}O_{49}$ nanowhisker was observed from the (010) direction during the sulfidation reaction as a series of lamellae. Based on these techniques, a hitherto undescribed growth mechanism, named “receding oxide core”, which complements the “surface-inwards” model, is observed and kinetically evaluated. Initially, the nanowhisker is passivated by several WS_2 layers via the surface-inwards reaction. At this point, the diffusion of H_2S through the already existing outer layers becomes exceedingly sluggish, and the surface-inwards reaction is slowed down appreciably. Subsequently, the tungsten suboxide core is anisotropically volatilized within the core close to its tips. The oxide vapors within the core lead to its partial out-diffuse out, partially, forming a cavity that expands with reaction time. Additionally, the oxide vapors react with the internalized H_2S gas, forming fresh WS_2 layers in the cavity of the nascent nanotube. The rate of the receding oxide core mode increases with temperatures above 900 °C. The growth of nanotubes in the atmospheric pressure flow reactor is carried out, as well, showing that the proposed growth model (receding oxide core) is also relevant under regular reaction parameters. The current study comprehensively explains the WS_2 nanotube growth mechanism, combining the known model with contemporary insight.

Keywords:

WS2-nanotube, in-situ, ex-situ, SEM, TEM

Reference:

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Data clustering for chemical and structural analysis of nanostructured films

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Poster Group 2

Background incl. aims
The progresses in data clustering algorithms permit nowadays to perform data analysis relatively fast, nearly on-the-fly. The EELS datasets are suitable 3D data to explore the effectiveness of unsupervised algorithms [1]. The aim is the precise chemical characterization of nanoparticles and thin films, by reducing possible bias from a manual data analysis.

Methods
We present two cases: 1) Spectroscopy (EELS): Cu nanoparticles (NPs) for plasmonic materials [2], 2) Imaging (HRTEM): Al:ZnO thin films (AZO) for new transparent conductive oxides (TCO) based on an amorphous structure [3]. In EELS, we have explored clustering algorithms of machine learning, such has k-means (KM), gaussian mixture models (GMM), and agglomerative clustering (AGC), all supported by principal components analysis (PCA), to explore the classification effectiveness in the latent space of the principal components with the highest variances (a-c). In the first case of Cu NPs, we have followed the formation of an oxide shell (Cu2O or CuO) surrounding a metallic Cu core, which is responsible for the plasmonic behavior and shift of the NPs film, after oxygen treatment in a plasma (d). We have quantified the shell extension and composition from EELS datasets containing O-K and Cu-L_{23} edges from individual NPs. In the second case of AZO films, we quantified the amount of amorphous content in the film, by analyzing a dataset of FFTs patches (~7.5k at 128×128 pixels with 32-pixel step) obtained from single HRTEM images of cross-view AZO (FIB lift-out) grown by RF magnetron sputtering on SiO_2, and by comparison with averaged SAED patterns from plan views (e-g). We have compared a full polycrystalline sample (obtained at 5 mTorr Ar pressure), with one expected to have reduced crystallinity (at 100 mTorr Ar pressure).

Results
With the help of simulated EELS data, have found that at realistic SNR as in the experiments (~1.5 or 3.5 dB in the average spectrum) AGC gives the best results (lowest RMS error) comparable with supervised methods, such as support vector machine (SVM), followed by k-means and GMM (the latter being robust due to the fitted normal probability functions that resembles latent space in an autoencoder). Experimentally, quantified core and shell composition of Cu NPs using clustering, finding progressive Cu(0) oxidation in Cu(I) and then in Cu(II). For the AZO films, we have found a reduction of crystallinity of ~33% from FFTs patches analysis in the sample grown at 100 mTorr Ar pressure.

Conclusion
Principal component analysis and data clustering are relatively fast algorithms to perform data analysis and classification also in electron microscopy and spectroscopy. Together with the recent deep learning algorithms, they may contribute to progressively automate the analysis and experiments, and to reduce biases when different users perform the analyses [4].

Graphic
(a-d) EELS data clustering. a) Synthetic EELS dataset of a Cu core/shell NP on carbon film for data clustering tests. The integrated counts are shown together with the true labels and the results from KM and AGC. b) A single spectrum and the average spectrum from the whole dataset. c) Example of labelling in the latent space of the first 2 PCA components. d) Evolution of metallic Cu NPs grown on SixNy under post-growth oxygen plasma treatment (time in seconds) and followed using data clustering of EELS spectra (insets) and ADF imaging. After an initial Cu(II) shell formation at 30 s, even the core is oxidized to Cu(I) at 60 s, while at 120 s all NPs are oxidized to Cu(II) with only few cores still visible in the ADF image (bright spots).
(e-g) FFTs data clustering. e) HRTEM images from AZO cross-sectional films obtained at different Ar pressures during growth. f) A set of ~7.5k (128×128) FFTs from the HRTEM images were first clustered in 3 classes (insets) and the set of FFTs in the AZO class were analyzed to produce brightness/orientation maps. From the brightness, a reduction of 33% in crystallinity in the 100 mTorr AZO was found. g) Integrated SAED profiles from samples in plan-view, confirming the results from FFTs patch analysis.

Keywords:

Data-Clustering, Machine-Learning, EELS, HRTEM, Nanostructures

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In-situ TEM Investigations of the electrical Breakdown of Nanotubes made of Misfit-Layered Compounds

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Poster Group 2

Background incl. aims
Misfit-layered compounds (MLCs) are complex material systems made up of two different layered materials [1]. The combination of the properties of both layered materials makes MLCs promising for numerous applications, e.g. in thermoelectricity. Due to the inherent asymmetry of MLCs, they tend to bend and form tubular structures. Micro- and nanotubes made of chalcogenide-based MLCs have been synthesized in copious amounts using the chemical vapour technique (CVT), for example for the MLC LaS-TaS_2 [2,3]. Transmission electron microscopy (TEM) characterization is crucial to study the structure and composition of MLC nanotubes in detail. In-situ TEM approaches can shed light on their behavior under external stimuli. Facilitated by the development of a sample preparation technique for electrical characterization by in-situ TEM with minimum damage and contamination [4,5], we studied the dynamic evolution of these MLC nanotubes under extremely high electrical currents.
Methods
Nanotubes made of LaS-TaS_2 were synthesized via the CVT technique [4]. To prepare an in-situ TEM sample for electrical studies, the nanotubes were first dispersed on a holey silicon nitride TEM grid. Suitable nanomaterials were selected in a conventional TEM analysis of the sample. The nanotubes sustained by the silicon nitride were subsequently transferred to an in-situ microchip assisted by focused ion beam (FIB) as described in [5]. Figure (a) shows a SEM image of one of the prepared devices consisting in a pair of MLC nanotubes. Using an in-situ sample holder (DENSsolutions Wildfire), the specimens were analyzed under the application of external heat and electrical currents in two aberration-corrected Titan microscopes (Thermo Fisher Scientific).
Results
Little changes in the nanotube specimens were observed when heating with an on-chip heating device and also for lower electrical currents. Only the application of considerable electrical currents, larger than 100 µA, led to structural modifications within the material. Electrical currents were applied as current ramps up to a maximum applied current followed by an abrupt stop of the current. During the ramps, the nanotubes are heated up smoothly to high temperatures followed by a rapid quenching to room temperature when the current was turned off. This rapid cooling led to fractures of the material, as seen exemplary in Figure (b), where two different breaking points for the inner and the outer part of the nanotube are observed. Already before the fracture, the LaS-TaS_2 MLC material starts to decompose and metallic tantalum is extracted, leaving La_2S_3 behind (Figure (c)). The investigations allow a detailed study of the chemical decomposition routes under extreme electrical currents. A clear difference between pure thermal annealing and electromigration is observed.
Conclusion

The conducted experiments allow an in-depth dynamical study of the behavior of micro- and nano-sized objects under high electrical currents. For example, for the investigated LaS-TaS_2 nanotubes, the TaS_2 first gets unstable and metallic Ta is extracted while released sulfur reacts with LaS to form La_2S_3. By specifically designing both the device geometry and the form of the applied electrical current, different chemical and physical processes can be studied at highest spatial resolution provided by the in-situ TEM approach.

Acknowledgments
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Figure caption
(a) SEM image of the prepared device. (b) TEM image of the fractured nanotube. (c) TEM image of the border of the nanotube showing extracted Ta and remaining La_2S_3.

Keywords:

Misfit-layered-compounds, in-situ-TEM, electrical-current, electrical-breakdown, high-temperatures

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Optimising a modern high performance FE-SEM for multimodal vEM

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Poster Group 1

The popularity of volume Electron Microscopy (vEM) has ballooned over the recent past. Noteworthy examples of the increased attention being paid to vEM include: large-scale funding investment initiatives (such as the Chan Zuckerberg Initiative's Imaging program(1)), influential opinion pieces in scientific journals (Seven technologies to watch in 2023 – Nature volume 613(2)) and the growing user base of informative community-run websites and discussion channels (such as the Volume EM community website(3), the Array Tomography community website(4), or the EMofCellsTissuesOrganisms Slack channel(5). One of the main advantages of the increased interest in vEM is the abundance of information to allow prospective vEM users to choose the workflow and methodology that best suits their needs, optimise their experimental approaches and interact with a rapidly expanding user base.

vEM can be accomplished in scanning electron microscopes (SEM), transmission electron microscopes (TEM) or with focused ion beam/ dual beam SEMs (FIB-SEM). This talk will focus on the two main techniques for vEM within a SEM; namely array tomography (AT) and serial block-face SEM (SBF-SEM). Each of these techniques has their own unique pros and cons in terms of the sample preparation requirements, achievable voxel resolution, and re-usability of sample material. Historically, one of the main drawbacks of vEM was that the requisite hardware often limited prospective vEM users to a single instrument choice, or worse yet, to a single vEM technique. Modern advances in hardware and software allows JEOL the ability to offer bespoke solutions for vEM workflows; whether the requirement is to choose a microscope best suited to the scientific questions, the flexibility to have access to both array tomography and serial block-face SEM within a single instrument, or both. The unique software and hardware advances, which allows multimodal vEM to be achieved, will be discussed in this talk.

Recent developments in FE gun and SEM column design makes it easy to optimize imaging conditions such as accelerating voltage, probe current, column mode and implementing beam deceleration. Hardware advances to allow this ease-of-use include an in-lens Schottky Plus FEG which integrates the electron gun with a low-aberration condenser lens improving brightness and reducing beam diameter, and an aperture angle control lens (ACL) (located beneath the condenser lens), that continuously suppresses the spread of the incident electrons maintaining the smallest possible probe size even when the probe current is increased.

Other hardware advances include the improved probe-forming capability of a field-free hybrid electromagnetic and electrostatic lens effect along with advancements in column optics for automated control and adjustment of the electron lenses and correction of electron trajectories in real time. Together these allow one to achieve optimized resolution under all imaging conditions. Another key to high quality data acquisition is a new scintillator-type back-scattered detector that is optimised for low kV imaging and allows high-resolution images to be acquired at fast speeds, perfect for the challenges faced by vEM sample preparation.

Transitioning our FEG-SEM from normal imaging mode to SBF-SEM mode is very straightforward through partnership with ConnectomX. All that is required is to vent the chamber, attach the stage-mounted Katana microtome, and pump down again; a process that can be done in just a few minutes. Capturing high quality 3D data has never been easier (Figure 1).

In addition to hardware advances, the evolution of software streamlines and simplifies automated data acquisition. For both AT and SBF-SEM experiments on JEOL microscopes, all of the hardware control (both microscope and microtome if in use), experiment setup and data acquisition is controlld through SEM Supporter software. Similar guided workflows exist for both techniques, which makes transitioning between the techniques straightforward. For example, the AT workflow complexity is easily condensed into three steps. First a low magnification montage is captured over the entire sample set. Then predictive ROI positioning is used to propagate the ROI over all serial sections. Finally image cross correlation is used to control image shift and scan rotation to keep the successive ROIs well aligned.
Following data acquisition significant advances have also been made to offer software solutions for image alignment, rendering and segmentation, to round out the solution for the whole vEM workflow.
In conclusion, JEOL offers bespoke solutions for different vEM applications, within a single SEM. Optimized solutions for all the steps in the vEM workflow – from high quality data collection, to easy to set up workflows, to intuitive and straightforward means for data processing and analyses, allow vEM to be within reach for researchers of any experience level.

Keywords:

Multimodal vEM, SEM, SBF, AT

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TEM and photocurrent response of FE BTO thin films heterostructure on silicon for Neuromorphic applications

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Poster Group 2

Ferroelectric systems continue to be of great interest in applications related to photonics and neuromorphic systems [1][2] due to their memristive properties [3]. The rapid polarization response to electrical stress and the large miniaturization capacity of a few nanometers make them ideal candidates for the development of storage devices, processing and sensors.
In this work we successful obtained thin epitaxial films of $BaTiO3$ (BTO) on silicon (Si), by means of Pulse Laser Deposition (PLD). Yttria-stabilised zirconia (YSZ), CeO_2 and a very thin layer of $La0.5Ca0.5MnO_3$ ($LCMO$) were used as buffer-layers. Reflection High-Energy Electron Diffraction (RHEED) confirmed the epitaxial nature of the heterostructure. X-ray diffraction (XRD) measurements showed the unique presence of index peaks (00l) for all layers, indicating the high crystalline quality of the heterostructure. Scanning Transmission Electron Microscopy with High Angular Annular Dark Field images (STEM-HAADF) were used to identify and characterize the continuous thin layer of LCMO at ~2.5nm. Ferroelectricity measurements using the PUND method show a large remaining polarization of ~13$\mu C/cm^2$ and a coercive field of ±700 kV/cm, much higher than other similar systems and obtained at lower temperatures [4]. This large remaining polarization is attributed to the thin layer of lower $LCMO$ that generate negative stresses in the BTO layer increasing its tetragonal character.
Photocurrent measurements with 403 nm light, show a large photocurrent response of ~ 15 $\mu A/cm^2$ with small voltage values. These values are also considerably large compared to other reports [5]. The generation of load carriers due to light, modifies the barrier heights at the $LCMO/BTO$ and BTO/Pt interfaces and the photocurrent levels were also highly dependent on the bias voltage level. The large polarization and coercive field values obtained are attributed to the low leakage currents and high resistance of the $LCMO$ layer due to its low thickness. These features make this heterostructure a very robust system, with the ability to tolerate high field values with barely lost ferroelectric properties and high levels of electrical stress. Its excellent photocurrent response also contributed to its high intrinsic properties as a UV detector/sensor. All the above features make it a very good candidate for exploration of properties in neuromorphic systems.

Keywords:

Thin films, ferroelectric, photocurrent, Neuromorphic

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Scanning electron diffraction reveals the molecular ordering of polysaccharides at the nanoscale

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PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Background

Cellulose is the most abundant biopolymer. Nature links glucan units to form polysaccharide chains which are organized in an intricate network of hydrogen bonds into crystalline cellulose fibers. These fibers are assembled into hierarchical structures that constitute the secondary cell wall of plants, forming the basis for their mechanical properties. The macroscopic attributes of these materials are determined by chemical interactions and mesoscopic organization spanning from the atomic scale to the macroscopic level. Understanding these assemblies is vital for grasping the properties of biological materials and for exploring ways to incorporate these naturally occurring components into hybrid materials.

Biopolymers are fundamentally challenging to study using electron microscopy due to their delicate and electron beam sensitive nature. Using scanning electron diffraction we can obtain unique nanoscale information about the molecular ordering of the polysaccharide chains, which can be used to understand e.g. their superior mechanical properties.

Methods

The strong interaction between an electron probe and matter enables the acquisition of scattering data from nanometer-sized volumes even from weakly scattering elements. Electron diffraction has proven to be an invaluable tool for the structural examination of a diverse range of nanomaterials. Scanning electron diffraction (SED) offers detailed maps that reveal the crystalline ordering at the nanometer scale, shedding light on the arrangement of polysaccharide chains within individual nanofibers and also their hierarchical assemblies in composite materials.

In this study, we are using a quasi-parallel electron beam, with a convergence angle of 0.1 mrad. The beam is scanned across the specimen to obtain a map containing information about the molecular scale ordering of the biomaterial. The high speed and sensitivity of hybrid detectors enable the acquisition of electron diffraction data from single cellulose nanofibers.

Results

Cellulose nanofibers (CNFs) can be extracted from various origins and stand out as a promising, sustainable building block with remarkable mechanical properties. Using SED data, the crystalline nanostructure within individual CNFs can be analyzed, offering insights into how the crystalline ordering persists. Data was obtained from twisted sections of the nanofibers and reveals that despite the strain this twisting introduces the crystalline structure remains intact.[1] Furthermore, SED data can be employed to investigate the hierarchical organization of cellulose fibrils within the plant cell walls as well as hybrid materials based on wood. One prominent example of this a composite mateial made from delignified and polymer-impregnated wood, the so-called transparent wood. SED data obtained from this material revealed the organization of cellulose fibrils into hierarchical helical structures across the various cell wall layers.[2] In most of the cell wall the cellulose fibers were organized along the extended dimension of the cells and towards the outermost part of the wall the fiber orientation gradually changed to a tangential orientation.

Conclusion
Using scanning electron diffraction we can for the first time reveal the molecular ordering of cellulose chains in twisting cellulose nanofibers as well as shed light on the mesoscale hierarchical ordering of cellulose fibers in hybrid materials and cell structures with nanometer resolution.

Keywords:

Scanning electron diffraction, biopolymer, cellulose,

Reference:

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2. M. Nero, H. Ali, Y. Li, and T. Willhammar, "The Nanoscale Ordering of Cellulose in a Hierarchically Structured Hybrid Material Revealed Using Scanning Electron Diffraction" Small Methods, Dec. 2023, doi: 10.1002/smtd.202301304.

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Two-photon line-scanning structured illumination microscopy (LIL-SIM) for super-resolution imaging in deep tissue

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LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
In the field of cellular imaging, the demand for sub-cellular resolution and three-dimensional (3D) visualization of biological specimens has been a driving force behind the development of various microscopy techniques. This study explores a novel approach of combining structured illumination microscopy (SIM)1,2 and two-photon line scanning (2P-LS) microscopy to provide super-resolution imaging in thick biological tissue. We apply our method, named LIL-SIM, to cardiovascular artery tissue and tree trunk to demonstrate the penetration capability of our imaging system.

Methods
Structured Illumination Microscopy (SIM) is a well-established super-resolution method, breaking the diffraction limit by introducing a spatially modulated light pattern to the sample. However, SIM's performance traditionally decreases in highly scattering media typical of biological tissues. Conversely, Two-photon (2P) microscopy provides exceptional depth penetration due to its employment of near-infrared light, reducing scattering, and targeting excitation to the focal volume, thus limiting photodamage. Despite the advantages gained by combining 2P microscopy and SIM, the necessity for camera-based detection lowers the modulation contrast of the pattern in thick biological specimens and reduces the signal to background ratio (SBR) of the acquired SIM patterns. This leads to artefacts in the reconstructed images due to insufficient modulation contrast of the generated patterns. Interestingly, the SBR can be increased by confocalization of the emitted fluorescence emission, restricting out of focus contributions of scattered photons. This leads to increased SBR and high modulation depth of the generated SIM patterns in thick biological tissue, enabling volumetric super-resolution imaging.

Results
We validate the super-resolution capability of our microscope by imaging tissue samples composed of pine wood and tree trunk, with z-depths of around 40 µm. For all experiments, we generated SIM patterns with five phase shifts to acquire homogeneous illumination of the sample and three rotation angles for an isotropic resolution enhancement. This amounts to a total of 15 acquired images for one SIM frame. Averaging of the 15 acquired frames leads to a homogeneously illuminated intensity distribution, which we refer to as the 2P widefield image in the following. As seen in Fig. 1a), LIL-SIM increases the resolution compared to diffraction limited 2P widefield imaging while simultaneously obtaining better optical sectioning by line confocalization. The 2P widefield image is blurred by the contribution of excited fluorophores close to the focal plane due to scattering. By the application of LIL-SIM and following computation of the acquired images with fairSIM3 , the resolution is effectively doubled. LIL-SIM insets 1c) and e) show clear resolution improvement of nanostructures over the acquired 2P widefield structures 1b) and d). The line plot comparison in Fig. 1f) shows a FWHM distance of 384 nm in the 2P widefield image and 150 nm for 2P-LS-SIM.

Conclusion
Our results demonstrate that the fusion of SIM and 2P-LS microscopy leads to an imaging modality capable of resolving structures down to 150 nm in thick biological specimens. We provide data analyzing imaging parameters, e.g. penetration depth, modulation depth of the illumination grating and phase stability of the illumination patterns and present reconstructed super-resolution images of thick tissue samples, e.g. cardiovascular artery and pine trunk.

Fig. 1 a) 2P widefield and LIL-SIM comparison. b-e) insets and f) line graph demonstrate resolution improvement. g-h) 2P widefield and LIL-SIM with increasing z-depth (10, 20 and 30 µm). Scale bars a) 10 µm, b) 2 µm, g) 5 µm.

Keywords:

Structured Illumination microscopy, Two-photon microscopy

Reference:

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Study of nuclear materials for silicon carbide composite fuel claddings via STEM, EDX and EELS

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Background incl. aims
Silicon carbide composites (SiC/SiC) are promising materials for fuel claddings in light-water reactors as their thermal, chemical and mechanical stability extend to very high temperatures, granting longer coping time in case of a loss-of-coolant accident. These materials would potentially allow to claim a power plant back even after many hours without active cooling of the core [1,2]. Despite their excellent performance under accidental conditions, during normal operating conditions, SiC/SiC composites are oxidized by the cooling water, forming silica (SiO_2). To prevent this, the application of a protective coating over the cladding is proposed. In this study, yttrium aluminum garnet (YAG) and chromium-doped yttrium oxide (Cr:Y_2O_3), deposited with pulsed laser deposition (PLD), were studied by scanning transmission electron microscopy (STEM). The coatings were observed before and after thermal treatment at temperatures resembling severe loss-of-coolant accidents.
Methods
Four samples were studied: 1) pristine YAG deposited on a silicon substrate; 2) YAG deposited on a stainless steel substrate that was annealed in air after deposition at 900 °C for 1 hour; 3) pristine Cr:Y_2O_3 deposited on a silicon substrate; and finally 4) Y_2O_3 with the same amount of Cr_2O_3 deposited on a zircalloy substrate and annealed at 1300 °C for 1 hour.
Electron-transparent samples for STEM were prepared by traditional low-angle Ar ion milling and by focused ion beam (FIB). The microstructure was investigated in the Linköping double corrected, monochromated FEI Titan3 60-300, operated at 300 kV. Energy dispersive x-ray spectroscopy (EDX) and electron energy loss spectroscopy (EELS) were employed to identify elements and density variations in the samples.
Results
STEM imaging of the samples shows that sample 1) is amorphous, however, apparent layering with an approximate period of 20 nm was observed in the sample. EELS spectrum imaging was performed to discern if the layering occurred as a consequence of elemental inhomogeneities or because of density variations within the coating, confirming the former. After annealing, the YAG assumed a crystalline nature with ~1 µm equiaxed grains (sample 2). The grain boundaries accommodate large voids and the original layers were removed, as can be observed in the graphic. The presence of these grain boundaries decorated by voids suggests that this material may not function as a diffusion barrier for water.
Sample 3) displays a predominantly amorphous structure, although crystalline nanoparticles are randomly distributed in the amorphous matrix. After annealing (sample 4), this coating presents different microstructures near the substrate and near the surface. The film has crystallized, and near the substrate, the film exhibits a dense structure. In contrast, the surface near region also exhibits a crystalline nature, although the grain boundaries appear to be porous and disordered. The origin of this presumably owes to chromium oxide segregating at the grain boundaries and evaporating during

the annealing process, leaving underdense boundaries behind that become swift routes for further chromium diffusion.
This is supported by the concentration of chromium, which is notably higher in the region near the substrate compared to near the surface, as measured by EDX. The interface between the two different microstructures shows apparent particles of segregated chromium. These presumably originate from migrating chromium that condenses into nanoparticles during the rapid cooldown. The gradual migration of chromium that occurs during annealing in the Cr:Y_2O_3 can help protect the integrity, or delay catastrophic failure, of the system as the inner phase maintains a diffusion barrier and effectively protects the fuel cladding.
Conclusion
This study shows that Cr:Y_2O_3 is a valid alternative material as a coating for SiC/SiC composites. This can be affirmed since the material acquires a microstructure that is gradually consumed during high-temperature processing.

Keywords:

Nuclear materials EELS STEM EDX

Reference:

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Functional chemistry of minimally altered organic matter in the meteorite Winchcombe probed by monochromated EELS

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background incl. aims
The composition of organic matter (OM) in meteorites such as carbonaceous chondrites is a useful indicator for the type of organic matter present on early, prebiotic earth [1]. This OM can be extracted with solvents and analysed with mass spectrometers, but these techniques lose local petrographic context, and the sometimes harsh chemical processes involved in the extraction have the potentially to unintentionally alter the detailed functional chemistry of the OM. Here, careful preparation of thin lamellas using focused ion beam (FIB) techniques, and correlative analysis by high-resolution electron energy loss spectroscopy (EELS) and scanning transmission x-ray microscopy (STXM) of the very same samples, allow for a detailed OM characterization at the nanoscale [2] within the OM's native petrographic context and with minimal alteration.

Methods
Using a Hitachi Ethos NX5000 triple-beam FIB system, several lamellas were prepared containing OM from the meteorite Winchcombe, with special care taken to minimize the impact from sample preparation and enable the study of minimally altered OM. Steps taken to minimize alteration include minimizing the use of protection layers where possible to avoid carbon contamination, backside milling to ensure FIB-deposited material is downstream of the thinned area, and rocking milling to improve uniformity. Where FIB deposition was necessary, metal-containing deposits only (Pt, W) were used, ensuring that any redeposition could be easily identified. Most lamellas were thinned to ≤ 40 nm, a thickness optimised both for sufficient EELS signal at low kV as well as for complementary STXM analysis of the same lamella. To minimize surface damage, crucial for thin samples, Ga+ milling was followed by 1 kV Ar+ polishing using the 'third beam' of the NX5000. Careful, targeted broad-beam low-energy Ar+ polishing, with repeated short exposures and multiple incident angles proved highly effective at reducing surface amorphization and Ga-implantation-related damage. The application of Ar+ polishing directly in the FIB offers here an additional degree of control during the final thinning procedure while minimizing sample handling, thus further lowering any risk of alteration to the OM. SEM imaging of thin areas was also limited and performed at low beam energies to mitigate electron beam alteration of the OM.

Monochromated EELS was carried out at 60 kV in a Nion UltraSTEM100MC-Hermes equipped with a Nion IRIS spectrometer and a DECTRIS ELA direct electron detector. With the samples in ultra-high vacuum, a probe size of 0.1 nm with low beam current of < 5pA, together with an energy resolution of 50-90 meV, makes it possible to distinguish local variations in the different bonds in carbon- and nitrogen-containing OM. Both nanoglobules (~ 300 nm) and 'diffuse' carbon infiltrated into the phyllosilicate matrix (< 100 nm) were analysed. Complementary X-ray absorption near-edge structure (XANES) analysis in the STXM was carried out at the 108 beam-line of Diamond Light Source, UK with a nominal beam size of ~ 40 nm and energy step sizes of 0.1 to 0.5 eV. Where possible, both XANES and EELS were carried out on the same lamella, allowing for direct comparison and highlighting the complementarity of these related techniques.

Results

Both STXM-XANES and STEM-EELS reveal distinctive C K absorption bands at 285 and 286.5 eV across all lamellas, relating to aromatic carbon and aromatic ketone/aldehyde bonds. The superior spatial resolution of the STEM probe highlights local variations in the 285 eV aromaticity-linked peak, with a generally higher ratio of aromatic/aliphatic content compared to CM chondrites, indicative of higher alteration. The low noise of direct electron detector-based EELS also enables probing the functional chemistry of the less abundant nitrogen (atomic N/C ratios of only a few %). N K distinct bands are at 398.8 eV and 399.8 eV, associated with C-N double and triple bonds, respectively. Applying statistical denoising methods enables the detection of energy bands beyond those distinguishable in the STXM data, pointing to the presence in nanoscale areas of amino acids such as L-alanine, which had been previously detected by soluble OM studies [3]. Here, the variation of this 402-403 eV fine structure at small length scales, together with the care taken to provide as unaltered a view of the OM as possible within its petrographic context, suggest these functional groups can be linked unambiguously to fluid processes within the OM.

Conclusion

The combination of STXM-XANES and high-resolution STEM-EELS on carefully prepared, minimally altered FIB lamellas allows for the analysis of OM at the smallest length scales. Different nanoscale textures such as nanoglobules and diffuse OM can be distinguished and compared to learn about the evolution and alteration mechanics of organics in carbonaceous chondrites. The direct correlation and excellent match between STXM and 60 kV STEM-EELS results demonstrate how this approach provides a powerful characterization tool for complex extraterrestrial samples with a unique combination of high spatial and energy resolutions.

Figure: (a) scanning electron micrograph of an extracted FIB lamella. The top is thinned to electron transparency and exhibits both a bigger irregular OM grain and smaller patches of OM. (b-c) STEM-EELS + STXM data of the C-K and N-K edges. EELS – 1 and 2 correspond to regions indicated in (a). Subtle changes in the EELS spectrum can be observed for OM areas, even in close proximity.

Keywords:

EELS, meteorite, Winchcombe, FIB

Reference:

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Elucidation of Structure-catalytic Activity of Nickel-based Nanomaterial for Electrocatalytic Water Splitting

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Poster Group 2

Background

The lack of satisfactory and cheaper catalysts for hydrogen production by electrocatalytic water splitting and hydrogen utilization in fuel cells is the major challenge for this clean energy technology [1]. New advanced nanostructured materials with special properties for green H2 production by water splitting are needed to address this major and urgent challenge [2]. Nanostructured materials have shown promising catalytic performance that exceeds the sum of their individual components [3-4]. Thus, they contribute to the development of industrially applicable and sustainable materials for new alternative clean electrochemical energy technologies. For the discovery of novel advanced energy materials that meet the requirements of emerging clean electrochemical energy technologies, advanced tools for the elucidation of structural (electro)catalytic activity are at the heart of future and sustainable nanomaterial development. These advanced materials require advanced scale-bridge spectroscopic technics characterization to understand the chemistry and the elemental species of nanostructured materials at the nanoscale. We have developed a rational design of easily scalable nickel boride (NixB)-derived catalyst to correlate with optimized catalytic activity through metal elements incorporation in a one-pot synthesis method. Our method overcomes the challenge of conventional annealing-dependent processes which have limited applicability on a large scale since they also require an inert processing environment. Furthermore, we elucidate a structure-activity relationship by utilizing scale-bridging correlative microscopy techniques leveraging transmission electron microscopy coupled with electrochemical characterization.

Methods

To elucidate the structure-electrocatalytic activity functionality of these nanostructures, we conducted a thorough electrochemical and TEM investigation on binary nickel boride and quaternary nickel boride-derived catalysts.

Results & Conclusion

A one-pot synthesis method enabled a rational design of non-noble metal, highly efficient, and durable electrocatalysts for hydrogen production at low-temperature electrolysis in harsh alkaline solutions. This improved catalytic performance is further corroborated by microstructural investigations using TEM. The nanoparticles obtained have an improved porous structure compared to conventionally synthesized NixB (Figure 1), providing more available sites for surface reactions and catalytic performance of this nanostructured material. Furthermore, we demonstrate that activation enables morphological and structural changes, while some transition metal elements act as sacrificial elements to provide more accessible and stable sites for oxygen-forming centers. This paves the way for a better understanding of metal boride-derived electrocatalysts for electrocatalytic water splitting

and contributes to future discoveries of non-noble metal catalysts, yet with highly efficient and stable nanostructured materials.

Acknowledgements:

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Keywords:

STEM-EELS, catalyst, nanoparticles, water electrolysis

Reference:

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Assessing the Accuracy of Strain Mapping using 4D-STEM

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Poster Group 2

Background
With the advent of sensitive direct electron detectors, coupled with ever-greater computational power, 4D-STEM has grown rapidly in popularity [1,2]. In particular, scanning electron diffraction (SED), a variant of 4D-STEM, is based on acquiring a set of diffraction patterns with low convergence (near-parallel) probes for each scan position such that subsequent computational data processing can retrieve nanoscale information regarding phase identification, crystallographic orientation, internal electric and magnetic fields and strains. This work assesses the impact of different acquisition conditions to identify optimal imaging conditions for 2D strain mapping.

Methods
A simulated strain distribution (quantum well phantom with an (unstrained) primitive unit cell of 5Å) that included areas with uniaxial and biaxial normal strain, shear strain, lattice rotation, and their combination, was used to generate a SED dataset for strain mapping. The simulated diffraction patterns, 'recorded' on detectors with four different pixel sizes, were generated using the kinematical diffraction module in Py4DSTEM python library [3], and a custom plotting code based on the pyxem python library [4]. The maximum extent of the simulated diffraction pattern on each detector (with the origin at the detector centre) is +/- 1.25 Å-1. Kinematical simulations can be justified here because strain mapping often uses precession electron diffraction, providing integrated 'near-kinematical' intensities and, importantly, integrates over any contrast seen within the diffraction disk ('rocking curve' contrast); such contrast is known to have a deleterious impact on the accuracy of strain mapping [5]. Noise is added to the diffraction data accounting for the statistical nature of electron arrival and to account for a background noise that represents readout noise of the detector. Strain analysis was performed on the SED dataset using both cross-correlation (CC) and centre-of-mass (COM) algorithms and the results compared with the strain phantom (ground truth) to assess the effect of each parameter on the accuracy of strain mapping. A diagram of the workflow is illustrated in Figure 1(a).

Results
The effect of number of detector pixels, signal to noise ratio (SNR), order of reflection / magnitude of g-vector, and probe convergence was investigated. In general, as expected, the higher the number of detector pixels, the higher the strain mapping accuracy. This is illustrated in Figure 1(b), where we see a significant improvement in the sum of squares of errors in strain mapping between 128 and 256 detector pixels, while increasing the number of pixels still further is beneficial, in the case studied it has diminishing returns. Increasing the order of the reflection, and thus increasing the g-vector magnitude will, in general lead to a lower SNR for each disc. For strain mapping it is intuitively the case that we would wish to use reflections with high SNR and large g-vector magnitudes (reflections of high order). However, lower order reflections tend to have higher SNR and therefore it is important to find a balance between SNR and g-vector magnitudes. The effect of beam convergence depends on which peak-finding algorithm is used. It is advantageous to use COM algorithms for

probes with low convergence, where it is difficult to fit the peak position due to the lack of pixels to accurately represent a circle. CC works best for higher convergence angles where the pixelation of the detector does not affect the fitting precision. CC also requires lower SNR to work precisely compared to the COM algorithm. The optimised conditions taken from the simulation were used to acquire an experimental strain mapping dataset of SiGe quantum wells in a MAG*I*CAL sample using CMOS camera (ThermoFisher Ceta) and hybrid pixel direct electron camera (Quantum Detectors Merlin) to evaluate the findings from the simulations in an experimental setting, see Figure 1(c).

Conclusions
We have assessed the accuracy of strain mapping using SED, a 4D-STEM variant, by considering key parameters such as the number of detector pixels and the SNR of the reflection used for strain mapping. A clear optimum is found that balances the SNR and g-vector magnitude for a strain phantom. We find it is advantageous to use a centre of mass algorithm for low convergence probes and a cross correlation algorithm for larger convergence probes. Using these findings we were able to reconstruct a high fidelity experimental strain map from a SiGe quantum well data set. The authors thank the EPSRC for funding under grant numbers EP/V007785/1, and EP/R008779/1.

Keywords:

Strain mapping, scanning electron diffraction

Reference:

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Cryogenic electron microscopy for native state analysis of soft- and nano-materials

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PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Background
Nanomaterials can potentially be used in a range of areas, including in consumer goods through to medical applications. In many areas, the nanomaterials are used while dispersed in a liquid, or contain hard-soft interfaces, which, while increasing their applicability to their end-use, can complicate characterisation and determination of structure-property relationships.
Electron microscopy, while ideally suited to the nanoscale imaging and analysis of these materials, can encounter limitations due to the vacuum requirements, which exclude many in situ or native state studies. In addition, necessary sample preparation can often lead to artefacts. A viable alternative is electron microscopy conducted on frozen hydrated samples, where the nanomaterials are 'captured' in the native state and any electron beam induced damage products are immobilised. This work aims to develop representative native state analysis of dispersed nanoparticles in soft materials using cryogenic electron microscopy approaches, with application shown here to a commercial sunblock sample containing metal oxide nanoparticles and Pickering emulsions (water-oil mixtures) designed to contain an active ingredient.

Methods
Results from two soft materials containing dispersed nanoparticles will be detailed. The first sample is a Pickering emulsion, comprised of oil in water droplets stabilised by ~5 nm platinum nanoparticles. The approaches are further developed by examining a commercial product, a sunscreen containing active ingredients of 4.5% of TiO_2 and 6.5% of ZnO nanoparticles.
All samples were prepared for cryo-TEM using an FEI Mark IV Vitrobot©. A 3.5 µl drop of suspension was loaded onto a lacey carbon-coated copper TEM grid (EM resolutions) before being blotted and then rapidly plunge frozen in liquid ethane. Transfer into the microscope was done using a Gatan-914 cryo TEM holder, and the temperature was maintained below −165 °C during analysis. Comparison was made to a static liquid cell (LC) commercially sold as a K-kit and supplied by Bio-Matek. S/TEM analysis was carried out using an FEI Titan3 Themis G2 equipped with a monochromator operating at 300 kV and fitted with 4 EDX silicon drift detectors and a Gatan One-View CMOS camera. The probe current was kept below 100 pA for all cryo and LC experiments. Samples were prepared for cryo-SEM using a Quorum Technologies PP3010 Cryo-SEM preparation system and examined in an FEI Helios G4 CX Dual beam FIB-SEM with a beam voltage of 1–10 kV and beam current 100 pA, while elemental mapping via an Oxford instruments EDX spectroscopy system was conducted at 15 kV.

Results
We have previously shown the advantage of cryogenic-EM approaches to the analysis of dispersed nanoparticles, including those in complex biological cell culture [1,2]. In this work we will show the advantages of using a cryogenic approach for more complex soft materials systems incorporating nanoparticles, with extension to the use of cryo-STEM-tomography and cryo-FIB-SEM.

Cryo preparation, transfer and analysis is essential for Pickering emulsions as the integrity of the sample is maintained as drying and the microscope vacuum results in bursting of the droplets. Undertaking higher magnification imaging with careful consideration of total electron fluence it is possible to examine the distribution of the nanoparticles using cryo-STEM [3], and we will show that utilising cryo-HAADF STEM over a ±60° tilt range permits 3D visualisation of the sample structure. This results in the confirmation of both the position of the stabilising nanoparticles and the overall droplet shape in 3D space. Using a combination of cryo-EDX and -EELS the elements in both the nanoparticles and oil droplets are confirmed.
Cryo-STEM and associated spectroscopies are also used to analyse the commercial sunscreen, with comparison to alternative in situ electron microscopy techniques – static liquid cell STEM and cryo-SEM [4]. While cryo-STEM does allow for higher resolution analysis, in this case both the concentration of dispersed particles and viscosity of the product causes complications with sample preparation. In analysis of a diluted product, both nanoparticle types are identified, something which was not possible in the static liquid cell due to electron beam artefacts causing dissolution of one nanoparticle type. Cryo-SEM was used to analyse the pure product without dilution but biased the characterisation to the larger fraction of nanoparticles and agglomerates.

Conclusions
Cryo electron microscopy offers route to the representative native state analysis of dispersed nanoparticles in soft materials. Complex systems, such as the soft hybrid inorganic-organic Pickering emulsions can be analysed by a combination of STEM-analytical techniques to provide nanoscale 3D information. Commercial products, with numerous components and required to be used at a set concentration can be more complicated, however with a combination of different in situ EM techniques an accurate, native state characterisation can be achieved.

Keywords:

cryo; STEM; cryo-SEM; nanomaterials

Reference:

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PVA/PEG-based nanofibers with Au nanoparticles for treatment of chronic woundss

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PS-05 (2), Lecture Theater 1, august 28, 2024, 14:00 - 16:00

Background incl. aims
Chronic wounds are a healthcare problem both for patients and for society. They are often contaminated with bacteria, and the infection impacts the healing of the wounds. Therefore, a tremendous effort has been made to develop wound dressings supporting wound healing and preventing or even eliminating bacterial infection. Due to increasing bacterial resistance to antibiotics, other research approaches are used [1]. The aim of this study was to prepare polyvinyl alcohol (PVA)/polyethylene glycol (PEG) – based wound dressing doped with gold nanoparticles, to analyze their physical and chemical properties, cytocompatibility, and antibacterial properties.
Methods
Sputtering of Au for nanoparticle preparation was carried out at 20 °C by the sputter coater device SCD 050 (Baltec, argon pressure of 8 Pa), with the current of 30 mA, sputtering time of 500 s, and the electrode distance of 50 mm. Immediately after deposition of the metal nanoparticles into PEG (polyethylene glycol, Mw 600), the mixture was diluted with 18 mL of distilled water. The ratio of PEG/H2O in this case was 1:9. PVA-based nanofibrous materials were prepared by DC electrospinning. Solution of nanoparticles (AuNPs) was added into a 10 % PVA solution; the ratio of PVA:PEG was 9:1 or 6:4. Electrospinning was carried out on a needleless electrospinning device NS 1WS500U (Elmarco, CZ) using high voltage on electrodes 40 and 10 kV. The temperature was set to 21 °C and the relative humidity was of 21 %. Some of the samples underwent heat treatment (HT) at 150 °C for 1 h. The presence, size and homogeneity of the AuNPs in nanofibers was proven by JEOL JSM-IT800 scanning electron microscope by the analyses of backscattered electrons. The theoretical Au concentration was 111 and 666 µg/g for 9:1 and 6:4 PVA:PEG ratio, respectively. The nanofibers were analyzed by energy dispersive spectroscopy, atomic force microscopy, scanning electron microscopy (VEGA Easy Probe (TESCAN, CR) as well. The PVA:PEG membranes with/without Au, with/without heat treatment and at both ratios (9:1, 6:4), 1 cm2 in size, were added into the culture of normal human dermal fibroblasts (NHDFs) or human umbilical vein endothelial cells (HUVECs), which were seeded at the density of 20 000 cells/well in a glass-bottom 24-well dish. Cell morphology was assessed by immunofluorescence staining of vinculin and F-actin staining using phalloidin-TRITC. Cell metabolic activity/viability was analyzed using resazurin assay on days 1, 3, and 7 after seeding. HUVECs were stained for von Willebrand factor, and NHDFs for type I collagen production on day 7. Antimicrobial properties of the prepared materials were characterized by contact tests of the

materials with two environmental bacterial strains of Gram-positive S. epidermidis and Gram-negative E. coli. The number of colony-forming units of both bacterial strains after contact tests were calculated using ImageJ. As a control, bacteria incubated in a physiological solution were used.
Results
Scanning electron microscopy revealed different surface morphology of the prepared nanofibers for particular PVA:PEG mixtures, i.e. 6:4 and 9:1 as well as the presence and relatively homogeneous distribution of Au on/inside the nanofibers. The density of nanofibers, and more importantly the intensity of so-called “clusters”, were different; the PVA:PEG nanofibers had a narrow distribution of thickness with small or large drops inside membranes. The EDX analysis exhibited different surface chemistry of the prepared samples based on the amount of added nanoparticles and PEG in the solution. Atomic force microscopy was used for the determination of surface roughness, morphology and effective surface area both on the fiber (detailed scan), and also generally over the surface (larger scan above 30x30 µm). The heat treatment also affected both the surface morphology and chemistry of the prepared samples as was revealed by SEM, EDX and AFM techniques. Heat treatment decreased both the dissolution and shrinkage of the nanofibers in the cell culture medium. On day 1 after seeding, both HUVECs and NHDFs were well spread, and in the following days, they proliferated in the presence of all tested nanofibers with/without Au. Metabolic activity/cell viability of the cells was similar to that growing on the control tissue culture polystyrene surface. The HUVECs were positively stained for von Willebrand factor and NHDFs were proven to produce collagen on day 7. The analysis of material antimicrobial properties showed a partial inhibition of bacterial growth, presented as a lower number of colony-forming units.
Conclusions
The PVA:PEG–based nanofibers with homogeneously dispersed AuNPs at different Au concentrations were prepared and some of them were further stabilized by heat treatment.
The material physical and chemical properties were evaluated by various physical methods, their cytocompatibility was proven in vitro culture using both HUVECs and NHDFs cells. The materials with higher Au content seem to be promising for the treatment of chronic wounds with/without bacterial infection.
Acknowledgements: Supported by Project No CZ.02.01.01/00/22_008/0004562 of the Ministry of Education, Youth and Sports, which was co-funded by the European Union, by the Praemium Academiae grant (No. AP2202) provided by the Czech Academy of Sciences, and by the European Union—Next Generation EU, the project National Institute for Research of Metabolic and Cardiovascular Diseases, Programme EXCELES, ID Project No. LX22NPO5104.
Graphics

Fig. 1. A. Immunofluorescence staining of von Willebrand factor in HUVECs cultured in medium with added PVA:PEG_6:4_Au_HT nanofibers on day 7 of culture, Olympus IX71 epifluorescence microscope, obj. × 20, B. SEM image of the same sample, JEOL JSM-IT800 microscope, magnif. × 60,000.

Keywords:

Gold nanoparticles, antimicrobial properties, cytocompatibility

Reference:

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Fine-tuning the Size of ZIF-L Nanosheets Through Controlled Synthesis

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PS-07 (1), Plenary, august 29, 2024, 15:00 - 16:00

Background incl. aims
To design novel hierarchical nanostructures with predefined properties and functionalities, e.g. for engineering and nanomedicine applications, we need to understand their bottom-up assembly processes as well as inherent structure-property relationships. This will open opportunities for precise synthesis of nanomaterials with targeted functionalities by simply controlling property-related structural characteristics in the bottom-up synthesis process, i.e. controlling size, morphology, crystallinity, etc. [1].
In this context, metal–organic frameworks (MOFs) have emerged as a customizable hierarchical material known for their high chemical and thermal stability. Controlled synthesis of these highly porous crystalline material is primarily about precise control of their chemistry, size, shape, and crystallinity. And, this ability has already been demonstrated, leading to controlled design of MOF structures with well-defined properties for various applications, such as gas storage & separation, wastewater treatment, and catalysis [2] [3]. For example, in catalysis applications where enhancing access of reactants to active sites is crucial, it is advantageous to design MOF nanosheets with precisely controlled thickness, as they offer a larger external surface area and decreased diffusion resistance [4].
Here, we investigate the synthesis process of zeolitic imidazolate frameworks (ZIF) with a leaf-like shape (ZIF-L nanosheets) to understand whether and how precisely we can control the nanosheet thickness (i.e. controlling the surface-to-volume ratio). We apply a series of photon- and electron-based characterization methods to understand the nucleation processes and growth mechanisms of ZIF-L nanosheets.

Methods
The ZIF-L nanosheets were prepared using a standard bottom-up synthesis method in which the synthesis parameters, including ligand-to-metal ratio, selection of additional ligands (modulators) as well as reaction time were systematically varied to synthesize uniformly thin nanosheets with controlled thickness.
A detailed structural characterization was conducted. Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), and X-ray diffraction (XRD) were used to confirm that ZIF-L was successfully synthesized. Crystallinity of the nanosheets was further determined by electron diffraction (ED). The size of the ZIF-L nanosheets (thickness and surface area) was determined using SEM and transmission electron microscopy (TEM).
ZIF-L are extremely sensitive to electron beams (critical dose <<100 e-/A2). Therefore, all TEM experiments were performed under low-dose conditions and/or using a Gatan cryo sample holder (cryo-TEM).

Results

ZIF-L nanosheets were synthesized by systematically varying the synthesis parameters to control their thicknesses. When the synthesis time was reduced from 4 hours to 2 hours at a constant ligand-to-metal ratio of <6, the nanosheet thickness decreased from ~ 135 nm to ~ 95 nm, as measured by SEM and TEM images. FTIR spectra, XRD as well as ED patterns confirmed that all nanosheets were ZIF-L single crystals. By further reducing the synthesis time to 20 minutes [5], the crystal thickness was further decreased to well below 90 nm. However, not all crystals exhibited nanosheet shapes. The particles showed nonuniform thickness and were agglomerated into flower-like shapes. Therefore, modulator ligands in various concentrations were added in the synthesis process to precisely control the balance between nucleation process and crystal growth. SEM imaging confirms that the addition of a modulator ligand resulted in formation of well-defined thin ZIF-L nanosheets even at low reaction times. Further (cryo) TEM experiments were performed to understand details of the ZIF-L growth kinetics.

Conclusion
The precise control of the morphology of a particular nanosheet MOF presented here is a great example of a promising strategy for designing nanostructures with predefined functionalities from the bottom up.
In detail, we provide an approach to design ZIF-L nanosheets with controllable size (i.e. thickness). The synthesis time as well as the concentration of modulator ligands were varied systematically. Their effects on nucleation, crystal formation, crystal growth, and the final leaf-like morphology were investigated by a detailed characterization study.

Keywords:

Metal-organic frameworks, nanosheets, FTIR, TEM

Reference:

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How to better understand ZrCu Thin Films Metallic Glasses recrystallization: a TEM in situ characterization?

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Evolution of nanostructured thin films is closely related to their intrinsic small scale. Therefore, adapted characterization tools and techniques have to be developed, to highlight such relationships. The talk will focus on an advanced generation of surfaces: the thin film metallic glasses (TFMGs), obtained by magnetron sputtering. In this field, the Zr-Cu system does represent a model film, quite well-known at a meso/macro-scopic scale. In particular, its functional properties strongly depend on its crystallization dynamics, which is not clearly established, and needs to be better understood. The objective of the talk is then to demonstrate how specific research strategies involving, in particular, advanced TEM mode (in situ HT) were able to determine the evolution of the films' microstructural evolution.
Metallic glasses (MGs) have been intensively studied since the 60's, for their amorphous structure and associated properties. However, applications of MGs have stayed limited due to the fast quenching imposed to limit the crystallization process, and leading to small pieces of multicomponent materials. The condensation from the vapor phase to form a solid film in PVD process is another way to design metallic glasses. TFMGs indeed may show, for instance, particular interest in terms of physico-chemical and bactericide behaviors, coupled with an enhanced ductility [1]. Thermal stability of such amorphous metals nevertheless remains an issue. A non-conventional in situ technique, high-temperature scanning indentation (HTSI) [2], was first used to monitor the physical changes occurring in ZrCu-TFMGs during heat treatment. A complementary study was also done in parallel with the same film exposed to the same thermal conditions, at the TEM scale using specific heating chip equipped with the Wildfire sample-holder from Dens Solutions. This part will be developed within the framework of the talk. To correlate local transformation with a more global approach, all results acquired at the nanometer-scale were systematically compared with High-temperature XRD characterization, using the same thermal treatment. It was shown, for the first time, that crystallization initiates at ZrO2 nanoclusters as a transformation front, propagating throughout the film (Fig. 1). Acquired every 30 seconds, the front progression was measured, leading to thermodynamic and kinetic key-parameters as coefficients of diffusion. Behind the front, recrystallization results in the formation of bco- Zr7Cu10 intermetallics, identified by HRTEM. The transformation kinetics was also deduced from JMAK' modeling, giving rise to a well-documented new transformation mechanism [3].
From a quantitative point of view, really close crystallization kinetics were observed with our dual global-local approach, with close reaction rate (6.2·10-4 s-1 1 and 9.3·10-4 s-1, respectively) and Avrami exponents (1.9 and 2.2, respectively). Both measurements also give similar activation energies for crystallization (441 kJ.mol-1 and 445 kJ.mol-1, respectively). It suggests that crystallization mechanisms are the same for both investigated scales, despite the different sample configurations (micrometric thin film for XRD versus nanometric thin film for TEM).

Keywords:

TEM, in-situ, recristallization, Metallic glass

Reference:

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combining global and local in situ approaches, Journal of Alloys and Compounds 987 (2024) 174233 doi:10.1016/j.jallcom.2024.174233

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Momentum resolved band gap measurement by high energy resolution electron energy loss spectroscopy

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Techniques based on light optics have been traditionally used for studying optical properties of semiconductors. But there are 2 fundamental limitations of photon sources – 1. Its spatial resolutions are limited to µm range and 2. Photons cannot transfer any momentum (q) to the material. Electron energy loss spectroscopy (EELS) has been used to measure optical band gap for a few decades which compensates the aforementioned two shortcomings of UV-visible spectroscopy with nanometer range resolution and momentum transfer. But the limitation of EELS has been the energy resolution of the electron source; an X-FEG without mono was only 1 eV of resolution. With improvements of monochromator design on Thermo Fisher Scientific Spectra platform microscopes, energy resolution < 20 meV is now possible. Also, the 3-condensor lens system allows for a large range of momentum resolution possible with an upper limit <200 µrad in STEM mode.

In this report, momentum resolved band gap measurement has been performed with an excited monochromator (energy resolution < 25 meV) with a momentum resolution of < 800 µrad using the Thermofisher Scientific EELS filter and spectrometer. Effort is put to automate the acquisition of EELS across the momentum space. In the literature, the modelling of EELS in the low-loss region has been commonly done by simplistic free electron gas model [1].We are developing an effective tight binding model approach to approximate the band gap and extract the low loss spectral function. The final state in the double differential scattering cross section formula has been described suing mixed dynamic form factor (MDFF) [2].

Figure caption: Figure 1. (a) The CBED pattern and the positions where the low-loss EELS measurements have been made. (b) The corresponding EELS spectra.

Keywords:

Extreme low-loss, band gap, q-resolved-EELS

Reference:

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Probing the Sensitive Reactions of Battery Cathodes Through (Cryogenic) Atom Probe Tomography

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
Understanding battery materials and their interfaces (e.g., lithium-containing electrode materials, organic liquid electrolytes, solid electrolytes Interphase (SEI)), is central for advancing the development in Li-ion batteries (LIBs). However, characterizing these materials at the atomic scale presents challenges due to their sensitivity to reactions and electron-beam irradiation, leading to occasional inaccuracies in the precise quantification of elements like Li, C, and N. Atom probe tomography (APT) emerges as an effective materials characterization technique, complementary to electron microscopy, capable of generating 3D chemical maps by imaging the individual atoms with equal sensitivity to all elements[1]. Recent advancements in cryogenic APT workflows have further enhanced its capability to study LIBs with greater precision [2].
Our aim is to understand LIBs with utmost precision and confidence using cryo-APT protocols. APT of LIBs is plagued with multiple problems, including including field-induced delithiation (deintercalation), premature sample failures due to weak interfaces at the grain boundaries and porous structure. Additionally, preventing beam induced damage during sample preparations is a critical concern. Through the innovative design of experiments and novel cryogenic focused ion beam (FIB) sample preparation techniques, we have gained new insights into battery cathode material via. APT. In my presentation, I will discuss the challenges encountered, the solutions developed and the eventual results obtained while studying LIBs through APT.

Methods
In this work, commercial NMC811 ($LiNi_{0.8}Co_{0.1}Mn_{0.1}O_2$) was procured from Targray (Kirkland, Canada). Sample preparation involved the use of dual-beam Secondary electron microscopy-FIB instrument, Helios 5CX Ga (Thermo-Fischer Scientific, Hillsboro, OR, USA). The APT measurements were conducted using local electrode atom probe (LEAP, Cameca Instruments Inc.), either straight flight path (LEAP 5000XS) or reflectron (LEAP 5000XR). Measurements were done at different conditions depending on the samples. The reconstruction of the three-dimensional atom maps and data analysis was carried out using the commercial software AP Suite 6.1.

Results
We have developed an innovative cryogenic specimen preparation technique for battery materials, avoiding beam induced damage transformation from FIB. This involves a novel “redeposition welding” technique, enabling the secure attachment of APT specimens to Si micro-posts without the use of gas-injection system (GIS), see figure 1a [3]. We supplemented the technique with FIB-enabled, in-situ metal coating on the prepared APT specimens, see figure 1b [4]. These methods were applied successfully to study commercial materials like NMC811, LMO and cycled carbon fibers, with thin coatings proving effective in mitigating deintercalation, see figure 1c. This enabled the accurate Li quantification in battery materials using APT, serving as a valuable template for future battery investigation.

In the next study, we will explore how cryogenic APT workflows can easily study thin reactive layers formed on cathode particles exposed to ambient atmosphere, fig. 1d&e [5]. Similar technique can be utilized to study the thin secondary electrolyte interfaces (SEI) layers forms on the active materials of the cycled batteries.

Conclusion

Battery studies using APT offer atomic scale understanding of Li segregation at defects, interfaces and the diffusional behavior of Li in the cycled LIBs. All this will be of huge importance for the development of batteries. We have developed a novel, versatile and robust cryogenic APT workflow, involving whole sample transfer under vacuum and cryogenic FIB sample preparation, yielding relevant near-atomic scale microstructural information on battery samples. Cryo-preparation prevents beam damage, while metallic coating facilitates heat dissipation and provides mechanical support to the specimens against the Maxwell stresses generated by the applied field, a common cause of atom probe specimen failure. This approach provided high-yield, repetitive and precise compositional information across multiple samples. These procedures are further being used to study nanoscale compositional changes happening in the aged battery cathodes or anodes. Additionally, we successfully studied the formation of thin passive layers on the surface of Ni-rich cathodes (NMC811), laying the groundwork for further exploration of SEI layers on cycled materials.

Keywords:

Batteries, Deintercalation, Cryo-FIB, APT

Reference:

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FOUR MICROSCOPY METHODS UNVEIL LIVER FENESTRATIONS: CORRELATIVE SEM, SIM, STED, AFM TARGETING SINGLE CELL TYPE

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LS-04 (1), Lecture Theater 4, august 29, 2024, 10:30 - 12:30

Liver sinusoidal endothelial cells (LSECs) are an excellent research target for developing high-resolution imaging methods due to their unique morphology [1]. LSEC membrane is perforated with multiple transcellular pores called fenestrations. Being in the range of 50-350 nm [2], fenestrations remain largely beyond the resolution limits of conventional optical microscopy, limited by light diffraction. LSECs play a crucial role in realizing the bidirectional transport of substances, especially lipoproteins, between blood and hepatocytes in the liver. Reduced LSEC porosity, called defenestration is often associated with ageing and chronic liver diseases such as hepatitis, steatosis, and cirrhosis. In vitro studies have shown that LSEC porosity can be altered pharmacologically [2,3]. These findings promise the development of a proper treatment allowing to reopen once closed fenestrations or to tune their diameters to achieve proper filtration properties within the liver [4]. Due to the great importance of fenestrations diameters, acting as a functional sieve for particles of a certain size into and out of the liver, the determination of the "true" fenestration size is extremely desirable.

Over the years various nanoscopy techniques have been challenged to disclose fenestrations in LSEC [5]. Obtained values of their diameters often significantly varied between the used techniques. The only way to understand these discrepancies was to visualise the exact same fenestrated areas using several techniques in a correlative manner. Each microscopy technique has its benefits and limitations originating e.g. from image acquisition, resolution, and level of sample preparation. We applied four different microscopy techniques, namely scanning electron microscopy (SEM), structured illumination microscopy (SIM), stimulated emission-depletion microscopy (STED), and atomic force microscopy (AFM), all to address the reported differences in fenestration size distribution.

A quantitative and qualitative description of the differences was made in a correlative manner [1]. Scanning electron microscopy (SEM) was combined with optical nanoscopy methods (STED, SIM). Additionally, atomic force microscopy (AFM) was used in combination with SEM (Figure) and STED, all in order to better understand the differences between literature reports on fenestration dimensions. The latter employed the prototype of a device in which AFM and STED were combined in one device, providing ease in directly overlaying obtained images. Finally, we showed the potential of correlative and comparative 4-dimensional imaging (3D + time) of living cells using AFM with fluorescence microscopy (conducted at the final stage of the experiment).
We concluded that dehydration needed in SEM causes fenestration enlargement, esspecially at the edges of so called sieve plates (Figure, arrowheads). Additionally, some fenestrations closed in hydrated samples, appear as opened after dehydration in SEM. An enlargement of fenestrations is observed when probing sample with an AFM tip if a sample is mildly fixed using formaldehyde. The effect vanishes after fixation using glutaraldehyde. The point of spread function (PSF) should be

considered when calculating fenestrations from images collected using optical nanoscopy. In our hands, smallest fenestrations, below 50 nm in diameter could not be disclosed with STED nor with SIM. Thanks to correlative studies and analysis of fenestrations in a one-to-one manner, we proposed the mathematical formula allowing for recalculation of fenestration size in order to compare data from LSEC imaged using different nanoscopy techniques. It will allow researchers to compare the results collected using different nanoscopy techniques. Finally, our results show promise of a better understanding of biological processes at the nanoscale, by combining the benefits of each method when used in a correlative way.

Research supported by the National Science Centre under the project "SYMFONIA 3", (UMO-2015/16/W/NZ4/00070), SONATA 15", (UMO-2019/35/D/NZ3/01804), and Research Council of Norway Nano2021 (288565).

Keywords:

fenestration, liver, correlative microscopy, AFM

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Investigating the formation of surface reconstruction layers in Ni-rich cathode materials using STEM

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
Lithium-ion batteries (LIB) are a key technology towards an emission-reduced transportation sector, in particular for electrical vehicles (EVs). One of the most promising cathode materials for LIBs are Ni-rich NMC cathode materials (LixNiyMnzCo1-y-zO2, with x > 0, y > 0.6). The main benefits of Ni-rich cathodes are an increased capacity, a high energy density and lower cost compared to conventional LIBs, which are needed to push the mileage limit and to make EVs more affordable. Although the advantages are great, Ni-rich NMC suffers from thermal and structural instabilities leading to a shorter life time and server capacity fading. The main degradation mechanisms occurring in NMC are: formation of surface reconstruction layer, formation of cathode-electrolyte interface (CEI), degradation of electrolyte and cracking of particles. [1,2]
This study focuses on the formation of surface reconstruction layer, specifically on the phase transition from layered structure (space group R-3m) to rocksalt phase (space group Fm-3m). [3] In the rocksalt phase the transition metals occupy the Li-positions and thus block the Li-diffusion paths. [4] A core-shell model describes the growth of this surface reconstruction layer, in which the degradation starts at the top of the surfaces and propagates into the bulk. [4] The aim of this study is to observe the phase change from layer structure to rocksalt structure and provide an answer to the questions: Does the rocksalt layer grow over time while the cell is kept under the harsh conditions of 4.5V and if so, is a trend in thickness growth of the rocksalt layer measurable?
Methods
A set of coin-half-cells against lithium was kept at high voltage of 4.5V for over 30 days. Every 2 days an electrochemical impedance spectroscopy (EIS) measurement was conducted. Afterwards, the cells were dissembled and the cathode sheets were rinsed with solvent. Using FIB-SEM allowed to cut very thin lamellas out of the cycled cathode sheets. However, preparing the sample for analysis presents several challenges. Firstly, the organic binder that surrounds the NMC particles melts faster under the ion-beam, leading to instabilities. Secondly, powder samples tend to redepositing and curtaining due to the pores in between. Finally, NMC is sensitive to the ion-beam, requiring lower voltages to be applied to reduce the amorphous layer on top. STEM was used to image the surface area of the particles down to atomic scale. Since the electron beam affects the NMC material and can lead to a phase transition from layered to rocksalt structure, it is very important to control the dose of the electron beam. [5] In addition, EEL spectra were acquired to provide a better understanding of the degradation process by monitoring the valance states of the transition metals. Moreover, further analytical methods such X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) were applied to investigate e.g. CEI formation.

Results
The EIS measurements showed an exponentially growing charge transfer resistance (Rct) which is an easily accessible indicator for degradation. Looking at the particles at atomic scale, the formation of rocksalt was present as depicted in Figure 1. The transition from layered to rocksalt appeared gradually and a typical transition region was visible. Interestingly, the variation of the thickness of rocksalt layer varied significantly within a lamella and even within one particle. It could be observed that the surrounding of the surface influenced the tendency to form rocksalt. A comparable, continuous trend in the growth of the surface reconstruction layer, as the Rct suggested, was not evident globally. However, the maximum thickness of degraded surface increased over time.
Conclusion
HRSTEM coupled with EELS provided a great insight into the rocksalt formation. The study indicates that while the core-shell model is generally a valid approximation for describing surface reconstruction in Ni-rich NMC, the real world is more complex. Therefore, it is crucial to comprehend the conditions under which a rocksalt structure is formed. Furthermore, understanding the time-correlation of different degradation mechanisms is crucial to improve battery materials.

Keywords:

Li-ion batteries, degradation, high voltage

Reference:

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Breaking the limits of functional Atomic Force Microscopy imaging using Focused Electron Beam Induced Deposition

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Atomic Force Microscopy (AFM) has evolved into an indispensable characterization method as it provides quantitative 3D surface information with spatial nanometer resolution. It also enables access to electric, magnetic, optical and thermal properties using specialized functional AFM tips. However, achieving this functionality necessitates additional coating on AFM tips, resulting in increased tip apex size and thus limiting resolution. Moreover, coating introduces the risk of delamination, which could compromise or entirely diminish the functionality of the tip. The aim of our research is to surpass the limitations of functional image quality. To be more precise, it was of interest to achieve (1) higher resolution for functional AFM tips, (2) higher sensitivity for e.g. EFM/MFM phase signal, (3) good signal to noise ratio, and (4) higher wear resistance, compared to commercially available tips.
We have utilized the additive direct-write technology Focused Electron Beam Induced Deposition (FEBID), which enables fabrication of all-functional nano-probes that do not require additional coating. Depending on the application, different requirements become relevant such as tip design, type of precursor (elemental composition), spring constants, and so on. The 3D nano-printing process [1] and a variety of advanced, FEBID-based tip concepts for CAFM, EFM [2], and MFM [3] will be briefly discussed.
Ideal fabrication parameters were individually identified with subsequent determination of post-processing steps based on the required precursor, if needed. The FEBIP tips were then compared with commercially available products in terms of image quality, including resolution, signal-to-noise ratio, wear resistance, among others. After thorough testing of magnetic (M) and conductive tips (C), we give an outlook on further expanding AFM tip capabilities by combining both functionalities, produced with a single precursor. While in the past, MFM and CAFM measurements are operated via different modes, the here introduced MC fusion-probes contain unique possibilities, as both techniques can be performed in a single AFM-CAFM-MFM experiment. We present first results, which form the basis of this new type of nanoprobes.
FEBID presents an ideal technique for pushing boundaries in functional AFM measurements. All probes share the coating-free character, thereby mitigating the delamination risks during operation. Furthermore, tip apexes are consistently within the sub-10 nm range, enabling high-resolution imaging beyond alternative products.

Keywords:

FEBID, AFM, EFM, CAFM, MFM

Reference:

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Novel polymer thin film fabrication for graphitization studied by in situ transmission electron microscopy

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Poster Group 1

Due to its excellent physical, chemical and electrochemical characteristics, pyrolytic carbon has emerged as a promising material for various technological applications [1]. Pyrolytic carbon can be obtained through the pyrolysis of a polymeric carbon precursor under controlled conditions at high temperatures and in inert atmosphere. By tuning the pyrolysis conditions, the hybridization of carbon atoms and thus the physicochemical properties of the derived carbon can be tailored. Although some researchers have attempted to investigate the graphitization process in atomic scale, a comprehensive understanding remains elusive. Transmission electron microscopy (TEM) is well-suited for investigating the graphitization of polymer thin films during the thermal treatment process at the nanoscale [2]. Indeed, TEM offers the advantages of in situ analysis capabilities which can reveal the nanostructure of pyrolytic carbon during the pyrolysis process. However, the preparation of polymer thin film samples for TEM remains a challenge. This work presents the microfabrication of suspended polymer thin film structures on MEMS-based TEM heating chips (DENSsolutions Wildfire), by two-photon polymerization (2PP) 3D printing technology [3]. We also report the results of the in situ TEM studies for tracing the graphitization of pyrolytic carbon.
A Nanoscribe Photonic Professional GT+ system from Nanoscribe was used to fabricate suspended polymer thin films using DENSsolutions chips as substrate. The microfabrication of thin films was done by direct laser writing of IP-Dip photoresist using 2PP with a Plan-APOCHROMAT 63×/1.40 Oil DIC objective. The optimized printing parameters were a laser power of 50%, a scan speed of 12000 µm/s, a hatching distance of 0.4 µm and a slicing distance of 0.1 µm. After printing, the samples were developed by a 20 min immersion in propylene glycol monomethyl ether acetate (PGMEA), followed by 20 min immersion in isopropanol. Finally, the structures were left to dry horizontally at room temperature.
TEM was performed with an FEI Titan 80–300 environmental transmission electron microscope. The microscope was operated at 300 kV at 10-8 Pa. In situ TEM was carried out with a TEM heating sample holder (Wildfire, DENSsolutions). Images were recorded on a Gatan OneView camera at an exposure time of 0.2 s. Images were post-processed using DigitalMicrograph (Gatan, Inc.).
To fabricate a thin film for visualizing the graphitization of carbon in the TEM, several designs and pyrolysis conditions were studied and optimized before selecting the optimal ones. The dimensions and integrity of the structure post-pyrolysis were assessed using SEM and the nanostructure of the resulting carbon was investigated by TEM. Based on these preliminary tests, we identified favorable parameters to resolve graphitic domain formation in the TEM. The identified conditions are a thin film with a thickness of around 700 nm pre-pyrolysis, a pyrolysis sequence reaching a maximum temperature of 1300°C and a minimum of 2 hours of dwell at this temperature. The final thin film design is presented in Figure 1a. The sample consists of a suspended ribbon with a center through-hole to facilitate imaging of the suspended thin film all around the edge of the ring. The central ring is 700 nm thick for TEM imaging (in dark grey in Figure1a) while the surrounding ribbon is thicker (900 nm) to provide support and strengthen the overall structure (in light grey in Figure1a).

Figure 1b & c show the structure after printing and after pyrolysis, respectively. From these images we see that the ribbon, the window, and the hole are well-defined both before and after pyrolysis. Three of the in situ TEM micrographs acquired during the dwell at 1300°C, are presented in Figure 1d to 1f. The images are acquired every 30 minutes, with the beam blanked between acquisitions. The formation of oriented carbon microdomains is visible, and their nature as graphitic carbon is affirmed by the measured inter-layer distance of 3.3 Å, a characteristic feature of graphitic materials. An increase in the number of graphene stacking layers was observed over dwell time. It is worth noting that since the beam is blanked in between acquisitions, these observations are valid independently of the beam influence.
To conclude, we demonstrated the successful 2PP fabrication of suspended polymer thin films for in situ TEM studies, as well as the visualization of graphitization on these films during in situ TEM heating studies.

Keywords:

in-situ TEM, pyrolysis, graphitization, two-photon-polymerization

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Single-cell pharmacology: atomic force microscopy and spectroscopy for multiparametric imaging of drug-induced alterations in vitro

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Since its first construction by Gerd Binnig, Calvin F. Quate, and Christoph Gerber in 1986 [1], it took only a few years to disclose the tremendous potential of atomic force microscopy (AFM) in the research of biological objects [2]. It is mainly because AFM does not need sample modification and labelling. Freedom of selection of conditions, including performing measurements in culture media, control of pH, and temperature combined with large x,y, and z piezo-scanner ranges all have led to the rapid development of live cell imaging. Finally, novel AFM imaging modes, based on a fast acquisition of force-distance curves, such as Quantitative Imaging (QI), PeakForce Tapping, or PinPoint have enabled 4D (3D plus time) imaging of changes in biological samples.

Here, the results of the development of so-called in vitro pharmacology on a single cell will be presented [3-5]. Briefly, the methodology allows tracking the single-cell morphology and its nanomechanical properties over time. Then, a drug is injected into the culture medium and the cell response to the drug is further observed. It allows monitoring of morphological features and the cytoskeleton remodelling, including its effect on alterations in Young's modulus distribution. Depending on the area of interest single frame is collected in the range of minutes to several seconds allowing observation of drug-induced changes in the morpho-mechanics of cells.

The main focus will be devoted to primary murine liver sinusoidal endothelial cells (LSEC) in vitro. LSEC have transcellular pores, called fenestrations, that are indicators of the healthy phenotype of the liver. These nanostructures – 50-350 nm in diameter – participate in the transport of lipoproteins and solutes (e.g. hormones) between the vascular system and the liver parenchyma. AFM remains the exclusive tool allowing monitoring of drug response in living LSEC for up to 6 hours. Fenestration number, diameter, lifespan, migration range, and deformability can be quantified [3]. Moreover, Young's modulus distribution over the whole cell can be calculated [5]. We show that fenestration lifespan varies from minutes to hours. During this time fenestration changes their diameters and migrates within the cell as far as several micrometers. We employed so-called loading force-dependent tomography to assess the deformability of fenestrations. We test the established methodology in several pharmacological strategies aiming to restore fenestrations in LSEC originating from wild-type animals and genetic knockouts.

We conclude that novel AFM provides multiple parameters for quantifying drug responses in LSECs and their fenestrations in real time. Introducing in vitro pharmacology on LSEC fenestrations will enhance understanding of the mechanisms underlying fenestration formation and function.

Research supported by the National Science Centre
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Keywords:

fenestration, live cell imaging, AFM

Reference:

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Segregation to Creep-induced Planar Faults in Ni-base Single Crystal Superalloys

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Nickel-base single crystal (SX) superalloys have long been served as indispensable materials for turbine blades in aerospace gas engines, owing to their outstanding creep properties. The unique mechanical resistance is attributed to the coherent γ/γ' microstructure in which the cuboidal γ' precipitates exhibit an ordered L12 crystal structure embedded in γ matrix channels, which is a solid solution with a face-centered cubic (FCC) structure. In severely harsh operation environments, the mechanical properties of superalloys are primarily controlled by the precipitate shearing events associated with elemental segregation to and away from dislocations and planar faults within the γ' phases [1].

The present work focuses on understanding the alloying segregation to dislocations and planar defects. Some key aspects under investigation are: a) Do the nano defect phases, formed through elemental segregation, contribute to the stabilizing defect phases within the γ' precipitate? b) Does the elemental segregation to defects result in a reduction of defect energy, thereby facilitating the cutting of the γ' precipitate by the defects? To address these aspects, we designed double creep shear specimens such that deformation takes place along a specific loading direction [11-2] to activate the slip system [11-2](111) with Schmid factor of 1. Local high-resolution energy dispersive X-ray (EDX) is used in scanning transmission electron microscopy (STEM) mode to measure the elemental segregation to planar defects as a function of creep strains (1% and 2%). Both creep-deformed sample states allow us to differentiate whether a compositional steady state, and consequently a nanophase, has formed in the vicinity of the crystal defects[2], or if transient states are measured. Transient states would indicate ongoing diffusion processes that potentially control the kinetics of shear processes [3].

Microstructural characterization post creep deformation with the low magnification high-angle annular dark-field (HAADF) STEM micrograph of the 1% crept sample in Figure 1(A), and the conventional dark field two-beam condition image of the 2% crept sample in Figure 1(C) both depict the termination of planar fault motion inside the precipitate, indicating a leading partial dislocation shearing segment within the γ' phase. High-resolution HAADF images in Figure 1(B) and (D), elucidate that along these leading segments, the nature of the largest stacking fault is a superlattice extrinsic stacking fault (SESF), while at the very tip, in front of SESF we observe a short complex intrinsic stacking fault (CISF). Both faults show the same defect configuration: a closely spaced pair of Shockley partial dislocations with identical Burgers vector1/6[11-2] glide on adjacent (111) planes. This process creates two energetically unfavourable CISFs, which are known to subsequently reshuffle Al-Al atom positions to convert the high-energy fault structure into a stable SESF with low planar fault energy [4].

The chemical distributions across the SESFs in Figure 2 illustrate almost identical segregation tendencies for 1% and 2% crept samples. The γ forming elements such as Cr, Co, W, or Re are enriched in the vicinity of the SESF, while γ' alloying elements Ni and Al are depleted. Quantitatively comparing the local alloying element concentration magnitudes at the SESFs show that the 1% crept sample exhibits the same local composition as the 2% crept sample, even though it experienced a longer creep time. This observation concludes that the segregation to the SESF has reached a steady state and establishes a stabilized nano-defect phase within the SESF after 1% creep strain. This result suggests that with a longer creep time, alloying elements do not further diffuse to the SESF.

Keywords:

superalloys, stacking fault, reshuffling, segregation

Reference:

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Polar textures in multiferroic BiFeO3-based superlattices

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Poster Group 1

Background incl. aims
Over the last few decades, the properties of ferroelectrics and their polar arrangements have been extensively studied in thin films or bulk systems. However, it is only recently that ferroelectric-based superlattices (SLs), i.e. an alternation of two layers with a chemical period Λ, have emerged and leaded for example to the discovery of exotic polar textures such as skyrmionic states or vortices in PbTiO3/SrTiO3 SLs. [1,2] Thus, SLs offer a unique and promising new platform to study these novel polar states which result mainly from the effects of strain, confinement, and competing interactions between the degrees of freedom (polar displacements, oxygen tilts) of these perovskite layers. BiFeO3/LaFeO3 (BFO/LFO) SLs, have also showed interesting features in recent work.[3] BFO is, in essence, the model multiferroic material which exhibits in its bulk form ferroelectric polar states coexisting with a complex magnetic state. However, when it's incorporated in such SLs, an unusual antiferroelectric like-state is observed. Here, the aim of our study is to understand, through scanning transmission electron microscopy (STEM) characterization, how the buffer (LaFeO3 or NdFeO3) layers influence the polar states of BFO.
Methods
BFO/LFO (or NFO) SLs were grown on cubic single crystal (001) STO substrate by pulsed laser deposition (MECA 2000 chamber) using a KrF laser (248 nm). BFO and LFO (or NFO) layers were grown at the same conditions under 0.5 mbar of oxygen pressure (PO2) at 740°C at 6 Hz repetition rate. Cross-section transmission electron microscopy (TEM) samples were prepared on an FEI ThermoFisher Helios Nanolab 660 by using the standard lift-out technique. The annular bright field (ABF) and high angle annular dark field (HAADF) STEM images were acquired by a FEI Titan3 G2 80-300 microscope, operated at 300 kV and equipped with a Cs probe corrector. The analysis of atomic displacements has been performed by the Python open-source library Atomap and Temul-Toolkit library for visualization.[4,5] In complement, STEM images were also analyzed by a "render-and-compare" (RAC) method based on Principle Component Analysis (PCA).
Results
We analyzed several BFO/LFO and BFO/NFO SLs, with various periods Λ (3, 6, 12 and 20 nm) and same BFO/LFO (or NFO) thickness ratio, deposited on STO substrates. We were able to reveal various polar textures within the BFO layers by analyzing the relative atomic displacements as well as the Fourier components with Atomap and RAC respectively. Among them, we unexpectedly observed in-plane and ~45° ferroelectric displacements, but also step-like polar arrangements resulting in a quadrupling of the unit cell confirmed by fast Fourier transform (FFT) images. While Atomap indicates an (up-up-right-right)-like shifts (see Figure), RAC rather suggests a more complex polar distribution with 4 different types of displacements. Moreover, some polar singularities showing hedgehog-like, i.e. skyrmionic textures, were also observed revealing the wild variety of the polarization in BFO.
Conclusions

STEM analysis shows that introducing BFO in SLs systems induces drastic changes of its polar textures, mainly by strain and confinement effects, thus showing the high potential for modulating its polarization. Besides, the comparison of data analysis methods shows that Atomap and RAC are complementary into revealing the nature of the observed displacements.

Keywords:

HR-STEM, ferroelectricity, polarization, data analysis

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Characterization of inorganic food additives and pearlescent pigments in sprays for food decoration by STEM-EDX

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Poster Group 2

Background and aims:
Food colorants are applied to enhance the appearance of food. To obtain specific hues, inorganic food additives containing (nano)particles are mixed in varying concentrations, and pearlescent pigments, consisting of mica platelets coated with a layer of titanium dioxide and/or iron oxide particles, are applied to provide glitter effects [1]. For assessing potential risks of multi-constituent substances and mixtures, characterisation of the fraction of small particles, including the particle size distribution, is needed for each single constituent or each component in the mixture [2]. This is challenging for control laboratories and only limited guidance is currently available [3].
This study developed electron microscopy-based methods to identify and characterize the particles of individual components in 8 commercially available food-decoration sprays of different colours, containing mixtures of food additives and pearlescent pigments.

Methods:
To avoid altering the properties of the particles consumers are exposed to, no extensive dispersion protocols or purifications steps were applied. Samples were prepared by spraying 5 mL in a glass vial and coated on EM-grids using the grid-on-drop method. Scanning transmission electron microscopy (STEM) combined with energy dispersive X-ray spectroscopy (EDX) and EDX-tomography were applied to image the various components. Subsequently, image processing using the ImageJ software was performed to obtain quantitative results.

Results:
The various components were identified based on their elemental composition, including potassium aluminum silicate-based pearlescent pigments, vegetable carbon, rutile titanium dioxide, iron oxide and aluminum containing (nano)particles. Their presence, size, and relative concentration varied among the different spray colours. Often the layer of titanium dioxide particles detached from the mica substrate, and titanium dioxide particles were also observed forming near-spherical aggregates. STEM-EDX tomography allowed identifying particles of overlapping components and examining the structure of the pearlescent pigments in 3D. This way, all components in the mixtures could be successfully identified, and the particle size distributions of each components in all but the most challenging mixtures could be obtained.

Conclusion:
The developed electron microscopy-based methods allowed identifying the different components in the mixtures, and the presence of a fraction of nanoparticles in each component was demonstrated based on their number-based particle size distributions. The methods and findings support regulatory bodies in assessing the potential health risks of mixtures of (nano)particles used in food-related applications.

Keywords:

pearlescent pigments, STEM-EDX, nanomaterials

Reference:

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Optimizing data acquisition and interpretation in atomically resolved STEM spectrum imaging

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Poster Group 1

Recent advances in instrumentation hardware have made chemical analysis, i.e. electron energy-loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDXS), at atomic resolution readily possible. However, the acquisition and interpretation of atomically resolved spectra can still be problematic due to, for instance, image distortions or poor signal-to-noise ratio (SNR) spectra, especially for investigating energy-loss near-edge fine structures. To achieve a decent SNR, STEM spectrum imaging (SI) requires much higher electron doses and longer dwell times (pixel exposure times) than for STEM imaging. Consequently, these distortions become more serious in SI data. Instabilities combined with long dwell times may create substantial image distortions, i.e. expansion, compression, and/or shearing of the lattice that limit the interpretability of the results.

In this contribution, we report the development of on/off-the-fly methods for optimizing atomically resolved STEM SI. The first technique is used to post correct (off-the-fly) image distortions in atomically resolved SI and diffraction imaging [1]. We demonstrate the correction of linear and nonlinear distortions, the feasibility of this tool for 4D-STEM diffraction imaging, and the application for maximizing the elemental mapping area. In the second technique, by combining multi-frame spectrum imaging and automatic energy-offset correction, we report an on-the-fly SI technique for minimizing image distortions and improving the SNR of EELS spectra [2]. The energy-offset correction technique significantly reduces correlated noise [3], as for successive spectra different camera pixels are exposed which precludes amplification of small gain normalization errors. We have implemented these techniques into STEM SI for atomically resolved EELS elemental and fine-structure mapping. Using practical examples, we demonstrate that multi-frame SI and post-alignment can effectively suppress image distortions and improve the final elemental-map quality. The energy-offset correction method reduces the correlated noise and helps to resolve weak features of the near-edge fine structures. The final SI with improved SNR enables the extraction of individual component maps of the Ti-L2,3 near-edge fine structure and of a Ti-O-Ti bonding direction map at atomic resolution in SrTiO3 (Figure 1), which has been theoretically predicted but extremely difficult to detect experimentally due to the poor SNR of the spectrum [4]. Combining multi-frame SI and automated energy-offset correction, we demonstrate that these techniques will open new opportunities for atomically-resolved EELS fine-structure mapping. Moreover, this multi-frame SI technique also paves the way for low-electron-dose imaging techniques to reduce damaging of the sample [5], as well as the possibility for atomically resolved imaging and SI at cryogenic temperatures. In the last, their application in battery and catalysis materials will be discussed.

Figure 1. Fine structure mapping of the Ti-L2,3 and O-K edges in SrTiO3 via multi-frame and energy-offset correction acquisition. (a) Schematic diagram showing the processing of the multi-frame SI. (b) The individual components map of the Ti-L2,3 edges: L3 t2g, L3 eg, L2 t2g, and L2 eg, respectively. (c) Structure of SrTiO3. Oxygen bonding direction maps extracted from multi-frame EELS SIs obtained by MLLS fitting to the O1- and O2 -K edge spectra.

Keywords:

electron energy-loss spectroscopy, fine-structure mapping

Reference:

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Progress in Magnon EELS simulations

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PS-08 (1), Lecture Theater 2, august 27, 2024, 10:30 - 12:30

Background incl. Aims

Magnons, the quanta of spin waves, play a pivotal role in various solid-state phenomena, including memory and information processing [1]. Understanding their behavior at the nanoscale is crucial for advancing magnetic technologies. Electron energy loss spectroscopy (EELS) holds promise for probing these excitations with high spatial resolution [2, 3], potentially enabling the exploration of magnon dispersions down to the atomic scale. However, experiments face substantial challenges due to the overlap in excitation energy ranges between phonons and magnons, with phonon signals typically overshadowing magnon signals significantly [3]. Hence, elucidating optimal conditions for discerning magnon EELS signals remains crucial.

In this work, we present our progress in simulating inelastic electron scattering signals of magnons in Scanning Transmission Electron Microscopy, discussing the feasibility of Magnon EELS detection.

Methods

We implement a new methodology [4] to compute magnon EELS from Pauli-multislice simulations. In particular, this methodology integrates state-of-the-art simulation techniques to model magnon spectra, including the Frozen Magnon Multislice Method with absorptive-potential-based phonon scattering [3].

Results

We present findings suggesting the potential for detecting statistically significant magnon signals by selecting combinations of temperature and sample thickness [3]. Additionally, we show the capabilities of our methodology by presenting angle-resolved magnon EELS simulations.

Conclusion

Our simulations suggest the existence of feasible conditions to differentiate magnon inelastic signals from phonon signals. Also, we show the potential of our developed methodology to simulate angle-resolved magnon EELS signals.

Keywords:

Magnons Phonons EELS Multislice

Reference:

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Study of Lithiation Dynamics in Cathode Materials by in situ TEM Electrochemical Liquid Technics.

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Poster Group 2

Lithium-ion (LIB) batteries' technology has evolved rapidly to be on par to the increasing demands of the market. The EV (Electric Vehicle) market requires batteries presenting high safety, higher energy density and duration. For instances, one of the cathode materials suitable for EV application is NMC 811, however, its accelerated degradation leads to a short battery life. For the further development of EV suited batteries, the understanding of NMC 811 degradation dynamics is crucial.
For a proper study of the dynamics of (de)lithiation in NMC 811 cathode material, an in-situ analysis at the primary particle scale is proposed. The electrochemical liquid TEM analysis configuration [1] allows the performance of battery cycling inside the TEM for imaging at different SoC (state of charge), inducing less of perturbations during data acquisition and imaging analysis. The information obtained could reveal the behavior of lithium inside the crystallographic, as well as other phenomena that lead to battery degradation.
One of the main causes of degradation in NMC 811 is due to the formation of a passivation layer during cycling, the CEI (cathode electrolyte interface) [2]. The CEI formation take place during the first cycles of the batterie, at the interface between the active material and the electrolyte, organic and inorganic compounds (LiF, LiOH, Li2Co3, etc.) are formed, consuming active Li which leads to the reduction of the batter capacity and degradation of the electrolyte. The CEI layer is not stable, dissolution and reformation of it occurs during each cycle, reducing the batterie life. STEM-EDX and 4D-STEM microscopy technics can be used to follow the formation of the CEI.
4D-STEM technique allows us to obtain structural information based on electron diffraction pattern collections (50k patterns) with a spatial resolution of 1-2 nm. [3-5]. Using this method, it is possible to reconstruct it via pattern matching, a phase mapping of the present inorganic components in a CEI as has already being done for ex situ analysis of NMC 811 (figure 1) [2]. Coupling 4D-STEM and STEM-EDX analysis for different SoC (State of Charge) will allow us to follow the behavior of the CEI layer and determine the dynamics behind it.
The obtained information could lead to the comprehension of the strains present at the primary particle level that could eventually lead to the fracture of secondary particles and the degradation of the material as reported several times in literature.

Keywords:

In-situ, 4D-STEM, NMC, CEI

Reference:

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Towards Quantitative Liquid Phase Electrochemistry for Understanding Electrochemical Processes

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Poster Group 1

Background incl. aims

Liquid phase transmission electron microscopy (LPTEM) has emerged as a powerful tool, presenting enormous potential for understanding the electrochemical processes behind electrolysis, batteries, and other technologies [1]. However, obtaining high-resolution structural and chemical information during these electrochemical processes remains a challenge due to the issue of liquid thickness. While utilizing a monolithic MEMS-based nanoreactor with integrated electrodes can be a potential solution, it too faces limitations from an electrochemical perspective. To ensure proper ion conduction to mirror processes occurring in macroscopic cells, LPTEM studies require a thicker liquid layer, which directly contradicts the need for high-resolution information. On the other hand, obtaining high-resolution information through post-mortem studies, by disassembling a typical two-chip nanoreactor, too is not a viable solution due to possible changes due to absence of the native liquid environment. Furthermore, ideally high-resolution information is required at different stages of in situ experiments. These necessitate the ability to dynamically control the liquid thickness. novel method that enables high-resolution and analytical electron microscopy studies within a liquid flow cell [2].

Methods

Smart micro electrical mechanical systems (MEMS) and holder designs have enabled us to control liquid flow and thickness, allowing us to create a stable and reproducible environment that is ideal for investigating the electrochemical processes of interest [2,3]. In particular thanks to the on-chip flow capability, the liquid in the field of view can be efficiently reduced by flowing gas, which is termed “purging” [4]. This purging method enables the acquisition of high-resolution TEM images, chemical composition and valence analysis through energy-dispersive X-ray spectroscopy (EDX) mapping and electron energy-loss spectroscopy (EELS). In addition, the purging approach is both reversible and reproducible, which therefore enables the alternation between a thick and a thin liquid configuration. This provide us the necessary dynamic control over the liquid thickness to perform the electrochemical experiments in a thick liquid configuration and then perform anlytical studies including 4D STEM at the thin liquid configuration to enable the best resolution without changing the state of the sample. This coupled with simple image processing that has allowed us to extract 3D information from the in-situ image series and a step towards live 4D STEM provides the pathway to developing new and more efficient energy technologies.

Results

Dendrite growth during cycling is one severe problem to harm durability and safety of energy applications, so many efforts have been studied to inhibit the dendrite growth. One example case is aqueous zinc (Zn) based battery chemistry, including redox flow battery, which has recently generated significant interest as an alternative battery technology for stationary applications beyond Li-ion batteries. The main driving force is the high volumetric capacity of aqueous zinc batteries, low cost, safety, and abundance of Zn metal. Despite these advantages, alongside electrode passivation, anode shape change, and H2 evolution, the problems of Zn dendrite growth cause premature battery failure and safety hazards, severely limiting the progress and further commercial exploitation of aqueous Zn batteries. Direct visualization of dendrites under operando conditions can lead to an in-depth understanding and the development of the most effective mitigation routes toward achieving a compact plating and smooth stripping of Zn during battery cycling, which is a prerequisite for battery safety, longevity, and viability. Thus we apply the developed method for dynamic control of liquid thickness and image processing to gain 3D growth along with crystallographic orientation information of the Zn deposits during electrodeposition using 0.1 M ZnSO4. Figure 1. Showcase dendrite formation and extracted 3D information from the in-situ image series.

Conclusion

The 3D visualization of electrodeposition at the nanoscale enabled us to study how different electrolyte additives affect growth across all three planes (XY, YZ, and XZ) and thus establish a workflow that researchers can utilize to optimize electrolytes to promote compact deposition and mitigate unwanted features like dendritic growth for Zn and other battery chemistries and electrodeposition processes in general.

Figure Caption

Figure 1: Investigation of Zn dendritic growth during plating in static 0.1M ZnSO4. (a-c) STEM images obtained at 7th, 8th and 9th seconds. (d) The plated Zn volume vs. time. (e-g) Processed images show the Zn dendritic growth between 7-8, 8-9, and 9-10 seconds. A color map on (g) represents the thickness [µm] at that second.

Acknowledgments

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Keywords:

liquid phase TEM, 4DSTEM, 3D

Reference:

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Correlative cryo-bioimaging to study coronavirus replication organelles

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background incl. aims
The Gammacoronavirus Infectious Bronchitis Virus (IBV) is a highly contagious pathogen of poultry and can be used as a model coronavirus (CoV) to study the formation and structure of CoV replication organelles (ROs). CoVs rearrange host cell membranes into ROs which act as platforms for the viral replication-transcription complex and are the sites of RNA genome replication. Conserved RO structures exist including double membrane vesicles (DMVs) and double membrane spherules (DMSs). The mechanisms of RO formation and the function of DMSs have not yet been elucidated. Using fluorescent recombinant viruses and correlative cryo-bioimaging, ROs will be marked and directly targeted for imaging using X-rays and electrons. These techniques will reveal RO structures over multiple scales, in 3D, to high resolution, and under near-native conditions. The aims are to develop a correlative workflow to observe the RO network in whole cells, RO formation events, and DMSs under cryogenic conditions. Subvolume averaging of electron tomograms will reveal membrane associated proteins which may give clues to RO formation mechanisms and functions.
Methods
Fluorescent tags were inserted at the N-terminus of nonstructural protein 2 (nsp2) using reverse genetics. The growth kinetics of recombinant IBVs were characterised in comparison to wild type. Viral stocks were concentrated to optimise the number of sites of nascent viral RNA synthesis able to be detected by immunofluorescence imaging. Resin-embedded correlative light and electron microscopy (CLEM) was used to confirm RO formation by the recombinant virus. Cryo-sample preparation by plunge freezing has been used to make grids for cryo-correlative light and soft X-ray tomography (SXT, B24, Diamond Light Source), correlative cryo-focused ion beam (FIB) milling, and cryo-electron tomography (ET, eBIC, Diamond Light Source).
Results
Recombinant GFP-nsp2-IBV does not significantly attenuate viral replication. GFP-nsp2 colocalises with sites of nascent viral RNA synthesis as seen by immunofluorescence imaging. GFP-nsp2 marks known IBV RO structures as seen by CLEM. Correlative cryo-SXT has revealed RO networks in whole cells to 30nm resolution. Plus, correlative fluorescence microscopy, FIB milling, and cryo-ET has resolved the structure of the DMS bilayer. Sub-volume averaging on cryo-electron tomograms will gives clues to the DMS protein composition and their functions.
Conclusion
Fluorescent-tagged viruses are being used in combination with advanced imaging to further understand CoV ROs. This work will significantly improve our knowledge about the sites of CoV RNA synthesis and this critical stage of the virus lifecycle. Consequently, novel replication mechanisms of current and emerging positive-sense single-strand RNA viruses may be revealed.

Keywords:

Correlative imaging, cryo-electron tomography, coronavirus

Reference:

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Enhancing tool performance with complex microscopy investigation of additively manufactured M2 steel and composites

Ing. Ph.d. Martina Koukolíková[1], Mr. Pavel Podaný[1], Mr. Miroslav Urbánek[1], Mr. Michal Brázda[1], Mrs. Ivana Poláková[1], Mr. Josef Hodek[1]
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Poster Group 2

Background incl. aims
The study explores the application of complex microscopy and imaging techniques to investigate the microstructure of M2 steel and carbide-reinforced composites additively manufactured (AM) using powder-based Directed Energy Deposition (DED). Aiming to understand the material's properties and behaviour under various conditions, the research emphasizes the role of microscopic analysis in enhancing tool and material performance. The emergence of additive manufacturing techniques, such as DED using powder feedstock, has opened new avenues for fabricating components with intricate geometries, complex material variations, and tailored properties. M2 steel is renowned for its exceptional mechanical properties, making it a preferred choice for high-speed steels. Carbide-reinforced composites investigate the impact of incorporating different concentrations of tungsten carbide (WC) particles into a high-temperature nickel-based superalloy Nimonic 80A for applications requiring improved wear resistance, such as forming tools. The role of microscopic analyses in the enhancement of manufacturing processes and material performance is emphasized by the research. These insights will serve as a basis for the digitalization of material testing and the development of material models using finite element methods.
Materials and Methods
The materials were fabricated by DED using a INSSTEK MX-600 system, which is equipped with a 2-kW yttrium fibre laser, under a protective atmosphere of argon 5.0 in a direct metal tooling (DMT) mode. By continuously monitoring and adjusting the laser power multiple times per second, the DMT system ensures a consistent layer by automatically reducing or increasing the laser power. The 800 and 1600 modules (laser-beam spot size: 800 µm and 1600µm) were applied. The powders were fed from separate feeders to the nozzle. The average particle size of the powders was declared by the producer to be 50-150 µm. Nevertheless, powder analyses were conducted adhering to the ISO 13320 standard. This methodology facilitated the precise quantification of particle size and particle fraction across designated particle size ranges through the utilization of laser scattering techniques. The metallographic analyses involved a detailed examination and comprehensive evaluation of the microstructural characteristics inherent in the as-built condition. The study delves into the utilization of advanced microscopy and imaging techniques, encompassing both light and electron microscopy techniques coupled with Energy Dispersive X-ray Spectroscopy (EDX) and Electron Backscatter Diffraction (EBSD). These sophisticated techniques were employed to scrutinize the intricate microstructure of additively manufactured tools, particularly with M2 steel and carbide-reinforced composites, enabling detailed analysis of microstructure, potential defects and phase distribution. The evaluation of the WC phase was conducted in accordance with ASTM E562 (2019) standard. Furthermore, confocal laser microscopy was applied to meticulously assess the wear rate.
Results
The microscopic and imaging analyses revealed significant insights into the correlation between the DED fabrication parameters and the resulting material microstructure. Key findings include the identification of optimal processing conditions for achieving desired mechanical properties and the

detection of critical microstructural features that influence the material's performance in engineering applications. The incorporation of M2 steel onto the operational surfaces of tools manifests a notable enhancement in tool longevity, predominantly ascribed to its exceptional hardness, resistance to wear, and capacity to endure mechanical stresses encountered during cold working cutting tools activities. Microstructural examination unveils a martensitic matrix with the presence of complex carbide particles containing Mo, V, Cr and W. Remarkably, these particles faithfully replicate the characteristic cellular structure inherent in additively manufactured materials in as-built states, i.e. after deposition. The resultant hardness attains a formidable 63 HRC.
The research also demonstrates a substantial improvement in tool life, wear resistance, mechanical properties, and microstructural stability with additively manufactured carbide-reinforced Nimonic 80A layer as a functional coating onto the tool steel. The gradual addition of particles (5%, 10%, 15%, and 20%) highlights the potential for WC-doped Nimonic 80A in multimaterial components, particularly in challenging environments of hot forming operations where wear resistance and mechanical strength are critical. The integration of WC into the Nimonic 80A matrix proved successful, yielding significant advantages for the material, while no defects were detected. Additionally, it was observed that the WC particles were uniformly dispersed within the matrix, contributing to the overall homogeneity of the material.
Conclusion
Microscopy and imaging techniques have proven to be pivotal in advancing the understanding of the microstructural characteristics of M2 high-speed steel and WC-doped Nimonic 80A produced via DED. This study not only highlights the importance of these techniques in material science but also sets the groundwork for future research aimed at optimizing metallic materials for various engineering applications. The investigation into the additively manufactured materials highlights its potential for high-performance applications. The findings not only contribute to the digitalization of material testing and the development of predictive material models but also pave the way for optimizing the manufacturing processes of high-performance tools, showcasing the role of microscopy in advancing materials design and application.

Keywords:

additive manufacturing,M2 steel,composites,complex microscopy

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Leveraging gas-cell in situ electron microscopy to track atmosphere-dependent reversible transformations in reducible oxides

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Poster Group 2

Background
Environmental catalysis has long been a subject of significant interest, particularly given the pressing need for renewable, eco-friendly energy sources amid the current energy crisis. O2 and/or H2 are commonly involved in these processes, either as reactants or products, with redox reactions playing a pivotal role in understanding the reaction pathways and their mechanisms. The performance of catalysts is closely linked to these mechanisms, making an in-depth study of these systems vital for enhancing catalytic activity and selectivity. However, catalysts are dynamic entities prone to undergoing structural and chemical transformations during reactions. Traditional electron microscopy techniques are limited in their ability to capture these changes, typically providing insights only into the catalyst's initial and final states1. In response to this challenge, gas-cell in situ electron microscopy has emerged as a crucial tool. This cutting-edge technique allows for the direct visualization of phenomena occurring at the nano and even atomic scale during catalytic reactions. A better understanding of the materials and their functionality during reaction will aid in designing rational catalysts with improved performance.
Yet, there is a big scale gap between conventional catalytic reactors and the in situ nanoreactor affecting phenomena such as mass or heat transport that should be given more consideration2,3. Aiming to investigate the potential disparities emerging between the macro and nano scales, we propose to in situ characterize the chemical behavior of reducible oxides, while contrasting it with the results obtained in regular catalytic reactors.
Methods
To this end, two families of model catalysts have been picked to track their evolution during reduction/oxidation reactions. The systems consist of CeO2 and Cu2O oxides with nanocubes morphology. A well-defined surface crystallography will contribute to generalizing about the phenomena taking place. These samples have been submitted to temperature-programmed reductions/oxidations (TPR and TPO) monitoring their reducibility.
Similarly, in situ redox reactions under H2 or O2 atmospheres have been carried out in a DENS Solutions Climate holder, using a double-corrected FEI Titan Cubed at 200 kV. Pressure inside the nanoreactor was about 950 mbar and gas flow was set to 0.01 ml/min with 50% of reactive gas (either H2 or O2) balanced with N2. The phase transformations related to temperature and gas flow in electron-transfer reactions entail chemical changes, like a shift in the valence state, and structural changes triggering a modification in the crystal lattice. Electron diffraction and electron energy loss spectroscopy (Gatan Quantum 966 ERS) have allowed us therefore to trace the modifications on the model Cu2O and CeO2 catalysts.
Results
Prior to investigate controlled-morphology Cu2O and CeO2 catalysts, a reference CuO sample has been tested to compare reduction degree and temperature between in situ and ex situ redox cycles. Their correlation determines a shift of about 50 ºC towards higher temperatures in the reduction at

the nano scale, spanning as well over a wider range. Deviations in the actual conditions of both experiments may point out differences in their kinetics that should be further considered. From a structural point of view, Figure 1A shows the drastic change suffered in commercial CuO crystals after interaction with H2, splitting apart into way smaller nanoparticles. The interpretation of electron diffraction patterns unveils that, in line with the TPR results, the morphological transformation is linked with the transition to metallic Cu.
Moving forward with our target systems, we have first explored the reduction of CeO2 nanocubes, where the ex situ TPR is mainly defined by two reduction events separated by at least 300 ºC. Figure 1B follows the evolution of ceria nanocubes under H2 when the temperature increases up to 950 ºC. The elemental mapping has been extracted from Ce-M4,5 EELS fine structure, showing the spatial distribution of Ce3+ (red color) and Ce4+ (green color) at 450 ºC and 950 ºC. The in situ reduction triggers the formation of a reduced cerium-based shell which propagates towards the core of the nanocubes at elevated temperatures. The presence of two reduction peaks muddles the direct correlation between the ex situ and in situ experiments, demanding therefore a quantitative analysis of both processes to match the temperatures and defined each of the two reduction events.
To clear out the roots of these and further experiments, a deeper investigation on the reduction/oxidation of these oxides is required. In addition, the Cu2O nanocubes will also be addressed comparing the results with the CuO reference and the details determined in the reduction of the CeO2 nanocubes approach.
Conclusion
The relationship between conventional quartz reactors and Si3N4-membrane nanoreactors has inferred a temperature deviation in the in situ measurement, although further experiments are mandatory to determine if this departure is material-related or just a fixed value. Despite the reduction of CeO2 nanocubes has been captured with the surface-to-core spreading of a reduced phase, the nature of this shell still remains unclear. Future experiments based on Cu2O nanocubes will be used to cross-check this singular observation.

Keywords:

in-situ, EELS, diffraction, reducible-oxides, catalysts

Reference:

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In-situ STEM-EELS observations on heating TiO2-x nanoparticles for solar and electrocatalytic applications.

Dr Khalil El Hajraoui[1,2], Dr Leonardo Lari[2,3], Mrs Fayzah Talbi[2,3], Prof. Richard Douthwaite[4], Prof Quentin. M Ramasse[1,5], Prof Vlado. K Lazarov[2,3], Dr Demie Kepaptsoglou[1,2]
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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Background incl. aims
Titanium dioxide (TiO2) nanoparticles (NPs) are extensively studied due to their versatile applications ranging from photocatalysis [1] and photovoltaics [2] to incorporation into food items as additives [3] . The reduction of these NPs to sub-stoichiometric TiO2-x, e.g., by solar light irradiation, exposure to inert or reducing atmospheres, or doping is garnering significant interest due to the reaction products superior physical properties compared to the unprocessed NPs. For instance, the introduction of Ti3+ species has been shown to prevent the recombination of photogenerated electron-hole pairs in TiO2 resulting in improved photoelectric properties. Similarly, Magneli TixOy based phases are highly desirable as electron transport layer (ETL) in dye-sensitised solar cells thanks to their high electrical conductivity, and chemical stability [4]. Here, we use state of the art in-situ electron microscopy to elucidate the evolution of the atomic structure, chemistry, and electronic structure of TiO2 anatase nanoparticles for ETL and photocatalysis applications.

Methods
We investigate at the atomic scale, the evolution of the microstructure in single-crystal anatase NPs, under variable temperature and in ultra-high vacuum environment, in an aberration-corrected monochromated Nion UltraSTEM100MC – Hermes Scanning Transmission Electron Microscope (STEM) operated at 60kV. The instrument is equipped with a cold field emission source, and can be monochromated down to an energy spread of around 6meV. STEM bright field (BF) and high-angle annular dark field (HAADF) images were acquired by rastering a 1Å corrected probe with a beam convergence half-angle of 30 mrad across the interfaces. The electron energy loss spectroscopy (EELS) spectrum images (SI) were acquired with a probe with an energy spread of 150 meV with a collection half-angle of 22 mrad using a Nion EEL spectrometer equipped with a Dectris ELA hybrid-pixel direct electron detector optimized for EELS acquisition at low acceleration voltages. The in-situ heating experiments were performed using a Protochips Fusion in-situ heating biasing adapter for Nion, and the temperature calibrated thermal E-chips from Protochips.

Results
STEM BF and HAADF imaging was used to observe the formation of extended defects during the annealing process of [111] oriented TiO2 NPs for which the {111} facets are known to exhibit high photocatalytic activity [5]. We observe the continuous growth of Ti2O3 trigonal structures (Fig.1) forming via Σ3 grain boundaries (yellow line) in the three corners of the {111} TiO2 anatase facet and between the Ti2O3 grains at a temperature as low as 300 ^{o}C. The formation of the Ti2O3 trigonal structures was further confirmed by spatially resolved monochromated electron energy loss

spectroscopy (EELS) measurements of the Ti L2,3 and O K edges at each stage of the annealing process. Specifically, we observed the change on the Ti L2,3 fine structure in the new domains, with the four characteristic multiplet peaks of the anatase near-edge structure (ELNES) broadening into two peaks more characteristic of a Ti2O3 trigonal structure alongside a shift toward a lower energy loss position: see Fig.(1,d-e). This ELNES change is known as a fingerprint of a Ti4+ to Ti3+ transition, confirming the imaging observations and the assignment of the newly formed domains to a Ti2O3 phase.

Conclusion
These observations are used to create realistic atomic models of the reduced and anatase phases, and of transitional structures observed during the experiments, which will be critical to develop a more fundamental understanding of possible unique electronic states forming at the Σ3 boundaries and their effect on the electron transport, including charge trapping, and other phenomena.

Keywords:

EELS, Photocatalysis, Photovoltaic, In-situ

Reference:

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ACOM characterization of phase transitions during overageing of aluminium alloys using a direct electron detector

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Poster Group 2

Aluminum alloys are notorious for the many phase transitions that can occur during precipitation heat treatment. Distinguishing these phases is challenging because many of them have only slight variations of their composition and lattice parameters. In this work, we characterized by TEM the evolution of the precipitation state during overageing (>1000h) of 2xxx series aluminium alloys. To identify the phases, a combination of automated crystal orientation mapping (ACOM) and energy dispersive X-ray spectroscopy (EDX) is implemented. Using standard ACOM setting (external CCD camera filming the phosphorescent screen) only one phase is identified in the heat-treated samples (the S phase), while using a direct electron detector, two phases are identified (the S and Q). The identification with the direct electron detector is consistent with the EDX observation. The potential benefit of direct electron detector cameras for phase recognition is discussed. We finally discuss how these observations improve the interpretation of complementary characterizations performed on this material, such as small angle X-ray scattering and hardness measurements.

Keywords:

direct electron camera; aluminium alloys

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Simulations of phonon and magnon EELS/EEGS including dynamical effects and multiple inelastic excitations

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background incl. aims
Phonons and magnons play important roles in various technological applications. Phonons are essential for understanding heat flow at the nanoscale, while magnons hold promise as an information carrier (quasi-)particle for a new generation of hardware in information technologies. Instrumental advances, bringing high spatial resolution offered by scanning transmission electron microscopes (STEMs) together with high energy resolution and high dynamic range offered by direct electron detectors, offer an exciting route for studying these quasiparticles down to atomic scale [1-2].
However, the strong interaction between the electron beam and the sample, resulting in multiple elastic and inelastic scattering events, complicates the interpretation of experimental data and reliable theoretical predictions are thus of high importance.

Methods
We extend the quantum excitation of phonons method [3] to the spectroscopic domain. This is achieved by deriving a time-autocorrelation function of the so-called auxiliary wave-function and showing that it represents the desired spectral information. The exact quantum-mechanical evolution of the auxiliary wave-functions is approximated by scattering on a structure model with the atomic coordinates (or magnetic moment directions in the case of magnon EELS) evolving classically. Motion of atoms or precession of magnetic moments is simulated by molecular dynamics or atomistic spin dynamics, respectively.

Results
We present a derivation of a new theoretical framework that treats elastic and inelastic scattering on equal footing, including energy loss and gain processes, and effects of multiple elastic and inelastic scattering events [4]. The resulting simulation method remains flexible and efficient, and in comparison with recently introduced frequency-resolved frozen phonon multislice method (FRFPMS; [5]) it includes multiple inelastic scattering, doesn't require knowledge of Debye-Waller factors, and effectively includes anisotropic vibrations (or precession of magnetic moments) and non-local absorption effects of the elastic channel. We present the method on simulations of phonon EELS/EEGS on crystalline silicon and magnon EELS/EEGS for bcc iron and compare them to available data.

Conclusion
A new approach to simulations of phonon and magnon EELS has been presented. Comparison with FRFPMS and adiabatic magnon spectra from spin dynamics shows good agreement.

Keywords:

phonon, magnon, EELS, EEGS, STEM

Reference:

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3. B D Forbes, A V Martin, S D Findlay, A J D'Alfonso, and L J Allen, Phys. Rev. B 82 (2010), 104103.
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Direct Visualization of Temperature Induced Phase Separation of Completely Miscible Au-Pd Alloy by In-Situ TEM

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Poster Group 2

Background incl. aims
Very recently, bimetallic nanoparticles (NPs) are getting a lot of attentions due to their superior efficiency in heterogeneous catalysis and potential to replace conventional fossil fuels. Bimetallic systems showed superior stability, and superior activity compared to any of their individual metallic counterpart. In this aspect, Au@Pd core@shell NPs and recently Au-Pd nano-alloys has been used for electrocatalytic production hydrogen peroxide (H2O2), ethanol oxidation reactions and they observed to show much better catalytic activity compared to bare Pd nanoparticles. However, Au NPs are less efficient for oxygen reduction but highly efficient for conversion of alcohols to aldehyde, which is completely opposite to the catalytic activity of Pd. These limitations were repressed by alloying both systems (Au and Pd), but the lower electrochemical potential of the metal alloy contrast to the individual metals decreases its efficiency towards alcohol to aldehyde conversion reaction. Thus, it should be highly beneficial to construct a bi-metallic Au-Pd system where both the phases are separated from each other in a single nanoparticle. In such systems, the oxidation and reduction reactions will take place at totally different locations of the same nanoparticle, leading to more superior catalytic activity and stability. Such systems are unique in this sense, as they retain the individual properties of separate constituents and also provide physical, chemical, optical interaction between them due to the presence of a common interface [1]. However, obtaining bi-metallic nanoparticle based on phase separation method depends on the fact that the lattice mismatch between two constituent metals must be large (>5%, ex. Au-Ni, Au-Cu). Metals with lower lattice mismatch tend to create an alloy system due to complete miscibility in their all composition range at all the temperatures according to their bulk phase diagram (i.e. Au-Pd) [2]. Though theoretical researchers predicted a miscibility gap of an Au-Pd bimetallic system, the conversion of Au-Pd nanoalloy to phase separated Au/Pd bi-metallic system with annealing could not be observed till now. Okamoto et. Al. observed Au-Pd alloy formation in face centered cubic (fcc) structure at high temperature for all Pd composition range [3]. Wu et. Al. observed formation of an alloyed nanoparticle upon in-situ heating of a core@shell Au@Pd nanostructure up to 600°C [4]. Precot et. al. studied the annealing effect of Au-Pd nanoparticles deposited on amorphous carbon at 873K and observed bi-modal distribution of nanoparticle upon annealing, where small nanoparticle and big nanoparticles became Au-rich and Pd-rich, resulting a bi-modal distribution of nanoparticles driven by Ostwald ripening mechanism [5].
We have shown by in-situ vacuum annealing high-resolution transmission electron microscopy (HRTEM) study of the generation of phase separated Au-Pd bimetallic nanoparticles from an initial Au nanotriangle (AuNT)@Pd core@shell nanoparticle system. We have observed through extensive aberration-corrected HRTEM, high-resolution scanning TEM (HRSTEM) and energy-dispersive X-ray spectroscopy (EDS) that the core@shell Au@Pd nanostructure forms an Au-Pd alloy during in-situ heating at 400°C and is very much stable up to 800°C, which is a completely normal behavior. Upon further heating at 900°C-1000°C range, the Au and Pd phase surprisingly got separated from each

other and the separation is observed to be much prominent for initial core@shell NPs with higher Pd loading.

Methods

Au nanotriangles (AuNT) were produced by a previously described seedless method. Thickness of Pd layer was controlled by controlling the amount of H2PdCl4 in the AuNT solutions. The nanoparticles were initially identified by UV-Visible and X-ray photoelectron spectroscopy (XPS) technique. The in-situ HRTEM and selected area diffraction (SAD) studies were performed in an image-corrected FEI TITAN operated at 300 kV. HRSTEM, energy dispersive X-ray spectroscopy (EDS) analyses were carried out in a probe-corrected FEI TITAN TEM equipped with high-brightness gun and an Oxford Instruments Ultim X-MaxN 100TLE detector for EDS measurements. A DENSsolutions Wildfire MEMS based double-tilt heating holders was used for the in-situ heating measurements. The heating circuits were fabricated on a SixNy membrane and it can measure temperature until 1300 °C. A very dilute solutions of the AuNT nanoparticles were drop casted on the heating chips and kept overnight for drying.

Results

We have extensively studied the interface and surface of bare AuNT and AuNT@Pd core@shell nanostructures and identified presence of number of defects at the Au/Pd interfaces (i.e. stacking-faults, dislocation pair etc.). Also, the melting point of bare AuNT is observed to be lower compared to the Pd doped sample and the melting point seemed to be increasing with increase in Pd content. Besides that, the relative change in lattice parameter with in-situ heating was also studied for bare NT and AuNT@Pd with different Pd thickness using fast Fourier transform (FFT) method and we observed the change in lattice parameter of (220) plane is much lesser for Au-Pd alloy compared to bare AuNT. However, the most important observation was the phase separation of Au-Pd alloy triangular nanostructure producing distinctive Au and Pd region at higher temperature which was verified by HRTEM and EDS measurement. The Pd region increased in size with higher Pd loading. The in-situ microscopy reveals the Pd atoms outward movement from the core due to their higher mobility at high temperature while Au atoms move inwards to finally produce a phase separated structure. This structure was also observed to be very stable as the EDS spectra taken from the sample at room temperature after 7 days revealed that the nanoparticle still remains in the phase separated form.

Conclusion

We have done in-situ heating experiment on bare AuNT and AuNT@Pd core@shell nanostructure up to 1000°C. The Pd deposited AuNT showed much more stability in terms of structure and thermal resistance as observed. Most importantly, though Au and Pd are miscible in all temperature range and all compositions. We observed when Pd content is higher, it can produce phase separated region at High temperature which was observed to be very much stable in ambient atmosphere over a month. This result will be of very much important to understand not only the catalytic activity of Au-Pd alloy but also to produce phase separated bi-metallic nanostructure consisted of separated Au and Pd region for superior catalytic activity.

Keywords:

Au-Pd alloy, Phase separation, In-situ

Reference:

Reference:

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4D-STEM Post measurement machine learning enhanced aberration correction for amorphous and magnetic samples

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Poster Group 2

High-resolution transmission electron microscopy is one technique to resolve the atomic structure of materials. However, the phase information of the exit-wave is lost. Therefore, 4D-STEM techniques such as ptychography were developed to retrieve the phase information and enhance the resolution. Even so electron ptychography is used frequently on different material systems, imaging the domain structure of magnetic samples remains challenging. This is due to the fact that for magnetic imaging the objective lens needs to be switched off and the aberration corrector becomes obsolete. This would decrease the resolution limit.
This work is a first step to achieve aberration free high-resolution imaging of magnetic domains in the field-free mode (objective lens is off). This is needed to avoid the interaction between the strong magnetic field of the objective lens with the magnetic sample.
It has been previously shown that 4D-STEM datasets can be used to remove residual aberrations in high resolution images of crystalline samples[1]. This work builds on the previous work and transfers it to objective lens free operations in which the probe corrector cannot be used. The largest issue in lens-free operations is that the spherical aberrations cannot be corrected. Additionally to field free measurements, this method can also be used for post processing 4D-data on amorphous samples like polymers or metallic glasses in which the probe correction was not ideal.
For the reconstruction of the aberrations, the Single-Side Band(SSB) ptychography method is used for gaining information about the aberrations. The SSB method is a direct reconstruction method, which is performed by making a Fourier transform of the real space. It should be noted that in the field-free mode, the Fourier transform of a brightfield image does not show sharp spots, but a more diffusely spread intensity distribution. As an example, a gold cross grating imaged at low magnification is shown in figure 1a and its Fourier transform in 1d. In order to remove the higher-order aberrations to improve the phase reconstruction, more SSB patterns need to be selected, in comparison to the method that relies on crystalline samples. This is due to the previously mentioned diffuse distribution of the intensity in the Fourier space which is then visible as noise in the SSB-patterns as shown in figure 1b. A minimum number of 20-30 patterns are needed to achieve good results. To index these patterns the knowledge of the position of the discs needs to be known see figure 1b. The position was accurately indexed by using artificial intelligence (AI), which measures the angle and the distance between the discs. The training data of the artificial intelligence was simulated to look as close as possible to real measurements. Since the amount of data for training an AI needs to be large and it needs a large amount of computational power to simulate thousands of 4D-STEM datasets. The SSB datasets have been calculated directly without running a full simulation. For this a list of object function was created, the distances, the angles between the discs and the aberrations were selected randomly. After the indexing of the patterns the aberrations are calculated in the same way as previously shown by Ning et al. in [1]. The resulting SSB phase images show that the aberrations have been corrected compare figure 1c and 1e. The resulting data can then be used to be further processed by an iterative ptychography method like the extended ptychographic iterative engine(ePIE).

Keywords:

Ptychography, aberration correction, AI

Reference:

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New insights into optical property characterization of 2D semiconductor materials through time-correlated photon/electron spectroscopies

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background: The optical properties of luminescent materials are intricately tied to their structural and chemical characteristics at the nanometric, or even atomic scale. Understanding this relationship has long relied on the combination of scanning transmission electron microscopy (STEM) with external optical techniques such as absorption or photoluminescence spectroscopies, probing the excitation and emission mechanisms at the micrometric scale. However, the significant gap in spatial resolution between these methods and phenomena of interest often leads to ambiguous or even impossible interpretations of the measurements. Recent advancements in nano-optics seek to address this resolution disparity by enabling observation of luminescent material properties at nanometric scales. In that sense, STEM has undergone substantial instrumental and theoretical progress, providing valuable insights into optical properties through conventional inelastic electron scattering (electron energy loss spectroscopy, EELS) and photon emission (cathodoluminescence, CL) spectroscopies [1]. However, both techniques are hindered by spectral resolution limitations and the broadband excitation process of the electron beam. This contribution investigates the possibilities presented by the latest advancements in STEM techniques, specifically focusing on electron-photon time-correlations.
Methods: To overcome these challenges, two techniques currently in development will be highlighted in this contribution:

- Cathodoluminescence Excitation Spectroscopy (CLE, Figure 1b), based on coincidence measurements between EELS and CL events. CLE offers insights into energy transfer pathways and excitation lifetimes at the nanometer scale, as previously reported [2,3].
- Electron Energy Gain Spectroscopy (EEGS, Figure 1c), an extension of the photon-induced near-field electron microscopy (PINEM) technique, investigates primary electron acceleration when the beam interacts with a strong optical field generated by a laser. By changing the energy of the photons, this technique can resolve ultra-fine optical resonances in the sub-meV range, revealing novel physical properties below the energy resolution achievable with state-of-the-art monochromated electron beams. So far, it has only been applied to photonic excitations, such as plasmons or surface polaritons [4].

Results and Conclusion: These techniques, with their distinct excitation and deexcitation processes (Figure 1a), are expected to be highly complementary, offering deeper insights into optical mechanisms at the nanometer scale. The present contribution focuses on using transition metal dichalcogenides (TMDs) as a model system of functional luminescent material to explore the insights provided by these correlated techniques.

Keywords:

Nano-optic, electron/photon spectroscopy, semiconductor TMD.

Reference:

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Laser particle acceleration in an ultrafast scanning electron microscope

Leon Brückner[1], Tomas Chlouba[1], Roy Shiloh[1], Stefanie Kraus[1], Julian Litzel[1], Peter Hommelhoff[1]
[1]Physics Department, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Poster Group 2

Background incl. aims

Ultrafast electron microscopy is rapidly approaching the limits of electron microscopes in terms of electron pulse length (typically hundreds of femtoseconds) and beam energy (up to 30 keV for a SEM and about 300 keV for a TEM). A miniaturized particle accelerator inside a microscope could overcome these limits by providing a beam of high-energy, ultrashort electron bunches. Dielectric laser acceleration (DLA) aims to achieve this by utilizing pulsed laser light for generating the accelerating field. The high damage threshold of dielectric materials could exceptionally high acceleration gradients of up to several GeV/m [1].
A DLA nanostructure typically consists of two rows of silicon pillars that are illuminated with an infrared laser pulse, which creates an oscillating nearfield mode with a phase velocity given by the period of the structure. Electrons are then injected into the channel in between the pillars with velocity (energy) corresponding to the phase velocity of the nearfield mode which ensures phase-matching and thus efficient energy transfer. By adding a buncher stage, additionally attosecond bunch trains can be generated over very short distances [2].

To allow acceleration over long distances, it is necessary to confine the particle beam transversally in order to prevent particle losses. This is realized by exploiting the transverse forces exerted by the nearfield. By incorporating periodic gaps in the structure design, we can instantly change the phase of the propagating electrons and thus alternate at will between transversally focusing and defocusing forces. With this scheme, beam transport over a distance of 78 µm has been demonstrated [3]. Combining guiding with a tapering of the structure period and the periodic gaps, we demonstrated coherent, phase-matched acceleration over long distances [4,5]. This enables significant energy gains, opening the door towards useful applications of these miniaturized accelerators.

Methods

We have designed and fabricated a 500 µm long acceleration structure with an average gradient of 22.7 MeV/m [4]. In the experiment, an ultrafast SEM serves as the electron source. Ultraviolet laser pulses with a duration of 150 fs are focused onto the SEM tip cathode, emitting electron pulses with a mean energy of 28.4 keV and a pulse length of the order of 700 fs at the sample. The DLA structure is illuminated with a 1.93 µm, 250 fs long laser pulse with a pulse energy of 2.2 µJ that is focused to an elongated spot of 640 µm beam waist along and 13 µm transverse to the structure, creating the design peak field strength of around 600 MV/m. The laser pulse front is tilted by 71° to ensure optimal temporal overlap between electrons and the light field along the propagation. The electron energy is measured with a magnetic spectrometer.

Results

The accelerator demonstrates successful capture, confinement and acceleration of electron bunches. The 500 µm long acceleration structure shows an energy gain of the accelerated bunch of 12.3 keV, meaning a relative gain of 43% and a total energy of 40.7 keV, as predicted by simulation [4].

Conclusion

With careful design and extension of the interaction length, this energy gain could be increased much further, potentially enabling high-energy TEM imaging or electron diffraction in SEMs, for example. Exploring different combinations of dielectric materials with high damage thresholds and excitation wavelengths, particularly in the mid-infrared, will give access to acceleration gradients far in excess of those demonstrated here.

Keywords:

ultrafast electron microscopy, light-matter-interaction, lasers

Reference:

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Direct Visualization of Temperature Induced Phase Separation of Completely Miscible Au-Pd Alloy by In-Situ TEM

Dr. Abhijit Roy[1,2], Dr. Simon Hettler[1,2], Dr. Raul Arenal[1,2,3]
[1]Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain, [2]Laboratorio de Microscopías Avanzadas (LMA), Universidad de Zaragoza, Zaragoza, Spain, [3]ARAID Foundation Zaragoza , Zaragoza, Spain

Poster Group 2

Background incl. aims
Very recently, bimetallic nanoparticles (NPs) are getting a lot of attentions due to their superior efficiency in heterogeneous catalysis and potential to replace conventional fossil fuels. Bimetallic systems showed superior stability, and superior activity compared to any of their individual metallic counterpart. In this aspect, Au@Pd core@shell NPs and recently Au-Pd nano-alloys has been used for electrocatalytic production hydrogen peroxide (H2O2), ethanol oxidation reactions and they observed to show much better catalytic activity compared to bare Pd nanoparticles. However, Au NPs are less efficient for oxygen reduction but highly efficient for conversion of alcohols to aldehyde, which is completely opposite to the catalytic activity of Pd. These limitations were repressed by alloying both systems (Au and Pd), but the lower electrochemical potential of the metal alloy contrast to the individual metals decreases its efficiency towards alcohol to aldehyde conversion reaction. Thus, it should be highly beneficial to construct a bi-metallic Au-Pd system where both the phases are separated from each other in a single nanoparticle. In such systems, the oxidation and reduction reactions will take place at totally different locations of the same nanoparticle, leading to more superior catalytic activity and stability. Such systems are unique in this sense, as they retain the individual properties of separate constituents and also provide physical, chemical, optical interaction between them due to the presence of a common interface [1]. However, obtaining bi-metallic nanoparticle based on phase separation method depends on the fact that the lattice mismatch between two constituent metals must be large (>5%, ex. Au-Ni, Au-Cu). Metals with lower lattice mismatch tend to create an alloy system due to complete miscibility in their all composition range at all the temperatures according to their bulk phase diagram (i.e. Au-Pd) [2]. Though theoretical researchers predicted a miscibility gap of an Au-Pd bimetallic system, the conversion of Au-Pd nanoalloy to phase separated Au/Pd bi-metallic system with annealing could not be observed till now. Okamoto et. Al. observed Au-Pd alloy formation in face centered cubic (fcc) structure at high temperature for all Pd composition range [3]. Wu et. Al. observed formation of an alloyed nanoparticle upon in-situ heating of a core@shell Au@Pd nanostructure up to 600°C [4]. Precot et. al. studied the annealing effect of Au-Pd nanoparticles deposited on amorphous carbon at 873K and observed bi-modal distribution of nanoparticle upon annealing, where small nanoparticle and big nanoparticles became Au-rich and Pd-rich, resulting a bi-modal distribution of nanoparticles driven by Ostwald ripening mechanism [5].
We have shown by in-situ vacuum annealing high-resolution transmission electron microscopy (HRTEM) study of the generation of phase separated Au-Pd bimetallic nanoparticles from an initial Au nanotriangle (AuNT)@Pd core@shell nanoparticle system. We have observed through extensive aberration-corrected HRTEM, high-resolution scanning TEM (HRSTEM) and energy-dispersive X-ray spectroscopy (EDS) that the core@shell Au@Pd nanostructure forms an Au-Pd alloy during in-situ heating at 400°C and is very much stable up to 800°C, which is a completely normal behavior. Upon further heating at 900°C-1000°C range, the Au and Pd phase surprisingly got separated from each

other and the separation is observed to be much prominent for initial core@shell NPs with higher Pd loading.

Methods

Au nanotriangles (AuNT) were produced by a previously described seedless method. Thickness of Pd layer was controlled by controlling the amount of H2PdCl4 in the AuNT solutions. The nanoparticles were initially identified by UV-Visible and X-ray photoelectron spectroscopy (XPS) technique. The in-situ HRTEM and selected area diffraction (SAD) studies were performed in an image-corrected FEI TITAN operated at 300 kV. HRSTEM, energy dispersive X-ray spectroscopy (EDS) analyses were carried out in a probe-corrected FEI TITAN TEM equipped with high-brightness gun and an Oxford Instruments Ultim X-MaxN 100TLE detector for EDS measurements. A DENSsolutions Wildfire MEMS based double-tilt heating holders was used for the in-situ heating measurements. The heating circuits were fabricated on a SixNy membrane and it can measure temperature until 1300 °C. A very dilute solutions of the AuNT nanoparticles were drop casted on the heating chips and kept overnight for drying.

Results

We have extensively studied the interface and surface of bare AuNT and AuNT@Pd core@shell nanostructures and identified presence of number of defects at the Au/Pd interfaces (i.e. stacking-faulst, dislocation pair etc.). Also, the melting point of bare AuNT is observed to be lower compared to the Pd doped sample and the melting point seemed to be increasing with increase in Pd content. Besides that, the relative change in lattice parameter with in-situ heating was also studied for bare NT and AuNT@Pd with different Pd thickness using fast Fourier transform (FFT) method and we observed the change in lattice parameter of (220) plane is much lesser for Au-Pd alloy compared to bare AuNT. However, the most important observation was the phase separation of Au-Pd alloy triangular nanostructure producing distinctive Au and Pd region at higher temperature which was verified by HRTEM and EDS measurement. The Pd region increased in size with higher Pd loading. The in-situ microscopy reveals the Pd atoms outward movement from the core due to their higher mobility at high temperature while Au atoms move inwards to finally produce a phase separated structure. This structure was also observed to be very stable as the EDS spectra taken from the sample at room temperature after 7 days revealed that the nanoparticle still remains in the phase separated form.

Conclusion

We have done in-situ heating experiment on bare AuNT and AuNT@Pd core@shell nanostructure up to 1000°C. The Pd deposited AuNT showed much more stability in terms of structure and thermal resistance as observed. Most importantly, though Au and Pd are miscible in all temperature range and all compositions. We observed when Pd content is higher, it can produce phase separated region at High temperature which was observed to be very much stable in ambient atmosphere over a month. This result will be of very much important to understand not only the catalytic activity of Au-Pd alloy but also to produce phase separated bi-metallic nanostructure consisted of separated Au and Pd region for superior catalytic activity.

Keywords:

Au-Pd alloy, Phase separation, in-situ

Reference:

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Influence of the loss function on gradient-based iterative ptychographic reconstructions in 4D-STEM

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IM-03 (4), Plenary, august 29, 2024, 14:00 - 15:00

Background incl. aims
The noise model of a detector's counting process is known as Poisson shot noise. However, direct reconstruction algorithms like single-sideband (SSB) or Wigner-distribution deconvolution (WDD) do not assume a specific noise model. This is not the case for gradient descent-based iterative methods that allow the free choice of the loss function and, therefore, the underlying noise model. In practice the mean square error (MSE), also known as L2 loss, is often used, which assumes Gaussian white noise. This is a good approximation for the Poissonian statistics for high counts.
In contrast, the (extended) iterative engine (e)PIE assumes no specific noise model. However, Melnyk showed that ePIE can be seen as a particular case of stochastic gradient descent using the MSE Loss on the amplitudes instead of the intensities [1], which we will call the Amplitude loss function in the following. The amplitude loss can be derived from the Poisson loss function via a Taylor expansion, assuming that the specimen guess is close to the original specimen [2] and, therefore, an approximation of the Poisson loss.
The interest in using STEM for low-dose applications has grown in recent years, e.g., to examine dose-sensitive, weakly scattering specimens like covalent or metal organic frameworks (COFs/MOFs), or 2D materials. Moreover, the interest rises to explore STEM for imaging proteins and lamellae of cells [3, 4, 5], which are very dose-sensitive. Therefore, we perform a detailed analysis of the influence of the loss function on the reconstruction accuracy using a gradient descent approach for the L1, MSE, amplitude and Poisson losses.
Methods
We used a MoS_2 monolayer as a test case to perform STEM simulations and experiments. Simulations were conducted with an acceleration voltage of 60 kV and 300 kV with a convergence semi-angle of 18 mrad, in focus with a dose of 10^6 electrons per diffraction pattern. The high-dose case is of particular interest because the dark field (DF) contains very few counts.
To better understand the loss functions, we compare different virtual detector geometries. Firstly, we compare the results of the full pixelated detector for 300 kV and 60 kV. Subsequently, only an acceleration voltage of 300 kV is used. Secondly, we use the pixelated bright field (BF) disc in combination with virtual ring detectors for the dark field. Thirdly, we use only the BF disc. The results suggest that the choice of the loss function makes a difference if a wide dynamic range of electron counts is measured. Therefore, we explore the BF disc again, but this time with more features in the BF disc introduced by an under-focused electron probe of 25 nm. Finally, we go back to the first case using 300 kV and stepwise reduce the dose to 10000 and 100 electrons per diffraction pattern for sparse-count dark field studies.
Results
Figure 1 depicts reconstruction results using different loss functions and high (left) as well as low (right panels) dose. The differences between the reconstructed objects using various loss functions are significant if the signal involves a wide dynamic range of electron counts. This applies to the high-

dose case (left) in which the signal levels of the BF disc and the DF differ significantly. It is best seen in the power spectra (c) where Poisson and Amplitude losses lead to reconstructed object transmission functions containing the highest spatial frequencies, followed by the L1 loss result. Note that only the Poisson loss leads to a flat spatial frequency transfer, whereas the other methods pronounce Fourier coefficients within a band of twice the probe-formin aperture radius. The line profile in (d) exhibits the sharpest peaks for the Poisson loss. Chemical contrast between a Mo monomer and S dimer is only represented in the phase of the Poisson and amplitude loss functions, respectively.
An electron dose of 100 electrons per diffraction pattern on the right of Fig. 1 appears as a limiting case for all losses considered here, with the Poisson loss still performing best. However, the difference to the MSE loss becomes smaller than for the high-dose case.
The counts in the DF are sparse, so we used virtual ring detectors for the DF to perform an azimuthal integration to get a more robust measurement. In this case, the differences between the loss functions became smaller and the chemical contrast accessible. Excluding the dark field counts and only using the BF, all loss functions lead to comparable reconstructions because the dynamic intensity range in the BF itself is rather small. Moreover, the introduction of more features into the BF disc by an under-focus increases the dynamic range slightly, but the variation is too small to pronounce one of the loss concepts against others.
Conclusion
Our results show that the differences between the loss functions become large for a broad dynamic range of electron counts, which is the case for high-dose measurements when the BF and DF are of interest at the same time, is relevant for chemical contrast found accurately represented in the phase of the Poisson loss based reconstruction. The MSE loss assumes a constant Gaussian noise distribution and the same standard deviation for all pixels. In contrast, the standard deviation of the Poisson loss scales with the square root of the number of counts. This makes a difference for a wide dynamic range in measurements and is consistent with our results. Our results suggest that the differences between the MSE and Poisson loss become small for dose-sensitive materials because sparse counts are measured and, as a result, a very low dynamic range. However, the Poisson loss is still beneficial at high frequencies and shows a lower variance of the reconstruction. In addition to the simulations, our study includes dose-dependent MoS_2 measuremts as well, which will be used to demonstrate the reliability of the theoretical findings in practice.
Funding
Funding from the European Research Council under Grant Agreement 101118656 (ERC Synergy project 4D-BioSTEM) is kindly acknowledged.

Keywords:

Loss functions, low-dose, gradient descent

Reference:

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The organization of organic/biominerals in the nanostructured 3D photonic crystals in insects' scales

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Poster Group 2

Background incl. aims
The colorful patterns displayed by longhorn beetles Doliops similis (Cerambycidae: Laminae) are formed by opalescent photonic structures confined within their cuticular scales [1]. The layers of packed nanospheres produce opalescent green colour. The morphogenesis of the nanoparticles in each scale and their self-assembly into opal-structures are unknown. We wish to employ correlative and multimodal imaging in our workflow that combines FIB/SEM volume imaging with super resolution microscopy of intact scales in order to monitoring the organic molecules distribution in the scale and in the photonic crystals.

Methods
Focus Ion Beam Scanning Electron Microscope (FIBSEM) was used to collect sequential images with voxel size of 5 nm. 3D model of 3580x 840x 270 voxels FIB-SEM stack at 5 nm resolution. Data were segmented by binarization after denoising (n2v [2]) and oriented domains were extracted using customized pipeline derived from the open source bmmltools [3].
With Fourier-transform Infrared Spectroscopy (FTIR), Small/Wide Angle X-ray Scattering and X-ray Fluorescence (SAXS/WAXS/XRF), we are able to measure the compositions in the scale, in the skin of the scale, and in the photonic crystals. Complemented with Depth-profiling Time-of-Flight SIMS (TOF-SIMS), X-ray Photoelectron Spectroscopy (XPS, NEXA G2, Thermofisher Scientific), we can understand the distribution and binding states of components with high resolution (<10 nm) in axial direction of an intact scale (60 x 40 x 5 um).

Results
We demonstrate that these needle-like scales contain a core-shell structure with a thin cuticular skin and an inner core of agglutinated amorphous calcium phosphate nanospheres (ACP, diameter of 160 nm). The spheres assemble into hexagonally close-packed layers along the surface normal of the scale, producing hence a uniform and intense green reflection colour. We successfully located the chitin fibers in the skin by applying chitin-binding proteins on sections of scales and visualized the distribution of chitin/proteins in the skin of the scales [4].

Conclusion
Our results show the D.similis longhorn beetles are able to produce highly-orderred arranged ACP nanoparticles within every micrometer-sized scale. From our preliminary results of IR, TOFSIMS, XPS, and super resolution microscope (Zeiss Elyra Sim2), we observed the co-existence of short chain lipids with the ACP nanoparticles in the core of the scale. Such observation seems an agreement with one of the hypotheses in biomineralization that the arrangement of chitin and (lipo-)protein at the interface of the cuticular shell and mineraloid core mediates the packing of the nanospheres [5]. To

address this hypothesis, we wish to employ tissue clearing methods with correlative imaging to observe the networks and conformations of organic molecules in the intact scale and their association with the ACP opal photonic crystals.

Keywords:

photonic crystals, biomineralization, volume imaging

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Multi-Convergence-Angle Ptychography with Simultaneous Strong Contrast and High Resolution

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Structural biology, which primarily focuses on the tertiary structure of biological molecules, has played a pivotal role in disease research, drug development, and the investigation of fundamental biological processes. Recently, the growing demand for more precise determination of three-dimensional biological structures using single particle analysis (SPA) or electron tomography (ET) necessitates high-quality two-dimensional projection images with both strong contrast and high resolution. Nevertheless, due to the fact that unstained biological samples are primarily composed of light elements and their structures are extremely radiation sensitive, which means that the electron dose used for imaging is limited, the contrast of biological samples in electron microscope images is very weak. To address this issue, phase contrast imaging at high defocus or novel phase plates is commonly employed. However, using high defocus corrupts information transfer at high spatial frequencies and the applications of phase plate attenuate signals from biological samples at high frequencies. Therefore, there is currently no technological means available to achieve imaging of non-stained biological samples with both strong contrast and high resolution simultaneously.
Electron ptychography has gained considerable interest in materials science and bioimaging, which has been recently reviewed1. However, the contrast transfer function of electron ptychography strongly relies on the selected convergence angles. In materials science, by employing a large convergence angle, ptychography has been demonstrated to achieve deep sub-angstrom resolution, whereas in the field of bioimaging, a small convergence angle tends to be used to enhance contrast of unstained biological samples at the expense of resolution2,3. In other words, conventional electron ptychography still fails to provide images with simultaneous strong contrast and high resolution.
Here, we introduce a novel ptychographic method, Multi-Convergence-Angle (MCA) Ptychography4. Our research addresses the current limitations of conventional ptychography and presents a promising approach for achieving high-quality two-dimensional images with improved clarity and precision.

The schematic experimental setup of MCA-ptychography is illustrated in Fig. 1(a). In contrast to conventional electron ptychography, which employs a single convergence angle (hereafter referred to as single-convergence-angle ptychography or SCA-ptychography), MCA-ptychography utilizes multiple condenser apertures with varying diameters to modify the convergence angles of the electron probe during the scanning process. However, such a device with the aperture-switching

capability is not currently available for electron microscopes. Therefore, as a proof of concept, we performed MCA-ptychography in series by acquiring the first dataset with one aperture for the entire scanning area and subsequently switching to the other aperture to acquire the second dataset for the same area. The two SCA-ptychographic datasets are mixed before reconstructing the object wavefunction via MCA-ptychography reconstruction algorithm.

A model of GroEL protein (PDB: 2eu1) suspended on a monolayer graphene substrate was simulated using the multislice method5. Two ptychographic datasets were generated at an accelerating voltage of 300 kV and convergence semi-angles of 3 mrad and 15 mrad, respectively. These two datasets were combined into a single dataset (mixed-angle dataset) and subsequently subjected to MCA-ptychographic reconstruction. The SCA-ptychographic reconstructed results at 3 mrad and 15 mrad, together with the MCA-ptychographic reconstructed result at different electron doses, are presented in Fig. 2. It is evident from the graph that SCA-ptychography at a smaller convergence angle provides strong contrast but limited resolution, while SCA-ptychography at a larger convergence angle achieves higher resolution albeit with weaker contrast. In contrast, MCA-ptychography not only provides strong contrast but also achieves high resolution. Furthermore, we found that even when the electron dose drops to as low as 11 e-/Å2, MCA-ptychography still maintain its robustness while providing simultaneous strong contrast and high resolution.

In conclusion, we propose a novel ptychographic method, MCA-ptychography, which incorporates multiple convergence angles during the data acquisition process and demonstrate its capability to provide simultaneous strong contrast and high resolution. Our research addresses the current limitations of conventional ptychography and in the future, we anticipate the integration of MCA-ptychography with cryo-electron microscopy, which will further enhance 2D projection images, leading to improved 3D reconstruction methods.

Keywords:

electron-ptychography, structural-biology, contrast-and resolution-enhancement

Reference:

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STEM-EELS unveils atomic stacking in 2D MoSe2 ECM memristors.

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Poster Group 2

Background incl. aims
Memristors, short for 'memory resistors', are two-terminal electronic devices whose resistance can be modulated by previous voltage and current inputs. As such, memristors can act as both processing and storage units, enabling computation within the memory subsystem itself, with high-speed switching and low power consumption [1],[2]. An electro-chemical metallization (ECM) memristor consists of a solid electrolyte layer (acting as an ion conductor) sandwiched between an electrochemically active electrode (usually Cu or Ag) and an inert electrode (such as Au or Pt) [3]. The device switches to an ON state when a positive voltage induces the formation of a metallic filament between the electrodes (SET), maintaining this state until a negative voltage dissolves the filament (RESET) [4]. Recently, 2D materials such as hexagonal boron nitride (h-BN) and transition metal dichalcogenides (TMDs, e.g.,MoSe2) have been integrated into memristor designs [5]. With their atomic thickness and suitable electrical/electronic properties, these materials have the potential to enable ultra-low switching voltages, a critical feature for neuromorphic computing and artificial synapses. In this work, we study a vertical ECM cell composed of chemical vapor deposited (CVD) few-layers MoSe2 sandwiched between a Au bottom electrode (BE) and a Cu top electrode (TE) in a cross-bar configuration (5×5 µm2). The metallic electrodes were patterned by ultra-violet (UV) lithography, while the MoSe2 film was transferred to the device and selectively etched by O2 plasma prior to the TE deposition. I-V measurements, performed using a two-terminal configuration, demonstrated bipolar resistive switching with SET and RESET voltages of 0.75V and - 0.95V, respectively, corresponding to the low and high resistive states (LRS, HRS) (see Fig. 1a). This macroscopic observation suggests the successful formation of conductive filaments (CFs) between the two electrodes.

Methods
To visualise the CFs formed in the cross-bar device, focused ion beam (FIB) lamellae were prepared from the operated devices (that demonstrated electrical switching) in a Hitachi Ethos NX5000 dual FIB-SEM instrument. Standard FIB protocol for low-kV STEM observations was followed to prepare these lamellae with extra care on reducing the ion milling time at 30kV to limit Ga+ damage on the MoSe2 film. Final 1kV argon (Ar+) polishing was applied for 4 min directly within the FIB-SEM instrument using a dedicated 'third beam' Ar+ source to remove remnant surface damage from the low-kV milling. These lamellae were investigated at atomic resolution in an aberration-corrected Nion UltraSTEM100 dedicated Scanning Transmission Electron Microscope (STEM) operated at 60kV. The instrument is equipped with a cold field emission source with a nominal energy spread of 0.3 eV. STEM High-angle annular dark field (HAADF) images were acquired by rastering a 1Å corrected probe with a beam convergence half-angle of 30 mrad across the interfaces. The EELS spectrum images (SI)

were acquired with a collection half-angle of 44 mrad using an Enfina EEL spectrometer retro-fitted with a MerlinEM direct electron detector optimized for EELS acquisition at low acceleration voltages.

Results

The HAADF images of the devices confirmed the presence of clean Cu/MoSe2/Au interfaces in most of the investigated regions, as displayed in Fig. 1b-d, where the atomic-scale crystalline structure of each material is apparent. This confirms the successful fabrication process and the absence of residues at the interfaces, resulting in the expected electrical contact between the layers [5]. Furthermore, the HAADF image in Fig. 1d shows the hexagonal order confirming the semiconducting character of the CVD-grown MoSe2 film, essential for a good ohmic-contact at the TE/MoSe2 interface in HRS. However, a number of sections of the interface between the MoSe2 and the respective BE and TE showed a non-perfect adhesion, resulting in 'gaps' seen as darker contrast along the interface between the materials (see Fig. 1b-d). EELS maps were acquired across the interfaces to clarify their chemistry at the atomic scale, and to identify the location of Cu after the biasing cycles undergone by the devices. The Cu and Se EELS maps in Fig. 1f, obtained from integration of the Cu and Se L2,3 edges, show that Cu appears to have diffused into the MoSe2 layers and beyond. A continuous Cu layer is observed at the BE surface, filling the 'gap' between the MoSe2 and the BE. The presence of Cu in MoSe2 layer could be linked to the formation of Cu CFs during the HRS and LRS cycles, while the Cu layer at the BE surface might be ascribed to the fabrication process. For the latter, the presence of discontinuities in the MoSe2 film (possibly connected to its morphology and/or transfer process) could allow the infiltration of Cu during the TE sputtering process.

Conclusion

Cu diffusivity is a big hurdle in device fabrication. Our results highlight its presence at the atomic scale, even in macroscopically functional devices. A number of solutions could be proposed to limit its effect starting by using a less energetic deposition process such as electron-beam deposition process, a low-cost process compatible with industrial large production. Furthermore, our findings show that atomic STEM-EELS characterisation of 'real-life' 2D MoSe2 based memristor devices is possible, providing new insights and paving the way towards a better understanding of memristor's working mechanisms and a potential optimisation in the device fabrication process.

Keywords:

EELS, Memristors, 2D materials, Semiconductor

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Study of metal powders oxidation by means of Energy Dispersion Spectroscopy (EDS)

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Poster Group 2

Background incl. aims
Energy Dispersion Spectroscopy (EDS) is a very common technique for elemental composition characterization based on the capacity of specimens to produce characteristic X-rays when illuminated by an electron beam.
Different works have already described the possibility of using EDS for the determination of the thickness of metal and oxide thin film. Such approaches usually rely on the simulation of EDS spectra by means of Monte Carlo method-based algorithms.
The aim of this work is to apply EDS for the determination of the thickness of the oxide layer formed onto the surface of copper alloy powder during its storage in order to provide a tool to rapidly assess the “goodness” of the powder and its compatibility with the laser-based additive manufacturing processes.

Methods
Powder of CuAg 3.4 alloy was prepared via a gas atomization process (VIGA) and sieved to obtain a size distribution compatible with additive manufacturing processes by laser bed powder fusion (LBPF) technique.
The powder was then divided into two batches and stored for 1 month under different conditions. In particular, the first batch was stored under dry Argon whereas the second was stored at room conditions.
The chemical composition of the surface of a CuAg 3.4 alloy powder was investigated by means of Raman spectroscopy using a Renishaw InVia Raman confocal microscope using an excitation wavelength of 633 nm. The optical response of the powder was recorded in diffuse reflectance mode (incidence at 0°) using a Shimadzu UV-2600 UV-visible spectrophotometer equipped with an ISR2700 plus integration sphere.
EDS spectra were recorded using a Zeiss EVO 15 SEM equipped with a Peltier-cooled Oxford Ultim Max 40 EDS detector.
EDS spectra of CuAg 3.4 particles coated with different thicknesses of cupric oxide (CuO) layer were simulated using the WinMCXRay.

Results
From UV-Vis and Raman spectra, it was concluded that the inert sample developed a mixed Cu2O/CuO oxide layer of 5.4 nm thickness whereas the oxidized sample exhibited a thicker layer (15.3 nm) composed mainly of CuO.
Using the WinMCXRay tool, the EDS spectra of CuAg 3.4 particles coated with an oxide layer of different was simulated, and the γ was defined as the ratio between the x-ray count at 0.525 keV (Cu Kα) and 0.931 keV (Cu Lα).

EDX spectra of the two samples were collected under different beam acceleration tension conditions in the range from 3 kV to 12 kV, using a beam current of 800 pA.
After background removal, the value γ was calculated on the experimental spectra and the obtained value was compared with the simulated data to determine the oxide layer thickness t_CuO. For simplicity, we assumed that the oxide layer is composed of CuO only.
We obtained a value of t_CuO=4.6 nm and t_CuO=16.1 nm for the inert and oxidized samples, respectively.

Conclusion

Energy dispersion spectroscopy was revealed to be a very useful and effective tool for assessing the extent of surface oxidation on metal powder. The oxide layer thickness evaluated by EDS are in good agreement with the estimation obtained from the UV-visible measurements and confirm the validity of the adopted procedure.
The possibility of measuring the oxide thickness without the need for more expensive and complicated instruments is of utmost importance in both academic and industrial contexts.

Keywords:

Metal powders, Energy Dispersion Spectroscopy

Reference:

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Solid state diffusion as the driving force in the selective oxidation of 2-propanol

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Transition metal oxides are excellent catalysts for the industrially relevant selective oxidation reactions, particularly of alcohols,[1] In the selectively catalyzed oxidation of 2-propanol to form acetone, the spinel cobalt(II, III) oxide (Co_3O_4) exhibits a peculiar behavior, which is expressed by changing the catalytic properties significantly. The changes depend on the temperature: below 200 °C, the catalyst shows high acetone selectivity, but deactivates rapidly, while above 200 °C, stable conversions and steady-state conditions can be reached, at the expense of lowered selectivity due to total oxidation (to form H_2O and CO_2).[2] In addition, once the high-temperature regime has been reached, the low-temperature region gets deactivated completely, but can be recovered by re-oxidation. Thus, it would be beneficial to stabilize the low-temperature regime. However, a prerequisite for being able to accomplish this challenging task is gaining an understanding of the underlying processes. As many phases and states formed during the reaction are metastable, this understanding can only be obtained when the sample is investigated under reaction conditions, i.e. operando methodologies are of great importance. In an operando experiment, the system is exposed to reaction conditions and its performance is measured simultaneously.[3]
Here, we use a combination of operando TEM (OTEM), as well as (operando) near-ambient pressure X-ray photoelectron spectroscopy (NAP-XPS) and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, combining the spatial resolving power of OTEM with the surface-sensitivity and energy-resolution of X-ray techniques. We employ this multimodal approach to elucidate a network of interconnected diffusional solid-state processes governing the catalytic activity of Co_3O_4 in the selective oxidation of 2-propanol towards acetone.
OTEM was performed on an image-corrected ThermoFisher Scientific Titan 80-300 instrument operated at 300 keV, equipped with a TVIPS TemCam-XF 416R CMOS camera. A DENSsolutions climate in situ holder, connected to a custom gas feeding and detection system,[4] was used. Using a gas saturator filled with anhydrous 2-propanol, and subsequent mixing it with oxygen in a 1:1 ratio, a total reactant pressure of 13.5 hPa was obtained inside the nanoreactor, which was, filled to 450 hPa with helium.
NAP-XPS and NEXAFS measurements were performed at the CAT endstation at the BESSY II electron storage ring operated by the Helmholtz-Zentrum Berlin für Materialien und Energie using the UE48-PGM (soft X-ray) and CPMU17-DCM (tender X-ray) branches of the EMIL beamline.[5] The chamber was backfilled with a 1:1 mixture of 2-propanol and O_2 to a total pressure of 0.5 hPa and photoelectrons were detected using a differentially pumped Specs Phoibos 150 NAP hemispherical analyzer.
The reductive chemical potential of the reaction feed (isopropanol/O2 1:1) induces the exsolution of amorphous CoOx nanoparticles from the spinel, which is already significantly reduced at room temperature. This leaves behind cation vacancies, which cause strong distortions of the Co_3O_4 lattice.

The exsolution is self-limiting as both the number of nanoparticles and the lattice parameter remain constant above 100 °C.
Increasing the temperature further causes the vacancies to collapse to voids inside the spinel particles, restoring the (mostly) undistorted lattice, as seen by the linear behavior of the lattice parameter above 200 °C. The exsolved nanoparticles also start crystallizing as rock-salt-structured CoO at temperatures above 200 °C, as evidenced by SAED and HRTEM. Furthermore, NAP-XPS and NEXAFS measurements reveal a maximum in the cobalt oxidation state at this temperature. Thus, the catalyst is in a frustrated state at 200 °C: at this temperature, the system transits from the low-temperature to the high-temperature activity regime and exhibits the highest selectivity towards acetone. Moreover, cobalt is in its highest oxidation state, the exsolved particles start to crystallize and the cation vacancies coalesce to voids. If the system is cooled down again and a second catalytic run is started, the exsolved and crystallized particles remain, but they are dissolved again during reoxidation.
Using a combined operando microscopic and spectroscopic investigation, it could be shown that the selective oxidation of 2-propanol on Co_3O_4 is accompanied by severe morphological changes and restructuring, which already start at room temperature. Furthermore, the catalyst is strongly controlled by solid state processes such as exsolution, vacancy agglomeration and crystallization. All together, they determine the catalytic properties and are responsible for the transition between the low and high temperature activity regimes.

Keywords:

operando; catalysis; cobalt; diffusion; exsolution

Reference:

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High-resolution investigation of rotavirus VLPs by cryo-EM

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Poster Group 2

Background:
Rotaviruses are enteric pathogens responsible for life threatening diarrhea in children. Despite several globally distributed vaccine programs, rotavirus-related deaths still number in the hundreds of thousands. Given the burden of these pathogens, the development of vaccine alternatives or direct-acting therapeutics is a necessity. Understanding rotavirus particle structure is fundamental to this pursuit. The rotavirus virion, ~90 nm in diameter, is comprised of three concentric protein layers each exhibiting icosahedral symmetry. The outermost layer is shed during cell entry, yielding a double-layered particle (DLP) of ~70 nm that initiates the infectious cycle. VP6 makes up the exposed T=13 layer of the DLP, which surrounds a T=1 core shell comprised of VP2. VP1 polymerases are encapsidated beneath the VP2 core shell, just off-center from each 5-fold axis. A virus-like particle (VLP) system is regularly used to study protein interactions critical for DLP assembly. In this system, recombinant VP2 and VP6 are expressed in insect cells using baculovirus vectors. These capsid proteins self-assemble into “double-layered” 2/6-VLPs, which can be purified from the culture media. Co-expression of VP1 along with VP2 and VP6 yields 1/2/6-VLPs. There are currently no high-resolution structures of rotavirus VLPs, hampering understanding of whether they truly mimic native DLPs. Therefore, we employed cryo-electron microscopy (cryo-EM) and single particle analysis (SPA) to solve high-resolution structures of rotavirus strain SA11 2/6-VLPs and 1/2/6-VLPs.
Methods:
Rotavirus strain SA11 VLPs were designed for expression using an established and well-characterized baculoviral vector system and subsequently expressed in SF9 cells. The VLPs were separated from cells by ultracentrifugation against a 35% sucrose cushion, and the resulting pellet was further purified using isopycnic ultracentrifugation in a cesium chloride (CsCl) gradient. The rotavirus SA11 DLP was produced by infection of MA104 cells with native rotavirus, followed by separation of virus particles from cell debris via centrifugation. Native triple-layered particles (TLPs) were rendered non-infectious by stripping of the outer capsid in ethylenediaminetetraacetic acid (EDTA), producing media containing DLPs. DLP particles were then purified under the same conditions as VLPs, using a sucrose cushion followed by isopycnic centrifugation in CsCl. Purified particles were frozen by plunge-freezing on copper (Cu) 200 mesh Quantifoil® grids, with 2µm holes and a hole-spacing of 1µm, using a Thermo Scientifc Vitrobot Mark IV. Particles were then imaged at 59,000 magnification using a Titan Krios cryo-EM and a Falcon 4 direct electron detector (DED). The resulting images were then processed using SPA workflows in the CryoSPARC v4.1.2 software package, where individual particles were isolated and computationally averaged to solve the 3D structures of each VLP. Atomic modeling of the rotavirus asymmetric unit was done using Phenix v1.21 and Coot v0.9.8.4 software.
Results:
Without icosahedral symmetry enforcement, 3D structures of 2/6- and 1/2/6-VLPs were solved to resolutions of 4.9Å and 4.6Å, respectively. Icosahedral symmetry (I1) enforcement allowed for improved structural resolution of 2/6- and 1/2/6-VLPs to 4.0Å and 2.7Å, respectively. The 2.7Å structure of the 1/2/6-VLP further allowed for isolation and de novo atomic modeling of the rotavirus

asymmetric unit as well as direct comparison to the rotavirus strain UK DLP X-ray crystal structure (3KZ4), demonstrating remarkable similarity and structural fidelity. The rotavirus SA11 DLP was resolved, without symmetry enforcement, to a resolution of 6.12Å. Icosahedral symmetry enforcement was not appropriate for the DLP structure, which contains asymmetric enzymes and genomic material.
Conclusions:
In achievement of the project aims, not only were the first high resolution structures of rotavirus VLPs produced, but also the first atomic model of a rotavirus VLP. This success significantly validates the rotavirus VLP system as an appropriate research tool, mimicking native rotavirus structure, and contributes to structural knowledge of the rotavirus VLP which is currently in development as a potential vaccine platform. Ongoing work seeks to (i) improve the 2/6-VLP structural resolution to ~2.7Å and (ii) to map structural differences, if any, between the particle types (i.e., 2/6-VLPs v. 1/2/6-VLPs v. DLPs).

Keywords:

rotavirus, virus-like particles, structural biology

Reference:

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Scale-bridging microscopic characterization of complex energy devices enabled by novel cross-sectional preparation routines

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Poster Group 1

Background incl. aims
Energy devices like batteries, fuel cells and solar cells are assembled from multiple functional components with different material properties. To gain a fundamental understanding of structure property relations, to systematically contribute to performance enhancement and to unravel device degradation and failure mechanisms, high quality cross-sections of entire devices or of as large regions as possible are required for complementary microscopic analysis.
Methods
Since many devices not only consist of different classes of materials but also of liquid and/or air sensitive components, working under cryogenic and/or inert conditions is essential to conserve their pristine state. To enable scale-bridging cross-sectional characterization of those devices all the way down to the atomic level, we utilize different preparation tools. A novel self-built cryo-cutter allows us to produce cross-sections of entire devices like pouch-cells in a one-step process and thus to investigate the inner structure in their pristine state by OM from the macroscopic scale to the optical resolution limitations. For characterization down to the atomic scale (cryo)ultramicrotomy is employed to generate high-quality and large-area cross-sections, e.g., of PEM-FCs and even highly reactive battery electrodes not only for investigation by OM/SEM (microtomic blockface) but also for (atomic resolution) TEM, as high-quality thin samples with thicknesses below 50 nm are provided.
Results
In this contribution, we demonstrate the scale-bridging capabilities of those preparation techniques applied to various devices and their components in conjunction with advanced (electron) microscopic and spectroscopic methods. Examples include devices like batteries (Fig. 1 a) and components thereof (Fig. 1 b) as well as complex fuel cells.
Conclusion
The applied techniques and developed routines are ideally suited to investigate even complete devices, individual components as well as materials. Aspects range from interfaces of individual layers and components in terms of their morphology and contact to the systematic identification of their composition and chemical bonding states.

Acknowledgement
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Keywords:

energy devices, ultramicrotomy, batteries, cryo

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Carbonaceous inclusions from the oldest sediments on Earth

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Poster Group 2

Background incl. aims:
The Isua Supracrustal Belt in Southwest Greenland contains rocks which are at least 3.7 billion years old, and are among the oldest rocks on Earth. Sediments found here display contiguous horizons of carbonaceous material parallel with sedimentary bedding planes (Rosing 1999). This material, present as graphitic carbon, has been proposed to be some of the oldest remnants of life on Earth, inferred based on its mode of occurrence, carbon isotope ratios, its association with heteroatoms such as nitrogen, oxygen, phosphorous and iron, and from the nanoscale crystal structure (Ohtomo et al. 2014, Hassenkam et al. 2017), evidence which is all compatible and together suggestive of an origin of the graphitic material as biogenic pelagic detritus.
In certain places, the graphite is enclosed in secondary metamorphic minerals such as garnets. This graphite occurring as inclusions has been targeted by previous studies, which also identified certain viscous fluid inclusions. The study presented attempts to characterize the chemistry of these fluid inclusions occurring alongside the graphitic material within garnet porphyroblasts.
Methods:
In this study, the liquid inclusions are accessed by breaking open the garnets and their residues are subsequently characterized using atomic force microscopy (AFM), as well as infrared spectroscopy coupled to AFM (NanoIR). Optical photothermal infrared spectroscopy (O-PTIR) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) are also used to characterize the chemistry of the inclusion residues and to assess their association with the solid graphitic inclusions.
Results:
Using AFM-based infrared spectroscopy, we obtain infrared absorption spectra from just the inclusion at a high spatial resolution. The liquid inclusion residues are ubiquitously characterized by infrared absorption at wavenumbers 1280 cm-1 and 1660 cm-1, interpreted as the C-N and C=O vibrational absorption from amide-like functional groups. Similarly, ToF-SIMS shows secondary species containing C, N and O from the inclusion residues. O-PTIR shows how the amide-like infrared absorption signal can be traced along horizons of graphitic material.
Conclusion:
This study shows how infrared spectroscopical methods coupled to optical and scanning probe microscopy methods can be used to characterize the chemistry of minute domains of ancient carbonaceous material occurring as inclusions in garnet porphyroblasts. The observations from NanoIR were corroborated by ToF-SIMS analyses. Together, these methods indicate that the carbonaceous material consists of C, N, and O-rich material in an amide-like configuration.
The chemistry of the inclusions is consistent with that which would be expected from degraded biogenic matter. Together with previous studies, this is highly suggestive of a biological origin for the carbonaceous material found in the oldest sediments on Earth.

Keywords:

AFM, IR-spectroscopy, ToF-SIMS, early life

Reference:

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Probing Chirality and Topology in Ferrimagnetic Multilayer Systems

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Poster Group 1

Background incl. aims:
Chiral ferrimagnets emerge as promising candidates for spintronics, especially for skyrmion-based device applications1,2. This ferrimagnet class lacks long-range crystalline-like structural order. Still, it exhibits a diverse set of appealing material properties, including bulk DMI of Néel type2, perpendicular magnetic anisotropy of bulk origin, and vanishing stray fields. Such characteristics of rare earth transition metal ferrimagnets contribute to a distinctive dual nature that integrates features from ferromagnets and antiferromagnets3. We aim to utilize advanced electron microscopy techniques to overcome the challenge of a volume-sensitive method for quantifying the strength of bulk DMI, particularly considering the crucial parameter of thickness.
Methods:
Ferrimagnetic Dy/Co-based multilayer films were prepared using the RF/DC Magnetron Sputtering technique for thin-film deposition techniques, ensuring precise control over film thickness and composition. In this study, we utilise JEOL NEOARM 200F in the LOW-MAG mode for Fresnel Lorentz Transmission Electron Microscopy (L-TEM) imaging, leveraging the objective lens as a magnetic field source. This facilitated to resolution of the chirality and topology of spin textures in thick ferrimagnetic Dy/Co multilayer films and quantification of the strength of Néel-type bulk DMI.
Results:
Under varying conditions, involving field cycling and tilting in the L-TEM microscope, we observe the coexistence of both skyrmions and topologically trivial bubbles within the material, indicating an intricate balance of micromagnetic interactions. In addition, we have encountered a fascinating underlying worm domain pattern that remains stable even after the saturation of chiral spin textures. In our exploration of DyCo monolayers, we encountered intriguing domains through high-precision MFM imaging, revealing a host layer beneath the normal domain pattern that guides the as-grown domain pattern. A similar background pattern, even higher periodic modulation appears during the L-TEM imaging, seemingly functions as a guiding pathway for normal domains, suggesting its potential role in influencing their behavior (see Fig. 1).
Conclusion:
Overall, the study highlights the crucial role of local real-space probes in comprehending the chirality of bulk DMI ferrimagnets. Furthermore, it demonstrates the capability of the Fresnel L-TEM technique in determining local saturation magnetization and revealing the imprinted

phenomenological structural phase under the magnetic domains with high resolution. Ultimately, these findings pave the way for further exploration and exploitation of these materials.

Keywords:

LTEM, chirality, magnetic, structural imaging

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Multidimensional Electron Ptychography

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Poster Group 1

Background
Over recent decades, scanning transmission electron microscopy (STEM) has become widely used for matierals charaterizations, achieving atomic-level detail in structural analysis across various scientific disciplines, including physical, chemical, and biological studies. With the recent development of high-acquisition-speed pixelated detectors and advanced computational methodologies, 4D-STEM is progressively transforming into a standardized modality in high-resolution EM. This method integrates a range of applications, from orientation mapping and strain measurement to electric and magnetic field mapping. Furthermore, it transcends the boundaries of conventional STEM imaging to include super-resolution techniques and to provide phase-based imaging, such as ptychography.
Methods
Ptychography, a technique for phase retrieval that extends from the concept of scanning in-line holography introduced by Hoppe, involves shining a light source on a specimen. It captures a sequence of diffraction patterns based on the position of the light source and utilizes iterative algorithms to reconstruct the wavefunction of the sample's exit plane.. Ptychography has attracted considerable interest from both X-ray and electron communities for its potential applications in super-resolution imaging, reaching a resolution of 0.39 ångströms. Electron ptychography has proven to have high-contrast light-element detection detection, high dose-efficiency efficient and 3D imaging [1,2]. Recently we have shown its potential to yield high-contrast image and high sensitivity in capturing phase information from biological samples [3,4]. The significance of phase imaging extends beyond the ability to detect light elements at super-resolution and minimal dosage, as it also promises to provide unique information associated with local variations in magnetic and electrostatic fields.
Results
In this paper, we explore the established multidimensional applications of electron ptychography, such as 3D tilted tomography [2], and single-particle analysis [4], conducted under both room and cryogenic temperatures. Specifically, we introduce a new STEM configuration illustrated in Fig. 1. This setup features an advanced hollow detector that is adeptly coupled with an EELS spectrometer [1]. The design facilitate simulatious, complementary analysis, leveraging the capabilities of sensitive phase and Z-contrast detection alongside comprehensive chemical mapping.
Conclusions
In ptychography, the image-forming optics of traditional imaging modes are replaced by computational methods that utilize a series of electron diffractions collected by rapid detectors. This technique has exhibited great potential in various important applications, including the high-contrast detection of light elements and low-dose imaging. The concept of multidimensional ptychography expands upon this by recording data more than the two dimensions. This expansion may involve capturing changes in the third dimension, electron energy loss, or time.

Keywords:

4D-STEM, Ptychography, Super-Resolution

Reference:

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Simulating electron energy-loss spectroscopy and cathodoluminescence for nanoparticles located on substrates

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Poster Group 2

Background
Electron energy-loss spectroscopy (EELS) and cathodoluminescence (CL) are widely used to study the plasmonic properties of individual nanoparticles. In the experiment, the sample is irradiated with relativistic electrons having energy of about 100 keV. After the interaction with the sample, electrons' energies are measured to obtain the energy loss spectrum (EELS spectrum). When interacting, the sample itself begins to emit photons. This light is collected by a parabolic mirror to obtain CL spectrum. The main advantage of EELS/CL over light scattering methods is the ability to study plasmonic properties with a spatial resolution of less than 1 nm (at wavelengths of visible light and infra-red).

Methods
We extended the capabilities of the discrete dipole approximation (DDA) to simulate EELS and CL. Until recently, there was only a theory for simulating the case of particles located in vacuum, which is never true in the experiment. In an electron microscope, the particle is always placed inside or on top of the substrate medium, which redshifts the plasmonic response of the sample. We extended our previously published DDA theory for particles inside an infinite medium[1] to simulate EELS and CL for particles on top of a semi-infinite medium, which successfully reproduces experimental results for particles deposited on TEM membranes of finite thickness.

Results
With the extended theory, the simulations successfully reproduce the results of EELS experiment where a gold nanoprism (209 nm side length, 10 nm thickness) was deposited on 30-nm-thick Si3N4 substrate.[2] A straightforward workaround for such simulations (with any DDA code) is to discretize a finite volume of the substrate below the particle, which would take 1 645 070 dipoles for both prism and the substrate (Fig. 1a), making it a heavy computational task. In the extended theory, however, the presence of substrate is accounted in the DDA formulation of the problem (by adjusting the dipole-dipole interaction function) requiring only 66 206 dipoles to discretize only the prism itself (Fig. 1b). This requires much less computational resources, leading to about 10 times faster simulations.
In the simulations of the prism, the Si3N4 refractive index was set to a constant 2.15 for all energies, and the refractive index of gold was taken from the data by Babar et al.[3] The redshift caused by the semi-infinite substrate in the simulated plasmon energy peaks matches the ones observed in the experiment by Griffin et al.[2]. Moreover, the simulated plasmon maps (Fig. 1c) also match the experimental ones,[2] in contrast to the simulations for a particle in vacuum in that paper.

The extended theory also successfully reproduces the CL experiment where a gold nanorod was placed on top of SiO2 membrane,[4] and even the CL experiment where a gold nanorod-shaped antenna was on top of gold substrate.[5]

Conclusion
Simulations with the semi-infinite substrate theory successfully reproduce experimental spectra and plasmon maps for both EELS and CL for samples on top of dielectric substrates, and for CL even in the case of a metallic substrate. Brief theory overview followed by simulation results in comparison with previously published and our own experiments will be presented at the conference.

Acknowledgements
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Keywords:

Plasmonics, EELS, cathodoluminescence, DDA

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Towards Atomic Resolution of Cryogenic Ptychography Single-Particle Analysis (Cryo-EPty SPA)

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

Background
Rapid advances in cryo-electron microscopy (cryo-EM) have made it possible to visualize three-dimensional structures of a wide range of biological macromolecules at near-atomic resolution in a close-to-native, frozen-hydrated state 1. However, the high electron irradiation sensitivity inherent in biological samples leads to a low signal-to-noise ratio and low contrast in EM images. While cryo-EM single particle analysis (SPA) usually records microscopic movies with high defocus to improve contrast, high-defocus condition attenuates low-frequency information and rapidly reverses high-frequency information in the phase contrast transfer function (PCTF), which causes difficulties in accurate identification, classification and alignment of particles to a common reference. Ptychographic diffractive imaging, a technique capable of reconstructing phase information from diffraction patterns using an iterative algorithm known as ePIE (Fig. 1a), holds great promise for achieving super-resolution, high-contrast, low-dose, and 3D imaging of biological samples in vitreous ice at low doses2. Moreover, ptychography utilizes the entire diffraction pattern , making it particularly dose efficient, especially when using direct electron detector data with a high signal-to-noise ratio at a low electron dose 3. Using cryogenic electron ptychography (cryo-EPty) [4], we have successfully reconstructed the 2D phase images of rotavirus double-layer particles (DLPs) measuring 76.5 nm in diameter at doses of 20 e/Å2, corresponding to a convergence semi-angle (CSA) of 4.83 mrad, employing approximately 300 particles. Additionally, we have demonstrated the visualization of 3D structures by integrating SPA 4. The resolution of the 3D map was estimated to be 1.86 nm at the nanometer level. These advancements underscore the potential for combining cryo-EPty and single-particle analysis to reach atomic-level resolution in imaging . We aim to increase the resolution achieved by not only increasing the number of particles used as is done in traditional SPA but by increasing the optically limited resolution with a larger convergence semi-angle.

Methods
We used apoferritin embedded in vitreous ice as a benchmark sample. Experimental cryo-EPty SPA datasets (Fig. 1b) were acquired in a scanning diffraction configuration, in which a defocused probe is scanned over a sample at cryogenic temperatures for probe overlap and information redundancy of approximately 85% as is commonly employed for defocused cryo-EPty 5. The use of a defocused probe enables a lower electron fluence than employed in physical sciences while maintaining full coverage of the scanned specimen area.

Results
We reconstructed 2D ptychographic phases of apoferritin particles with varied CSAs (i.e., 4.64 and 8.01 mrad) as shown in Fig. 2a-b to obtain information transfer over a greater range of spatial frequencies. We will demonstrate that using the SPA pipeline with the ptychographic phase, high-resolution 3D density maps of apoferritin can be reconstructed from the stack of particle phases.

Using a Fourier Shell Correlation, we anticipate that the 3D density maps will approach a resolution of a few angstroms with a greater number of particles. Our findings show that larger convergence angle datasets produce higher-resolution information that can be combined with essential lower spatial frequency information for data across a greater range of spatial frequency.

Conclusion
Our findings suggest that the promise of cryo-EPty combination with SPA will pave an alternative method for high-resolution 3D reconstructions of biological samples, potentially reaching atomic resolution .

Keywords:

4D-STEM, Ptychography, SPA, Cryo-EM

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eCHORD crystalline orientation maps: channeling contrast at interfaces

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Poster Group 2

Background incl. aims
Every signal acquired by a scanning electron microscope (SEM) originates from a certain interaction volume below the surface, making localization of the received information challenging. For orientation maps obtained by EBSD, the Kikuchi patterns acquired at the interface between two grains contain contributions from both crystals. This impacts the precise localization of the interfaces on the generated map and thus, influences the spatial resolution when dealing with objects of a size comparable to the interaction volume [1]. For EBSD, this interaction volume can be hardly reduced, as Kikuchi patterns significantly weaken at accelerating voltages of 5kV and lower.
The same “blending” effects are expected in eCHORD orientation maps obtained using SEM since the channeling contrast also arises from a certain interaction volume beneath the sample surface [2, 3]. However, at such low accelerating voltages, electron channeling contrast can still be accomplished, hence orientation maps with a reduced interaction volume could potentially be obtained via the eCHORD approach.

Methods
When considering the orientation maps obtained using the eCHORD approach [2, 3], the raw data comprises of an image series captured by rotating the region of interest (ROI) within the SEM, with the sample being tilted at approximately 10-15°. Such an image series constitutes a datacube from which intensity profiles can be extracted, one for each beam position on the ROI, representing the variation of the backscattered electron (BSE) signal as the sample rotates. Similar to Kikuchi patterns, these intensity profiles are also influenced by the respective contributions of two neighbouring crystals. To evaluate how this effect affects the eCHORD orientation maps, acquisitions were carried out on a copper thin film of 3 µm thickness exhibiting submicronic twins with 80 nm in thickness. The acquisition conditions are outlined as follows: 120 images (2048*1536) were captured using a frame time of 20.3s per image, at an accelerating voltage of 5kV, with a sample tilt of 15°, and utilizing a below-the-lens BSE detector. It is worth noting that an accelerating voltage of 5kV is now a routine condition for eCHORD experiments.

Results
Experimental evidences of a hybridisation effect are presented, showing that the intensity profile at a given interface is a blend between the intensity profiles of the two neighbouring grains. In addition to the interaction volume, various other factors explaining these hybridisation effects are presented and discussed: CHORD geometrical setup, image alignment, denoising, and indexing by pattern matching. Numerical simulations were also performed to quantitatively assess the effect of this hybridisation on the results of indexing intensity profiles, leading to a proposed adjustment in the indexing algorithm for the eCHORD method. This adjustment involves deriving the experimental as well as the theoretical profiles of the CHORD database prior to indexation, aiming to limit the effect of hybridisation on the final orientation maps. We demonstrate that this new algorithm has beneficial

effects on indexation quality in general, compensating for differences between simulated and experimental profiles.

Conclusion
An effect of hybridization at interfaces has been observed in eCHORD orientation maps, which is limited by the adjustment of the indexing algorithm, thus demonstrating its robustness against degraded data.
This work represents an intermediary step in a more comprehensive approach aiming to enhance the spatial resolution in orientation maps obtained by eCHORD. This is achieved through the reduction of the accelerating voltage, improving the acquisition procedure, and by enhancing the post-treatment operations.

Keywords:

eCHORD, orientation mapping, interfaces, SpatialResolution,

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Coupling Clustering and Channeling Contrast in the Scanning Electron Microscope

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Poster Group 2

Background incl. aims
Channeling contrast in the Scanning Electron Microscope (SEM) facilitates the imaging of crystals and their defects. A name for this approach has been coined as ECCI for “Electron Channeling Contrast Imaging”. This contrast arises from the diffraction of the electron beam as it traverses through the crystalline sample, and is intricately linked to the crystalline orientation of the underlying crystal. Within a grain, the channeling contrast hence aids in the recognition of crystalline defect. In a polycrystalline sample, neighbouring grains can be differentiated by the channeling contrast due to their difference in orientation. However, obtaining multiple ECCI images with different angles of electron beam incidence on the crystal is necessary: (i) to identify the defect character (such as screw or edge dislocations) and (ii) to ensure that all grains are detected within a polycrystalline region of interest. Eventually, the acquired ECCI image series have to be treated quantitatively to extract valuable information about the microstructure. In this context, machine learning, particularly clustering algorithms, is extremely beneficial for analyzing ECCI data. As an example, defects like dislocations and stacking faults can be detected in an ECCI image series by clustering [1-2]. In the present work, the idea is to process ECCI image series by clustering: (i) to extract the grain size distribution rapidly in an automatic and reproducible manner, (ii) to extract meaningful information about the microstructure, i.e., recrystallized fraction in an aluminum alloy [3], and (iii) to reduce dramatically the computation time of orientation maps using the eCHORD approach [4].

Methods
The raw dataset consists of a series of ECCI images acquired by rotating the region of interest (ROI) in the SEM, with the sample being tilted to~10-15°. Such an image series constitutes a datacube from which intensity profiles can be extracted at each ROI position, reflecting the variation of the backscattered electron (BSE) signal due to the sample rotation. To demonstrate the effectiveness of clustering algorithms for estimating grain size distributions, a copper thin film of 3 µm thickness exhibiting submicronic twins as thin as 80 nm in thickness is employed as a test sample. For determining the recrystallisation fraction, a 6XXX aluminum sample is considered and subjected to heat treatment to achieve a ~75% recrystallised microstructure. Finally, to explore the possibilities for fast-indexing within the framework of the eCHORD approach, duplex steel with coexisting austenite and ferrite phases is adopted.

Results
The intensity profiles defined at each place in the ROI will be used to cluster the data for grain size distribution calculations. The HDBSCAN algorithm [5] was selected for clustering, as it does not require the number of clusters (i.e., of grains) as an input. Furthermore, the algorithm automatically determines a reasonable criterion for defining a cluster. The influence of the intensity profiles pre-treatments is discussed, as well as the minimal number of images required for the clustering is explored, which is found to be between 10 to 20 ECCI images only.

For the recrystallisation fraction, it appears that the non-recrystallised area in the ROI corresponds to pixels that are left apart within the “noisy” class during the clustering using HDBSCAN. The results show good agreement with the recrystallised fractions as determined by EBSD on the same ROI. Concerning eCHORD orientation mapping, the approach involves grouping similar intensity profiles into clusters, computing the mean profile for each cluster, and subsequently indexing it by comparing it to an eCHORD database. In this case, over-clustering is not a problem, and the KMEANS algorithm has been used with a significantly higher number of clusters than the apparent grain count, ensuring a reasonable spatial resolution is maintained. If some clusters belong to the same grain, the indexing operation will yield the same crystallographic orientation in the final map. This approach has been applied to the image series of duplex steel, allowing it to discriminate between the austenite and ferrite phases. Due to the tremendous GPU-computed indexing speed, it is possible to compare all the cluster profiles to both austenite and ferrite databases in order to perform phase mapping and retrieve the correct orientations.

Conclusion
This work demonstrates that clustering the ECCI image series using several types of clustering algorithms (HDBSCAN, KMEANS) can be extremely beneficial for several applications such as grain size distribution calculation, recrystallization fraction determination, phase discrimination, and orientation map computation.

Keywords:

HDBSCAN, KMEANS, eCHORD, orientation mapping

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Water condensation on core-shell nanofibers studied using multiscale environmental electron microscopy

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Poster Group 2

Background incl. aims
Superhydrophobic, omniphobic, icephobic and self-cleaning mean advanced properties of a surface when precise control of the roughness combining different levels of topography, from micro- to-nanostructures for the called hierarchical surface [1-4]. Particularly one-dimensional nanostructured matrix allows the integration of nanowires with dimensions around several micrometers with the nanofiber surface roughness at the nanoscale. We study here hierarchical photoactive core-shell nanostructured surfaces, which wetting behaviour can be easily tuned. Understanding the mechanism of water condensation on such nanofibers at the nanoscale is crucial to design efficient systems. In this presentation, we identify their hydrophobic/hydrophilic behaviour and discuss the water condensation mechanisms using multiscale environmental electron microscopy.
Methods
Liquid-phase electron microscopy can be carried out with different systems. We used a dedicated environmental scanning electron microscope (ESEM) and an environmental transmission electron microscope (ETEM), in which different hydration states can be reached by controlling the sample temperature and the water vapor pressure.
TiO_2 core-shell nanowires were fabricated by vacuum and plasma assisted deposition techniques according to the soft template method [5]. In order to obtain the water repellent character, surface functionalization by fluorine-based grafting of the oxide surface has been employed [1]. Hydrophobic nanowires were collected by scratching the substrate and were dry-deposited onto a grid with a holey carbon membrane. The fibers were either analyzed as such, or plasma-treated to become hydrophilic. During the experiments, care was taken to minimize irradiation damage and preserve the hydrophilic/hydrophobic nature. An image processing method was developed to highlight the changes brought about by water condensation.
Results
Experiments in ESEM were performed in the STEM mode (see Figures 1a and d). Under similar conditions, where water condensates on the holey carbon membrane, the hydrophilic nature of the plasma-treated nanowires can clearly be evidenced: they are systematically surrounded by water. On the contrary, water droplets are visible on the carbon membrane but not on hydrophobic nanostructures. The presence of water onto hydrophilic surfaces but not on hydrophobic ones could be confirmed at the nanoscale using similar thermodynamic conditions in ETEM (see Figures 1b and e). The experiments were continued until water was visible around both types of nanowires (see Figures 1c and f).
Conclusion
ESEM images, obtained on a large number of core-shell nanowires, enable us to assess the reproducibility of results obtained at nanometric scale in ETEM on isolated fibers. Irradiation of the

nanostructures with the electron beam during the experiments did not modify their hydrophilic/hydrophobic character. In fact, clear differences in behavior towards water condensation are revealed at the nanometric scale. These differences will be discussed as a function of nanostructure and chemistry.

Acknowledgements
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Figure caption
water condensation on core-shell TiO2 fibers. a, b, c) hydrophilic fibers. d, e, f) hydrophobic fibers. a, d) BF-STEM images in ESEM. b, e) BF-TEM images in ETEM at the initial state. c, f) BF-TEM images in ETEM on hydrated fibers.

Keywords:

ESEM, ETEM, liquid, in-situ, nanowire

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Glacios 2 Cryo-TEM and Smart EPU Software streamline Cryo-EM for drug design with higher throughput

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Poster Group 2

Background incl. aims

Cryo-EM has significantly impacted the field of structural biology due to its capabilities to resolve the three-dimensional structure of proteins, protein complexes and other biological macromolecules at high or even atomic resolution. However, acquiring high-quality data still largely depends on the expertise of the microscope operator. This limits the speed of adoption as researchers must invest considerable time into understanding microscopy and the technicalities of the workflow. Here, we show how the Thermo Scientific™ Glacios 2™ cryo transmission electron microscope (Cryo-TEM) with low-energy-spread Cold Field Emission Gun (E-CFEG) and combined with Thermo Scientific™ Smart EPU Software enables users of all expertise levels to acquire high-quality cryo-EM data.

Methods
In collaboration with the Greber lab (Institute for Cancer Research), several high-resolution structures of the 85 kDa Human CDK-activating kinase (CAK) were determined. CAK is a master regulator of cell growth and division and is a promising target for cancer therapeutics.

Results
Structures of CAK were rapidly determine in free and nucleotide-bound states as well as in complex with 14 inhibitors(1). In addition to achieving high-resolution structures from large datasets, ~4 Å and ~3 Å-resolution structures of ligand-bound complexes were determined using from only 1 hour and 4 hours of data collection respectively. Furthermore, in combination with an E-CFEG we have been able to generate a 1.5 A reconstruction of Apoferritin; the highest resolution 200 kV structure to date.

Conclusion
These results show the use of cryo-EM to enable structure-based drug design.

Keywords:

Cryo-EM; Cold FEG; automation; throughput

Reference:

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Use of micron sized silicon particles as anode by capacity controlled cycling

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Poster Group 1

Background and Aims:
Silicon offers an extremely high gravimetric capacity of 3579 mAh /g, about 10 times greater than graphite. Making it a promising next-generation anode material for lithium-ion batteries (LiB). However, throughout charge-discharge cycles, silicon experiences significant volume fluctuations, leading to material cracking and pulverization. This leads to solid electrolyte interphase (SEI) layer breaking, necessitating the formation of a new SEI layer, which results in capacity loss and poor cycle performance. Partial lithiation or capacity-limited charging presents a promising strategy for addressing the challenges associated with severe volume expansion in lithium-ion batteries. [1,2] Nonetheless, further investigation into the morphology of the silicon particles is necessary to enhance battery performance and durability. This study aims to delve deeper into the morphology of partially lithiated porous and non-porous silicon microparticles, as little literature exists on the subject. [3,4]

Methods:
The samples were prepared by Focused Ion Beam (FIB). Employing post-mortem analysis, we examined the morphology of partially lithiated silicon microparticles after being cycled. Selected area electron diffraction (SAED) and energy dispersive X-ray spectroscopy (EDX) allow distinguishing between amorphous and crystalline silicon phases present after the lithiation, resulting in a very valuable tool. Furthermore, Techniques like computed tomography and in-situ transmission electron microscopy (TEM) experiments shed further light on porous silicon microparticles and their behaviour upon lithiation.

Results:
Lithiation of the non-porous microparticles leads to the amorphization of the pristine crystalline silicon, leading to changes detectable with TEM. TEM analysis revealed the absence of particle pulverization across all samples despite evidence of lithiation within the particle interiors This highlights the positive influence of lower cutoff potentials upon morphological changes. In-situ studies performed on both the porous and non-porous Si samples further show the reason for long-term stability.

Conclusions:
Our findings provide insights into the complex interplay between silicon and lithium during partial lithiation shedding light on the morphology of silicon microparticles used in anodes crucial for anode applications in lithium-ion battery technology . Furthermore, using capacity-controlled charging with porous silicon microparticles has demonstrated the potential for achieving high long-term capacity, paving the way for realizing the full potential of lithium-ion batteries.

Figure caption:
Bright field (BF) TEM image and corresponding diffraction images of silicon particles. The image on the left a) corresponds to the pristine silicon anode, and on the right b) is an example of the anode after being lithiated.

Keywords:

Lithium-ion-batteries, silicon microparticles, partial lithiation,

Reference:

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Analyzing conformational and compositional heterogeneity of macromolecular complexes by cryo Electron Microscopy: Zernike3Deep and HetSIREN

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

BACKGROUND INCL. AIMS
Biological macromolecules have varying degrees of conformational flexibility and compositional heterogeneity that complicates the image processing of large sets of cryo Electron Microscopy images, especially when flexibility is continuous or there are many compositional options. New approaches like HetSIREN (1) and the Zernike3D family of algorithms (2) are capable to generate conformational landscapes representing complex heterogeneity cases while also producing higher resolution maps of the different states. Additionally, novel meta-approaches, like the ones pioneered by Scipion Flexibility Hub (3,4), start bringing consistency and reliability to this emerging field of flexibility analysis and map quality improvement.

METHODS
HetSIREN and Zernike3Deep are two cutting-edge deep learning algorithms meticulously designed to address the analysis of structural heterogeneity in cryoEM single particle analysis. These new algorithms not only excel at segregating particles into distinct 3D conformational states but also places special focus on the representation of structural features and map quality to enhance the biological interpretability. These new algorithms are distributed as part of the Scipion Flexibility Hub (4), together with new tools designed to analyze and integrate findings across different conformational landscapes.

HetSIREN performs a constrained heterogeneous reconstruction or refinement, relying on a structural prior in real space. This approach decodes high-resolution stable and non-stable structural states, providing a more sensible picture of the structural landscape explored by a biomolecule.

In contrast, Zernike3Deep relies on a semi-classical neural network that is specifically designed to learn mathematically meaningful latent spaces. These spaces are to be used in conjunction with the Zernike3D basis to accurately expand molecular motions. The decoded motions are suitable not only for modeling high-resolution structural states captured in the conformational landscape but also for applying as a correction for motion blur artifacts during the reconstruction process. This application increases the resolution of molecular regions that are moving and difficult to resolve.

RESULTS
HetSIREN and Zernike3Deep have proven themselves as versatile tools for the heterogeneity analysis of cryoEM particle datasets with varying characteristics, emphasizing the importance of accurately representing structural features to enhance biological interpretation. To illustrate the capabilities of these methods, we present an analysis of the Gr-HsP90-FKBP51 complex, a highly dynamic and biologically significant biomolecule. Its understanding is hindered by motion blurring, which obscures both its structural characteristics and dynamic behavior.

The accompanying graphic summarizes the analysis conducted by Zernike3Deep, depicting the PCA representation of the conformational landscape, along with the primary motion described along the first principal component. Additionally, the graphic also compares the motion-corrected map derived from Zernike3Deep heterogeneity analysis with the one obtained without correction by a classical method in the field (CryoSPARC (5)). The results underscore the importance of incorporating advanced heterogeneity analysis into cryoEM workflows, enabling the recovery of structural information that would otherwise be overlooked by the conventional assumptions of more traditional methods regarding reduced and discrete structural heterogeneity.

CONCLUSION

CryoEM structural analysis is undergoing a revolutionary shift towards extracting richer and more accurate conformational landscapes. The advent of new heterogeneity algorithms moves beyond the limiting assumptions of classical methods, significantly reducing the amount of structural information discarded during estimation processes. In this work, we introduce two innovative deep learning methods for advanced heterogeneity analysis, named HetSIREN and Zernike3Deep, as well as a meta-analysis environment named Scipion Flexibility Hub. These methods are designed not only to accurately estimate conformational landscapes and structural states but also to place a strong emphasis on the representation of structural features. These approaches aim to enhance the biological interpretability of the estimated structural states and improve the resolvability of highly dynamic molecular regions.

Keywords:

cryoEM, Image Processing, Conformational Variability

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Multiscale characterization of plaster setting using operando multiscale liquid-phase Electron Microscopies

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Poster Group 1

Background incl. aims
Gypsum has been traditionally used as a building material, but can also be a model for the development of injectable biomaterials for bone substitution. Set plaster, or gypsum ($CaSO_4.2H_2O$), is prepared by mixing dry hemihydrate powder ($CaSO_4.0.5H_2O$) with water. Its properties as a solid binder are largely influenced by the setting reaction occurring when $CaSO_4.0.5H_2O$ dissolve and crystals of gypsum precipitate. It is well known that the resulting solid is formed by an interlocked network of gypsum needles and platelets, but the exact setting process is not well understood yet. The objective of our study is therefore to monitor the evolution of crystals and understand the entire setting process, using operando observations [1], using Liquid-phase scanning electron microscopy.
Methods
For Scanning Electron Microscopy (SEM) experiments, the hemihydrate powder is manually mixed with an aqueous solution containing 5.2 mM citric acid. Plaster paste is then deposited onto the window of a Quantomix cell placed in a Zeiss Supra 55VP SEM microscope. Such cells are indeed water - and airtight, and therefore plaster evolves under the same conditions as in real use, especially in terms of water-to-plaster ratio. Gypsum crystallization is followed by taking SE and BSE images every 30 s.
In situ Environmental Transmission Electron Microscopy (ETEM) experiments are performed on a Titan ETEM from FEI/TFS. The hemihydrate powder is dry deposited onto a grid covered with a holey carbon membrane, then placed on a cryo-holder from Gatan/Ametek. The temperature is slowly decreased to 0°C and once stabilized, the partial pressure of H_2O is increased until liquid water covers the hemihydrate particles.
Results
The dissolution of hemihydrates and crystallization of gypsum is followed operando in SEM. Image processing and analysis procedures are developed to segment the gypsum crystals and follow their growth vs time. Based on geometric information and the knowledge of the equilibrium morphologies [2], we propose an indexation of the crystal facets (see example in Figure 1). The growth rates of the different planes, including unstable ones, is measured.
In situ experiments in ETEM give a more precise view of the onset of gypsum nucleation, with the formation and evolution of facets at the nanometer scale.
Conclusion
The use of liquid-phase electron microscopy at different scales allows the investigation of gypsum growth in a real plaster mixture. The formation of unstable facets and their transformation into stable ones are evidenced from the onset to full plaster setting. Moreover, the growth rates for all types of facets can be measured and compared with the few values found in the literature. The influence of several parameters is discussed (addition of a retardant, presence of a membrane, image acquisition conditions and irradiation damage) [3].
Acknowledgements

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Figure caption
Growth of the gypsum crystals during plaster setting and indexation of a crystal for two different times.

Keywords:

operando, liquid, crystal, growth

Reference:

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Beyond Ribosomes: In Situ Structural Biology of a Challenging Target in C. Reinhardtii

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Poster Group 2

Background
Understanding high-resolution protein structure in the context of the whole cellular environment is the vision of visual proteomics. With the advent of high-throughput cryo-FIB and cryo-electron tomography (CryoET), paired with cutting-edge computational techniques, achieving such an ambitious goal is no longer a far-reaching dream. The new generation of cryo-FIB from Thermo Fisher Scientific uses plasma ions, which reduce redeposition and ion beam damage, substantially improving throughput. In preparation for a large-scale effort towards visual proteomics of the model organism Chlamydomonas reinhardtii, we have prepared enough lamellae to allow for acquisition of more than 1800 tomograms of different cellular compartments. Initial results demonstrate that the data is of sufficient quality to achieve sub-nanometer resolution (6Å) for the 80S ribosome using a fraction of the dataset (six tomograms). Together with collaborators, multiple proteins were selected as targets for sub-tomogram averaging (STA). One of these proteins required the development of a novel workflow. Photosystem II (PSII), which is present in very high abundance in the chloroplast, proved to be a challenging target as it is a membrane embedded protein with only a small region protruding into the lumen of the thylakoid membrane. The denoising neural network cryoCARE was implemented on all tomograms in the dataset to increase template matching accuracy. Unfortunately, in the case of PSII, denoising tended to blur or erase the small density that projects from the membrane which led to significant under picking when template matching was performed.
Methods
An alternative strategy that paired a regression denoising UNet with a semantic segmentation UNet was employed to generate candidate coordinates for further averaging and classification. Regression denoising: Synthetic training data for regression UNet was generated using CryoTomoSim. Using a box of 400x400x50 pixels, a mixture of medium and small proteins were modeled in four layers at 7.84 Å /pixel, exact protein identity is not important to training. 10 membrane vesicles were modeled. Iterations for each layer were 500, 500, 4000, 8000, and particle density was 0.8. Finally, the vitreous ice option was used. The output or ideal tomogram was simulated at -1 micron defocus, -89 to 89 tilt, 0.5 degree tilt increment and total dose = 0. The input or noisy tomogram was simulated at -3 micron defocus, -60 to 60 tilt, 3 degree tilt increment, total dose 80. Both datasets are loaded into Dragonfly 2022.2 (Object Research Systems) and used as training input and output for a regression 2.5D UNet with architecture: depth level 5, initial filter count 64, slice count 5, patch size 128, Loss function ORSMixedGradientLoss. Training proceeded for 46 epochs using a total of 15,840 patches. Segmentation: 2.5D Segmentation UNet for PSII was trained using Dragonfly 2022.2 according to protocol. In brief, Segmentation Wizard was used for manual annotation of 5-6 training slices and a generic UNet of the following architecture was trained: (2.5D: 3 slice, depth level: 5,

initial filter count: 64, patch size: 128). Training data included slices from tomogram numbers: 24, 373, 473, and 900. Aside from patch size, all hyperparameters are left as default. Training labels were Membrane, ATP Synthase, Ribosomes, PSII and Background. Post segmentation, PSII class was extracted for each tomogram, split into connected components, and center of mass X, Y, Z coordinates were calculated for each label and exported to CSV for subtomogram extraction.
Once trained, both UNets are applied to 41 tomograms to generate 52,000 PSII candidates which can be extracted for the STA workflow.

Results

With the initial set of 52,000 candidate coordinates, one high-quality class emerged during classification and as of the writing of this abstract an 18Å structure has been achieved (Fig 1). Ongoing work is in progress to apply this workflow to an additional 200 tomograms. With more candidate coordinates, optimized alignment of sub-tomograms, and further post-processing, we are confident we can improve the resolution of the in situ structure of PSII.

Conclusion

The scale of this dataset is exciting, but the huge number of molecular complexes within living cells makes it difficult to identify, confirm the identity of, and determine each structure by just one group. Achieving a full visual proteome of C. reinhardtii will necessitate a large collaborative effort. Challenging targets such as PSII demonstrate the need for new, creative methods or combinations of techniques to facilitate in situ structural determination. To that end, we would like to create an open access database for C. reinhardtii to accelerate annotation and curation, enable further cell biology research, and develop new computational tools for in situ cryo-ET. Along with sharing the raw data, reconstructed tomograms, denoised datasets, and structural determinations, we will provide high quality segmentations of selected datasets created using 2.5D (Dragonfly) and 3D (MemBrain Seg) UNets. This project has the potential to provide invaluable insights into cellular processes and will hopefully lay the foundation for future large-scale studies of other species.

Keywords:

CryoET, Deep Learning, Visual Proteomics

Reference:

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Cryogenic large volume 3D and TEM sample preparation with multiple ion species plasma FIB

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Poster Group 2

Direct quantitative investigation of the inner morphology and structure of soft materials is of critical importance to provide profound insights for properties evaluation. The scanning electron microscope (SEM) and focused ion beam (FIB), combined known as FIB-SEM or DualBeam, are conventionally recognized as a highly effective method to acquire 3D volume information. FIB-SEM together with integrated serial sectioning software has made the automated 3D data acquisition and analysis possible. However, slicing using Ga ion beam at room temperature has been found inducing severe damage to the beam sensitive soft materials, resulting in significant deterioration of the 3D data quality. Cutting edge cryogenic FIB-SEM technique provide a fully automated workflow which allows large volume, damage-free ion beam slicing and high spatial resolution SEM acquisition during serial sectioning under cryogenic conditions. With automated 3D reconstruction of the micrograph stacks, we can subsequently recover the comprehensive volume information of such beam sensitive materials. In addition, cryogenic multiple ion source PFIB has been confirmed to be capable of fabricating high quality large area TEM lamellae without damaging the beam sensitive bulk samples. Coupled with cryogenic in-situ nanomanipulator, the TEM samples can be easily lifted out under cryogenic conditions and subsequently transferred to TEM for further investigation.
In this paper we present a series of large volume 3D imaging results and TEM sample preparation examples of extremely beam sensitive soft samples. The samples were processed on Thermo Scientific Helios 5 Hydra Plasma FIB platform with multiple ion species (Xenon, Argon, Oxygen and Nitrogen) combined with integrated state-of-the-art rotatable cryo stage. The slicing and imaging acquisition was achieved using the latest generation Automated Slice and View software and the subsequent data processing was conducted using Avizo 3D analysis and visualization software. The unique technical experiment set up and comprehensive application experience will be discussed in this presentation. In addition, inert gas transfer workflow of the beam sensitive materials from the plasma FIB to TEM will be discussed.

Keywords:

Cryogenic, beam sensitive, soft materials

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Formation of MgSi grain boundary precipitates with core-shell structure in fcc-Al

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
Aluminum (Al) shows promise as an excellent conductor, but its usefulness in electrical and electronic industries is often limited by its relatively low strength. Alloying pure Al with other metals like Mg, Cu, or Ag can increase its strength, but this also results in a decrease in its electrical conductivity [1]. Another method to strengthen Al is by reducing the grain size through severe plastic deformation such as high pressure torsion extrusion. However, grain growth can occur at elevated temperatures [2]. Adding small amounts of Mg and Si can stabilize grain growth by forming MgSi precipitates, which hinder grain boundary migration. This phenomenon is known as the solute-drag effect [3]. The impact of these precipitates on electrical conductivity is primarily influenced by their size, shape, atomic structure, and composition. All these parameters depend on the thermomechanical treatment and this work aims to bridge between structural and physical properties including the influence of individual mechanism during the thermomechanical preparation of the material.
Methods
In this study, Al - 0.94 at.%Mg-1 at.%Si solid solution were plastically deformed at 100°C using high pressure torsion extrusion (HPTE) and subsequently aged at temperature between 130°C and 160°C for up to 48 hours to generate MgSi precipitates. Tensile tests and Vickers hardness measurments were carried out to get information about the mechanical strength at different areas of the sample. Electrical conductivitz was measured on the sample surface by the Eddy current method using a Sigmascope device.
To investigate the overall microstructure, scanning electron microscopy (SEM) in combination with electron backscatter diffraction (EBSD) was performed using a Yeiss Auriga 60 equipped with EDAX Digiview EBSD camera. Information about dislocation density and crystal size was recorded bz X-ray diffraction experiments.
The nanometer sized precipitates were studied using high resolution transmission electron microscopy (HRTEM) including geometric phase analysis (GPA) as well as advanced scanning transmission electron microscopy (STEM) techniques such as energy dispersive X-ray spectroscopy (EDS), electron energy loss spectroscopy (EELS) and 4D-STEM were conducted on a double corrected Titan Themis Z operated at 300 kV. Atom probe tomography was conducted correlatively for accurate quantification of the composition of the precipitates.

Results
The initial polycrystalline Al - 0.94 at.%Mg - 1 at.%Si was about 100 um. Following high-pressure torsion extrusion (HPTE), the grain size reduced by a factor of 20. Subsequent aging processes did not result in significant grain growth but rather induced texturing, as illustrated in the SEM-EBSD inverse pole figure (Figure 1a). STEM-EDS measurements detected the formation of magnesium silicide

(MgSi) precipitates at both grain boundaries (GBs) and within the grain interiors. It is noteworthy that the morphology of GB precipitates differs from those within the grains. Within the grains, precipitates primarily exhibit an elongated rod-like morphology, while at the GBs, precipitates appear as symmetrical spherical caps (see Figure 1b). This difference in morphology can be attributed to the higher energy of the GB, which attracts solute magnesium and silicon atoms more strongly. As an illustration, Figure 1c depicts a low-angle boundary with a misorientation angle of approximately 4^{o} between adjacent grains. Such a low-angle GB would be composed of a series of dislocations with spacings typically falling within the range of 11nm. Notably, GB precipitates were observed to preferentially form at the initial positions of these edge dislocations. Atomic-resolution imaging of a single precipitate revealed a core-shell structure with a partially disordered shell. 4D-STEM was utilized to determine the phase of the precipitate core, elucidating the presence of a complex U2-$MgSi_2$ phase.

Conclusion

The formation of MgSi-precipitates by HPTE is responsible for a higher strength and better electrical conductivity. Our electron microscopy studies reveal that the morphology, crystal structure and composition of GB precipitates differs from precipitates formed within the grains. The GB precipitate shows a core-shell structure with higher concentration of Si in the outer shell. This can be attributed to the higher segregation energy of Mg in Al than Si. Finally, the GB precipitate could mainly attribute to the better performance during conductivity measurements. HPTE can be used to increase the fraction of GB precipitates and this may open the path to engineer fcc-Al for better performance as electrical conductor.

Keywords:

Grain boundary, precipitation, STEM, APT

Reference:

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Controlling heterostructures with atomic precision in III-V nanowires using microheaters in an in-situ TEM

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PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

Precision in heterostructure control within III-V nanowires is essential for the advancement of future technologies, especially quantum computing and quantum internet. The ability to embed one crystal phase, e.g., zincblende, within another, e.g., wurtzite, can be used to determine the optical and electrical properties of so-called crystal phase quantum dots. This crystal phase engineering offers a sharp transition between phases, superior to compositional quantum dots, but its control remains challenging within traditional ex-situ growth environments. Integrated electron microscopy with gas handling capabilities offers a unique in-situ perspective, revealing the dynamics of crystal phase formation and the parameters influencing it. Our research study is focused on how changing the growth conditions can be used to enable atomically precise crystal phase control. We also look into the growth dynamics and the commonly assumed control parameter: the contact angle, to unravel its actual impact on crystal phase determination in GaAs nanowires.
We use a cantilever based microheater system for GaAs Metalorganic Vapor Phase Epitaxy (MOVPE) growth within a Transmission Electron Microscope (TEM) as depicted in the figure to the left. This setup enables us to map the crystal phase formation at different growth conditions, specifically varying temperature and group V precursor flow. Instantaneous adjustments in temperature and precursor flow allow for the real-time analysis of the dependencies between these growth parameters and the phase transitions as illustrated in the figure to the right. Additionally, we introduce a field perturbation technique to the catalytic droplet to decouple its shape from the underlying growth conditions, further investigating the mechanisms driving the phase formation.
Our findings reveal that crystal phase transitions can be controlled with atomic precision using temperature shift in-situ. The cantilever-based microheaters offer rapid temperature changes, achieving 100^{o}C variations within milliseconds, significantly outperforming the slower response of precursor flow adjustments, taking tens of seconds. This rapid temperature control is instrumental in creating atomically precise crystal phase quantum dots. We conduct an in-depth study of droplet geometry during these phase transitions, revealing critical insights into the growth dynamics. Furthermore, by applying an external electric field, we successfully deform the catalytic droplet, effectively decoupling the contact angle from the crystal phase formation process. This experimental approach underscores the complex interplay between physical conditions and nanowire growth mechanisms.
In conclusion, using microheaters within a TEM with an integrated gas handling system has enabled a detailed study of the growth dynamics and heterostructure formation in III-V nanowires. By having the advantage of instantaneous temperature changes, we have demonstrated the ability to form crystal phase quantum dots with atomic precision. Furthermore, our findings challenge the traditional coupling of the contact angle as a driving force for the crystal phase formation. This research paves the way for new methodologies in nanowire synthesis, potentially revolutionizing the approach to designing materials for quantum and nanotechnological applications.

Keywords:

MEMS chips, III-V-Nanowires, Contact angle

Reference:

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acquiCHORD : track the rotating ROI !

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background and aims:
Acquiring images automatically in a SEM opens up a multitude of possibilities: temporal tracking of surface evolution (mechanical tests, environmental SEM...), image mosaics to cover a large field of view, drift correction, 3D acquisition with FIB... This is not new, but often this aspect of microscopy has been the preserve of microscope suppliers or equipment manufacturers (EDX, EBSD...). With the progressive opening of control APIs, and more particularly the advent of the Python language as an interface with the microscope, it is becoming increasingly easy to take full control of the machine. We present here the software acquiCHORD dedicated to the acquisition of images of a sample rotating in the microscope around an axis perpendicular to the stage. Such series of images are used in the laboratory for two main subjects: 1/ the 3D reconstruction of the surface of objects in the micrometric range [1], and 2/ the use of channeling contrast in crystalline materials for the study of crystalline defects by ECCI [2], for the obtaining of crystallographic orientation maps by the eCHORD approach [3], and for the automatic determination of grain size distributions by Machine Learning [4].

Methods:
Despite the relative simplicity of the idea (rotation - image acquisition - rotation, etc.), several locks must be released to allow this type of acquisition. We present in this work how the different solutions have been implemented in the acquiCHORD software to manage i/ the significant geometric offset between the area of interest and the rotation axis used, ii) the tilt between the optical axis and the rotation axis, which is necessary to obtain usable contrast variations, and iii) the mechanical imprecision of the rotation itself. Moreover, in order to be able to implement the acquiCHORD software on different brands of scanning electron microscopes, the program was designed to place on one side the computational calculations and drift correction, and on the other side the application of movement commands to the microscope. Indeed, the geometric references and the senses of displacement are not always the same from one brand of microscope to another. The program, coded in Python language, has been successfully implemented on ZEISS and ThermoFisher brand SEMs, with automatic machine recognition and notably of the APIs available (old OCX for ZEISS on a SUPRA model; full Python API for ThermoFisher QUATTRO SEM).

Results:
Two examples are presented. The first concerns the surface reconstruction of an OstraCode from the collection of the Laboratory of Geology of Lyon (Ecole Normale Supérieure). The particularity is the micrometric size of the object which makes impossible a 3D reconstruction of the surface by classical methods of optical photogrammetry. The second example concerns the characterization of the microstructure of a copper film for microelectronics with twins of a few tens of nanometers. Obtaining series of images in rotation on objects as small as these adds experimental challenges that have been overcome thanks to the use of a piezo-electric control stage fixed in the microscope, also controlled by the acquiCHORD software. Two examples of the use of the corresponding image series (orientation mapping and grain size determination by clustering) are explained

Conclusion:
The acquiCHORD software now makes it possible to obtain series of images in rotation in a SEM that can be used for several objectives, ranging from 3D surface reconstruction to fine characterization of the microstructure of polycrystalline materials. The software is under CeCILL license (from CEA CNRS INRIA Free Software) which is a free software license adapted to both international and French legal matters, in the spirit of and retaining compatibility with the GNU General Public License (GPL). AcquiCHORD is available on request.

Keywords:

API, Python, SEM, channeling contrast

Reference:

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Novel tools for the Spatio-Temporal Photocontrol of protein-protein interactions

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LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

Background incl. aims
The dynamic regulation of AMPA glutamate receptors (AMPARs) trafficking into and out of the synapse is a major mechanism underlying synaptic plasticity, a fundamental basis of learning and memory. However, the detailed molecular mechanisms governing the dynamics of these postsynaptic receptors are still not fully understood, stressing the need for new methods to modulate and monitor these processes. With the purpose of controlling locally, acutely and in a reversible manner the interaction between proteins involved in AMPAR trafficking we have developed original biomolecular tools based on optogenetics. These approaches will be effectively applied with spatial and temporal precision on selectively targeted individual cells and they will work based on two strategies: by reversibly immobilizing proteins by light, or light-controlling the binding state (association / dissociation) of proteins. The design and production of these tools have involved molecular/cell biology, biochemistry and protein engineering for later on, validating them in heterologous cells and neurons by Advanced Fluorescence Techniques. Our tools effectively address current technical limitations in neuroscience and could allow investigating questions from further biological disciplines.

Methods
The design and production of these optotools have involved molecular/cell biology, protein engineering and biophysical characterization, for later on, validated them in heterologous cells by Advanced Fluorescence Techniques. In order to characterize these approaches in transfected eukaryotic cells as a model of complex cellular context, a new red shifted FRET pair has been developed to monitor protein interactions. Besides heterologous cells, the approaches have been also validated by u-PAINT in neurons.

Results
On one hand, we have designed Photoactivatable competing ligands to destabilize AMPARs by disruption of the natural interaction between Stargazin and PSD-95. On the other hand, we have engineered photoswitchable crosslinkers to specifically recognize AMPAR subunits and control with spatio-temporal resolution the mobility and local number of AMPARs.

Conclusions
The approaches we have engineered should most importantly allow tackling fundamental questions related to AMPARs mobility but also be of high impact on a large scientific community as they could be transferable to many systems.

Keywords:

Optogenetics, super resolution, FLIM-FRET, PPI,

Reference:

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Progress in STEM instrumentation: atomic-resolution SE imaging and meV-level energy resolution EELS

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IM-04 (1), Lecture Theater 1, august 27, 2024, 10:30 - 12:30

Background
Scanning transmission electron microscopy (STEM) has recently advanced in two major directions: atomic resolution imaging of surfaces using the Secondary Electron (SE) signal [1, 2], and ultra-high energy resolution electron energy loss spectroscopy (UHR-EELS) [3]. In this contribution, we review the progress at Bruker AXS (formerly Nion R&D) in these two fields.

Methods
A new SE detector for the Nion STEM has been developed, in the form of a compact Everhart-Thornley detector located within the objective lens polepiece, less than 20 mm from the sample. It uses an electrostatic deflector to direct slow electrons that came from the sample in an off-axis direction. The deflected electrons go through a wire mesh window and are accelerated towards a scintillator. Light produced by the scintillator is guided by a glass rod towards a fast and efficient photomultiplier tube (PMT). The design is UHV-compatible and bakeable. It avoids injecting any first or second-order aberrations, or significant instabilities into the primary beam traveling towards the sample, and the attainable spatial resolution of the STEM remains the same when the detector is turned on.

The generation of the SE signal is strongly affected by surface contamination on the sample, and we use a laser illumination system we have developed to clean samples in-situ. Samples cleaned in this way typically stay clean in the UHV sample chamber of our microscope. The microscope is normally equipped with an ultra-high energy resolution electron energy-loss spectroscopy (EELS) system, which can be used both for phonon studies and for general-purpose low loss and core-loss EELS, and elemental mapping. Combining SE imaging with EELS typically allows a more complete characterization of the sample than either technique on its own. Another advantage of our solution is that the Nion STEM is able to produce atom-sized electron probes at primary energies of 20-60 keV, for which the SE cross sections are larger, and the delocalization of the SE signal slightly smaller than for 100-300 keV operation.

Results
We have used SE imaging to examine several types of samples with many interesting results, which will be shown at the meeting. Monolayer and few-layer samples such as graphene, BN and MoS2 are especially interesting, as their thinness simplifies the modeling of how the incident fast electron beam interacts with the sample to produce SE signal.

As an example, in Figure 1 we show a medium-angle annular dark field image (MAADF), secondary electron image (SE) and an EELS map of the same area of monolayer graphene that contains single-atom substitutional impurities. All the images detect the single-atom substitutions, and provide further information. The MAADF image essentially "weighs" the atomic nuclei, the SE image allows atomic resolution to be reached on the surfaces of thick and even bulk samples, and the EELS map demonstrates that the chemical species of individual atoms can be readily identified by their energy loss signal.

On a parallel track, we continue to improve the energy resolution of our Ultra-High-Energy Resolution Monochromated EELS-STEM (U-HERMES) system. The best resolution we have attained so far is 2.6 meV (full width at half-maximum (FWHM) of the zero-loss peak (ZLP)), at 20 keV primary energy. These results and the challenges encountered when trying to push the resolution further will be presented at the meeting.

Figure caption:
a) MAADF image, b) SE image (Gaussian filtered), c) EELS elemental maps of the same area of monolayer graphene with B and N substitutions. Eo = 60 keV.

Keywords:

SE imaging, atomic resolution, meV-level-EELS

Reference:

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Joint Ptychographic Tomography of Frozen Hydrated Proteins

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

Single particle analysis (SPA) of frozen-hydrated proteins using cryogenic electron microscopy (cryo-EM) enables the three-dimensional structure determination of biomolecules with ångström resolution. Despite the remarkable advances enabled by cryo-EM SPA, the technique requires extensive data acquisition and processing and suffers from size limitations. Cryo-EM techniques are limited for very large biomolecules and very small proteins, due to the presence of multiple-scattering and poor contrast arising from the low electron fluence necessary to prevent sample damage respectively.
Scanning transmission electron microscopy (STEM) techniques have traditionally not been applied to the study of biological samples, due to the high fluence requirements of the most easily interpretable imaging modality using high angle annular detectors (HAADF). However, considerable efforts have recently been employed to apply phase-contrast STEM methods to study biological structures [1,2,3]. Among these techniques electron ptychography, where one iteratively reconstructs the scattering potential using a set of converged beam diffraction patterns (4D-STEM), stands out due to its high dose-efficiency and relaxed sampling requirements [4].
Cryogenic electron ptychography has recently been used to obtain sub-nanometer resolution of apoferritin samples using a relatively small number (~11,000) of high signal-to-noise reconstructions [5]. This “serial” approach, where one uses the 4D diffraction datasets to reconstruct 2D projection images which are then subsequently used to reconstruct a 3D volume using standard cryo-EM methods, is not maximally dose efficient. In this talk, I will propose an alternative technique we term “joint” ptychographic tomography SPA, where the 3D volume is reconstructed directly from the 4D data. This has multiple advantages over 2D projection-based techniques: first, nonlinearities arising from multiple scattering in the sample can be accurately modeled; second, it enables 3D regularization directly which can more effectively fill-in information from missing projection directions; and finally, it can more accurately capture amplitude and phase variations of the scattering potential.
Figure 1 illustrates the technique on small (1728 particles) simulated datasets of virus-like particles (PDB ID:1dwn) and apoferritin (PDB ID: 8rqb). Representative reconstructed micrographs are shown in Fig. 1b for the two proteins using electron fluences of 45e/Å2 and 35 e/Å2 respectively. These are used to reconstruct 3D volumes “serially” using the commonly used SPA software cryosparc with and without imposing symmetry. Alternatively, the volumes can be directly reconstructed using our joint ptychographic-tomography implementation in the open-source software py4DSTEM [4]. The resulting 3D maps, together with the respective docked models are shown in Fig. 1c.
We estimate that, for properly oriented poses, joint ptychographic-tomography offers a 10-20% improvement in resolution, as assessed by gold-standard Fourier shell correlation. Finally, we

illustrate how joint ptychographic tomography can be used to estimate the unknown tilt orientations directly from the 4D data and show progress towards experimental results.

Keywords:

single-particle analysis, ptychography, tomography, phase-retrieval

Reference:

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Iterative Phase Retrieval Methods for Weakly Scattering Signals: Transfer of Information and Efficient Regularization

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

When a converged electron probe is scanned across a thin sample, it acquires phase-shifts due to sample interactions which scatter the incident electron wavefunction. Reconstructing these various scattering sources from phase-less measurements of the intensity at far-field detectors is a high-dimensional, non-convex, inverse scattering problem. Iterative electron ptychography is a phase-retrieval technique which attempts to solve this inverse problem using the redundant information in a set of converged-beam diffraction intensities with sufficient real-space illumination overlap [1], e.g., using defocused-probe 4DSTEM measurements [2].
We have recently introduced a general computational framework, implemented in the open-source analysis toolkit py4DSTEM [3], to reconstruct common coherent scattering sources using physically inspired forward and adjoint operators as-well as a suite of regularization constraints robust against common experimental artifacts. Here, we present recent experimental results using the ptychographic framework on a number of materials-science samples, including atomic defects in few-layer hBN, post-acquisition aberration correction on Au nanoparticles, few-layer twisted SrTiO3 moirés, and strain measurements in upconverting core-shell nanoparticles [4], as-well as biological samples, including single-particle analysis of frozen hydrated proteins at sub-nanometer resolution [5].
Moreover, we present simulated results on how the depth-resolution of these phase-retrieval methods can be extended by solving a joint inverse problem for orthogonal tilt-series directly to obtain the three-dimensional nature of scalar and vector scattering sources such as electrostatic (Figure 1a) and magnetic vector potentials (Figure 1b), respectively [3]. In contrast to "serial" ptychographic-tomography, where one performs 2D ptychographic reconstructions for each tilt projection before reconstructing the 3D object using standard tomographic methods, "joint" ptychographic tomography leverages the ability of multislice-ptychography to capture non-linear propagation, together with three-dimensional regularizations, to recover some information inside the "missing-wedge" due to sample-geometry limitations.
Finally, we discuss the transfer of information of iterative electron ptychography and derive various analytical expressions and numerical results for a white-noise model. We compare the results against other common iterative phase retrieval methods, notably differential phase contrast and tilt-corrected BF-STEM [3], to arrive at experiment design recommendations as a function of electron fluence and defocus (Figure 1c).
Phase-retrieval methods in STEM offer particular promise due to their remarkable dose-efficiency, enabling the observation of otherwise imperceptible signals, such as fields inside materials, and of radiation-sensitive materials, such as hybrid organic materials and biological samples.

Keywords:

phase-retrieval, ptychography, tomography, single-particle analysis

Reference:

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Electron microscopy study of energy materials growth

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Poster Group 2

Background incl. aims
Different energy systems have diverse requirements for energy materials, ranging from single-atom materials to crystalline materials. The synthesis of various energy materials tailored to specific applications is a pressing research area. Our aim is to investigate the real-time growth mechanisms of both crystalline and single-atom materials using in situ aberration-corrected electron microscopy. This exploration aims to uncover the nucleation and growth processes of metal nanomaterial systems and elucidate strategies for manipulating their phases.
Methods
In this study, we employed in situ aberration-corrected electron microscopy to observe the real-time growth of energy materials. We synthesized desired crystal catalyst materials and transformed precursors into targeted single-atom materials. Additionally, we investigated the sintering mechanism of Pt-based catalysts and proposed a sulfur anchoring strategy to guide the fabrication of high-loading single-atom and ultra-small metal intermetallic compounds. We also utilized the Oswald mechanism to synthesize various single atoms and developed a thermal printing method for single atoms. The mechanism behind thermal printing was scrutinized using in situ electron microscopy.
Results
Our study revealed insights into the nucleation and growth mechanism of metal nanomaterial systems, facilitating the manipulation of multiple metastable phases. We also proposed a sulfur anchoring strategy for the fabrication of high-loading single-atom and ultra-small metal intermetallic compounds. Furthermore, we successfully synthesized various single atoms using the Oswald mechanism and developed a thermal printing method for single atoms.
Conclusions
Through our research, we have advanced the understanding of the growth mechanisms of single atoms and crystalline materials, as well as the control of their synthesis. These findings contribute significantly to the field of energy materials and hold promise for future applications.

Keywords:

in situ TEM, growth mechanism

Reference:

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Nanoendoscopy-AFM: A new technique to explore Focal Adhesions in living cells

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Introduction: Focal Adhesions (FAs) are complex structures containing several adapter proteins such as paxillin, vinculin, FAK, talin, and zyxin, which together create a dynamic nanostructure that mechanically links intracellular actin bundles to the extracellular matrix (ECM). They play important roles in various cellular processes including cell adhesion, cell migration, force transmission between the intracellular structures and ECM, or vice versa. Although several microscopic techniques have been employed to explore FA architecture, many of these methods face limitations when applied to living cells or involve complex sample preparation that can impact the natural function of FAs. Consequently, there is a strong demand to investigate various aspects including the dynamics of these multiprotein complexes in living cells using new techniques. A recent advancement in this regard is Nanoendoscopy-Atomic Force Microscopy (AFM), a technique that measures intracellular structures of living cells without disassembling the cell, maintaining intracellular structures in their physiological environment [1]. Here we use this advanced nanoendoscopy-AFM technique to visualize and study the dynamics behavior of FAs in living cells.
Methods: Fig. 1a shows the schematic of the 3D nanoendoscopy-AFM technique. A long and sharp AFM nanoprobe fabricated by electron beam deposition (EBD) or focused ion beam (FIB) milling is inserted into living cells to visualize FAs by collecting force versus distance (F-z) curve maps. The continuous penetration of the cell by this ultrathin nanoprobe does not damage the cell or negatively impact cell viability.
Results: We succeeded in imaging FA structure directly within living fibroblast cells by nanoendoscopy-AFM, as confirmed by complementary confocal microscopy (Fig. 1b,c). FA indentation or penetration by the AFM nanoprobe does not trigger FA disassembly. Using this approach we can monitor FA growth over time, as well as the dynamic rearrangement of stress fibers connecting to individual FAs (Fig. 1d,e). Moreover, FA thickness is reduced when it matures or grows from the cell periphery to the center. In addition, this technique enables us to obtain insights into the mechanical characteristics of FAs. Our findings reveal that FAs exhibit increased stiffness during maturation and undergo softening upon disassembly.
Conclusion: Our results provide a new nanoscale look at FA ultrastructure inside living cells and at the dynamic processes regulating stress fiber attachment to these structures. In the future, we aim to gain more detailed information on the structural and biomechanical intricacies of FAs in living cells.
Fig. 1. (a) 3D nanoendoscopy-AFM measurement of FAs. (b) Confocal image of actin stress fibers (green) connected to FAs (paxillin; adapter protein in red) and (c) a corresponding AFM image of an individual highlighted FA. (d) and (e) represent contact point mapping images of the same FA at 0 and 46 minutes of imaging, indicating the maturation of the FA.

Keywords:

focal adhesion, nanoendoscopy-atomic force microscopy,

Reference:

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Advancing Ultrafast Transmission Electron Microscopy with Dielectric Metalenses

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims
In 2005, Ahmed H. Zewail merged ultrafast femtosecond laser with femtosecond precision with TEM's sub-nanometer spatial resolution developing Ultrafast Transmission Electron Microscopy (UTEM). This technique combines the sub-nm spatial resolution of TEMs with the fs temporal resolution of ultrafast lasers and is paving the way to the study of ultrafast fundamental processes at the nanoscale. In the last years, a new paradigm for the arbitrary modulation of the probing-electron wave function phase through quantized photon-electron interaction was put forward and has the potential for enhanced microscopic sensitivities to material properties [1]. The quality and the extent of the electron beam shaping strongly depend on matching the transverse coherence of the electron beam (~ 1 µm) with the spot size of the laser beam, which generally exceeds the former by more than one order of magnitude. This mismatch prevents the modulation of the electron wave function phase, which is crucial for the ability to probe specific materials degree of freedom (such as chirality of materials when adopting a vortex electron beam). In order to address this issue, we propose the integration of a crystallin silicon metalens in the platform that enables electron-light interaction.

Methods
The electron beam shaping inside the UTEM is facilitated by a Photonic free-ELectron Modulator (PELM). An external Spatial Light Modulator (SLM) imprints an arbitrary amplitude and phase pattern on an ultrafast optical field. The light is then projected on a flat electron-transparent metallic thin film on the PELM platform, where it interacts with the electron beam. The integration of a dielectric metalens into the PELM enables the focusing of incident light down to a few micrometers before its interaction with the electron beam. We fabricated a metalens on a free-standing silicon membrane kept in place by a silicon window. Then, we integrated the silicon windows to the PELM platelet positioned at 45° with respect to the metallic film. The focal distance of the metalens is on the order of several hundreds of µm's. This geometry allows the focusing of the light arriving horizontally from the side of the microscope on the metallic film, where the electron-photon interaction will occur. A metalens is composed of properly arranged nano antennas (also called meta-atoms) [2]. We designed the meta-atoms geometric parameters by performing extensive finite element method (FEM) simulations through the wave optics module of the COMSOL Multiphysics® software. The position and orientation of the meta-atoms on the metalens surface are then arranged to obtain the geometric phase spatial variation needed to focalize the incident light beam.
Results

From the Comsol simulations we obtained the meta-atoms dimension that optimizes the transmission and the phase difference condition, as well as mechanical constraints. The obtained theoretical transmission is equal to 44%. The metalens-integrated PELM geometry requires a focal length of 0.7 mm to let the electron beam interact with the focused laser spot and then proceed along the microscope. For that focal length and a 500 µm metalens diameter, the wave propagation simulations provide the focusing of the incident laser down to 2.5 µm.
We have also computationally characterized the performance of the designed dielectric metalens by simulating the propagation of a gaussian plane. Moreover, we explored the metalens robustness to non-idealities by applying a random noise on the phase and we verified that a transversely-patterned light beam, such as a Hermite-Gaussian profile, is correctly focused without distortions.
Conclusions
This setup is a compact solution that can be inserted in the UTEM to allow matching the laser spot size with the transverse coherence length of the electron beam, providing access to a wide range of excitation schemes and pump-probe geometries. Currently, we are working on the optical characterization of the metalens, in order to experimentally confirm its performances. The next step will be to mount the metalens windows in the PELM and characterize its performance inside the UTEM.

Funding
This work is part of the SMART-electron Project that has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 964591.

Keywords:

Metalens, electron-photon interaction, Ultrafast TEM

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PFIB-preparation and STEM-characterization of electrochemically plated lithium at the interface to the solid electrolyte Li6PS5Cl

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Solid-state batteries are of particular interest when it comes to safe high-energy storage as they potentially enable the usage of lithium anodes. Theoretically, a lithium metal anode has a more than 10 times higher specific charge capacity than graphite [1], which is typically used as anode material in commercially available lithium-ion batteries. But, industrial production of highly reactive lithium metal anodes is challenging. One approach is to electrochemically deposit lithium in a so-called anode-free cell. The lithium is plated between a current collector and the solid electrolyte during charging. In the half-cell assembly investigated throughout this study, the current collector consists of stainless steel and the solid electrolyte of argyrodite-type Li6PS5Cl. The latter is a promising solid electrolyte candidate due to its high ionic conductivity, but comes along with a solid electrolyte interphase (SEI) formation when in contact with lithium metal [2]. Therefore, the characterization of interfacial side reactions at the micro- and nanoscale is crucial.

Scanning transmission electron microscopy (STEM) offers structural insights at atomic resolution. Still, it comes with a challenging sample preparation that is further complicated by moisture and ion beam sensitivity of the battery materials. Furthermore, lithium metal and sulfide electrolytes like Li6PS5Cl require cryogenic conditions during ion- and electron-beam exposure to withstand the beam doses. The plasma focused ion beam (PFIB) system Helios 5 Hydra CX is capable of an inert-gas transfer from and to the glove box as well as sample preparation at temperatures around -190 °C. In the PFIB, the sample is thinned to electron-transparency (< 100 nm), from where it is transferred to the glove box and mounted in a sealed transfer holder (Mel-Build) for further investigation in a STEM (JEOL JEM-2200FS). Low-dose electron imaging and STEM-EDX (energy dispersive X-ray spectroscopy) at cryogenic temperatures are performed.

The lamella preparation of the highly sensitive lithium metal and Li6PS5Cl requires adapted preparation steps: low ion currents to preserve the Li | Li6PS5Cl interface as well as eliminating tungsten deposition. Usually, tungsten deposition is used to protect the sample during thinning and to attach the sample to the manipulator needle for lift-out and attachment of the lamella to the copper TEM grid. The high mobility of ions in Li6PS5Cl makes it impossible to distribute the tungsten atoms of the precursor gas locally. Hence, a complete gas-free workflow is needed. The manipulator needle is attached to the lamella gas-free using redeposition of the needle material. Also, the lamella is attached to the copper grid using redeposition of the grid. The steel current collector serves as a protection layer during thinning.
The thinned lamella is investigated in the STEM. A cryo-STEM EDX map and spectrum are depicted in Figure 1. The dark blue area (Figure 1b) encircles Li6PS5Cl with its characteristic peak relation of phosphorus, sulfur, and chlorine, as seen in the corresponding EDX spectrum (dark blue graph Figure 1c). Between lithium and Li6PS5Cl is a roughly 200 nm thick SEI, colored in light blue in Figure 1b, showing chlorine enrichment in the EDX map (Figure 1a) as well as in the corresponding EDX

spectrum (light blue graph Figure 1c). Lithium is not detectable using EDX, as it's characteristic X-rays have too low energy and are absorbed by the detector's window.

The preparation of electrochemically plated lithium and Li6PS5Cl using the PFIB is highly complex and requires the absence of air as well as cryogenic conditions. Nonetheless, the effort is necessary to gain structural and chemical information on the SEI. An approximately 200 nm thick chlorine-rich layer between plated lithium and argyrodite-type Li6PS5Cl is already observed. Further measurements like STEM-EELS (electron energy loss spectroscopy) to detect lithium and TEM-PED (precession electron diffraction) for structural information need to be carried out.

Keywords:

Plasma-FIB, Battery, Inert-gas-transfer, Anode-free-cell

Reference:

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Image fusion for 3D reconstruction of SEM images

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Poster Group 1

Background
Image fusion is commonly used in photography and optical microscopy to merge a stack of images taken with different focal lengths to produce a single in-focus image. It is necessary in applications where details of a single image are required to be as sharp as possible (e.g., medical imaging and surveillance), and where acquisition conditions are limited (e.g., when the scene depth is much larger than the system's focal length). There are several approaches and algorithms to determine the in-focus regions in each image of the stack. The algorithms can be grouped into three main categories: spatial domain, transform domain, and neural network [Bhat2021]. Furthermore, when the focus depth of each image is known, a depth map of the scene can also be estimated as a byproduct of the fusion algorithm. This depth map will contain information about the apparent distance of each object in the image.
Scanning electron microscopes are known for their improved depth of field over light microscopes and perhaps that is the reason why image fusion has seen little use with scanning electron microscopy images [Ersoy2008, Marturi2013]. However, the focal depth in SEM is known with high precision and the depth of field can be adjusted with the operating parameters of the microscope.
Methods
The focus of our work is twofold: 1) create an in-focus image using multiple SEM images acquired with different focal lengths and 2) use that information to estimate a depth value for each pixel of the resulting in-focus image. To do that, spatial domain image fusion algorithms are the most suitable for the task, because each output pixel is selected from one of the corresponding input pixels, and thus its focus depth is unequivocally determined by the focus depth of the image from which the pixel is chosen. Transform domain algorithms do not offer this possibility because the output image is usually a weighted sum of the input images in a particular transform domain. Furthermore, because the algorithm must be accessible, fast, and independent from the input data, we have decided not to use a neural network-based algorithm.
Our approach is based on the algorithm proposed by Haghighat et al., where the in-focus image is built by finding, pixel by pixel, which input image has the maximum local variance. This algorithm assumes that local variance and sharpness are directly correlated.
Our method can be divided into three mains steps:
1. Compute the magnitude of each input image gradient, that will then be used to estimate each input image local sharpness.
2. Run a sliding window of size 8x8 across all the gradient images; for each block we compute its variance, and we find and store in a 2D array the index of the input image with the largest local sharpness (i.e. largest local variance).
3. Smooth the array of indices with a low pass filter to make the output more consistent.

Results
The output from the above algorithm is a map that contains, for each output pixel, the index of the sharpest input image. From this index map we can construct the output in-focus image and its associated depth map (from focus).

Because we process the image block-wise, some blocky artifacts might occur at the borders of objects. To mitigate this problem, we propose an additional post-processing filtering of the depth map using the guided filter proposed by Sun et al. This filter aims at refining the depth map using the in-focus image as a pilot (like a bi-lateral filter).
The in-focus image and its depth map can finally be used to create a 3D point cloud of the imaged sample for the user to inspect it in three dimensions.
Conclusion
Our results indicate that this is a promising technique for visualization of SEM data and offers the possibility to determine the surface profile of samples. We will present our protocol for obtaining the image stacks, the procedure to create an in-focus image, depth map and a 3D point cloud from the data using the in-house built application.

Keywords:

SEM, Image fusion, 3D-reconstruction, Profilometry

Reference:

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Dynamics at phase boundaries of active catalyst studied by multi-scale operando EM

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Catalysis is a highly complex phenomenon and intricately linked to the interplay between processes that dominate at different time and length scales. It is therefore impossible to understand the emergence of catalytic function solely based on the study of static atomic arrangements. Up to now, the full-scale complexity of catalysis is only poorly understood because the way in which atomic-scale processes influence the behavior and dynamics at larger scale is not well documented experimentally. The result is that we still, to a large extent, develop new catalysts on the basis of iterative trial-and-error approaches. Elucidating the link between atomic-scale structural dynamics, feedback mechanisms, and collective behaviour is the key to a deeper understanding and further optimisation of catalysts and processes. From imaging of quasi-static low- energy configurations through gas-phase-induced state switching to observation of complex non-equilibrium dynamics and oscillatory behaviour (Figure 1), electron microscopy has provided novel insights across several length and time scales and has meanwhile matured from a service tool for catalyst researchers to a driving force in catalysis research [1,2]. In my presentation, I will show that consideration of dynamics and processes taking place at different time and lateral scales as well as the consideration of non-linear and complex behaviour is essential for our understanding of working catalyst.

Keywords:

catalysis, non-equilibrium dynamics, multi-scale

Reference:

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Phenomenology of the dealumination in Faujasite Y zeolitic cataysts

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Background incl. aims
The dealumination is one of the most efficient strategies for designing optimized catalysts of zeolite-type [1]. In aluminosilicates such as the zeolite Y, which is a widely employed material in the field of the heterogeneous catalysis, the dealumination involves the removal of Al atoms in the 4-fold coordination (with oxygen) from the crystalline zeolitic framework thus leading to an acquired hydrothermal stability and an interconnected hierarchical porosity [2], which are both beneficial properties in catalytic reactions. Although, the mechanism of structural rearrangement after the extraction of the framework Al atoms (FAL) from their tetrahedral sites turning into extra-framework Al atoms (EFAL), the evolution of the mesopores (pores from 2 to 50 nm of diameter), the elemental distribution of Si and Al as well as the chemical speciation between the FAL and EFAL are still considered as hot topics in the subject [3]. Our study aims indeed to provide an understanding of the phenomenology of the dealumination at the nanometric space resolution by combining different TEM-based approaches.
Methods
For this research work, a series of 4 zeolites Y at subsequent stages of a multi-step dealumination process, mainly obtained by alternating thermal treatments under steam and chemical treatments, was considered. A first hydrothermal treatment (steaming 1, 620°C and 80% humidity) is performed on the commercial CBV300 (Zeolyst) zeolite Y in a reactor and followed by a NH4 ionic bath, a second hydrothermal treatment (steaming 2, 700°C and 80% humidity) and a HNO3 (1M) leaching. These zeolites undergoing such protocol were characterized by Electron Tomography, STEM EDX, STEM EELS together with synchrotron STXM XAS in order to locate the chemical signatures of Si and Al within the grain and correlate with morphological modifications.
Results
The results of the electron tomography highlight the presence of a well developed system of mesopores that is structured in cavities and channels converging towards the core of the grains of zeolites. The facets of the zeolites Y are mainly belonging to the {111} smmetry and the results show the preferential elongation of the mesopores parallelly to the basal facets. Moreover, most of the channeling mesopores propagate at the intersection between two plans of the {111} simmetry. At more advanced steps of the dealumination, the mesoporous network is observed in conjunction with some extra material on the edges of the zeolite, inside its microporous matrix and into the mesopores. This extra-framework material, that is suspected to be amorphous, is associated to a phase enriched in Al as it is observed with the STEM EDX analysis. The characterization by STEM EELS on the K-edge of Al and Si reveals the gradient of these two elements on the edge of the grain and at the mesopore mouth. The Si/Al ratio is provided for this extra-framework material, that we consider as EFSiAl (Extra-Framework Silica Alumina) and, for the first time, related to morphological aspects at the nanometric scale. Moreover, the analysis by STXM XAS on the Al K-edge shows that the signal arising from such areas is mainly tetrahedral, in contrast with the current idea that the EFAl sites are

octahedral α-alumina type. These findings could be interpreted by assuming the flexibility of the the framework and its reversibility to 4-fold Al under experimental vacuum conditions. Although, small traces of 6-fold Al persist in the XAS spectrum and might provide a proof of the difference in terms of structure and stochiometry of the EFSiAl phase.

Conclusion

This study has evidenced the role of the {111} family of plans as preferential symmetry for the development of the mesoporous network within Faujasite Y zeolites during dealumination treatments. At the single crystal scale, the removal of material inside the grain is coupled in parallel with the presence of areas enriched in Al. This is termed as the EFSiAl phase, and it is considered as a collateral product of the dealumination. The EFSiAl is mainly observed at the edges of the grain and inside the mesopore. Contrarily to the expectations arising from the literature which tend to assign to the Al sites of the extra-framework material the 6-coordination, our results show a principal tetrahedral signal even for the Al on the EFSiAl phase.

Keywords:

Zeolites, Hierarchical porosity, 3D-TEM, Spectro-Microscopy

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Development and applications of backscattered electron and X-ray detector

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Poster Group 1

Electron microscopical analysis frequently suffers from the criticism that we're only investigating a small region, a small percentage, of the whole material. When we only investigate minute regions of the sample, how can we make sure we're not biased by what we find or haven't found? How can we make sure that we're achieving a realistic overview of the sample? One strategy to increase the sampling is to acquire a large area mapping, where secondary electron (SE), backscattered electron (BSE) and X-ray signals are collected over 100 to 10,000's of fields by moving the stage to cover the entire sample. The rate limiting factor is usually the throughput and sensitivity of the energy dispersive spectrometry (EDS) detector(s) to collect enough X-rays to achieve an acceptable signal-to-noise ratio (SNR) for all elements in the sample.

One solution is to increase the solid angle of the EDS detector, by moving it under the pole piece of the scanning electron microscope (SEM), while keeping the ability to acquire SE and BSE signals simultaneously. In 2023, we introduced a detector which combines both X-ray and BSE sensors into one device (Unity, Oxford Instruments, UK). The head features two circular silicon drift detector X-ray sensors, two custom shaped BSE sensors and two cutouts to give unobstructed line-of-sight to conventional EDS detectors. Here, we will lift the lid on some of the important developments required to bring together the backscattered electron and X-ray (BEX) detector system. Figure 1 shows the type of data which achievable with this detector.

Firstly, the integrated BSE. Having two distinct sensors means both Z-contrast and topography can be produced. The unusual shape of the detectors maximises the collection area of the detector and therefore SNR, whilst still maintaining compatibility with additional standard EDS detectors. The Z-contrast is possible because the sensors are symmetrically positioned around the pole piece and cover a large solid angle. Combined experiments with a BSE signal is important because this has a much higher signal to noise and better resolution than x-ray elemental maps. In the overlaid results, Figure 1, the BSE is providing a sharpness to the image that wouldn't be present in the x-ray maps alone.

Secondly, the Unity detector is designed to work in parallel with a standard EDS. The Unity detector has a protective membrane over each of the X-ray sensors to help extend their life and cope with the high count rates an under pole piece detector is exposed to. Due to this membrane, there is limited detection of light elements, combining with a traditional EDS detector resolves this. Counterintuitively, having a second detector with a lower count rate is much better for mapping light elements, as it allows for the use of a longer process time, leading to improved energy resolution and therefore by association improved deconvolution of the lower energy X-ray lines. The BEX detector does not need to process low energy signals which means it can run faster and be optimised for the higher energies. This set up is technologically slightly tricky as it means the combination of two X-ray sensors which differ in size, shape, and sensitivity, in addition to the simultaneously acquired BSE signal. Unity is designed to maximise the throughput and achieve the optimal mapping resolution,

whilst the ideal process time for the conventional EDS detector can be selected based on the relative solid angle, detector sensitivity and geometry. A similar ratio and comparison of the variance optimizes the selection of the optimum X-ray map for each element. Map selection has also been optimized for a multidetector system, making sure the best results are being displayed, for each element.

BSE experiments are typically run with a constant dwell time where are EDS experiments are run with a variable dwell time but maintaining and constant live time. This must be taken into account when designing a synchronized experiment. We will present on the technology behind an improved workflow for synchronized multi-sensor and multi-signal experiments, including the importance of acquisition synchronization, combined processing and displaying of the final data.

Figure 1: Showing example results taken with a BEX detector, with a combined overlay of the BSE and several elemental maps (a). A larger view of (a) is shown in (b) emphasizing the sharpness of contrast which is achievable by this technique.

Keywords:

BSE, EDS, Multi-signal, Synchronization, hyperspectroscopy

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Uranium reduction by magnetite and mechanism of UO2 formation monitored by low-dose STEM-EELS

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Poster Group 1

Background incl. aims
Uranium (U) is a ubiquitous element in the Earth's crust, and its biogeochemical behavior is primarily constrained by its redox transformation from soluble uranium (U) hexavalent species (U(VI)) to sparingly soluble tetravalent species (U(IV)) under anoxic conditions. U(VI) reduction by mineral phases, most commonly ferrous iron-bearing minerals, has been shown to produce crystalline U in the form of UO_2 as the end-product at circumneutral pH values. However, there is increasing evidence for the presence of pentavalent U (U(V)) in these minerals. Our work [1] has evidenced the presence of U(V) during U(VI) reduction by preformed magnetite and its persistence for days to weeks prior to the formation of fully reduced U(IV)O_2 nanoparticles. Despite this evidence, uncertainty about the mechanistic role of U(V) in uranium reduction pathways remains.
We reported the formation of single U oxide nanocrystals (1-5 nm) followed by the formation of nanowires that extended from the magnetite surface outward. Over time, the nanowires collapsed into ordered UO_2 nanoclusters, resembling those previously reported for the final product after U(VI) reduction by magnetite [2]. U(IV) was suggested as the dominant valence state in the nanowires based on both Fast Fourier Transform (FFT) on specific regions of HAADF-STEM images of the nanowires and the branching ratios (BR) obtained from M4/M5 peaks from U TEM-EELS spectra. However, due to the sensitivity of U(V) under the beam, the presence of mixed valence states may be overlooked by using the BR acquired from U TEM EELS spectra under high beam current. Differences between UO_2 and uranium oxides with mixed valence states, such as U_3O_8, are too small to be robustly differentiated with FFT analysis of HRSTEM images. Current work aims to measure O K-edge EELS STEM spectra of the nanoparticles within the nanowires and compare the edge feature to that of U oxides reference standards to characterize the valence state of nanocrystals.
Methods
We gathered low-loss and high-loss spectrum images of O-K edge and U N edges for UO_2+x standards and time-resolved U(VI) reduction samples. Solutions with magnetite (0.25 or 5 mM Fe) and U(VI) (30 or 200 uM) were prepared anoxically at neutral pH, and samples at various reduction stages were collected. Both U oxide standards and U nanowires formed during the reduction process are susceptible to electron beam-induced damage and reduction, changing the electronic structure of the samples. Thus, it is necessary to use ultra-low beam currents of 10-50 pAs, and we adapted the Gatan Continuum-K3 spectrometer-detector alignments to optimize sensitivity, dose rate per pixel, and the signal-to-noise ratio (SNR) at such low beam doses. We processed the EELS Data cubes with zero loss alignment, denoising, deconvolution, background subtraction, and spectral smoothing. Then, spectra from nanocrystals within the nanowires and in nanoclusters were extracted and

compared with those from UO_2+x standards. We also fit the O K-edge of these spectra with data from UO_2+x standards to determine the valency and state of sample reduction.

Results

STEM-EELS measurements were performed for the following U oxide standards that contain the three main U oxidation states or mixtures thereof: UO_2 U(IV), U_4O_9 2xU(IV)+2xU(V), U_3O_7 U(IV)+2xU(V), KUO_3 U(V), and $BaUO_4$ U(VI). The O K-edges of standards were compared with the corresponding simulations based on DFT calculations, see Fig.1(a). The U(IV) containing standards O K-edge (UO_2, U_4O_9, and U_3O_7) have a lower intensity at peak 1 (533 eV), whereas peak 2 (539 eV) has a higher intensity. In contrast, U(V)- and U(VI)-containing standards O K-edges (KUO_3 and $BaUO_4$) have a different electronic structure, with a higher intensity of peak (labeled 1) at 534 eV and a lower intensity peak (2) at 541 eV. The measured U oxides standards correspond well with their simulations, and averaged spectra of each standard (at least 8 measurements) are used for the multiple linear least fitting within the region of O K-edge (520-620 eV) in nanowire samples. Figure 1(b-f) shows that the experimental setup allows us to obtain high-quality spectra from a single pixel even though the thickness of the nanowires does not exceed 10 nm. Fitting shows the presence of mixed valence state oxides U_4O_9 and U_3O_7 within the nanowires, thus confirming the presence of U(V).

Conclusion

We measured O K-edges of U oxide standards with various valence states and samples comprising nanowires composed of 1-5 nm size particles with as low as a 50 pA dose. The fits of reduced nanowire samples and comparison UO2+x standards O K-edge spectra confirm the presence of mixed valence state particles in nanowires, thus evidencing the presence of U(V).

Fig.1: (a) Comparison of O K-edge spectra for measured (M) U oxides standards and its respective simulations (S) that show varying peak 1 and 2 heights and their eV shift that reflects distinct U oxidation states. (b) Sample containing nanowires. (c) Detail of approximately 30 nm long nanowire sitting between 2 magnetite grains. (d) High loss spectrum imaging (SI) of the selected region with background subtraction, the red square represents 8 angstrom-sized pixels. (e) EELS spectrum from selected pixel (red square) of SI shows the quality of data. (f) Multiple linear least square fitting (MLLS) map shows the presence of mixed valence state U oxides U_4O_9 and U_3O_7.

Keywords:

Uranium reduction, low-dose STEM-EELS

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Atomic-scale microscopy of different materials by ultrashort THz-driven Atom Probe Tomography

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Poster Group 1

Background
Terahertz (THz) radiations with low energetic photons (meV) are used today in a wide range of applications such as imaging, sensing or spectroscopy. The low photon energy of THz radiation has the advantage of inducing low damages on fragile materials by reducing heating encountered when a visible wavelength is used.
Our research is based on the combination of a single-cycle THz source with atom probe tomography (APT) to study THz-assisted field evaporation on a wide range of materials, including metals, ceramics, semiconductors (crystalline silicon), and insulators (amorphous silica). Using our homemade THz-assisted APT setup - “TERA-SAT” - we aim to explore the new impacts of THz pulses on materials characterization at the atomic level.
Methods
Atom Probe Tomography (APT) is an imaging technique based on controlled field evaporation of atoms from a nanometric needle-shaped sample under a strong electric field. Evaporation of atoms is triggered by a laser pulse in the UV or THz domain. The ions are then directed onto a time- and position-sensitive detector. The 3D atomic positions are reconstructed through a back-projection algorithm and the chemical nature is determined by calculating the mass-to-charge ratio.
The experimental setup used (fig. 1(a)) in this study is composed of a pulsed THz source based on the two-color plasma generation process coupled with an APT chamber.
Results
THz radiation has been proven to be beneficial for the reduction of thermal effects in field evaporation of metals and low band-gap materials such as LaB6.
Further tests have also been performed on pure silicon nanotips, where the presence of thermal effect is strongly dependent on the spectrum of the THz radiation.
Finally, we analysed nanotips made of sol-gel amorphous silica, which is chosen as matrix for bio-molecules embedding for future studies. First, we demonstrated the possibility of field-evaporating such material in a well-controlled way using THz pulses. A more detailed comparison of the mass-to-charge ratio spectra obtained with THz-APT and UV-assisted APT (fig.1 (b)) reveals an increase of the signal to noise as well as an improvement in the mass resolving power which is inversely proportional to the full width of the peak at 1 and 10% of the maximum.
Conclusion
We have analyzed different materials using our TERA-SAT setup, demonstrating its ability to trigger field evaporation. The use of THz radiation is proven to reduce local heating phenomena, especially in the case of metals, allowing a-thermal field evaporation.

We have obtained encouraging results also for non-metallic samples, but further investigation will be necessary to find out the optimal parameters (THz amplitude, DC field) to improve the accuracy of compositional analyses, thus to increase the signal to noise ratio and the mass resolution power of the mass spectra.

Keywords:

Atomic-scale-microscopy; Atom-Probe-Tomography; pulsed-THz; field-ion-emission

Reference:

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From structural evolution and interfacial dynamics to manipulation of surface plasmon resonances in Bi-Sb-Te systems

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Poster Group 2

Layered chalcogenides ternary alloys and heterostructures are known as great thermoelectric, topological insulators as well as non-metallic plasmonic building blocks. We present on a multimodal approach based on in situ transmission electron microscopy (in situ TEM), monochromated electron energy loss spectroscopy (mono-EELS) in conjunction with density functional theory (DFT) calculations and electron-driven discrete dipole approximation (e-DDA) simulations to establish a holistic understanding spanning across nanoscale dynamics, interfacial properties and ultimately correlating structural evolution and heterogeneities to plasmonic modulations in Bi-Sb-Te ternary alloys and heterostructrues.

Using in situ heating TEM and DFT calculations, we identify the sublimation pathways, the role of native point defects and edge configurations in anisotropic sublimations of Bi2Te3-Sb2Te3 in-plane heterostructure and Sb2-xBixTe3 alloy. Structural evolution consisted of formation, growth, and coalescence of thermally induced nanopores and ultimately preferential sublimation initiating at reactive regions with structural heterogeneities. The preferential sublimation sites are center, heterointerface and outer edges for the heterostructure and only outer edges for the alloy counterpart. Excessive Te in the center of the structure and high density of defects on the heterointerface are anticipated to be the main driving force for preferential depletion of chalcogenide (Te) from these reactive sites. Additionally, The DFT calculations provide a mechanistic understanding on the role of native defects and edge formation energies, revealing the antisite defects including TeBi and TeSb to be the dominant native defect, promoting preferential Te sublimation and playing a key role on the defects assisted sublimation.

To understand defect-plasmon interactions, we perform mono-EELS, e-DDA simulation and SVD analysis to understand the electron-driven plasmon excitations in the ternary heterostructures. We successfully resolved the nanoscale spatial variation of surface plasmon resonances in the pristine heterostructure as well as in the presence of intrinsic or extrinsic defects (i.e. thermally induced defects). Furthermore, we have used singular value decomposition (SVD) analysis to disentangle the spectral data and identify the individual spectral contributions of various corner, edge, and face modes. Finally, the experimental mono-EELS findings are validated with e-DDA numerical simulations. Layered chalcogenide ternary systems as a less explored plasmonic system holds great promises as emerging platform for integrated plasmonic. Understanding the sublimation pathways, nanoscale interfacial dynamics and ultimately introducing controlled structural defects can open avenues for defect engineering and ultralocal modifications of plasmonic properties in these non-metallic plasmonic systems.

Keywords:

sublimation, defects, surface plasmon resonances

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Following low and high-temperature electrolysis processes with in-situ and cryo electron microscopy

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
Electrochemistry plays a crucial role in various hydrogen production technologies [1], yet understanding the intricate nanoscale processes that govern these reactions remains a significant challenge. To address this gap, we employ advanced electron microscopy techniques to visualize and unravel the nanoscale mechanisms behind electrochemical phenomena.
Methods
While the Plasma Focused Ion Beam (PFIB) tomography at room temperature allows us to visualize the evolution of solid oxide fuel cell (SOFC) [2] electrodes due to operation, and the cryo PFIB tomography allows characterizing proton exchange membrane (PEM) [3] of low-temperature water electrolysis. This coupled with the in-situ TEM allows us probing the mechanisms of low and high-temperature electrolysis.
Results
PFIB tomography enables us to generate high-resolution 3D reconstructions of SOFC materials, revealing the evolution of triple-phase boundaries and potential degradation sites that influence ion transport and catalytic activity. In situ TEM experiments, utilizing MEMS chips, allow us to monitor electrochemical processes in real time. By studying $Sr_{0.95}Fe_{0.9}Mo_{0.1}O_{3-\delta}$ (SFM) [4] and related materials, we demonstrate exsolution mechanisms that can impact the performance of SOFCs. Further, we showcase the application of MEMS-based chips in visualizing power-to-x processes, exemplified by methane generation on Ni catalysts.
For PEM MEAs, maintaining the original hydration level is crucial to understand degradation mechanisms. Our cryo workflow which includes largescale surface investigation using a laser scanning microscopy (LSM), preserves hydration throughout sample preparation, FIB tomography, TEM lamella preparation, and TEM characterization, ensuring optimal sample preservation for TEM imaging.
Conclusions
Our research highlights the transformative power of electron microscopy in unraveling the nanoscale secrets of hydrogen production. By visualizing these intricate processes, we gain a deeper understanding of the factors influencing material performance and optimize material design to enhance the efficiency and durability of hydrogen production technologies. This approach holds immense promise for advancing the H_2 value chain and driving the transition towards a clean energy future.
Acknowledgments
Authors acknowledge the funding provided by the BMBF (German Research Foundation) through the project DERIEL (03HY122C).

Keywords:

FIB-tomography, in-situ, and cryo TEM

Reference:

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Real-time undersampling optimization during electron tomography of beam-sensitive samples using golden ratio scanning and RECAST3D

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Poster Group 1

Background
In recent years, nanomaterials have seen widespread usage in fields as diverse as biomedicines, energy storage and catalysis due to their unique chemical and physical properties. Hence, in-depth 3D characterization of these properties is critical to improving our understanding of nanomaterials and allows researchers to design nanomaterials better suited towards application. Electron tomography allows researchers to characterize the 3D volume of nanomaterials by acquiring a series of 2D projections at various angles (tilt series).1 Unfortunately, the acquisition of multiple projections to acquire a single 3D volume necessitates long acquisition times and electron beam exposure which can damage sensitive samples. To minimize such damage, researchers often try acquire fewer projections through tilt undersampling schemes, which can induce additional imaging artefacts. Therefore, it is important to determine the optimal number of projections that minimizes both beam exposure and undersampling artifacts for accurate reconstructions of beam-sensitive samples. Current methods for determining this optimal number of projections involve acquiring and post-processing multiple reconstructions with different numbers of projections, which can be time-consuming and requires multiple samples due to sample damage.2 Herein, we propose a novel workflow3 that combines a continuous acquisition scheme known as golden ratio scanning (GRS)4 with a quasi-3D real-time electron tomography software (RECAST3D)5 to simplify the process of tilt undersampling optimization.

Methods
To determine the optimum number of projections, a tilt series is collected continuously using GRS and a quasi-3D reconstruction is performed in real-time with RECAST3D. The reconstruction quality is determined using two metrics: i.) the signal to noise ratio and ii.) the change in the reconstruction as new projections are added. When the optimum reconstruction quality is achieved the acquisition is terminated. To validate this approach, the resulting optimum reconstruction is compared to a standard tomographic procedure for both a simulated structure undergoing beam damage and a Au nanoparticle. Finally, the application of this technique is highlighted by applying the technique to two beam sensitive metal-organic framework complexes: Au@NU-1000 and Au/Pd@ZIF-8.

Results
In both the simulated and experimental cases, the use of this methodology resulted in a significant improvement in reconstruction quality when compared to standard tomographic acquisition and comparable results to conventionally optimized tomography. However, whilst typical tilt-undersampling optimizing takes several acquisitions and post processing to perform, this workflow can achieve similar results during a single acquisition saving significant time for the microscopist.

Conclusions

Herein, we present a novel workflow for optimizing tilt undersampling during a single acquisition using GRS and real-time quasi-3D reconstruction. Simulated and experimental studies demonstrate that reconstructions of beam-sensitive samples optimized using this workflow have higher fidelity with the pre-damaged sample than reconstructions using standard incremental acquisition. Through application to MOFs, we demonstrate that this approach can be used to effectively optimize imaging conditions of beam sensitive samples during a single acquisition.

Figure 1. Golden ratio scanning is used to acquire new projections which can be continuously added to the tilt series increasing sampling density. The reconstruction quality is monitored using a quasi-3D reconstruction software (RECAST3D) which quickly reconstructs 3 orthoslices during the acquisition. The acquisition can be arbitrarily terminated when the optimum reconstruction is obtained.

Keywords:

Electron Tomography, Beam Damage, MOFs

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A new approach for direct visualization of unlabeled lipid nanoparticles for intracellular pathway analysis

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LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
Lipid Nanoparticles (LNPs) are the most clinically advanced delivery vector for both small molecules (liposomes) and genetic material payloads, such as siRNA or mRNA (ionizable cationic LNPs).[1] Their recent development has unlocked a wide range of therapeutic approaches, overcoming the limitations of naked genetic payloads and of previous delivery strategies.[2] This is testified by the fact that the majority of clinically approved nano-systems are of lipidic nature.[3] However, much is still unclear about the release of genetic material in the cytoplasm upon LNPs cell internalization and trafficking in the endo-lysosomal compartment. This process, known as endosomal escape, is a crucial step of the delivery process but to date is inefficient and often considered the bottleneck for the clinical efficacy of LNPs.[4] Most of the methods for detection and quantification of endosomal escape are either indirect assays, usually based on detection of endosome damage indicators, or rely on fluorescent labelling of LNPs. The latter, however, has proven to significantly alter the surface properties of nanoparticles and thus their biodistribution and cell interaction properties.[5] While the direct imaging of LNPs in biological samples is impossible given the nature and elemental composition of LNPs, which are practically identical to the composition of cells. To overcome these limitations, a new hybrid Liposomal-Gold nano-system (namely Lipo-Gold) has been here developed for the direct analysis of cellular interaction and intracellular trafficking of LNPs.
Methods
The Lipo-Gold have been synthetized by a modified thin lipid film hydration method: specifically, the lipid film composed by saturated phospholipid DPPC, cholesterol and PEGylated lipid DSPE-PEG (2000 Da), has been hydrated with an aqueous solution of biocompatible reducing agent, namely ascorbic acid (vitamin C). The vesicles encapsulating the reducing agent have been extruded to the desired dimension and a size-exclusion chromatography column has been employed for the quick removal of free ascorbic acid. Upon addition of metal precursor, HAuCl4, AuNPs spontaneously and quickly formed in the LNPs aqueous compartment. The formation of hybrid Lipid-Gold nanoparticles has been optimized and characterized by quantification of encapsulated ascorbic acid, UV/Vis spectroscopy, dynamic light scattering (DLS), nanoparticles tracking analysis (NTA) and electron microscopy techniques. Then, MTS cytotoxicity assay, flow cytometry, confocal and hyperspectral darkfield microscopy, as well as TEM, have been used to evaluate the cellular biocompatibility and cell interaction properties of the nanoparticles and to verify whether the hybrid Lipo-Gold system could be used as a model for the study of LNPs.
Results
The optimization of the methods resulted in formation of very monodispersed ~130nm LNPs encapsulating 15-35nm AuNPs. The resulting nano-system has proven to be highly biocompatible through MTS cytotoxicity assay after 24 or 48 hours even at very high concentrations. Unlabeled hybrid Lipid-Gold nanoparticles have been employed for the analysis of cellular internalization by

TEM and hyperspectral darkfield microscopy, while fluorescent labeling with DiD dye (Ex/Em: 647/663nm) have been used to compare the properties of the hybrid system in comparison with empty LNPs. Both flow cytometry and confocal microscopy clearly showed that the cellular internalization and intracellular localization is not altered upon AuNPs synthesis as compared to empty LNPs, both quantitatively and qualitatively. This proves that the hybrid Lipid-Gold nano-system is a potential good model for the direct analysis of LNPs interaction with cells, taking advantage of AuNPs optical properties.

Conclusion

This work resulted in the development of a hybrid Lipo-Gold nano-system that showed promising results towards its employment as a reporter system for the visualization of LNPs cellular internalization and intracellular trafficking. This could enable further and easier optimization of Lipid Nanoparticle platform, enhancing the efficacy of the endosomal escape and thus the overall clinical relevance. Furthermore, Dynamic TEM measurements are ongoing on this system, with the aim of getting real-time visualization of the intracellular path of nanoparticles in response to an external optical/thermal trigger. The method will be developed in standard biological samples prepared for electron microscopy analysis (i.e. dehydrated cells included with epoxy resin), thus with a low biological relevance. However, the method would be a proof of concept that could then be employed in state-of-the-art, and more biologically relevant, techniques such as Cryo-TEM for the time-resolved, high-resolution analysis of intracellular trafficking of LNPs in biological samples.

Keywords:

Lipid-Nanoparticles, Cellular internalization, Endosomal escape

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3D visualization of in situ nanoscale dynamics in transmission electron microscopy via self-supervised deep learning

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Background
In recent years, nanomaterials have seen widespread usage in fields as diverse as biomedicines, energy storage and catalysis due to their unique properties. The efficacy of nanomaterials for an application are significantly dependent on their physical and chemical properties. Electron tomography is an invaluable technique, which allows researchers to determine these properties in 3D by reconstructing a series of 2D projections collected at various angles (tilt series).1 However, the 3D spatial properties alone are insufficient to fully characterize nanomaterials and their application. In real world conditions, material properties are rarely static and they change as a result of environmental conditions such as pressure, heat and chemical reactions.2,3 To fully characterize the dynamic behaviour of materials, researchers must move towards 3D volume + time characterizations. Unfortunately, collecting a single tilt series for electron tomography can take about an hour, resulting in loss of temporal information and motion blurring artefacts in the resulting reconstructed volume. Herein, we propose a novel reconstruction method that uses a deep image prior self-supervised neural network (DIP-NN)4 to determine the 3D volume as a function of time. This allows researchers to collect a series of 3D volumes with a temporal resolution of less than a minute.

Methods
To reconstruct a volume time series, 1D slices of the tilt series were used as an input to the DIP-NN. Each slice was mapped to a depth and time coordinate. A 2D orthoslice of the reconstructed volume-time series was predicted for the specified coordinates. To train the network, the orthoslice was forward projected and compared back to the original tilt series. During the reconstruction, the volume-time series is reconstructed slice-by-slice, until the full volume-time series is acquired (Figure 1). Hence, for every 2D image collected in the tilt series a full 3D volume was acquired at the same time of acquisition. This methodology was used to reconstruct simulated nanoparticles with morphological and compositional changes and experimental Au/Ag nanoparticles that were subject to changes in both shape and alloying as a result of in situ heating during electron tomography.

Results
In both the simulated and experimental cases, a significant improvement was observed in temporal resolution compared to conventional tomography. In the simulated case, a set of 100 images in the tilt series was used to reconstruct a set of 100 volumes, where only one would be acquired using conventional electron tomography. In the experimental case, we were able to obtain a frame rate of approximately 1 volume per minute, far outpacing even fast tomography. In both experimental and simulated cases, the reconstruction quality, determined based on the signal-to-noise ratio and the structural similarity index, were comparable to conventional tomography.

Conclusions

Herein, a machine learning method is presented which allows the reconstruction of a series of 3D volumes with a temporal resolution of less than a minute. Unlike supervised machine learning approaches, this method can be trained solely from the acquired tilt series. This method was validated with both simulated and experiment studies on Au and Ag nanoparticles during heating.

Figure 1. DIP-NN algorithm takes in 1D slices of the tilt series using the coordinates for the depth (z) and time (t) to predict a 2D orthoslice of the reconstruction at the same coordinates. For training, this 2D orthoslice is forward projected and compared back to the tilt series. To reconstruct a full volume time series, the 2D orthoslices are stacked slice by slice for every z and t value.

Keywords:

Tomography, Deep Image Prior, Dynamics

Reference:

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Structural and mechanical adaptation of brachiopod shells

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background incl. aims
For hundreds of millions of years, nature has evolved a large assortment of organic-inorganic hybrid materials that are optimally adapted for a wide range of functions, including navigation, protection and mechanical support1. These materials not only exhibit exceptional material properties but also display multifunctionality, including features such as adapting, sensing and self-healing2. Among the most remarkable biominerals found in nature is the shell of the brachiopods Discinisca tenuis and Lingula anatina. These shells are constituted of an inorganic phase, composed of carbonate-substituted fluorapatite in the form of francolite that comprises ~68 % of the shell weight and an organic fraction that is composed of proteins, glycosaminoglycans and chitin and comprises the remaining 32% of the shell weight3. We previously reported in D. tenuis that absorption of water by the shells causes, within minutes, structural changes at the molecular, nano- and micron-scales, resulting in the material switching from harder than bone when dry, to flexible when hydrated, to the point that the shell can be folded in 2 without fracturing4. Although the L. anatina shells (Fig. 1a) exhibits similar behaviour upon hydration, they have different structures to D. tenuis and as such, the mechanisms that underpin such changes in mechanical properties are still unknown.
The aim of this research is to determine how the structure of the L. anatina shell changes as a result of hydration, resulting in the shell going from stiff when dry to flexible when hydrated. Our starting point was to characterise at the nanoscale the structure of the shell, in particular the interface between the francolite crystals and the organic matrix components. This was done using transmission electron microscopy (TEM) techniques, including high-resolution TEM (HR-TEM), electron diffraction, scanning-transmission electron microscopy (STEM), and electron energy loss spectroscopy (EELS) on thin sections of the shell, prepared using the focused-ion beam-scanning electron microscope (FIB-SEM).
Methods
We used FIB-SEM to prepare a ~70 nm-thick lamella from the L. anatina shell. The lamella was then transferred to a transmission electron microscope equipped with an energy filter, STEM and EDX detectors. HR-TEM and electron diffraction combined with dark-field imaging were used to determine the crystallographic orientation of the francolite nanocrystals, together with the size of the single crystalline domains. High-angle annular dark field STEM (HAADF-STEM), together with EELS, were used to identify and image the organic matrix components (mainly chitin) within the bundles of francolite crystals. Micro-computed X-ray tomography (microCT) and cryo-FIB-SEM were used to determine structural changes to the shell, caused by the absorption of water.
Results
TEM imaging of the FIB-SEM section showed that the shell consists of fancolite rods that are ca. 200 nm in length and 60 nm in width (Fig. 1b). Using electron diffraction and dark-field imaging, it can be

seen that these rods are polycrystalline containing domains with different crystallographic orientations (Figs. 1c-d). Within these domains, individual crystals measure ca. 2-4 nm in thickness and 15-20 nm in length. Lattice images obtained with HR-TEM show that the angular spread in the crystallographic orientation of each domain is in the order of 10-15 °, showing slight misalignment in the orientation of the individual crystals that compose such regions (Figs. 1e-f). Using HAADF-STEM and EELS, we could identify the presence of organic matrix, mainly chitin, intermixed with the crystalline domains within the francolite rods and between individual rods (Figs. 1g-h). Taken together, this is the first nanoscale characterisation of the structure of the shell of the brachiopod L. anatina. This characterisation will pave the way to understanding how hydration changes the shell structure, resulting in different mechanical properties. In this regard, considering that the interaction between chitin and the mineral weakens with hydration, it is conceivable that the intercalation of the two components – chitin and francolite single crystals - at the nanoscale plays a critical role in the responsiveness of the mechanical properties of the material to hydration and dehydration. MicroCT demonstrated that regions of the shell that are predominantly organic swell upon hydration. Additonally, cryo-FIB-SEM showed the presence of water in voids at the organic-inorganic interface.
Conclusion
We show that the shell of the brachiopod L. anatina, at the nanoscale, is composed of polycrystalline rods of francolite nanocrystals. Within the rods, the crystals are organized into domains with similar crystallographic orientations that are closely associated with chitin fibers. We speculate that this close association between these two components is an important factor that underpins the changes in mechanical properties that the shell undergoes as a response to hydration. We also speculate that chitin has an important role to template the orientation of the francolite crystals during their formation. Finally, the organic components of the shell play a key role in the absorption of water.

Keywords:

Biomineralization, calcium phosphate, TEM, FIB-SEM

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Enhanced Free-Electron Wavefunction Modulation via Photon-Induced Near-Field Electron Microscopy (PINEM) with Shaped Light Fields

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Poster Group 2

Background and aims
Photon Induced Near-Field Electron Microscopy (PINEM) is a novel imaging technique that relies on the inelastic interaction between a free-electron beam and laser light, mediated by a thin film or a nanostructure [1]. This technique is generally employed to study the ultrafast dynamics of local fields generated by light-induced polaritonic excitations in nanoscale systems. Recently, we further exploited the quantized nature of electron-light interaction, that is intrinsic to PINEM, to achieve coherent control of the electron wavefunction [2-4]. So far, light-induced shaping has relied on Gaussian laser beams illuminating plasmonic nanostructures. In contrast, on-demand electron modulation for dynamic and versatile control has not yet been fully explored. Here, we show that shaped light can be used to introduce an arbitrary modulation on the transverse electron beam profile [5]. In fact, it is already possible to arbitrarily control light fields through spatial light modulators (SLMs) and our goal is to exploit PINEM to transfer this tunability to electron beams, thereby broadening the capabilities of ultrafast TEMs (UTEMs).

Methods
Our experiments are conducted in a UTEM, specifically a JEOL 2100 TEM modified as shown in Fig. 1. A 200 keV electron beam interacts with a femtosecond IR laser beam within a Photonic-based ELectron Modulator (PELM), an additional module of our TEM with an extra sample holder. The laser profile is shaped using a HOLOEYE Phase-Only SLM. We probe the electron-light interaction either by energy filtering imaging (UTEM @ EPFL) or by imaging the momentum distribution of the electron beam in High Dispersion Diffraction (HDD) mode with a 100-m camera length on a direct electron detector (UTEM @ UNIMIB).

Results
We used the SLM to induce a Hermite-Gaussian (HG) shaping of the laser profile and, through energy filtering, we imaged at different focal planes only the electrons that interacted with light. By comparing the obtained data with simulations of an analytically modeled system (Fig. 2), we determined the coherence length δ of the electron beam to be larger than 50 nm. This is a crucial parameter that sets an upper limit to the laser phase profile that can be completely transferred to the electron beam for coherent electron modulation. Additionally, we have directly imaged the momentum distribution of the electron beam and probed the transverse-momentum exchanges at the PELM plane following the electron interactions with quanta of the IR photon beam. In these measurements, we have reached values of transverse coherence on the order of $\delta \sim 1\ \mu m$. By acquiring momentum images in HDD mode with a micrometric aperture at the image plane, we have obtained simultaneous momentum-and-space-resolved information at the PELM plane. Fig. 3 shows

the light-modulated electron-beam profile. The inset illustrates the reconstruction of the SLM-modulated laser-beam profile at the PELM plane.

Conclusions

In conclusion, our findings demonstrate the potential of using shaped light fields in PINEM for enhanced tunability of electron modulation. The ability to control the electron wavefunction with such precision opens up new possibilities for the study of electron-light interactions. This could have far-reaching implications for various scientific domains, including life sciences, materials science, and nanotechnology. The rich dimensionality of light-electron interaction, spanning the transverse, longitudinal, temporal, and energy domains, harbors untapped potential and paves the way for future investigations in this dynamic field.

Funding

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Keywords:

Photon-Induced Near-Field Electron Microscopy PINEM

Reference:

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Uncovering microscopic details of shearing mechanisms in the $L1_2$ structure by unambiguous stacking fault analysis

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background incl. aims
γ/γ'-strengthened Ni- and Co-base superalloys for applications at high temperatures rely on the precipitation-strengthening effect of the $L1_2$-ordered γ' phase embedded into the face-centered cubic γ matrix phase. Therefore, the microscopic mechanisms by which the γ' phase can be sheared crucially influence the mechanical properties of the alloy, and their understanding is of key importance. In this context, an established method to discriminate between intrinsic and extrinsic stacking faults (SFs) is their edge-on imaging in high-resolution scanning transmission electron microscopy (HRSTEM) in ⟨110⟩ projection. The superlattice ordering within the $L1_2$ phase gives rise to a larger variety of fault structures – namely, complex and superlattice variants of both intrinsic and extrinsic stacking faults. Notably, whether a given SF is complex or not may not be reliably revealed by studying one ⟨110⟩ projection alone [1, 2]. We present an experimentally feasible approach that enables this differentiation in a reliable and unambiguous way. This approach entails examining the fault structure not only in ⟨110⟩ projection, but also in a neighboring ⟨211⟩ projection, which is achieved by tilting the specimen by 30° in the TEM. Two applications of this analysis technique are discussed in this contribution: the first is the experimental validation of microscopic details of a well-known formation mechanism for superlattice extrinsic stacking faults, and the second concerns the shear-based γ'-to-χ phase transformation often observed in superalloys based on the Co-Al-W system, in which χ is an equilibrium phase [3].

Methods
Fault structures in the single crystalline Co-base superalloys ERBOCo-4 (composition in at.%: Co43.2-Ni32.0-Al8.0-Cr6.0-Ti2.8-Si0.4-Hf0.1-Ta1.8-W5.7) and ERBOCo-VF60 (composition in at.%: Co79.8-Al8.9-Ta2.3-W9.0) are analyzed. HRSTEM imaging was performed at a double Cs-corrected FEI Titan³ Themis at 300 kV as well as a probe-corrected Thermo Fisher Scientific Spectra 200 C-FEG at 200 kV. On the latter, spatially resolved energy-dispersive X-ray spectroscopy (EDXS) was conducted using the Super-X G2 detector.

Results
The first example concerns a high-temperature deformation mechanism of the γ' phase which involves the formation of SFs. Details of this study can be found in Ref. [2] and are briefly summarized in the following. Under [001] compression, a common deformation mode resulting in the formation of superlattice extrinsic SFs (SESFs) is the well-known Kolbe mechanism [4]. Here, two identical a/6⟨112⟩ Shockley partial dislocations shear into γ' on neighboring planes, leaving behind two neighboring complex intrinsic SFs (CISFs) which together constitute a complex extrinsic SF (CESF).

Complex variants of SFs typically possess high SF energies due to nearest-neighbor violations in the superlattice ordering; therefore, the central fault plane of the CESF undergoes a diffusion-mediated reordering step which effectively translates that plane by a/2⟨110⟩ and transforms the fault into a lower-energy SESF. While numerous observations of this mechanism have been made in Ni- and Co-base superalloys since its model description in 2001, two key aspects have previously eluded experimental verification: firstly, a direct observation of the complex nature of the intrinsic segment between the two leading partials, and secondly, the occurrence of reordering. By imaging the fault structure and leading partials in a compressively deformed sample of Co-base superalloy ERBOCo-4 not only in ⟨110⟩ projection (which may be ambiguous regarding the complex or superlattice character of a fault), but also in ⟨211⟩ projection, we were able to reveal the intrinsic and extrinsic segments as CISF and SESF, respectively, directly confirming both aforementioned aspects [2].

In the second example, microscopic details of the γ'($L1_2$)-to-χ($D0_{19}$) phase transformation were elucidated in the Co-base superalloy ERBOCo-VF60 (see figure). The accelerated formation of the χ phase during annealing (850 °C, 20 h) was prompted by the diffusion of Cr into the superalloy, destabilizing the γ/γ' microstructure. Coherent, plate-shaped χ precipitates have formed on {111} planes. Based on the fcc-to-hcp transformation, the transformation of the $L1_2$ structure (ABC stacking) to the $D0_{19}$ structure (AB stacking) is shear-based and can be achieved by the introduction of an SISF after every other plane of the $L1_2$ structure. By studying the leading partials driving the transformation at the tip of a precipitate in HRSTEM, details of the transformation process were uncovered [5]. In [110] projection (bordered in red), the formation of SFs is evident, causing a change in stacking. Furthermore, a change in the alternating bright/dark contrast of atomic columns on the planes marked by dashed red circles implies the involvement of a diffusion-mediated reordering process. By additionally imaging the fault structure in [211] projection (bordered in black), the Burgers vectors of the leading partials were determined unambiguously (from superlattice shifts made visible by Fourier filtering in this projection) as noted in the schematic drawing. Notably, the transformation does not progress directly through shearing by a/3⟨112⟩ partials necessary to form SISFs, but rather by a/6⟨112⟩ partials which leave behind CISFs. The smaller Burgers vectors facilitate shearing and reduce elastic strains at the transformation front. At the same time, the high-energy CISFs are transformed into lower-energy SISFs by reordering processes. As illustrated in the schematic drawing, this fault configuration can serve to transform the ABC stacking of γ into the AB stacking of χ. The sum of all translation vectors in this configuration, and therefore the net strain introduced into the microstructure, is zero. Fault configurations following this pattern of three a/6⟨112⟩ partials creating CISFs followed by reordering to SISFs were observed repeatedly at the transformation fronts, indicating that this may be the preferred transformation mechanism. Besides the change in crystal structure, the formation of the χ phase also involves a significant enrichment of W and Ta supplied by diffusion from the surrounding microstructure. An in-depth characterization and discussion of elemental distributions associated with this phase transformation was conducted based on EDXS measurements down to the atomic scale.

Conclusion

We have presented two experimental applications of a novel HRSTEM-based method to reliably and unambiguously identify the complex or superlattice nature of both intrinsic and extrinsic SFs in the $L1_2$ structure. In both cases, microscopic details of shearing mechanisms in the γ' phase of high-temperature superalloys were elucidated. In the future, this method may help uncover the details behind other $L1_2$ shearing processes.

Keywords:

Scanning-transmission-electron-microscopy, superalloys, stacking-faults, deformation-mechanisms, phase-transformations

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Understanding CuO/Al2O3 Interactions during Thermochemical Redox Reactions: TEM, X-ray Microscopy, and XAS Study

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Poster Group 2

Background
Chemical Looping Combustion (CLC) presents a midterm solution for fossil fuel utilization with inherent carbon dioxide capture, utilizing oxygen carrier materials. These carriers replace air to provide oxygen for combustion across a wide range of fuels, operating through reduction/oxidation cycles in a circulating fluidized bed reactor at high temperatures [1]. Copper oxide supported on alumina grain (CuO/Al2O3) is widely considered a promising oxygen carrier (OC) for industrial CLC use due to its benign nature and flexible redox behavior, ensuring high reactivity and oxygen transfer capacity. However, successive high-temperature (800-900°C) reduction (combustion) and oxidation (regeneration of oxide phase) cycles induce chemical and morphological changes in the material, leading to degradation in its oxygen-carrying properties. The evolution in the cycled material is attributed to the diffusion of Cu-phases at the grain scale [2]. Herein, we bridge the gap in understanding between the observed µm-scale migration of Cu-based phases and nanoscale transformations of Cu nanoparticles (NPs) by employing a multi-scale characterization approach, both temporally and spatially, using Scanning Transmission X-ray (STXM) and Scanning Transmission Electron (STEM) Microscopies, and operando Quick X-ray Absorption Spectroscopy.

Methods
We conducted a study on CuO supported on 50-100 µm sized γ-Al2O3 grains, synthesized via incipient wetness impregnation and calcined at 900°C. To replicate the cyclic nature of (CLC), we subjected the fresh samples to oxidation and reduction under air and H2 at 900°C using a thermogravimetric analyzer (TGA). Ultramicrotomy sections with a thickness of 100 nm were prepared for SEM, STXM, and TEM characterizations. Energy stacks and mappings were generated at the Cu L-edge and Al K-edge to identify specific spectral features of each compound. In situ TEM analysis was conducted using a probe Cs-corrected microscope equipped with closed-cell in situ gas setup featuring a sealed environmental cell (e-Cell), operating at atmospheric pressure. Finally, operando XAS measurements were performed in a capillary tube under identical reaction conditions as in the TGA. By acquiring relevant reference material spectra and utilizing chemometric data processing, we extracted chemical-structural phase distribution over the course of 50 redox cycles at 900°C.

Results
The microscopic study reveals significant transformations in the CuO/γ-Al2O3 system during redox cycling (Figure 1 a and b) [3]. Initially, fresh grains consist of γ-Al2O3 with uniformly dispersed CuO nanoparticles (10-20 nm). After 50 redox cycles, observation at the grain scale (µm) demonstrates the progression of the gamma to alpha reaction front within the solids. This front exhibits a distinct structural-chemical gradient, characterized by three zones: zone 1 displaying non-stoichiometric Cu (II) aluminate, zone 2 with an intermediate thin layer (< 200 nm) of Cu (II) aluminate enriched in Cu,

and zone 3 composed of α-Al2O3 phase containing large CuO particles. The proportion of copper varies significantly across these zones, ranging from 10 wt% Cu in zone 1 to ~25 wt% at the edge of the reaction front in zone 2. In situ STEM observation at 900°C under H2-reduction reveals the migration of copper, forming copper nanoparticles from a starting oxidized sample predominantly composed of homogeneous Cu (II) aluminate. This suggests that copper mobility during redox cycling is associated with the phase transition of γ to α-Al2O3.

Furthermore, the spectral evolution during oxidation and reduction reactions, depicted in Figure 1c, highlights changes in the concentration of different Cu-Al species [4]. The oxidized state initially consists of CuxAlyO4, gradually transforming into the metallic copper phase with increasing redox cycles. Notably, a threshold circa 25 cycles indicate a drastic conversion from the aluminate phase to copper oxide. At the end of the redox cycles, the oxygen carrier predominantly comprises α- Al2O3 and CuO. The transition point is linked to the growth and propagation of α- Al2O3, suggesting a mechanism involving particle size influencing the alpha alumina phase transition, proceeding through an initial slow seeding phase followed by rapid propagation.

Conclusion
The integration of spectroscopic and microscopic techniques, both in situ and post-mortem modes, provided a comprehensive understanding of the material evolution in terms of morphology and the diverse phase interactions across spatial dimensions and over extended reaction durations.

Keywords:

STEM, STXM, Quick XAS, Copper

Reference:

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Operating a model Solid Oxide Fuel Cell in the Environmental Transmission Electron Microscope

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Solid oxide fuel cells (SOFC) are a class of solid-state electrochemical conversion devices that produce electricity directly by oxidizing a fuel gas. They consist in an anode-cathode duet separated by a solid electrolyte, i.e., a material conducting oxygen ions. The anode is fed with hydrogen or other fuels whereas the cathode is in contact with air, meaning oxygen. Overall, a SOFC operates thanks to the combined action of two external stimuli: a gaseous environment and temperature. Owing to the recent advances in in situ and operando transmission electron microscopy (TEM), we have set up an experiment to operate a SOFC inside an environmental TEM to identify how the device microstructure determines its electrical properties. To do so, an elementary anode-electrolyte-cathode sandwich was prepared by focused ion beam (FIB) and mounted on a heating and biasing microelectromechanical (MEMS)-based specimen holder (DENSsolutions) and inserted in an Environmental TEM (FEI Titan ETEM), as shown in Figure 1 a,b.
Methods
Standard SOFC materials were investigated: the cathode was strontium-doped lanthanum manganite (LSM) co-sintered with yttria-stabilized zirconia (YSZ), the electrolyte was YSZ, and the anode a cermet of NiO co-sintered with YSZ. NiO was first reduced to Ni, leaving pores in the structure due to the volume loss and hence enabling the penetration of the fuel to the triple phase boundaries Ni/YSZ/porosity at the anode side. For practical reasons, we used a single chamber configuration to trigger the operation the cell: the anode and cathode were exposed simultaneously to the oxidant and reducing gases. Due to a difference in the catalytic activity between the electrodes, O2 should reduce at the cathode, while H2 should oxidize at the anode, thus leading to a voltage difference between the two terminals.
Results
The reduction of NiO was first performed under a forming gas N2:H2 in the ratio 20:1 under 15 mbar up to 750°C (N2 was constantly used as a mixing gas for safety reasons due to the need of mixing O2 and H2 in the single-chamber configuration). The O2 to H2 ratio was then increased to trigger the operation of the cell. A small quantity of O2 was introduced into the microscope, leading to a total pressure of about 16 mbar at 600°C. At this point, the variation of voltage between the anode and cathode was correlated to the gas composition and the anode microstructure (see Figure 1 c-g). The latter was analyzed by means of conventional and high-resolution imaging, diffraction, and EELS (electron energy-loss spectroscopy). The system was cycled several times by decreasing and re-

increasing the O2 concentration in the gas flow, and correlations between microstructure, gas composition, and cell voltage were established, as it will be discussed at the conference. Results were further confirmed by macroscopic ex situ tests in an oven using the same materials [1].

Conclusion

The operation of a SOFC in a single chamber configuration was demonstrated using operando ETEM. Such operando experiments open numerous perspectives to investigate the root cause of failure pathways affecting SOFCs, like poisoning of active sites or coarsening of the Ni catalyst [2].

Keywords:

SOFC, in situ, operando, ETEM

Reference:

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[2] The authors acknowledge the French microscopy network METSA for funding and the consortium Lyon-St-Etienne de microscopie for ETEM access. The FIB preparation was performed at the facilities for analysis, characterization, testing and simulations (FACTS, Nanyang Technological University). Additional support was provided by the INSTANT project (France-Singapore MERLION program 2019-2021) and the start-up grant M4081924 at Nanyang Technological University.

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Prospects of nanofluidic cavities for cryo-EM sample preparation

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IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

Cryogenic electron microscopy (cryo-EM) has become an essential tool for 3D structure determination of biological macromolecules. Despite many technical advances, the difficulty to reliably prepare samples with uniform ice thickness and the excessive sample loss during grid preparation still present a barrier for routine high-resolution imaging and limit the current throughput of the technique [1,2]. Nanofabrication techniques employed for Micro-/Nanoelectromechanical Systems (M/NEMS) provide new opportunities to miniaturise and automate cryo-EM sample preparation. We have recently shown that MEMS-based nanofluidic sample supports with well-defined geometry can be used to prepare cryo-EM specimens with uniform ice thickness from picolitre sample volumes and allow for high-resolution structure determination [3]. Despite these promising prospects, several key challenges remain to be addressed to transform this approach into a viable alternative for widely used holey support films, most prominently mitigation of beam-induced specimen movement and putative interaction with the water-support film interface. I report on the present status of our developments, describe recent efforts in addressing some of the outstanding challenges and elaborate on others that require additional work to resolve. I will also showcase recent results demonstrating the potential of new chip generations to provide new capabilities for further automation of the cryo-EM workflow, and to explore new frontiers for cryo-EM applications such as time-resolved imaging and high-throughput screening.

Keywords:

Cryo-EM, sample preparation, MEMS, nanofluidics

Reference:

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Creating free-standing nanostructures with plasmonic properties via Focused Electron Beam Induced Deposition

Di Di Verena Reisecker[1], David Kuhness[1,2], Georg Haberfehlner[1,3], Michele Brugger-Hatzl[3], Robert Winkler[1,2], Anna Weitzer[1], David Loibner[1,2], Martina Dienstleder[3], Gerald Kothleitner[1,3], Harald Plank[1,2,3]

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Poster Group 2

Over the past decades, significant interest was directed towards the optical properties of nanoscale structures. As they are highly sensitive to slight changes in material, geometry, size and arrangement, controlling these aspects in every detail allows to tailor their plasmonic response according to the targeted operation and opens up access to a multitude of applications in research and development. With miniaturization reaching its limits, expanding the nanostructures into the third dimension could give access to horizontal and vertical mode coupling, reduce substrate damping, increase device performances and allow for the investigation of novel optical effects. Herein, we present how Focused Electron Beam Induced Deposition (FEBID), a 3D manufacturing technique capable of printing nanostructures with feature sizes down to around 10 nm, can be applied to fabricate and tune the plasmonic properties of planar and free-standing gold nanostructures.[1] Therefore, planar gold nanowires as well as free-standing nanotips of varying geometries are printed and purified with a focused electron beam and their plasmonic response investigated through STEM-EELS mapping measurements. Corresponding plasmon simulations provide additional backing to our experimental discoveries, displaying excellent agreement (see Figure 1). This study lays the foundation for on-demand spectral tuning of the plasmonic response in 3D systems through upfront modeling and design of tailored nanostructures, thereby unlocking opportunities for innovative plasmonic applications in 3D space.

Keywords:

Nanoplasmonics, Spectral Tuning, Additive Manufacturing,

Reference:

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Atomic-resolution STEM analysis of polar states in Sr1-xBaxMnO3 thin films

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Poster Group 1

Background incl. aims
Subtle changes in stoichiometry and crystal symmetry govern the fundamental physics of perovskite oxides. Particularly in thin films, the interplay between chemistry and structure can be altered by fine-tuning the conditions for epitaxial growth. A good example is the family of multiferroics $AMnO_3$ (A = Ca, Sr, Ba) perovskite antiferromagnets in which ferroelectricity driven by the off centering of the magnetic cation Mn4+ can emerge through selective crystal distortions [1]. In this talk we will explore how the polar states of the family of epitaxial Sr1-xBaxMnO3-δ (SBMO), thin films [2] can be controlled by careful selection of the substrate, growth conditions and post-deposition annealing parameters.

Methods
Epitaxial Sr1-xBaxMnO3-δ ($0 \le x \le 0.5$) thin films with thickness in the range 7–18 nm were grown by pulsed laser deposition (PLD) using a KrF excimer laser, a fluence of 1 J/cm², and a pulse repetition rate of 10 Hz on single-crystalline (001)-oriented (LaAlO3)0.3(Sr2TaAlO6)0.7 (LSAT) substrates. The crystal structure and thickness of the films were studied by X-ray diffraction (XRD) and X-ray reflectivity (XRR), while atomic-level analysis of the SBMO films was performed by scanning transmission electron microscopy (STEM). The atomic displacements were measured from the annular bright field (ABF) and high-angle annular dark field (HAADF) images by determining the displacement of the Mn and O sublattices with respect to the centrosymmetric positions defined by the Sr/Ba sublattice, from which the film polarization was evaluated. Electron energy loss spectroscopy (EELS) was performed to assess the oxygen content of the films. The fine structure of the background-subtracted O–K edge was fitted to a double Gaussian to quantify the Mn valence following the procedure described by Varela et al. [3]

Results
A combination of macroscopic XRD characterization and atomic scale STEM of the SBMO films has evidenced that the polar displacements are extremely dependent on the oxygen stoichiometry, Ba content and epitaxial strain. The parent compound $SrMnO_3$ grown on LSAT upon tensile strain is known to present in-plane polar displacements along the pseudocubic <110> directions [4]. On the other hand, this scenario can be altered by tuning both the Ba doping and oxygen stoichiometry. As an example, in the case of x = 0.4, the epitaxial strain shifts from tensile to compressive when the annealing conditions change from 550 ºC at 400 Torr O_2 to 650 ºC at 350 Torr O_2 (see Fig. 1a). The EELS fine structure of the O-K edge shows that this phenomenon is accompanied by a remarkable variation of the oxygen content (from δ=0.15 to 0.25), and therefore of the nominal valence of the Mn cation (Fig.1b). Atomic resolution ABF enables to resolve the Mn and O sublattices, revealing that structural and chemical variations induce a drastic change in the axis of polar displacements of Mn

and O. While highly oxygenated SBMO (x = 0.4) presents in-plane atomic displacements with an estimated value of in-plane polarization of approximately 30 µC/cm² (Fig. 1c), the more oxygen deficient and compressively strained film with the same Ba content, shows a predominantly out-of-plane polarization with a deduced polarization as high as 95 µC/cm² (Fig. 1d) [5].

Conclusion

Polarization is intimately linked to the sign and magnitude of epitaxial strain, film thickness and oxygen stoichiometry. It can be tuned either in-plane or out-of-plane with respect to the substrate plane by the adequate choice of the substrate-induced strain, Ba doping and O content –induced by controlled annealing. This chemistry-mediated engineering of the polarization orientation and magnitude of oxide thin films opens new venues for the design of functional multiferroic architectures and the exploration of novel physics and applications of ferroelectric textures with exotic topological properties.

Keywords:

Oxides, multiferroics, STEM, films, polarization

Reference:

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Multi-Dimensional Data Restoration from Subsampled EBSD Data

Zoe Broad[1], Jack Wells[2], Daniel Nicholls[3], Alex W. Robinson[3], Amirafshar Moshtaghpour[4], Robert Masters[5], Louise Hughes[5], Angus I. Kirkland[4], Nigel D. Browning[1,3]
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Poster Group 2

Background incl. aims

Electron backscatter diffraction (EBSD) is a popular technique for the identification of sample morphology, providing information about the crystal orientation and grain boundaries present [1]. Although detector technology has significantly improved, analysis times can be long, on the order of hours, depending on sample type and size. Additionally, long dwell times are often needed to enable sufficient signal to be collected, making the technique unviable for beam sensitive samples.

Recently, compressive sensing techniques have been applied to electron microscopy, wherein low-dose and fast acquisition methods are enabled through the use of subsampling. To date, subsampling has been successfully applied in STEM and FIB-SEM [2-4].

EBSD datasets are 4-dimensional (4-D), meaning multiple applications of subsampling and reconstruction are possible. Demonstrated here is the application of probe subsampling in EBSD datasets. For reconstruction 2-D, 3-D and 4-D data volumes were investigated.

Methods

A low noise Ni-superalloy dataset consisting of 416 x 512 probe locations was used, with a mask applied to simulate 25% subsampling. The reconstruction methods investigated used 2-D, 3-D and 4-D data volumes to reconstruct the full dataset.

Probe location maps are formed by taking each pixel from the full set of EBSD patterns at a single probe location, forming an image similar to a pattern quality map. 2-D reconstruction consists of inpainting the probe positions on an image by image basis. In 3-D reconstruction the dataset is vectorised. In 4-D reconstruction the data is inpainted in the position it was acquired in.

Results

The results of inpainting the 2-D and 3-D data volumes are shown in Figure 1 (Reconstruction quality of 25% sampled EBSD datasets using 2-D or 3-D data volumes for inpainting).

For 2-D inpainting a minor decrease in hit rate is observed, dropping from 99.80% to 99.53%. The band contrast map is less defined at grain boundaries than in the fully sampled dataset and an overall smoother map being output with the surface texture being lost.

For 3-D inpainting the hit rate drops to 97.87%. This is evident in the IPF Z map, where significant zero solution pixels can be seen around the grain boundaries. By using a 3-D data volume for inpainting there is a greater overlap of EBSD patterns between grains which results in a less distinct EBSD pattern being inpainted. Despite this, the band contrast map is slightly sharper than the 2-D reconstruction, although some grain boundaries are no longer evident.

Although 4-D reconstruction shows promising results on a heavily cropped dataset (8x10 pixels), the memory currently required for this method limits its applicability. Due to this cropping, the datasets cannot be indexed in AZtec and hit rate is not recorded. Further work looking into optimising the datasets and inpainting parameters may help to improve this.

Conclusion

Pre-indexing reconstruction has been demonstrated. 2-D reconstruction is thus far the most effective method, with 3-D reconstruction through probe positions providing valid but slightly lower quality reconstructed patterns. Although the output quality of 4-D reconstruction is good it is currently unfeasible given the memory requirements.

Keywords:

EBSD, SEM, compressive sensing

Reference:

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Reliable tomographic reconstructions of (sub)-nm gaps in plasmonic gold dimers for correlation to optical properties

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IM-01, Lecture Theater 1, august 26, 2024, 14:00 - 16:00

Different decay paths govern plasmon damping. Among them, direct charge transfer has incredible potential in several applications from quantum optics to biosensing, but its exact mechanisms are still an open question [1][2][3]. We investigate the optical properties of plasmonic gold nanosphere-dimers separated by nanometer sized gaps towards the optimization of direct charge transfer. The dimers are synthetized with a molecular junction of conductive dithiol molecules in the gap. The optical response of metal nanoparticles is ruled by their exact three-dimensional morphology [1]. This is particularly critical for investigating charge transfer, which depends on the exact gap size. Different techniques are available to investigate the shape of nanomaterials. Among them, transmission electron microscopy (TEM) allows for atomic resolution. However, TEM images are 2D projections of 3D objects, resulting in the loss of valuable three-dimensional information. This loss can be especially detrimental for extracting quantitative information for non-symmetric structures or in case of objects that do not lie perfectly flat on the substrate. Three-dimensional information can be obtained by electron tomography (ET).

In this work, we begin by addressing the limitations of 2D high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and 3D ET, with particular focus on the gap reconstruction. We discuss the difficulties that emerge, both in 2D and 3D, when defining thresholds for extracting the quantitative morphology. We then combine experiments and simulations to address ET's challenges in reconstructing (sub)- nanometer gaps in gold dimers. We introduce a model for fitting sub-nanometer interparticle gaps. The proposed model is based on the convolution of a Gaussian function and a step function. Finally, the results are correlated with optical measurements. Optical properties are investigated using a homemade confocal dark-field spectroscopy setup and electron energy loss spectroscopy (EELS).

We start by highlighting the limitations of 2D STEM for gap reconstructions. Different experimental STEM images of the exact same dimer taken at different tilt angles resulted in different gap sizes and strongly varied with the chosen image threshold. By changing these two parameters, the gap size can vary between 0.1 nm and 2 nm. Such large differences in gap sizes have tremendous effects on the optical properties. As shown in Fig.1, even rather minor changes in morphology significantly impacted the optical properties of the system. The inaccuracy in determining the gap sizes from the 2D STEM images (Fig. 1b) made it also impossible to simulate the optical properties of the dimers. In Fig. 1c the scattering cross sections were simulated based on the 2D morphology information. For that, the STEM images were thresholded with the Otsu method to extrapolate the morphological parameters, which were then used as input for the optical simulations. The simulations were performed using the MATLAB toolbox MNPBEM[4]. In Fig.1c, the experimental and simulated

scattering spectra of four different dimers are displayed. The discrepancies between simulated and experimental scattering spectra suggest that 2D STEM images alone are insufficient for retrieving the morphology of the dimers.
Even when moving to electron tomography, we observed that the choice of threshold remained critical for the final gap reconstruction. Therefore, we developed a model to fit the data and retrieve the gap size without running into the thresholding problem. The proposed model is based on the convolution of a Gaussian function and a step function. The validity of the model was first investigated on simulations and them applied to fit experimental data.
We are currently working towards extending the convolutional model to analyse 2D STEM data by including the thickness-dependent HAADF-STEM intensity[5] in the fitting procedure. This will enable gap size extrapolation without relying on thresholding methods.

In conclusion, in this work we highlight the limitations of 2D imaging for retrieving the morphology of plasmonic particles. We then advocate the use of electron tomography to achieve a better reconstruction of the system morphology. We introduce a model tailored to fit experimental data from electron tomography and extrapolate nanogap sizes with one pixel accuracy. Finally, by correlating optical properties and morphology, we demonstrate the importance of this accurate three-dimensional reconstruction. Being able to accurately simulate the optical properties and experimentally correlate it to the exact morphology will finally allow us to design plasmonic systems with fine-tuned properties. This can be crucial for applications in catalysis, sensing and quantum optics.

Keywords:

plasmon, electron-tomography, correlation, optical properties

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Correlative transmission electron microscopy and photoluminescence microscopy revealing enhanced fluorescence in nitrogen vacancy containing nanodiamond

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Fluorescent nanodiamonds (FNDs) are diamond nanoparticles containing color centers that emit visible light at room temperature. Among the color centers in FNDs, the nitrogen-vacancy centers (NV) have drawn the most attention due to its exceptionally stable optical properties and great prospect in sensing and biomedical diagnostic applications [1]. The current understanding of the optical properties of the FNDs relies largely on the optical measurement methods from either ensemble of materials or from few single particles. As most of the FND fabrication is a top-down process where larger diamond crystals are milled to desired nanometer size, FNDs generally have broad size distribution and irregular shapes [2]. Therefore understanding of FNDs structure-property relationship using ensemble measurements only is insufficient.

We have developed a new method based on correlative transmission electron microscopy and photoluminescence (TEMPL) [3]. TEMPL allows a direct correlation of the fluorescence brightness and three-dimensional size and shape of individual nanoparticles. PL provides optical information with exquisite energy resolution, and TEM provides structural information with exquisite spatial resolution. Unsupervised machine learning (ML) with the generalized 3D shape descriptors, is used to analyse correlations between the PL brightness and 3D shape of FND particles. The automation provided by machine learning allows TEMPL to be applied to large sample areas (2-3 orders of magnitude larger than a typical TEM field of view) containing a statistically significant number of particles.

Using this new method, we directly reveal that the volume-averaged PL brightness of thin, flake-like nanodiamond particles is up to several times greater than that of three-dimensional-shaped, thicker particles provided the particle diameter is less than the sub- wavelength limit. With the assumption that the number of NVs within a particle is proportional to its volume, this implies that individual NVs within thinner particles are brighter. This experimental observation is supported by the theoretical simulations on simplified particle geometries on a range of supporting substrate and surrounding medium. The simulations indicates that the comparative brightness of thinner particles, either on a thin supporting substrate or in a low- index medium, is attributable, at least in part, to the constructive interference of partial light waves in these particles. With increasing particle thickness, such an effect becomes damped. The sub-wavelength dimension of the substrate plays an important role, as it results in higher effective reflectivity comparable to diamond particles in a low-index medium (e.g., a low- index solution).

We have introduced a machine-learning-assisted correlative TEMPL method, which enables direct elucidation of the 3D morphological−fluorescence relationship of fluorescent nanoparticles. We emphasize that the method provides three-dimensional morphological information, which is potentially crucial for a variety of fluorescent nanoparticle systems but rarely achieved. Another significant advantage is that, in contrast to ensemble-based methods, TEMPL is performed ultimately at the level of individual particles. Notwithstanding this, machine learning assistance permits the analysis of a statistically meaningful number of particles. By using the TEMPL method to analyse the 3D morphology-fluorescence relationship of NV-containing nanodiamonds, we directly reveal that the volume-averaged brightness of thin, flake-like nanodiamond particles is up to several times greater than that of three-dimensional-shaped, thicker particles.

Keywords:

Correlative microscopy, Machine Learning, fluorescence

Reference:

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Electron holography of a vortex-type magnetic domain wall in a cobalt nanotube

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Background incl. aims
Three-dimensional nanomagnets and curved magnetism are nowadays exciting topics in Nanomagnetism. The appearance of novel spin textures and topologically protected magnetization states may be the key to develop 3D spintronic devices for magnetic data storage, magnetic logics and sensing [1]. One of these exciting architectures are ferromagnetic nanotubes (NTs), in which the predicted core-less spin states are ideal for stable and fast domain wall (DW) motion beyond the Walker limit [2]. Even though they have modelled and predicted theoretically, a detailed experimental observation of these DWs is missing. In this work we aim at revealing the nature of magnetic DWs in cobalt NTs.

Methods
Focused electron beam induced deposition (FEBID) [3] has been used to synthesize 3D cobalt NTs. FEBID is an exceptional nanolithography technique to produce high-quality magnetic architectures. It is a direct one-step nanolithography technique that allows 2D and 3D growth of architectures of almost arbitrary shape on virtually any substrate or surface geometry. It is based on the decomposition of an organometallic gas precursor by the electron beam to produce a deposit. In the case of 3D nanostructures of magnetic materials, such as cobalt or iron, the deposits are mixed with carbon and oxygen residues of the precursor. This contamination reduces its purity and therefore its magnetization. Thermal annealing procedures in high vacuum of an environmental SEM have been performed to increase the metallic content of as-grown cobalt layer [4]. Structural and chemical characterization of the as-grown and annealed NTs have been carried out by STEM-EELS in a probe-corrected Titan. Quantitative magnetic imaging of the remanent state and DW structure of a 3D ferromagnetic cylindrical NT has been achieved by off-axis Electron Holography (EH) in aberration-corrected Lorentz (field-free) transmission electron microscope (TEM) Hitachi I2TEM. Magnetic and electrostatic phase images have been obtained through holograms obtained before and after flipping the specimen. Micromagnetic simulations of the cobalt NTs have been carried out using the OOMMF software.

Results
12-nm-thick cobalt layers have been deposited on non-magnetic vertical Pt-C nanowire templates with a diameter of ≃70 nm, and then annealed at 450 ºC in high vacuum to produce high-purity, chemically and structurally homogeneous cobalt NTs (see Fig. 1a,b). They present a good cylindrical symmetry and homogeneous cobalt coverage over the Pt-C template, with a very thin (1-3 nm)

oxidation layer at the surface caused by exposure to ambient atmosphere. While as-grown NTs present a low cobalt content (< 70% at.), the thermally annealed ones present very high purity (approx. 95% at.). The magnetization state of the FEBID cobalt NTs determined by EH evidences a magnetic induction value near bulk values (>1.55 T), comparable to the magnetic induction measured in homogeneous cobalt nanowires annealed in similar conditions [3]. The thermally annealed cylindrical NTs give evidence for the nucleation and pinning of a DW during the magnetization reversal process induced by a excitation of the objective lens of the microscope. A quantitative analysis of the magnetic phase image and its comparison with detailed OOMMF micromagnetic simulations have confirmed that the magnetic contrast corresponds to a core-less head-to-head vortex DW, , as shown in Fig. 1c-e. In this coreless magnetic structure, the magnetization is confined in the nanotube plane with the spins rotating perpendicular to radial direction of the cylindrical structure.

Conclusion

Thermally annealed NTs with high-purity cobalt have been successfully grown by FEBID. EH performed in an aberration-corrected Lorentz TEM gives evidence for a high magnetization of the NTs in agreement with the increased metallic content achieved by high vacuum thermal annealing. A DW has been nucleated, imaged and quantitatively characterized for the first time. A comparison of phase images with micromagnetic simulations demonstrates the formation of a head-to-head vortex DW, as predicted in the literature according to the nanotube geometrical dimensions.

Keywords:

Holography, magnetism, nanotubes, vortex, spintronics

Reference:

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Towards atomic-resolution electron energy loss spectroscopy in an uncorrected 30kV scanning electron microscope

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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background incl. aims
As an era-defining technological advancement in the field of nanoscience and beyond, the effective implementation of aberration correction has allowed electron microscopy to routinely reach deep sub-angstrom-level spatial resolution. Recently, ultra-high-resolution monochromators relying on related electron-optical designs have further enabled electron energy loss spectroscopy (EELS) in the meV regime. Among many impactful consequences, these developments have seen the widespread adoption of low-voltage instruments, which can maintain very high spatial resolutions thanks to their aberration correctors, even down to 20kV, with 2-dimensional materials often providing the ideal sandbox and test objects, leading to the advent of practical 'single-atom microscopy' [1].
Beyond single-atom sensitivity, low-voltage operation is highly sought-after for reasons such as reduced knock-on damage to samples or increased inelastic cross-sections resulting in a high signal for spectroscopy. However, for a large number (perhaps even a majority) of practical materials science applications, the complexity and price of such instrumentation, especially when analytical capabilities are added, can be a drawback. In contrast, high-throughput capabilities with lower entry barriers in terms of cost and complexity, but which maintain a relatively high-resolution (but not always single-atom sensitivity), can often be preferable in order to address numerous scientific questions. These may in turn reveal further avenues for investigation that more complex instrumentation may then be used to explore.
Methods
One possible approach in recent years has led to the emergence of (low-voltage) scanning electron microscopes (SEMs) operated in a transmission geometry – or (T)SEMs [2]. When equipped with cold field emission sources, these instruments have been shown to reach 0.2nm information transfer in bright-field STEM imaging [3], and to provide remarkable flexibility for surface and spectroscopic investigations of functional materials [4].
Here, we show how the capabilities of such a high-resolution (T)SEM can be pushed even further towards near-atomic resolution. We use a Hitachi SU9000EA microscope, a low-kV (≤30kV) uncorrected (T)SEM equipped with a diffraction camera and a Hitachi electron energy-loss spectrometer developed for this instrument, which thanks to its cold-field emitter has a native energy resolution of ~0.3eV. In the optical configuration chosen for the experiments, the estimated probe size at 30kV acceleration voltage was below 0.4nm, sufficient to demonstrate atomic-resolution imaging and spectroscopy in carefully selected materials systems.
Results
Figure 1a shows a high-angle annular dark field (HAADF) image of a La1/3NbO3 ceramic (LNO) observed in [001] zone axis. The sample was prepared by crushing pellets of the sintered material into fine crystallites, and dispersing a chloroform suspension onto a lacey carbon support film, as described elsewhere [5]. The overall structure of this highly promising candidate thermoelectric

material is that of a perovskite, but it exhibits two distinct alternating A-site planes: one fully occupied by La ions, while the alternate position is fully La-deficient: a model is overlaid on figure 1a. The Fourier transform of the image, inset, demonstrates information transfer down to 0.26nm in HAADF (similar performance was also observed in bright field images, while atomic plane resolution was also observed in secondary images).
In this projection, the distance between non-deficient La cation planes is 0.8nm, making it an ideal test sample to use EELS to map with atomic-plane resolution the location of La in the structure. The observed oscillations, peaks and troughs, of the integrated intensity of the La M4,5 edge in an EELS linescan acquired along the indicated orange segment, figure 1b, follow exactly those of the simultaneously acquired HAADF signal – with the darker layers corresponding to La-deficient positions. The use of the La M4,5 edge, whose onset sits at the relatively high energy loss of 832eV, also serves to highlight the applicability of EELS in this uncorrected 30kV system, even at high energy losses.
Conclusion
These results demonstrate unambiguously atomic-plane resolution in EELS mapping in an uncorrected SEM, used in a transmission geometry. Together with high spatial resolution imaging capabilities across multiple signal channels (HAADF, BF, SE), this further demonstrates the versatility of these microscopes, whose advanced capabilities as (T)SEM-EELS instruments belie their relative operational simplicity and low cost.

Keywords:

SEM, EELS, HAADF imaging, instrumentation

Reference:

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Simultaneous acquisitions and applications of DPC/OBF STEM, EDS and EELS

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Poster Group 2

Background incl. aims
The use of a segmented detector has become standard for various STEM observations, particularly for Differential Phase Contrast (DPC) STEM[1] and Optimum Bright Field (OBF) STEM[2]. DPC STEM can visualize weak electromagnetic fields such as p-n junction interfaces[3] and magnetic skyrmions[4]. In low-dose experiments with beam-sensitive materials, like zeolites and metal-organic frameworks (MOFs)[5], OBF STEM method achieves noticeably better contrast during live imaging. The applications of a segmented detector are further extending across a variety of material and life science fields. As an example, this research shows a combined analysis of these advanced imaging techniques with elemental analysis methods, EDS and EELS, simultaneously acquired in our new FEMTUS platform.
Methods
The sample was a semiconductor memory. The experiment was performed using JEM-F200, equipped with SAAF-Quad detector (an annular four-segmented detector), Dual SDD detector for EDS, CEOS Energy Filtering and Imaging Device (CEFID) with Dectris ELA hybrid-pixel electron detector, and integrated analysis platform FEMTUS developed by JEOL. In the FEMTUS platform, all detectors and cameras can be synchronized and simultaneous acquisition becomes possible with easy operation. For all experiments we chose an accelerating voltage of 200 kV, STEM mapping was performed with a dwell time of 10ms, convergence semi-angle of 6.6 mrad, and EELS collection semi-angle of 2.2 mrad limited by the central hole of SAAF-Quad detector.
Results
Figure 1 shows the result of DPC STEM and EDS/EELS elemental mapping, acquired simultaneously in a single scan. Fig. 1a shows the x-components of the center of mass (COM) DPC STEM derived from the signals of four SAAF-Quad detector channels. It can be seen that the COMx image reveals thin line contrasts around the regions indicated by the arrows. The DPC STEM method has better sensitivity for differences in projected potential, originating from both electromagnetic field and/or local chemical composition. Fig. 1b and 1c represent a magnified view of the EDS and EELS count maps, respectively. EDS mapping has an advantage in detecting heavy elements such as tungsten and titanium, which are difficult to access using the phase imaging method (DPC or OBF STEM) and EELS. As complementary information, the EELS mapping shows clear contrast for light elements (oxygen, nitrogen, and silicon) with higher S/N ratio compared to EDS. All of this information can be used to analyze the origin of DPC STEM contrast. Fig. 1d shows intensity profiles of COMx and EELS data extracted from the area indicated by the white square. The peaks of COMx intensity correspond to the increase of oxygen component, whereas the amount of nitrogen decreases in the interface region. Such combined information is very helpful to investigate the origin of phase contrast images, such as the composition difference between SiOx film and SiNx bulk region shown here.
Conclusion
In summary, we acquired DPC STEM, EDS, and EELS data of semiconductor samples simultaneously and revealed that the origin of DPC STEM signals was due to changes in the local chemical composition. Without the additional information from EDS and EELS, it was difficult to clarify

whether the obtained phase contrast represents chemical composition, electromagnetic field, or just a difference in local thickness. Such simultaneous acquisition of DPC, EDS, and EELS enables us to directly understand the origin of the observed phase image contrast. Furthermore, since compared to EDS and EELS mappings, DPC STEM is very sensitive to changes in the projected potential, it will be possible to clarify compositional differences by integrating the EDS and EELS signals of regions where phase contrast differences could be observed, even under low-dose conditions. This should also be useful for the composition analysis of electron beam-sensitive materials whose structures are destroyed with just a few scans. On the day of the presentation, we will show the details of the experimental results and additional instances of simultaneous data acquisition including OBF STEM.

Keywords:

Segmented-Detector, DPC-STEM, OBF-STEM, EDS, EELS

Reference:

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Imaging Atomic Processes in Catalysts using a New High-Order Imaged-Corrected Environmental-TEM

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Poster Group 1

Background incl. aims:
The current quest for sustainable energy and environmental technologies calls for a new view on catalysis. Today, catalysis of chemical reactions is commonly perceived as a complex surface phenomenon that inescapably links structural dynamics and functionality of the catalytic nanomaterials [1]. However, insight into this intricate relation between catalytic active surface sites and their mechanistic actions has remained limited due to the lack of suitable atomic-resolution imaging competences. The Center of Visualizing Catalytic Processes (VISION) addresses this core scientific challenge by introducing new electron microscopy technology and applications. In this contribution, we demonstrate a new-generation environmental transmission electron microscopy (ETEM) enabling three-dimensional atomic-resolution imaging of catalytic nanomaterials under exposure to reactive gas atmosphere in a chemical meaningful way.

Methods:
The VISION PRIME microscope is based on an ultra-stable Thermo Fisher Scientific SPECTRA ULTRA platform and designed for in-line holography to retrieve time-resolved exit-wave functions of catalyst nanomaterials during exposure to reactive gas environments at pressures of up to 10-20 mbar. The microscope is equipped with (i) a new 5th order aberration-corrector for the objective lens in broad-beam mode (CETCOR Prime), (ii) a four-stage differential pumping system for confining a reactive gas environment in the mbar range to the vicinity of the sample (ETEM), (iii) a monochromator setup to extend the information limit below 50 pm and (iv) direct electron detection acquisition (Falcon 4i) for low-dose-rate imaging to suppress electron-beam-induced sample alterations.

Results:
Here we present the design of this new-generation ETEM as well as selected performances and applications. Specifically, the ultra-stable electron optics allow the high-resolution transmission electron microscopy (HRTEM) mode to reach 60 pm resolutions routinely, and to further extend the resolution toward 50 pm, in both high vacuum and environmental modes. This is achieved by exploiting the high stability base and optics of the Spectra platform and integrating the ETEM module into it.

Using the flexible microscope illumination system enables to generate Nelsonian illumination with a rapid and flexible electron dose-rate control. This is key to suppress electron-beam-induced sample alterations [2,3]. Furthermore, direct electron detection capability in HRTEM mode helps to achieve the largest image signal for the fewest number electrons. In conjunction with the ultra-stable electron optics direct detection enables the recovery of the electron exit-wave functions at the highest sensitivity from focal image series acquisitions. This uncovers the three-dimensional atomic structure of the catalyst nanomaterial [3,4].

Conclusions:
The VISION PRIME electron microscope offers a sample-limited rather than optical-limited resolution under high vacuum and environmental conditions. This atomic scale visualization capability offers new means to address the three-dimensional atomic structure and dynamic behavior of catalytic nanomaterials during exposure to relevant reaction conditions. The VISION PRIME is therefore key for uncovering the role of gas-surface interactions in complex catalytic nanomaterials at the atomic-scale and for advancing new catalyst design strategies.

Keywords:

Catalysis, high-resolution-transmission electron microscopy, ETEM

Reference:

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Investigation of Ferroelectricity using Advanced Microscopy

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Poster Group 1

Backgrounds:
Ferroelectric materials, characterized by their ability to exhibit spontaneous electric polarization switchable under applied electric fields, manifest intricate interactions among electrical, thermal, and mechanical properties. These materials, pivotal in various applications such as spintronics, computing, communications, memories, actuators, motors, and sensors, undergo changes in behavior and properties in response to factors like temperature, electric field, pressure, and strain. To explore ferroelectricity, some basic techniques, such as single-crystal X-ray diffraction, second harmonic generation measurement and dielectric measurement, were used to investigate the crystal structure, symmetry and dielectric anomaly. However, it is difficult to accurately determine whether the crystal structure is centrosymmetric or non-centrosymmetric down to nanoscale level by virtue of these basic measurement. This has hindered the progress of investigating the ferroelectricity related properties in the past.
Methods:
Advanced microscopies including Piezoresponse Force Microscopy (PFM) and Scanning Transmission Electron Microscopy (STEM), have been regarded as powerful approaches for evaluating the physical properties of materials down to nano, or even atomic scale. PFM has already been an essential method to investigate materials with piezoelectric properties. Especially, PFM shows its superiority in the studies of local non-destructive visualization of ferroelectric domain structures. In addition, Scanning Transmission Electron Microscopy (STEM), a fundamental tool in nanoscience, facilitates the acquisition of atomic-scale images and spectra. These images and spectra provide information on the structural and chemical properties of the studied nanostructures. As a result, STEM aids in a comprehensive understanding of the physical properties of functional materials at the atomic level. Consequently, combining advanced microscopies such as PFM and STEM has been shown as a powerful approach for evaluating the ferroelectric properties down to nanoscale even atomic level.
Results:
This study delves into a comprehensive examination of several ferroelectrics including organic-inorganic hybrid perovskites and traditional inorganic ferroelectrics utilizing advanced microscopies including PFM and STEM. These approaches enable a profound understanding of the intricate relationship between domain structure, electric polarization, lattice structure, and electronic states, thereby offering control over the nuanced mechanisms of ferroelectrics. Leveraging advanced microscopy including PFM and high-resolution STEM, we precisely investigated ferroelectric domain structures, the atomic and electronic structures of ferroelectric materials, shedding light on the impacts of domain properties, atomic displacements, and electronic configurations on ferroelectrics.
Conclusions:
The findings of this research hold significant implications for deepening the understanding on the ferroelectric properties, including changes in the ferroelectric domain structure and atomic level displacements, potentially paving the way for the creation of more efficient and reliable memory

storage, sensors, and energy converters. We anticipate that our research will establish a robust foundation for the examination of ferroelectricity and other complex functional materials down to atomic level.

Keywords:

Ferroelectrics, thin Films, Advanced Microscopy

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In-situ TEM Investigation of Degradation Process in Ni-Rich Cathodes.

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Lithium-ion batteries have become integral for energy storage in various applications, including electric vehicles, and portable electronics [1]. Cathode materials such as LiNi1−x−yMnxCoyO2 (NMC), LiNixCoyAl1−x−yO2 (NCA), LiCoO2 (LCO), and LiMn2O4 (LMO) are widely used [2]. However, challenges like fast capacity fade, high cost, and poor cycling performance persist. Layered LiNiO2 (LNO) shows promise but suffers from structural changes and oxygen evolution during cycling [3]. During cycling we can identify three distinct stages where, the layered O3 stacking sequence is reduced from trigonal structure with rhombohedral symmetry $R\bar{3}m$ to monoclinic C2/m, then into a layered mixture of O3 and O1 (H2-H3) stacking and further to a rocksalt phase ($Fm\bar{3}m$) [4]. Among all phases, the H2 to H3 transformation results in poor structural stability especially while operating at high voltages [5]. This study aims to investigate the degradation mechanism of LNO cathodes, focusing on its initial O3 stacking sequence.

Methods
The degradation dynamics of LNO cathodes were investigated using in situ electron microscopy, electron energy loss spectroscopy (EELS), and four-dimensional scanning transmission electron microscopy (4D-STEM). This degradation has been monitored in real-time, which traces for the structural changes taking place for Li+ diffusion and degradation taking place with the chemical composition. A constant potential of +4.3 V was applied and the first delithiation cycle was examined via in situ TEM. On the other hand, EELS contributed to valuable insights regarding the changes of chemical composition during cycling, while 4D-STEM aided in obtaining high-resolution images and analysis of diffraction in order to complete the characterization of structural transformations taking place within the material of the cathode.

Results
Observations revealed the layered structure of pristine LNO, with cation mixing layers rapidly forming new Ni-rich phases mixed with Li2O phase near the reaction front and pure NiO species further away. During the first delithiation cycle, expansion occurred as the particle was charged versus a Li metal source as the anode, resulting in increased c lattice parameters and phase transitions from H1 to monoclinic, then to H2 and H3 (representing collapse of the layered structure).

Conclusion
The study sheds light on the degradation mechanism of LNO cathodes with the O3 stacking sequence, elucidating structural and compositional changes during cycling. Future work will focus on comparing simulated and experimental phase reconstruction mechanisms from layered to spinel phases and investigating oxygen loss in LNO cathodes during cycling.

Keywords
Lithium-ion batteries, Ni-rich cathodes, degradation mechanisms, in situ TEM.

Keywords:

Lithium-ion, Ni-rich, degradation, in-situ TEM.

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Identification of Chemical Segregation and Surface Twinning Structures in Electro-deposited Al Dendrites

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
Lithium-ion batteries (LIBs) dominate the industry, but limited lithium supplies and safety concerns with their electrolytes push the search for alternatives. Rechargeable aluminium batteries (RABs) are promising among post-lithium battery chemistries, which exhibit considerable potential as candidates for future applications due to the low cost of component materials and excellent theoretical energy density. Most research on aluminium batteries (AlBs) has focused on enhancing cathode materials [1] and ionic liquid electrolytes [2], overlooking attention to Al anodes [3]. It is crucial to note that the inherent metallic nature of aluminium increases susceptibility to dendrite formation during reversible plating and stripping processes [4]. Dendrites pose safety risks by breaching separators, leading to anode disintegration and cell failure. Uneven Al deposition, resulting in dendrite formation, can cause cell short circuits, particularly under repeated cycling at high capacities [5]. Therefore, it is of great importance to obtain in-depth understanding of the Al dendrite growth mechanism to increase battery safety. Multiple approaches were adopted to restrain the Al dendrite growth and identify the dendrite growth mechanism. Whilst most studies are preferred to reveal the mechanisms from the perspective of electrochemistry, electron microscopy is able to provide more straightforward approaches from crystallographic perspectives to understand the dendrite growth mechanism.
Methods
Chronoamperometry (CA) was performed to electroplate Al on Al foil from the [EMImCl]/AlCl3 electrolyte. The electrodeposited Al dendrite morphology was observed by a ThermoFisher Helios 5CX FEG FIB-SEM. The grain growth and orientation of the dendrite were determined by Transmission Kikuchi Diffraction (TKD) techniques, using an Oxford Instrument Symmetry S3 Electron Backscatter Diffraction (EBSD) detector. The scanning transmission electron microscopy (STEM) and corresponding EDX elemental analysis were carried out using a ThermoFisher Scientific Spectra 300 aberration-corrected microscope, operated at 200kV. Samples for STEM were fabricated by the ThermoFisher Helios 5CX FIB.
Results
In this work, we employed Focused Ion Beam-Transmission Kikuchi Diffraction (FIB-TKD) and STEM techniques, to investigate the crystallographic structure of the Al dendrite and the chemical species formed during the Al-dendrite growth. Figure 1 shows a tree-shaped Al dendrite, with clear primary dendrite feature and secondary dendrite arms. The corresponding STEM EDX and FIB-TKD maps from the cross sections parallel and perpendicular to the growth direction enable us to identify the

chemical segregation inside the dendrite and the grain growth along the dendrite. In combination with the detailed aberration-corrected STEM images and the corresponding spectroscopy study, we are able to determine the detailed chemical species inside the dendrite arms and thereby investigate how they affect the Al dendrite growth, including growth orientation and preference.

Conclusion

This investigation provides new understanding of Al dendrite growth and offers insight into the reaction mechanism between the Al and electrolyte, which could guide the future development of new rechargeable Al batteries.

Keywords:

Dendrite; Aluminium battery; TEM; TKD.

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ACOM-TEM Investigation of the white etching layer formation in rail track

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

The growing concern to reduce the environmental impact of freight and passenger transport has increased the importance of rail as an energy-efficient mode of transport. Rail track maintenance has often been carried out on a preventive basis. In order to control costs and primary-source and primary consumption, while taking into account the increase in traffic, a more optimized approach to maintenance is necessary. This involves better anticipating the appearance of rail defects and modeling their evolution. The aim of the OPTIMRAIL(*) Project is to make maintenance predictive, by determining behavioral laws for the evolution of rail microstructure leading to the formation of defects, such as "squat", which accounts for 70% of rail defects following the survey conducted by SNCF in 2021 [1]. The squat defect is characterized by a collapse of the rail tread and a more or less pronounced network of cracks. [Figure 1 (a)]. It is linked to a surface tribological transformation (STT) leading to crack initiation. The latter is called the white etching layer (WEL) because of its appearance under the optical microscope after chemical etching [Figure 1 (b)]. The mechanisms by which the initial perlitic microstructure of railway steels evolves into the white phase are still debated in the literature. Two mechanisms are generally proposed. The first involves a thermally activated mechanism leading to martensite formation following austenitization followed by rapid quenching. The second mechanism is driven by an accumulation of plastic deformation that ultimately leads to nanograins of ferritic structure supersaturated with carbon atoms.

In the aim to clarify the mechanisms, samples of rails that had been in service under real traffic conditions were collected, and metallurgical analysis of the rolling band and the near surface layer was performed [2]. In particular, thin foils have been prepared by FIB at the near surface, allowing to study detailed microstructures from the surface up to 30 microns bellow. Automated crystal orientation and phase maps were produced using ASTAR technique [3] to investigate microstructure evolution (phases, orientation and disorientation). Perlitic structure remains fine and well organized 30 micron below the surface [Figure 1 (c)]. 20 microns below the surface, optical microscopy revealed a transformation in white phase, and ACOM-TEM maps start to show disorganization of perlitic structure, and only Fe3C and ferrite phases. At the surface, the organized perlitic structure is totally lost [Figure 1 (d)]. In some area, richer in carbides, ferrite quality of indexation is poor. Austenite phase seems to have a better reliability in this area, still with some discrepancy between modeled and real diffraction pattern, probably due to strain field induced by the many carbides. To verify the presence of austenite, orientation and phases maps were conducted at two different tilt (0° and 15°) along X axis. Austenite was still better recognize in similar region for the two maps, and coherency of orientation between the two maps was verified. We can then conclude that retained austenite was formed during rail track service, meaning that the mechanism at the origin of the WEL involves austenitization followed by rapid quenching. This information also provide clues of the temperature reached during friction. Martensite and carbide formations are currently under investigation.
(*)The work carried out as part of the OPTIMRAIL project is supported by the Carnot Institute Ingénierie@Lyon.

Keywords:

Steel; ACOM, Phase transformation

Reference:

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Defect engineering of core-shell systems based on 2D transition metal dichocalgenides

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background
One of the biggest challenges facing the development and profitability of new ways to obtain H_2 by water splitting method is to find new materials for Hydrogen Evolution Reaction (HER) electrocatalysis that are not based on Pt, with the aim to obtain electrodes which are both performant and sustainable. The synthesis of TMD(Transition metal dichalcogenide)-based materials is one of the most promising strategies. MoS_2-Au hybrid systems have shown high HER activity, and the plasmonic properties of gold makes it an interesting proposal in photoelectrocatalysis. In this work, we report the synthesis and complete physical and chemical characterization of both Au@MoS_2 and Au@Mo(W)S_2 core-shell nanostructures. In addition, we have studied the influence of thermal reduction treatment in hydrogen atmosphere to increase the activity of these systems via a precise control of the number of shell layers and associated defects.

Methods
Electrochemical HER behavior of the samples was determined by using linear sweep voltammetry (LSV), cyclic voltammetry (CV) and vhronoamperometry, the last one in combination with a light source to study the plasmonic properties. The measurements were carried out in a three-electrode standard set-up in acid conditions (H_2SO_4 0.5M) by using a Biologic potentiostat/galvanostat (SP-150e) with platinum, Ag/AgCl and glassy carbon electrodes as a counter, reference and work electrodes. Structural characterization was performed in a double-corrected Transmission electron microscope (FEI Titan Cubed) in combination with in-situ TEM studies of the evolution of the sample under thermal hydrogen conditions carried out in environmental gas-cell (Climate Holder, DENS Solutions). Other complementary techniques, such as XPS and Raman spectroscopies, were performed to give a complete insight on the structure and chemistry of the samples.

Results
Au@Mo(W)S_2 systems with different W/Mo ratios (including W/Mo=0) were synthesized. TEM analyses highlight the presence of W atoms in the shell layers of the nanostructures. Moreover, a reduction in the number of shell layers additional thermal treatment can be observed by applying a thermal treatment in reducing conditions, as evidenced by both ex situ and in situ TEM studies. The incorporation of such defects induce to a change in the electrochemical properties of the material: low W/Mo ratios enhance the HER activity with respect to the pure Mo sample. However, the thermal treatment affects differently each sample: the best activity being observed for the pure Mo sample heated at 800°C whereas the ternary Au@Mo(W)S_2 samples show an improvement at lower temperature, highlighting thus the interplay between heteroatoms and structural defects on the HER

properties. Moreover, plasmonic properties of the sample was evidenced, by both light-assisted electrochemical studies and low-loss STEM-EELS analyses.

Conclusion
This work provide a complete overview on both the integration of W hetetoatoms and the incorporation of structural defects by thermal treatment into 2D-based core-shell structures. The detailed structural and electrochemical characterization of these processes allow us to link the structural and electrochemical properties as well to fine-tune the chemistry of theses systems.

Keywords:

TMD core-shell plasmonic-material HER

Reference:

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Electron energy-gain spectroscopy of optical excitations in integrated photonic structures

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Poster Group 2

Background
Optical shaping of electron beams, e.g. in the form of longitudinal attosecond bunching that promises increased temporal resolution [1], significantly extends the range of experiments possible in transmission electron microscopes (TEM). Based on inelastic scattering with an optical field, the momentum and energy of the electron are modified by absorption or emission of photons [2]. This inelastic interaction can, in turn, be employed to investigate the nano-optical response of samples with high spatial resolution in photon-induced near-field microscopy (PINEM) [3]. However, due to the weak coupling of free electrons and photons, inelastic field probing and beam shaping techniques so far required intense optical pulses and short electron pulses available in ultrafast TEMs.
Methods & Results
Here, we present the efficient modulation of a continuous electron beam by integrated photonics microresonators made from silicon nitride (Si3N4) that are optically pumped with a continuous-wave (CW) laser [4]. The fiber-coupled, chip-based resonator is placed inside a TEM, as illustrated in Figure 1a, such that the continuous electron beam can pass over the chip parallel to its surface before being analysed with an imaging spectrometer. Swift electrons interacting with the resonator's guided optical mode can absorb or emit photons from the laser field coupled to the resonator. This leads to the formation of electron energy sidebands spaced by the photon energy (~0.8eV, corresponding to ~1550nm) in the spectrum as shown in Figure 1b. The inelastic electron-light scattering is facilitated by the velocity matching of the electrons to the optical phase velocity as well as the high-Q resonant field enhancement. We characterise the latter by employing electron energy-gain spectroscopy (EEGS). To this end, the frequency of the CW pump laser is scanned across the cavity resonance at a low input power while electron spectra are recorded in parallel. We retrieve the laser detuning-dependent electron-light coupling strength (Fig. 1c) that exhibits a linewidth of 390 MHz corresponding to a spectral feature of only 3.1µeV width. From this EEGS trace, we infer a cavity quality factor of 7.7*105.
Increasing the optical pump power coupled to the microring resonator, the inherent nonlinearity and anomalous dispersion cause the parametric generation of new optical frequencies via four-wave mixing. We observe the formation of various nonlinear optical intracavity states whose spectral and temporal properties strongly depend on the laser detuning from the cavity resonance frequency. When scanning the laser across the resonance at a power > 100mW, we can thus perform an EEGS measurement on the nonlinear optical states by recording electron energy spectra in parallel [5]. The resulting electron spectral trace, shown in Figure 1d, exhibits prominent changes when entering different nonlinear optical states (marked by dashed white lines). For stable and chaotic intensity modulations (regions 1 and 2), resulting from the superposition of different optical wavelengths,

averaging over different instantaneous interaction strengths leads to a smoothening of the electron spectra. However, the interaction of electrons with dissipative Kerr solitons (region 3), self-stable short optical pulses with a broad spectrum, yields a broad, low-intensity plateau and a strong central peak since only a fraction of electrons interacts with the high-intensity pulse and scatters to high energy changes.

Conclusions

In conclusion, we characterise the inelastic interaction between electrons and the optical mode of an integrated photonics microresonator. By performing EEGS on one of the cavity resonances, we achieve an unprecedented energy resolution that might be transferred to both the study of material excitations as well as the probing of quantum optical excitations with free electrons. The observed strong interaction of a continuous electron beam with a low-power CW laser, moreover, enables efficient longitudinal electron beam modulation with optical fields in a conventional TEM setup. Harnessing the toolbox of optical waveform shaping in integrated photonics, we employ the multicolour fields of optical frequency combs and their impact on the electron energy spectra upon interaction to further extend these beam-shaping capabilities.

Keywords:

EEGS, UTEM, Inelastic Electron-Light Scattering

Reference:

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Rapid and large FOV mapping of 60° grains in epitaxial MX2 with a segmented detector

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Poster Group 2

Owing to the rapid development in growth of synthetic MX_2 (where M is the metal and X is the chalcogenide) materials in recent years, their average grain sizes are fast approaching several tens to hundreds of micrometers [1]. Epitaxial growth on a single crystalline template is the favored approach to achieve wafer-scale and industrially compatible growth of high-quality single crystalline MX_2 monolayers. Despite the control over orientation provided by templated approaches, selective growth of domains with a single orientation while avoiding the anti-parallel orientation (or 60^o rotated domains) has proven to be challenging [2]. Inclusion of such anti-parallel domains inevitably leads to the formation of 60^o grain boundaries which are detrimental to electron mobility due to the presence of mid-gap states [3]. It is therefore necessary to develop methods to enable rapid and large field of view (FOV) mapping of the anti-parallel domains in epitaxial MX_2. We present a novel approach using segmented detector geometry to rapidly map the 60^o grain boundaries over a large FOV in epitaxially grown MX_2. This relies on the asymmetry of diffraction peak intensities which split into two families Ka and Kb due to reduced symmetry from 6-fold to 3-fold in a monolayer of MX_2 [4]. The specimens are prepared by transferring the MoS_2 from sapphire to a carbon coated TEM grid using a tape assisted method described elsewhere in detail [3]. The conventional dark-field TEM (DF-TEM) is carried out on a Thermofisher Metrios 80-200 TEM operating at 200 kV by placing the smallest objective aperture on any first order diffraction spot. A large number of images with a certain overlap are automatically acquired in a grid pattern, preprocessed, and stitched together to form a montage with significantly larger FOV. This process is repeated for the opposite diffraction spot to obtain the complementary image. The novel segmented detector approach is carried out in a Helios5 FX dual beam operated in a “STEM in SEM” setup at 2kV and equipped with a segmented annular detector. The six segments of the detector are operated such that only 3 of the 6 segments are active for the acquisition and transmitted beam is centered as shown in schematic Fig.1 a. In this mode, as the three segments are separated from each other by 120^o, the detector segments selectively collect the intensities from either the Ka or the Kb spots depending on the orientation of the grains resulting in a contrast which helps differentiate between 60^o grains in monolayer MX_2. The segmented detector approach is first tested on a small grain sample with high density of 60° twins. Fig.1(b) shows a color overlay of the two complementary images wherein the contrast in a monolayer is reversed between the two images making the layer appear either more green (0°) or more red (60^o). The boundary separating these two regions can be either a β or γ grain boundary [3]. The images are segmented using a gaussian mixture model and assigned classes to obtain statistics on 60° twin inclusion in monolayer as well as various stacking orders in bilayer MoS_2 (Fig.1(c)). The method is then applied on large grain samples and compared with the more conventional montage DF-TEM approach used so far. Fig.1(d) shows a similar color overlay obtained by montaging several DF-TEM images acquired at 200kV. The contrast between the main orientation and 60^o rotated domains is rather weak and images are noisier compared to those obtained with a segmented detector at 2kV shown in Fig. 1(e). Moreover, the stacking in bilayer cannot be uniquely identified with the conventional DF-TEM due to influence of unintended specimen tilt [4] which in case of the

segmented detectors is overcome by design through radial integration. A drawback, however, is that 2kV electrons don't have enough energy to see through the carbon support.
The segmented detector in SEM provides superior results from a large FOV in a fraction of the time it takes for montage acquisition and processing, i.e., few minutes compared to several hours or days. In addition. in bilayer, the stacking order can be easily assigned and not influenced by sample mistilt.

Keywords:

Segmented, Grains, Epitaxial, MX_2, Stacking.

Reference:

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Stability insights of MnO2 electrocatalysts from identical location and in-situ electrochemical liquid cell electron microscopy

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
Electrocatalysts require high activity as well as long-term stability to ensure their practical and economic viability. Nevertheless, although critical, the stability consideration is oftentimes overlooked in research[1]. For instance, manganese dioxides are considered promising electrocatalysts to substitute expensive noble metals due to their high abundance, low price and oxygen evolution reaction (OER) activity. However, their electrochemical and structural stability during operation is still controversial[2]. Herein, we will address the stability of MnO_2 using identical location (IL-EM) and electrochemical liquid phase electron microscopy (ec-LPTEM), which offer an excellent platform to investigate the electrocatalysts performance in-situ[3].

Methods
The structure and morphology evolution of the MnO_2 electrocatalysts are characterized using SEM and (S)TEM imaging and associated spectroscopy techniques such as EDS and EELS in ex-situ, in identical locations and in-situ in ec-LPTEM. This combination approach allows the dynamic study of structural changes within the liquid as well as the effects of longer electrochemical operation.

Results
Our results address the debated existence of a stability window for the operation of MnO_2 catalysts for the acidic OER. Measurements based on IL-EM and ec-LPTEM are presented on the specific potential range and conditions of operation within the stability of the catalyst. Beyond the stability window, the structural evolution of the catalyst from its synthesis will be directly correlated through time-resolved imaging to the electrochemical stability during OER operation to resolve mechanisms governing the catalyst instability.

Conclusion
We present a thorough study addressing the growth and electrochemical and structural stability of MnO_2 electrocatalysts for the acidic OER using IL-EM and ec-LPTEM. Such results are key for the design of durable non-noble electrocatalysts.

Keywords:

IL-(S)TEM, ec-LPTEM, in-situ, electrocatalysis, stability

Reference:

0 The Center for Visualizing Catalytic Processes is sponsored by the Danish National Research Foundation (DNRF1462)
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New generation environmental in situ TEM holder for gas cell studies across multiple platforms

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Poster Group 2

In situ (scanning) transmission electron microscopy ((S)TEM) experiments in gases became widely possible with the development of sandwiched MEMS-based samples carriers (e.g. Nano-Chips) – gas Nano-Reactors. Various stimuli like heating or biasing in gaseous environments have been employed to study materials synthesis [1], catalyst [2], ferroelectrics [3], resistive switching [4] and more. The majority of the commercial in situ (S)TEM sample holders, however utilize only four electrical contacts that limit the combination of stimuli that the user can simultaneously apply. Real life applications, like proton exchange membranes, fuel cells, and non-volatile memory, etc, on the other hand, require combined application of thermal and electrical stimuli at ambient environmental conditions and thus, higher number of electrical signals.

In this work we present our new platform for in situ and operando environmental (S)TEM experiments. The heart of this platform is a newly designed holder that has eight electrical connections and allows the application of thermal and electrical stimuli in ambient environmental conditions. This is achieved through a newly designed dual chip environmental cell with an increased number of contacts. Additional electrodes are used to characterize the electrical performance of a FIB lamella sample either in 2- or 4-contact mode. The holder has a removable tip that has generic design and fits different brands of TEM, which substantially improves the correlation of the same sample between different TEM platforms. Furthermore, the design of the holder allows to rotate the tip making it suitable to both, TEM and STEM operation.

Using the new holder, it is possible to achieve high resolution at ambient gas pressure as obvious from the TEM image below. When rotating the tip by 180 degrees, the sample will become on top, which is beneficial for the high resolution STEM imaging. Since the tip flipping is done without the Nano-Reactor disassembly, it is possible to observe the same sample in different modes and easily trace the same region of interest. We will also demonstrate the capability of the holder in terms of elemental analysis using EDS and EELS analysis. We will show a few experimental examples of the new environmental operando system and explain how our new platform can be used in correlative studies involving different experimental methods like in situ TEM, SEM and beamlines.

Graphic
During the in-situ experiments, the user has full flexibility to define if the sample should be on top (better for STEM imaging mode) or on the bottom (better for TEM imaging mode). Therefore, the user can simply flip the tip 180 degrees (as seen in Figure a-c). Despite the rotation of the tip, the user can always preserve the same environmental and stimuli conditions constant. Figures d-f) show that the same tip is mounted on Thermo Fisher and JEOL holder bodies. The high resolution image of a nano-particle at ambient pressure in TEM (left) and STEM(right) imaging modes. The inset in the TEM image correspond to the sample place of the sample imaged in the STEM mode.

Keywords:

In situ, cross-platform, TEM, beamline

Reference:

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Cryo lift-out technique to study host-pathogen interaction on cell monolayer

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Poster Group 2

Cryo Lift-out Technique to Study Host-Pathogen Interaction on Cell Monolayer
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Background:

Cryo lift-out technique is an advanced sample preparation technique for cryo-TEM microscopy. Here, a bulk from the sample surface is lifted out in a vertical orientation using a cryo-needle and thinned using FIB milling, allowing the study of tissue or thicker samples in TEM. In addition to that, the recent development of serial lift-out technique would allow us to understand the information along the volume of the sample. However, in cell biology researc, traditional cryo-FIB that yields horizontal lamella is used, which poses a bottleneck to study the vertical structures in the cells (missing wedge issue). Lift-out is not possible on cell monolayers because of the smaller thickness of the samples. Therefore, we developed a sample preparation technique using hydrogels to grow cell monolayers, followed by high-pressure freezing (HPF) and then lift-out to study the vertical (apico-basal oriented) structures in cells.

Methods:

Optimized hydrogel preparation is vital for performing lift-out on cell monolayer. Here, we used acrylamide gels that have better water content which suits cryo-microscopy. HUVEC cells are used as a model because of our interest in studying a novel (unpublished) actin cytoskeleton structure that is formed from the apical side of the cell to the basal (through the cell volume), due to an extracellular bacterial infection. The hydrogel-Cell-Bacteria sample is frozen using HPF technique, and the lift-out and FIB thinning were performed on Aquilos-2 FIB SEM. Tomograms were acquired using Glacious or Titan TEM.

Results:

Hydrogel preparation, including parameters of polymer concentration, height, and width of the gels, is optimized. The lift-out, which is a completely manual process, has been semi-automated with the help of the IFLM module to perform correlation microscopy using the Maps software. At present, we are replacing the half-moon grids with a rectangular track to perform serial lift-out with the cell samples. The ongoing efforts are to obtain the region of interest with the help of Cryo-CLEM and IFLM techniques to build tomograms.

Conclusion:

Cryo-EM's contribution to cell biology research provides a huge potential to understand the proteins' structures, arrangements, and their microenvironment in their native state. On the contrary, apico-basal polarity and corresponding structures are common in cell biology. For instance, structures such as cell-cell junctions, intestine villus, pedestals, podosomes have specific functions and unique orientation. To study these structures at their native orientation at TEM is physically not possible, due to its alignment along the beam path and missing wedge issue. Therefore, we have developed a sample preparation technique that would allow the researchers to use cell monolayers to perform lift-out techniques to study the vertically aligned structure in a horizontal lamella.

Keywords:

Cell monolayer, Hydrogel, HPF, Lift-out

Reference:

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Revealing atomic structure and composition in ultrahigh energy storage density ferroelectric thin-films

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Poster Group 1

Ferroelectric thin film material is positioned to be a strong candidate for nano/microelectronics component with ultra-high energy storage density with low electric field [1]. One of the key strategies to achieve such high energy storage density is by creating a secondary phase nanostructure around the morphotropic phase boundary.

Here we show detailed characterisation of (Bi, Na)TiO3 (BNBT) thin films grown on SrTiO3 single crystal substrate. The nanostructures in the thin films as well as their electronic structures and composition variations were characterised using 4D STEM. Due to the presence of Na, it has been found that the BNBT structure can change under the electron beam, causing by the migration of Na. The ultra-fast ARINA detector allows acquisition of 4D STEM data with only few pA current, and short dwell time to avoid the electron beam damage to the BNBT film. Moreover, electron energy loss spectroscopy (EELS) was acquired using the K3 detector, to probe the composition and the electronic structure across the nanostructured domains.

We found that there is Bi segregation forming strips of half unit cell wide Bi2O3 layers. Such composition is confirmed by the atomic resolution EELS maps, showing no presence of Ti within the Bi2O3 bright strips. The effect of the Bi2O3 layers is to laterally displace the lattice above the defect by half a unit cell, resulting a morphotropic anti-phase domain boundary. In addition, the Bi2O3 segregation layer gives about 22% longer c-axis for the 3 unit cell around the defect. Such large expansion of c-axis is attributed to the presence of the super-tetragonal phase (super-T) [2-3]. The electronic structures of Ti and O sites adjacent to the Bi2O3, measured using EELS Ti L edge and O K edge, is consistent with the lattice distortions. Density functional theory calculations were carried out to probe that the origin of the super-T structure is likely due to the oxygen vacancies around the Bi2O3 strips.

Such complex nanostructures with multiple structure phases con-existent forming nanodomains is found to be the key for the ultra-high performance of such ferroelectric thin films.

Keywords:

4D-STEM, EELS, ferroelectric

Reference:

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Nanotubes of (SmxY1-x)S-TaS2 based on Quaternary Misfit Layered Compounds (MLCs)

Mr Mohammad Furqan[1,2], Dr Simon Hettler[1,2], Dr MB Sreedhara[3], Dr Reshef Tenne[4], Dr Raul Arenal[1,2,5]
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Poster Group 2

Background including aims.
Misfit layered compounds (MLCs) have garnered considerable attention due to their fascinating chemistry and properties [1]. MLCs consist of two different layered oxides or chalcogenides that are stacked alternately along their c direction. The MLC stack is composed of metal chalcogenide (MX) which possesses a distorted rock salt structure and a transition metal dichalcogenide (TX2) which crystallizes in a hexagonal structure [1,2]. The properties of MLCs are determined by the chemical and structural interplay between MX and TX2. MLC-nanotubes (NTs) synthesized via the chemical vapor technique (CVT) offer potential applications in thermoelectrics due to the complementary properties of the two layered compounds [1]. Recently, a modified synthesis method of MLC-NTs has permitted the introduction of additional elements to form a quaternary compound starting from LaS-TaS2 [3,4]. Here, we present an in-depth electron microscopy analysis of the novel family of (SmxY1-x)S-TaS2 nanostructures [5]. In this novel family, the partial exchange of Sm(S) by Y(S) provides a pathway for the fine control of the MLC structure and its properties.

Methods
MLC-NTs made of (SmxY1-x)S-TaS2 were synthesized via the CVT technique [1-3] by varying the precursor proportions of Sm vs Y between x=0 to x=1. The samples will be designated by the Sm percentage, Sm20 corresponds to (Sm0.2Y0.8)S-TaS2 and similarly. To analyze these NTs, different TEM techniques (high-resolution (scanning)TEM (HR(S)TEM) imaging, selected area electron diffraction (SAED), electron energy loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDS)) were performed. These TEM studies were developed using two aberration corrected Thermo Fisher Scientific Titan microscopes. Raman spectroscopy has also been employed to study these NTs.

Results
The detailed analysis of the NTs by electron microscopy and different spectroscopies verifies the partial substitution of Sm by Y in the (Sm,Y)S subsystem and also reveals the structural changes when compared to the pure SmS- or YS-TaS2 MLC-NTs. These structural changes can be linked to the slight difference in the lattice parameters of SmS and YS. Figure (a) corresponds to the SAED pattern of Sm40 (Sm0.4Y0.6)S-TaS2 sample where the reflections marked by the green dotted circles represent the reflections from (Sm,Y)S and reflections marked by red dotted circles represent the TaS2 subsystems, respectively. The c-axis periodicity (1.13 nm) is indicated by yellow arrows. The c-axis is perpendicular to the tube axis, which is given by the purple double arrow. A high-angle annular dark-field STEM image of a Sm80 NT is shown in Figure (b) and illustrates the repetitive layer stacking of (Sm,Y)S and TaS2. The inset of (b) shows a low-magnification TEM image of the Sm80 NT. Figure (c)

depicts a Raman spectrum acquired from an individual Sm80 NT, which was fitted using a set of Lorentz functions. In a vibrational spectroscopy such as Raman, a Lorentz line shape is used to model pure vibrational modes, which only undergo homogeneous line broadening. Raman spectroscopy measurements of the whole set of samples reveal the tunability of the vibrational properties of these NTs. The study of the elemental composition of these NTs by EDS is shown exemplary for a NT of the Sm80 sample in Fig 1(d), which reveals an average atomic weight percentage of around 17 at. % Ta, 24 at. % Sm+Y, and 59 at. % S. The EDS results obtained from all the samples show that the substitution of Sm by Y is homogeneous and in-phase with structural changes in the lattice parameter. Further low-loss EELS studies and electric properties of these samples are in progress. These investigations will also provide a more complete understanding of these systems including their electronic/optoelectronic properties.

Conclusion

In summary, through comprehensive electron microscopy and spectroscopy analysis, we have shown the successful synthesis of an unexplored group of quaternary MLC-nanotubes. The MLC structure is observed in all samples and the compositional analysis shows a homogeneous substitution of Sm by Y.

Keywords:

Inorganic-nanotubes, misfit-layered compounds-(MLC), Structural Analysis

Reference:

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In situ TEM holder for liquid cell research with combined electrochemical and thermal stimuli control

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Poster Group 1

In situ (scanning) transmission electron microscopy ((S)TEM) experiments in liquid phase gain momentum due to the development of sandwiched MEMS-based samples carriers (e.g. Nano-Chips) – liquid Nano-Cells. Various stimuli like heating or biasing in liquid environments have been employed to study materials nucleation and growth [1], ion batteries [2], electrocatalysts [3] and more. The majority of the commercial in situ (S)TEM sample holders utilize only four electrical contacts that limit the combination of stimuli that the user can simultaneously apply. However, to mimic the real-world operation conditions in applications like proton exchange membranes, Zn-ion batteries, etc require combined application of thermal and electrical stimuli and thus, higher number of electrical signals.

In this work we present our new platform for in situ and operando environmental (S)TEM experiments. The heart of this platform is a newly designed holder with eight electrical contacts and multiple liquid inlets that allows the application of thermal and electrical stimuli next to liquid flow and mixing capability. The specially designed liquid Nano-Cells with eight electrical contacts contain a heater and four biasing (Working, Reference, Counter + 1) electrodes for combined electrochemical and thermal experiments. The holder has a removable tip that has a generic design and is compatible with different TEM vendors. Additionally, the design of the holder allows to rotate the tip making it suitable for both TEM and STEM operation.

We will demonstrate the capability of the holder in terms of TEM and STEM imaging, elemental analysis using EDS and 4DSTEM. We will show a few experimental examples of the new environmental operando system, including electroplating and stripping of metals as a function of temperature and the flow.

Keywords:

In situ TEM, liquid phase

Reference:

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Self-supervised deep learning method for in-cell cryo-electron tomography

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IM-10 (2), Lecture Theater 3, august 29, 2024, 14:00 - 16:00

Cryo-electron tomography (cryo-ET) is a powerful technique for visualizing and analyzing macromolecular complexes within their native cellular context. However, cryo-ET analysis is hindered by the low signal-to-noise ratio (SNR) inherent to cryo-ET data and the lack of ground truth data, which pose significant challenges for supervised automated mining of molecular patterns within the cellular environment. As a result, the accurate localization and identification of macromolecular structures of interest, particularly those that are small and less abundant, continue to be a major challenge in the analysis of cryo-ET data.
To address these challenges, we developed a new self-supervised deep learning approach tailored for the dense (voxel-wise) representation of in-cell cryo-ET data. This method generates high-resolution representations of cellular information at the voxel level, facilitating precise segmentation of structural details within tomograms, including particles (globular macromolecular complexes) and filaments (for example, DNA). To evaluate the performance of the model, we created an extensive simulated dataset (Purnell, 2023) that closely mimics a crowded cellular environment, featuring membranes, actin and microtubule filaments, and over 100 PDB protein structure entries of varying sizes. Additionally, we enhanced the dataset by simulating the presence of DNA structures.
Experimental results from the simulated data from the 2021 SHREC competition (Gubins, 2020) and our new simulated crowded dataset demonstrate the efficiency of our method in extracting detailed information about membranes, actin, microtubules, particles, and filaments on a voxel level. To achieve further separation of different types of particles, we conducted an experiment in which we extracted subtomograms for each detected particle and generated embedding representations for every subtomogram. This approach resulted in a new representation space, where distinct clusters were formed using unsupervised clustering, effectively separating different types of particles. The ability to distinguish and separate different types of structural information highlights the potential of our method for advancing the analysis of complex cellular structures in cryo-ET data.
Furthermore, we applied our method to tomograms of Mycoplasma pneumoniae (O'Reilly, 2020), which capture the entire cell in a single tomogram. We successfully extracted structural information of the membrane, particles, and putative DNA filaments, creating a comprehensive 3D structural cell model.
In conclusion, our novel self-supervised deep learning approach demonstrates significant potential in overcoming challenges associated with ground truth generation and accelerating biological discoveries from cryo-ET data. By enabling accurate segmentation and extraction of macromolecular and filament information, even in cases with limited or missing annotations, our method advances the analysis of cryo-ET data. Furthermore, the proposed approach can be utilized to construct a comprehensive 3D Mycoplasma pneumoniae cell model from in situ tomograms, showcasing its potential for diverse applications in the field.

Keywords:

self-supervised deep learning, cryo-ET, segmentation

Reference:

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Deep image prior for limited-angle electron tomography

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Poster Group 1

Background incl. aims
Electron Tomography (ET) is a widely used technique for the 3D characterization of nanomaterials. To obtain faithful reconstructions, high-quality projection data must be acquired over the full 180° tilt range and at tilt increments of 1-2°. However, for slab-like sample geometries, the tilt range is often limited due to geometric restrictions within the TEM, and a large tilt increment is used to minimize the total electron dose. These restrictions lead to blurring and distortions in reconstructions obtained with conventional algorithms such as simultaneous iterative reconstruction technique (SIRT). Compressed sensing (CS) approaches have been introduced to improve the quality of reconstructions. Although CS techniques outperform conventional methods in sparse-view acquisition scenarios, they fail to correct artifacts due to the limited angular range. Data-driven deep learning (DL) approaches have been proposed for denoising and distortion correction of X-ray tomography and ET reconstructions. Although promising, these approaches are highly dependent on the availability of high-quality data sets for neural network training. Such data sets are often not available in practice, making it difficult to apply DL methods to a wide range of samples. Recently, Deep Image Prior (DIP) has been proposed for several image restoration problems, and adapted to limited-angle X-ray tomography. The method uses a neural network as a prior for the reconstruction, but has the advantage of not requiring any training data set, making it suitable for ET applications.
Methods
In this work, we apply DIP with total variation regularization (DIP-TV) to a sample consisting of carbon-supported platinum nanoparticles (NPs) dispersed on a carbon grid. ADF-STEM and HAADF-STEM tilt series were acquired from -60° to +60° with an increment of 2°, using an FEI Titan Themis operating at 200kV. In HAADF-STEM projections, only Pt NPs were visible, while in ADF-STEM mode, both Pt NPs and C support were visible but the images were affected by diffraction contrast. DIP-TV was applied for 3D reconstruction in both imaging modes, using increments of 2°, 10° and 20°. Results were compared with those obtained with SIRT and CS with TV regularization (CS-TV).
Results
Figure 1 shows the reconstruction of a slice from ADF-STEM and HAADF-STEM tilt series, obtained with SIRT, CS-TV and DIP-TV. The tilt range is [-60°:+60°] for all reconstructions, with the missing wedge in the horizontal direction, while the tilt increment is set to 2°, 10° and 20°. In HAADF-STEM reconstructions (left), only Pt NPs are visible. SIRT suffers from elongation artifacts and ‘star artifacts’ as the tilt increment increases. CS-TV slightly improves the quality of the reconstructions but suffers from oil painting effects. DIP-TV reconstructions appear consistent under all acquisition conditions, with near-spherical, well-contrasted particles. The ADF projections are more complex, as the carbon support is visible and superposed on the Pt NPs. SIRT reconstructions suffer from significant artifacts in the support, which increase as the number of projections is reduced. CS-TV still manages to reconstruct the particles, but the carbon is not well retrieved. In particular, for both SIRT and CS-TV reconstructions, the carbon appears heterogeneous and porous below and above each NP. This is probably due to the non-linear intensity in ADF images (diffraction contrast). In contrast, DIP-TV retrieves near-spherical NPs and a homogeneous carbon support, under all acquisition conditions.

Using 20° increment (7 projections in total), the shape of the carbon is slightly distorted on the right-hand side, but the technique largely outperfoms SIRT and CS-TV.

Conclusion

This work shows the robustness of DIP-TV for ET using a limited number of projections and a restricted tilt range. The method benefits from the power of neural network architecture, but requires no training data set, making it suitable for ET and extremely versatile since it can be used under different acquisition conditions. Moreover, the method could be applied to different modalities of the same material and potentially directly for 3D reconstruction.

Keywords:

Electron Tomography, Reconstruction, Deep Learning

Reference:

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Characterisation of red fluorescent protein FLIM properties and comparison with novel StayGold live cell imaging

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LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

Red fluorescent proteins (RFPs) have become indispensable tools in molecular and cell biology, facilitating a wide range of applications including live cell imaging, protein tracking, and gene expression analysis. However, the increasing availability of RFP variants poses a challenge in selecting the most suitable probe for specific experimental requirements. In this study, we conducted a comprehensive comparative analysis of eight commonly used RFPs: mCherry, mCherry2, mCarmine, mKate2, TagRFP, mScarlet, mScarlet-I, and mScarlet-H, with a focus on their fluorescence lifetime properties.
We employed time-correlated single photon counting (TCSPC) and fluorescence lifetime imaging microscopy (FLIM) techniques to characterize the fluorescence lifetimes of these RFPs. Our results revealed significant variations in fluorescence lifetimes among the tested RFPs. mCherry exhibited the shortest fluorescence lifetime, followed by mCherry2 and mCarmine. In contrast, mKate2, TagRFP, mScarlet, mScarlet-I, and mScarlet-H displayed longer fluorescence lifetimes, with mScarlet-H demonstrating the longest lifetime among the tested variants.
Furthermore, we investigated the potential of fluorescence lifetime imaging (FLIM) as a tool to distinguish between these RFPs in live cell imaging experiments. By analyzing the fluorescence decay curves and calculating the average fluorescence lifetimes for each RFP, we observed distinct lifetime signatures that enable discrimination between the RFP variants. Additionally, we evaluated the photostability and brightness of these RFPs under similar experimental conditions, providing comprehensive insights into their performance characteristics.
Our findings highlight the importance of considering fluorescence lifetime properties when selecting RFPs for specific imaging applications. The ability to distinguish between RFP variants based on their fluorescence lifetimes expands the toolkit for multicolor imaging and facilitates the development of advanced imaging techniques such as fluorescence lifetime imaging microscopy (FLIM) and Förster resonance energy transfer (FRET) assays. Moreover, the availability of RFPs with diverse fluorescence lifetime characteristics enhances the versatility and applicability of fluorescence-based imaging approaches in biological research.
In conclusion, this comparative analysis provides valuable insights into the fluorescence lifetime properties of commonly used RFPs, paving the way for informed selection of RFP probes tailored to the requirements of specific imaging experiments.
Subsequently, we extended our investigation to compare the performance of tagRFP against a novel StayGold fluorescent protein (FP), despite its established suitability for super resolution spinning disc laser scanning microscopy. Our comparative analysis revealed the superiority of StayGold over tagRFP in several key aspects.
Firstly, StayGold exhibited enhanced photostability compared to tagRFP, enabling prolonged imaging sessions with minimal photobleaching effects. This attribute is particularly advantageous for long-term live cell imaging studies where maintaining fluorescence signal intensity over extended periods is critical.

Secondly, StayGold demonstrated superior brightness and signal-to-noise ratio (SNR) when visualized under super resolution spinning disc laser scanning microscopy. The improved brightness of StayGold allowed for enhanced image clarity and resolution, enabling the detection of finer cellular structures and dynamics with greater precision.
Furthermore, StayGold exhibited favorable fluorescence lifetime properties, with a distinct fluorescence decay profile that distinguishes it from tagRFP. This unique characteristic not only facilitates multiplexing experiments but also provides additional flexibility in fluorescence imaging applications.
Moreover, we explored the functional capabilities of tagRFP and StayGold by tagging them to different actin markers. TagRFP was attached to Lifeact as an actin marker, while StayGold was either tagged to F-tractin or utrophin as an actin skeleton marker. Our results demonstrated the suitability of StayGold for visualizing actin structures with high specificity and resolution, surpassing the performance of tagRFP in this context.
In conclusion, while red fluorescent proteins (RFPs) are traditionally favored for imaging deeper tissue layers due to their longer wavelength, our study suggests that StayGold may emerge as the superior alternative, particularly for long-term live cell or organoid imaging using super resolution spinning disc laser scanning microscopy (LSM).
Despite the conventional advantage of RFPs in imaging deeper tissues, StayGold offers distinct advantages that make it a compelling choice for specific applications. Its enhanced photostability, superior brightness, and favorable fluorescence lifetime properties, as demonstrated in our comparative analysis, position StayGold as an ideal candidate for long-term imaging studies where sustained signal intensity and minimal photobleaching are critical factors.
Moreover, StayGold's ability to achieve high-resolution imaging of cellular structures, such as the actin cytoskeleton, coupled with its suitability for super resolution spinning disc LSM, further enhances its utility in dynamic imaging scenarios. The precise and specific labeling provided by StayGold, particularly when tagged to actin markers like F-tractin or utrophin, underscores its potential for visualizing intricate cellular processes with exceptional clarity and resolution.
While RFPs remain valuable tools for certain imaging applications, our findings suggest that StayGold may offer unique advantages in the context of long-term live cell or organoid imaging, especially when coupled with advanced microscopy techniques such as super resolution spinning disc LSM. Continued exploration and optimization of StayGold FP hold promise for further advancements in fluorescence microscopy and biomedical imaging, providing researchers with enhanced capabilities for studying complex biological systems with unprecedented detail and precision.

Keywords:

SR-SpinningDisc-LSM
FLIM
Red-and-Green-FPs

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Preparation of biological samples for cryo-electron microscopy using the HPF "Waffle" method

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Poster Group 1

Background incl. aims
Cryo-electron microscopy (cryo-EM) has emerged as a pivotal technique in structural biology, offering unparalleled insights into the architecture of macromolecules at near-atomic resolution.
A crucial requirement for acquisition and collection of high quality data is properly vitrified and highly concentrated specimen. However, sample preparation still presents challenges in thicker specimens as bigger cells or cellular clusters, those are frozen by conventional plunge freezing method and may suffer with improper vitrification. Another problems could be low concentration or inadequate distribution of sample on electron microscopy grid or preferred orientation of the specific sample [1]. Here, we focus on the recently introduced "Waffle" method [1] and show it potential for preparation of various types of sample used in cryo-EM.
Methods
The waffle method is based on sample vitrification within the thickness of the TEM grid bars and it combines plunge freezing on the electron microscopy grid with a technique of high pressure freezing, that provides an advantage of proper vitrification of specimens thicker than 15um.
Thus, a 20-30um thick layer is prepared which needs to be further processed by cryo-focused ion beam micromaching (cryo-FIBM) to final thickness ~200nm before cryo-EM imaging.
Results and conclusions
We show benefits and limitations of the waffle method for vitrification of purified proteins, protein crystals, bacterial cell suspensions and eukaryotic cells.

Keywords:

waffle, vitrification, electron microscopy

Reference:

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Elemental segregation at substrate/metal interface to manipulate heterogeneous nucleation

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background incl. aims
Heterogeneous nucleation on a substrate in a metallic melt is a fundamental step in tailoring the microstructure of engineering materials with desired properties, and therefore it is a research topic of critical scientific and technological importance to meet the goals of the 'circular economy' [1]. The structural and chemical compatibility at a substrate/metal-liquid interface dictates the heterogeneous nucleation process. This can be altered by elemental interfacial segregation, affecting the nucleation behaviour accordingly [2]. Atomic-scale interfacial segregation has been linked to the successful application of TiB2-based grain refiners for aluminium casting, also explaining some of their limitations depending on the nature of segregation structure [2,3]. Interfacial segregation has also been applied as a strategy to modify native oxides in aluminium and magnesium alloys, whereby naturally occurring oxides can then be harnessed for grain refinement [2,4]. Studies of the microstructure and composition across the substrate/metal interface, down to the atomic scale, are thus essential in determining nucleation potency.
Advances in aberration-corrected scanning transmission electron microscopy (STEM) and electron energy-loss spectroscopy (EELS) now routinely allow for both atomic-resolution imaging and compositional analysis of materials. In this work, we use advanced STEM-EELS to investigate different substrate/metal interfaces including TiB2/Al, MgO/Mg, and γ-Al2O3/Al in the corresponding casting alloy ingots of different grain refinement performances, aiming to provide an overview of our atomic-level understanding of the behaviour of interfacial segregation and its resultant effect on heterogeneous nucleation and grain refinement.
Methods
Casting experiments were used to evaluate the grain refinement. Pressurized melt filtration was applied to different alloy melts to collect the inoculant particles, by which the possibility of substrate/metal interface appearing in a TEM foil specimen is greatly increased for characterization. STEM imaging and EELS acquisition were performed on a Nion UltraSTEM100 scanning transmission electron microscope, equipped with a Gatan Enfina EELS spectrometer retrofitted with a MerlinEELS direct electron detector. The microscope was operated at an accelerating voltage of either 100 or 60kV, depending on the beam sensitivity of the observed structures, with the probe-forming optics configured for a 31mrad convergence semi-angle and a probe size of 1Å or smaller.
Results
High-precision high-angle annular dark field (HAADF) STEM imaging and EELS mapping offer an atomic-level view of how interfacial segregation affects the grain refining performance in model alloy systems, with an example of the TiB2/Al system shown in Figure 1. Rigid- or non-rigid registration were applied to series of (spectrum) images, taken with short dwell times and along different zone axes, to reveal the precise structure and chemistry of an atomically thin, so-called Al3Ti 2-dimensional compound (2DC) segregation layer at the interface between inoculant TiB2 particles and Al matrix: figure 1d. This structure was shown to account for the grain refinement achieved in an Al-

0.2Al5Ti1B alloy. Similarly, a Zr-segregation-induced Ti2Zr 2DC (figure 1e) is atomically resolved at the interface, which poisons the grain-refining effect of TiB2: a coarse and columnar grain structure forms in Al-0.1Zr-0.2Al5Ti1B. Interestingly, the grain refinement behaviour is rejuvenated after the addition of Mg (Al-0.1Zr-0.2Al5Ti1B-1Mg), which results in the dissolution of the poisoning Ti2Zr 2DC and the formation of hitherto never-observed Mg-rich layers adopting a local structure similar to bulk Al, and thus termed Al-like layers (figure 1f-h). Analysis of the electron energy loss near-edge fine structure (ELNES) provides additional information regarding the valence state and/or electronic structure of these interfacial structures, for instance confirming the nature of the 'Y-O' bonding within a Y-rich segregation layer at the MgO/Mg interface [4].

Conclusion

Atomic-resolution STEM-EELS has provided conclusive evidence to clarify the role of interfacial 2DCs in determining the nucleation potency of TiB2 and thus the grain refining performance. With the high spatial resolution and single-electron-sensitive EELS detector, advanced STEM-EELS is expected to be a powerful tool in understanding heterogeneous nucleation, designing grain refiners, and controlling solidification structures and mechanical properties across a wide range of metallic materials.

Keywords:

Interfacial segregation, STEM/EELS, Solidification

Reference:

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Consistency and reliability of ptychographic deconvolution approaches

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

Background incl. aims
Control over and knowledge of the electron probe is important in all scanning transmission electron microscopy (STEM) techniques. This is emphasized even more in ptychography where the exact electron probe is required to deconvolve its effects from that of the specimen. Here the introduction of probe retrieval into iterative schemes has greatly increased their capability [1] and today iterative optimization of the electron probe is standard in most ptychographic schemes such as the extended ptychographic iterative engine (ePIE) [2]. However, in most cases the electron probe is reconstructed on a pixelated grid and thereby not limited to solutions physically realizable by the optical system. Here we present a method to characterize reconstructed probes by conventional lens aberrations. The fitted aberrations are then used to investigate the quality of the retrieved probes and their consistency is examined in a systematic study using 4D-STEM focal series recorded for a thin SnS2 2D-flake. Additionally, the influence of partial coherence and limited electron dose on the retrieved probes is analysed and the usefulness of the retrieved probes for different ptychographic methods, such as single sideband ptychography (SSB) and gradient descent-based schemes, is elucidated.

Methods
As a basis for the study 4D-STEM focal series were chosen as these datasets should only differ in defocus with the specimen and other aberrations being constant. 4D-STEM focal series were recorded of a thin SnS2 2D-flake using a Medipix3 detector and a probe corrected FEI Titan Themis STEM. ePIE reconstructions were performed using a GPU accelerated implementation in the pytorch framework. A probe fitting procedure was developed to fit axial lens aberrations up to third order by minimising the mean square error between the fitted probe and the ptychographic probe.

Results
The reconstructed phase gratings and the obtained probes are shown in Fig. 1. All phase gratings show the hexagonal atomic structure of the 2D-material with the heavy tin and lighter sulphur columns. Direct comparison is however hindered by specimen drift. The probes all show a strong contribution of coma. The excellent agreement between fitted and ptychographic probe is visible in both the real space amplitude and the reciprocal space phase plate (Fig. 1, bottom left). Here fitting against the complex wave function proved to be more successful than fitting the unwrapped reciprocal space phase plate. While fitting against only the real space intensity also worked it can result in the complex conjugate wave function i.e. a probe with opposite defocus.
Plotting the fitted defocus versus the defocus applied at the microscope shows the expected linear relationship (Fig. 1, middle). The other aberration coefficients are in very good agreement with each other (Fig. 1, right). The standard deviations for the mean aberration coefficients throughout the series are comparable to the ones obtained using a conventional Zemlin-tableau.

The obtained aberration coefficients are then applied to other ptychographic methods e.g. single-sideband ptychography, where only the correction of higher order aberration allowed the imaging of the atomic structure, or as a start for gradient-based maximum likelihood methods where multiple scattering and partial coherence are considered (Fig. 2).
Despite the good match between fitted and ptychographic probe some deviations appear, in comparison with the fitted probe the fine oscillations of the ptychographic probe vanish earlier into a noise background. Additionally, the reciprocal space amplitude deviates from theory, it is not flat inside and contains amplitude outside the apparent aperture. Simulations show that in our experiment these deviations are mainly produced by the limited electron dose while the effect of partial coherence is less important (Fig. 3). Experiments using CsPbBr3 nanocubes and gold nanoparticles additionally showed that the fitting procedure also performs well for thicker, strongly scattering specimen.

Conclusion
The successful fitting and good agreement of the aberration coefficients with each other indicate that the ptychographic deconvolution was effective and that the retrieved probes are physically meaningful. The good agreement of the aberration coefficients throughout the focal series suggests the use of the fitted aberrations to correct the alignment of the microscope during a session without requiring a dedicated alignment grid.

K.M.-C. and T.L. acknowledge funding from the DFG, contract EXC 2089/1-390776260 (Excellence Cluster e-conversion).

Keywords:

Ptychography, 4D-STEM, Aberrations

Reference:

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Atomic-resolution mapping of phonon modes across Magnéli structures in thermoelectric (Al,Nb)-doped TiO2

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Phonons play a critical role in many physical properties of a materials including their thermal and electrical conductivities. Changes in normal phonon mode frequencies occur in the presence of defects. In thermoelectric materials (TE), such defect-induced localised modification of the vibrational response is widely used to tailor the thermal conductivity [1]. In previous work [2,3] it was shown that atomic-level defect engineering resulted in the enhancement of the TE performance of (Al,Nb)-doped TiO2. The introduction of crystallographic shear (CS) structures leads to the reduction of lattice thermal conductivity, which is believed to occur through enhanced phonon scattering. Therefore, it becomes important to measure the spatial distribution and dispersion of the localized vibrational response across the CS structure, in order to gain insight into the heat-conduction process. Recent advances in scanning transmission electron microscopy (STEM) and electron energy-loss spectroscopy (EELS) have provided powerful and flexible tools to study phonons at high spatial resolution, down to single atom sensitivity [4]. In this work, we use STEM-EELS to probe the localised phonon response in CS structures at atomic resolution in the polar (Al,Nb)-doped TiO2.

Methods
EELS measurements were performed on a Nion UltraSTEM100MC 'Hermes' scanning transmission electron microscope, equipped with a Nion IRIS high energy resolution EELS spectrometer with a Dectris ELA direct electron detector. The acceleration voltage was 60 kV and the probe convergence semi angle was 31.5 mrad, resulting in a 1 Å probe size. The experimental optical geometry follows the conditions in Ref [4], in which the off-axis , or dark-field EELS (DF-EELS) geometry significantly reduces the contribution to the EELS signal of electrons having undergone delocalized dipole scattering, while promoting that of localized impact phonon scattering. This approach enables in principle single-atom sensitivity of phonon scattering.

Results
Fig. 1(a-b) shows a HAADF STEM image of the {120}-type CS structure in (Al,Nb)-doped TiO2 acquired along the [001] (rutile-equivalent) zone axis. The CS structure, which results in complex atomic lattice re-arrangements, is accompanied by the segregation of Al along the shear planes, with Al-rich columns preferentially occupying the Ti sites indicated by white arrows on Fig. 1b. Atomically resolved VEELS measurements performed across (Fig. 1(c-d)) reveal atomic scale variations localised at the CS structure boundaries. Changes in both the intensity and the frequency of the main modes

are observed at the boundaries, with localised effects on the Al-rich columns. Results are discussed in context of the local structure and chemistry, determined at the same position through careful chemical mapping, while phonon map simulations using the frequency-resolved frozen phonon multislice method [5] are used to rationalize the experimental findings.

Keywords:

vibrational EELS, phonon, thermoelectric materials

Reference:

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Innovative Designs For Enhancing the Functionality of MEMS-Based Phase Plates through Numerical Simulation and Optimisation

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Poster Group 2

Background incl. aims:
MEMS-based phase plates play an important role in shaping electron beams within transmission electron microscopes, opening avenues for diverse applications such as generating vortex beams [1], orbital angular momentum (OAM) sorter [2], aberration corrector phase plates, and more [3]. Despite their significance, designing and modeling phase plates pose challenges due to the difficulties encountered in obtaining analytical solutions. In the design process of phase plates, simulation using numerical methods such as Finite-Difference Time-Domain (FDTD) or Finite Element Method (FEM) stands as a crucial step. This capability allows us to compare numerical results with the expected phase shift produced by a phase plate. While simulation proves fundamental in designing phase plates, finding an optimal topology and parameters can often be challenging due to the complexity of the design space. Consequently, a significant portion of the potential of a MEMS phase plate remains unexplored. In recent years, the emergence of Machine Learning and computational tools has revolutionized design optimization processes. In this study, we leveraged the potential of utilizing numerical simulation alongside optimization tools to enhance the functionality of different types of phase plates. A crucial aspect of our investigation involved simulating, analyzing, and enhancing the proposed MEMS-based phase plate devices, scrutinizing the interaction of the generated field by the phase plate with the electron beam prior to fabrication and experimental testing.
Methods :
In our study, we utilized COMSOL Multiphysics software, a powerful FEM tool renowned for its versatile capabilities in simulating MEMS devices, complemented by other numerical methods such as Deep Neural Networks (DNN). COMSOL provides various Finite Element simulation techniques alongside optimization approaches such as Parameters, Topology, and Shape optimization. Moreover, we investigated alternative methods, including Inverse design using DNN, to explore new avenues for advancing phase plate design. In certain studies, to navigate the complexity barrier and enhance the design process, we employed a hybrid optimization method that combines inverse design of phase plates with additional forward optimization. In these specific instances, we utilized a Hybrid Machine Learning (HML) approach, leveraging the TensorFlow library in Python, coupled with forward optimization tools available in COMSOL.
Results :
In this paper, we present an analysis of three distinct phase plates, detailing their simulation and optimization methodologies.
In the first design, we investigated the performance of an Electrostatic Spiral Phase Plate. The primary objective of this phase plate is to generate a linear distribution of phase to produce a vortex beam. Upon optimizing the parameters, it was observed that the applied potential should take on the form of a rounded curve, with minimal bias variation in the direction facing the 'chopstick electrodes' and a steep gradient in their proximity. Forward optimization of parameters was employed, utilizing the absolute difference between simulation results and the aimed phase shift as the objective function (Fig a).

In the second example, we investigated the electrical and thermal properties of OAM sorter phase plates. Unlike the Spiral PP, which requires a linear phase distribution on the boundary, the boundary conditions in this case are more complex. We initialized our optimization study with a circular symmetric design involving a needle and 42 boundary electrodes. However, due to the limitation of the number of bias connections in a real TEM holder, having 42 biases would not be feasible. Nevertheless, this optimization gives us a broad understanding of the possible design and can later serve as an initial guess for Topology Optimization. To reduce the number of electrodes, the design underwent topology optimization, which suggested a change in the geometry to a non-symmetric design. Additionally, we simulated the Joule Heating effect of the OAM sorter phase plate, as heating effect can help avoid contamination. Figure b illustrates the simulation results of the electrical and thermal response of the OAM sorter phase plate, along with the optimized boundary potential. Figure c showcases the simulation and optimization of an aberration corrector phase plate. This thin-film phase plate aimed to compensate for the aberration of the electron beam by imposing a continuous quartic function as a phase shift. In addition to precise simulation, we enhanced the optimization process with DNN machine learning. A HML approach using the TensorFlow library in Python was employed to determine the optimal set of electrode potentials that result in the desired quartic phase shift. A Convolutional Neural Network (CNN) was trained using over 100000 2D simulations with random bias voltages applied to six electrodes as inputs, and the resulting total phase shift as the output. The output of the HML model was subsequently used in a parameter optimization process within COMSOL Multiphysics® to address any inaccuracies in the machine's predictions.

Conclusion:

In this study, we demonstrated how numerical and computational methods can enhance the functionality of MEMS phase plates. Utilizing powerful simulation tools allowed us to predict results before fabrication and hands-on testing. By leveraging optimization tools, we were able to explore new design possibilities and optimize phase plate geometries to achieve enhanced performance and functionality. Our findings underscore the importance of employing advanced computational techniques in MEMS design and pave the way for further research in this field.

Figures Caption :

a) Model for Optimization of boundary conditions electrodes for spiral phase plate with a large number of adjustable electrodes. Simulation results show phase shift (left). Projected potential before and after optimization (right).

b) Optimized potential of boundary electrodes for the OAM sorter (left). Thermoelectric Simulation (right).

c) Bias potential of the phase plate and a 3D surface representing the corresponding phase shift (left). The outcome details the total phase shift (right).

Keywords:

Electron_Microscopy Electron_beam_shaping Phase_plate Simulation Optimization

Reference:

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Enhanced nanoscale phase characterisation in modern steels using precession electron diffraction and energy filtering

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background incl. aims
The development of lightweight and ductile Advanced High-Strength Steel hinges upon a comprehensive understanding of phase change kinetics during production. In addition to various techniques for chemical characterisation, transmission electron microscopy (TEM) is an essential tool for the structural analysis of examined phases. In this work, we showcase a significant enhancement in the acquisition of diffraction patterns (DPs) for nanostructured phases in modern steel systems, such as quenched and partitioned (Q&P) steels. We combined zero-loss energy filtering (EF) with precession electron diffraction (PED) to access crystallographic information of nano-inclusions inaccessible by standard diffraction techniques.

Methods
Selected area electron diffraction (SAED) is usually not suitable for the challenging task of nano-inclusions crystallography, which requires information about different zone axes for unambiguous phase identification. Due to the large aperture size, dynamic diffraction effects and, in particular, the intricate steel matrix containing arbitrarily oriented, strained and ferromagnetic grains, an in-depth analysis of SAED patterns is limited. To address these limitations and achieve a high lateral resolution, we employed site-specific nanobeam diffraction in PED mode with a beam diameter of approximately 1 nm and a precession angle of 1 - 3° around the central axis. Unlike standard DPs, where dynamical effects are unavoidable, PED provides quasi-kinematical results and a larger number of reflections through pattern integration, thus compensating for small variations in sample thickness or misorientation. The influence of inelastic and multiple scattering was mitigated through additional zero-loss filtering, noticeably enhancing the signal-to-noise ratio of our PED patterns. The experiments were performed with a JEOL JEM-2200FS equipped with an in-column Ω-filter and a TVIPS universal scan generator using a TemCam-XF416 CMOS camera.

Results
One application example is the investigation of iron carbides embedded in Q&P steels with varying Si or Al content, annealed at 320°C and 420°C. Our site-specific PED patterns provided enhanced clarity, revealing a multitude of carbide diffraction spots and enabling a more reliable analysis compared to results obtained via SAED. By conducting sample tilt series towards different crystallographic zone axes (Fig. 1), we achieved unambiguous identification of the carbide phase, its orientation relationship to the martensitic matrix, and the lattice parameters. The presence of orthorhombic θ-Fe_3C [2] was confirmed, in line with prior X-ray diffraction (XRD) measurements in the steel alloys annealed at 420°C. In alloys annealed at 320°C, where XRD results were inconclusive, the tilt series approach revealed the presence of transition carbides ε-Fe_3C (hexagonal) and η-Fe_2C (orthorhombic/quasi-hexagonal) [3][4]. Utilising the tilt series approach alongside the improved

accuracy of the PED patterns allowed for a definitive distinction between ε- and η-transition carbides, overcoming previous challenges [1]. Further examples of PED applications are shown.

Conclusions
Our findings underscore the benefits of employing PED together with EF for characterising the crystallography of nanostructured phases in modern steel systems. Through comparison of DP quality, we demonstrate the insights attainable for complex samples by PED. The rising availability of commercial PED solutions and the convenient applicability of the PED method enhanced through EF, demonstrated on our examples, may incentivize the broader scientific community to embrace this method for investigating metals and alloys.

Keywords:

PED, steel, nano-inclusions, phase characterisation

Reference:

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Accessible low-cost, open-source, single-shot phase imaging implemented on an openFrame-based microscope

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Poster Group 2

Background incl. aims
We have previously presented polarisation-resolved differential phase contrast microscopy (pDPC) - a robust, low-cost single-shot open-source technique utilising "PolCam" (a polarisation-sensitive camera with a Sony Polarsens™ sensor) to simultaneously acquire 4 images from which semi-quantitative phase images can be calculated [1]. While pDPC microscopy can be easily implemented on a commercial microscope, as we have demonstrated with an automated multiwell plate fluorescence microscope (based on an Olympus IX-83 frame), the implementation of pDPC on an openFrame-based microscope [2] controlled by µManager [3] greatly widens access to phase imaging – with facile integration with fluorescence imaging modalities – at a whole system component cost below €20,000 (<€3000 for only the pDPC components). openFrame describes our specific open-source modular hardware for light microscopy that enables users to assemble a wide range of instruments with no constraints on software or other hardware components that can be self-built or commercial products. Examples of openFrame-based instruments and other open-source instrumentation can be reached through www.openScopes.com.
Here we present an improved pDPC software plug-in for µManager that performs analysis of pDPC data and provides real-time phase imaging. We also report the extension to higher magnification in a new condenser-less geometry that we have applied to imaging biological samples including mycobacteria.
Methods
An openFrame microscope stand is configured for brightfield transillumination imaging including a Köhler transillumination arm utilising a low-cost white LED and a condenser lens that provides a maximum illumination numerical aperture (NA) of 0.55. pDPC is initially implemented by mounting a diffuser and quadrant polariser (QP) mask at the condenser back focal plane. This QP utilises low-cost polarising polymer film and is implemented in a custom 3D-printed mount. Because differential phase microscopy requires a higher illumination NA relative to the imaging NA [4], this condenser-based pDPC system is limited to a spatial resolution of ~λ/1.1. To overcome this limit, we have developed a condenser-less geometry using a simple extended LED array that provides approximately uniform illumination. This approach is simpler and at lower cost compared to approaches based on electronically switched microLED arrays, e.g. [4] and enables the use of higher NA objective lenses.
The pDPC image data processing is managed by a new µManager plug-in (MM2_pDPC) that reads the four polarization resolved images from the PolCam raw images and uses a pre-determined calibration matrix to correct unbalanced illumination and crosstalk between the different polarising quadrants. The phase component of the optical transmission function of the sample is calculated, and a preview of the resulting phase image can be displayed in real time within the µManager graphical user interface (GUI).

The single-shot capability of pDPC makes it suitable for dynamic subjects including timelapse imaging of microbiology and we have extended the capabilities of openFrame-based microscopes by developing a low-cost 3D-printed stage top incubator with temperature stabilization, which we have applied to support the long-term imaging of live mycobacteria.

Results

Our condenser-based pDPC implementation on openFrame provides robust and rapid (>60 frames/s) phase imaging up to ~0.5 NA. The condenser lens efficiently couples the LED radiation to the sample and the new pDPC µManager plug-in provides real-time phase images. We have applied this to extended time-lapse imaging of mycobacteria in a low-cost (<€500 components) stage-top incubator that provided stable operation at ~34°C over >4 hours with <3°C fluctuation and rapid (~30 minutes) warm-up time.

The condenser-less pDPC system can be realised at even lower cost – we initially used a domestic LED light panel (<€20) to provide 60x, 0.8 NA imaging of mammalian cells HEK-293 and (M. smegmatis) mycobacteria. The figure shows a phase image of a HEK-293 cell acquired with condenser-less pDPC at 60x magnification. However, the highly diffuse illumination of this set-up resulted in lower illumination intensity at the sample and therefore requires longer (~500 ms) image data acquisition times. We are currently upgrading this to a higher transillumination power and higher NA pDPC system that will be optimised for imaging mycobacteria and particularly for the study of M. tuberculosis.

Conclusion

We have demonstrated that research-grade semi-quantitative phase microscopy capabilities can be realised using open-source modular hardware and software to widen access to this important imaging modality. pDPC is particularly convenient to integrate with other modalities as it is wavelength agnostic (within the detection sensitivity range of the Polarsens™ camera). The modular openFrame microscope platform supports the straightforward integration of pDPC with other imaging modalities, including fluorescence microscopy and single molecule localisation microscopy (e.g., easySTORM [2]), which can also be open-source and cost-effective. The compact size, robust design and relatively low-cost of openFrame-based microscopes make them suitable for biosafety containment facilities, e.g., for the study of infectious diseases.

Keywords:

Polarisation differential phase contrast microscopy

Reference:

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Beyond Ribosomes: In Situ Structural Biology of Diverse Targets in C. reinhardtii

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

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Understanding high resolution protein structure in the context of the whole cell is the vision of visual proteomics. With the advent of high-throughput cryo-electron tomography and cryo-FIB milling, paired with cutting edge computational techniques, achieving such an ambitious goal is no longer a far-reaching dream. The new generation of cryo-FIB from Thermo Fisher Scientific uses plasma to generate focus ion beam, which reduces redeposition and ion beam damage, substantially improving throughput. In preparation for a large-scale effort towards visual proteomics of the model organism Chlamydomonas reinhardtii, we have prepared enough lamellae to allow for acquisition of more than 1800 tomograms of different cellular compartments (Fig 1).

Initial results indicate that the data is of sufficient quality to achieve sub-nanometer resolution for selected complexes using a fraction of the dataset. Six tomograms from the same lamella were used to determine the structure of 80S ribosome at 6Å using template matching1 and subtomogram averaging. Encouraged by this, a number of other targets were selected among the collaborators for further study such as ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), ATP synthase, nucleosomes, photosystem II, and microtubules.

While some proteins were in such high abundance that template matching alone was sufficient to obtain a sub-nanometer structure, as in the case of Rubisco where we were able to determine a 7Å structure from within the pyrenoid compartment, other targets proved more of a challenge and necessitated a combination of computational techniques to achieve results. The denoising neural network cryoCARE was implemented on all datasets to increase template matching accuracy. In the case of microtubules, which are rare in C. reinhardtii, filament tracing in Amira (Thermo Fisher Scientific) was necessary to determine initial centerlines for reconstruction along individual protofilaments.

Photosystem II (PSII), which is present in very high abundance in the chloroplast, proved to be a challenging target as it is a membrane embedded protein with only a small region protruding into the lumen of the thylakoid membrane. Multiple template matching methods failed to identify most of the visible particles and unfortunately noise2noise denoising tended to blur or erase the small densities along the membrane. We therefore employed a novel approach that paired two deep learning networks to generate PSII candidate coordinates which could be further refined for classification and subtomogram averaging. The first network is a regression UNet trained on purely synthetic data2 to denoise cryo-tomographic data. The denoised data was then used to train a 2.5D UNet to segment membrane, ribosomes, and all densities that protrude into the thylakoid lumen as PSII (Dragonfly 2022.2, Object Research Systems). Coordinates (without any angular information) were extracted from the PSII class and used as particle picks, which were then aligned using the surrounding membrane. Work is ongoing, but initial results from 24 datasets are very promising.

The scale of this dataset is exciting, but the huge number of molecular complexes within living cells makes it difficult to identify, confirm the identity of, and determine each structure by just one group. Achieving a full visual proteome of C. reinhardtii will necessitate a large collaborative effort. To that end, we would like to create an open access database for C. reinhardtii to accelerate annotation and curation, enable further cell biology research, and develop new computational tools for in situ cryo-ET. Along with sharing the raw data, reconstructed tomograms, denoised datasets, and structural determinations, we will provide high quality segmentations of selected datasets created using 2.5D (Dragonfly) and 3D (MemBrain Seg3) UNets. This project has the potential to provide invaluable insights into cellular processes and will hopefully lay the foundation for other large-scale studies of other species.

Keywords:

CryoET, FIB-milling, plasmaFIB, visual proteomics

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Quantitative analysis of single-atom support interactions by Deep Learning techniques

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Background
The utilization of single atom catalysts (SACs) has captured significant interest due to their superior reactivity compared to nanoparticle counterparts, especially in Environmental Catalysis and Clean Energy applications [1].
SACs can be supported on non-porous structures like oxides or carbon-based materials, or within crystalline porous materials such as zeolites or metal-organic frameworks (MOFs). Their performance is heavily influenced by the local atomic environment and structural characteristics, particularly notable in non-porous materials where surface heterogeneity and defects offer diverse anchoring sites with distinct catalytic performances. Tailoring these characteristics is essential for modulating catalyst reactivity, prompting significant efforts in synthetic methods development and characterization techniques advancement [2]. Notably, High Resolution High Angle Annular Dark Field Scanning Transmission Electron Microscopy (HR HAADF-STEM) emerges as a crucial tool for directly visualizing single metal atoms. However, while its qualitative usage is widespread, quantitative extraction of structural features remains limited [3].
This gap motivates the integration of deep learning algorithms, particularly convolutional neural networks (CNNs), with DFT calculations for a comprehensive and quantitative assessment of metal-support interactions in SACs. In this study, we focus on Pd SACs anchored onto MgO (100) nanoplates, aiming to provide a detailed understanding of their structural features and interactions with the support, crucial for advancing heterogeneous catalysis knowledge and applications in Environmental Catalysis.

Methods
The synthesis of morphology-controlled MgO nanoplates involved a hydrothermal method followed by thermal treatment, resulting in $Mg(OH)_2$ nanoplates that were subsequently heated in a H_2(5%)/Ar flow. Pd-based catalysts were synthesized using two precursors: $Pd(NO_3)_2$ in acetone and an aqueous solution of the coordination complex $[H_3PdL]^{5}$+, with both catalysts prepared via semi-wetness impregnation methods and treated to remove nitrates. Macroscopic characterization included X-ray diffraction using a Bruker D8 Advanced A25 diffractometer, X-ray photoelectron spectroscopy analysis using a Kratos Axis Ultra DLD spectrometer, and electron tomography experiments conducted on a Talos FX200 G2 microscope operated at 200 kV. Transmission and Scanning Transmission Electron Microscopy measurements were carried out on a FEI Titan3 Themis 60-300 double aberration-corrected microscope operated at 200 kV. In particular, HR-HAADF images were acquired with a beam current of 30 pA and a dwell time of 1.25 µs. The Deep Learning algorithm, implemented using Tensor Flow and Keras, involved segmentation using a residual U-net structure for HR-HAADF STEM image analysis. Additionally, DFT calculations using Quantum Espresso with Perdew-Burke-Ernzerhof functional and Hubbard U parameter were performed to assess structural properties

Results

Figure 1a illustrates a representative HR-HAADF STEM images acquired on the Pd catalyst supported on MgO {001} nanoplates, recorded along <211> zone axis. In this orientation, the atomic columns comprise only either Mg or O atoms, allowing to distinguish the nature of the interaction at play.
To precisely quantify these interactions, a smart segmentation procedure leveraging Deep Learning techniques was developed, enabling the accurate discrimination of atomic columns containing Pd single atoms from those containing only Mg. The segmentation process, guided by convolutional neural networks employing the U-net architecture, effectively separates contrasts in HR-HAADF images, Figure 1b. In particular, 900 Pd-support interactions, revealing Pd species adjacency to cationic columns, suggesting substantial interaction with Mg. XPS signals indicate Pd^{2}+ oxidation state, implying Pd species may substitute Mg^{2}+ ions on {001} surfaces, contrasting previous reports of Pd atoms stabilizing on oxygen vacancies.
To unravel the mechanisms underlying Pd-support interactions, we conducted density functional theory (DFT) calculations. These calculations provide an energy differences which suggest a sequence wherein Pd first interacts with V-centers (Mg-vacancies), then likely occupies F-centers (O-vacancies), and finally resides atop the surface, aligning with experimental observations, indicating Pd species localized within Mg atomic columns.
Detailed structural analysis of relaxed models reveals that Pd atoms tend to move slightly into the surface or outside the center in the case of F-centers, while in V-centers, the displacement is subtler, highlighting distinct behaviors in Pd localization within defects. A method, using AtomSegNet, achieves sub-pixel precision, measuring displacements accurately. This approach maps displacement vectors and modulus, enabling precise measurement of shifts from 0.1 to 0.6 Å, Figure 1c. The histogram fitting reveals peaks at 0.17±0.07 Å and 0.45±0.12 Å, matching DFT-calculated values for V and F centers. A 3:1 ratio in Gaussian curve areas indicates more atoms with smaller displacement, consistent with theoretical predictions. Considering MgO support properties, metal loading, and V-to-F ratio, an estimated 1% cationic vacancies align with literature findings for powder MgO.

Conclusion

The methodology's strong validation is evidenced by the close agreement between experimental image analysis and computational predictions, providing unprecedented insights into metal-support interactions in high-surface area powder catalysts. Direct imaging reveals that Pd species preferentially stabilize within surface-located V-centers on MgO supports, a significant advancement in SAC structural analysis. The method's robustness is further underscored by alignment with macroscopic techniques like XPS, enhancing confidence in conclusions drawn from the synergistic integration of advanced AC-STEM, AI analysis, and computational approaches. Its application to diverse catalytic materials promises to uncover general principles governing metal-support interactions, advancing both fundamental and applied catalysis research.

Keywords:

Single-atoms, HR-HAADF, Deep Learning, DFT

Reference:

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Probing photonic resonant modes in InAs semiconductor nanostructures by STEM-EELS

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Dielectric and semiconductor nanostructures are garnering increased interest due to their low radiative loss properties. This makes them a promising alternative to metal plasmonic structures for manipulating light fields at the nanoscale. The flexibility to manipulate optical resonance can be further enhanced by electrically doping and gating the mobile carrier density in semiconductors. In this study, we present the experimental observation of distinct optical resonances in one-dimensional InAs nanostructures, achieved through high-energy electron excitation.
Methods
The nanowires were grown with molecular beam epitaxy (MBE) system. Their length and width were fined tuned by modifying the growth conditions. The STEM-EELS investigation was conducted using a double-aberration corrected microscope (Thermo Fisher Scientific Themis Z) fitted with a high-resolution monochromator and a Gatan 1066 continuum spectrometer. The energy resolution is approximately 200 meV.
Results
Figure 1 presents the two-dimensional EELS mapping of an individual InAs nanowire across various energy windows. It's evident that as the energy loss increases from 0.94 eV to 1.17 eV and further to 1.43 eV, the EELS intensity distributions display different nodal patterns. These patterns correspond to resonant modes from m = 2 to 4 and can be recognized as standing wave Fabry–Perot type resonances. Figure 1(b) shows the intensity line profiles along the direction of the nanowire's growth, taken from the central area. To fully understand the experimetnal observation, we applied the quasi-normal mode theory and boundary element methods to simulate the resonate excitations in siminar geometric structures. The simulated results succssfully interprate the subtle changes in the EELS reponse across different energy windows within a given geometric confinment.
Conclusion
Our STEM EELS investigation and simulation show that InAs nanostructures are a promising candidate for local light field manipulation covering the near infrared-visible-ultraviolet (NIR-vis-UV) spectral range.

Keywords:

Optical resonance, InAs nanowire, STEM-EELS

Reference:

Duncan T. L. Alexander, Valentin Flauraud, and Frank Demming-Janssen, Near-Field Mapping of Photonic Eigenmodes in Patterned Silicon Nanocavities by Electron Energy-Loss Spectroscopy, ACS Nano 15 (10) (2021) 16501-16514. DOI: 10.1021/acsnano.1c06065.

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Application of cryogenic in situ biasing (S)TEM holder to study phase transitions in complex oxides

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Cryo scanning transmission electron microscopy (STEM) is becoming an indispensable tool for studying phase transitions in a vast range of applications in the field of quantum materials [1], magnetic materials and nanostructures, ferroelectrics [2], topological insulators, etc. at the atomic scale. A detailed characterization of a sample's structural and electronic properties across phase transitions necessitates a sample holder with double tilt capability and a continuous temperature control of the specimen while maintaining a sample stability that enables atomic resolution imaging.

In this contribution, we will share our latest developments of a combined in situ cooling, biasing, and heating holder able to achieve atomic resolution imaging in a wide temperature range [3]. The holder, cooled by liquid nitrogen, allows to set any user-defined temperature (Figure 1). The temperature control is achieved using microelectromechanical systems (MEMS)-based heating and biasing chips [4, 5] in combination with a dedicated cryo TEM sample holder. Due to the low power consumption of the microheater, it is possible to sweep the temperature of the sample from -175oC to +800oC, while maintaining the holder at liquid nitrogen temperatures. It was found that atomic resolution imaging can be attained while continuously varying the temperature over a thousand degrees with marginal focus and image shift.

We will present several application examples applied to ferroelectrics that that include thermal and electrical cycling including cooling conditions.

Keywords:

In Situ, cooling, phase transitions

Reference:

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Multiscale study of water condensation on aerosols using in-situ Environmental Scanning and Transmission Electron Microscopies

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background incl. aims
Predicting cloud formation and the arrival of precipitation has always been a hot topic in meteorology. With global warming and the many ongoing drought events, this has become a major issue. Environmental scanning and transmission electron microscopy (respectively ESEM and ETEM) can provide valuable information to understand how clouds are formed by condensation of water on submicronic aerosol particles. Indeed, Environmental Electron Microscopy allows following the structural changes during the condensation of water droplets, while controlling the relative humidity, from the dry state up to fully hydrated conditions.
Methods
Sodium chloride particles are used as model marine aerosols. The deliquescence of crystals in the size range 100 nm – 1 µm is studied in ESEM (QuattroS from TFS) equipped with either a commercial Peltier stage (SE imaging) or a home-made tomographic stage (STEM imaging in both BF and DF modes) [1]. The deliquescence of smaller crystals is studied in ETEM (Titan ETEM from FEI/TFS) using either a commercial cryo-holder from Gatan/Ametek or a home-made system based on a Peltier micro-cooler [2]. More realistic aerosols, such as (non-hygroscopic) Arizona dust decorated with (hygroscopic) sodium chloride, are then studied.
Results
The evolution of the crystal facets and corners as a function of relative humidity is studied in ESEM in the SE mode, and is compared with the literature [3]. The measurement of the relative humidity allows a calibration of the other microscopy set-ups. Interestingly, oscillating behaviors at the beginning of deliquescence could be reproduced and followed in real time in STEM and TEM on smaller crystals (see Figure 1). They were found to match 2D simulations based on local fluctuations of the solute content. The distribution of sodium chloride during hydration/dehydration cycles is also analyzed.
Electron tomography is also carried out at multi-scale, in ESEM and ETEM on hydrated samples and quantitative data can be extracted: crystal shape and size as well as water droplet shape, and the aggregate shape and compound distribution in the case of salted Arizona dust.
Conclusion
The multi-scale approach combining ESEM and ETEM enable the analysis of salt particles not only in 2D, but also in 3D, for different values of relative humidity. As irradiation damage can have large influence on the observed behavior and kinetics, the electron dose received by the sample is quantified and its effects discussed.
Acknowledgements
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microscope. José Ferreira, Erwan Allain, Christophe Goudin, Victor Trillaud (MATEIS) and Mimoun Aouine (IRCELYON) are acknowledged for their experimental contribution.

Figure caption

water condensation on aerosols.(a) XY slice of a tomogram obtained in ESEM, showing NaCl crystals within water droplets. (b) Hygroscopic behavior of realistic aerosol (mixed phase Arizona dust and NaCl) with spatial localization and chemical identification of soluble phases during hydration (several T cycles).

Keywords:

ETEM, ESEM, in-situ, liquid

Reference:

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[2] Vas, J. et al., Microsc. Microanal., 28, S1 (2022), 818.
[3] Langlet, M. et al., Chemical Engineering Science 86 (2013) 78.

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In- and ex-situ implantation of helium to characterise faults in titanium beryllide

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Poster Group 2

Background incl. aims
Transition metal beryllides, MBe_{12}, are an important material class in tokamak fusion reactors for use in tritium breeder modules as a neutron multiplier to maintain the refuelling cycle. During the neutron multiplying reaction chain, helium and tritium are also produced as well as knock-on radiation damage. Beryllium metal is a commonly used candidate neutron multiplier but develops large helium bubbles at breeder module operating temperatures which retain tritium on their inner surfaces (Zimber, et al., 2023), which complicates end-of-life recycling and would be a radiation and explosion hazard in an accident scenario. Titanium beryllide retains less tritium and is tougher (Kim, et al., 2021), so has been chosen as a candidate neutron breeder for EU-DEMO and JA-DEMO. There are still many material unknowns, however; what crystal defects develop, what do they do at high temperature, and how do helium and tritium/hydrogen behave in the microstructure?

Methods
In this study we used the University of Huddersfield's MIAMI-2 ion beam/TEM to implant 300kV He+ ions into 2µm thick sections ("bulk ex situ" sections for the purposes of containing the He peak) of TiBe12 at temperatures 387°C, 480°C, 600°C and 900°C to fluence of 10^{17} ions/cm². From this energy we expect a He peak approximately 900nm under the surface. We then re-sectioned the samples to produce cross sections parallel to the irradiation direction, with the He peak present as a horizontal band of helium approximately 900nm down the re-section. To begin investigating the effect of free surface proximity, a 100nm sample was also irradiated in-situ with 20keV He+ at 550°C. This produces a uniform low level of He (50x less than ex situ case) implantation, and a gently increasing comparable level of knock-on damage going down through the sample thickness.

Results
The thick "quasi ex situ" samples developed faults on {110} planes at temperatures up to 600°C; at 900°C the fault plane had changed to {111}. Displacement vectors were obtained using g.R analysis; some were found to be from the ½<110> or ½<011> families, some from neither previously observed displacement vector type (Banerjee, Jacobson, Zindell, & Mitchell, 1991). Bubbles of size 1-10nm were found at the He peak in samples irradiated at 480°C and 600°C. At 900°C, large bubbles (>200nm) evolved on grain boundaries and smaller (50-100nm) bubbles in grain interiors at the He peak (Sharp, et al., 2024). The {111} faults were preferentially associated with the grain interior bubbles, implying some kind of free surface or He sink effect at work.
The thin in situ sample contained some faults at room temperature before irradiation, which experienced very little change as the temperature was increased to 550°C. A high density of new faults began to appear almost as soon as He+ irradiation was started. Analysis of prior and irradiation induced faults in the in situ specimen is presented and compared with ex situ work to give an initial step to explaining the relationship between bubbles and faults.

Conclusion
Planar crystal faults are present in unirradiated $TiBe_{12}$; new faults are created on irradiation with high energy (20-300keV) helium ions. At lower temperatures, faults lie on {110} planes, transitioning to {111} planes at some temperature between 600°C and 900°C in constrained material. In the same temperature interval, helium bubbles change from single nm size, to 50-100nm in grain interiors and larger on grain boundaries. Bubbles and {111} faults created at high temperature are preferentially associated.
Next steps in the subject will be to pinpoint the fault transition temperature, and to carry out and simulations and compare with experimental results to find the atomic configurations at {110} and {111} fault types, if there is a volume change between the two types that affects their relationship with bubbles, and whether/how they are sinks for interstitial helium atoms. The same process of understanding must then follow for hydrogen in the structure.

Image caption: ADF-STEM image from sample irradiated ex situ at 900C. White lines are {111} planar faults; small black objects are grain interior bubbles. At bottom is a larger bubble on a grain boundary. Image is part of a montage presented in Sharp et. al. 2024.

Keywords:

In-situ, irradiation, fusion, beryllide, defects

Reference:

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Zimber, N., Lammer, J., Vladimirov, P., Kothleitner, G., Keast, V. J., Dürrschnabel, M., & Klimenkov, M. (2023). Hydrogen and helium trapping in hcp beryllium. Communications Chemistry, 6, 76.

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Scale-Bridging Analysis of Hierarchical Mesoporous Transition Metal Foams

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Poster Group 1

Mesoporous materials have attracted tremendous attention in recent decades due to their unique property portfolio and potential applications in various fields such as energy storage, catalysis and sensoring. Mesoporous copper for example has proven suitable as a material for large-scale production due to its properties, cost efficiency and excellent accessibility. Mesoporous transition metals comprise an enormous surface area, high electrical conductivity, improved catalytic activity, and long-term mechanical stability. Those properties are strongly influenced by the material's morphology specifically the pore size, pore distribution as well as connectivity. The chemical composition of the surface particularly governs the material's catalytic properties.
Dealloying as a technique utilises the differences in the galvanic series. A porous structure is created by selectively etching out an element of an alloy. Dealloying has been established as a facile method to tailor hierarchical porous structures with extensive pore networks of varying sizes dependent on the depth and location in the material. It is, therefore, a robust, scalable and cost-effective approach for the synthesis of mesoporous transition metals and their alloys [1].

In this study, dealloying is applied under various process parameters to investigate their influence on the porous structures formed using a copper-manganese alloy sample. The dealloying process is conducted under acidic conditions (hydrochloric acid). To unravel structure formation dependent on the various process parameters, e.g., pore size and distribution, morphology of pores, composition etc., advanced 3D imaging techniques including micro-computed tomography (µCT), focused ion beam (FIB), and transmission electron microscopy (TEM) tomography are employed along different length scales. To gain a deeper understanding of how morphology and composition influence catalytic activity and stability, we evaluated the performance of mesoporous copper foams as electrodes for the hydrogen evolution reaction (HER).

The resulting structures exhibit systematic channel networks and variations in pore size depending on depth and location in the material. We found that even small changes in the synthesis process can have a significant impact. For example, very small concentrations (≤1 at%) of other elements may lead to strongly altered morphologies with, e.g. an order of magnitude smaller pores and higher overall mechanical stability; moreover, the catalytic properties and overall morphology were affected.

We successfully have fabricated mesoporous copper for HER reaction. Multiscale analysis of the structures of the dealloyed material from the millimetre to the nanometre range facilitate the understanding of structure formation and unravels the complex structure-property relationship. The project, therefore, contributes to the detailed understanding of the impact of the various reaction parameters of the dealloying process to the structures formed, and subsequently their impact on the performance of the material.

Keywords:

Dealloying, Nanostructured-material, Hierarchical, Scale-bridging-analysis, Catalyst

Reference:

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Intralayer modification on Ti3C2Tz MXene Multilayer by Tailoring Surface Terminations

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background incl. aims
MXenes are a class of two-dimensional materials whose properties can be tuned by tailoring surface terminations during the etching of the parent MAX phase precursors (1). It can be affirmed that the repetition of a MXene multilayer structure is the sum of interlayer width and intralayer thicknesses. Notably, conventional measurement techniques like X-ray diffraction (XRD) do not distinguish between these dimensions. However, it is crucial to recognize that the interlayer spacing holds particular significance in numerous applications, especially those reliant on swift diffusion of species in between MXene sheets, for example energy storage, material separation and interception. Consequently, understanding the determining variables for the interlayer spacing requires a thorough interpretation of intralayer spacing. Through theoretical simulations it has been identified that the surface terminations of Ti_3C_2Tz MXene affect the sheet thickness. As is evident from a theoretical study which is shown in the graphic below, the sheet thickness varies for different surface terminations. Therefore, the aim of this study is to experimentally verify the structural changes that occur as a function of surface termination chemistry.

Methods
Ti_3C_2Tz MXene multilayer structures with a mix of Oxygen (-O), hydroxide (-OH), and fluorine (-F) surface terminations have been used in this study. It is well known from the previous studies that F desorbs from the surface at around 500 °C and is removed completely while heating upon 700 °C (2). Double aberration corrected Scanning Transmission Electron Micrsscope (STEM) was used to observe the changes occurring within the MXene sheets at the atomic scale during in situ heating, to these temperatures. Images were obtained at ambient conditions after maintaining 15 minutes at every 100 °C up to a maximum temperature of 800 °C. Electron Energy Loss Spectroscopy (EELS) was used to explore the chemistry of the MXene.

Results
Experiments were conducted under vacuum between the temperature range of 100 °C to 800 °C. For every 100 °C increment, the structural and compositional changes in MXene multilayers were investigated. As expected F starts to leaves the surface after 500 °C, which was confirmed by EELS and accordingly changes the surface termination chemistry. From High Angle Annular Dark Field (HAADF) imaging in STEM mode the MXene intralayer corresponds to 0.482 ± 0.002 nm at 100 °C. This thickness is maintained until 500 °C after which it is reduced and reaches a spacing of 0.457 ± 0.002 nm at 700 °C arises as a result of fluorine removal from the surface. These results clearly reveal the impact of surface terminations on the sheet thickness. In addition, as the intralayer spacing decreases, the interlayer spacing increases by 0.026 nm at 700 ^{o}C (0.597 nm) in comparision to the ambient MXene conditions (0.571 nm).

Conclusions

In conclusion, it is evident that the surface terminations significantly influence the MXene sheet thickness. This variation is proposed to originate as a consequence of the strength of the interaction between the electron cloud of Titanium atoms and the surface terminations. This intralayer tuning can be widely applied in any MXene applications where intercalation plays a vital role.

Keywords:

MXenes, Surface terminations, in-situ STEM

Reference:

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Analytical Phase-Shifting Electron Holography using Fresnel-corrected holograms

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Poster Group 2

Background incl. aims
Unlike conventional Transmission Electron Microscopy (TEM) techniques, Electron Holography (EH) gives an access to the phase of the electron wave, which makes it suited for imaging electromagnetic fields with fine sensitivity and a spatial resolution in the range of nanometers [1].
When performing a measurement, the complex wave contribution of the sample is contained in the object band of the Fourier transform of the recorded hologram. For this reason, the most common way of extracting the sample phase contribution consists in applying a circular mask around the object band [2]. This low-pass filtering cuts off excessive noise and undesirable Fresnel diffraction effects, but also limits the final spatial resolution of the recovered phase map. While this limit may not be an issue depending on the scale of interest, such a loss of precision can lead to artefacts in the phase maps realignment process when working with finer details, which remains a critical step in separating the electric and magnetic contributions.
Other approaches overcome this by combining two holograms to suppress the zero-order band [3], effectively doubling the maximum mask size available and typically getting the spatial resolution down to 2 - 3 nm. This may not be enough in some cases though. A solution to this problem is the Phase Shifting (PS) method, which uses linear combinations of a greater amount of holograms to mathematically isolate the object band [4], thus negating the necessity to apply a mask in Fourier space and allowing for a theoretical pixel precision. Implementations tend to use specific initial phase values in order to get back to an ideal diagonal matrix system, which greatly facilitates the calculation. When working with arbitrary initial phase values, it is still possible to avoid the complex matrix inversion by performing sine fittings in each pixel, using an even greater amount of hologram recordings [5]. Due to the absence of any filtering however, Fresnel modulation effects become a source of errors in the reconstruction process in both cases. However, this effect can be rendered ineffective by adjusting the hologram fringes spacing to match that of the modulation pattern [5].
In this work, we aim for the pixel-precise reconstruction of phase maps using a modification of the PS method, so that the recovered sharp atomic details can lead to better phase maps alignments. This study focuses primarily on a more general analytical solution to the PS equation in the case of arbitrary chosen initial phase values, which eliminates the need to conform to an ideal case. We also investigate the use of biprism voltage as a suitable way to minimize Fresnel modulation effects and related calculation errors. Finally, we apply these methods to both computer simulations and TEM measurements from specifically prepared magnetic samples.
Methods
The general analytic solution we derived to the PS matrix equation works for any set of non-redundant initial phases containing at least three elements. While more measurements ultimately yield less noise in the final phase map, we typically used 5 or 7 hologram recordings for each manipulation up to this point. As common as it is for this matter, we used beam tilting to tune each initial phase value. Under the small angle approximation and disregarding the Fresnel modulation pattern, this is equivalent to an overall phase offset in the fringe pattern. The experimental values

can then be extracted for each hologram from the center of the object band in Fourier space and be used as parameters in the solution expression.
Since there is no requirement regarding the fringe spacing in the holograms, we also choose to tune the biprism voltage low enough to give a sufficiently good contrast while still cancelling out the Fresnel effect. Up to this point, all of our measurements have used this particular setting.
Results
Our computer simulations use artificially generated phase and amplitude maps from which holograms are calculated. We first tested our method on square-shaped phase maps with various apodizations, noise levels and Fresnel diffraction effects. Using our analytical method on Fresnel-free holograms, we see that the reconstructed phase maps show accurate reconstructions of the initial wavefront regardless of the spatial details [Fig.1].
Additionally, we measured the Fresnel modulation effect on holograms for various biprism voltage values in order to minimize this effect. So far, all of our experimental results have thus been using a voltage of 21 V instead of the usual 200V, which corresponds to 24nm-wide fringes for an image pixel resolution of 0.12 nm.
Conclusions
We were able to reconstruct phase maps using our generalized PS analytical method for reasonable noise and Fresnel modulation levels. While this method works best on perfectly generated holograms, we also have shown that it is possible to maximize the fringe contrast and correct the Fresnel modulation effect by adjusting the biprism voltage. Likewise, other parameters such as the initial phase do not need to match ideal values since our method takes the experimental measurements into account.
Using this modified phase shifting method, we expect that further testing done on specifically prepared samples can give satisfactory results with an effective spatial resolution approaching the theoretical pixel size.

Keywords:

Holography, phase-shifting, biprism, Fresnel, modulation

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Effects of electron cascade and lamella preparation on InGaN quantum well recombination dynamics

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Poster Group 2

Background: InGaN/GaN quantum wells (QWs) have garnered significant attention for LED applications due to their high quantum efficiencies in the ultraviolet/visible range and emission tunability with indium content, among other factors. However, the growth of QWs with high indium content encounters issues with indium phase separation, resulting in structural changes and inhomogeneous light properties. This can be controlled via growth stopping, forming quantum dot like islands that behave as localized emitters [1,2]. Furthermore, subsequent etching reduces the strain-induced quantum Stark effect present in majority of such structures. Due to its deep subwavelength spatial resolution and broadband frequency components enabling coupling to high-energy optical transitions, cathodoluminescence nanoscopy (CL) has emerged as the ideal tool to study such high-bandgap semiconductor systems [3] and characterize their nm-scale features and defects. Despite it being a popular characterisation technique, the impact of the electron cascade on the sample, as well as the optoelectronic effects of thinning bulk samples into lamella, as is commonly performed for studies in a transmission electron microscope (TEM), have hitherto not been studied systematically. In this work we aim to confront this using a comprehensive study comparing time-resolved (TR) scanning electron microscope- (SEM) based CL, TEM-CL, and TR-photoluminescence (PL) measurements.
Methods: We utilized CL imaging with both 5 and 150 keV electron beams to acquire hyperspectral maps on InGaN/GaN QWs. Additionally, employing a beam blanked SEM with pulse lengths ranging between 30 – 100 ps, we conducted time correlated single photon counting and extracted luminescence decay maps as a function of position, temperature, and wavelength. The samples subsequently underwent preparation as lamella using Ga-based focused ion beam milling, after which the same measurements were performed. These measurements were compared to the decay maps obtained via an ultrafast photoemission TEM, along with decay traces from analogous samples acquired using TR- PL at liquid helium to room temperatures.
Results and Conclusions: We show that despite the energy density deposited by a 5 keV electron beam being an order of magnitude larger than that deposited by a 150 keV beam, the probability of exciting higher energy localized emitters, seen in the RGB fitted hyperspectral map in the graphic, is reduced due to the size of the electron cascade. The recombination dynamics of carriers, however, are comparable, with two major lifetime components of around 100 ps and 1.5 ns, as well as a delay in the rise time of the lower energy component of the emission. Interestingly, the TR-PL results indicate shorter lifetime components of 30 and 120 ps, when usually lifetimes in PL are reportedly longer than in CL. We present a model describing the recombination dynamics measured and the nuances between the different experimental techniques and respective results.

Keywords:

InGaN heterostructure, recombination dynamics, cathodoluminescence

Reference:

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Magnetic layers reversal in new MRAM devices measured with operando Electron Holography

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Poster Group 1

Background incl. aims
Recent improvements in spintronics led to new types of non-volatile Magnetoresistive Random-Access Memories (MRAM) with low power consumption and short switching times, such as the current-induced Spin-Orbit Torque (SOT). So far, studies of such devices mostly rely on the measurement of their electrical and magnetic characteristics using external electrical methods [1]. However, their optimization ultimately requires a deeper understanding of the internal processes through direct observation. Given the usual dimensions of these components (<60 nm lateral, <1nm in thickness), observing magnetization textures and reversal during their activation requires the use of large-scale instruments [2].
In particular, Transmission Electron Microscopy (TEM) is a tool of choice for this matter, offering the combination of nm-scale spatial resolution and magnetic sensitivity. Indeed, Electron Holography (EH) gives access to 2D projected electric field and magnetic induction maps with a sensitivity in the nm.T range [3], which can lead to information about various electrical properties of the sample.
This work aims for a better understanding of the switching processes taking place inside these devices upon writing and reading data by performing an operando analysis (e.g the quantitative dynamic imaging of their inner magnetic structure) on electrically biased samples using EH [4]. We will present here the study of the free layer reversal process inside Magnetic Tunnel Junctions (MTJ) in both isolated and array-like SOT devices. Given that the zone of interest is a ferromagnetic layer less than 1nm-thick with weak internal fields close to the detection limit, we also chose to study the reversal process in a wider in-plane MTJ as preliminary results less sensitive to the measurement quality.
Methods
Most of the EH measurements presented here use Phase Shifting (PS) by combining several holograms recorded with different beam tilting angles as a mean to enhance both spatial resolution and magnetic sensitivity. While this method gives an exact analytical solution to the PS matrix equation, it comes at the expense of limiting ourselves to study the reversal processes in a pseudo-static regime.
In any case, separating the electric and magnetic components of the electron wave phase requires two holograms to begin with. We perform all image alignments using points with strong Mean Inner Potential (MIP) gradient and theoretical zero magnetic field in an iterative correction process.
In order to ensure the best measurement quality regardless, the device under observation must be sufficiently thinned down below 100 nm to limit the electron beam absorption through heavy materials, while remaining intact regarding its electrical behavior. Additionally, the injection of an external electrical bias on the sample inside the microscope chamber remains a technological challenge [5]; these conditions for accurate measurements require a highly controlled preparation process that will be presented.
Results

We first performed PS electron holography on a 8nm-wide in-plane double MTJ without any electrical bias, where a controlled external magnetic field acted as the trigger for the free layers switching. In this experiment, we were able to witness the reversal on phase maps approaching the pixel resolution for an external field of around 9mT [Fig.1]
We also conducted experimentations leading to a complete preparation process for the electrical connections of SOT samples inside the TEM chamber in order to perform PS on them, this time with a voltage bias for triggering the layer switching. Although the MTJ in SOT are typically smaller than that of our previous experiment, they still exceed the 0.12 nm spatial resolution limit of our image pixel size.
Conclusions
These first results on unbiased samples show that a pixel-precision magnetic induction map is obtainable through the PS method with some image realignments and additional precautions. Given our advancement on the SOT preparation, we thus expect to be able to measure the free layer switching in these particular devices with a similar spatial resolution, keeping in mind the study of domain wall motion as the next step towards a deeper understanding of the SOT mechanisms.

Keywords:

Phase-Shifting Holography, operando, MTJ, SOT

Reference:

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[5] M. Mecklenburg, M. Brodie, W. Hubbard, et al. Fabrication of a Lift-Out Grid with Electrical Contacts for Focused Ion Beam Preparation of Lamella for In Situ Transmission Electron Microscopy. Microscopy and Microanalysis 19, 458–459 (2013).

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Methodological development of nanoscopic defects characterization in nuclear materials: contribution of TEM-APT correlative microscopy

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Background

The structural steels used in the primary circuit of nuclear power plants are subjected to irradiation and/or temperature conditions that lead to changes in their mechanical properties. These mechanical properties are directly linked to changes in microstructure due to ageing, particularly on a nanometric scale (segregation, precipitation, clusters of point defects, etc.). To predict these changes, it is necessary to describe the nano-objects present as precisely as possible, i.e. to correlate their chemistry, morphology and crystallography. In the nuclear materials scientist community, two complementary instruments are often used to characterize nano-features: Atom Probe Tomography (APT) and Transmission Electron Microscopy (TEM).
APT enables characterization of nm-scale chemical heterogeneities in terms of size, density, composition, and morphology in the 3D of the real space. However, it does not provide a full crystallographic description.
TEM related techniques can provide access to the crystalline structure of the matrix and nano-objects, as well as their orientation relationships. Its wider field of view also allows us to quantify the number density of objects with better accuracy. Chemistry can also be studied in analytical STEM, although the matrix signal can be convoluted with that of the precipitates. Contrary to APT the collected data are only in 2D.

Methods

The aim of TEM-APT correlative microscopy is to characterize the same nano-objects using both techniques for the most complete possible description [1]. Since APT is a destructive techniques, correlative microscopy here consists in analyzing an APT thin needle with TEM first, and then to analyze the same sample by APT. It allows to remove ambiguities about the nature and/or location of nano-objects and thus provide new insights into the mechanisms involved in defect formation.
This work therefore involves developing a methodological approach that makes maximum use of the data collected from each technique (such as MultiVariate Statistical Analysis processing for chemical analysis in STEM) and combination of data accessible by only one of each technique obtained on the same sample. To this end, a thermally aged austeno-ferritic model alloy, in which the ferrite undergoes two phase transformations: spinodal decomposition and the formation of G-phase particles, was studied in an effort to optimize sample preparation for correlative microscopy and data processing. The challenges that have been faced are as follows: improving the contrast of electron microscopy analyses, improving the yields of TEM-APT correlative microscopy analyses, understanding the artefacts associated with the physics of field evaporation and 3D reconstruction for APT, and optimization of data processing of EDS through statistical methods (using Principal Components Analysis in particular) in order to improve EDS quantification and correlation with atom probe data.

Results

The aim of the first part of the study is to extract more quantitative data from TEM analysis (imaging and elemental analysis). Contrasts are difficult to interpret in TEM experiments because of the shape of APT samples, the amorphous layer induced by FIB preparation, the presence of oxide layers hindering diffraction patterns and the contribution of the background (i.e. vacuum) in images. In order to reduce the amorphous layer, the addition of a cleaning step has been investigated with the use of an Ar-beam polishing at low-voltage with a Precision Ion Polishing System II (PIPS II) [2]. In addition to a systematic plasma cleaning before and after every S/TEM analysis, this step would also permit to address the yielding of tips that have been investigated in EM by removing the high-field carbon contamination and the oxide layers simultaneously. Quantification in Analytical TEM is not straightforward when trying to isolate the signal from the precipitates from that of the matrix. Compositions of the α/α' domains as well as G-phase precipitates measured thanks to APT have been used to improve the data processing of EDS data. On one hand, EDS spectrum imaging supported by PCA denoising as well as sample preparation improvement, helped to optimize the detection of nano-objects. On the other hand, thanks to correlative microscopy and PCA, we were able to extract their composition from EDS maps.

The second part of the study is to use TEM images as additional information to obtain reliable 3D reconstructions with atom probe data, even without the presence of crystallographic poles. Through the comparison of different 3D reconstruction methods that exist on an experimental dataset from TEM-APT correlative microscopy: static and dynamic voltage-based reconstructions [3], shank angle or tip profile, we suggest a methodology to reconstruct a volume properly. This is done by adjusting the spatial distribution of the G-phase particles and their shape. This correlation also enabled to highlight distortion artefacts observed on APT volumes.

Conclusion and Perspectives

This work aims to present a methodology for extracting maximum information from samples thanks to correlative TEM/APT microscopy. Leveraging the strengths of each instrument, we addressed the limitations of the other, enabling successful characterization of particle shapes, sizes, crystallography and chemical composition. Efforts have been dedicated to improve the sample preparation procedure with low voltage ion beams, allowing improved image contrast and better identification of objects and therefore facilitating quantitative analysis. The optimization of the data processing of EDS spectrum, assisted by APT data, has been undertaken. PCA has demonstrated its effectiveness in reducing the background noise and thus improving the detection of poor signals (e.g. low counts experiments, from nano-particles embedded in the matrix).

Other future developments currently under investigation include:
- Exploring alternative methods to enhance contrast in diffraction-based EM imaging, such as through flash-polishing techniques. [4]
- Expanding application of the methodology to analyze more complex materials that contain a wider range of nano-objects, such as irradiated steels.

Keywords:

TEM-APT correlative microscopy, nuclear metallurgy

Reference:

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Image Restoration from Subsampled STEM Measurements using Deep Learning

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Poster Group 2

Background
Scanning Transmission Electron Microscopy (STEM) has been shown to be a powerful tool for observing the atomic structure of complex materials. However, the radiation damage induced by the electron beam limits imaging beam-sensitive materials with acceptable signal-to-noise ratio. Subsampled STEM [1] has recently been investigated as an approach for reducing the radiation damage without compromising the level of signal per each measurement (or probe position). It is achieved by subsampling the grid of probe positions, which results in an incomplete set of measurements. A complete STEM image is then recovered from those subsampled measurements through an inpainting process.
A myriad of inpainting methods have been introduced based on, e.g., variational [2], Plug-and-Play (PnP), and deep learning frameworks [3]. In this work, we focus on PnP methods, which have been widely used for solving various imaging problems by using an off-the-shelf denoiser as an image prior.
Methods
We propose a deep learning-based inpainting method. Inspired by the work on Deep Denoiser Prior (DDP) for image restoration [4], we utilize a pre-trained deep neural network as an implicit image prior and then integrate that pre-trained network into an iterative inpainting algorithm. We discuss how the training of a DDP can be improved using synthetic and experimental STEM images corrupted by different sources, such as detector noise, scan distortion, sample movement, and aberrations. Therefore, we also introduce a new tool for the fast generation of synthetic STEM images. Additionally, inspired by recent work on invariant priors [5], we demonstrate that enforcing equivariance to certain transformations, such as rotations, reflections, and translations, during the denoising step, improves the quality of inpainting.
Results
The results of our inpainting method are shown in Figure 1. The images tested in Figure 1 were not used for the training of the denoiser neural network. Ground truth synthetic images of SrTiO3 and Si were generated using our image generation tool. These images were then randomly subsampled with respect to 25% of probe positions. Despite slight imperfections around the boundary of the images, marked by a yellow arrow, the reconstructed images are of very high quality, with Signal-to-Reconstruction Error Ratios (SREs) greater than 43 dB.
Conclusion
This work presents an inpainting method for subsampled STEM data that leverages the power of both variational and deep learning methods. Given the flexibility of PnP methods, any neural network architecture can be used as a DDP. In the future, we plan to extend this work to inpainting subsampled data in different modes of electron microscopy, such as scanning electron microscopy and 4-dimensional STEM.

Keywords:

Deep Learning, STEM, Inpainting, Low-Dose

Reference:

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Multi Element ELNES Mapping of Compounds

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Scanning transmission electron microscopy in combination with electron energy loss spectroscopy is a powerful method which provides very local spectroscopic information[1]. The local spectroscopic data contains elemental and electronic information. However, the elemental quantification needs a rather large energy window to have simultaneous quantification on multiple elements. Whereas the electronic information needs high energy resolution which limits the size of the energy window due to the limited amount of pixels in an electron detector. This makes the simultaneous elemental quantification and fine structure analysis almost impossible when multiple elements are of interest. One possible solution would be to scan multiple STEM-EELS map and changing the drift tube or magnetic prism between each frame. The difficulty is that sample drift, damage and real time user interaction, such as refocusing, make this procedure very cumbersome. Another solution is doing DualEELS which applies two drift tube voltages at each probe position which solves the problems discussed above[2]. However only two snippets are available which is still insufficient for most materials where multiple edges occur.
A novel method was developed which overcomes these challenges by applying any number of offsets to the drift tube without adding dead time of the detector. Moreover, the defocus for the different drift tube voltages is corrected for to maintain a good energy resolution. This methodology provides easy correlation of elemental abundances with the fine structure for multiple edges in a sample.
In this work, the methodology is applied to multiple samples providing new insights into solving materials science questions. For example in Fig. 1, the core-loss edges of K-L, Ti-L, O-K, Eu-L and Ta-M together with the low loss are mapped quasi-simultaneously on the interface of EuTiO3 (ETO) and KTaO3 (KTO) which are interesting in the research on 2D electron gas systems[3]. At the top of ETO (purple box), a different oxidation state is observed from the fine structure of Ti, O and Eu simultaneously when comparing it to the fine structure of the ETO layer (red box). With this example we show the power of the new approach for quality, speed and ease of use in ELNES applications.

Figure 1. A series of 2d spectrum image maps converted into a 1d line maps across an interface between ETO (top) and KTO (bottom) where the low loss and different fine structures are shown for different positions with respect to the interface. The information from the different ELNES profiles can be directly correlated since they are acquired quasi-instantaneous.

Keywords:

EELS, Elemental quantification, Fine structure

Reference:

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pyEELSMODEL: python library for model-based EELS quantification

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Poster Group 2

Electron energy loss spectroscopy is a powerful method used to investigate the elemental abundance and electronic structure of a material. Extracting this information from the experimental data is a complex process and multiple methods exist to get the results where each of them have their own advantages and disadvantages. Moreover, each method has free input parameters which influences the final results and can lead to experiments bias and reproducibility issues, especially in the common case where all parameters and the exact workflow is not shared.

In this work, an open-source python package (pyEELSMODEL) is presented which offers multiple alternative EELS quantification methodologies[1] . The library allows a transparent way to share a specific data processing workflow from raw data to a resulting plot that can appear in a paper. pyEELSMODEL expands upon the former EELSMODEL (c++) software which introduced the model-based philosophy in the EELS community [2]. This method attempts to describe the experimental data with a physical model and optimizes the parameters of this model via a minimization scheme such as least squares or maximum likelihood. The values of these optimized parameters can be used to estimate information on the material such as eg. elemental abundance. The new pyEELSMODEL package is written in python making it, in general, easier to integrate and extend as compared to the former c++ code. Multiple robust quantification workflows are available and can be easily used by the novice EELS user via eg. Jupyter notebooks.
This package is also particularly useful for testing and validating novel data processing methodologies since its results can easily be benchmarked against more common methodologies and could act as a test standard against which to make performance claims.

In this presentation, we will demonstrate the use of pyEELSMODEL on several experimental STEM-EELS maps showcasing the robustness and speed of the model-based quantification methodology for modern large size datasets. In Fig. 1, EELS quantification is used to get elemental maps on a mix of copper and silver nanoparticles on top of a carbon substrate. The lower plots show the resulting fitted model on silver (a) and copper (b).

Fig 1. Elemental maps of copper and silver nanoparticles on top of a carbon substrate. (a) Shows the fitted model on a silver nanoparticle whereas (b) shows a fit on the copper nanoparticle.

Keywords:

EELS, Quantification, Model-based

Reference:

[1] pyEELSMODEL repository: https://github.com/joverbee/pyEELSMODEL

[2] Verbeeck J. et al; Model based quantification of EELS spectra; Ultramicroscopy; 2004; doi:10.1016/j.ultramic.2006.05.006

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Tilted multislice approach for quantitative STEM simulation

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Poster Group 2

Background incl. aims
Scanning transmission electron microscopy (STEM) is a powerful tool for studying materials down to the atomic scale. Although qualitative STEM imaging offers plentiful information about atom locations and identification, quantitative analysis is becoming increasingly valuable for insights into composition, atom counting and thickness determination [1]. However, due to the strong dynamical scattering in crystals, directly solving the inverse problem from scattered electron intensity in STEM is nearly impossible. As a result, STEM imaging simulation is crucial for quantitative STEM analysis. In this study, we develop a new multislice algorithm that can be applied to nonorthogonal crystal structures, a task generally challenging in conventional multislice simulation [2].
Methods
For nonorthogonal unit cells, such as a triclinic unit cell, we first use an iterative algorithm to find a new unit cell. We aim to make the angles between the three new basis vectors as close to 90° as possible, while the c-axis keeps along the electron beam direction. The nonorthogonal supercell is then divided into a series of tilted slices parallel to the a-b vector plane. The Coloumb potential of the crystal is then projected onto the tilted slices based on a nonorthogonal mesh, and a tilted Fresnel propagation is performed between subsequent slices. The generated STEM image based on nonorthogonal mesh is then re-mapped into conventional format. By using this tilting-slice operation, we can effeiciently model the electron dynamical scattering in nonorthogonal structures.
Results
We implemented the method in our in-house STEM simulation code and performed high angle annular dark field (HAADF) STEM simulations on Gd2B4O9 [0 0 1], as shown in Figure 1.
Conclusion
We develop a tilted multislice approach to simulate focused electron beam propagation in nonorthogonal structures. This effectively extends quantitative STEM analysis to compound materials with more complex structures.

Keywords:

Quantitative STEM, multislice, nonorthogonal crystal

Reference:

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Cellular in-situ Assessment of Complex Tissue Environments in 3D

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Poster Group 2

The tissue microenvironment has emerged as a critical determinant of immune cell function and inflammatory disease outcome. However, assessing complex multicellular niches within the intact anatomy of a tissue and linking them to cellular in-situ function with the necessary volumetric dimension and spatial resolution remains a challenge. Our goal is to develop workflows that enable quantitative and functional volumetric imaging of intact tissue compartments during the course of inflammation. We use computational reconstruction to correlate the 3D positioning of immune and non-immune cells with microanatomical patterns of tissue architecture. In this way, we seek to define functional niches and establish comprehensive phenotyping of local environments during the onset, progression and resolution of inflammatory disease.

Keywords:

Quantitative and functional volumetric imaging

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4DSTEM-in-SEM by placing a pixelated detector below the sample

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Poster Group 2

Background
Four-dimensional scanning transmission electron microscopy (4DSTEM) is a powerful diffraction-based technique to probe local crystallinity, orientation, strain, and many more properties of electron-transparent samples [1]. Furthermore, various open source Python packages exist to analyze the large amounts of data generated by 4DSTEM, for instance LiberTEM, py4DSTEM, and pyxem. 4DSTEM can also be employed in scanning electron microscopes (SEMs) for example by placing an electron-sensitive pixelated detector (camera) below the sample similar to on-axis transmission Kikuchi systems [2-5]. The lower accelerating voltage of SEMs leads to strong scattering even from monolayers. 4DSTEM-in-SEM is ideally suited to characterize 2D-like materials due to their inherent thickness of a few nms.
Compared to dedicated (scanning) transmission electron microscopes ((S)TEMs), the large sample chamber of SEMs with their various flanges as well as the large area around the sample give enough space to integrate cameras and other equipment into the sample chamber without compromising on the SEM's original functionality. The large field of view of SEMs of several mm^2 combined with their resolution in the sub-nm range are ideal to map properties on different length scales. Furthermore, using SEMs as a base platform allows for simpler, more easily customizable, and more economical experimental setups. On the other hand, the lower accelerating voltage of SEMs require thinner samples and the native spatial resolution doesn't allow STEM-imaging at atomic resolution.

Methods
We built two transmission diffraction substages that fit between the pole piece and the SEM stage, and can be mounted on top of the original SEM stage. One substage utilizes a fiber-coupled scintillator-based CMOS camera and the other substage a Medipix 3 hybrid-pixel detector with single electron sensitivity (X-Spectrum GmbH, Germany). Both substages with their corresponding cameras are shown in Fig. 1 a) and b). The compact 2.5x2.5x1.1cm^3 CMOS camera is integrated inside a hexapod stage and mounted to a linear stage, enabling us to physically adjust the distance between camera and sample (the camera length). The camera length is limited to the distance between the sample and the camera because there are no active lenses after the sample. The substages have sample holders for standard TEM grids and are kept inside an external vacuum chamber when not inserted into the SEM to reduce carbon contamination.
Hardware synchronization between each camera, the GeminiSEM 500 (Zeiss), our in-house scan generator, and the electrostatic beam blanker makes it possible to only expose the sample during a camera frame and to utilize the maximum possible frame rate of each camera. The scan generator also acquires the signals of the SEM's secondary electron (SE) detectors during a 4DSTEM scan. We integrated all hard- and software in-house, providing full control over the 4DSTEM-in-SEM systems allowing us to explore new and existing methods.

Results
Fig. 1 c) shows maps of the in-plane orientation and the coverage of both components of a C60/MoS2 van der Waals heterostructure [5]. The in-plane orientation map of MoS2 obtained by

gold-mediated exfoliation shown in Fig. 1 d) reveals the single crystallinity of the MoS2 over several mm² [5]. The data shown in Figs. 1 c) and d) were obtained using the above-mentioned CMOS camera-based system and are exemplary applications of 4DSTEM-in-SEM.

Conclusion
4DSTEM-in-SEM is feasible and adds to the SEM's wide range of applications. The large scan area of several mm² and the nm sized spatial resolution of SEMs is ideal to employ 4DSTEM in SEMs to characterize low-dimensional materials and thin bulk samples. Furthermore, SEMs are an excellent platform for method development due to their simplicity compared to dedicated (S)TEMs, their spacious sample chamber, the convenient access to the sample area, and their good imaging performance.

Acknowledgements
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Keywords:

4D-STEM, 4D-STEM-in-SEM, transmission-electron-diffraction, 2D-materials

Reference:

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Structural and optoelectronic properties of layered halide perovskites

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Halide perovskites have recently risen into the spotlight due to their exceptional optoelectronic properties and compositional flexibility, enabling a wide range of choices for each component in the archetypical ABX3 formulation (A: cation, B: typically Pb or Sn, X: halide anion). The basic structural unit is the [BX6]4- octahedron, sharing corners in a 3D lattice. A new class of perovskites, featuring layers of corner-sharing octahedra alternating with layers of organic spacers, has been demonstrated to have enhanced fluorescent emission compared to their 3D counterparts. Understanding local composition, crystallography and optoelectronic properties is fundamental to develop an understanding of these materials and their engineering.
In this work we showcase the combination of various electron microscopy techniques (EDX, cathodoluminescence, 4DSTEM, monochromated EELS) to unravel the interplay between local compositional, structural and optoelectronic properties in layered halide perovskites. We also study systems where the halide component is replaced, obtaining a lateral heterostructure.
Methods
Materials (PEA2PbBr4, PEA2PbI4, PEA2MA2Pb3I10) were synthesised using a wet chemistry route described in [1]. The resulting materials were dispersed on silicon for CL measurements and lacey carbon or silicon nitride grids (20 nm thick) for STEM measurements. SEM-EDX was carried out in a ZEISS GeminiSEM 560 using an Oxford Instruments detector. SEM-CL was done in an Attolight Allalin 4027 Chronos SEM-CL. Spectra were acquired with an iHR320 spectrometer (focal length of 320 mm, 150 gratings per mm blazed at 500 nm, 700 µm entrance slit) and an Andor 1024-pixel charge-coupled device (acquisition time 100 ms/pixel). All the measurements were performed at room temperature under high vacuum (<10 – 7 mbar) with acceleration voltage of 10 kV and a pulsed beam with a frequency of 80 MHz, a pulse duration of ~7ps, at a current of ~100 pA as measured by a Faraday cup. STEM measurements were done in a ThermoFisher Spectra300 with probe and image correctors, a monochromated source and a dual-X EDX detector (1.7 sr total solid angle).
Results
We considered layered perovskites with different thicknesses and composition. Thick flakes were used for CL measurements. In PEA2PbBr4 and PEA2PbI4, a multivariate analysis decomposition highlights the presence of an emission originating from the bulk of the material and a blue-shifted emission visible along the edges in the CL hyperspectral maps. The analysis was extended to lateral PEA2PbBr4 - PEA2PbI4 heterostructures (for which the elemental distribution is shown in the figure), identifying emission from both the phase in the bulk (original phase of the flake) and the one along the edge (dominated by the replaced halide). The emissions were correlated with local compositional changes.
Furthermore, we have carried out a 4DSTEM analysis to verify local crystallinity in thin PEA2MA2Pb3I10 flakes at low electron doses. The flakes were good single crystals, with loss of crystallinity observed at the edges. The monochromated EELS signal was integrated over large areas

to minimise dose and used to calculate optoelectronic properties, finding good agreement of the calculated bandgap with optical measurements.
Conclusion
We were able to obtain spatially resolved CL measurements on layered halide perovskites, identifying emission heterogeneities. We also characterised local crystallinity and demonstrated the ability of measuring the local bandgap in individual flakes using EELS. These approaches will enable tailoring of the material engineering, leading to better light emitting and management devices.

Keywords:

Cathodoluminescence EELS halides perovskites

Reference:

[1] Andrea Griesi et al 2024 Nanotechnology 35 105204, DOI 10.1088/1361-6528/ad12ec

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Elucidating the effect of silicon on Fe-Zn phase formation in galvanized steel via advanced TEM

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Introduction

Raising the bar for weight reduction, safety, and environmental protection in modern automotive and construction materials can be barely achieved without new types of Advanced High-Strength Steel (AHSS) [1] and must be guided by in-detail structural and chemical characterization. To meet safety and durability requirements, modern AHSS products additionally need effective corrosion protection, and the galvannealing process, which involves hot-dip galvanizing in a Zn bath followed by annealing, is considered to be one of the most efficient methods for achieving this goal. Such a well-balanced but complex process of interfacial interactions can be significantly affected by alloying elements, which are added in AHSS to augment its mechanical properties and achieve a superior combination of high tensile strength and good formability [1,2]. For instance, silicon affects the steel bulk properties enhancing the Fe-liquid-Zn interfacial reaction by solute Si in the α-Fe phase. An additional effect is attributed to the formation of surface oxides on the steel sheet during annealing before immersion in the Zn bath [3,4]. One can conclude that the structural and chemical peculiarities of the steel/coating interface region including the so-called inhibition layer, e.g. [3], must be considered as a key factor governing the phase formation kinetics. Advanced transmission electron microscopy (TEM) can help us collect morphological, structural, and elemental information at the nanoscale. For a deeper understanding of phase evolution, it is necessary to study not only fully galvanized samples processed at different annealing times but also steel sheets at the outlet of the bath, coated with almost pure Zn.

Methods

We optimized various TEM sample preparation techniques and showed that low-temperature FIB is a method of choice for dependable Zn-coated steel preparation [3], which can be further enhanced utilizing a plasma P-FIB operated with, for instance, Xe ions. Cross-sectional TEM lamellae were prepared using a CrossBeam 1540 XB SEM (Zeiss, Germany) Ga-FIB using W to form a protection capping layer. The final thinning was performed at -60°C applying a Micro Heating Cooling Stage (MHCS) (Kleindiek Nanotechnik GmbH, Germany) based on the thermoelectric effect and 5 kV acceleration voltage to minimize the invasive influence of the Zn-Ga eutectic formation. The investigation was carried out in a JEOL JEM-2200FS (JEOL, Japan) operated at an acceleration voltage of 200 kV. The TEM is equipped with an in-column Ω-filter and a TemCam-XF416 (TVIPS, Germany) CMOS-based camera. HRTEM data processing was done with Gatan Microscopy Suite. Crystal structure simulations were performed via JEMS software. STEM EDX analysis was fulfilled in a scanning (S)TEM mode for qualitative elemental characterization of the specimens with an X-MaxN 80 T detector from Oxford Instruments (United Kingdom).

Results

Employing complementary TEM techniques (HRTEM, SAED, STEM EDX), we traced the formation of Zn-Fe phases in AHSS with high Si content at different stages of galvannealling process along with as-galvanized reference specimen [4]. In particular, it has been disclosed that the Si-based surface oxide layer, formed on steel during the recrystallization annealing step before dipping into the Zn bath

(460°C) remains stable, separating the coating from the reaction zone (Figure I). This efficiently hinders the desired reaction of Fe with Al in a Zn bath at the early stage of hot-dip galvanizing and complicates the formation of the Fe2Al5-xZnx inhibition layer. One can reveal that liquid Zn can penetrate local disruptions in this film forming a δ phase layer below, while Fe diffusion into the coating is suppressed. The δ layer supersaturates with Fe and Si from the steel during long-term annealing at 480°C and decomposes forming a δ phase [5] matrix with Fe-Si-Al-based nanoprecipitates with a cubic structure (Figure II). The phases were identified via HRTEM and STEM EDX. Based on experimental STEM EDX data, the achieved phase configuration was refined via Thermo-Calc Fe–Zn–Si–Al system simulation. It must be emphasized again that the observed Si-based oxide layer remains stable even after long-term annealing, thus Si, which constitutes this membrane, was not actively involved in the Fe-Zn reaction.

Conclusion

Our results indicate the following evolution of phases at the steel/coating interface of AHSS subjected to an industrial continuous hot-dip galvannealing process. During dipping in a liquid Zn bath, Zn penetrates the gaps in an existing oxide film and forms a layer below directly reacting with the Fe. This layer was identified as the δ phase. Thus, the desired Fe2A5-based inhibition layer cannot be formed as intended. Mentioned δ phase layer can grow during subsequent annealing being, however, efficiently constrained by the mixed oxide membrane. While long-term annealing, Si dissolved in the steel destabilizes the δ phase supersaturated with Fe and Al, triggering its decomposition into the δ phase and Fe-Si-Al-based nanoprecipitates. The challenges and solutions on the way toward a fruitful and dependable TEM analysis of galvanized industrial steels will be also discussed in detail.

Acknowledgments: We would like to gratefully acknowledge the financial support by the Austrian Federal Ministry of Labour and Economy, the National Foundation for Research, Technology and Development and the Christian Doppler Research Association.

Keywords:

Fe-Zn, phase formation, galvanized steel

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Correlative characterization by X-ray tomography, SEM/FIB and TEM using reference markers: bridging imaging with micro-structuring

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Correlative characterization is the spatial registration of several imaging modalities with the aim to combine complementary information on the same region of interest [1]. The imaging modalities are furthermore supplemented by micro-structuring tools such as focused-ion beam (FIB) technology for selective extraction of the region of interest (ROI), and femtosecond-laser milling for material removal.
Within the same length scale, analyzing identical ROIs using several modalities is well-established in electron microscopy, e.g. various STEM imaging modalities (BF, DF, HAADF) with EELS and EDX spectroscopy or also the combination of APT and TEM. Across length-scales, the analysis of identical ROIs requires combination of different instruments. Without a correlative approach, the characterization of the full length-scale hierarchy relies on statistically random sample extracts, which only provides a good representation for very homogeneous samples, with similar ROIs uniformly distributed within the sample. However, for macroscopically inhomogeneous samples, such as heterogeneous catalysts, a correlative approach is indispensable to characterize the individual components of the sample. Therefore, for linking the local micro-structure to the macroscopic level of the sample, a full correlation of all applied techniques on the part of the sample containing the ROI is desired.
We present a correlative workflow, which allows the combination of 3D analysis across several length scales from the bulk sample with defined laser pre-structuring for micro-CT at the sub-mm level, identification of regions of interest on the 10-100 µm scale in a FIB, for 2D/3D surface and tomography visualization by SEM/EDX/EBSD and light microscopy, followed by defined sample extraction and transfer to nano-CT and TEM.
For this purpose, we developed a dedicated sample carrier, enabling large tilt angles for FIB work, as well as flexibility for direct transfer to micro-CT. The carrier is equipped with global three-dimensional reference markers for accurate determination of the rotation, tilt, and translation of the carrier with the sample. Additionally, local markers, in the form of Pt-deposited or FIB-milled patterns, are placed directly on the sample in the vicinity of the ROI. These facilitate local navigation and accurate translational positioning around the ROI for FIB-extraction, after the rotation and tilt has been aligned by means of global markers.
The workflow, with the use of the correlative sample carrier, global and local markers, as well as a series of geometrical calculations, gave a two-fold result. Firstly, an accurate image correlation in 3D was enabled, overlapping 3D tomographic and 2D projection images virtually in image-processing software, e.g. Dragonfly. Secondly, step-by-step instructions, in the form of a simulated FIB projection image, were generated for real-time overlap with the FIB image, leading to an accurate FIB-extraction of the ROI for nano-CT and/or TEM. We verified this workflow on samples from metallurgy [2] and catalysis [3].

Using global and local reference markers, and tracking them at different length scales, we achieved accurate ROI-targeting for FIB-extraction, and covering multiple length scales and imaging techniques. The workflow is aimed at routine multi-scale, multi-modal correlative investigations on samples from materials science, targeting specific ROIs, thereby gaining local chemical and structural information from bulk samples.

Keywords:

correlative workflow; tomography; microscopy, FIB

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Domain structures in ferroelectric epitaxial WO3 thin films

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Poster Group 1

Background incl. aims
Tungsten oxides (WO3-δ) have in the past received attention for their gas sensing, catalytic and electrochromic properties. Recently, ferro- and piezoelectric properties123 coupled to local mechanical strain and even supercondutivity45 have been reported in tungsten (tri)oxide as well, inspiring interest in the electronic properties of these materials. However, this behavior is not yet fully understood and investigations of epitaxial films of tungsten oxides are particularly scarce. In this work, we aim to provide some insight into the nature and formation of ferroelectric domains and domain walls and their structural and electronic properties.
Methods
Epitaxial films of tungsten (tri)oxide were grown by pulsed laser deposition at different thicknesses on a number of different substrates to reveal the effects of (epitaxial) strain. The substrates used are yttrium aluminate, lanthanum aluminate and strontium titanate. Atomic force microscopy and piezoresponse force microscopy were used to visualize the surface topography of the resulting films and to image the ferroelectric domain structures, respectively. In addition, conducting atomic force microscopy was utilized to show local electrical conductivity. The nanoscale structure of the films was probed using aberration-corrected scanning transmission electron microscopy (STEM). Differential phase contrast imaging enables the imaging of the lighter oxygen anions. Real-space analysis of the STEM images using an in-house developed software tool provides a sensitive means for visualizing local strain gradients and polarization.
Results
Our results suggest an intricate coupling between the epitaxial strain imposed by the substrate, film thickness and the ferroelectric domain structure in the film. Low-strain films grown on yttrium aluminate show a fairly traditional domain structure consisting of a combination of purely polar, non-ferroelastic and ferroelastic domain walls, distinct from the type of domain structure reported for thicker films on the same substrate1 in which the polar nature of the film is localized to the domain walls, as well as those reported for bulk tungsten trioxide based on theory2. Electrical conductivity is found to be enhanced in the vicinity of the domain walls. In contrast, a higher degree of epitaxial tensile strain suppresses the formation of domains altogether, favoring strain accommodation through an extended planar defect.
Conclusion
We provide some new insights into the formation of polar domain walls and strain accommodation in epitaxial thin films of tungsten trioxide under different conditions of epitaxial strain and show that there is potential for the functionalization of domain walls therein.

Keywords:

ferroelectrics, strain, STEM, PFM, epitaxy

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Enabling atomic resolution electron microscopy at elevated temperature and beyond pressures of a few bar

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PS-01 (1), Lecture Theater 3, august 28, 2024, 10:30 - 12:30

Background
Transmission electron microscopy (TEM) is an invaluable tool in knowledge-driven optimization of catalysts. Over the years, important mechanistic insights in chemical processes have been gained by combining the atomic resolution of TEM with miniaturized reactors. This type of reactors confines gas or liquid phase reaction environments to the vicinity of the catalyst between pairs of thin electron-transparent windows and has opened up for in situ and operando studies of catalysts during reactions conditions mimicking industrial catalysis. However, the electron beam has to traverse the reaction environment as well as the windows which, in turn, have to remain impermeable and mechanical stable to sustain pressure differences of up to several bars and therefore typically consist of 15-30 nm thick Si-based membranes. In practical terms, such membranes reduce signal contrast, and eventually resolution. Thus, thinner windows of similar strength and impermeability are needed to further improve imaging conditions.

The class of two-dimensional (2D) materials, and especially graphene, are interesting in this regard. It has been observed that graphene membranes sealing small volumes in a substrate can sustain pressure differences beyond 15 bars before delaminating from the substrate, and in addition, even a single layer of graphene is highly impermeable. 2D materials adhere to substrates via the Van der Waals force, and so graphene's ability to seal off a substrate, is of utmost importance, if graphene is to be employed as window material. At this stage, the performance of graphene-based seals at room temperature, i.e. leakage circumventing the graphene-based seal, exhibits large variation and allows gasses to leave sealed volumes through the interface between seal and substrate, making graphene-based windows impractical. Furthermore, the temperature dependence of the leakage have until now been unknown.

Methods
To investigate the leak from graphene-sealed volumes, we prepared electron transparent cavities and sealed them by mechanically exfoliating few-layer-graphene sheets on top. After exfoliation, the samples were cut to fit a GATAN heating holder. Prior to inserting the sample into the holder and subsequently the electron microscope, the cavities were filled with argon in a pressure chamber. Inside the vacuum of the TEM, the gradual decrease in argon content of the cavities was monitored as a function of time and temperature with Electron Energy Loss spectroscopy using a bottom-mounted Gatan Image Filter.

Results

At elevated temperature, the argon content of the cavities was found to decrease exponentially with time in accordance with what has been found at room temperature in previous studies. Furthermore, the exponential rate constant of the decay was itself exponentially dependent on the temperature, i.e. exhibiting an Arrhenius dependency. This indicates that the diffusion from the graphene-sealed cavities is a thermally activated process in which the argon atoms are surpassing an energy barrier. This behavior was found across different samples and cavities sealed by the same flake, showing the generality of the phenomenon. Surprisingly, repeated emptying and filling of the cavities at elevated temperature drastically improved the leak tightness of the seals, and in one case, the leak rate at room temperature improved 6 orders of magnitude from an already decent origin, as compared to previously reported leak rates. Consequently, some graphene-sealed cavities could after repeated experiments sustain pressures up to approximately 10 bars at 500 °C, while exhibiting a remarkably low leakage.

Conclusion
Our investigations reveal that graphene-based windows can be used for in situ and operando high resolution electron microscopy at elevated temperature and beyond pressures of a few bar. However, thermal engineering of the graphene seal is needed to tune the graphene-based seals' performance properly.

Keywords:

Graphene, Thermal, Seal, TEM, Heating

Reference:

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ACS Applied Materials and Interfaces, 2020, 12, 33, 37750–37756
Nanoscale, 2023, 15, 16896-16903

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Deep convolutional neural networks for atomic imaging in STEM

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Scanning Transmission Electron Microscopy (STEM) is a well-established method for looking into the physical properties of complex nanostructures. However, a major drawback is that acquiring very high-resolution images may lead to negative effects such as radiolysis and knock-on damage [1]. It has been shown that by lowering the electron beam dose, sample damage is reduced, however this leads to a lower signal-to-noise ratio (SNR) reducing the final quality of the image [2].
Convolutional Neural Networks (CNN) are a type of feed-forward neural network that is used as a powerful tool for improving image SNR through methods such as denoising. Recently, aberration-corrected STEM using CNNs has shown promising results, achieving resolutions below 0.1 nm. Specifically, improved SNR without using high-dose electron beams has been achieved by using CNNs trained on large datasets of microscopy data [3]. These methods have demonstrated the capability of CNNs to reduce damage to samples by improving image quality of low-dose STEM below 0.1 nm.
Inspired by the successful use of deep learning-based convolution for noise reduction [4] outside of STEM, we propose the use of self-supervised deep CNNs trained on both real and synthetic high-dose data to improve the SNR of low-dose data. We aim to create a robust network that is portable to methods outside of denoising by using the high-dose data to retrain the network for a variety of conditions [5] (such as hysteresis, defocus, and image blur).
This talk will present the results of utilizing self-supervised deep learning CNNs to improve the quality of low-dose data below 0.1 nm. We will also compare our proposed method to the STEM neural network autoencoder [3] and SDnDTI for Magnetic Resonance Imaging (MRI) [4] and discuss the potential of our method to improve upon on current methods within STEM as well as other domains by analyzing the SNR with the final image structural similarity (SSIM).

Keywords:

STEM, Deep Learning, Aberration Correction

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Analyzing Lithiation Dynamics in LiFePO4 Cathode : Insights from TEM Experiments and Phase Field Modeling

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Lithium-ion batteries have revolutionized the field of portable electronics and electric vehicles by providing efficient and compact energy storage solutions. Despite advancements, several challenges continue to persist, such as limited battery lifespan, capacity degradation, and safety concerns. To address these issues and gain deeper insights into the underlying mechanisms, we present a comprehensive study involving an electrochemical liquid cell for in situ transmission electron microscopy (TEM) experiments. The utilization of the Dark Field technique (Figure 1) in this device enables us to directly observe the lithiation/delithiation process [1]. Through electron diffraction, we can track the modification of lattice parameters induced by the lithiation process.

In this study, we can observe the dynamic evolution of the delithiation interface between the LiFePO4 (LFP) and FePO4 (FP) phases inside a single crystal. This real-time monitoring provides valuable insights into the fundamental processes occurring during charge and discharge cycles. Additionally, we aim to evaluate the local displacement field extracted from the 4D-STEM ACOM (Acquisition of Crystal Orientation Maps) datasets [2-4]. Indeed, the lithiation process induces modifications in the lattice parameters of the FePO4 host, necessitating the calculation of the symmetric strain tensor at the LFP/FP interface and the estimation of the elastic free energy density. By integrating 4D-STEM data treatments and analysis, we can accurately characterize the strain distribution and its influence on the phase transformation dynamics.

To gain a comprehensive understanding of the lithiation/delithiation dynamics and further elucidate the underlying mechanisms, we employ phase field model to simulate the diffusion phenomena. The phase-field model has proven to be a powerful tool for studying phase transformations and microstructure evolution in materials. Here, we use an Allen-Cahn type phase-field approach to investigate the phase transition from LiFePO4 to FePO4 cathode crystals in the presence of Li-ion chemical potential difference with the surrounding liquid electrolyte. The solid-solid phase boundary between LFP and FP phases modeled as a diffuse interface of finite width [5], and its motion is implicitly governed by the phase-field variable. We numerically solve a set of partial equations describing the temporal evolution of the phase-field variable and the chemical potential [6, 12]. By incorporating the real particle morphology, obtained by TEM imaging, and considering the smoothed boundary method (SBM) [10], our phase field model provides a more realistic representation of the lithiation/delithiation dynamics within LiFePO4 crystals.

Initially, we employ a simple 2D model incorporating the chemical free energy and the interfacial free energy with a double-well potential approach [7]. By studying the kinetics of the lithiation/delithiation process within the LFP particle, which follows a one-dimensional Li-ion

diffusion path, we observe two distinct regimes known as Surface Reaction Limited (SRL) and Bulk Diffusion Limited (BDL), as described in [8]. Our simulations are obtained by varying a kinetic parameter l in our equations, this parameter is inversely proportional to the net rate of insertion R that we find in [11].
Furthermore, we extend our model by incorporating the elastic free energy to consider its influence on the phase growth direction and shape during the LFP/FP phase transition [9] within a realistic LiFePO4 single particle morphology. The elastic properties are adjusted using strain maps based on 4D-STEM data treatments and adaptative diffraction image registration.

In conclusion, this research explores the Li-ion insertion (or extraction) mechanisms into (or from) LiFePO4 crystals using in situ TEM experiments combined with phase-field simulations. The correlation between the phase-field model and the 4D-STEM results provides valuable insights into the complex dynamics of phase transformations and microstructure evolution, contributing to the understanding of key factors affecting the performance and lifetime of Li-ion batteries. Our approach contributes to the elucidation of the intricate process that drives the dynamics of lithiation inside individual cathode materials during the electrochemical process.

Keywords:

Phase-field model, Allen-Cahn, LiFePO4, 4D-STEM

Reference:

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Characterisation of 2-D chalcogenides utilising Electron Microscopy techniques and Density Functional Theory.

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Poster Group 2

Two-dimensional material systems have garnered much attention since the first isolation of graphene[1]. Due to their low dimensionality, these materials exhibit interesting properties such as thickness dependent bandgaps [2] and magic twist-angle superconductivity [3], making them attractive for both experimentalists and theorists alike with much promise for industry application. In this study we focus on the utilization of electron microscopy techniques to experimentally characterise various chalcogen based 2D systems with the intent to compare it to Density Functional Theory (DFT) simulations.

Of particular interest for this study is the effect of various structural changes that influence the electronic structure. Firstly, a twist-angle induced Rashba splitting at the VBM in van der Waals heterostructures and secondly, the influence of various point defect complexes and their effect on the electronic band structure.

Herein, the angular dependence of GaS/GaSe heterostructures are theoretically studied via DFT calculations and compared to preliminary experimental studies. Electronic band structure calculations were performed on a series of twisted GaS/GaSe heterostructures with and without spin-orbit coupling (SOC) to investigate the effect of band splitting. The mobility of point defects in exfoliated PtSe2 was correlated using the climbing image Nudged Elastic Band method incorporated in the FHI-aims all-electron code. The atomistic models observed experimentally were geometrically optimized using FHI-vibes.

Bulk GaS and GaSe crystals (2D Semiconductors, Inc.) were exfoliated using the typical scotch-tape method. Flakes were carefully stacked and transferred using an all-dry viscoelastic stamping technique. In the study of point defect analysis and mobility, PtSe2 (HQ Graphene) was exfoliated through mechanical exfoliation to achieve few layer flakes. Scanning Transmission Electron Microscopy (STEM) imaging was performed using a FEI Titan 80-300 at 300 kV and a Nion UltraSTEM at 60 kV and 200 kV. STEM images were recorded with a high angle annular dark-field (HAADF) detector on a Nion UltraSTEM. Multi-frame imaging was utilised to reduced beam damage, while custom scan patterns minimised beam induced defect motion.

Results from first-principles calculations predict that GaS/GaSe heterostructures demonstrate the presence of Rashba spin splitting effects and an angular dependent band-gap shift. Rashba splitting was found to be asymmetric along different high symmetry paths. Heterostructures were fabricated at various twist angles and imaged using HAADF STEM with thickness measurements being performed via PACBED simulation. Low voltage HAADF-STEM was further utilised to investigate point defects inherent in exfoliated PtSe2. Many examples of Se/Pt vacancies and antisites were present

along with various combinations of the two. Mobile defects were observed in few layer regions. Fast frame image series were utilized to study the hopping nature of the defects to determine energetically favorable pathways.

The collaborative use of theoretical modeling via DFT and experimental characterisation through TEM was used to characterise novel 2D material systems.

Keywords:

2-D Materials, Heterostructures, STEM, DFT

Reference:

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THE INVESTIGATION OF THE PRESENCE OF TELOCYTE CELLS IN THE HUMAN OVARIAN STROMA

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Poster Group 1

Background: Telocytes (TCs) have been identified as a rather new and fascinating type of interstitial cell. Previously known as interstitial Cajal-like cells (ICLCs), these cells are now distinguished in the literature by their extremely long and thin telopods (Tp) and by the various sized vesicles they secrete into their environment. To date, these cells have been detected in several organs of humans and many other species. TCs are also found in female reproductive organs, although no data on the existence or morphological characterisation of TCs in human ovaries have been identified in the literature to yet. Furthermore, studies on human ovaries have shown the existence of many stromal cell clusters. However, there is still a significant lack of detailed research describing these cell types. This emphasizes the need for more research into understanding and identifying ovarian stromal cells, including the possible discovery of telocytes (TCs) in human ovaries. The ability of TCs for cell-to-cell signaling, plays an essential role in the regulation of homeostasis, immune surveillance and tissue morphogenesis. It has also been shown that these cells play important roles in angiogenesis, embryogenesis, and various pathologies or even tumorigenesis, under the guidance of tissue-resident stem/progenitor cell self-renewal and differentiation. Expressions or co-expressions of different markers of TCs have been reported in certain organs, but in general, CD34, vimentin, PDGRF-α and PDGRF-β, c-kit and α-SMA are primarily used in TC studies and these are so far considered as reliable TC markers. A combination of double immunofluorescence and electron microscopy is the definitive approach for identifying these cells, setting the gold standard in terms of accuracy and precision.
Aims: This research aims to advance our understanding of ovarian stromal cells and detect the presence of TCs by employing techniques such as immunohistochemistry and electron microscopy. Through the investigation of their morphology and identification of telocytes in the human ovarian stroma, this study endeavors to provide novel insights into ovarian architecture and its implications for reproductive health.
Methods: Tissue samples were obtained from women aged 18 to 65 undergoing Total Hysterectomy and Bilateral Salpingo-Oophorectomy (THBSO) for uterine reasons, excluding those who had received chemotherapy. After tissue preparation procedures for light microscopy, immunohistochemical and double immunofluorescence staining used CD34/c-kit, CD34/vimentin, CD34/PDGFR-β, and CD34/α-SMA markers for telocyte identification. Transmission electron microscopy and the immunogold technique, utilizing the CD34 marker, facilitated precise telocyte identification. This comprehensive approach aimed to illuminate telocyte presence and distribution in the ovarian stroma, offering future insights into ovarian physiology and pathophysiology.
Results: Our study confirms the presence of telocyte cells within the human ovary, characterized by their distinctive morphology. We detected telocyte cells in the ovarian stroma through

immunohistochemical analysis, labeling specifically with CD34 which is the most commonly used telocyte marker. Additionally, we utilized immunofluorescence analysis, employing double labeling with CD34/c-kit, CD34/vimentin, CD34/PDGFR-β, and CD34/α-SMA to further characterize these cells and distinguish them from fibroblasts and pericytes. We found out that TCs were strongly positive for CD34, PDGFR-β, vimentin, and weakly positive for α-SMA. On the other hand fibroblasts were found to be CD34 negative, while strongly positive for vimentin and PDGFR-β. Lastly pericytes were found to be CD34 negative and strongly positive for α-SMA and PDGFR-β. Notably, our findings delineate telocyte cells from fibroblasts and pericytes, underscoring their unique cellular identity within the ovarian microenvironment. Moreover, transmission electron microscopy provided detailed insights into the ultrastructural features of these cells, enriching our understanding of their morphology. TCs were recognized for their long and thin prolongations and small cell bodies primarily situated around blood vessels and dispersed throughout the ovarian stroma. Additionally, the use of the immunogold technique in conjunction with the CD34 marker facilitated the precise identification of telocyte cells, increasing the validity of our findings.
Conclusion: Overall, our study significantly contributes to the understanding of telocyte biology within the context of the ovarian stroma, shedding light on their presence, distribution, and morphological attributes, thus paving the way for further exploration of their functional significance in ovarian physiology and pathology.

Keywords:

Human Ovary, Telocytes, Immunofluorescence, TEM.

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Ptychography Optimization for Atomic Analysis of Bending Mode in Bilayer Transition Metal Dichalcogenide Translational Motion

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Poster Group 2

Background incl. aims
Twisted two-dimensional transition metal dichalcogenides, have exhibited a variety of interlayer coupling phenomena and novel structural reconstructions, leading to modifications in electronic properties. However, the atomic-scale transitions within these reconstructed structures could be observed only on scales of a few nanometers, with transitional motions requiring differentiation at sub-nanometer scales. Thus, atomic scanning transmission electron microscopy-based methods are indispensable. However, due to the beam sensitivity and instability of structures, acquiring noise-free images has posed a challenge. In this study, we utilized ptychography as a fitting experimental methodology to investigate these twisted structures.
Methods
In our study, we employed suspended homobilayer WSe2 as the substrate for sample preparation. The fabrication process involved a tear and stack method, utilizing monolayers derived via the Scotch tape technique. For the analysis, we utilized ptychographic algorithms available in the abTEM and Py4DSTEM software packages. Both multislice and single-slice calculations were performed to analyze the data. This methodology allowed for a comprehensive examination of the atomic transitions within the suspended twisted 2D structures of the WSe2 samples.
Results
By adjusting various parameters for ptychography, we have identified the most stable conditions for examining suspended bilayer bending mode samples. The optimization of ptychographic conditions, including dose, focus, scan size, and other experimental parameters, has been pursued to secure the most effective imaging results. Under these optimal conditions, an atomistic analysis of the suspended bending mode twisted samples was conducted. Remarkably, the rippling domain boundaries, which had been theoretically anticipated for the bending mode, were directly observed through ptychography under low dose conditions, facilitating stable image acquisition. Furthermore, we have delineated the transition from the vertex AA core to the saddle point across varying angles, a phenomenon previously unobserved in High-Angle Annular Dark-Field (HAADF) imaging.
Conclusions
In conclusion, this study has leveraged ptychography to unveil novel insights into the atomic-scale transition of twisted two-dimensional transition metal dichalcogenides, achieving imaging clarity under optimized conditions. Our methodical approach in adjusting ptychographic parameters has enabled the direct observation of rippling domain boundaries and the intricate transition from the vertex AA core to the saddle point. These findings not only overcome previous limitations posed by beam sensitivity and structural instability but also significantly enhance our understanding of the structural and electronic properties of these complex materials, paving the way for future explorations and applications.

Keywords:

Ptychography, Twisted 2D materials, 4DSTEM

Reference:

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Comparative Analysis of Self-Supervised Learning Techniques for Electron Microscopy Images

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Poster Group 2

Background incl. aims
Deep learning has revolutionized a wide array of tasks across different domains, including electron microscopy (EM) image analysis, by leveraging large labeled datasets for training. However, the scarcity of such labeled datasets in EM necessitates the exploration of alternative methods. Self-supervised learning (SSL) emerges as a promising approach to leverage unlabeled data, featuring techniques such as, e.g., masked image modeling (MIM) — which predicts missing parts of the input data, as well as contrastive learning — which learns by distinguishing between similar and dissimilar pairs of data. This study aims to investigate the impact of these SSL techniques on EM images, providing a case study on the effectiveness of leveraging unlabeled data in a domain where labeled datasets are limited and expensive to create.
Methods
Utilizing the “NFFA dataset”, which comprises 21,169 Scanning EM images across 10 categories, we established a baseline by training models from scratch with random weight initialization. We then pre-trained models using two SSL approaches: Masked Autoencoders (MAE) for MIM and Momentum Contrast V3 (MoCoV3) for contrastive learning, followed by fine-tuning on the NFFA dataset. Another pixel-based MIM technique, Multi-level Feature Fusion (MFF), was also tested. The performance of each SSL technique was evaluated based on accuracy improvements and convergence speeds relative to the baseline. Our analysis highlights the distinctions between MIM and contrastive learning approaches in handling EM images.
Results
The baseline model yielded an accuracy of 77.42%. Upon employing SSL techniques, significant improvements were observed: finetuning with MAE weights achieved an accuracy of 92.84%, MFF led to 93.86%, and MoCoV3 led to an accuracy of 92.56%. MFF, in particular, demonstrated a superior ability to enhance feature learning from unlabeled data, indicating its impact in the task of EM image classification. Furthermore, all SSL-pretrained models showcased accelerated convergence rates compared to the baseline.
Conclusion
This study confirms the viability and potential of SSL techniques in EM images. MIM, exemplified by MFF, outperformed contrastive learning in this domain, suggesting that methods focusing on reconstructing or predicting unseen parts of the image are particularly beneficial for EM tasks. The results advocate for a targeted selection of SSL strategies based on specific dataset characteristics and task requirements, highlighting a path forward for efficient model training in EM image analysis and beyond. The influence of SSL pretraining was studied in this research and experiments were conducted on SEM image classification. Further research in this direction includes investigating the influence of SSL pretraining on dense, pixel-wise classification (i.e., semantic segmentation) tasks in EM.

Keywords:

Self-Supervised-Learning, Electron-Microscopy, Image-Classification, Masked-Image-Modeling, Contrastive-Learning

Reference:

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EDX-based annotation of biological features in large-scale EM

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Poster Group 2

Background
Advances in electron microscopy (EM) of bio-samples now enable ultrastructure to be inspected at biologically relevant scales. Capabilities in data analysis have however not kept pace with the throughput increase offered by novel acquisition approaches and automation. Moreover, the greyscale nature of the electron micrographs complicates a comprehensive analysis of all that is hidden within. The analysis of EM datasets is typically based on manual annotations, allowing in-depth analysis by segmenting the data into biologically meaningful features. However, such annotations are laborious and subject to the annotator's interpretation of the data. Furthermore, deep learning can used to automate segmentation but the need for manually annotated ground 'truth' remains. Energy-dispersive X-ray (EDX) imaging, or ColorEM, provides elemental context to the recorded ultrastructure, allowing variations in elemental concentrations to be reflected in color [1]. In this study we further tailored acquisitions to ColorEM and explored its potential to highlight biological features in a data-driven manner.

Methods
Pancreatic tissue composed of hormone-containing endocrine cells and zymogen-containing exocrine cells was epoxy-embedded and subjected to ultramicrotomy. A Thermo Fisher Scientific Talos S/TEM equipped with two Bruker XFlash 6-100 EDX detectors was used to interrogate the sectioned sample. The spectral richness was promoted by improving the acquisition parameters necessary for heterogenous cellular material. Greater context was provided through a mosaic acquisition of a full islet of Langerhans.

Results
ColorEM has been further improved and successfully implemented on pancreatic tissue. The high-resolution and energy-dispersed recordings allow the ultrastructure of biological tissue to be visualized in its elemental context. Various biological features such as heterochromatin, hormone- and zymogen-containing granules and lysosomes, can be readily discerned in the elemental images. The final large stitched image not only provides elemental context to the ultrastructure, but also places this in the larger context of the tissue.

Conclusions
Here we implement large-scale ColorEM, supplementing the spatial EM data with spectral EDX data. We leverage the addition of the spectral dimension to provided elemental context to the recorded biological ultrastructure, with the tiled acquisition providing the context of the tissue. Selection of which elements to visualize is based on a data-driven approach where the dissimilarity in terms of elements, and combinations thereof, amongst the biological features are identified.

Keywords:

STEM, EDX, ColorEM, Label-free, Hyperspectral

Reference:

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Improving segmentation of FIB tomography data

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Poster Group 2

Background incl. aims
Accurate reconstruction of nanostructures using focused ion beam (FIB) tomography data is challenging due to slicing and imaging artefacts, as well as intensity ambiguities in the scanning electron microscope backscattered electron (BSE) images. We propose a multimodal machine learning approach that combines intensity information obtained at multiple electron beam accelerating voltages (multiV) to improve the three-dimensional (3D) reconstruction of hierarchical nanoporous gold (HNPG) structures. The proposed method significantly improves segmentation accuracy and leads to more precise 3D reconstructions for real FIB tomography data.
Methods
MultiV FIB tomography of epoxy infiltrated HNPG with ligament sizes of 15 nm and 110 nm was performed using a Dual Beam FEI Helios NanoLab G3 system and its ASV4 control software for automated tomography. During multiV tomography, each slice was imaged using a BSE detector three times with accelerating voltages of 1, 2, and 4 kV and a beam current of 50 pA. To compensate for drift during the process, 2 fiducial markers were prepared and positioned on the cross-section and on top of it. A ruler system was implemented also on top of the cross-section to monitor and measure the thickness of each slice. We developed 3 multimodal architectures for 3D nanostructure reconstruction with machine learning, employing different data fusion techniques: early fusion, intermediate fusion and late fusion.
Results
Our results indicate that the late fusion architecture excelled among the three options. Remarkably, the intermediate fusion architecture exhibited significantly poorer metrics than the late fusion architecture. This drop in performance can be attributed to the large size of the ML model, which posed challenges for optimization given a limited amount of training data. However, the effectivity of training data may be improved using domain adaptation. In a comparative study, confronting our ML-multiV method with a cluster-based k-means clustering algorithm and also ML models trained using individual single kV datasets, the multiV model outperformed all other segmentation techniques.
Conclusion
FIB-SEM tomography data are affected by artifacts and ambiguities in image intensities. These effects make it difficult to use cluster-based segmentation methods. More advanced ML-based methods can efficiently suppress the effects, even when trained only on a single set of synthetic FIB tomography images. The multimodal ML method with a late fusion architecture using multiV imaging data will further improve segmentation accuracy.

Keywords:

FIB tomography, multimodal ML, segmentation

Reference:

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Phase formation pathways of Me2+ oxides on sapphire (α-Al2O3) substrates

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Background
Aluminate structures present phase formation anomalies irrespective of the initial stoichiometry of the oxide powder mixes. In metal oxide systems (Me2+ or Me3+), multiphases form at different cation to aluminum (Mex+/Al) ratios. Singh and his colleagues observed multiple phase formation in starting mixture ratios of CaO to Al2O3; 1:1, 1:2 and 3:1. Even if the stoichiometry started from calcium or aluminum rich compositions, CA was present in the diffractograms (Singh et al. 1990. p.873). Previous studies on calcium aluminates suggested that large cation size of Ca2+ may create a difficulty of diffusion into the Al2O3 (Tian et al. 2016. p.104). Tian and his coworkers demonstrated the distribution of Ca-rich phases with backscattered electron (BSE) images towards the outer layers of the core shell structure. In the center of this core shell structure Al2O3/CA6 was present. We observed that the first forming phase in strontium, calcium and yttrium aluminate systems is 1:1 oxide compound SA (SrAl2O4), CA (CaAl2O4), and YAP (YAlO3) irrespective of the starting Mex+/Al3+. This study claims that the Mex+/Al ratio at the interface determines the first forming incipient phase. Also, it appeared that having a single type of Al-O coordination polyhedra is facilitating the phase formation in aluminate systems.

Methods
SrO-Al2O3 binary system have 6 compounds: Sr4Al2O7, Sr3Al2O6, SrAl2O4, Sr4Al14O25, SrAl4O7, and SrAl12O19 (Fig.1). Phase pure aluminate powders were synthesized using Pechini method. Crystallization temperatures of aluminate powders were determined with thermogravimetric analysis. Phase distribution above 800-900 °C, the first crystallization, was recorded with ex-situ XRD by 50-100 °C temperature slots. Isothermal heating of 1 hour was applied to compounds. The temperature was increased until there was no change in the phase distribution of each compound. These powders were used to acquire Al-L2,3 and O-K ELNES edges of phase pure aluminate compounds with different stoichiometry to be used as a fingerprint of the compound.
Heating temperatures of deposited substrates were selected for each compound in a way that samples were XRD phase pure of desired stoichiometry. Amorphous or crystalline precursors having different Ca/Al ratio were deposited on sapphire substrates with different orientations. The effect of changing stoichiometry at the interface of the reaction couple was adjusted as a coating. Each deposited substrate was characterized with scanning electron microscopy (SEM) and X-Ray diffractometry (XRD) before the sample preparation for transmission electron microscopy (TEM). Then, the first structure to form at the interface was analyzed with the help of high-resolution TEM (HRTEM), HR-STEM, and EELS-ELNES. Different aluminate compounds at the interphase between reaction couples were compared with ELNES fingerprint of each pure aluminate compound.

Results

All sapphire substrates were analyzed with X-ray diffractometry (XRD). Four strontium aluminates were identified with ELNES edges (Fig.2 a-d). Al-L2,3 edge at an energy of 73.1 eV was chosen as the fingerprint for each aluminate compound. K edge of oxygen is at 532.0 eV. We tried to identify the incipient structures at the interface of sapphire-aluminate film with different Sr2+/Al3+ ratio with the Al-L and O-K fingerprints of phase pure compounds.

Conclusion
Our study was a first attempt to clarify what kinetic constraints are determining the incipient metastable aluminate phase in Sr-, Ca-, Y- aluminate systems.

Graphic

Figure 1: Equilibrium binary phase diagram of SrO-Al2O3 (Van der Heggen, D. et al. 2022. p.3).

Figure 2. Al L2,3 ELNES edges of a) Sr4Al14O25 (S4A7), b) SrAl12O19 (SA6), c) SrAl4O7 (SA2) and d) Sr3Al2O6 (S3A), respectively.

Keywords:

Interface reactions, aluminates, interphase, EELS

Reference:

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A new transgenic mouse model for functional tracing of circulation via albumin-tagged fluorescent probes

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LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

Liver-secreted albumin is the most abundant protein in blood plasma and cerebrospinal fluid. We have previously developed liver-targeting adeno-associated viral vectors (AAV8-P3-*) that express fluorescent protein-tagged albumin to visualize blood plasma in adult mice (DOI: 10.1016/j.crmeth.2022.100302). We have also established a virally induced CRISPR/Cas9-based knock-in of green fluorescent albumin in neonatal mice (DOI: 10.1101/2023.07.10.548084). Here, we have generated a new transgenic mouse model by CRISPR/Cas9 in which the bright red fluorescent protein mScarlet is knocked into the albumin locus to produce mScarlet-tagged albumin (Alb-mSc). In adult heterozygous knock-in mice, the plasma fluorescence signal intensity recorded an order of magnitude higher than a standard AAV-expressed Alb-mSc (AAV8-P3-Alb-mSc, 2E11 vg). This strong plasma fluorescence allowed for the imaging of the entire depth of the cortical vasculature reaching to the white matter (up to ~1mm in depth) by two-photon microscopy. Thin skull preparation enabled the visualization of dura mater by “shadow imaging”, where Alb-mSc presumably infiltrated into the interstitial space from dural vessels. Furthermore, Alb-mSc mice were crossed with Prox1-eGFP mice to delineate lymphatic structures. Alb-mSc signals were subdural interstitial space were clearly attenuated reflecting the composition of cerebrospinal fluid. Nonetheless, shadow imaging was possible in the cortical parenchyma, whereby cortical neurons are detected by the relatively high fluorescence of the extracellular space. The knock-in mouse line offers multi-purpose utilities for studying morphological and functional changes in the brain and other organs.

Keywords:

Circulation, Vasculature, Blood flow, Albumin

Reference:

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Influence of low-pressure atmosphere in the pores formed in hexagonal boron nitride under electron irradiation

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Poster Group 2

Background incl. aims
During (scanning) transmission electron microscopy ((S)TEM), the energetic electrons used for imaging can lead to damage in the sample. Electron knock-on damage caused by elastically scattered electrons is the best known of such damaging mechanisms, and has been studied extensively also for 2D materials. In contrast, less is known about the damage mechanisms in insulating materials, such as hexagonal boron nitride (hBN) . In early studies, it was shown that extended irradiation leads predominantly to the formation of triangular nanopores. These pores and their triangular shape were suggested to arise from direct interaction between the electrons and the material, and it was shown that the dominated zig-zag edges were nitrogen terminated.
Methods
The Nion UltraSTEM 100 microscope integrated into our experimental setup at the University of Vienna allows experiments at low pressure atmosphere between ultra-high-vacuum (UHV) ~ 1x10-10 mbar and 4x10-6 mbar by carefully leaking the desired gas into the column. Measurements at varying pressures have already shown that the partial pressure of different gases can have an influence in the damage observed in graphene. Specifically, it was shown that different graphene edges dominate under an oxygen atmosphere and in UHV.
Results
Here hBN was imaged at UHV and up to an oxygen pressure of around 2x10-8 mbar. The most prominent finding was that the shape of pores that appeared under electron irradiation depends on the oxygen partial pressure. As an example, medium angle annular dark field (MAADF) images are shown in figure. In UHV the pore shape is round with no preference of either boron or nitrogen termination, whereas triangular pores emerge with rising oxygen partial pressure, with edges dominated by nitrogen atoms. The rate of pore growth also increases with increase in oxygen partial pressure in microscope column, and no any pore growth was observed when the sample was exposed to air without electron irradiation.
Conclusion
The results suggest that the shape of the pores observed in the earlier studies was determined by the composition of the microscope vacuum. The pores grow only under electron irradiation and their shape depends on the vacuum conditions.

Keywords:

hBN, in-situ STEM, 2D Materials

Reference:

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Unsupervised Machine Learning-based STEM diffraction pattern denoising for enhanced grain visualization in phase change materials

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background
Phase change materials (PCM) are an emerging class of materials in which different phases of the same material may have different optical, electric, or magnetic properties and can be used as a phase change memory [1]. Phase-change memory materials, exemplified by (Ag, In)-doped Sb2Te (AIST) in this research, have several advantages, including high-speed read and write operations, non-volatility, and a long lifespan [2]. PCMs are able to switch between amorphous and crystalline phases when subjected to heat or electrical current. However, the full understanding of PCMs depends heavily on accurate characterization, often through techniques such as scanning transmission electron microscopy (STEM).
In the field of materials science and nanotechnology, the analysis of STEM diffraction patterns is crucial for understanding the structural characteristics of materials, especially in the context of PCMs. Accurate interpretation of diffraction patterns is essential for crystallographic analysis, phase identification, and grain visualization during an in-situ switching experiment. However, the analysis of STEM diffraction patterns in PCMs can be challenging due to the presence of noise and weak signals (Fig.1 left).
Methods
In this study, we present a solution to address the challenge of grain visualization in PCMs. We propose an unsupervised machine learning (ML) approach that employs an autoencoder to denoise STEM diffraction patterns.
Autoencoders are neural network architectures that have the ability to learn in an unsupervised manner and that are able to represent complex data in a lower-dimensional, noise-reduced form [3]. By applying this technique, we enhance the quality of diffraction patterns, improving the signal-to-noise ratio, which is highly beneficial for further analysis and visualization.
Results
Our results demonstrate a significant enhancement in the clustering and visualization [4] of crystalline grains within STEM diffraction patterns of phase change materials. By reducing noise and enhancing signal clarity, the unsupervised ML-based denoising technique allows for more precise discrimination between different crystallographic orientations and refines the identification of grain boundaries.
Furthermore, we employed clustering based on the non-zero order peak position. Nnotably, this approach yielded significantly improved results for the denoised data (Fig. 2).
Additionally, the proposed denoising enhances pattern-matching quality in commercial orientation mapping software (ACOM ASTAR), indicated by higher average index values and more visible structure in index maps, as illustrated in Fig. 3), facilitating precise analysis of crystallographic orientations and grain boundaries.
Conclusion

The proposed approach paves the way for a deeper understanding of phase change behavior, aiding in designing and optimizing PCMs for various applications, from thermal energy storage to non-volatile memory technology.

As an unsupervised method, it does not require the laborious production of specific training data and, therefore, can serve as a universal tool for STEM diffraction pattern denoising and signal enhancement. Last but not least, the proposed denoising technique is not limited to PCMs; therefore, our work can be understood as a general strategy for enhancing diffraction patterns.

Keywords:

4D-STEM, Denoising, Machine Learning

Reference:

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[2] Zhai, et al., 2013, Physica B-condensed Matter, 408, pp. 12-15.
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Investigation of Production of Boron Nanotubes by Ultrasonication

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Poster Group 2

Background
Since the first report of carbon nanotubes (CNTs), formidable efforts have been devoted to the synthesis of nanoscale tubular one-dimensional structures. Interest is due to their future applications, such as nanoelectronics, optoelectronic and biochemicalsensing devices. Despite their outstanding mechanical and electrical properties, CNT's success fueled the interest in alternative one-dimensional materials that can be mass-produced with high purity. Boron, positioned adjacent to carbon on the left side of the periodic table, exhibits a diverse chemistry comparable only to that of carbon. Because of electron-deficient nature of elemental boron, the BNTs can be identified as promising candidate materials. Recent experiments demonstrating the synthesis of boron nanotubes have heightened interest and expectations regarding their potential applications in various fields. The aim of our study is to develop an understanding for new process that allows the production of BNTs from boron spheres and hollows under milder process conditions.
Methods
All the chemicals were purchased and applied as received without further purification. The experimental materials used in this work were boron powder (purity ≥95%) and isopropanol (purity ≥99,5%). The Boron powder precursor was first mixed in isopropanol. The mixture sonicated with a prope-type sonicator at 400 W under nitrogen atmosphere for 4 h, followed by centrifugation at 4750 rpm for 40 min. The supernatant was collected and thermally treated at 200˚ C in the autoclave with a PTFE container for 12 h. Then 0.05 mL of this sample was dropped on a TEM grid, and the solvent was evaporated prior to transmission electron microscopy (TEM) analysis. The TEM results were obtained using the JEOL JEM-ARM200CFEG UHR-TEM (equipped with STEM, Cs corrected STEM, EDS, Gatan Quantum GIF and Digital CCD Camera) at 200 keV.
Results
When the TEM image at low magnification is examined, the presence of round-shaped amorphous particles with diameters ranging between approximately 50nm and 150 nm is observed, as well as tubes with a length of approximately 200nm and a diameter of 40nm (Fig1). The hallow-like structure can be clearly discerned from the TEM observation (Fig2, Fig3 and Fig4). We believe that, all of these happenings during the production process in the reaction vessels and by the centrifugation depending based on separation of the different structures. We are trying to understand the factors that influence the formation or transformation of different boron structures in particular boron nanotubes. EDS analysis of selected regions shows that the structures are composed of boron with some other impurities (Fig 5). When the ELNES shared in Figure 6 is examined, it is seen that the results in terms of boron element coincide with the EDS data, suggesting that boron is oxidized.
Conclusion
In the method we have developed, it has been seen that boron nanotubes can be produced by ultrasonication. However, it is clear that sonication time, sonication power, thermal treatment and centrifugation parameters should be further optimized. In addition, our studies will continue to increase the nanotube production efficiency and to realize pure production by separating from round-shaped amorphous structures. The fluorine contamination revealed in the EDS analysis is

thought to be due to the PTFE container inside the autoclave where the thermal treatment was carried out. Sodium, calcium, iron and sulfur are estimated to be impurities originating from the boron precursor used in the experiments. It is considered that there is a strong possibility that the detected Titanium may be caused by the ultrasonic probe. Based on the results obtained, the effects of the elements detected and evaluated as impurities at this stage on the formation of boron nanotubes continue to be examined.

Keywords:

Boron Nanotubes, Ultrasonication, TEM

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On how to tame the beast: Towards a high-throughput plasma FIB pipeline

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

Background incl. Aims: Recent advances in data acquisition, processing and sample preparation [1,2,3] have made cryo-electron tomography (cryo-ET) a powerful technology for investigating subcellular processes in situ. However, its usage for clinically relevant systems such as tissues, organoids and other 3D cell cultures remains limited due to the increased sample preparation complexity: With increasing sample thickness, direct milling on its substrate is not always possible. Alternatively, region-of-interests can be removed from the bulk, lifted to a new sample substrate and thinned finally to an electron-transparent lamella of less than 300 nm thickness. Both approaches, the so-called waffle [1] as well as the lift-out [2] method, are typically performed using a gallium ion beam for milling. Due to the low sputtering yield of gallium and the low ion beam currents available in conventional focused ion beam (FIB) microscopes, the throughput of both techniques is limited and the resulting slow lamella preparation poses a major bottleneck in clinical cryo-ET applications. Substantially faster sample preparation is expected with the recently introduced plasma FIB microscopes [4] because of the higher sputtering yields of the different ion beam species and the higher available ion beam currents offered. However, it is currently not characterised how the different ion species and higher currents of the ion beam might impact the sample integrity and how such structural damage propagates through the sample material. Here, we systematically investigate the structural damage caused by different ion beam species and currents. For this, we compare the structural preservation of proteins (e.g. ribosomes) with increasing distance to the impact centre for increasing beam currents by serial lift-outs [5] (see figures A, B). Our findings can be directly used to improve the efficiency of both standard as well as advanced sample preparation approaches, thereby enabling cryo-ET investigations of clinically relevant patient tissue.
Methods: To ensure uniform sample vitrification, S. cerevisiae were high-pressure frozen with a thickness of 20 µm in 20% dextran. To quantify the impact of strong ion beam currents, 100 µm long lift-out blocks were exposed to standard 1 nA and only one side was treated with a high current (15, 60, 200, 500, 1000, 2500 nA; figures A, B). The lift-out block was then cut into a series of 5 µm thin slices, therefore increasing distance to the high-current impact side with each slice. Each slice was then thinned to less than 300 nm. For detailed damage evaluation, tomograms were acquired and reconstructed followed by ribosome picking and subtomogram averaging (Thermo Scientific Tomography 5, AreTomo, crYOLO and Relion 3.1.2).
Results: We examine in total three different types of damage induced by high-current milling: 1) specimen level damage that is already evident by FIB/SEM imaging, 2) damage affecting lamella quality, such as devitrification and curtaining, that is visible by low-magnification TEM imaging and 3) structural damage on molecular level that is only apparent after tomogram acquisition and subtomogram averaging. Damage at specimen level (figure B) and lamella level (figure C) affect both the quantity and quality of lamellae that can be milled in a given time frame. The third type of damage will elucidate which currents can be used for sample preparation to still achieve high resolution after subtomogram averaging (figure D). The usage of serial lift-out allows us to trace the

expected damage gradients after high-current milling for up to 100 µm from the area of impact and gives unprecedented insights into milling-induced damage in biological samples.
Conclusion: Our research provides the first systematic analysis of plasma FIB currents and their induced damage. Having these profound insights into sample-beam interaction will be of great value for developing high-throughput sample preparation pipelines for bulk specimens.

Keywords:

Serial-lift-out, plasma-focussed-ion-beam/scanning-electron-microscopy (PFIB/FIB/SEM), cryo-electron-tomography (cryo-ET)

Reference:

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Imaging the three-dimensional morphology of granular superconductors with energy-filtered TEM tomography

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Poster Group 1

Background incl. aims
This work aims to resolve the structure - property relationship in granular Aluminum (grAl) thin films. GrAl is currently applied in microwave kinetic inductance detectors, parametric amplifiers, fluxonium qubits, resonators and filters. The granular structure and crystallite size distribution of the Aluminum particles have been known for a long time through dark field TEM imaging. The disadvantage of dark-field TEM is that it shows a 2D-projection of a 3D-network. In order to resolve the interconnection between the Al particles, 3D-STEM tomography was applied to resolve the morphology of the grAl. Combination with accurate models for simulations will advance the understanding of granular superconductors and their properties.
GrAl is produced by physical vapor deposition of Aluminum in presence of partial Oxygen pressure. It is a so-called ‘dirty superconductor’. The emerging granular Aluminum nanostructure that is embedded in amorphous Aluminum oxide features tunable non linearity, kinetic inductance and low AC losses in its superconducting state. The Aluminum grains are assumed to form Josephson junctions between each other. A phase shift is induced on the superconducting current while tunneling through a small insulating Aluminum oxide gap between grains. Tunability of the non-linear properties is achieved by adjusting the room temperature resistivity of the film and varying the number of junctions in the network by changing the volume of a grAl component.
The critical temperature for superconductivity TC over the room temperature resistivity of the films reveals a dome shaped relationship with its maximum of 3 K at around 500 µΩcm while TC for pure aluminum is at 1.2 K. Depositing the films on cold substrates decreases Aluminum crystallite size and raises TC even beyond 3 K.
Methods
Two samples are investigated with 120 µΩcm and 25000 µΩcm resistivity. The films of 20 nm thickness are deposited on lacey Carbon TEM grids for easy nanowire templating. Gold fiducial markers are drop cast on the grid. The coated Carbon laces are investigated by energy-filtered TEM (EFTEM) on a Thermo Fisher Scientific Titan Themis Z at 80 kV with a Gatan image filter. EFTEM tomography was applied to reveal changes in al plasmon peak. The tilt series is aligned with the help of fiducial marker positions and is reconstructed using the Discrete Algebraic Reconstruction Technique (DART). Contrast levels are being estimated from an initial Simultaneous Iterative Reconstruction Technique (SIRT) reconstruction.
Results

STEM and TEM imaging show minimal contrast variations between Aluminum Oxide and metallic Aluminum due to similar atomic masses. Image corrected HRTEM shows partially polycrystalline grains in projection. EFTEM at 14 to 16 eV shows good contrast of the Aluminum volume plasmon which is confined to the Aluminum particles. Aluminum Oxide and Carbon give minimal intensity contributions. The low resistivity sample with 120 µΩcm is reconstructed as a network of interconnected 5 nm Aluminum particles with a surface oxide cover of 3 nm. An oxide barrier separating close grains cannot be resolved. The non-linear inductive behavior can be explained with constrictions in the conductive path through the grains instead of oxide barriers as initially assumed. The high resistivity sample with 25000 µΩcm is reconstructed with 3 nm grains a notably bigger surface oxide layer of 12 nm. Many grains are still interconnected but there are oxide barriers separating clusters of grains from each other. The interconnection of grains at high resistivity is either a reconstruction artefact or shows that already a small amount of oxide barriers is sufficient for high non-linearity.

Conclusions

This study provides a novel insight into the 3D morphology of granular Aluminum (grAl) thin films, challenging previous assumptions about the structure-property relationship in these materials. The advanced electron microscopy techniques have revealed that the non-linear properties of grAl are more likely due to constrictions in the conductive path through the grains rather than oxide barriers. Furthermore, the study has shown that even a small number of oxide barriers can result in high non-linearity, particularly in high resistivity samples. These findings pave the way for more accurate simulations and could potentially enhance the performance of devices that utilize grAl. Further research is needed to fully understand the implications of these results and to explore the influence of substrate temperature during deposition on the structure of these films.

Keywords:

EFTEM Tomography Granular-Superconductor

Reference:

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Nanodiamond-based quantum sensing of mechanoregulated metabolic plasticity of cardiac fibroblasts

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background incl. aims

Quantum sensing is one of the most advanced quantum technologies. Quantum sensors based on single electronic spins, or small spin ensembles, can deliver nanoscale spatial resolution in detecting magnetic fields. This property has been exploited in breakthrough experiments in the biomedical field ranging from localised detection of free radicals [1] or temperature [2] inside living cells, the demonstration of magnetic resonance imaging on picoliter samples [3], to the identification of HIV with >10^5 better sensitivity than classical methods [4]. However, we have only just started scratching the surface of the application potential of quantum technology into biomedical research and development. Our group has been developing novel nanodiamond-based quantum sensing protocols to investigate mechanoregulation of metabolism at sub-cellular level. In this study, we have focused on the mechanoregulation in the heart scar formation. The myocardial scar formation known as cardiac fibrosis is a key contributor to heart failure. Anti-fibrotic therapies are still under development due to limited understanding of molecular processes behind scar formation. The cardiac fibrosis starts with changes in the mechanical properties of a heart extracellular matrix (ECM), which leads to transdifferentiation of cardiac fibroblasts (quiescent stage) into myofibroblasts (activated stage). We have very little knowledge about how mechanical stimuli govern fibroblasts metabolic plasticity. Free radicals (FRs), a class of reactive molecules with an unpaired electron, have emerged to be crucial for intracellular signalling. However, as FRs are short lived and difficult to detect with the state-of-the art-methods, therefore their role in cardiac metabolic plasticity remained unknown. In our studies, we aimed to reveal the role of FRs in lipid plasticity of cardiac fibroblasts in response to mechanical stimuli with quantum sensors.

Methods

The experiments were performed on primary human cardiac fibroblasts cultured on stiff (Young modulus >10 kPa) and soft (Young modulus <10 kPa) substrates to stimulate their activation. All experiments were performed for young (2nd passage) and aged cells (>5th passage). We used a quantum sensing technique called T1 relaxometry [5] to detect changes in free radical generation by mitochondrial networks. Fluorescent nanodiamonds (FNDs) with NV- centers were used as sensors. We modified the surface of the nanodiamonds with antibody for targeting their delivery to mitochondria. Then we checked the uptake efficiency and colocalization of FNDs with mitochondria and transporting vesicles inside the cells. In these studies, the T1 relaxometry was for the first time combined with optical trapping of FNDs to simultaneously measure FRs and viscoelastic properties of mitochondrial networks. We further evaluated changes in lipidome with single cell lipidomics using MALDI mass spectrometry imaging and fluorescent imaging of stained lipids.

Results

Our studies have demonstrated that uptake of the nanodiamond sensors depends on the age of cells and stiffness of the substrate. We have shown successful targeting of FNDs to mitochondria, which was independent of age of cells and substrate stiffness. We have found that FR generation by mitochondrial networks shows some dependence on the age of the cells and also indicate mechanoregulation. Furthermore, the mechanical stimulation and age of cardiac fibroblasts influence heterogeneity of lipotypes and their distribution across the population of quiescent and activated cells. We have shown lipotype correlation with the FRs level and viscoelastic properties of mitochondrial networks.

Conclusion

As a result, our research shed light on the mechanobiology of the heart scarring process. We have proven that quantum sensing combined with optical trapping can be used to study mechanoregulation of metabolic plasticity of cardiac fibroblasts. Our simple quantum sensing model of mechanoregulated cardiac fibrosis could be further developed towards 3D cell cultures and serve as screening platform for metabolic drugs.

Keywords:

Quantum sensing, optical trapping, fibrosis

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FIB-SEM and automatic segmentation for investigation of mitochondrial organization in cells of urinary bladder urothelium

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
Mitochondria are the main source of ATP and their ultrastructural features as well as their subcellular distribution depend on cell type and metabolism. In urothelial superficial umbrella cells, ATP is required for the synthesis and delivery of urothelial plaques by fusiform vesicles to the apical plasma membrane where they form a tight permeability barrier between urine and body fluids [1]. On the other hand, mechanical stretch of urothelium during bladder filling promotes ATP production and excretion into extracellular space [2]. ATP activates purinergic receptors on afferent nerves and interstitial cells in the lamina propria and on smooth muscle cells of detrusor. This ATP signalling pathway induces detrusor contraction and thus emptying of the bladder. Mitochondrial structural changes in urothelium were reported in aging organisms and some bladder disorders [3]. Our aims were to preserve the ultrastructure of umbrella cells close to native state in the living organism and to develop a method for the automatic segmentation of mitochondria.

Materials and Methods
Mouse urothelium was high pressure frozen (Balzers HPM010), freeze-substituted (Leica AFS) and embedded in Epon. Ultrastructural features of umbrella cells were examined on ultrathin sections (Philips CM100). The volumetric data from the umbrella cells was generated by Ga-ions milling and imaging in FIB-SEM (Helios NanoLab 650). For automatic segmentation of mitochondria from FIB-SEM data, we propose a pipeline based on a volumetric convolutional neural network with mechanisms that reduce the impact of noisy and inconsistent input data. The main features of the proposed pipeline are a contrast enhancement technique, usage of segmentation masks, and zero-mean convolutions.

Results and Discussion
Ultrathin sections showed numerous flattened fusiform vesicles (Figure 1a) that were composed of two parallel urothelial plaques, which confirmed superior preservation of umbrella cells ultrastructure [4]. Mitochondria with smooth outer membrane were oval to elongate (Figure 1b). Sometimes narrowing was observed (Figure 1c), which point to fissions and fusions of mitochondria [3]. Our approach produced state-of-the-art results for extraction of mitochondria from FIB-SEM volumes [5]. 3D visualization of segmented volumes presented a global distribution of numerous mitochondria within umbrella cells (Figure 1d). Some mitochondria are tubular or globular, but many of them form complex structures (Figure 1e), indicating dynamical changes possibly associated with their various functions [4].

Conclusion
We believe that our approach can be used to better understand the role of mitochondria in normal and in pathological conditions in urothelium as well as other tissues.

Figure 1: Ultrastructure of the umbrella cell and 3D model of their mitochondria. A) Cytoplasm of high pressure frozen – freeze substituted umbrella cell contains mitochondria (M) and tissue specific fusiform vesicles (FV), which could also be organized into stacks (FVs). B) The shape of mitochondria on a two dimensional ultrathin section varied from being oval (Mo) to elongated (Me). C) Narrowing of the mitochondria (arrow). D) Visualization of mitochondria from segmented FIB-SEM volumes (256×256×256 voxels). E) A part of complex mitochondria with a bifurcation and a membrane connection. Bars: 500 nm – A, 250 nm – B, C.

Keywords:

FIB-SEM, segmentation, mitochondria, urothelium, bladder

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Investigating microstructural phenomena in Additive Manufactured metals through high temporal thermal cycles in-situ heating

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Poster Group 2

Background incl. aims:
Additive manufacturing (AM) of metal components holds significant potential for delivering customized design, improved flexibility, and cost-effective production. However, controlling the microstructure-defined properties of AM components is a major challenge due to the inherent complexity of extreme thermal processes that form the AM microstructures. Material solidification and solid-solid phase transformation occur under far-from-equilibrium [1] conditions in cyclic layer-by-layer processes. Accordingly, established phase diagram predictions are not significantly relevant for understanding and predicting the phase formation and their consequences for the component properties. Real-time observation of microstructure evolution during AM thermal cycles at sub-µm resolution is therefore crucial for controlling microstructure related properties of AM components, as well as for exploring “unusual” microstructure formations, leading to new and exciting properties.
Methods:
In-situ electron microscopy studies offer the potential to investigate such microstructure phenomena with resolution ranging from mm down to sub-nm. However, achieving extreme thermal profiles, such as those occurring during AM process inside a microscope are not trivial.
Micro-heating devices, based on microelectromechanical systems (MEMS) combine very fast (10^3 ^{o}C/s) heating and cooling rates with high stability [2] and are potentially able to reproduce the thermal cycles of AM process. However, they were primary developed for studies of electron transparent samples smaller than 20 µm in transmission electron microscopy (TEM).
Results:
In this work we will present our efforts in exploring the high heating rates of MEMS heaters to mimic the thermal cycles of AM process inside the scanning electron microscope (SEM) and in the TEM. Supported by computational modelling (COMSOL) and indirect measurements such as Raman spectroscopy, we investigated the limits and the accuracy of MEMS heaters depending on e.g. sample size, environment, and heating rate and will discuss the prospect and limits of this approach. Furthermore, we will present the first electron backscatter diffraction (EBSD) and transmission Kikuchi diffraction (TKD) results demonstrating the potential of correlating microstructure characteristics with non-equilibrium phase transition during AM process.
Conclusion:
Our findings provide deeper understanding and highlight the potential of simulating AM thermal cycles inside the electron microscope using MEMS heater both in the TEM and in the SEM. Specially for metallic specimens, our results shows the capability of replicating ramping rates and temperature gradients inherent to AM processes within the electron microscope, thereby enabling the correlation of microstructural characteristics with phase transitions with spatial resolution varying from sub-nm to µm. This work contributes to advancing the understanding of simulating AM thermal cycles within the electron microscope, offering opportunities for enhanced characterization and analysis of materials under extreme heating conditions.

Keywords:

In-situ, MEMS-heater, Additive Manufacturing, SEM

Reference:

Reference
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The effects of solution processing methods on halide perovskite nanostructure

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Poster Group 2

Background:

Over the last decade, hybrid lead halide perovskite (LHP) materials have emerged as auspicious candidates for next-generation thin-film photovoltaics. This is largely due to both impressive optoelectronic properties and photovoltaic power conversion efficiencies, making them ideal for next-generation solar applications. Within this class of materials, formamidinium lead triiodide ($FAPbI_3$)-based perovskites are of particular interest due to their near-ideal bandgaps and improved thermal stability [1]. However, the photoactive (α-) phase of $FAPbI_3$ is only meta-stable at room temperature, and a variety of compositional tuning and additive-based approaches have therefore been employed to stabilise this phase.

While a range of methods can be used to fabricate LHP thin films, the most common of these is one-step spin coating. Precursor salts may be dissolved in a range of solvents to form a precursor ink, into which additives that improve film properties and stability may be incorporated. However, little is known about the effect of both solvent choice and additives on film nanostructure. This ultimately limits the practical application of LHPs in devices, as higher performance materials tend to be less stable. Understanding how precursor ink chemistry affects thin film crystallisation can thus inform improvements in long-term stability of films and devices, and is pivotal to improving this technology moving forward.

Methods:

In this work, we examine 'neat' $FAPbI_3$ films prepared via different solution processed routes including films prepared from the conventional precursor salts formamidinium iodide and PbI_2 and single-crystal precursors in solutions of 4:1 DMF/DMSO and 4:1 DMF/NMP, both with and without the addition of a 40% MACl additive.

High-resolution transmission electron microscopy (HRTEM) is used to elucidate the nanoscale structure of these films at the atomic scale. However, $FAPbI_3$ is highly susceptible to electron beam-induced damage; doses in excess of 100e-/$Å^2$ induce degradation into PbI_2 indistinguishable from that natively present in the film [2]. Here, all HRTEM is performed at doses below this critical threshold to ensure beam-induced effects are minimised.

Results:

While bulk characterisation data suggests that these varied approaches to $FAPbI_3$ film formation result in structurally the same material (albeit with varying levels of electronic defects and morphological film changes) [3], HRTEM shows at the nanoscale these films exhibit significant

structural and compositional differences. Figure 1(a) shows a typical low-dose HRTEM image of a $FAPbI_3$film prepared from conventional precursor salts in DMF/DMSO. The FFT of this image (Figure 1(b)) shows poor agreement with spacings expected for α-$FAPbI_3$(red rings) and the 2H-polytype of PbI_2 (blue rings). In comparison, the HRTEM image of a single-crystal precursor sample in the same solvent system and the corresponding FFT (Figure 1c,d) shows good agreement with the spacings expected for the photoactive perovskite phase. We attribute these differences to the presence of unreacted precursor material and the presence of non-photoactive $FAPbI_3$ polytypes.

It has been previously suggested that in some more structurally stable compositions, degradation in these materials occurs as a result of the inclusion of various unwanted $FAPbI_3$ polytypes during crystallisation [4]. Our HRTEM images show direct evidence of these phases, and in combination with other characterisation techniques support the suggestion that the inclusion of these undesirable phases can be controlled and ultimately eliminated through careful modulation of the precursor ink chemistry. By using a single crystal precursor we have been able to achieve better precursor ink purity, which results in films with significantly reduced structural disorder (evidenced by lower Urbach energies, as shown in Figure 1e,f). In this work, we link these observations directly to nanostructural changes visible in the HRTEM data.

Conclusions:

Precursor ink chemistry undoubtably affects LHP thin-film composition and structure. We show a direct link between these properties and laboratory-based results through the use of low-dose HRTEM. These links provide an important stepping stone on the path to truly understanding precursor ink chemistry, which ultimately may be the key to perfecting the crystallisation of LHPs.

Keywords:

perovskites, photovoltaics, low-dose, HRTEM, STEM

Reference:

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Multimodality and correlative low-voltage electron microscopy: powerful tool for imaging in life and material sciences

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Poster Group 1

Background incl. aims
Low-voltage electron microscopes (LVEMs) offer distinct advantages over conventional high-voltage instruments: visualization of samples with high contrast and resolution, and multimodal imaging and five modes available in one instrument (TEM, STEM, SEM, ED, EDS) providing an easy-to-use solution for multimodal imaging and correlation. Performing multimodal and correlative imaging on several instruments is time-consuming and expensive and increases the risk of sample damage or contamination during the transport from one analytical device to the other. LVEM combines several imaging and analytical methods available during one imaging session, which decreases this risk and allows for quick and easy analysis.

Methods
LVEMs are versatile in imaging (TEM, SEM, STEM) and analytical (ED, EDS) modes. In TEM mode, LVEMs enable the users to observe and explore thin specimens' internal structures from low magnification for a quick overview to high magnifications with high resolution. While STEM mode enhances the high-resolution imaging capability even for samples with higher-than-standard thickness and, in combination with EDS, provides the option to obtain elemental mapping of the sample. SEM mode provides surface imaging of bulkier samples, obtaining topographical information. LVEMs are equipped with backscattered electrons (BSE) detectors enhancing material contrast. Furthermore, the capabilities of ED allow researchers to obtain crystallographic information from nanomaterials and crystalline samples, lattice parameters, and information about phases present in the sample.

Multiple imaging techniques of multimodal or correlative microscopy are necessary for a more comprehensive sample understanding in the application fields such as biosensors, immunolabelling, or nanoparticles development. The LVEMs allow researchers to study the structural, chemical, and morphological properties of various material and biological specimens by correlating data from TEM, SEM, STEM, EDS, and ED modes in different magnifications, and for material science samples also the dark-field TEM and STEM, where appropriate.

Results
Analytical chemistry trends are generally moving forward, and modern scientific instruments are designed to gain more and more information as possible from the interaction between the electrons and the sample. That corresponds with the new development of 4D-STEM – a combination of imaging and ED mode.
While multimodality involves acquiring multiple signals from one analysis, correlation should find a way to find a relation between these signals. TEM-ED, STEM-EDS, and SEM-STEM correlations are quite popular and can be easily done on single instruments.

Correlation between TEM and SEM brings information about sample inner structures from TEM and morphology from SEM. If combined with EDS, we can do the elemental mapping in STEM-EDS, and if wanted, ED can be done quickly with all these modes together to see the crystallinity and lattice parameters. In addition to ED, imaging in dark-field TEM delivers other information, including inner structures, which are not achievable by SEM mode. LVEMs' uniqueness lies in connecting all these analytical modes in one device.
LVEMs also help in cases where the sample cannot be prepared as a sample specimen of one kind. For complex studies, it is very common for one part to be a typical sample for TEM, but the other part of the investigated object is more suitable for SEM mode. In this case, LVEMs offer the opportunity to perform these measurements with one instrument.

Conclusion
LVEMs represent a unique solution in correlative and multimodal electron microscopy, offering versatility and the ability to analyze life and material science samples in several imaging and analytical modes in one imaging session in one device.

Keywords:

Low-voltage electron microscopy, multimodal imaging

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Mean inner potential change of latex sphere with temperature measured using off-axis electron holography

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

Background incl. aims
Electron-beam-induced specimen charging is frequently encountered in transmission electron microscopy (TEM), in particular when studying insulating or biological materials. Polystyrene latex is a widely-used material for immunodiagnostic reagents and is often studied using cryo-TEM. Both a highly energetic electron beam and a low specimen temperature can change the intrinsic properties of materials. The mean inner potential (MIP) is an intrinsic parameter of a material, which is defined as its volume-averaged electrostatic potential with respect to a distant vacuum reference. The MIPs of many materials have been measured at room temperature using off-axis electron holography [1]. However, few low temperature measurements of MIPs and charging phenomena have been performed.
Methods
The TEM mode of off-axis electron holography involves the use of an electron biprism to overlap a reference electron wave that has passed through vacuum with an electron wave that has passed through a sample. Analysis of the resulting interference fringe pattern can be used to recover both amplitude and phase information. In the absence of dynamical diffraction and magnetic fields, the projected phase shift of the electron wave that has passed through the sample can be expressed in the form $\phi(x,y)=C\,V(x,y)\,t$, where C is a constant that depends on the microscope accelerating voltage (6.53×10^6 rad/Vm at 300 kV), V(x,y) is the total projected potential (including the MIP V_o and the electrostatic potential V_1 induced by electron-beam-induced charge redistribution) and t is the sample thickness. For a spherical solid of radius R, $\phi_o(x, y)=2C\,V_o\,(x,y)\,\sqrt{(R^2-(x^2+y^2))}$. The contribution to the phase from the electron-beam-induced charging outside and inside the sphere are $\phi_1(x,y)=C\,Q/(4\pi\varepsilon_o)\,\ln(((x-x_o)^2+y^2)/(x^2+y^2))$ $(r \geq R)$ and $\phi_1(x,y)=2C\,Q/(4\pi\varepsilon_o)\,(\ln(\sqrt{((x-x_o)^2+y^2)}/\sqrt{(R^2-(x^2+y^2))}+\sqrt{(R^2-(x^2+y^2)}/R+(\sqrt{(R^2-(x^2+y^2))^3}/3R^3)$ $(r < R)$, respectively [2], where ε_o is vacuum permittivity, $(x_o, 0)$ is the position of image charge. In order to quantitatively measure the charge on a latex sphere, we applied a model-independent (MI) approach [3, 4], which involves the measurement of the total charge enclosed by a chosen contour (Figs 1a and 1b). In this way, the contribution to the phase shift from the MIP and charge redistribution can be separated.
Off-axis electron holograms were recorded at 300 kV on an FEI Titan G2 TEM equipped with a 4K Gatan K2 camera. Each hologram was acquired by using a total exposure time of 6 s sub-divided into 30 frames of 0.2 s. Phase reconstruction was performed using HoloWorks software. Reduced specimen temperatures were reached by using a liquid helium holder from CondenZero (down to 5 K) and a liquid nitrogen cooling holder from Gatan (98 K).
Results
Figures 1a and 1b show a reconstructed phase image of a latex sphere with a diameter of ~220 nm attached to the end of a W tip. The hologram was recorded at room temperature (RT) and the charge was measured by performing a loop integral (marked using a yellow frame). The black arrow in Fig. 1a indicates the calculation direction. The total amount of charge on the latex sphere is calculated to

be ~273 e. Figure 1c shows phase images of a latex sphere with a diameter of ~430 nm on a 50-nm-thin Au film recorded at RT and 5.3, 29 and 70 K. Figure 1d shows equiphase contours generated from differences between the RT and lower temperature phase images. The total amount of charge is calculated to be 705, 633, 600 and 555 e, respectively. The differences result from both a change in electron-beam-induced charging and a change in MIP with temperature. In the surrounding vacuum region, phase changes are present due to the charge on the sphere and the substrate. By using this approach, 20 sets of data were recorded from spheres with sizes of 220, 430 and 604 nm at temperatures of 5.3, 23, 26, 29, 70, 98 and 298 K. The MIP value at each temperature was measured from these results. Figure 1e shows the relationship between temperature and MIP, with the measurements shown as black squares and error bars and a blue curve showing a parabolic fit with an R-squared value of 0.95732. The fitted parabola gives an intercept of 5.96 ± 0.16 V. The change in MIP from RT (5.17 ± 0.18 V) to that extrapolated to 0 K is ~16%.

Conclusions

Electron phase images of latex spheres of different sizes have been recorded at different specimen temperatures using off-axis electron holography. A model-independent approach has been used to measure the charge on each sphere. The MIP is also found to change, especially at the lowest specimen temperature.

Keywords:

Mean-inner-potential, e-beam-induced charging, nanoparticle

Reference:

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Shedding light on the birth of hybrid perovskites by In-Situ TEM and Synchrotron X-ray scattering

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background

Organic-inorganic halide perovskites (OIHPs) have emerged as promising candidates for a broad range of optoelectronic devices thanks to their unique physical properties[1]. In the current framework of energy transition, OIHPs have demonstrated great potential in the photovoltaic field with the development of OIHPs-based solar cell reaching, in 2022, a power conversion efficiency of 25.6%[2] similar to its silicon-based analog. Methylammonium lead iodide (MAPI) has been the most commonly studied LHPs due to its very promising optoelectronic properties. One of the main explored pathways for obtaining MAPI perovskite is the Ligand-Assisted Reprecipitation (LARP) approach[3]; however, this method is not totally understood from a phenomenological point of view. Especially, the use of DMF as a polar solvent for the preparation of the precursor mixture leads to the formation of intermediate DMF-solvated phases[4] before the obtention, by annealing, of the final MAPI phase.
While a large amount of the community is focused on the OIHPs' physical properties with the aim of its application in photovoltaic, very less tackles this subject with an in-situ approach for the study of its growth processes. Thus, we decided to adopt this specific research angle to provides a better understanding of the LARP approach for synthesizing hybrid perovskites.

Methods

In one of our study[5], we took advantage of the development of microchip-based systems which enabled to study dynamic phenomena in their native environment, e.g. gas or liquid, directly in the TEM vacuum column. By replicating the LARP synthesis protocol in a Liquid-Phase TEM (LPTEM) cell (Figure 1.A), we were able to dynamically track the growth in solution of an intermediate DMF-solvated phase during the LARP process. These observations were correlated with synchrotron-based X-Ray scattering measurements performed on the SWING beamline of the SOLEIL synchrotron. The second part of the study is dedicated to the study of the transition by annealing in air from the intermediate phase toward the final optoelectronically interesting MAPI perovskite material. For this purpose, we have carried out annealing at 80°C in a Gas-Phase TEM (GPTEM) cell using a similar microchip system. We correlated the TEM observations with Temperature-resolved X-Ray diffraction using a chamber dedicated to temperature measurements.

Results

First, we monitored the nucleation and the growth processes of a DMF-solvated-intermediate phase by directly visualizing the reaction medium using LPTEM (Figure 1. B-F). The LPTEM images revealed

the formation of roughly spherical objects (Figure 1. C-D) in the early stages of the process transitioning to elongated ribbon-like morphology particles (Figure 1. E-F) after a few seconds, through a crystallization process. These local observations, in direct space, were correlated with more global information brought by synchrotron-based X-ray scattering measurements (Figure 1. G and H). With excellent time consistency with LPTEM imaging (Figure 1. B-F), the X-ray scattering measurements enabled us to identify similar morphology and confirm the local observations from LPTEM. This combined analysis, conducted in real-time and in representative conditions, allowed us to decipher the structural evolution of the emerging phases.

Secondly, we tracked in-situ the transition, occurring in air by annealing at 80°C, of the as-obtained intermediate phase towards the MAPI perovskite, using a combination of GPTEM, in image and diffraction modes (Figure 1. I and J), and temperature-resolved X-Ray diffraction associated with Rietveld refinement. We provided direct and unprecedented evidence of the fragmentation of the crystals, occurring simultaneously with the structural transformation between the intermediate and final phases. While GPTEM evidenced the fragmentation of the ribbon-like crystals into platelets, the Rietveld refinement explained this morphological transition based on a crystalline structure that already exhibited a preferential orientation towards (hk0) and (0k0) family planes.

Conclusion

We have, for the first time, directly visualized the growth of an intermediate phase of hybrid perovskites in liquid media during LARP synthesis using TEM. We show that combining LPTEM observations with synchrotron X-Ray scattering measurements is particularly effective for investigating such phenomena across various scales. Additionally, we investigate the transition of this intermediate phase through annealing and demonstrate how a correlative approach, combining TEM in Gas-Phase and X-Ray measurements, provides new insights into the mechanisms underlying structural changes during crystalline transitions.

Our approach, which required the challenging development of new correlative methods, underscores the significant value of in-situ correlative studies for enhancing our understanding of the synthesis of emerging hybrid materials. There remains a gap in understanding the growth mechanism of these materials in solution and we firmly believe that in-situ TEM-based correlative studies can contribute significantly to addressing these shortcomings.

Keywords:

Hybrid perovskite, In-Situ TEM, Correlative

Reference:

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Ptychography at finite dose in SrTiO3

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Poster Group 2

Many important materials are unstable under the electron beam and easily damaged by high-dose imaging, leading to a blurred image of a damaged sample. Ptychography is one of the most dose-efficient electron microscopy techniques currently available, providing a route to high-resolution images of undamaged samples.

The aim of this research is to develop, implement and apply new dose-efficient 4D-STEM imaging methods, based around existing algorithms for electron ptychography to improve imaging of beam-sensitive materials. Here, we lay the groundwork for an in-depth discussion of finite dose ptychography, towards the development of new low dose algorithms for ptychographic imaging to improve achievable contrast at low electron dose.

Previous studies have explored ptychographic techniques theoretically for infinite dose (Clark et al. 2023). In this study, we are investigating the thickness dependence of ptychographic imaging methods, in particular we are interested in the dependence on varied electron probe dose to see just what the dose limits are for an accurate reconstruction.

We simulate a Strontium Titanate (STO) thickness series of 1-55 unit cells (3.905 – 215 Å) along the [1 0 0] axis with a 26.6 mrad electron probe at 300 keV with a midplane focus, using μSTEM (Allen et al. 2015). We then model a dose-series across the thicknesses, by applying Poisson statistics. The resulting 4D datasets were then used to form images via a range of algorithms, including central Bright-Field (cBF), Annular Dark-Field (ADF), integrated Center of Mass (iCoM), Single Sideband (SSB), Wigner Distribution Deconvolution (WDD), and extended Ptychographic Iterative Engine (ePIE).

With these data, we then find lowest-feasible dose limits for imaging samples in realistic imaging conditions, detecting image features and atomic column positions, to provide guidelines for successful experimental data collection, advice for expected most-successful imaging approaches, and data collection parameters.

Keywords:

Low dose, Ptychography, 4D-STEM

Reference:

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Reactivity of High Entropy Nanoalloys under O2 and CO Oxidation reaction studied by in-situ TEM

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Carbon monoxide (CO) contamination has a major input in climate change. An efficient way to reduce its contribution is to oxidize CO into CO2. Therefore, heterogenous catalysis of CO oxidation reaction has been widely studied and many noble metal nanoparticles has been proposed as efficient catalysts. However, considering the price increase of noble metals and their rarity, there is a need to come up with new catalysts that involve less of noble metals. In this perspective, High Entropy NanoAlloys (HENA) represent a new class of catalysts with promising tunable properties. The presence of 5 metals or more crystalized in a solid solution and the mix of noble and non-noble metals allow easily tuning the reactivity and significantly reducing the cost. Moreover, the existence of different reaction sites on the same NP allows HENA to simultaneously activate CO and O2 molecules. Thanks to the synergy of the different elements, these NPs seem to be active at low temperatures but the relation between their complex atomic structure and their reactivity under O2 and CO remains unknown.

In this contribution, we exploit aberration-corrected in situ STEM investigations to reveal the structural and chemical evolution of single CoNiCuPtAu NPs between 100°C and 600° C under O2. The HENA were directly synthesized on the silicon nitride membrane of a MEMS-based gas cell, using pulsed laser deposition, allowing control of both particle composition and size1. Our in-situ gas experiments performed at atmospheric pressure combined atomic-scale STEM imaging with chemical analysis performed by STEM-EDS in an aberration-corrected JEOL ARM 200F TEM, using a Protochips Atmosphere gas/heating holder.

In comparison with our previous in-situ heating experiments of the same HENA performed under vacuum, gas in-situ experiments show that the NP growth, that is governed by coalescence, is slowed down with the presence of O2 and that the NPs are more stable. Unlike NPs annealed under vacuum, under O2 no evaporation was observed and the 5 elements remain in the NPs at high temperature. Nevertheless, we reported structural and morphological changes of the NPs. At 100°C and under 1 atm of O2, we can see reshaping of NP facets as shown in figure 1a. The NPs keep their CFC structure in the core and only the external layer will be oxidized (figure 1a) but, interestingly, at higher temperatures (up to 600°C), the oxide layer will disappear (see figure 1b). The oxidation of HENA also causes the formation of nanovoids in NPs, phenomena known as Kirkendall effect, that we can see in figure 1d. This effect will still be present at higher temperatures and for some NPs, even the coalescence would not allow refilling the holes.

These preliminary results allow us to identify that 100°C is the efficient working temperature of HENA under O2 and showcase that the oxidation occurs only on the external layer of the NPs.
Besides the unexpected Kirkendall effect has motivated an ongoing study of the influence of nanovoids on NPs' reactivity towards CO oxidation.

Keywords:

Oxidation, in-situ microscopy, kirkendall effect

Reference:

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Attosecond electron microscopy using free-electron homodyne detection

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

The desire to map material structures and dynamics at intrinsic scales of ångströms and attoseconds is driving methodological advances in condensed matter science. While X-ray and electron methods provide structural detail, attosecond temporal resolution is being achieved by evolving optical spectroscopy techniques [1]. Photon-induced near-field electron microscopy (PINEM) enables the imaging of near-field intensities with nanometric resolution [2]. However, access to the evolution of nanoscale fields and structures within the light cycle requires sensitivity to the optical phase, e.g. by phase-contrast Lorentz PINEM [3], interferometric detection [4], or electron pulse bunching [5].
Here, we introduce Free-Electron Homodyne Detection (FREHD) as a universally applicable approach to electron microscopy of phase-resolved optical responses at high spatiotemporal resolution [4]. In this scheme, a phase-controlled reference interaction (Fig. 1a) serves as the local oscillator to extract arbitrary sample-induced modulations of a free-electron wavefunction. We demonstrate this principle through the phase-resolved imaging of plasmonic fields of a gold nanoprism (Fig. 1c) with few-nanometer spatial and sub-cycle temporal resolutions. The near field at a sample modulates the phase of the electron wavefunction, and this wavefunction modulation is amplified or attenuated for in-phase and anti-phase reference interactions, respectively, which allows for a coherent read-out of a position-dependent phase (Fig. 1b). The characterization of both the amplitude and phase of the near-field allows to image the electric field at different phases in a time-resolved movie (Fig. 1d).
In the area of phase-resolved near-field imaging, including scanning probe techniques and photoelectron emission, FREHD has distinct strengths. It is non-invasive, exhibits perfect linearity in response, and provides consistently high spatial resolution that is virtually independent of the sample and the optical wavelength. This resolution can be correlated with detailed structural characterization in transmission electron microscopy down to the atomic scale.
The method goes beyond imaging electromagnetic fields, and is also capable of detecting modulations imprinted on an electron beam by electronic or structural material responses. In particular, it encompasses attosecond charge density dynamics, including subtle light-induced changes in the structure factor at both the fundamental frequency and its harmonics.
In conclusion, we introduce Free-Electron Homodyne Detection that generalizes the high-resolution measurement of attosecond materials responses in electron microscopy, without a need for electron density bunching, and offers fascinating new possibilities to image local attosecond and phase-resolved responses on the nanometer scale.

Keywords:

Attosecond Lorentz phase-microscopy homodyne-detection holography

Reference:

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Multimodal Low Voltage Transmission Electron Microscopy Analysis of Iodine Nanoparticles for Stroke Theranostics

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Poster Group 1

Background incl. aims
Ischemic stroke stands as the second leading cause of mortality globally, necessitating innovative approaches for its diagnosis and treatment. This project delivers a validated pipeline for assessing the kinetics and dynamics of iodine nanoparticles (IoNPs) within the organism, specifically targeting critical areas like the brain, blood-brain barrier, blood, and organs like the spleen.
Understanding thrombus characteristics, including size, composition, and origin, holds significant promise in guiding treatment strategies not only for stroke but also for related cardiovascular diseases. Current imaging modalities are limited in their ability to visualize various clot types effectively, hindering precise diagnosis and tailored treatment.
Biodegradable IoNPs have the potential to become the basis of a modern theranostics (therapy and diagnostics) approach to improve the treatment. The afore-mentioned IoNPs made of monomer MAOETIB (2-(Methacryloyloxy)ethyl-2,3,5-triiodobenzoate) have been described as desired contrasting agents. Furthermore, they can be used as drug carriers.

Methods
The essential method for the characterization of nanoparticles is undoubtedly electron microscopy, offering intricate insights into nanoparticle behavior within tissue matrices. LVEM operates at significantly lower accelerating voltages compared to conventional TEM, typically below 25 kV. This lower voltage regime offers several distinct advantages, including increased contrast and gentle electron-sample interaction.
Low voltage provides improved contrast mechanisms, which are particularly invaluable for samples composed of light elements. The reduced electron energy results in increased electron scattering, enhancing image contrast and revealing finer details within specimens. This capability is especially important in imaging biological sections and generally low-contrast materials, where conventional TEM techniques may yield limited contrast.
The application of LVEM in nanoparticle characterization involves various aspects, including morphology, size distribution, crystallinity, surface properties, and interparticle interactions. Through advanced imaging techniques such as dark-field imaging and electron diffraction, LVEM facilitates a deeper understanding of NPs structure and behavior at the nanoscale level.
The unique combination of TEM, STEM, and SEM imaging modes in LVEM instruments provides researchers with a comprehensive toolkit for investigating nanoparticles integrated into the tissue structure. Furthermore, LVEM's versatility extends beyond imaging, offering advanced analytical capabilities such as energy-dispersive X-ray Spectroscopy (EDS) and Electron Diffraction.

Results

Utilizing a multimodal approach, comprehensive insights were gathered regarding the characteristics and behavior of IoNPs, spanning from initial nanoparticle characterization and quality assessment post-fabrication to their dynamics within blood circulation and targeted organs. A rat served as the model organism in this study, providing a relevant and translational platform for investigating IoNPs interactions in vivo. Nanoparticles were imaged throughout their entire lifecycle, starting from their presence in a native solution, followed by intravenous injection into the bloodstream, and subsequently deposited within the relevant organs. The examination of nanoparticles using low-voltage transmission electron microscopy was integrated to a comprehensive characterization utilizing various analytical methods.

Conclusion
The integration of LVEM into nanoparticle research promises to advance our understanding of nanoparticle behavior within biological systems. LVEM's unique imaging capabilities, particularly well-suited for biological sections, make it an invaluable tool for the development of theranostic strategies and personalized treatments. With its multimodal capabilities, LVEM emerges as a pivotal technology for the comprehensive analysis of biodegradable iodine nanoparticles, aiming towards improved healthcare outcomes.

Keywords:

Low-Voltage Electron Microscopy, Stroke theranostics

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Mitigation of beam damage on MoS2 using electrostatic beam blanking in TEM

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Poster Group 1

Background
Transmission electron microscopy (TEM) has allowed for impressive opportunities to visualize matter at the atomic scale. In such experiments, the constituent atoms typically respond dynamically to the electron beam irradiation, alter the sample and, hence, modulate image contrast and intensities. While mitigation strategies have focused much on electron energy and dose variation, there is a growing emphasis on the role of the electron dose rate.

That is, for a broad class of materials, the electrons incident on the specimen is considered to cause phonon or electron excitations because the impingement rate is higher than the subsequent relaxation rate. Consequently, accumulation of energy and or charge leads to bond rupture and atom displacements. In this picture, pulsing the electron illumination seems beneficial as it enables a sharply defined maximum electron impingement rate to match the onset causing sample alterations. Here we show how a structured electron beam can suppress beam-induced alterations of a two-dimensional MoS2 by 50% compared to the continuous mode.
Method
The present experiments are conducted on a Thermo Fisher Scientific SPECTRA ULTRA microscope operated at 300 kV. An electrostatic beam blanker (ESBB) system developed by Thermo Fisher Scientific, is used to structure the electron beam with a 10 ns temporal electron delivery window with repetition rate of 1MHz. The microscope was operated in pulsed and continuous mode. In each mode, an electron diffractogram of the MoS2 was recorded at similar electron dose-rate and total dose. In pulsed mode the beam blanker was tuned to generate 1 electron per pulse. The area exposed was kept the same during acquisition to compare the behavior of the specimen in both modes.

Results
The electron diffractograms of MoS2 show for increasing electron dose and dose-rate had different evolution of the central diffraction peak intensities in pulsed and continuous modes. In continuous mode the diffraction peaks faded to ~60% of the original intensity due to loss of crystallinity caused by beam damage. In pulsed mode the diffraction peak intensities faded only by ~1%. A similar effect was observed by (1,2) delivering dose electron-by-electron and allowing relaxation time reduces beam damage. Further we found that the effect of pulsing decreased when increasing the number of

electrons per packet. This is the first time that this effect is shown with only a beam blanker and on a nanosecond time scale.

Conclusion
Thus, the comparison of pulsed and continuous electron beams suggests that the electron-induced degradation of MoS2 is a multi-electron excitation rather than a single-electron scattering event, consistent with ref. (3,4) and that excitations on the microsecond timescale must be circumvented to maintain structural integrity of the MoS2 sample. We anticipate that beam blanker pulsed electron delivery may benefit the stability of other materials and enable novel experiments with beam sensitive materials in general.(5)

Keywords:

Beam damage, structured electron illumination

Reference:

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5. We thank Felix van Uden for preparing the microscope and Maarten Waaijer, Erik Kieft, Boy Markus, Bert Freitag and the rest of the Thermo Fisher Scientific team for participating in the initial discussions. Further we thank Tim Booth from the 2DTU group at DTU for assistance in providing MoS2 samples. The Center for Visualizing Catalytic Processes is sponsored by the Danish National Research Foundation (DNRF1462).

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Deep Learning assisted denoising of in situ liquid STEM-movies of nanoparticle nucleation and growth

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Poster Group 2

Over the recent years, the comprehension of the formation mechanism of metallic nanoparticles (NPs) has greatly benefited from in situ liquid TEM that enables imaging of the nucleation and growth of individual NPs in liquid media. However, these experiments pose challenges regarding both data acquisition and analysis. In particular, substantial scattering from the liquid around the NPs and the electron transparent SiN membranes significantly diminish the signal quality [1]. One solution would be to increase the electron dose rate, but this can lead to undesirable effects due to radiolysis. The other one is to use post-experiment techniques to remove the noise while keeping the signal of interest. Herein, we present an innovative approach that combines deep-learning (DL) and scanning transmission electron microscopy kinematic simulations [2] to denoise in situ liquid STEM-movies of NPs during their nucleation and growth in liquid media, a complex task where multiple information is intertwined.
Within a commercial liquid cell, we can visualize the formation of gold NPs, induced by the interactions between the electron probe and the precursor. We acquired a video of these events using a double corrected JEOL ARM 2100. To analyze and understand the growth mechanism, each frame was denoised by a homemade convolutional neural network similar to U-Net [3]. However, the quality of the results obtained is correlated to the quality of the training data. Hence, numerical simulations are a solution in the case of liquid electron microscopy where we cannot acquire data experimentally.
Our work consists of the development and optimization of the dataset and the CNN architecture. Besides considering the size and shape dispersions of nanoparticles, kinematic simulations account for a significant obstacle in studying NP growth by liquid cell TEM which is the formation of NPs on the opposite membrane of the cell. The latter contributes to the random background fluctuations because they are imaged way out-focus. Once we considered these challenges, our method effectively denoises low and high magnification videos, thus elevating the signal-to-noise ratio from 1 to 8 [Figure 1], above the threshold value of 5 set by the Rose criterion [4]. Consequently, our analysis pipeline facilitates the study of NP growth mechanisms with improved statistics and fewer acquisition constraints. We will show the application of this methodology to investigate surface site attractiveness on both gold nanocubes and nanorods within the context of NP synthesis.
In this work, we demonstrate the application of deep learning to the specific case of the denoising of in situ liquid microscopy. Thanks to this, we were able to get a better visualization and, thus, a better understanding of the mechanisms of the formation of nanoparticles.

Keywords:

Liquid TEM, AI, denoising

Reference:

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In situ insights into the thermal stability of high-entropy nanoalloys

Syrine Krouna[1], Anissa Acheche[2], Jaysen Nelayah[1], Christian Ricolleau[1], Guillaume Wang[1], Hakim Amara[2], Damien Alloyeau[1]
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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

The structural stability of high entropy nanoalloys (HENA) brings hope to developing more stable nanomaterials for high-temperature applications in different fields such as catalysis or mechanics. Nevertheless, the enhanced thermal stability of nearly equiatomic nanoalloys containing at least 5 metals is nothing more than a theoretical speculation about the impact of thermodynamic contributions and sluggish diffusion kinetics on their structural properties and remains to be proven. In this context, studying the thermal behavior of HENA is a necessary first step to understand their stuctural properties and evalutate their structural stability with the view to better target their potential high-temperature applications.

In the present work, FCC AuCoCuNiPt NPs were directly synthesized on the silicon nitride (SiN) membrane of a MEMS-based heating chip, using pulsed laser deposition, allowing control of both particle composition and size [1]. Then, we used in situ scanning transmission electron microscopy (STEM), corroborated with atomistic simulations, to study in real time and at the atomic scale the structural and compositional evolution of from 298 K to 973 K. We combined atomic-scale STEM imaging with chemical analysis performed by STEM-EDS in an aberration-corrected JEOL ARM 200F TEM, using a Protochips heating holder. Molecular Dynamic (MD) simulations are performed using the open source Large-scale Atomic/Molecular Massively Parallel Simulators (LAMMPS) package. In order to investigate Cu-Au-Co-Ni-Pt NPs, the Embedded Atom Method (EAM) potential derived by Zhou et al. is used to describe the interaction between different atomic pairs [2].

The growth of FCC AuCoCuNiPt NPs is mainly driven by coalescence observable from 623 K (Fig. 1a). Furthermore, both in situ STEM and MD simulations reveal strong structural and chemical evolutions in the NPs with the formation and melting of an AuCu layer at the surface of NPs at high temperature (figure 1b and 1c). This phase separation that appears progressively with temperature is driven by surface effects and pronounced atomic diffusion that is surprisingly more active in these quinary nanoalloys than in monometallic and bimetallic subsystems. Besides ruling out the existence of sluggish diffusion in AuCoCuNiPt nanoalloys, our study allows distinguishing kinetic and thermodynamic effects on their structural properties, which is an essential prerequisite to better control the synthesis of complex nanomaterials. From a practical point of view, this study calls into question the use of AuCoCuNiPt HENA for high-temperature applications and more generally reveals the necessity to investigate the thermal behavior of HENA to determine if and how surface effects govern their structural properties, to evaluate their stability and adapt their potential applications.

Keywords:

In-situ microscopy, atomistic simulations, thermodynamics

Reference:

1. A. Barbero, C. Moreira Da Silva, N. O. Peña, N. Kefane, A. Jaffar, M. Thorey, H. Bouaia, J. Nelayah, G. Wang, H. Amara, C. Ricolleau, V. Huc and D. Alloyeau, Faraday Discuss. 242, 129–143 (2023)
2. Zhou, X. W., Johnson, R. A. & Wadley, H. N. G. Phys. Rev. B 69, 144113 (2004)

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Role of Miro1 adaptor protein in mitochondrial mobility between cancer and stromal cells

MSc. Jaromír Novák[1,2], Zuzana Nahacka[1], Gabriela Oliveira[1,3,4,5], Petra Brisudova[1,2], Sarka Dvorakova[1], Marketa Dalecka[2], Verena Puttrich[1], Lenka Grycova[1], Ludek Stepanek[6], Renata Zobalova[1], Mikkel Terp[7], Zdenek Lansky[1], Paulo Oliveira[3,4], Henrik Ditzel[7,8], Michael Berridge[9], Jakub Rohlena[1], Jiri Neuzil[1,2,10,11]

[1]Institute of Biotechnology, Czech Academy of Sciences, Vestec, Prague-West, Czech Republic, [2]Faculty of Science, Charles University, Prague, Czech Republic, [3]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [4]Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal, [5]Institute for Interdisciplinary Research, Doctorate Program in Experimental Biology and Biomedicine (PDBEB), University of Coimbra, Coimbra, Portugal, [6]Czech Center for Phenogenomics, Institute of Molecular Genetics, Czech Academy of Sciences, Vestec, Prague-West, Czech Republic, [7]Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, [8]Department of Oncology, Odense University Hospital, Odense, Denmark, [9]Malaghan Institute of Medical Research, Wellington, New Zealand , [10]School of Pharmacy and Medical Science, Griffith University, Southport, Australia, [11]1st Faculty of Medicine, Charles University, Prague, Czech Republic

LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

Background
Cancer cells are in close contact with healthy tissue to maintain proliferation and secure the protumorigenic microenvironment. An example of this intimate interaction is the ability of cancer cells to 'steal' functional mitochondria from surrounding stromal cells to compensate for damage to their own organelles, whether resulting from mutational burden or therapy intervention. This process, called horizontal mitochondrial transfer (HMT), occurs mostly via tunneling nanotubes (TNTs), thin membranous protrusions that connect the cytoplasm of donor and acceptor cell. Mitochondria move between cells over a distance of tens to hundreds of micrometres of TNT length using a microtubule-associated molecular adaptor and motor proteins. Here, we aim to elucidate the role of Miro1, an adaptor protein that is located in the outer mitochondrial membrane and is known to be involved in mitochondrial mobility and the HMT.

Methods
To elucidate the role of Miro1 in HMT in vivo, we have developed a mouse with inducible Miro1 knockout (Miro1KO) and with endogenous expression of the mitochondrial targeted mKate2 red fluorescence protein (mito::mKate2). We subcutaneously injected syngenic GFP expressing B16 cancer cells lacking mitochondrial DNA (so called ρ0 cells), representing a model of severe mitochondrial damage. This allows us to follow tumor growth with respect to Miro1 expression and identify HMT based on the presence of mito::mKate2 (stromal origin) mitochondria in GFP (ρ0 cancer cells) in vivo and in vitro. To further elucidate the role of Miro1 in cancer and stromal cells, we have used live cell time-lapse microscopy to capture the dynamics of mitochondrial movement as well as Sholl analysis to describe their location within cells. Finally, we have optimized cell-free reconstituted system of isolated microtubules, mitochondria, adaptor, and motor proteins, allowing us in combination with interference reflectance microscopy to track and identify alternations in movement of single organelles along microtubules.

Results
First, in vivo we observed a delay in mtDNA acquisition and tumor growth in Miro1KO mice. Furthermore, we elucidated that Miro1 is necessary for microtubule-mediated mobility and organelle relocalization toward the cell periphery and into TNTs. At the molecular level, our data suggest that Miro1 is necessary for the initial phase of mitochondrial movement, the association of organelles with microtubular tracks.

Conclusions
In summary, our results illustrate the importance of mitochondrial transfer in the context of cancer cells experiencing mitochondrial damage (e.g. as result of mutations or treatment). In particular, we have elucidated the role of Miro1 in this process, supporting the model in which it recruits mitochondria on microtubules, secures their movement towards the cell periphery and inside TNTs, and ultimately into the recipient cancer cell.

Keywords:

Horizontal mitochondrial transfer, Miro1, cancer

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In situ insights into the thermal stability of high-entropy nanoalloys

Syrine Krouna[1], Anissa Acheche[2], Jaysen Nelayah[1], Christian Ricolleau[1], Guillaume Wang[1], Hakim Amara[2], Damien Alloyeau[1]
[1]Université Paris Cité, Paris, France, [2]ONERA, Châtillon , France

Poster Group 2

The structural stability of high entropy nanoalloys (HENA) brings hope to developing more stable nanomaterials for high-temperature applications in different fields such as catalysis or mechanics. Nevertheless, the enhanced thermal stability of nearly equiatomic nanoalloys containing at least 5 metals is nothing more than a theoretical speculation about the impact of thermodynamic contributions and sluggish diffusion kinetics on their structural properties and remains to be proven. In this context, studying the thermal behavior of HENA is a necessary first step to understand their stuctural properties and evalutate their structural stability with the view to better target their potential high-temperature applications.

In the present work, FCC AuCoCuNiPt NPs were directly synthesized on the silicon nitride (SiN) membrane of a MEMS-based heating chip, using pulsed laser deposition, allowing control of both particle composition and size [1]. Then, we used in situ scanning transmission electron microscopy (STEM), corroborated with atomistic simulations, to study in real time and at the atomic scale the structural and compositional evolution of from 298 K to 973 K. We combined atomic-scale STEM imaging with chemical analysis performed by STEM-EDS in an aberration-corrected JEOL ARM 200F TEM, using a Protochips heating holder. Molecular Dynamic (MD) simulations are performed using the open source Large-scale Atomic/Molecular Massively Parallel Simulators (LAMMPS) package. In order to investigate Cu-Au-Co-Ni-Pt NPs, the Embedded Atom Method (EAM) potential derived by Zhou et al. is used to describe the interaction between different atomic pairs [2].

The growth of FCC AuCoCuNiPt NPs is mainly driven by coalescence observable from 623 K (Fig. 1a). Furthermore, both in situ STEM and MD simulations reveal strong structural and chemical evolutions in the NPs with the formation and melting of an AuCu layer at the surface of NPs at high temperature (figure 1b and 1c). This phase separation that appears progressively with temperature is driven by surface effects and pronounced atomic diffusion that is surprisingly more active in these quinary nanoalloys than in monometallic and bimetallic subsystems. Besides ruling out the existence of sluggish diffusion in AuCoCuNiPt nanoalloys, our study allows distinguishing kinetic and thermodynamic effects on their structural properties, which is an essential prerequisite to better control the synthesis of complex nanomaterials. From a practical point of view, this study calls into question the use of AuCoCuNiPt HENA for high-temperature applications and more generally reveals the necessity to investigate the thermal behavior of HENA to determine if and how surface effects govern their structural properties, to evaluate their stability and adapt their potential applications.

Keywords:

In-situ microscopy, atomistic simulations, thermodynamics

Reference:

1. A. Barbero, C. Moreira Da Silva, N. O. Peña, N. Kefane, A. Jaffar, M. Thorey, H. Bouaia, J. Nelayah, G. Wang, H. Amara, C. Ricolleau, V. Huc and D. Alloyeau, Faraday Discuss. 242, 129–143 (2023)
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Recent developments and future trends in time-resolved cathodoluminescence: measuring dynamics at the nanoscale

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Poster Group 1

Background
Cathodoluminescence spectroscopy is a well-established technique utilized to extract detailed information about the optical properties of materials [1, 2] at spatial resolutions surpassing the diffraction limit of light. By employing an electron beam as a broadband excitation source, materials can be brought into an excited state. The subsequent relaxation of these excited states typically follows intricate pathways involving both radiative and nonradiative recombination mechanisms. Gaining a comprehensive understanding of these dynamic processes is essential for the design and optimization of nanomaterials and devices.

Results
We will explore various experimental implementations of time-resolved cathodoluminescence (TRCL) that are applicable for lifetime mapping or g(2) imaging. In the former, a decay trace is measured following excitation with a pulsed electron beam, enabling the determination of the excited state's lifetime [3]. In the case of g(2) imaging, photon statistics are instead utilized to observe bunching and anti-bunching effects [4, 5]. By analyzing the bunching behavior in extended material systems, it becomes possible to extract valuable information about emission lifetime and excitation efficiency. An important advantage of this approach is its compatibility with a continuous electron beam, eliminating the need for modifications to the electron microscope.

Conclusions
Here we describe the latest advancements in time-resolved cathodoluminescence and future directions, drawing examples from semiconductor research to highlight the remarkable capabilities and value that TRCL brings as an advanced nanoscale characterization technique.

Keywords:

Time-resolved cathodoluminescence, spectroscopy, dynamics, ultrafast

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Investigating the chemical oxidative polymerization of 1,8 - dihydroxynaphthalene using a correlative in-situ approach

Dr Nivedita Sudheer[1,2], Kevin Ziegler[3], Emmanuel Maisonhaute[4], Vincent Ball[3], Cédric Boissiére[5], Tom Ferte[2], Dris Ihiawakrim[2], Clement Sanchez[1,5], Ovidiu Ersen[2]
[1]Collège de France, Paris, France, [2]Institut de physique et chimie des Matériaux de Strasbourg (IPCMS), Strasbourg, France, [3]Institut national de la santé et de la recherche médicale (INSERM), Strasbourg, France, [4]Laboratoire Interfaces et Systèmes Électrochimiques (LISE), Paris, France, [5]Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), Paris, France

PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

Melanins, ubiquitous natural pigments found across various life forms, possess diverse biological functions such as camouflage and radioprotection. Notably, allomelanins derived from 1,8 - dihydroxynaphthalene (1,8-DHN) in Ascomycetes demonstrate robustness, offering protection against hostile environments. Despite their structural complexity and insolubility in solvents, allomelanins exhibit unique physical and chemical properties, including broad-band visible-light absorption, free radical characteristics, water-dependent conductivity, and redox behavior, albeit their exact structure remains incompletely defined[1], [2]. Thin films derived using chemical oxidative polymerization from physiologically active chemicals such as polyphenols have been studied to exhibit remarkable versatility across diverse substrates[3], [4], [5]. This study presents an in-depth investigation utilizing in-situ correlative microscopy and surface electrochemical methodologies to scrutinize thin films formed via the oxidative polymerization of 1,8-DHN molecules in aqueous electrolytes.

The study showcases the electrodeposition of 1,8 DHN onto gold electrodes and glassy carbon electrodes through cyclic voltammetry under in-situ liquid TEM using a miniaturized electrochemical cell. The resulting films demonstrate adjustable thickness and swelling properties, modulated by varying the potential sweep rate (20mV/s, 200mV/s and 1000mV/s). Analysis of the films' surface characteristics is conducted through Raman-coupled electrochemical spectroscopy and in-situ ellipsometry, complemented by cyclic voltammetry to explore their electrochemical behavior. Additionally, employing in-situ TEM provides insights at the nanoscale, in direct space into the electrochemical mechanisms occurring during the film formation.

Through the application of in-situ liquid TEM using an electrochemical cell, the real-time mechanism of thin film formation during electrodeposition has been elucidated, marking a significant advancement in the field. A proposed mechanism for the oxidative polymerization of 1,8 DHN is supported by Raman-coupled electrochemical spectroscopy, indicating dimer formation during the initial stages. Additionally, concurrent analysis with in-situ ellipsometry has allowed for the simultaneous investigation of the relationship between film thickness, scan rates, and optical properties, while quasi-in-situ impedance spectroscopy has provided insights into the electrical and conducting properties of these polymers in relation to the sweep rates. Notably, these films exhibit inherent antioxidant properties with antioxidant components distributed throughout the films' structure within a restricted energy range of -1 V to +1 V versus Ag/AgCl.

From a material point of view, this correlative study which includes in-situ liquid TEM analyses highlights the considerable potential of employing mild electrochemical conditions to fabricate

electropolymerized thin films via the Chimie-Douce mechanism utilizing fundamental organic constituents. Moreover, combining several in-situ techniques including electrochemical TEM, Raman coupled electrochemical spectroscopy, and ellipsometry, we bring new insights into chemical oxidative polymerization at different scales and more generally a structural evolution during electrochemical phenomena. Given their insolubility in commonly used solvents and favorable mechanical properties, these films hold promise as protective coatings. Moreover, their electroactive and antioxidant properties render them highly suitable for a wide range of applications, including environmental remediation, biomedicine, and catalysis.

Keywords:

In-situ-liquid TEM, Raman-coupled electrochemical spectroscopy

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Correlative TOF-SIMS/SEM for subcellular investigation of microalgae in extreme environment

Claire Seydoux[1], Jade Ezzedine[2], Camille Beaulier[2], Grégory Si Larbi[2], Stéphane Ravanel[2], Eric Maréchal[2], Jean-Paul Barnes[3], Pierre-Henri Jouneau[1]
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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

Visualizing the metabolism within its structural context is key to understanding the biological phenomena at stake, especially at the subcellular scale. While biochemical purification may function for specific organelles of model species, this is tedious and often impossible for atypical or less studied organisms. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables the combined visualization of elements and molecules and is becoming more and more popular in the field of life sciences, especially for the investigation of tissue samples. Even though the ultimate lateral resolution of TOF-SIMS ought to be sufficient for subcellular imaging, very few studies have yet pushed the lateral resolution that far. In this context, we have investigated how the subcellular architecture and molecular composition of microalgae thriving in extreme environments is affected by the harsh conditions they live in.
For this purpose, we have performed TOF-SIMS imaging, combining both high lateral resolution (up to 100 nm) and high mass resolution (up to 9,000) over resin-embedded specimen. We also performed over the same zone correlative SEM (10 nm) in order to identify subcellular compartments and used this image to segment the hyperspectral TOF-SIMS dataset. We are thus combining the strength of both techniques and increasing the resolution of TOF-SIMS imaging using SEM-derived information.
We investigated the snow alga Sanguina nivaloides that blooms over lasting snow in the spring season and was recently been identified in the Alps, as well as the uranium-tolerant Coelastrella sp. PCV that has been shown to tolerate extremely high doses of uranium. In these algae, we have been able to perform molecular profiling of several subcellular features, including cell walls as thin as ~ 100 nm, nuclei, pyrenoid, starch granules and platelets and chloroplasts. In the case of S. nivaloides especially, the environmental samples hint at a likely interaction between the alga, mineral particles and bacteria that could explain how the alga is able to collect micronutrients in its hostile snow environment. Coelastrella on the other hand is able to remodel its compartments and their metabolic content when submitted to uranium-related stress.
Our TOF-SIMS images almost meet NanoSIMS images (that is however restricted to purely elemental mass spectrometry imaging) in term of resolution. Correlative TOF-SIMS/SEM allows a remarkable enhancement of the comprehensibility of TOF-SIMS images by adding in extra structural information. We reveal for every different organelle a different metabolic footprint and hints into their adaption to their specific environment.

Keywords:

Metabolic imaging, Microalgae, Correlative microscopy

Reference:

Ezzedine, Jade A., et al. "Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging." Nature Communications 14.1 (2023): 7500.

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Multimodal Scanning X-ray Spectromicroscopy of 2D layered Titanium Carbide MXenes

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Poster Group 1

Two-dimensional layered materials, such as transition metal carbides or nitrides, known as MXenes, offer an ideal platform to investigate charge transfer processes in confined environments, relevant for energy conversion and storage applications, such as supercapacitors and Li-ion batteries. Their rich and tunable surface chemistry plays an essential role in the pseudocapacitive behavior of Ti3C2Tx MXenes, where Tx=O, OH, F, Cl etc. However, the local distribution of surface functional groups over single flakes and within few- or multilayered flakes remains unclear. In this presentation, we introduce synchrotron-based scanning X-ray microscopy (SXM) with simultaneous transmission and electron yield detection (EY), enabling multimodal nanoscale chemical imaging with bulk and surface sensitivity, respectively, of individual MXene flakes. Soft X-rays absorption spectroscopy (XAS) allows the probing of fine electronic structure of the metal valence electrons and light elements present in the layered transition metal compounds and electrolytes. Specifically, for transition metal structures with termination groups found in MXenes, the crystal field interaction determines the shape, strength, and occupancy of electronic orbitals. Transmission imaging techniques with soft X-rays are ideal for monitoring changes in the local chemistry of few-layered materials, with guest-species between the interlayers or exposed to different environments. Microscopy techniques, other than SXM, offer limited spatial resolution (optical microscopy), only surface sensitivity (Scanning probe microscopy, XPEEM), only bulk sensitivity (TEM, TXM) or lower energy resolution (EELS). Single and few-layered Ti3C2Tx MXenes, synthesized with hydrofluoric acid (HF-etched), and multilayered Ti3C2Tx MXenes, synthesized via the molten salt route (MS-etched) [5], are imaged by multimodal SXM at the Ti L- and O K-edge using the BESSY II synchrotron light source. Transmission detection mode is used for bulk sensitivity, required to probe the surface chemistry in the MXene interlayer, while EY mode allows high surface sensitivity for probing the top MXene layer. SXM allows the monitoring of MXene flake thickness and electron emission properties at the sub-flake level. Local XAS measurements have a high chemical sensitivity to the Ti and O bonding configurations, hence to the surface chemistry of MXene. The Ti chemical bonding differs dramatically between HF-etched and MS-etched Ti3C2Tx MXenes explained by the larger content of O-termination in MS-Ti3C2Tx. Thus, different surface chemistry and electronic properties are identified for MXenes, synthesized via different routes. SXM also enables the chemical identification of the different components found in post-mortem analysis of battery components cycled in a Li-ion battery with Ti3C2Tx MXene, as working electrode. Adsorbed electrolyte (surface) and electrolyte-electrode interaction (bulk) can be probed with high chemical sensitivity. Changes in the chemical bonding configuration of electrochemically cycled MXene electrode are recorded in the bulk of MXene, related to interaction of MXene with intercalated lithium cations, and its top layer, due to interaction with the electrolyte. Specifically, upon comparison of XA spectra at Ti L- and O K-edge between cycled and pristine MXene in bulk, we identify increased Ti oxidation state in cycled MXene, related to the bonding of lithium cations with oxygen surface termination groups. Furthermore, surface-sensitive TEY measurements

hint at surface reactions between the MXene and the electrolyte during the electrochemical cycling process. This distinction underscores the complex interplay of bulk intercalation and surface phenomena in these materials. Multimodal SXM can be applied to other laminated materials, such as graphene, layered transition metal dichalcogenides, thin film solar cells and multilayered polymer films, thereby opening new insights into intercalation and surface redox processes.

Keywords:

MXenes, STXM, Batteries, Surface Chemistry

Reference:

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Coulomb-correlated multi-electron states in a transmission electron microscope beam

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Poster Group 2

Background incl. aims
In contrast to possibilities arising from electron correlations in condensed matter systems, stochastic Coulomb interactions in free electron beams are usually considered detrimental. In electron microscopy, electron repulsion leads to stochastic longitudinal and transverse emittance growth and limits the brightness of state-of-the-art electron sources. However, in a regime where few-body interactions dominate, Coulomb interactions can also have beneficial effects based on strong inter-electron repulsion, such as non-Poissonian electron beam statistics [1]. A powerful approach to induce such strong electron-electron interactions is femtosecond-triggered photoemission from nanotips [2, 3].
In this work [4], we characterize the strong Coulomb correlations of multi-electron states from a laser-triggered nanoscale field emitter, with applications in correlated probing applications, shot-noise reduction, and electron heralding schemes.

Methods
The experimental work is carried out in transmission electron microscopes that are modified for photoexcitation of the electron source [4, 5]. A laser pulse train with 2 MHz repetition rate and 160 fs pulse length generates ultrashort electron pulses by close-to-threshold laser-triggered Schottky emission [5]. The photoelectrons are analyzed with an event-based electron detector, which is mounted behind an imaging energy filter and characterizes the spectral and spatial properties of the electrons. The detected electrons are matched to the generating photoemission laser pulse, and the number of electrons n=1,2,3,4 is assigned to each pulse (see Fig. a).

Results
The single electron state spectrum exhibits a single peak at the beam energy of 200 keV (see Fig. b). Strikingly, the electron pulses with n>1 exhibit a distinctive spectral shape with the number of peaks identical to the number of electrons in the pulse (see Fig. c-e), indicating a strong interaction between the electrons in the pulse. The n=2,3,4 -states are plotted with respect to the state-averaged energy. For n=2, a projection of the energies of electrons A and B onto a two-dimensional energy pair histogram (Fig. f) shows a characteristic pair-correlation energy of about 2 eV. The strong inter-particle energy exchange is caused by acceleration-enhanced inter-particle Coulomb interaction, as confirmed by trajectory simulations. State-sorted beam caustics reveal a discrete increase in virtual source size, a longitudinal source shift, and a pronounced angular distribution of the few-electron states compared to single-electron pulses. As the correlation primarily emerges in the initial acceleration stages of the electron gun, controlling the electrostatic configuration enables control over the ratios of transverse and longitudinal Coulomb correlations. Using apertures along the optical axis, which limit the maximum transverse momentum of electrons transmitted into the

column, we can decrease the current-current correlation function by lowering the extraction voltage applied to the nanotip cathode.

Conclusion
The high fidelity of few-electron pulses in conjunction with single-particle detection enables the preparation of distinctive electron states for correlated probing, as well as enhanced microscopy and lithography. Specifically, we propose schemes to generate an electron beam with sub- or super-Poissonian two-electron statistics. By means of spatial filtering with an annular aperture or spectral filtering with either a slit or a beam stop, the two-electron state is suppressed or enhanced, resulting in a shot-noise- (annular aperture, slit) or singlet-state-reduced (beam stop) electron beam, respectively. Applications of the two-electron state-enhanced electron beam include heralded single-electron sources and may foster new developments in free-electron quantum optics and quantum-enhanced electron microscopy.

Keywords:

UTEM, Electron correlations, Electron sources

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Operando TEM Reveals Oscillatory Surface and Bulk Dynamics of Nickel Nanoparticles in Ethylene Partial Oxidation

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Poster Group 2

Background

Heterogeneous catalysis, particularly employing transition metal nanoparticles as catalysts, has emerged as a promising avenue for facilitating the partial oxidation (POX) of hydrocarbons (methane, ethane, propane) to syngas.[1] Syngas, a mixture of carbon monoxide (CO) and hydrogen (H_2), serves as a crucial precursor for a wide range of industrial processes including the synthesis of methanol, ammonia, and various other essential carbon based chemicals.[2] Recent efforts have focused on unravelling the catalytic mechanisms at play in these reactions aiming to improve efficiency and selectivity while minimizing unwanted byproducts. One intriguing phenomenon observed in such catalytic systems is the self-sustained oscillatory behaviour, where the catalytic activity undergoes periodic fluctuations over time.[2] These oscillations can lead to enhanced reaction rates and selectivity, offering specific insights into the dynamic nature of catalytic processes. This phenomenon have been observed in many heterogenous catalytic systems including oxidation of hydrogen or ammonia, oxidation or reduction of carbon monoxide, dry reforming of methane, nitric oxide, various saturated and unsaturated hydrocarbons and alcohols.[2] In the context of transforming ethylene to syngas, POX presents one of possible pathways for production of valuable chemicals and fuels. Our study investigates the role of nickel nanoparticles (Ni NPs) as catalysts in the POX of ethylene, aiming to understand the oscillatory dynamics observed during the reaction. Utilizing operando transmission electron microscopy (TEM), we attempt to gain valuable insights into structural and morphological changes of catalyst at high spatial (sub-nm) and temporal resolution (milliseconds), thus contributing to the development of more efficient and sustainable catalytic systems.

Methods

For this study, Ni NPs were sputtered onto the heater of a Microelectromechanical Systems (MEMS) chip of the nanoreactor.[3] The operando TEM experiments were conducted using an aberration-corrected JEOL GRAND ARM Vortex TEM microscope, operating at 300 kV, combined with a DENSsolution Climate gas system and a HIDEN quadrupole mass spectrometer (QMS) 3F PIC for real-time gas analysis. Before the gas mixture for the POX of ethylene was introduced, the Ni NPs underwent reduction in a gas mixture of H_2:He (1:4 ratio) at 500°C for 3 hours. The POX of ethylene was then carried out at a pressure of 1 bar, with a gas ratio of C_2H_4:O_2 = 1:1, and at a temperature of 950°C.[4]

Results

Using operando TEM, we were able to directly observe the structural and morphological changes of Ni NPs in a self-sustained oscillatory regime during the POX of ethylene to syngas. Despite the Ni NPs varying in shape and size, ranging from several tens to up to 200 nm (Figure 1a), mass spectrometry (MS) data have demonstrated that the vast majority of Ni NPs exhibited synchronized behaviour, oscillating between a high activity (metallic state) and a low activity regime (nickel-oxide state) shown in Figure 1b.[4] More importantly, the MS data allows direct correlations between the structural and morphological changes of the NPs and alterations in the gas phase, thus providing a comprehensive understanding of the catalytic process at play.[4]
Furthermore, the observed dynamics of Ni NPs, though some of them unsynchronized, can be categorized into two distinct types: bulk dynamics and surface dynamics.[4] In bulk dynamics, as depicted in the Figure 1c, the entire particle undergoes a structural transformation when transitioning between the high activity and low activity regimes. On the other hand, the nanoparticles that exhibit surface dynamic only show minor morphological changes to their overall shape.[4] The time-lapse series of TEM images (Figure 1c) showing a single Ni NP undergoing bulk dynamics under specific reaction conditions. The transition period between the Ni and NiO states varies, ranging from 2 to 100 seconds within a single cycle. The sequence initiates with the particle in a metallic, high activity regime. Within approximately 1 second, it swiftly transitions to the oxidic state, low activity regime, indicating a rapid structural change. Ultimately, as one cycle concludes, the particle returns to the high activity regime, during which it shifts from an irregular oxide shape to a more rounded, metallic configuration.[4]

Conclusions
In conclusion, our investigation into the POX of ethylene using Ni NPs as catalysts reveals intricate dynamics underpinning the catalytic process. Utilizing advanced operando TEM, we observed both the structural and morphological transformations of Ni NPs in a self-sustained oscillatory regime. Through detailed analysis and characterization by operando TEM, we pave the way for optimizing catalyst design and operational parameters, aiming for enhanced efficiency, selectivity, and a deeper mechanistic understanding of catalytic processes at the nanoscale.

Figure 1: Oscillatory dynamics of nickel nanoparticles during ethylene partial oxidation. (a) Overview TEM image highlighting size and shape variations of Ni NPs. (b) Mass Spectrometry (MS) data depicting a complete cycle, illustrating the catalyst's high and low activity regimes. (c) Time-lapse evolution showcasing morphological changes in a single Ni NP under reaction conditions: C_2H_4:O_2 gas mixture ratio of 1:1, at a pressure of 1 bar and a temperature of 950°C.

Keywords:

operando TEM, oscillations, catalysis, Nickel

Reference:

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Low dose and in situ 4D-STEM powered by real-time sparse-array analytics on an event-driven detector

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Poster Group 2

Background incl. aims
Four-dimensional scanning transmission electron microscopy (4D STEM) measures 2D reciprocal space scattering information from an electron probe laterally shifted across a 2D real space specimen area [1]. This high-dimensional dataset allows much richer information to be retrieved than conventional STEM, ranging from crystallographic orientation and phase, strain, short and medium range ordering, to differential phase contrast (DPC), ptychography as well as other new imaging modes. The past decade has particularly witnessed the advance of this technique because of the introduction of fast direct electron-counting detectors. These detectors usually run at 1-10 kHz continuous readout, which corresponds to 1000-100 µs pixel dwell time in 4D STEM measurements, while the conventional STEM usually runs in few µs pixel dwell time. Such a 2-3 order-of-magnitude gap in speed would limit the application of the versatile 4D STEM measurements to at least two fields, including low-dose atomic-resolution high-contrast imaging of sensitive specimens as well as in situ time-resolved studies of dynamic behaviour upon external stimuli.
Methods
In order to tackle this challenge, we installed an event-driven CheeTah T3 detector with 2x2 chips (in total 512x512 pixels) from Amsterdam Scientific Instruments (ASI) on a monochromated and probe-corrected Thermo Fisher Scientific Titan STEM for 4D STEM applications. This detector retrieves precise timing of scanning line trigger signal from the microscope with the same clock as the electron impact events on the detector, which facilitates a reliable assignment of the electron events to each scanned pixel. Thanks to its 1.56 ns Time of Arrival resolution, this detector also permits recording electron events continuously in an effectively much higher frame rate for 4D STEM. Due to its inherent sparse nature, this high-dimensional data can be compressed and computed in real time efficiently in the compressed sparse row (CSR) format based on the collaborative development between ASI and LiberTEM team [2].
Results
4D STEM measurements at effectively MHz frame rate with live image reconstruction has been demonstrated based on these developments (see one example in the enclosed figure). This unique workflow enables the user to navigate the specimen, adjust focus or astigmatism, and eventually visualise sensitive specimens or dynamics without changing between different detectors or optical modes. A few application examples will be discussed during this presentation. This includes, but not limited to,
- Fast and low-dose phase-contrast imaging at nanometer to atomic resolution based on integrated centre-of-mass imaging, ptychography, and other 4D STEM modes,
- In situ 4D STEM measurement of both electromagnetic fields and strain/orientation/phase on magnetic and semiconductor specimens under tensile loading and unloading conditions,

- In situ 4D STEM mapping of electron-beam-induced charging on dielectrics and vitrified ice.

Conclusions

Effectively MHz frame rate event-driven detector has been successfully demonstrated for low dose and in situ time-resolved 4D STEM characterization. Sparse-array data format was implemented for real-time feedback and efficient analysis with compact data size. Versatile applications on low dose imaging of sensitive specimens and time-resolved studies of dynamic processes will be presented during this contribution. Further improvement and other potential applications for this workflow will also be discussed.

Keywords:

4D-STEM, low-dose, in-situ, event-driven detector

Reference:

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Unveiling degradation mechanisms in layered Li-rich cathode materials using combined operando neutron diffraction and 4D-STEM

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PS-04 (2), Plenary, august 26, 2024, 14:00 - 16:00

Background incl. aims
Layered Li-rich nickel-cobalt-manganese (LRM) oxides are promising materials for applications as cathodes in next-generation high-capacity power batteries.[1] However, the commercialization of LRMs is challenging due to their cycling instability, which originates from their intrinsic structural characteristics and deterioration processes.[2] Until now, neither their structures, which can comprise two-phase nanocomposites or only single-phase solid solutions, nor their degradation pathways, have been clarified. It is noteworthy that existing studies are based on assumptions and inferences regarding the structure under specific conditions and do not truly reflect the actual structural evolution of LRM cathodes under operational conditions.[3-5] Moreover, due to the lack of systematic research on typical failure structures in LRM cathodes, the structural evolution throughout the entire lifecycle of LRM cathodes remains obscured. Therefore, it's of great importance to reveal the actual evolution process of the structure and the degradation mechanism of LRM cathodes unfer operational conditions comprehensively. The aim is to unveil initial lattice structure and the complete structural evolution process, encompassing transition metal rearrangement, phase structure evolution, defect structure growth, and ultimate structural failure, throughout the entire lifecycle of rich lithium manganese cathodes, with the hope of providing insights for the design of LRM materials.
Methods:
This study employs in operandon neutron diffraction technology with characteristics such as sensitivity to light elements and resolution of TMs. the study combines advanced pression-assisted 4D-STEM technology with comprehensive phase analysis capabilities, supplemented by atomic-resolution aberration-corrected electron microscopy (HAADF-STEM), focusing on both short and long-range structures of LRM cathode materials.
Results:
In this work, We prepared a LR-NCM | graphite full cell and carried out both in operando neutron diffraction and low dose and precession-assisted 4D-STEM to map spatially-averaged structural evolution in real time over a large field of view and visualize light (O, Li) and heavy (TM) atoms simultaneously with atomic spatial resolution before, during and after cycling. Our work provides detailed insights into dynamic nanostructure evolution and local structural interactions through multiscale characterizations. The structure of pristine LRM is demonstrated to be a solid solution based on C2/m symmetry, resembling a thin layer of spinel. In the initial cycling, irreversible extraction of Li ions from the transition metal layers leads to the formation of vacancies within the layers, causing irreversible structural transformations within the material. The in operando neutron diffraction technique further tracks the formation of oxygen vacancies after the first discharge.

Despite surface coating with a spinel layer to prevent oxygen loss, the effectiveness is limited. In subsequent extended cycles, continuous loss of excess Li ions and migration of TMs within the layers result in the irreversible transformation of the internal structure from a lithium-rich phase to a conventional layered phase. This transformation leaves numerous vacancies within the layers, leading to the formation of internal nanoscale defects. These defects obstruct the channels for Li-ion transport within the layers, consequently causing a decline in material performance.
Conclusion:
Our work provides a new perspective on material failure mechanisms, emphasizing the importance of intrinsic modification methods in stabilizing the internal structure of layered high-energy cathodes, beyond conventional surface modifications.

Keywords:

Degradation-mechanism, Li-rich cathode, neutron-diffraction, 4D-STEM

Reference:

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In-Situ insights into Multivalent Metal Energy Storage: A model system for calcium sulfur batteries

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Poster Group 1

As we globally realize large-scale electrical energy storage coupled with the rapid increase in global electromobility, the challenge of securing a consistent supply of critical raw materials utilized in lithium-ion batteries (LIB) continues to grow. Consequently, the search for alternative systems delivering comparable energy densities has become an important topic. Multivalent metals, renowned for their long-term stability, emerge as promising candidates in this regard.

The combination of calcium and sulfur represents a potent alternative, attributed to their abundant availability and recent research delineating the appreciable stability of their electrolyte system. [1] Nevertheless, to enhance fabrication efficiency, performance, and stability, a deeper understanding of the electrochemical dynamics at the anode surface, where the electrolyte interfaces with calcium, is essential. The work in the Casino project [2] aims for an understanding and optimization of key aspects towards its application.

The electrochemistry happens at the surface between the electrodes and electrolyte system. Understanding the details is what makes improvements possible. A novel in-situ model system is developed to mimic the behavior of the macroscopic symmetric Ca-Ca battery system while allowing atomic-level observations. Direct transfer of 2D materials enables the formation of liquid pockets and therefore high-resolution studies. [3,4] Calcium and electrolyte solution is encapsulated between 2D materials, and In-situ investigations are employed combined with EELS and EDX to uncover key mechanisms.

Keywords:

in-situ TEM, Multivalent Batteries

Reference:

The project was financially supported by the Bundesministerium für Bildung und Forschung (BMBF), under project CaSino, Grant number (Förderkennzeichen) 03XP0487D.
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TEM study of neutron radiation damage in tungsten

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Poster Group 2

Nuclear fusion provides a safe and abundant source of energy for a long period of time and offers several important additional advantages. These include: fusion does not contribute to greenhouse gas emissions or global warming (a), reduction of long-lived radioactive waste compare to the nuclear fission reactors (b) and inherent safety features (c). Tungsten (W) is considered a promising plasma-facing material for future fusion reactors since it has number of advantages such as high melting temperature, excellent thermal conductivity, high strength and low sputtering yield. Its application in the International Thermonuclear Experimental Reactor (ITER), which is being built in Cadarache, France, and in the future Demonstartion Power Plant (DEMO) has significantly increased interest in W behavior under neutron irradiation. Knowledge about the defect formation and their evolution in neutron irradiated W is essential not only for assessing its applicability as a structural material in fusion reactors, but also for understanding numerous irradiation experiments in fission reactors.
ITER grade W was neutron irradiated in the BR2 material test reactor (Mol, Belgium) at 600°C, 800°C, 900°C and 1200°C to a damage dose of 0.1 dpa, 0.2 dpa, 0.5 dpa and 0.8 dpa. The microstructure of the irradiated material was analyzed using the Talos F200X transmission electron microscope (TEM), which is equipped with four energy-dispersive X-ray detectors (Super-X). The precipitates were detected either by high resolution TEM or by EDX elemental mapping, where they are visible in elongated shape.
The detailed TEM characterization of the radiation-induced materials shows the formation of three types of defects, e.g. voids, dislocation loops and nano-sized precipitates. The source of the precipitates are the transmutation-induced elements such as rhenium (Re) and osmium (Os), which accumulate with the damage dose [1]. In addition, a dose-dependent segregation of Re and Os, i.e. the formation of clusters around defects, was observed [2].
The results of the TEM analysis are summarized in Figure 1. The parts (a) and (b) show the voids and dislocation loops formed in W under neutron irradiation. The voids have a round or almost round shape, but in a few cases faceted voids with a size of 3-4 nm have been observed (a). Dislocation loops are typically visible in DF images obtained using a defined g-vector with a “coffee bean” contrast (b). Most loops are between 3 and 10 nm, but individual loops can be up to 20 nm. The number density of dislocation loops at 0.1 dpa and 0.2 dpa is comparable to the number density of voids (about 1022 m-3), while for material irradiated to 0.5 dpa and higher, the number density is reduced by about two orders of magnitude.
Spatial distribution of Re and Os segregation was studied by STEM-EDX element mapping in the TEM, where their local presence was visualized by intensity variations of the different colors. The part (c) demonstrates the distribution of Re (green) in representative areas in the material. The clear segregation was observed at the grain boundaries, line dislocations and radiation-induced voids and loops. At 0.1 dpa and 0.2 dpa, the Re distribution was detected only around the voids, while the Re signal at the dislocation loops was seemingly below the detection limit. There was no Os signal

detected in 0.1 dpa damage, while a weak Os signal was detectable in the material with 0.2 dpa. At 0.5 dpa and 0.8 dpa, Re and Os were detected around voids and loops.
The detailed analysis of Re and Os segregation on defects in the sample irradiated to 0.8 dpa is shown in Figure 1d-g. The distribution of Re (green) and Os (blue) are visualized in corresponding elemental maps (d and e) and shown overlapped in the part (f). The overlapping clearly show that Os tends to form small rich areas inside Re clouds which mostly have a spherical shape. Os-rich areas often have a needle-like shape, indicating the formation of precipitates or their precursor. These Os-rich areas are formed on both voids and dislocation loops.
To provide a detailed visualization of the Os distribution, the intensity profiles were recorded across voids and loops. The example of segregation around a void marked by a yellow arrow is shown in part (d-e). The profile (g) was taken through the void marked with the arrow. It shows the typical reduction in Re-intensity at the void position and the formation of elongated, Os-rich regions adjacent to the void on one side. In the few cases the crystallinity of the precipitate was detected. The atomic spacing of 0.23 nm could correspond to the [111] atomic plane of the □-WRe2 phase.
The study includes a detailed TEM characterization of the radiation-induced defects, which can be divided into three types, e.g. voids, dislocation loops and precipitates. In addition, a dose-dependent segregation of Re and Os, i.e. the formation of Re rich clusters around defects, was observed.

Figure 1: Extensive TEM analysis of radiation damage in W. TEM images of voids and dislocation loops a shown in parts (a) and (b) correspondingly. Part (c) visualize the Re-segregation on defects, dislocations and grain boundaries. Parts (d-g) demonstrate the analysis of Re- and Os-segregation on defects.

Keywords:

fusion, tungsten, radiation damage, transmutation

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Electron-photon quantum interaction enables novel ultrafast electron imaging approaches

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

Background incl. aims
Coherently controlling the electronic and structural properties of new generation materials and devices down to the sub-nm level with femtosecond precision is one of the ultimate challenges in quantum technology, and has the potential to engineer novel states of matter with exceptional macroscopic functionalities. Here, we respond to such pressing need by introducing a new paradigm where the interaction between light and free-electrons can be exploited for coherently manipulating electron beams [1,2], enabling dynamical investigation of materials with enhanced sensitivities to specific materials degrees of freedom, unattainable using conventional schemes. We develop innovative ultrafast electron microscopy (UEM) methods based on light-induced electron modulation aiming to observe, understand, and control the non-equilibrium behavior of low-dimensional systems at such ultrasmall and ultrafast scales, which would be otherwise hidden in standard UEM observations.

Methods
The playground for these experiments is a modified Ultrafast Transmission Electron Microscope (UTEM) with multiple optical accesses for: i) electron pulse generation, ii) electron wavefunction modulation, and iii) sample excitation. Arbitrary longitudinal and transverse modulation of a free-electron wave function is obtained using light fields properly modulated in space and time. In particular, a femtosecond electron pulse interacts with the chosen configuration of the optical distribution as generated via a spatial light modulator (SLM) (see schematics in the left panel of the figure), and the energy-momentum quantum exchanges between electron and photons resulting from such coupling are mapped in the electron multidimensional phase space. [3]

Results
In this contribution, we will first demonstrate how to coherently modulate the spatial, momentum, energy, and temporal distributions of the electron wave function with high versatility on different modulation basis. This derives from the technological development of a new device: a photonic-based modulator for dynamic multidimensional control of electrons. Then, we will show how our approach for versatile transverse and longitudinal electron modulation at the fs-timescale is crucial for the first time implementation of new imaging techniques (see schematics in the right panel of the figure), such as Ramsey-like holography [4] – for phase- and time-resolved imaging of hybridized low-

energy modes in strongly-correlated systems – and Single Pixel Imaging [5] – that has been only recently proposed for optical detection and now is possible also in electron microscopy permitting lower electron doses, faster acquisition rates for individual nanoscale objects in their natural environment.

Conclusion
In conclusion, we report on the realization of an all-optical rapidly-programmable phase mask for electrons, which allows us to implement new electron imaging approaches with enhanced sensitivities and performance, thus radically changing how matter is investigated in electron microscopy. This is based on a specific sensitivity to materials' inner coherence when light-modulated structured electron waves are adopted. Our results pave the way for achieving unprecedented insights into new generation materials and their dynamic behavior, impacting also future photonics and electronics applications. More generally, unlocking this potential will play a decisive role in our ability to address the grand challenges that the world is facing nowadays, especially regarding 'energy', 'information' and 'health'.

Funding:
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Keywords:

UTEM; electron-wavefunction modulation; low-dimensional materials.

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ePattern: an adaptive 4D-STEM Pattern Registration Algorithm to Optimize ACOM Pattern Matching

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Poster Group 2

The technique known as four-dimensional scanning transmission electron microscopy (4D-STEM)1 has recently emerged as a potent tool for the local characterization of crystalline structures in various materials, including cathode materials for lithium-ion batteries and perovskite materials for photovoltaics.
This 4D-STEM data analysis method based on the ACOM system of NanoMegas (Astar)2,3 uses pattern matching of a scanning nano-diffraction dataset with libraries of diffraction patterns simulated from known structures extracted from CIF files. This method enables to construct crystalline phase and orientation maps to determine crystallinity4,5, microstructures6, structural deformation7, and grain boundaries8 using scanning nano-diffraction with precession mode in a nanometer resolution9,10. However, the utilization of new detectors specifically designed for electron diffraction patterns and other advanced techniques necessitates the continuous adaptation of methodologies to address the challenges associated with crystalline materials.

The goal of the data preparation methods proposed here is to improve the quality of Astar pattern-matching using a dataset of diffraction patterns acquired with a CMOS Oneview camera. The high sensitivity of the CMOS camera and the data filtering developed here modify the diffraction images leading to a compromise between improving image quality and optimizing template-matching results11.

Our strategy employs a data reduction technique that utilizes registration methods to identify electron diffraction spots within patterns. By doing so, we are able to filter and capture the diffraction signal and subsequently reconstruct the patterns. These reconstructed patterns are then inputted into the Astar suite pattern-matching software. This process allows us to record the essential information of each reflection in a dataset, including intensity, size, and position, with a high level of accuracy (sub-pixelar) for the position, typically on the order of 10-3px. Additionally, this technique achieves a significant reduction in data size, typically by a factor of 103. This means that the essential information of the diffraction pattern is stored on a disk with 100 to 1000 times less space required. The adaptative nature of this method not only reduces noise and compresses nanodiffraction scanning data for ACOM and strain mapping analysis but also extends its applicability to other techniques such as 3D electron diffraction (3DED).

To evaluate our approach, we employ dedicated metrics that clearly demonstrate a substantial improvement in phase recognition. Our results indicate that this data preparation method not only enhances the quality of the resulting image but also boosts confidence levels in the analysis of crystal orientation and phase determination outcomes. Furthermore, it helps mitigate the potential impact of user bias that may arise when manipulating parameters during the application of this method.

Firstly, modifications on inside diffraction patterns are estimated through image quality metrics such as peak signal-over-noise (PSNR), structural similarity index measure (SSIM), and root-mean-square error (RMSE). Secondly, the quality of the pattern-matching process on filtered and reconstructed images obtained by the proposed experimental data preparation method is evaluated using index and orientation reliability, as defined in the Astar software. We demonstrate that the experimental data preparation helps to improve the pattern-matching quality result, as it reduces noise overfitting, improves structural similarity index measure, and increases the orientation reliability.

Keywords:

4D-STEM ACOM, ePattern software, diffraction-pattern

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In situ ESEM study of supported noble metal nanoparticles dynamics under reaction conditions

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Supported noble metal nanoparticles (NPs), including Pt, Pd, and PtPd alloys on Al2O3 or CeO2, are pivotal in heterogeneous catalysis for processes like the water gas shift reaction, methanation, and hydrocarbon oxidation/partial oxidation. Maximizing the economic efficiency of these costly metals necessitates catalysts with high activity over their lifetime. A primary challenge to maintaining activity is thermal deactivation via sintering. Two fundamental sintering mechanisms, namely Oswald ripening (OR) and particle migration and coalescence (PMC), have already been identified in the literature. However, recently there has been an ongoing debate about surface vs gas-phase mediated sintering pathways [Ple-16][Oh-23]. To help address this open question we aim to capture real-time, statistically significant data to provide insights into the dynamics of chosen NP/support systems under reactive conditions on a broad micrometer scale using electron microscopy.

While in situ catalysis experiments using electron microscopy were previously carried out only in transmission electron microscopes (TEM) equipped with special holders for heating or gas experiments, allowing the study of single nanoparticles with atomic resolution, recently environmental scanning electron microscopes (ESEM) have become a popular tool to study dynamic catalytic behavior on a broader micrometer scale [Bar-19].
In this work we will introduce our novel in situ experimental setup inside an ESEM using a heating stage, capable of temperatures of up to 1000°C and a self-build gas injection system, thereby enabling the study of large populations of supported NPs and their reaction to experimental stimuli. This experimental setup coupled with an automatic post-processing routine based on computer vision algorithms allows the analysis of long time series of recorded images and permits a statistical evaluation of the NP motion trajectories, thus providing insight into the sintering behavior of these NPs as a function of temperature and gas atmosphere.

Figure 1 shows the main steps of our post-processing routine, which is based on computer vision algorithms and an approach developed by Faraz et al [Far-22] for the environmental transmission electron microscope (ETEM). After acquiring long time series in step 1, NPs are detected in each frame and segmented in step 2. To overcome signal-to-noise limitations, the shape and center of mass of the nanoparticles are approximated in step 3 to better compute motion trajectories by comparing differences in nanoparticle positions from frame to frame in step 4. Not only the motion trajectories can be analyzed in step 5, but also the evolution of the NP size distribution over time.
In contrast to the work of Faraz et al., our routine works on a larger length scale and processes more nanoparticles under the additional constraint that the nanoparticle support has a more homogeneous contrast, making drift compensation based on background registration more difficult.

We will present the first preliminary results of our experimental setup under simplified oxidation conditions of 10mol-% O2 in N2, as well as reduction conditions of 5mol-% H2 in N2 at various temperatures for different NP/support systems. Following up from a previous study in which we analyzed the redispersion mechanism of Pt/CeO2 under alternating oxidation/reduction cycles ex situ, we will present the results of this redispersion experiment in our new ESEM setup, as well as ex situ characterization of the CeO2 support substrates used in this work [Sch-24]. In addition, we will present our data post-processing routines and discuss some challenges.

Keywords:

In situ, ESEM, Sintering, post-processing

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In-Situ Charging and Charging Map for Characterization of Electronic Materials

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Electronic materials have garnered significant interest due to their potential applications in a wide array of important electronic devices, including transistors, sensors, solar cells, and more. Achieving high-performance devices and developing new electronic materials for future technologies relies fundamentally on understanding electronic processes through advanced characterization techniques. Currently, the characterization of the active layer in a device can offer either global functional information, such as with the most current-potential methods, which only demonstrate the impact of defects on performance without detailing the quantity and distribution of defects, or provide localized details, as seen with techniques like atomic force microscopy and conventional scanning electron microscopy (SEM), which may not capture the entirety of the case. Therefore, we present a novel characterization method capable of acquiring both kinds of information from the active layer, which would be highly beneficial for comprehending the structure-morphology-performance relationship.

Methods
The use of Ultra-low voltage (ULV) SEM has become increasingly crucial for investigating beam-sensitive materials, particularly organic molecules and polymers, due to its ability to significantly suppress beam damage. Additionally, the charging behavior under ULV conditions becomes more material-characteristic compared to conventionally used beam energies (e.g., < 500 eV). Fine-tuning the beam energy within the range of 10-200 eV can result in either negative or positive surface charging [ref.1], depending on factors such as beam energy, beam current, charging density, and the conductivity of the materials. This capability allows us to simulate the working state of a p- or n-type transistor by in-situ generating the required charge type. Moreover, the surface potential resulting from charge flow anomalies enables us to visualize morphological defects along the pathways. Through post-imaging processing, we can calculate the real working pathway for charge-carrier transport, subsequently correcting the charge-carrier mobility [ref. 2].
Furthermore, under ULV conditions, we can collect the energy distribution spectrum of emitted electrons, which can be transformed into a surface charging map. The gradient of the charging attenuation provides structural information within (poly)crystalline domains [ref. 3].

Results
Utilizing the first method mentioned above, we successfully corrected 11 organic thin-film transistor devices, resulting in highly reliable mobility values that accurately reflect the material's charge transport capability. With the latter method, we can estimate the charge-carrier mobility from polycrystalline thin films, which align well with the calculated values. Of particular interest is our ability to distinguish between various crystalline domains with different molecular orientations. Furthermore, we can determine molecular orientation by observing the gradient of the charging

attenuation, a technique which, to the best of our knowledge, represents the first application of SEM for this purpose.

Conclusion
By employing in-situ charging and charging maps via ULV-SEM, we can gather information regarding various morphological phenomena. This enables us to directly correlate device performance with the chemical structure of the active material and its morphology, thereby facilitating the design of novel structures and the optimization of the active layer in electronic devices.

Keywords:

charging map, molecular orientation, mobility

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3D Operando Monitoring of lithiation spatial composition in NMC-cathode electrode by X-ray nano-CT & XANES-techniques

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PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

Over the last decades, with increasing energy demand along with the shift towards greener energy solutions, Li-ion batteries have gained attraction for energy storage applications.[1] Due to the high complexity in the hierarchical architecture of composite electrodes, it is extremely challenging to ensure a homogenous electrode structure. The non-uniform distribution of phases, leading to microstructural heterogeneities, has been shown to induce a non-uniform electrochemical behavior, which can deteriorate the performance, and cause macroscopic failures.[2,3] Therefore, the identification of these microstructural heterogeneities and understanding their effects on the dynamics of (de)lithiation is crucial to improve electrode design.

Lithium compositional distributions in composite electrodes can also be investigated using various techniques such as Raman microscopy,[4] X-ray diffraction (XRD),[5] or 2D X-ray absorption near-edge structure spectroscopy (XANES).[6] However, these methods have been restricted to low spatial resolution or spatially integrated signal along the depth direction. These methods hence cannot accurately capture the spatial distributions and can lead to a limited interpretation of the mechanism. Recently, Finegan et al.[7] used Operando X-ray diffraction-computed tomography (XRD-CT) for characterizing, in 3D, the dynamic crystallographic structure between and within LMO particles during electrochemical operation.

Several works have combined XANES and X-ray tomography to generate a rich data set that allows a direct correlation of the chemical information and the microstructure. Meirer et al.[8] used 3D XANES to probe the conversion of NiO to Ni metal in a partly reduced electrode. They found significant inhomogeneities in terms of chemical states across particles as well as evidence of fracturing caused by volume expansion during the reaction. More recently, Wang et al. [9] collected a 46-point spectrum across the iron K-edge of a LiFePO4 cathode particle and developed a run-out correction system to enable automated tomography.

Here, we perform XANES coupled with transmission X-ray computed tomography (TXM-CT) at nanoscale in Operando mode to investigate LiNi0.5Mn0.3Co0.2O2 (NMC) positive electrodes[12]. The ability to probe battery materials in an operando mode provides significant advantages over ex situ approaches and allow characterizing the microstructure evolution while capturing the electrochemical reaction kinetics inside electrode and active materials (AM). In this work, we demonstrate that Operando XANES-TXM nanoCT allows access to the Li distribution (local Li concentration) at nanoscale within the LiB electrodes during the operation. We also proposed a method that relies on just three energy levels located at pre-edge, edge, post-edge for minimization of radiation damage to the sample. Also, a specific electrochemical cell and a sample preparation process were introduced, which make the use of electrodes typical to those used in real-life applications possible.

Our experimental results unveil the non-uniformity of the Li repartition throughout the LiB porous electrodes during operation [10]. As a result, there are regions being more active than the others as well as preference spots on the particle surface where the reactions are more likely to occur. The Li repartition across the electrode depth is adequately uniform, which indicates a negligible effect from porous electrode. This is expected as we used EC:DMC solvent-based electrolyte at 1.2 M which reduces the limitation from ionic transport in liquid phase within the porous electrode. Thus, we can conclude that mass transport in the electrolyte is not rate limiting factor in this electrode under the applied cycling conditions. In addition, some of the reconstructed slices also revealed that the lithiation process could also happen from internal pores of the AM particles. This observation also implies that the electrolyte along with the carbon conductive can penetrate some internal pores through the AM grain boundaries and allow the (de)lithiation process to occur from the inside of the NMC secondary particles.

Finally, we expect that our analysis technique will provide a direct method to unveil the effects of the electrode microstructure on its electrochemical performance [11,13]. This multiscale insight can shed light to the optimization of the electrode design to improve the electrode performance.

Keywords:

nano-XCT, operando experiments, lithiation dynamics

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Coherent inelastic scattering probed by holographic scanning transmission electron microscopy

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IM-03 (2), Plenary, august 28, 2024, 14:00 - 16:00

In today's nanoscale technology, a detailed understanding of the structures and induced fields is fundamental. Electron microscopy has proven a powerful method for quantitative phase-contrast imaging of electric fields in nanostructures. One successful approach is based on off-axis holography which employs the interference of a complex object wave with a reference wave. However, this technique requires a wide, coherent electron beam emitted from a highly coherent electron source, and cannot be transferred straightforwardly to access ultrafast dynamics in the femtosecond-nanometer range.
Nonetheless, phase-resolved measurements of local optical responses have recently been revealed using Lorentz microscopy [1] and free-electron homodyne detection [2]. These measurements rely on the inelastic scattering of electrons off optical near-fields in so-called Photon-Induced Near-field Electron Microscopy (PINEM) [3,4]. Pulsed electrons generated in an ultrafast transmission electron microscope (UTEM) gain or loose energy upon interaction with the optical field of the driving laser pulse. While both approaches for phase-resolved measurements have proven powerful in terms of resolution, sensitivity and robustness, their stringent requirements on electron beam coherence or a designated sample holder, respectively, render the general experimental implementation challenging.
In this contribution, we present a method based on scanning transmission electron miscroscopy (STEM) with spatially separated coherent probes [5], that yields holographic information on inelastic interactions at reduced electron beam coherence requirements. Specifically, we insert an amplitude grating in the electron microscope condenser system and can, thereby, prepare a tailored electron probe composed of a coherent superposition of spatially separated STEM foci corresponding to given diffraction orders. The far field of these probes interferes to form an image of the grating pattern. Distinct elastic and inelastic interactions experienced by the separate probes will modify the interference condition and affect the recorded far field distribution.
Experiments are carried out in a UTEM based on a Schottky field-emission JEOL JEM-F200 instrument, which we modified to allow for a laser-triggered photoelectron mode and optical sample excitation. As a first application of STEM holography, we perform 2D-raster scans at nm-scale spatial resolution to visualize elastic and inelastic interactions at an optically excited heterostructure.
In the simplest case of only elastic interactions, a difference in, for example, sample thickness between the separate probes would lead to a translation of the interference pattern for different scanning positions. Imaging of inelastic scattering processes is achieved by employing a single-electron-sensitive hybrid pixel detector behind an energy filter. Moreover, by adjusting the electron microscope imaging spectrometer to collapse one of the momentum space dimensions on the detector and adding energy dispersion along this direction, we anticipate recording two-dimensional images simultaneously, encoding holographic information both on relative phases between the probes and on the energy spectrum.
In conclusion, we demonstrate STEM holography adapted to the characterization of coherent inelastic scattering. A particular advantage of this technique is the relaxed coherence requirements,

which could be central to overcoming common challenges in ultrafast transmission electron microscopy and improving time-resolved imaging of electric and magnetic fields on the nanoscale.

Keywords:

STEM-holography, inelastic scattering, optical near-fields

Reference:

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Improving electron tomography of mesoporous silica structures by Ga intrusion

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Poster Group 1

Background incl. aims:
Mesoporous silica structures are widely used in the field of nanotechnology, e.g. as substrate in heterogeneous catalysis [1] and as stationary phase material in size-exclusion chromatography [2]. To understand and improve the material's properties, a precise characterization of the pore network is indispensable. Common approaches to quantitatively analyse the mesoporous space are averaging sorption/intrusion techniques [3] and three-dimensional imaging techniques such as electron tomography in high-angle annular dark-field (HAADF) scanning transmission electron microscopy (STEM) mode [4]. While the first mentioned techniques lack the localized spatial information, HAADF-STEM tomography resolves the mesoporous structure with a spatial resolution in the nm-range, but suffers from poor contrast between pore space and network in case of low atomic number (Z) materials, since the imaging contrast scales with ~Z^2 [1]. In this study, we uniquely combine Ga intrusion of the pore space in mesoporous silica with HAADF-STEM tomography to enhance the Z-contrast between pores and silica significantly by a factor of ~9x. This enables a reliable quantitative analysis and visualizes localized information about the pore network and the process of Ga intrusion. In particular, we apply 360° HAADF-STEM tomography on pillar-shaped specimen prepared from the intruded mesoporous silica. This allows for precise 3D reconstruction of the tilt series without missing-wedge artifacts to accurately obtain important pore characteristics such as size distribution, tortuosity, and connectivity [5].

Methods:
Mesoporous silica with an average pore size of 20nm is intruded with Ga up to different filling degrees (50%, 100%) by a modified Hg intrusion porosimetry technique. From the intruded sample systems pillar-shaped specimen are prepared on a tip by scanning electron microscopy (SEM)/focused ion beam (FIB) techniques using a Helios NanoLab 660 DualBeam SEM-FIB. The samples are analysed by 360° HAADF-STEM tomography in a double Cs-corrected FEI Titan³ Themis at 300 kV.

Results:
Backscattered electron (BSE) imaging on a FIB prepared cross-section of the fully intruded silica reveals that the method of Ga intrusion leads to a homogeneous filling of the pore space in a representative volume required for STEM tomography (Figure 1a - left). The homogeneous distribution of the infiltrated Ga phase is crucial for a reliable quantitative analysis by STEM tomography. If the pressure during the intrusion process is reduced, a partial filling (50%) of the porous network can be achieved with the empty pockets being evenly distributed in the entire

sample (Figure 1a - right). This finding resembles the homogenous and narrow pore size distribution of the material and hints towards smaller pore channels being present within the unfilled spaces, since the infiltration of smaller pore channels requires in general higher pressures.
From the fully- and half-intruded silica, pillar-shaped specimen can be prepared on tomography tips by common FIB lift-out technique (Figure 1b). In contrast, imaging and pillar preparation from pure silica is challenging, since the material is electron-beam sensitive and non-conductive. This leads to sample charging and causes sample drift and uneven milling during FIB preparation. Therefore, a charge neutralizer is indispensable in case of silica, which is, however, not available on any FIB instrument. Also during STEM tomography, charging effects occurring on pure silica easily cause complications resulting in bending and structure deformation, which strongly worsen the 3D reconstruction quality. Moreover, the build-up of carbon contamination during tilt series acquisition worsens the image contrast over time, due to the low atomic number difference of Si, O, empty space and C contamination in HAADF-STEM imaging mode. In contrast, specimen intruded with Ga feature a ~9x stronger contrast, so that C contamination is negligible and the pore and silica space can clearly be resolved throughout the entire tilt series (Figure 1c). As outlined, Ga intrusion of mesoporous silica features multiple advantages: (1) it improves the imaging contrast in HAADF-STEM mode, (2) increases the sample integrity, (3) mechanically stabilizes the pillar, and (4) forms a conductive pathway through the sample eliminating charging effects during preparation and STEM tomography. These benefits improve significantly the reconstruction quality of the tomographic tilt series in the fully intruded specimen (Figure 1d - left), facilitate the phase segmentation between Ga and silica (Figure 1d - middle) and enable a reliable quantitative analysis of the porosity and pore size distribution by the method of maximum sphere inscription (MSI) (Figure 1d - right, Figure 1e). For a benchmark comparison, the mesopores of the silica structures were investigated with N_2 physisorption, evaluated with the non-local density functional theory (NLDFT) method. Moreover, we investigated the 3D reconstruction of the half-intruded silica specimen (Figure 1f) to improve our knowledge regarding the Ga intrusion process and to study contrast differences of pores, Ga and silica in more detail. While the filled channels are clearly visible, the unfilled channels, which indicate bottlenecks in the pore pathways, are weakly distinguishable from the surrounding silica network (Figure 1f - left). The respective segmentation is challenging, but helps to identify bottlenecks in the pore pathways (Figure 1f - right). In sum, the findings from electron tomography can further elucidate the gallium intrusion process on the nanoscale and contribute to the development of methods for textural characterization based on liquid intrusion.

Conclusion:
This study highlights the potential of Ga intrusion for contrast enhancement of the pore space in mesoporous silica during 360° HAADF-STEM tomography to study quantitatively important pore characteristics. Vice versa, the study further helps to understand the process of Ga intrusion. The intrusion process leads to a homogeneous Ga distribution and enhances the mechanical stability of the non-conductive silica during both FIB sample preparation and acquisition of the STEM tilt series by minimizing electrical charging. The Ga intrusion process can be regarded as a potential common technique to improve electron tomography not just on mesoporous silica but also on various other porous materials.

Keywords:

360° ET, quantitative pore analysis

Reference:

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The significance of the phonon polarization vector in vibrational EELS

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Poster Group 1

Background incl. aims
Phonons are the quasiparticles of lattice vibrations. They influence a variety of material properties such as for example the heat conductivity and play a major role in physical effects of technological importance such as for example superconductivity and the phonon Hall effect. Phonons are thereby wave excitations of the entire lattice of a material in the solid state, so-called normal modes, which can be distinguished by the wave vector q and the phonon branch v. Each mode (q, v) has a definite frequeny ω(q,v) and phonon polarization vector e(q,v). Knowledge of both of these quantities fully characterizes a phonon mode (q, v).
Vibrational Electron Energy Loss Spectroscopy (EELS) is establishing itself as an important tool to characterize phonons at the nano- and atomic-scale [1]. In this contribution we discuss the role of the phonon polarization vector in vibrational EELS from different perspectives.
Methods
As the first step we consider the analytical expression for the vibrational EELS cross section in first-order Born approximation, which scales with the phonon frequency ω(q,v) in a simple manner and depends on the scalar product of the momentum transfer Q and phonon polarization vector e(q,v). Here we focus on the richer dependence with respect to the phonon polarization e(q,v). In the second step we consider explicit simulations of vibrational EELS with the Frequency-Resolved Frozen Phonon MultiSlice method and comparison with experiments.
Results
The scalar product dependence of vibrational EELS is the key to the phonon polarization vector. It governs the visibility of phonon modes and is responsible for an interesting modulation of the intensity of the phonon EELS as a function of the detector placements, both for small and large off-axis detectors. The effects have been observed in recent experiments [3,4].
Conclusion
The phonon polarization vector leads to an intricate modulation of vibrational EELS, which can be exploited to gather information on the direction of the phonon polarization vector.

Keywords:

phonon polarization, EELS, simulation, theory

Reference:

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2. P M Zeiger and J Rusz, Physical Review B 104 (2021), 104301.
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Direct imaging of deformation in metallic glasses using precession nanodiffraction mapping

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

Background:
Despite having unique attractive properties, non-crystalline/amorphous materials suffer from catastrophic failure after reaching their yield strength. The ability to improve the mechanical and functional properties of metallic glasses depends on understanding their inherent strain state. Recently, nanoscale strain mapping was used to unravel shear band formation in a monolithic metallic glass [1]. The effect of structural changes induced by the introduction of secondary crystalline phases, highly rejuvenated regions or other defects renders transformation mechanisms in metallic glasses even more complex. Due to the emergence of novel dedicated specimen holders, in-situ transmission electron microscopic measurements are a powerful tool allowing to directly measure the deformation behavior of these tailored nanocomposites [2].
Aim:
In this work, we want to directly image the local elastic strain fields at heterogeneities in metallic glasses using precession nanodiffraction mapping.
Methods:
The study was carried out on a CuZr-based metal alloy, which is a well-known glass former. The introduction of heterogeneities included thermal processing techniques and solid state amorphization. Nanosized specimens with an electron transparent window were fabricated through focused ion beam milling. A unique push-to-pull device, which is a micro-electromechanical systems-fabricated flexure device from Bruker was used for in-situ tensile testing in the transmission electron microscope. Precession nanodiffraction mapping was integrated with an in-column energy filter and a direct electron detector from QuantumDetectors. This combinatorial approach enabled rapid acquisition of high quality diffraction patterns. It also allowed us to follow the structural response during in-situ deformation on the nanoscale.
Results:
The results of precession nanodiffraction mapping were able to describe local events of cooperative shear in metallic glasses. From acquired in-plane atomic level strain maps the interaction between heterogeneities could be described quantitatively. Different enthalpy states of the metallic glass showed different shear band mechanisms, which contradicts the model of a uniform disordered material after processing. It was shown, that high interfacial stresses between heterogeneities cause complex strain concentrations preventing strain localization in a single shear band. We were also able to show that crystalline phases strongly influence the stress field in the amorphous phase. Recorded strain maps from elastostatic loaded samples and during in-situ tensile deformation revealed early stages of shear nucleation and propagation on the nanoscale. As a result, a direct link could be established between the structure and properties of these tailored metallic glass composites.
Conclusions:
We demonstrated the experimental measurement of local quantitative strains on the nanoscale. For this purpose, structurally altered metallic glasses were investigated. It could be shown how

heterogeneities influence the deformation behavior and mechanical properties. These new findings will improve the understanding of the interplay between structural heterogeneities and carriers of plastic deformation. With these conclusions, our work contributes significantly to the development of new high-performance materials.
Acknowledgments:
The authors gratefully acknowledge the financial support from the Austrian Science Fund (FWF): Y1236-N37.

Keywords:

nanodiffraction mapping, metallic glass, deformation

Reference:

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[2] H. Sheng, et al. Materials Research Letters, 9 (2021) 190-195.

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Unveiling moiré-induced topological polar structures in freestanding ferroelectric membranes

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Poster Group 1

Complex correlated oxides are quantum materials characterized by unshielded d-electrons. Their interaction across competing energy scales leads to diverse functionalities, which can be altered by slight changes of their structure, composition, or boundary conditions [1]. In this context, recent studies on ferroelectric oxides have shown the formation of complex polar topologies, which are related to a delicate interplay between the intrinsic tendency of the material towards a uniform polarization and the electrical and mechanical constraints placed upon them [2]. However, the cube-on-cube epitaxial structure of these materials forces the use of single crystalline substrates for their growth, which restricts the possible mechanical boundary conditions and, therefore, the formation of new topological structures. To overcome this limitation, we have isolated the ferroelectric materials from their parent substrate by a selective chemical exfoliation method, thus obtaining freestanding layers of just a few unit cells in thickness. This new approach vastly increases the potential for novel hetero-integration approaches by stacking various freestanding layers and avoiding the chemical, structural, or thermal limitations of conventional synthesis and growth processes [3].

In this work, we show how a polar vortex pattern is induced by stacking twisted freestanding ferroelectric $BaTiO_3$ layers [4]. We study the formation of these topological polar structures in relation to the twisted angle and layer thickness by aberration-corrected scanning transmission electron microscopy and density-functional theory calculations.

These results show an exciting opportunity to create novel polar topologies using the unique modulations that are possible in moiré bilayers and pave the way for potential applications in future high-density ferroelectric memory devices.

Keywords:

ferroelectric, freestanding membranes, topological structures

Reference:

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[5] This work has been supported by the Regional Government of Madrid CAM through SINERGICO project CAIRO-CM (Y2020/NMT-6661). Authors acknowledge received funding from the EU FLAG-ERA project To2Dox (JTC-2019-009) and the Spanish Ministry of Science and Innovation (grant PID2020-118078RB-I00 and fellowship PRE2018-084818). G.S.-S. acknowledges financial support from the Spanish MCI grant nos. RTI2018-099054-J-I00 (MCI/AEI/FEDER, UE) and IJC2018-038164-I. V.R. was supported by the V PRICIT (Regional Programme of Research and Technological Innovation).

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Oxygen vacancy imaging in complex oxides by a 4D-STEM optimized virtual detector

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Background incl. aims
The interactions between the spin, charge, orbital and lattice degrees of freedom in transition metal oxides (TMO) give rise to a wide range of emergent phenomena, such as high temperature superconductivity, colossal magnetoresistance, or metal-insulator transitions, to name a few. Small variations in local chemical or electronic doping resulting from the presence of O vacancies can lead to drastic changes in these physical properties, since the macroscopic response is ultimately determined by the interplay between the oxygen s-like and transition metal d-like electrons. Thus, the in-depth comprehension of such phenomena relies on being able to quantify the presence of very reduced densities of these elusive defects. However, the detection of very low O vacancy concentrations remains extremely challenging. Aberration-corrected electron probes in the scanning transmission electron microscope (STEM) can bear on this task. In this work we propose a new method aimed to detect single O vacancies in a TMO such as SrTiO3 (STO), exploiting the capabilities of 4D-STEM techniques.
Methods
Density-functional theory (DFT) calculations were used to obtain reference relaxed STO supercells containing a single oxygen vacancy per 3x3x9 unit cells in order to mimic very low deoxygenation levels. Simulations of 4D-STEM data based on these models were carried out using the py_multislice code. Convergent beam electron diffraction (CBED) patterns of fully oxygenated O columns and columns with a single oxygen vacancy were obtained. Simulations have been performed implementing the multislice method in combination with the frozen phonon approximation, to account for multiple and thermal diffuse scattering. Analysis of the scattered intensity distribution, as a function of scattering angle and specimen thickness, allows identification of the characteristic fingerprints associated with the presence of single oxygen vacancies in atomic resolution diffraction images. A virtual detector can be used post-acquisition to generate images from 4D-STEM datasets. The imaging geometry was optimized here by selectively picking a distribution of inner/outer angles of annular detectors. Angular ranges were chosen to maximize intensity variations when comparing individual O atomic columns, and a virtual imaging mode was engineered to maximize the contrast between fully oxygenated columns and atomic columns where single O vacancies were present. Such simulations were then used for comparison with experimental 4D-STEM data obtained from ultrathin, freestanding SrTiO3 membranes 16 nm thick with O vacancy concentrations bellow the sensitivity threshold of complementary techniques such as electron energy-loss spectroscopy (EELS).
Results
The contribution to the electron scattered intensity distribution associated with O columns where a single oxygen vacancy is located can be quantified by subtracting the CBED pattern from a fully oxygenated column to a vacancy containing column, as illustrated in Fig. 1. The analysis of the resulting scattered intensity distribution reveals two angular regions of interest: (1) a central region

inside the bright field disk where fully oxygenated columns scatter in a more intense fashion due to beam channeling and (2) an external region in the bright field disk where the scattering intensity is increased due to a dechanneling effect. This behavior is robust and easily interpretable upon sample thickness variations. Such well-defined angular dependence of the scattered intensity constitutes a characteristic fingerprint of single oxygen vacancies.
Experimental data can be then examined with these features in mind. Experimental 4D-STEM datasets were analyzed using the virtual detector previously discussed in order to track spatially any O column intensity variation. Optimized detector images were produced by integrating the relevant angular ranges in experimental data. The resulting images exhibit, indeed, variations in O column intensity that are consistent with small amounts of O vacancies, which can be identified well above the noise threshold. Results will be shown to prove that both image contrast difference and single CBED pattern features demonstrate single vacancy detectability. The contrast difference between fully oxygenated and vacancy containing columns in the optimized virtual detector imaging mode will also be discussed in the light of conventional annular dark field (ADF) and annular bright field (ABF) images.
Conclusion
We demonstrate the detection of single oxygen vacancies in SrTiO3. Combining DFT calculations, atomic resolution 4D-STEM simulations and data analysis, the characteristic fingerprints of single oxygen vacancies can be identified in both simulations and experimental data, which indeed replicate the predicted fingerprints both in image intensity and CBED patterns. This capability enables for the first time the experimental identification of single oxygen vacancy columns in a freestanding STO membrane. This method opens a new path in the field of advanced materials characterization by electron probes, highlighting the advantages of 4D-STEM techniques and the unique insight they provide.

Keywords:

4D-STEM, oxygen-vacancies, complex-oxides, vacancy ordering

Reference:

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Correlative cryo soft x-ray tomography and fluorescent microscopy of biological samples in the laboratory

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Poster Group 2

Background incl. aims
Soft x-ray tomography (SXT) uses low energy x-rays to image frozen-hydrated biological specimens such as entire mammalian cells or thick tissue slabs with a few tens of nanometers of spatial resolution. The technique takes advantage of the biological water window whereby water is relatively transparent to x-rays from 284 to 543 eV (2.34 to 4.4 nm) but carbon-based organelles are absorbing. This native contrast imaging allows high resolution imaging of bulk specimens with minimal sample preparation. While SXT has traditionally been confined to synchrotrons the recent development of a laboratory scale SXT microscope opens the possibility of integrating this novel technique into light and electron imaging workflows. The SXT microscope features an integrated light microscope for overview imaging and fluorescence targeting, allows for swift acquisition of 2D and 3D x-ray images covering extensive areas on the specimen, and enables efficient and rapid identification of cells of interest. The utility of the microscope is currently being demonstrated through 2 EU-funded consortia (https://cocid.eu/ and https://clexm.eu/). Recent case studies have included correlative imaging of whole, virus infected cells treated with anti-viral drugs; imaging the 3D distribution of therapeutic-laden nanoparticles in whole cells; and the development of correlative workflows for tissue imaging across scales. An alternative sample carrier in the form of a thin-walled glass capillary was also developed, further enhancing the novelty of the lab-based microscope.
Methods
Integrated cryo fluorescence was used to screen an entire EM grid for cell selection and correlation with SXT. Low magnification/large field of view 2D x-ray mosaics were then acquired over large areas of the grid before acquiring tilt series from ±60° on selected targets. Data from both modalities were then overlaid to provide the location of fluorescent proteins in the context of whole cell ultrastructure. For cells suspended in glass capillaries the tilt series was acquired over ±90°.
Results
Resulting cryo-SXT tomograms and fluorescent light images of cellular organelles and nanoparticles provide proof of concept for the lab-based microscope and associated workflows.
Conclusion.
Workflows of correlative light, electron and soft x-ray microscopy combines the strengths of each modality. The recent availability of a compact soft x-ray microscope will accelerate the development of novel workflows and biological imaging applications that can benefit from this technique. We will present our microscope workflows and application data.

Keywords:

Soft x-ray tomography, fluorescent microscopy

Reference:

Acknowledgement
We acknowledge funding from the European Union's Horizon 2020 Research and Innovation programme (No. 101120151, project CLEXM and No. 101017116, project CoCID) as well as the Irish Research Council (No. EBPPG-2020-278).

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Understanding the effect of Al:ZnO coating on the structural and chemical stabilities of LiNi1/3Mn1/3Co1/3O2 electrode

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Poster Group 1

LiNi1/3Mn1/3Co1/3O2 (NMC-111) is one of the most promising cathode materials in Li-ion batteries. However, structural and chemical instabilities of the electrode / electrolyte interface at high charge cut-off voltages lead to capacity fading. It is necessary to study the structure and chemistry of electrodes and electrode / electrolyte interfaces in Li-ion batteries since the interfaces determine the local Li-ion transport kinetics and the electrochemical performance. Surface modification using metal oxides is one of the best approaches to suppress capacity fading. In this work, a systematic study on the degradation mechanism of an uncoated NMC-111 powder electrode was presented. Moreover, the effect of an Al-doped ZnO (Al:ZnO) coating layer on the chemical and structural stabilities of NMC-111 electrode cycled at high charge cut-off voltages was analyzed using scanning electron microscopy, analytical transmission electron microscopy, and X-ray photoelectron spectroscopy. The objective of this work is to study the effect of electrode / electrolyte interface on the degradation mechanism and also the effect of coating layer on the stability of electrode / electrolyte interface. The coating layer was applied to commercial NMC-111 powder using a microwave-assisted sol-gel synthesis method. High-resolution TEM imaging, STEM imaging, nano-beam electron diffraction (NBD), electron-energy-loss spectroscopy (EELS), and energy dispersive X-ray spectroscopy (EDX) were carried out using a FEI Titan Themis G3 60-300 TEM. Cross-sectional TEM samples were prepared using a ZEISS cross-beam 340 FIB-SEM. In the case of uncoated NMC-111 electrode, cation mixing, pitting corrosion due to hydrofluoric acid attacking the electrode surface, and an irreversible phase transformation from a trigonal layered to a rock-salt phase occurred, leading to capacity fading. While, in the case of Al:ZnO – coated NMC-111 electrode, cation mixing, pitting corrosion, and the irreversible phase transformation were mitigated. Therefore, the rate capability and capacity retention were improved since the coating layer protected the electrode surface from the direct electrolyte exposure.

Keywords:

EELS, TEM, EDX, NBD, SEM

Reference:

Makvandi, A., Wolff, M., Lobe, S., Heidrich, B., Peterlechner, M., Gammer, C., Uhlenbruck, S., Winter, M., Wilde, G. Al-doped ZnO-Coated LiNi1/3Mn1/3Co1/3O2 Powder Electrodes: The Effect of a

Coating Layer on The Structural and Chemical Stability of The Electrode / Electrolyte Interface. Adv. Mater. Interfaces 11, 2300668 (2024).

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Momentum-resolved STEM Tomography of Gold-Silver core-shell Nanoparticles

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Poster Group 1

Background
Precisely tailored Gold-Silver core-shell (Ag@Au) Nanoparticles (NPs) can have outstanding optical properties due to localized surface plasmon resonances, making them suitable candidates for advanced senors or for application in photothermal therapy [1]. Exact control over the nanocrystal growth however, demands for suitable analytic methods to confirm the quality and reproducibility of the synthesis. Facetted core-shell NPs like this especially benefit from techniques like Electron Tomography and STEM Tomography in particular, which are able to reconstruct their nanoscopic structures in all three dimensions, via acquisition of a tilt series of projections [2].
Depending on the detectors used, STEM offers a great variety of suitable signals for these projections [3]. For example the commonly used Bright-field (BF), annular dark-field (ADF) and high-angle annular dark-field (HAADF) signals each cover rings with different radii, corresponding to certain magnitudes in momentum space. However, the exact radii of the detector in momentum space additionally vary with the chosen camera length and the choice of those radii in the experiment has significant impact on the fidelity of the tomographic reconstruction, since most techniques rely on the linearity of the signal during projection and might even have different optima for detecting different materials. To understand the impact of the detector choice and to overcome its limitations, Momentum-resolved STEM (MR-STEM or 4D-STEM) has been applied, where for each scan position of a 2D scan raster an individual 2D diffraction pattern is recorded with the help of a fast direct electron detector and signals for each magnitude of momentum have been extracted from this data set. This allows to apply and compare arbitrary virtual detectors post-experimentally. In combination this enables MR-STEM Tomography, where the best suitable signal with respect to its performance in tomographic reconstruction of the Ag@Au NPs can be determined in hindsight.

Methods
The MR STEM experiments were conducted using a probe corrected FEI Titan Themis operated at 300kV and equipped with a Quantum Detectors MerlinEM direct electron detector (based on Medipix 3).
Acquisition of the 4D tilt series has been done using an in-house developed software written in python, utilizing the FEI Scripting COM interface. A 4D tilt series with a tilt range of -75° .. +72.5° in 2.5° steps has been recorded with 256 x 256 scan points and 256 x 256 pixel diffraction patterns at an semi-convergence angle of 18mrad. For reconstruction, a WSIRT reconstruction algorithm has been used with 5 to 10 iterations.

Results
The performance of different detector radii in momentum space in regards of tomographic reconstruction has been investigated on reconstructions of selected slices. Additionally an magnitude of momentum resolved WSIRT reconstruction of the whole volume has been explored, leading to a

4D dataset (x, y, z,|k|), which can then be evaluated e.g. using Principal component analysis (PCA). Furthermore the second moment in momentum space has been calculated, where ring segments with radius k have been weighted with k^2, summing up from a chosen minimum radius to a cut-of radius. Figure 1 exemplary shows the reconstructed Second Moment tomogram (c) of four self-assembled Ag@Au NPs (b), clearly depicting the octahedrally faceted Au cores encapsulated by truncated cubically faceted Ag shells.

Conclusion
Momentum-resolved STEM has been explored to find the most suitable signal for STEM Tomography, comparing different ADF / HAADF signals. For the Ag@Au NPs, the second moment has proven to quickly converge to similar results as the HAADF signal reconstructions, if the outer detector radius is chosen large enough, however the less strict dependence on the inner radius might still be advantageous for some applications.

Figure 1:
Momentum Resolved STEM Tomography of Gold-Silver Core-Shell Nanoparticles:
a) HAADF STEM overview image
b) HAADF STEM image of the region of interest, highlighted in (a)
c) Second moment tomogram as an example of momentum resolved STEM tomography.

Keywords:

STEM Tomography, 4D STEM

Reference:

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[2] M WEYLAND, et al. Materials Today, 2004,
[3] H ROBERT, et al. Ultramicroscopy, 2022,

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Phase Stability and Evolution of Defect Structure in (CoCuMgNiZn)-Entropy Stabilized Oxide

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Poster Group 1

Continuously diminishing fossil fuel resources, growing environmental concerns and changing geo-political scenario has incentivized the search for new energy resources and technologies. It has been well understood that future sustainable and green energy innovations will be mostly driven by the innovations of materials. Multicomponent high entropy oxides (HEOs) are perceived to have the potential to bring about a paradigm shift in catalysis, electro-chemical energy conversion and storage, electronics etc. HEOs may be looked at as a recent vintage of the high entropy alloys (HEAs), which was brought into existence by innovative thinking with a dash of serendipity by Profs. Cantor and Yeh independently. High entropy oxides were discovered following the line of high entropy alloys, however much later. The first high entropy oxide, redefined as entropy stabilized oxide (ESO), was discovered in 2015 in CoCuMgNiZn-oxide by Prof. Maria and coworkers. Since then a number of HEO/ESO systems have been discovered, and concept extended to carbides, borides, nitrides etc. HEOs are slightly different than HEAs as in HEOs there are more than one cation and anion sublattices and substitutions may happen in either of them. The calculation of configurational entropy is also different and much more robust than HEA counterpart. In addition, these systems are sensitive to environment in order to sustain its defect equilibria and electrical neutrality. It has been believed so far that all HEOs are single-phase solid-solutions where cations randomly fill up a disordered sublattice while oxygen decorate the ordered anion-sublattice, with little or no reference to their stability.

CoCuMgNiZn-oxide was synthesized using powder metallurgy route. The pure (>99.9%) precursor oxide powders were taken in stoichiometric proportions, mixed thoroughly in mortar and pestle, consolidated to a green compact of 12mm dia. pellets, which were sintered at 1323K for 10hours. Couple of other sintering schedules were also adopted to address phase stability and subsequent microstructural evolution. The processed pellets were metallographically polished for SEM and XRD analysis. TEM of the pellets were done from both the bulk and its crushed powder. Powders from the pellet were sonicated in ethanol before drop-casting it on carbon-coated copper grids, whereas chunks taken out of the pellet were mounted on a 3mm dia. hollow steel tube, dimpled and ion-milled to produce electron transparent thin foils.

The as-synthesized pellets produced clean hyperspectral maps when subjected to SEM-EDS, and formation of micron-sized grains separated by grain boundaries. The XRD pattern from high-resolution diffractometer also gave a clean signature of a cF8 rock salt phase, however with finite peak broadening and shoulder peaks to the principle reflections. Moreover, the ratio of intensity of the (111) and (200) reflections were reversed when the same composition was examined, one in its bulk (pellet) form and other in its powder form. Prolonged holding time at 1323K resulted in the fall of intensities of all the reflections along with broadening of peaks. Low-temperature ageing treatment revealed highly broadened and asymmetric peaks, with significant deviation in intensity ratios. Systematic TEM experiments were carried out to probe the system. The ion-milled samples

occasionally show nano-crystalline regions with spotty-ring diffraction pattern along the edge of the thin foils. However, tweed-contrast in the grain interior can clearly be discerned which show streaking in the reciprocal space. Powder TEM warrants the exact same clue where fine scale tweed-contrast could be seen in dark-field imaging from the [001] zone axis pattern. Moreover, fine parallel fault lines can be seen in the matrix phase and its corresponding diffraction pattern from [011] show spot-splitting and diffused streaking. Upon ageing, the tweed-contrast develops into an orderly arrangement of defect structure and sharp streaks centered around primary reflections appear. Centered-dark field imaging with s>o under two-beam condition clearly reveal the defect structure. Along with this, several regions in the matrix reveal a superimposed diffraction pattern where a major rock salt phase shares its signatures with high and low-symmetry phases of the spinel crystal structure, hence developing an orientation relationship with each other.

In conclusion, there seems to be an unexplored energy landscape where the system likes to jump from one local minima to another. There also seems to be oriented growth of phases, structurally derived or similar but chemically separate. The tweed morphology may act as template for growth of the defect structures under suitable conditions. Minimization of strain in the geometrically frustrated sublattices seems to drive such microstructural nuances. Models of such transformation will be presented in detail.

Keywords:

High-Entropy Oxides; TEM; Phase-pure SS

Reference:

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The study of spatial relationship between Restrictor complex and RNA-Pol II through Expansion Microscopy

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LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
In recent years, there have been continuous research efforts to optimize the experimental Expansion Microscopy (ExM) protocol for studying spatial correlation among different proteins. Our focus has been on developing an effective method to relocate the same cells acquired in pre-expansion onto expanded gels, enabling precise calculation of expansion and deformation factors. We then applied ExM and colocalization analysis to uncover the spatial relationship between the Restrictor complex and RNA-Pol II. The Restrictor complex, comprising ZC3H4, a putative RNA-binding protein, and WDR82, an adapter protein that interacts with RNA-Pol II Ser5-p, terminates extragenic transcription arising from active enhancers and promoters, thereby mitigating the pervasive capacity of transcription of the genome, which may lead to genomic instability (1). However, the exact molecular mechanism of action of the Restrictor complex remains poorly understood.

Methods
HCT-116 WT and HA-WDR82 HCT-116 cells were seeded on 35 mm MatTek dishes before being processed for indirect immunofluorescence. ZC3H4, WDR82, and RNA-Pol II Ser5-p or RNA-Pol II Ser2-p were fluorescently labeled. A scratch with no symmetry for rotation and reflection on adhered cells, together with a pre-expansion overview of the sample, were used to identify pre-expansion imaging areas. Samples were then processed for ExM according to (2). After expansion, the same areas were identified under a stereo microscope based on their relative position with respect to the scratch (Fig.1). Both pre- and post-expansion images were acquired using a Yokogawa spinning disk confocal system (CSU-W1, Nikon Europe B.V.). Colocalization was assessed using Pearson's coefficient calculation and object-based colocalization analysis.

Results
Expansion factor, deformation, fluorescence intensity decrease upon expansion, and both 2D and 3D colocalization were evaluated. Across three experiments, an expansion factor up to 6 and an average deformation factor of 9% were obtained. Overall, the colocalization results obtained with ExM revealed a stronger spatial correlation between the Restrictor complex and RNA-Pol II Ser5-p compared to RNA-Pol II Ser2-p, consistent with molecular, genomic, STocastical Optical Reconstruction microscopy (3) and Structured Illumination Microscopy observations previously obtained in our laboratory.

Conclusion
Our ExM imaging workflow efficiently facilitated the acquisition of identical areas both before and after expansion, thus providing essential control over the expansion protocol. Considering that the expansion factor directly influences the ultimate resolution of any ExM experiment, it is crucial to consistently account for the precise expansion factor of each sample to ensure fair comparisons among different samples. The colocalization analysis of ExM images further supported the concept

that Restrictor-mediated transcription termination primarily affects initiating and early-elongating RNA-Pol II complexes.

Keywords:

ExM, colocalization, extragenic transcription

Reference:

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Developing cryo-Volume Electron Microscopy using the JEOL 4700 cryo-FIBSEM to address biological ultrastructural questions

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

The Focused Ion Beam Scanning Electron Microscope (FIBSEM) is well suited for site-specific sectioning where the FIB milling exposes a surface that can be imaged either with the FIB or SEM to examine the microstructure within each layer and the interfacial structure between layers. By collecting ultrastructural data from a large volume, to be interrogated in silico; novel understandings of the interconnectivity between many individual cell types can be deduced. These volume Electron Microscopy studies (vEM) overcome two principal limitations of EM: the loss of 3D understanding of ultrastructure and the possibility of "missing" rare ultrastructural events through the limited volume of tissue examined. However, to date, this has only been successfully applied to chemically processed tissue, with their associated artefacts. Non-chemical fixation using low temperature allows cells and tissues to be studied close to their native biological state and has been applied to cryo electron tomography (cryoET) of thin sections of cells and tissues. With cryo lamellae produced from vitrified material by controlled milling using focussed ion beam milling in a dual beam scanning electron microscope at cryogenic temperatures (cryoFIBSEM).

Here we develop cryo vEM (cvEM) to generate larger 3D volumes than possible with cryoET by a sequence of sectioning in a cryo FIBSEM at high resolution and close to the biological native state. By successfully overcoming the high beam sensitivity of vitrified material, lack of heavy metal contrast and significant challenges to the generation of sufficient signal to noise it has been possible to demonstrate the potential of cvEM as a complementary approach to cryo ET capable of generating larger volumes (e.g., an entire cell), and at the same time retaining resolution sufficient to clearly define ultrastructural details at the level of the membrane bilayer (Figure 1). We have incorporated novel scan approaches and evaluated in-painting techniques to manage charge and improve signal to noise (1,2). We also show the workflow can be successfully extended to multicellular volumes by increasing vitrification depth with high pressure freezing, the "waffle" method. By integrating a cryo correlative capability (cryoCLEM) into the workflow, we are able to target regions of interest for cvEM. Data detailing differences between two Euglenoids at the subcellular level has been generated. Clear differences between Eulgena longa and Euglena gracilis Paramylon storage granules and key organelle were observed.

Future development aim to incorporate lamella lift out with cryo CLEM, cvEM to a predetermined region of interest with cryoET of recovered lamella. A complete and flexible full cryo FIBSEM workflow, the circle would be completed for cryogenic samples for complex biological studies.

Keywords:

cryo FIBSEM, vEM

Reference:

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Femtosecond extreme-ultraviolet imaging of magnetic domains during ultrafast demagnetization

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Background incl. aims
Understanding femtosecond dynamics of magnetic domains such as photoinduced ultrafast demagnetization and magneto-optical switching is important for the development of optoelectronics such as ultrafast optical-magnetic memory devices. Despite intensive research, the extraction and elucidation of those ultrafast magnetization dynamics remains highly challenging due to the multitude of the electronic, phonon, and spin processes involved. Direct observation of nanometer magnetic domain dynamics in real space, in particular, the domain wall profile, can yield valuable information for determining the mechanism of magnetization dynamics (1, 2) yet it remains elusive due to the simultaneous requirement of nanometer and femtosecond spatiotemporal resolution. In this work, we utilize femtosecond extreme ultraviolet (XUV) imaging with high-harmonic light source (3) to visualize the magnetic domain dynamics during the demagnetization of a Co0.75Tb0.25 ferrimagnetic thin film in real time and extract the local changes of the domain walls at each wall position.
Methods
Coherent diffractive imaging of the Co0.75Tb0.25 thin film produced with magnetron sputtering is conducted with circularly polarized femtosecond XUV pulses at 59 eV (Co M2,3 edge), which is time-delayed with a 35 fs laser pulse centered at 800 nm exciting the sample. The circularly polarized XUV pulses are produced with high harmonic generation of bi-circular 800 nm and 400 nm laser pulses in He with a MAZEL-TOV configuration (4). The amplitude and phase of the transmitted XUV field is reconstructed using an iterative phase retrieval algorithm and the local magnetization is extracted from the phase difference between the left and right circularly polarized field exiting the sample (5).
Results
Using femtosecond imaging at the Co M2,3 edge with circularly polarized XUV pulses we obtained images of the magnetic domains in Co0.75Tb0.25 thin films with 13.5 nm spatial resolution, which is confirmed with analysis of the phase retrieval transfer function. The positions of the domain walls are determined by the local maxima of magnetization gradient. At each domain wall position, the wall width is extracted by fitting the magnetization as a Bloch-type domain wall function of a cutout perpendicular to the domain wall. By fitting the domain wall profile as a function of time delays between the optical pump and XUV probe, we show that while the domain wall height exhibits a subpicosecond decrease and recovery due to demagnetization, the widths of the domain walls remain largely unchanged throughout 0-20 ps delays with an average of approximately 25 nm.
Conclusion
In summary, we demonstrate femtosecond XUV imaging of magnetic domain dynamics of a Co0.75Tb0.25 alloy thin film with 13.5 nm spatial resolution and for the first time extract the real-time changes of domain wall widths. The observed real-space domain dynamics can be further utilized to elucidate the mechanisms of the demagnetization process. Our method can be readily

applied to other magnetic thin film materials to understand and control ultrafast magnetization dynamics.

Keywords:

Ultrafast magnetization dynamics, EUV imaging

Reference:

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Evaluation of SXES on different kind of materials : successes and failures

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Poster Group 1

First, a brief reminder of the main aspects of wavelength dispersion spectrometry (WDS) will be given, with examples illustrating the contribution of WDS analysis compared to EDS analysis. Then, we will introduce the problem of detecting low energy X-ray photons (less than 1 keV) and the useful information on the sample that can be derived from it.
Among others, the “SXES” type detector (Soft X-ray Emission Spectroscopy) is one of the technical solutions developed by manufacturers to enable the detection and analysis of X-ray photons in this energy range. Their principle is based on the use of a variable pitch array (VLS) and a CCD camera instead of the analyzer crystals and proportional counters of traditional WDS spectrometers. The lithium detection capacity, getting information on the chemical environment at the local scale or even the very good detection sensitivity for light elements are the main advantages of this device. Examples, successful (or not!) will be presented and compared to data accessible by WDS crystal spectrometry.
To conclude, other technological solutions (already marketed or in development) will be presented such as new EDS spectrometers or new WDS analytical crystals for the detection of Li, as well as RZP (Reflective Zone Plate) type grating spectrometers for example.

Keywords:

EPMA SXES WDS EDS

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Nanowire Triple-Junction GaInP/InP/InAsP Solar Cells Realized

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PS-04 (1), Plenary, august 26, 2024, 10:30 - 12:30

Background
Planar multi-junction solar cells, based on III-V semiconductors, matched to the solar spectrum, are today's world-record efficiency solar cells1. Multi-junction III-V nanowire (NW) solar cells with equal performance would require significantly less material and is more sustainable at lower cost than planar solar cells. The NW geometry allows more material combinations along the NW axis than for current multijunction solar cells. We report the design and proof-of-principle demonstration of axially defined GaInP/InP/InAsP triple-junction photovoltaic NWs optimized for light absorption exhibiting an open-circuit voltage (VOC) of up to 2.37 V. The open-circuit voltage amounts to 94% of the sum of the respective single-junction NWs.
Methods
Hexagonal Au seed particle arrays on 2 inch InP:S (111)B wafers were defined2 using displacement Talbot lithography, Au evaporation, and lift-off. We grew the NWs in a MOVPE reactor at 440°C. We used phosphine (PH3), arsine (AsH3), trimethylindium (TMIn), and triethylgallium (TEGa) as precursors, and hydrogen sulfide (H2S) and diethylzinc (DEZn) as n- and p dopants, respectively. Throughout NW growth, hydrogen chloride (HCl) was used to prevent tapering in situ. HAADF-STEM and XEDS was used to verify the composition and structure of the 12 different segments constituting the triple-junction solar cell, together with a LED-illuminated EBIC.
Results and conclusions
We used an EBIC set-up with a tungsten probe to electrically contact single NWs. The NWs can be excited by a LED source. For the triple-junction NWs, their voltage is the sum of the subcell voltages. We measured an average VOC of the triple-junction photovoltaic NWs of 94% of the sum of the VOC of single-junction photovoltaic NWs. The highest measured VOC of the triple junction photovoltaic NW, 2.37 V, corresponds to the same value as by adding the highest measured VOC of the single junctions.

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2 L. Hrachowina, N. Anttu, M.T. Borgstr€om, Wafer-scale synthesis and optical characterization of InP nanowire arrays for solar cells, Nano Lett. 21 (2021). 7347e7353.
ACKNOWLEDGMENTS: Nano Lund, the K A Wallenberg Foundation and the Swedish Research Council

Fig. 1 Energy-dispersive X-ray spectroscopy line scans and STEM images of the whole NW (a) and the top junction (b). (c, d) EDXS line scans along the NWs from (a) and (b), respectively. A schematic drawing of the composition of the segments is inserted in (a) The arrows in (b, d) emphasize the position of the InP segment used in the Esaki tunnel diode.

Keywords:

HREM, HAADF-STEM, Solar cell, EBIC

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Machine Learning-Enabled 3D Visualization for Microstructural Risk Assessment in Creep Strength Enhanced Ferritic (CSEF) Steels

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Poster Group 1

Background incl. aims
Modern power plants, especially coal-fired and combined cycle facilities, rely heavily on Creep Strength Enhanced Ferritic (CSEF) steels for their thick components. These components face extreme stress due to operating temperatures reaching 650°C. CSEF steels excel in this environment because their unique tempered martensite microstructure offers exceptional strength. However, the long-term creep resistance, which refers to a material's ability to resist deformation under constant stress, can be a challenge for CSEF steels. Factors like nitrides, inclusions, and uncontrolled particle formation within the steel can significantly weaken it. These disrupt the carefully designed microstructure, creating potential weak spots for cracks to start and grow.
Introducing Tantalum (Ta) into the steel's composition offers a solution. Ta forms tiny MX precipitates within the microstructure. These strategically placed precipitates act like roadblocks, hindering the movement of dislocations (defects in the crystal structure) and migration of grain boundaries. This ultimately slows down the creep process and extends the time it takes for the steel to deform. While Ta is beneficial for creep resistance, achieving optimal performance requires precise control over the size and distribution of these Ta-containing precipitates. Large Ta-rich particles or inclusions have the opposite effect, introducing weak points within the microstructure. These weak points become prime locations for cavities to form, which further accelerate creep and compromise the material's integrity.
Understanding how damage develops in these steels requires a multi-faceted approach. We need to link pre- and post-testing conditions, assess damage in the base metal, develop consistent testing procedures, and gather statistically relevant data. This will allow us to optimize the use of Ta and ensure the long-term strength and reliability of CSEF steels in power plant applications.

Methods
Microscopic analysis of as-received and creep-exposed CSEF steel samples utilized a suite of complementary techniques: focused ion beam (FIB) microscopy, field emission gun scanning electron microscopy (FEG-SEM), transmission electron microscopy (TEM), and electron backscattered diffraction (EBSD). To further enhance microstructural risk assessment, a framework integrating machine learning with 3D visualization was also developed for analysing the microscopic data.

Results
A meticulous investigation employed various characterization techniques, including focused ion beam (FIB) serial sectioning and electron microscopy, to track and quantify the distribution of second-phase particles and inclusions in CSEF steels. The key focus was on the interactions between these features and creep cavities, particularly during the early stages of creep.
Our findings reveal a critical link between Ta-containing particles and creep damage. The close association suggests these particles act as preferred sites for cavity nucleation, potentially impacting the overall creep behavior of CSEF steels. Conversely, no significant correlation was observed between Laves phase particles and creep damage. The adoption of 3D tomography proved to be

instrumental. It provided a detailed and accurate analysis of the spatial distribution of microstructural features, particularly the interactions between Ta-containing particles and cavities. This 3D perspective offered a deeper understanding of the mechanisms governing creep behavior in CSEF steels.
This research underscores the critical role of advanced characterization techniques like 3D tomography and machine learning in understanding the intricate relationships between phases within materials. By leveraging 3D analysis, we were able to identify the crucial role of Ta-containing particles in creep damage initiation. These insights pave the way for targeted interventions in steel design and processing to optimize the performance of CSEF steels under creep conditions in power generation applications.

Conclusion
This study investigated the role of Tantalum (Ta)-containing phases in creep behaviour of CSEF steels used in power plants. By employing advanced microscopy techniques, including 3D characterization, we identified a strong correlation between the presence and size of these phases and the formation of creep cavities at early stages. This finding highlights a potential metallurgical risk factor for creep in CSEF steels. These insights pave the way for targeted interventions in steelmaking practices. By optimizing Ta content and processing techniques, we can potentially improve creep resistance across various steel alloys. Furthermore, the established methodology using 3D characterization offers a valuable tool for future research and development of high-performance CSEF steels for power generation applications.

Keywords:

CSEF steels, high temperature creep

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Single-Layer Transition Metal Dichalcogenides: Unveiling Excitonic Processes with Multimodal Microscopy

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

Background
Single-layer transition metal dichalcogenides (TMDs) exhibit remarkable light-matter interaction, making them prime candidates for next-generation optoelectronic devices. When thinned down to a single monolayer, the bandgap of TMDs changes from an indirect to a direct bandgap, resulting in efficient light emission. However, harnessing their full potential requires a deep understanding of their nanoscale optical properties. Probing these properties is challenging due to the highly confined nature of excitons in TMDs [1]. Advanced techniques like cathodoluminescence (CL) and electron energy loss spectroscopy (EELS) offer powerful tools for such investigations, but successful implementation hinges on meticulous control over sample preparation. Integration with hexagonal boron nitride (hBN) encapsulation offers a promising solution, potentially leading to improved optical properties and the emergence of moiré potentials for further functionalities [2, 3].

Results
This work addresses the aforementioned challenges in studying excitonic processes within single-layer TMDs. We leverage meticulous sample preparation techniques, including polymer-assisted stamping and encapsulation in hBN flakes, to ensure well-defined interfaces and controlled heterostructure thickness [4]. This meticulous approach paves the way for reliable analysis using advanced microscopy techniques.
We employ a multimodal approach, combining Scanning Transmission Electron Microscopy (STEM)-CL and STEM-EELS for comprehensive characterization. While STEM-CL allows for precise mapping of excitonic recombination processes, STEM-EELS measurements with monochromated electron beams provide information about the absorption mechanisms within the heterostructure at a high spatial resolution. Our investigation highlights the crucial role of hBN encapsulation in enhancing the intensity of cathodoluminescence. By comparing SEM-CL and STEM-CL on the same sample across a wide range of acceleration voltages, we directly assess the effectiveness of each technique. Additionally, we explore the impact of different substrates like SiO2/Si on emission characteristics and spatial resolution, emphasizing the importance of substrate selection for optimal analysis.

Furthermore, we leverage Monte Carlo simulations to understand the relationship between energy deposition within the heterostructure, influenced by acceleration voltage and sample thickness, and the resulting CL intensity. We observe a proportional relationship between these parameters, further validating our experimental findings.
Conclusions

This study offers valuable insights into the influence of material interfaces and heterostructure thickness on the optical properties of TMD monolayers. By elucidating the role of hBN encapsulation in enhancing CL intensity, we pave the way for the development of high-performance optoelectronic devices. Furthermore, our multimodal approach, combining STEM-CL and STEM-EELS, provides a comprehensive understanding of excitonic processes in TMD monolayers. This knowledge is crucial for the future design of complex nanostructures with tailored optical properties and the development of controlled quantum emitters. The ability to correlate CL and EELS measurements offers an unparalleled perspective on light emission processes, laying the groundwork for fundamental advancements in understanding excitons in 2D materials.

Figure caption: Results from SEM-CL and STEM-EELS. (a) Normalized SEM-CL intensity map integrated over the spectral range from 775 to 805 nm, as indicated by the dashed lines in (b). Bubbles corresponding to residues from sample preparation can be observed as dark patches in the intensity map. (b) Normalized CL spectrum extracted from the SEM-CL intensity map in (a) exhibiting a single CL peak originating from the A-exciton (XA) of the TMD monolayer. (c) Monochromated STEM-EELS spectra taken at a beam accelartion of 100 keV. The EELS spectra are displayed for different dispersion so that absorption features from both the TMD monolayer as well as the hBN encapsulation layer can be observed.

Keywords:

TMD, CL, EELS, Excitons, encapsulation

Reference:

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Atomic Resolved In situ mechanical Microscopy leads to realize the negative mixing enthalpy solid solutions

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Poster Group 2

The advancements of atomic resolved in situ mechanical microscopy (ARISMM) helped to understand the grain boundary sliding mechanisms [1], the grain boundary phase transformation mechanisms [2] and the grain boundary migration as well as rotation [3]. Recently, by ARISMM, it was uncovered that a face-centered cubic (FCC) disordered solid solution AuCu alloy nanowires (NWs) exhibit superplasticity of ~260% and ultrahigh strength of ~6 GPa, overcome the trade-off between strength and ductility [4]. With these, we further propose the concepts of ultra-high strength and ductile metals by leveraging negative mixing enthalpy solid solutions. Mixing enthalpy, a pivotal parameter in the thermodynamic state of materials, reflects the chemical affinity among distinct atoms and plays an essential role in quantifying the internal energy of a system. The introduction of elements characterized by negative mixing enthalpy disrupts this entropic solid solution framework, leading to the formation of localized negative enthalpy chemical affinity clusters, characterized by compositional fluctuations and local ordering. Aberration corrected atomic resolved microscopy helps us to realize the distribution of configurational mixing entropy and enthalpy in a solid solution. Pursuing the proposed strategy, we incorporated Al into the nearly ideal solid solution alloy HfNbTiV, thereby modulating the solid solution architecture of alloy. Given that Al and other alloying elements (Al-M, where M = Hf, Nb, Ti, V) exhibit negative mixing enthalpies, the integration of Al fosters the emergence of multilevel nanoscale heterogeneous structures within the alloy, culminating in the creation of the HfNbTiVAl10 negative enthalpy solid solution alloy. Finally, the HfNbTiVAl10 alloy shows remarkable tensile ductility (~ 20%) and ultrahigh yield strength (~ 1,390 megapascals) [5].This study highlights the importance of atomic resolved in situ mechanical microscopy for designing advanced structural materials.

Keywords:

negative mixing enthalpy, solid solutions

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Ultra-fast and facile synthesis of cathodes for rechargeable batteries using an organic syntehsis route

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Poster Group 1

The development of new promising cathode materials for lithium-ion batteries and sodium-ion batteries must go hand-in-hand with the development of new, more efficient, and more environmentally friendly synthesis routes. Several strategies have been reported in the literature to try to overcome the current limitations that prevent a broader deployment of promising cathode materials for intercalation batteries. Among them, the synthesis of electrode materials via an emulsion procedure demonstrated to be beneficial to obtain materials with controlled morphology [1].

In particular, a novel synthesis route [2], which involves the use of metal acetates, water, and oleic acid to form an emulsion is specially promising since only organic precursors are used, and the side reaction products are not contaminant,which makes it significantly more environmentally friendly.

Here we present a structural and electrochemical characterisation of the cathodes synthesised using a modification of the aforementioned route. This modification reduces the mixing times of all the precursors required down to a few minutes, which is a very significant improvement compared with the tradicional sol-gel or ball-milling routes used at the laboratory levels. Furthermore, the modification makes this route very easily upscalable at the industrial level.

We have synthesised a large variety of promising cathodes for the families of lithium-ion and sodium-ion batteries. Transmission electron microscopy and related techniques were used to evaluate the quality of the particles fabricated using this novel synthesis route, and the results are compared with particles fabricated using the more stablished synthesis methods. The particle size and distribution were characterised by means of conventional transmission electron microscopy, while X-ray energy dispersion spectroscopy maps in scanning-transmission electron microscopy (STEM) was used to visualize the elemental distribution in individual particles. Aberration-corrected STEM corroborated that the expected atomic structure had been achieved in individual particles.

In a subsequent step, the particles were analysed at the fully charged and several-times cycled states to visualize the atomic rearrangements and chemical changes that may lead to battery failure. In those cases, the electron dose was carefully controlled to avoid irreversible beam damage, especially in the charged particles. Additionally, multi-frame acquisition followed by non-rigid registration and averaging was used to further reduce the damage due to exposure to the electron beam.

All the results obtained using the ultra-fast synthesis route described here are in very good agreement with those obtained for the same materials using the more conventional sol-gel or mechanical dry-mixing methos, whereas the times involved are considerably reduced.

Keywords:

Cathodes, Li-ion, Na-ion, STEM, Organic

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Evaluating spherical models of EBSD patterns for forward modelling indexing

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Poster Group 2

The application of full pattern matching-based forward modelling indexing techniques of EBSD and transmission EBSD patterns has shown significant improvements over conventional indexing methods that are based on the detection of individual bands in the EBSD patterns [1,2]. Forward modelling provides superior robustness on poor quality patterns, improved orientation precision, and can resolve common indexing challenges including pseudo-symmetry, non-centrosymmetric point groups (polarity), and overlapping patterns. Furthermore, new GPU implementations of spherical harmonic indexing and real space orientation refinement can both achieve speeds of thousands of patterns per second on modest hardware making its application accessible for routine EBSD analyses.
A remaining challenge for these indexing methods, both dictionary indexing and spherical indexing, is that they rely on detailed knowledge of the crystal structure of the phase(s) that are being indexed. The dynamic simulations that are the basis of the forward-model indexing methods are critically dependent on the crystal symmetry, atomic positions, occupancy, and electron scattering parameters. This is especially important in more complex phases such as solid solutions, intermetallic phases, and minerals. In many cases this detailed information is not available.
Alternative master patterns that do not require the same detailed knowledge of the crystal structure can be created using a kinematical model or using actual EBSD patterns. For these methods the information that is already available in the more basic EBSD structure files that are used for Hough transform-based indexing is sufficient. With the lattice parameters, the point group symmetry, a list of expected reflectors or bands, and the relative intensity of the bands in the EBSD patterns a kinematic spherical intensity model can be constructed. Alternatively, Kikuchi spheres may be created using actual EBSD patterns, provided that their phase and orientation is known by conventional EBSD indexing [3]. These spheres can then be applied as experimental master patterns for spherical indexing.
The advantage of using the experimental master patterns is that the required calculation time is less than 1 minute while dynamic simulations may take multiple hours to generate. Experimental patterns will also exhibit the same contrast range as the experimental patterns which may change with specific pattern image processing methods. With a closer match between the experimental patterns and the master pattern, improved indexing results may be expected. In this presentation the requirements for successful generation of master pattern simulations for pattern matching will be discussed together with application examples.

Keywords:

EBSD, Forward-model, spherical-indexing, master-pattern

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The role of atomic displacements in high-resolution scanning transmission electron microscopy imaging of strained nanoparticles

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Poster Group 2

Background
Strain in nanoparticles (NPs) plays an important role in the control of catalytic or chemical reactivity and its impact on the atomic level structure is essential for applications in many areas such as biomedicine or soil remediation. Hence, atomic level displacement analysis is greatly important with Z-contrast imaging in high-resolution scanning transmission electron microscopy (HRSTEM) being at the forefront of its exploration [1]. However, analyzing atomic level strain in NPs with HRSTEM or HRTEM faces numerous challenges such as the impact of strain on the image formation since strain induces modifications of column intensities and intensity distributions and hence affects the interpretation of image contrast and apparent column positions. Thus, knowledge of the 3D strain distribution in NPs is required. To investigate this, we employed a workflow combining finite volume strain calculation and image simulation to study the impact of strain on image formation for an iron oxide (magnetite) segment like those found in cluster source deposited cubic and partially oxidized iron NPs to be compared with experimental Z-contrast images.

Methods
Our approach was inspired by our previous work which shows that lattice strain is induced by stress at the oxide domain boundaries in iron-iron oxide core-shell NPs [1]. To investigate how strain affects Z-contrast image formation, we constructed an atomistic model of the oxide shell, then applied a finite element calculated strain field to this model to be used for simulating HRSTEM images of such strained domains employing the QSTEM simulation software [2] including thermal diffuse scattering. Subsequently we compared our findings with experimental data obtained using aberration corrected Z-contrast imaging.

Results
We find that lattice strain has various effects on the HRSTEM images including a significant change of Fe(II) column intensity distribution within the domains in comparison to the unstrained as well as streak formation due to strain-induced atomic displacements, which is in-line with our experimental observations. We further compared the atomic displacement fields derived from experimental images with those resulting from our image simulations showing a significant maximum displacement at the oxide surfaces and a linear strain gradient between the core/oxide boundary and the oxide surface, which is in good agreement with experimental observations with high-resolution STEM.

Conclusion
Overall, we find that atomic level displacements due to strain fields strongly affect HRSTEM image formation and need to be carefully considered when quantitatively analyzing such data e.g. for the extraction of strain field information. We find that strain mapping from 2D images cannot necessarily be taken at face value but require a sufficient understanding of the 3D strain field distribution. Furthermore it becomes evident that evaluation of atomic column intensities for e.g. atom number determination is non-trivial in the presence of strain fields since the column intensities and positions

determined from HRSTEM images can be strongly affected by atomic displacements in beam direction leading to significant local variations of electron scattering.

Keywords:

Z-contrast imaging, Strained Nanoparticles

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Unravelling the collagen mineralization using multiscale in situ X-ray-scattering/Raman spectroscopy and ex situ electron microscopy/nano-Xray-fluorescence

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IM-05 (2), Lecture Theater 3, august 26, 2024, 14:00 - 16:00

Background
The process of collagen mineralization is key to numerous biological processes including bone and tooth formation, arteriosclerosis as well as tendon and cartilage calcification and other forms of ectopic calcification. Therefore, understanding the processes that govern the formation of a hard inorganic calcium phosphate phase (usually hydroxyapatite) within a soft matrix of collagen is central to identify the physico-chemical processes involved in collagen calcification, which occurs in close interaction with the collagen template. E.g. for bone it is known that the hydroxyapatite mineral is organized, like collagen, in a hierarchical fractal-like fashion [1]. A key problem in this context is the dynamics of the mineralization and the possible presence of intermediate phases before a full mineralization with hydroxyapatite occurs. Using the polymer-induced liquid precursor (PILP) method for in vitro collagen mineralization [2], we employed state-of-the art characterization techniques, a combination of in situ X-ray scattering, in situ Raman spectroscopy, nano X-ray fluorescence (nXRF) with 50 nm resolution and transmission electron microscopy (TEM) to monitor the mineralization process at different length scales. In our studies we focused on the impact of mineralization on the collagen response and the possible occurrence of intermediate phosphate phases apart from hydroxyapatite.

Methods
A combination of in situ characterization with Raman spectroscopy and small and wide-angle X-ray scattering (SAXS, WAXS) was used with the same flow cell, which was heatable and kept at 37 ˚ C during the mineralization process. We applied PILP using osteopontin, fetuin-A and poly aspartic acid as active protein polymers to mimic this process in vitro using rat-tail derived collagen type I fibers. Raman spectroscopy enables a molecular level analysis of the mineralizing collagen, SAXS provides information on the particle evolution and impact of mineral growth on the collagen D-banding (a periodic pattern caused by repeat sequences of overlap and gap regions in the staggered collagen molecule organization) and WAXS permits the study of the time dependent evolution of crystallinity monitoring the {002} lattice planes in the hydroxyapatite mineral phase. The resulting mineralized collagen fibers were subsequently analyzed using nano - XRF microscopy to map the distribution of Ca and P within the fibers and TEM and electron diffraction for microstructural investigations and analysis of the mineral phases.

Results
In situ Raman spectroscopy reveals intermediate phosphate phases during the initial mineralization stage confirmed by electron diffraction in TEM. In accordance with these findings, we observe three stages of mineralization with SAXS showing an initial expansion of the collagen D-spacing, associated with the infiltration phase, a subsequent contraction of the D-spacing, associated with the beginning of nucleation and crystallization and finally a continuous growth phase, correlated with an expansion of the D-spacing. Analyses of the mineralized collagen ex situ using TEM and nXRF indicates the

presence of two morphological features, which we associate with octa calcium phosphate and hydroxyapatite, respectively, and an inhomogeneous pattern of tessellation in accordance with reported patterns for bone mineralization [3].

Conclusion
Our in vitro studies of collagen mineralization shows a remarkable resemblance of observations made in biological systems indicating that the PILP process is an excellent proxy to study relevant physico-chemical processes e.g. in bone formation. The joint application of multiscale and multiplatform methods to monitor the progression of mineralization as well as the resulting mineralization patterns allows for unique insights into the evolution of the mineral phase and the interaction between the hard mineral phase with the soft collagen template.

Keywords:

SAXS/WAXS, nano-XRF, Raman, TEM/STEM, Collagen-Mineralization

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Visualization of binder mixtures in hard carbon composite electrodes using OsO4 and uranyl acetate staining

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Poster Group 1

Background and aims
Aqueous processable binders, such as a sodium carboxymethyl cellulose (CMC) - styrene butadiene rubber (SBR) mixture are crucial constituents in composite battery electrodes ensuring good adhesion properties, high elasticity as well as improved cyclability and thermal stability (Alvira et al. 2022). The uniform distribution of the binder, active material, and conductive carbon is a prerequisite. Inhomogeneous distribution that may occur during the fabrication and processing of the electrode, such as coating, drying, and calendaring can lead to segregation, delamination or solidification which directly influence the overall battery performance (Jaiser et al. 2016). Therefore, its crucial to assess the distribution of the constituents, particularly the binder as agglomeration or segregation of the binder during longer cycling may lead to capacity loss.
The aim of the study is the visualization of CMC and SBR binder components next to hard carbon (HC) and conductive nano carbon particles using osmium tetroxide (OsO4) and uranyl acetate (UA) staining. OsO4 stains the double bonds present within SBR (Lee et al. 2022), whereas UA reacts with the carboxy groups (Stoeckenius 1961) of the CMC. As CMC is soluble in water, the sample preparation techniques must be adapted. In this study, we applied the embedding procedure introduced by Eswara-Moorthy et al., where Pt is deposited within a several µm deep volume of the sample using a 30 kV electron beam. This method avoids mechanical polishing using aqueous solutions and provides excellent contrast within the sample.

Methods
HC electrodes were prepared by doctor blading of a water-based slurry consisting of 80 wt.% HC, 10 wt.% SuperP, 5 wt.% Na-CMC and 5 wt.% SBR. The sample was first stained with OsO4 vapour by placing the sample in a sealed container together with a couple of droplets of 4 % aqueous OsO4 solution for 6 days, whereby the droplets were replenished after 3 days. In a second step, a droplet of 2 % uranyl acetate dissolved in ethanol was placed on the sample, removed after 30 min, and the sample was then rinsed with ethanol. The samples were investigated using a Helios Nanolab 600 and a Quanta 3D FEG (both Thermo Fisher Scientific Inc., USA). To achieve better contrast, avoid shine through artefacts, and reduce curtaining effects during FIB milling, the samples were embedded in Pt using electron beam induced deposition (EBID) with methylcyclopentadienyl trimethyl platinum as precursor and an electron beam operated at 30 kV, 32 nA. EDX mapping was conducted using an Element EDX detector attached to the Quanta and Apex standard software package (both EDAX Inc., Germany). The microscope was operated at 6 kV and 16 nA with a dwell time of 50 µs and 64 frames.

Results
Compared to the non-stained sample, secondary electron (SE) images of FIB cross-sections of the EBID Pt embedded region of the stained sample show reasonable contrast between the binder, HC particle and Pt (Fig.1 a & b). The contrast is sufficient to easily segment the 3 phases in FIB-SEM

tomography data sets. It is also possible to distinguish between the binder matrix and conductive carbon nano particle within the binder. Although pores larger than approximately 1 m in diameter were not completely filled with Pt, the delicate structure of the binder phase was well preserved. Regions containing binder material are completely embedded and therefore provide volumes suitable for FIB-SEM tomography studies due to the enhanced contrast. EDX maps reveal the successful staining of both components, SBR with OsO4 and CMC with UA (Fig1. d & e). However, it should be noted, that remnants of the UA staining procedure may occur within the sample, but UA particles can be clearly distinguished. They appear brighter than the binder components in the SE images (e.g., Fig1 c & d, marked with red star) and in EDX measurements, as they contain less C but significant amounts of Na.

Conclusion

In this contribution, we show the potential of OsO4 and UA staining of SBR and CMC binder mixtures to reveal the 3D distribution of the binder phase within HC composite electrodes. EBID Pt embedding results in improved contrast and avoids artefacts as well as protect the delicate binder structures. The here described sample preparation method is suitable for FIB-SEM tomography studies and EDX mappings of FIB cross-sections, as the Pt surrounding the binder also limits the extend of the excitation volume compared to epoxy resin embedding. As both binder components react with different staining agents, namely CMC with UA and SBR with OsO4, both components can be tracked individually using EDX. For TEM-tomography studies EBID Pt embedding may not be suitable and should be replaced by classic epoxy resin embedding.

Fig.1: SE images and EDX maps of FIB cross-sections of EBID Pt embedded HC composite electrodes, red arrows point at binder components, hard carbon particles are exemplarily marked with “HC” a) SE image of non-stained electrode; b) SE image of the stained electrode with the stained binder components revealing clear difference in grey value relative to the HC particle and Pt embedding; c) SE image of ROI for EDX mapping; d) Os distribution; e) U distribution, red star marks possible UA remnant from the UA staining step.

Keywords:

OsO4/UA staining, FIB-SEM-tomography, SBR-CMC binder

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Analysis of molecular packing and nanoscale atomic variation in polymer semiconductors

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Poster Group 2

Background incl. aims
Conjugated polymers are an important class of organic light emitting diodes (OLEDs) and organic solar cells (OSCs). These materials are predominantly semi-crystalline or amorphous with intricate molecular packing and mixed variety of structural orders and disorders [1]. The susceptibility of these materials to 'burn-in degradation' [2] can induce blend-demixing and photo-induced ordering/disordering [3], thereby resulting in the performance losses of the devices [4]. Controlling this performance degradation during operation necessitates an understanding in changes in chemical structures and structural disorders at the nanoscale – the length scale commensurate with the transport of charge carriers. Yet direct nanoscale characterisation is limited for polymer semiconductors and their associated devices due to the irreversible changes in these materials structure when exposed to high-energy ion and electron beam conditions [5]. Here, we advance the structural characterisation of polymer semiconductors, whether in the form of free-standing films or cross-sectioned lamella, using low-dose four-dimesion scanning transmission electron microscopy (4D-STEM), enabling the analysis of the molecular packing, crystallinity, and atomic arrangement in the polymer semiconductors in response to temperature and ion milling-induced damage.

Methods
Low-dose 4D-STEM analysis was conducted using established nanobeam scanning electron diffraction alignment at electron Physical Science Imaging Centre (ePSIC), Diamond Light Source. In particular, Merlin-Medipix detector and <1 mrad convergence semi-angle with 1-2 pA in probe current at 300 kV were used to minimize radiolytic damage. We obtained data at a range of camera lengths to enable both mapping of crystalline domains from Bragg scattering as well as reciprocal space (variance measures) and real space electron Pair Distribution Function (ePDF) analysis of disordered and amorphous regions. The materials under examination were free-standing polymer films (F8:F8BT, 1:1), prepared by spin-coating onto PDOT:PSS/ITO/Glass substrates. Subsequently, the multi-layered sample was submerged in deionized water, and the F8:F8BT films were floated onto carbon support films for 4D-STEM analysis. Additionally, we developed cryo-Focused Ion Beam (cryo-FIB) protocols to facilitate the structural examination of the cross-sectioned device model, Glass/ITO/PDOT:PSS/F8:F8BT (1:1).

Results
The developed techniques reveal the formation of nano-crystalline domains in the F8:F8BT films after heat treatment. These domains are attributed to the crystallisation of F8 polymers, as evidenced by indexing some diffraction patterns aligning along the zone axis. Additionally, ePDF analysis allows us to characterise the atomic structures in amorphous areas with varying contrast. The analysis indicates that there were no chemical changes in the F8:F8BT blends induced by temperature. However, partial phase segregation occurred, as also supported by low-dose EELS analysis. We extended these analyses to a cross-sectioned device model prepared by cryo-FIB, and the findings

demonstrate that our cryo-FIB protocol preserves the crystalinity of the polymer blends. ePDF shows that cryo-FIB milling does not alter the chemical structures of the films, i.e. intramolecular structure, but does affect the intermolecular arrangement.

Conclusion
The developed electron microscopy techniques enable the characterisation of microstructures and nanoscale atomic arrangements in beam-sensitive polymer semiconductors, paving a pathway for examining phase segregation and chemical changes resulting from the burn-in degradation. By doing so, effective strategies can be developed to minimise structural degradation in polymer semiconductors, thereby preventing performance losses during operation.

Keywords:

Polymer semiconductors, 4D-STEM, ePDF, cryo-FIB

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Toward 3D imaging of the PEMFC electrode microstructure

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Poster Group 1

Background incl. aims
Proton Exchange Membrane Fuel Cells (PEMFCs) are being developed mainly for transport applications. Significant progress has been made over the last decade, leading to the commercialization of the first generation of fuel cell cars. More recently, R&D efforts have shifted to heavy-duty vehicles, where PEMFCs are more competitive with Li-ion batteries. However, their cost and durability are still the two barriers limiting the expansion of this market. To overcome these obstacles, we still need to optimise the components and structure of the electrodes and better understand their degradation mechanisms.
PEMFC electrodes are a nano-porous material composed of carbon particles (30 nm) supporting platinum nanoparticles (3 nm) - the catalysts of electrochemical reactions - and linked together by ionomer (proton conducting polymer).
Scanning and transmission electron microscopies (SEM and TEM) are widely used for investigating the microstructure of PEMFC electrode. They have played a significant role in the improvement of electrode components and the understanding of their main degradation mechanisms. However, the currently available descriptions of the electrode microstructure do not allow yet to unambiguously identify the key microstructural parameters that limit gas and proton transport within the electrode. These factors are crucial as they govern PEMFC performance, especially at high current densities, and are therefore the subject of numerous studies, using either fine electrochemical measurements or modeling approaches.
The aim of our work is to provide an improved description of the electrode microstructure in 3D to give quantitative information on its porosity, Pt nanoparticle (NP) spatial distribution and ionomer distribution. For this purpose, we are developing 3D characterization techniques applied to the PEMFC electrodes, such as electron tomography and 3D FIB-SEM.
Methods
The studied cathode electrode was prepared using TEC10E50E catalyst from Tanaka that is 50 wt % loading of Pt deposited on high-specific area carbon (HSAC), and Nafion D2020 as the electrode ionomer. The Pt loading of the electrode is 0.2 mgPtcm-2 and the ionomer/carbon ratio is 0.8.
The electrode was analyzed using electron tomography and 3D FIB-SEM.
Results
As a first step, electron tomography analyses were performed on the Pt/HSAC powder to determine the distribution of Pt NPs which can be located either on the carbon surface (outer NPs) or inside the carbon porosity (inner NPs)[1-2]. Our strategy is to record tilt image series by using two annular detectors: i) a high angle annular detector avoiding Pt NP diffraction contrast for the 3D image reconstruction of Pt NPs (without diffraction artifact) and ii) a lower angle annular detector that enhances carbon contrast for the 3D image reconstruction of the carbon phase. These two segmented images were then combined (Figure 1), allowing the calculation of the NP size histogram but also of the volume and the surface area of the two NP populations (inner and outer NPs).

On the second step, the porosity of the electrode was analyzed by 3D FIB/SEM. A volume of around 10 X 10 x 5 µm3 was imaged with a cubic voxel size of 5 × 5 × 5 nm3. The solid phase (carbon, Pt NPs and ionomer) segmentation was based on a composite image created from two electron detectors (secondary and backscattered electrons) and using a machine learning software. Quantitative data such as the total porosity and the pore size distribution were extracted from this volume. Figure 2a shows a 2D slice of this segmented volume where the solid phase is in black and the porosity in white.

In order to analyze the electrode porosity with a higher spatial resolution, electron tomography experiment was also performed on a 150 nm thick slice of the electrode cut by ultramicrotomy after being embedded in an epoxy resin. The tilt image series were recorded with a pixel size of 0.5 nm and a field of view of 2 x 2 µm2. The 2D slice of the reconstructed volume (Figure 2b) shows that the largest solid agglomerates detected on the 3D FIB/SEM image can be identified as an agglomeration of numerous carbon particles. The challenge now is to quantify the porosity created between these carbon particles, which cannot be detected by 3D FIB-SEM.

Finally, the distribution of the ionomer was analyzed within the electrode similarly to [3]. For this analysis, a 150 nm slice of the electrode was cut by cryo-ultramicrotomy without embedding in epoxy resin as described in [4]. TEM images show that the ionomer is not homogeneously distributed on the surface of the entire carbon particles but is often observed in the concave surface created between the carbon particles (Figure 3). These observations suggest a ionomer distribution in agreement with the heterogeneous ionomer coating model proposed by Inoue et al. [5]. We also suggest that a part of the ionomer could be localized in the small pores located inside the large carbon agglomerates described in the previous paragraph. This could explain why the ionomer is so difficult to detect on TEM images.

Conclusion

The different 3D electron microscopy techniques make it possible to obtain information on the distribution of the different components of the PEMFC electrode. This 3D description of the electrode can then be incorporated into recent models that calculate electrochemical performance as a function of microstructural parameters, in an attempt to identify the key parameters that limit cell performance. Similar studies are also being carried out on aged electrodes after the fuel cell operation.

This work has been done in the frame of the FURTHER-FC project. This project has received funding from the Fuel Cells and Hydrogen 2 Joint Undertaking (now Clean Hydrogen Partnership) under Grant Agreement No 875025. This Joint Undertaking receives support from the European Union's Horizon 2020 Research and Innovation program, Hydrogen Europe and Hydrogen Europe Research.

Keywords:

PEMFC, Electrode, e-tomography, 3D FIB-SEM

Reference:

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Correlating atomic-resolution structure to the properties of transition metal nitride coatings

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Introduction
The industrial applications of transition metal nitrides require an in-depth understanding of the structures and their correlations to properties. This has led to numerous experimental and theoretical research [1-5]. Utilizing modern electron microscopy and spectroscopy, our understanding of nitride structure and property relations and its deformation has been significantly advanced. This presentation will briefly summarize our recent atomic-resolution studies on transition metal nitride coatings using spherical aberration-corrected transmission electron microscopy (TEM).
Methods
The film used in this study was prepared by direct current magnetron sputtering using a custom-made lab-scale magnetron sputter deposition system. The film was grown on MgO(001) or Si substrate at 700 ℃, and the potential was kept floating during the deposition. Specific deposition conditions for different films are slightly different. Nanoindentation tests were conducted on a KLA Nanoindenter G200 equipped with a diamond cube-corner indenter tip to obtain H and E by the conventional analysis first proposed by Oliver and Pharr. The tests applied the continuous stiffness measurement method with a constant indentation strain rate □ of 0.05 s−1. The cross-sectional TEM lamella of the as-deposited and indented films was machined by focused ion beam (FIB) using an FEI Helios NanoLab 660 workstation. The lamella was cut along the <110> direction of the substrate and then transferred to a Cu TEM grid. The lamella was polished using accelerating voltages from 30 to 2 kV and ion currents ranging from 20 nA to 7 pA. Some TEM specimens were also prepared using a standard approach, i.e., grinding, polishing, dimpling, and Ar ion-milling.
For high-resolution TEM (HRTEM) characterization, a JEOL 2100F and FEI Titan Themis 60-300 cubed TEM, both equipped with an image-side Cs-corrector, were adopted. The scanning TEM (STEM) images and energy-dispersive X-ray spectroscopy (EDXS) were acquired by a 300 kV field emission TEM (JEOL ARM300F) equipped with double Cs-correctors. The microscope has two windowless detectors, each with an active area of 100 mm2.
Results
The extensive high-resolution transmission electron microscopy (HRTEM) observations on TaN/TiN multilayer reveal that the dissociation of full dislocations results in the network of stacking faults (SFs) and the formation of Lomer-Cottrell lock arrays inside the TaN layer. Consequently, the high density of stacking faults dramatically strengthens the TaN/TiN multilayer [1]. Using valence electrons and inner shell electron spectroscopy, a combined experimental analysis on a multilayered structure of CrN/AlN allowed mapping of the multilayer's mechanical properties (bulk modulus) at the nanometer scale [2]. Moreover, we found that the presence of oxygen impurities causes a remarkable reduction of the bulk modulus of rs-CrN while having no significant effect on the bulk modulus of the stable wurtzite structure wz-AlN layers. The findings have been validated by theoretical calculations [2].
We observed an atomic-scale intermixing phenomenon in the nanoscale TiN/AlN multilayer by coupling the cross-sectional FIB cutting with detailed atomic resolution electron microscopy analyses. A new solid solution phase was created during nanoindentation, which could be unambiguously

shown by mapping the electronic structure differences (i.e., using Ti-L2,3 edge). This also raises some concerns about understanding experimentally measured hardness values in multilayers using the nanoindentation method. By using atomic-resolution energy dispersive x-ray spectroscopy, we further corroborated that such a homogenous solid solution zone formed upon loads [3]. We recently found a new deformation mechanism of a high vacancy-mediated WNx/TiN multilayer through quantitative electron microscopy analysis, theoretical calculations enabled by ab initio molecular dynamics, and combined with mechanical testing [5].

Conclusions

Advanced electron microscopy methods reveal the atomic and electronic structures of transition metal nitride coatings, which allows correlating the structure with properties at the atomic scale and leads to new findings. These exciting findings shed light on the design of novel multilayer coatings for applications.

Keywords:

Nitride, HRTEM/STEM, EELS, deformation

Reference:

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[2] Zaoli Zhang et al., Acta Materialia, 194(2020) 343
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Cross-sectioning of adherent cells on thin plastic substrate for serial block-face imaging

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Poster Group 1

Background

The single-celled enteric parasite Giardia lambia (Figure 1A) uses a ventral disc [1], consisting of a spiral-formed microtubule apparatus, for adhesion to their host duodenal epithelium. For analyzing the phenotype of various mutations of the ventral disc proteins, we started with conventional thin sectioning of the parasites cultivated on thin plastic bottom of plastic culture dishes (Figure 1B). The complexity of the observed phenotypes prompted us to consider volume imaging for a more comprehensive analysis of the ventral disc morphology. Instead of performing an additional cultivation and processing for volume imaging, we tested, if the already available sample blocks, prepared with our standard protocol for thin sectioning (osmium tetroxide, tannic acid, uranyl acetate/UA-Zero, Epon) [2], were suitable for serial block-face (SBF) scanning electron microscopy (SEM) at sufficient resolution. To our surprise, the resulting data sets were of sufficient quality (Fig. 1C) to reconstruct the entire ventral disc microtubule apparatus of the parasite in 3D (Figure 1D), but limited in resolution. SBF SEM usually requires a high-contrast en bloc staining of the sample with various heavy metals and reduction of the charging by improving the electrical conductivity (e.g. conductive filler) or by charge compensation (e.g. gas injection). As a consequence, the time of sample preparation usually is much longer for SBF imaging than for standard thin section imaging. Moreover, the introduction of more heavy metals changes the ultrastructural appearance of the sample. With our study, we wanted to find a generic sample preparation workflow for adherent cells which provides sufficient contrast and image quality in SBF SEM.

Methods

For the experiments we used adherent HeLa cell cultures grown in plastic dishes which were equipped with small silicone inserts on their plastic bottom (ibid µ-dishes, with microinserts). To stepwise improve the image contrast and quality, we introduced various simple modifications to our standard embedding protocol: minimal resin embedding, addition of silver colloids, higher processing temperature during heavy metal treatment, higher concentration of osmium tetroxide, additional heavy metal incubation steps (osmium tetroxide, lead aspartate). Cells were embedded in situ on their substrate in Epon, extracted with a hot scalpel and mounted on an aluminum stub using conductive epoxy glue. After trimming to a size of 1x0.25x0.25 mm, SBF SEM was performed with a Teneo Volumescope at high and low vacuum conditions.

Results

The addition of further incubation steps with heavy metals to our standard sample preparation protocol resulted in improved resolution and image quality in block-face imaging. The contrast of the

samples which were prepared with a single osmium tetroxide incubation and an additional en bloc treatment with lead aspartate (Figure 1E) allowed a proper discrimination of the main organelles and cytoplasmic structures already at low magnification and was very similar to the appearance of cells in thin sections taken from samples prepared by our standard protocol after on-section staining. The increased heavy metal load introduced by an additional osmium tetroxide incubation provided slightly higher lateral resolution at the cost of a rather unusual contrast. SBF imaging of adherent cells at low vacuum resulted in more stable image series at higher resolution in all directions than at high vacuum, regardless of the heavy metal load. The addition of silver colloid improved only the imaging at high vacuum with less significance at highest heavy metal load tested.

Conclusion

Our cross-sectioning workflow for SBF SEM of adherent cells on plastic substrate offers the possibility of direct investigation of cell-substrate interactions. The upright mounting of the sample allows us to collect complete volumes of many cells and large total sample volume. Finally, we could find a protocol which provides comparable ultrastructural appearance than in conventional thin section EM and with only limited additional effort compared to our standard embedding protocol.

Keywords:

conductivity, cell culture, sample mounting

Reference:

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Direct visualization of chemical transport in solid-state chemical reactions by time-of-flight secondary ion mass spectrometry

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Background incl. aims
Systematic control and design of solid-state chemical reactions are essential for modifying material properties and pioneering novel synthesis techniques. Enhanced chemical transport and accelerated kinetics of active elements and compounds across interfaces are known to drive solid-state chemical reactions [1]. Monitoring temperature-dependent chemical dynamics at micro- to nanometer length scales in real-time is crucial for providing valuable feedback to tailor the properties, performance, and stability of multi-material structures. Conventional methods for studying solid-state reactions, such as in-situ TEM heating, can identify formed phases and their relative weights but may overlook transient and minor phases [2, 3]. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) at nanometer resolution offers a solution to these challenges. This technique provides information about element distribution and bonding encoded in multiatom ions from the near-surface region of solids [4], making it ideal for studying complex chemical processes in energy storage and hot corrosion. In this study, we combine focused ion beam–scanning electron microscopy (FIB-SEM) and TOF-SIMS to track the migration of active chemical elements from a glass coating to the oxide scale during in-situ hot corrosion experiments. Our findings demonstrate the distinct potential of TOF-SIMS in exploring nano-scale chemical dynamics of materials in response to temperature, optical, or electromagnetic stimulations.

Methods
TOF-SIMS analysis was performed using a Ga primary ion beam at an accelerating voltage of 30 kV and a current of 0.23 nA, scanned continuously across the sample surface throughout in-situ heating using MEMS heating chip. The lateral resolution of the resulting images was estimated as better than 290 nm at an abrupt step in the 23Na+ signal (20%–80% of the maximum intensity). Isochronal heating was applied from 50 to 850 °C (1 °C/s), producing a Z-direction (direction of successive two-dimensional scans) that directly tracks the temperature. Each frame's acquisition time was 1.4 s, allowing for real-time observation of chemical dynamics. The examined sample comprises a sodium borate coating as the uppermost layer, followed by the intermediate oxide layers with the dendritic iron oxide grown above the continuous iron–chromium oxide layer. The sample was cross-sectioned using FIB, and the cross-sectioned specimen was lifted out and deposited on a MEMS heating chip (flat on the chip, perpendicular to the ion beam).

Results
This advance in nanoscale chemical analysis with TOF-SIMS reveals corrosion induced by sodium diffusion below the coating's glass transition. These findings unveil sodium diffusion decoupled from boron at much lower temperatures than previously known. Moreover, selective dissolution of iron oxides in solid-state corrosion was observed, with iron moving into the borate coating while chromium remains in the steel oxide, elucidating a part of the nanoscale electrochemical mechanism. Additionally, this technique enables the detection of transient phases and impurities during hot

corrosion reactions between sodium ion and oxide layers, allowing us to track the formation of early-stage corrosion reaction products and lithium impurities in the oxide layers. Ultimately, we can employ image-based determination of the sodium diffusion coefficient by combining our established Fickian diffusion model with microscopic measurements of the moving sodium front, extracting the activation energy for diffusion and the temperature-dependent diffusion coefficient through FIB-based TOF-SIMS analysis.

Conclusion
In summary, the developed in-situ TOF-SIMS experiments provide valuable inputs for the study of chemical dynamics occurring in the hot corrosion processes of inorganic glasses with complex compositions, offering insights for mitigating corrosion reactivity at high temperatures. More widely, the developed TOF-SIMS techniques open the exploration of chemical dynamics at high temperatures in applications from metal-forming and engine lubricants to nuclear reactor components to advance the understanding of performance degradation through to new materials synthesis routes.

Keywords:

Coatings, TOF-SIMS, Sodium, Borate, Corrosion

Reference:

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HRSTEM study on the phase formations and transitions in nanolaminated MAB phases

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Poster Group 2

Background incl. aims
MAB (M = transition metal, A = A group element, B = Boron) phases possess a nanolaminated structure, which gives rise to desirable properties from both metals and ceramics due to the combination of metallic and covalent bonding. The metallically bound A elements can be selectively removed leading to the formation of 2D graphene-like MBenes. The most studied MAB phase system, MoAlB, exhibits an oxidation resistance in line with other alumina formers. Upon addition of Cr, the quaternary (Mo1-xCrx)AlB phase is reported to form for high Mo contents, while the experimental data reported for high Cr contents are limited. To gain a deeper understanding of the system including phase formation and transition, a high-resolution transmission electron microscopy (TEM) study was conducted, whereby the data were correlated with in-situ as well as macroscopic results.

Methods
MAB thin films were synthesized by magnetron sputtering at temperatures up to 600 °C. In order to gain an understanding on the phase formation in the MAB samples, HRSTEM analysis was employed in combination with energy dispersive X-ray (EDX) analysis, used for chemical analysis. To confirm the TEM results, multiple analysis techniques were used. Structural data were acquired by X-ray diffraction, while time-of-flight elastic recoil detection analysis (ToF-ERDA) was used for the macroscopic chemical composition. Additionally, electrical resistance measurements and differential scanning calorimetry (DSC) was performed. To further analyze the mechanisms of phase formation and transition, MAB thin films were deposited on SiN heating chips (Wildfire nano-chips, DENS solutions) and investigated in situ with STEM imaging as well as in situ selected area electron diffraction (SAED). Gibbs energy of formation calculations obtained by DFT revealed the energetically favorable phase formations.

Results
HRSTEM and STEM-EDX analysis revealed the formation of the quaternary $(Mo,Cr)_2AlB_2$ phase in a Cr-rich (Mo0.24Cr0.76)0.40Al0.32B0.28 thin film, which had not been reported beforehand. These results were confirmed on a macroscopic scale by XRD, ERDA and EDX. This in agreement with the Gibbs energy of formation calculations obtained by DFT, which show, that the energetic barriers for phase formation of the quaternary phase can be overcome in the employed synthesis scenario. Additionally, the formation of the Cr_3AlB_4 phase as it is energetically favorable in comparison to the corresponding quaternary phase, as well as the formation of CrB_4 and Mo were observed. The Cr_3AlB_4 and the Cr_2AlB_2 phase could furthermore be found in thin films of the Cr-Al-B system, which

was confirmed by means of HRSTEM, SAED and EDX. Thereby, inclusions of an Al-rich phase within the MAB phase consisting of up to 15 Al layers were revealed.

Conclusions
The phase formations and transformations of MAB systems were identified by in situ and ex situ TEM analysis. In addition to the unreported $(Mo,Cr)_2AlB_2$ phase, further formation of Cr_2AlB_2 and Cr_3AlB_4 MAB phases was reported. The results were confirmed by DSC, ERDA, XRD and resistance measurements. The experimental description of the shown phase formations in the quaternary (Mo,Cr)AlB and ternary CrAlB system allow for a deeper understanding and contribute to a more sustainable usage of the material.

Keywords:

Phase formation, MAB phases, HRSTEM

Reference:

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Mechanics of morphogenesis – The re-invention of cell sheet folding

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LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

Mechanical constraints impact the way that organs and tissues develop. Yet, we are only beginning to understand the physical principles that shape tissues. The folding of cellular monolayers, as seen in gastrulation, neurulation and organogenesis, serves as a model for understanding the shaping of three-dimensional tissues. Within the algal family Volvocaceae a range of complexity in cell sheet folding has evolved only 200MYA, providing an excellent model system to study the underlying mechanics. Volvox globator embryos consist of a spherical cellular monolayer which turns itself inside-out in a gastrulation-like process called inversion.
We have been using a combination of advanced light sheet and 2-photon time lapse imaging, biophysical perturbations, and mathematical modelling to understand the forces underlying inversion. We found that different ways to turn a sphere inside out have evolved in different volvocacean species, corresponding to deviations in geometrical constraints [1-4]. We have developed a framework to predict out-of-plane forces in dynamic three-dimensional cell sheets. Model-based analyses of orthogonal laser ablations allow us to infer out-of-plane forces and stresses (Fig. 1). [5]. We found that inversion is driven by spatio-temporally concerted changes in cell shapes, cell connections, and tissue properties. Our findings suggest that these green algae have likely evolved mechano-chemical signalling mechanisms, equivalent to those found in the animal kingdom.

Keywords:

Morphogenesis, mechanobiology, lightsheet-imaging, Volvox inversion

Reference:

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Investigating solar degradation mechanisms of the Ta3N5 photoelectrode by in-situ transmission electron microscopy

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Poster Group 1

Background incl. aims
Photoelectrochemical water splitting is a clean and renewable method to directly convert green energy carrier from solar light into hydrogen. Ta3N5 is a promising photocatalytic material for use as a photoanode, with suitable bandgap (2.2 eV), and with the conduction band (CB) and valence band (VB) energy levels appropriately straddling the water redox potentials [1]. By applying only 1.23 V of overpotential, the photocurrent density under 1 Sun illumination reaches the theoretical limit of approx. 13 mA.cm2 [2, 3]. However, during operation when the photoelectrode is exposed to water, sunlight, and biasing conditions, photocorrosion has shown to occur. This reduces the stability of the device significantly and degrades the material by oxidizing the surface. In order to utilize this material as a stable and efficient photoanode, it is important to understand and prevent the solar degradation process. We have previously investigated the stability of the electronic band structure of Ta3N5 using operando ambient pressure X-ray photoelectron spectroscopy (AP-XPS) at the HIPPIE beamline at the MAX IV synchrotron in Lund using the “dip and pull” setup for pure Ta3N5 as well as NiOx coated materials and found Fermi-level pinning to occur, and a significant improvement was made by stabilizing the surface. We then wanted to investigate the dynamic structural changes that occur to Ta3N5 during the degradation process when Ta3N5 is exposed to water vapor, biasing conditions, and light.

Methods
Ta3N5 thin films on glass substrates were made with pulsed vapor deposition of metal Ta, following a nitridation process. Some samples were also made with a protective NiOx coating. The ex-situ characterization work was done using a FEI Titan G2 transmission electron microscope (TEM), DCOR Cs probe corrector, Super-X EDS detector, and Gatan GIF Quantum 965 electron energy loss (EELS) Spectrometer at SINTEF in Oslo. While the in-situ TEM characterization was carried out using an environmental transmission electron microscope a Titan E-Cell 80-300ST TEM at DTU. The TEM sample was made with a focused ion beam and connected to a heating and biasing chip with a diode placed on top of the extra contacts.

Results
During the degradation process, an amorphous layer is formed at the surface. The surface layer formation is highest when combining water, light, and biasing of the sample. The process seems to first make small nanocrystals of Ta3N5. After some time, they are amorphized, and new nanocrystals are formed. This process continues till we have a stable uniform surface layer.

Conclusion

The surface degradation process creates an amorphous layer, and is most pronounced when combining water, light, and bias. The degradation first makes small nanocrystals of Ta3N5, for then to amorphized them separately.

Keywords:

Degradation, TEM, operando, Ta3N5, Hydrogen

Reference:

Reference:
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Band gap measurements of aluminum and indium doped Ga2O3 multilayers

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PS-03 (3), Lecture Theater 2, august 30, 2024, 14:00 - 16:00

Background incl. aims
Power electronics (PE) are integral to the acquisition, distribution, and storage of energy, serving as a pivotal facilitator for enhancing energy efficiency, leveraging renewable resources, and advancing smart grid technologies. While silicon-based PE components have reached a peak in optimization, further enhancements are constrained by intrinsic material properties. This limitation underscores the appeal of wide bandgap semiconductors in PE applications, offering the potential for superior speed, compactness, and energy efficiency compared to their silicon counterparts [1]. One promising avenue lies in the development of High-Electron-Mobility Transistors (HEMTs) based on novel (MxGa1-x)2O3 thin film heterostructures (where M represents Al or In), which exhibit the promise of heightened operational speeds, reduced footprint, and increased power capabilities owing to their ultra-high breakdown field [2]. Ga2O3, capable of existing in various polymorphs such as α, β, γ, δ, and ε phases, boasts a bandgap ranging from 4.5 to 4.9 eV depending on crystallographic orientation [3]. κ-Ga2O3, notably, demonstrates a substantial polarization effect (23 µC/cm2), indicative of its potential to harbor an interface-localized two-dimensional electron gas (2DEG), making it a viable candidate for HEMTs [4, 5]. Through controlled alloying with group III metals, (MxGa1-x)2O3 presents a tunable framework for adjusting critical properties like bandgap and carrier concentrations, thereby tailoring its suitability for specific applications. The band gap range obtained by using Al and In is spanned out by In2O3 (Eg= 2.9 eV) [6] and Al2O3 (Eg= 8.8 eV) [3], and the values can be calculated by eq1: Eg = 4.91 + 2.10x for κ-(AlxGa1-x)2O3 and eq2: Eg = 4.90 – 1.95y for κ-(InyGa1-y)2O3.

Methods
In this work we have investigated (MxGa1-x)2O3 thin film heterostructures (M = Al, In) with various doping concentrations, thickness of layers, and substrate deposition, made using pulsed laser deposition (PLD) and vertical (quasi-) continuous composition spread (VCCS) PLD. The morphology, crystal structure, element composition and band gaps have been measured by transmission electron microscopy (TEM) for three types of samples. For the analysis, we have used a FEI Titan G2 operated at 60 kV with a monochromator, DCOR Cs probe corrector, Super-X EDS detector, and Gatan GIF Quantum 965 electron energy loss (EELS) Spectrometer. The analysis of the EELS spectrum images (SI) and individual spectra (such as the band gap fitting) are performed by our own python-based EELS fitting program combined with Gatan Digital Micrograph and Hyperspy.

Results
Scanning TEM (STEM) image of one type of sample is shown in Figure 1A. Where the top layer is (Al0.3Ga0.7)2O3, second layer is (In1.8Ga0.82)2O3, and the third layer Ga2O3, where the substrate is indium tin oxide (ITO). The band gap has been fitted for each spectrum in the spectrum image (SI) in Figure 1B. The average band gap for each row with the variations (in gray) is plotted in Figure 1C. The band gaps have been fitted to be 5.1 eV, 4.3 eV, and 4.8 eV, for (Al0.3Ga0.7)2O3, (In1.8Ga0.82)2O3, and Ga2O3 respectively. The band gap value for (Al0.3Ga0.7)2O3 and (In1.8Ga0.82)2O3is lower than what can be estimated theoretically, which is 5.54 eV and 4.45 eV respectively. We also observe a

decreasing band gap across the interfaces. Is this due to a decrease in band gap or because of the delocalization of the electron beam? The data has then been analyzed in detail and compared to the structural properties across the interface and compared with two other samples of (MxGa1-x)2O3 deposited on ZnO substrates with different compositions and thinner layer thicknesses.

Conclusion
The results have shown that the reduced band gap values are most likely due to strain in the layers and was not found in layers with high amounts of defects. The decrease in band gap across the interface is a real decrease in value, probably due to lattice mismatch-strain.

Keywords:

Band gap, Ga2O3, TEM

Reference:

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The fatigue response of the IN939 superalloy prepared by additive manufacturing

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Poster Group 2

Background incl. aims
Ni-based superalloys have become a time-honored high-temperature material. The first steps in their development were directed towards optimizing the manufacturing processes, chemical composition, and microstructure. The additive manufacturing (AM) technologies, also commonly called 3D printing, emerged and changed some classical approach. AM offers several advantages such as design freedom, very good efficiency of material usage, possibility to create hierarchical structures, crystallographic texture etc. Recently, the parameters of Laser Powder Bed Fusion (L-PBF) were optimized for the nickel based superalloy IN939. The main aim of the contribution is to address fatigue and thermomechanical fatigue (TMF) properties to the specific microstructure of the IN939 prepared by the L-PBF.

Methods
Microstructural analysis was performed by the mean of scanning and transmission electron microscopy. The Tescan Lyra 3 FEG/FIB scanning electron microscope equipped by the energy dispersive spectroscopy and backscattered electron diffraction detector (EBSD) was used the microstructural analysis, texture measurement and grain size evaluation. The Talos 200i transmission electron microscope equipped by the energy dispersive spectroscopy was adopted for the analysis of typical cell structure and strengthening particles.
The material was prepared by AM method. To achieve ideal microstructure, the IN939 was exposed to three-step heat treatment: solution annealing at 1175°C for 45 minutes followed by two step precipitation hardening 1000°C/6h + 800°C/4h.
The isothermal fatigue at 800 °C with symmetrical push-pull cycle was measure. The two modes of TMF, in-phase and out-of-phase loading were used for testing. During in-phase loading, the maximum mechanical loading corresponds to the maximum temperature, while in the out-of-phase mode, the maximum temperature corresponds to the minimum loading.

Results
Microstructural analysis revealed elongated grains with a preferential orientation <001> parallel to building. IN939 incorporates a considerable amount of a reinforced gamma prime phase that is embedded coherently within the matrix.
The fatigue properties at 800 °C and response to the TMF loading were measured. Obviously, the TMF is more detrimental than pure fatigue in very low cycle region, especially the in-phase TMF loading. The differences between particular loading modes decreases with decreasing strain amplitude. The EBSD measurement revealed no grain coarsening or changes in crystallographic texture. The fatigue cracks propagated predominantly along grain boundaries.

Conclusion
Following conclusion based on obtained results can be drawn :

- The typical AM microstructure consist of dislocation cells with crystalographic texture was revealed.
- The In-phase TMF mode seems to be most damaging in very high strain amplitude region, while the diffences in fatigue life in low strain amplitude region are almost negligible.
- The crack propagation path along grain boundaries was documented.

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Keywords:

IN939 superalloy, fatigue, additive manufacturing

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Revealing the vacancy ordering in Prussian blue analogues through serial electron diffraction

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Poster Group 2

The irregular arrangements of the vacancies, interstitials or impurities on the lattice sites in a crystal structure can cause diffuse scattering in diffraction patterns, causing deviation from Bragg diffraction spots. Such diffuse scattering features can be utilized and back-calculated to explore the arrangements of defects within the structure. The Prussian blue analogues (PBAs) are composed of two transition metals coordinated with cyanide ligands, resulting in a specific porous structure with diverse applications such as electrochemical catalysis, ion transportation, and gas absorption. Some researcher elucidated the vacancy networks in series of PBAs using single-crystal synchrotron diffraction. However, above mentioned techniques require large single-crystals (>500 um), which is not suitable for some groups of PBAs.
Here, contributed by high spatial-resolution, transmission electron microscope was applied. We focus on the vacancies ordering in Cu[Co(CN)6] PBAs nanoparticles, which is hard to collecting single crystal x-ray or synchrotron data. Additionally, the presence of cyanide ligands and zeolitic water inside structure makes PBAs can be easily damaged by electron beam within seconds, which hindering structural study using 3D electron diffraction. To address these challenges, the serial electron diffraction (serialED) was introduced to capture single-frame exposure electron diffraction for thousands of nanocrystals. Following by clustering and indexing, the diffuse scattering patterns were analysed from serval main zone-axis diffraction patterns. The diffuse scattering features from HK0 slice closely resembled those reported in the literature obtained by synchrotron methods, confirming the consistency and reliability of our method. The delta pair-distribution function also provides insights into the structural ordering in PBAs.

Keywords:

PBAs, electron diffraction, diffuse scattering

Reference:

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3.Quantitative three-dimensional local order analysis of nanomaterials through electron diffraction. EM Schmidt, PB Klar, Y Krysiak, P Svora, AL Goodwin… - Nature Communications, 2023

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Characterization of 2DEG on WG semiconductors through sub-sampled 4DSTEM

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Poster Group 2

Wide-bandgap (WBG) materials such as gallium nitride (GaN) are better candidates for fabricating power switches and high-frequency transistors than conventional Silicon-based technology. At the interfaces of a high mobility transistor heterojunction (HEMTs) such as that between AlGaN/GaN, in addition to the formation of a strained zone which increases the electronic mobility of the charges located there, a strong polarization effect is created as well. Such polarization causes band bending, including the Fermi level, at the AlGaN/GaN interface resulting in the accumulation of electric charges and the formation of a very electron-rich two-dimensional gas (2DEG). 2DEG contributes to the outstanding performance of AlGaN/GaN based HEMTs and can achieve a sheet carrier concentration close to the interface, well in excess of those observed in other III – V material systems.[1]. The ability to reveal the 2DEG in real space by TEM's conventional techniques would be desirable, but up to now has been very challenging to accomplish due to an unavoidable strong diffraction contrast formation at the heterointerfaces. [2]. Recently a new microscopy technique called 4D scanning transmission electron microscopy (4D-STEM) is gaining momentum for probing materials at sub-Angstrom resolution with the full electron−atom scattering interactions recorded in a convergent beam electron diffraction pattern (CBED) [3]. The 4DSTEM has been demonstrated that sub-Å measurements of electric fields and electrostatic potentials in materials is feasible [4].

In this work we studied a AlGaN/GaN based device where the 2DEG has been kept on and off in two different regions of the device, the latter obtained by means a p-GaN structure. In our opinion, this is the best system to test this technique to directly visualize the 2DEG in real space. The acquisition of a full set of CBED on the entire field of view could be very time and data consuming. For this reason, in this study, we acquired a sub-sampled 4DSTEM dataset by sampling only the 10% of the entire field of views [5]. As for the detector we used a QD MerlinEM Direct Electron Detector (DED) 256x256 and with 2x12bit count depth able to work as fast as 2000fps. The experiments have been carried out on a probe corrected ARM200F operated at 200keV. Data analysis was performed by center of mass (CoM) field shift data analysis method.

The 2DEG has been revealed in the regions in the proximity of the interface between GaN/AlGaN and in both regions. We discovered that where we expect 2DEG to be off it appears still visible instead but shifted far away by the interface due probably to the counter field of the p-GaN located at the opposite interface.

In this work we have shown that 4DSTEM through its analysis methods, such as CoM, can show the exact location of 2DEG as a function of interface electric fields.

The localization of 2DEG in the real space is essential for understanding its properties and to improve device designs aimed at optimizing both properties and miniaturization.

Keywords:

WBS, HEMT, 4DSTEM, sub-sampling

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Scanning precession electron diffraction tilt series for orientation analysis

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

Identifying the orientation of crystalline phases at the nanometer scale is relevant for understanding materials properties. Here we will demonstrate that collecting and processing scanning precession electron diffraction (SPED) datasets collected at a few different specimen tilts can improve the accuracy of template-based orientation mapping [1,2]. In addition, the tilt series allows complete determination of the relation between the specimen crystallographic setting and the goniometer axes. This insight, combined with the orientation map, can be used in a convenient semi-automatic approach to predict the tilts required to reach a target specimen orientation for further structure analysis.
SPED of different polycrystalline systems (Si, Ag, and oxides) were recorded in a JEOL JEM2100F with a NanoMegas DigiStar precession system and a Quantum Detectors MerlinEM direct electron detector. Scans were taken over areas up to 15 x 15 µm containing 10's of grains using a nominal precession angle of 1˚. Tilts series using one or two axes contained 3-5 tilts in the range 0 - 20˚ from the initial flat specimen position. For data analysis and visualization, we used primarily the open-source python library pyxem [3].
The indexed frames, taken at different tilts, were compared, after manually aligning the frames, using the set tilt as expected misorientation. Together with considering as well the best 5 to 25 normalized cross correlations between the experimental patterns and the simulated pattern bank, orientation-dependent misindexations can be identified and the orientation estimate refined. Compared to the standard approach of collecting SPED using only a single specimen tilt and the best correlation scores for each pattern, the tilt series approach reduces indexation variations within grains. This gives a more uniform representation of the grains in the final orientation maps.
The accuracy of the refined orientation analysis can be determined with a known orientation relation, here for example Σ3 twins in face-centered cubic and diamond crystal systems. The misorientation deviation between the measured and expected misorientation between twin domains is used as the metric [4]. The found accuracy is below the used precession angle.
The refined orientation mapping based on a small tilt series has a further practical use. From a single axis tilt series, the position of the two perpendicular tilt axes can be determined. As the tilt series is small and over a limited angular range, sufficient probe positions must be used to accurately determine the tilt axis position. Misaligned between frames and areas with overlap, such as grain boundary areas, were excluded through thresholding. A second tilt axis series or grains correctly indexed in different frames can be used to verify the found axes positions. Using the determined orientation of a grain together with the deduced axes positions, the tilts to reach a target zone for a grain can be predicted. Based on tests on different TEMs and holders, the target zone was within 2˚. In the tests the specimen was placed at approximately the same rotation relative to the holder axes. However, should the specimen be differently placed compared to where the orientation was mapped

out, an additional transformation matrix can be included in the navigation tool to recalculate the target tilts for the given specimen placing. This correction is based on a manual estimation of misorientation from the tilts to the actual target zone, diffraction (using the Laue circle), or imaging (assuming in-plane rotation).
To conclude, template matching based on multiple SPED scans at a few varying specimen tilts improves the accuracy and the final orientation visualization. In addition, the approach is used to make a practical navigator tool that widens to use of template matching results for subsequent lattice imaging and further crystallographic analysis. With the advancements in automatic scan controls, faster detectors and optimized transparent open-source routines, the benefits gained will more than compensate for the drawbacks of acquiring and processing multiple scans.

Keywords:

Texture, diffraction, open-source, template-matching, ACOM

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Deep Learning assisted X-ray Microscopy Characterization of Nickel based Metal Matrix composite reinforced with TiC

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Poster Group 1

Background
Metal Matrix Composite (MMC) are a class of materials with remarkable mechanical, thermal, and electrical properties. These composites consist of a metallic matrix reinforced with ceramic or other non-metallic phases. Reinforced MMC are of interest in many applications due to their multifunctionality, which yields combinations of properties such as high specific strength, stiffness and toughness, and a low coefficient of thermal expansion [1]. Ni-Ti-C based metal matrix composites explored in this paper is produced using the laser engineered net shaping (LENS) process presenting a unique hierarchical microstructure consisting of an in situ formed and homogeneously distributed titanium carbide (TiC) phase reinforcing the nickel matrix [2]. Additionally, by tailoring the Ti/C ratio in these composites, an additional graphitic phase can also be engineered into the microstructure. The resulting three-phase Ni-TiC-C composites were comprehensively characterized using Deep learning assisted X-ray Microscopy (XRM). By analyzing the microstructure at the sub-micron level, XRM enables the understanding of the spatial distribution and alignment of reinforcing phases within the metal matrix.
Method
Ni-Ti-C-based MMCs were fabricated using the laser engineered net shaping (LENS) process. The LENS process involves the deposition of metal powder layers using a laser beam, allowing precise control over the composition and microstructure. XRM, with its high-resolution and non-destructive capabilities, provides valuable insights into MMCs. Notably, certain phases within the titanium carbide (TiC) domains push the limits of X-ray microscopy detection, relying on X-ray-to-visible-light conversion via scintillators coupled with optical magnification objectives. To overcome previous limitations, a unique combination of specially designed high-resolution objectives and deep-learning-based 3D reconstruction was utilized. The combination of new generation scintillator technology and integrated deep-learning reconstruction known as DeepRecon [3] enable sub-micron resolutions in dense and large MMC sample.
Results
Ni-Ti-C based MMC exhibits intricate microstructures that span a wide range of length scales, with various phases playing a critical role in determining its exceptional properties. Leveraging the high-resolution and non-destructive capabilities of X-ray microscopy the microstructure of the MMC is studied and analyzed in 3D at different length scales. The X-ray microscopy images reveal the spatial arrangement of TiC phases within the metal matrix. Features of <.0.1µm were previously undetectable in large and dense samples due to their small size and due to the noise produced in a tomography scan from these samples were observed using the DeepRecon – Deep Learning based reconstruction algorithm. The findings from XRM data significantly enhance the understanding of MMCs.
Conclusion
These composites hold promise for various engineering applications, including aerospace, automotive, and structural components. The study of Ni-Ti-C-based MMCs not only provides insights

into the relationship between microstructure and properties but also set forth a vital tool and technology needed to understand these intricate structures. X-ray microscopy emerges as a powerful tool for MMC analysis, offering high resolution and non-destructive capabilities. Its application enhances the comprehension of MMC performance, thus opening new avenues for their optimized design and engineering.

Keywords:

Metal Matrix Composites, X-ray Microscopes

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Cryo-FIB as a preparation tool for soft X-Ray Tomography: Analysis beyond EM

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Poster Group 1

Background incl. aims
Focused Ion Beam Scanning Electron Microscopes (FIB-SEM) used at Cryogenic (cryo) conditions have been used as a preparation tool for soft-matter samples since 2006 with a recent rapid expansion of their use in the case of the 'on-grid' thinning approach for cryo-electron tomography (cryo-ET). Less well developed is the site-specific cryo-FIB preparation of samples that cannot be grown (or deposited) and thinned on a TEM grid, such as tissue or other soft matter samples e.g. battery materials. Site-specific FIB preparation followed by extraction (lift-out) is a route to achieving the thickness requirements of the TEM prior to transfer. By adapting this approach to meet the dimensions and requirements of other analytical equipment it is possible to expand beyond the limit of electron microscopy.
Methods
This work utilised a Crossbeam 550 FIB-SEM (Carl Zeiss) equipped with a Quorum 3010 (Quorum Technologies) cryo-system. The micromanipulator was an Omniprobe 200-cryo (Oxford Instruments). Samples were frozen by slushy nitrogen, metal mirror (MM) freezing or high pressure freezing (HPF) before transfer to the cryo-system. Prior to FIB-SEM the samples were platinum sputter coated in the preparation chamber and subsequently by the platinum organometallic precursor of the gas injection system (GIS) of the FIB. Gallium FIB preparation was performed at 30kV accelerating voltage currents of between 15nA (initial larger volume milling) and 50 pA (final polishing of samples). SEM of the samples was performed at 2-5 kV. Cryo-redeposition milling was used to attach samples to the micromanipulator and support substrates.
Results
Building on pre-existing protocols for the preparation of TEM compliant samples (lamellae) we increased the dimensions of the volumes prepared to meet the requirements of a soft-x-ray tomography (sRT) microscope which to approx. 45µm and a thickness of 5µm which we term 'slabs' (fig 1a). The sXT can penetrate a sample up to 10 µm thick however, we accounted for the sample needing to be tilted in the X-Ray beam (+/- 55°). These slabs were transferred to specially modified copper support structures (fig 1 b-d).

Fig 1. (A) Schematic showing the relative dimensions of the lamella and slab, the tip of a micromanipulator is included for scale. (B) FIB image showing the micromanipulator with attached slab during slab extraction. (C&D) Micrographs of the slabs following deposition onto the support structure and subsequent polishing using the FIB. Scale bars 10 µm (B&C) 4 µm (D).

Conclusion
Cryogenic FIB preparation combined with lift-out using a cryogenically cooled in situ micromanipulator is demonstrated as a viable preparation tool for the preparation of soft-matter sample (~45x5 µm) slabs for sXT. We have presented recent efforts to use Cryogenic Focused Ion Beam Scanning Electron Microscopes (cryo-FIB-SEM) as a preparation tool for cryogenic analysis

beyond the FIB itself, embracing emerging themes in multi-length-scale analysis and correlative microscopy.

Keywords:

Cryo-FIB, soft-X-Ray Tomography, correlative microscopy

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A new protocol for fluorescent quantitative labeling of individual proteins for live cell internalization assays

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Poster Group 1

Background
A single protein can be chemically labeled with different fluorescent dyes in order to get insights about its cellular localization and molecular mechanism of action [1]. Overall, an ideal fluorescent dye should be specific and stable, and should not interfere with the physicochemical and conformational properties of the protein. For internalization assays in living cells, organic fluorophores such as fluorescein (FITC) and rhodamine (TRITC) are the dyes of choice since they have a short lifetime (<5 ns), and they are brighter and more photostable than other fluorescent labels [2]. They also have a small size, thus interfering less with the biological system (i.e. protein). The main disadvantages of these compounds are their low specificity and unknown binding stoichiometry. In this work, we have tailored a minimal labeling protocol, originally developed for proteomic applications, in order to tag single proteins for being used in live cell internalization assays.

Methods
The original 2D-DIGE minimal labeling protocol ensures that only 1–2% of the available Lys is labeled. Thus, this protocol facilitates successful labeling of an individual protein at a specific location (ε amino group of Lys) and with a well-defined stoichiometry (1:1). Moreover, this modification only adds just about 500 Da to the protein total molecular weight, thus minimizing perturbations of the physicochemical and conformational properties of the protein. Overall, the full standard protocol is accomplished in 4-5 hours and comprises three different steps (Figure 1). Firstly, the protein of interest is resuspended in the reconstitution buffer and labeled with the CyDye fluorophore of choice. After incubation, labeling is stopped by adding an excess of L-lysine (Lys) to the reaction mixture. Secondly, the excess of free Lys-bound CyDye is removed to minimize background in the internalization experiments. At this point, the Cy-labeled protein can be concentrated if necessary, by filtration through a size exclusion membrane of suitable cutoff. Finally, in the last step, the quality of the labeling reaction is tested by SDS-PAGE.

Results
To validate experimentally our protocol, we carried out the fluorescent labeling of IBB1, a major soybean protease isoinhibitor of the Bowman-Birk family that is currently being investigated as colorectal chemopreventive agent [3], with two different CyDye fluors. A single protein band of about 12 kDa was visible after scanning the polyacrylamide gel at 488 nm (IBB1-Cy2) or 635 nm (IBB1-Cy5), respectively, proving the success of the minimal labeling reaction. Then, we monitored the in vivo internalization dynamics of the labeled IBB1 protein in human colorectal adenocarcinoma HT29 cells. We observed that the CyDye-labeled IBB1 proteins crossed the plasma membrane of HT29 cells after a few minutes and was gradually accumulated, forming fluorescent patches randomly distributed across the cytoplasm. We also tested the suitability of this method for carrying out multiplex

experiments. Thus, two different combinations of labels were tested. Firstly, IBB1-Cy2 internalization was combined with nuclei staining using Hoechst 33342 dye. The fluorescent signal of IBB1 did not overlap with Hoechst-specific labeling, indicating that the protein did not internalize into the cell nucleus. Secondly, IBB1-Cy2 internalization was combined with the fluorescent probe FM 4-64, an endocytic tracer [4]. We observed that the fluorescent signal from the endosome marker colocalized with the Cy2-labeled IBB1 protein in the cytoplasm of HT29 cells, suggesting that IBB1 is internalized through one of the existing endocytosis pathways.

Conclusions
In this work, we have adapted a protocol that was originally developed for two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) applications, to label proteins with CyDye fluors for single-molecule internalization assays in living cells. This protocol is suitable for both proteins purified from their natural sources and recombinant proteins expressed in heterologous systems (e.g. E. coli, P. pastoris, etc.). This “minimal labeling” method offers a number of advantages including specificity and known stoichiometry, simplicity, high reproducibility, and sensitivity and allows multiplexing while minimizing perturbations of the biological system. Moreover, the minimal labeling protocol is suitable for multiplex experiments, either combining up to three different proteins labeled with Cy2, Cy3, and Cy5, respectively, or using CyDye fluors together with other fluorescent labels. Finally, since only a single lysine (Lys) residue per protein molecule is labeled, this method is also quantitative.

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Keywords:

CyDye-fluors, IBB1, internalization-assays, minimal fluorescent-labeling

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New protein fluorescent labeling methods for carbonylation and S-acylation studies in plants

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Poster Group 1

Background
Pollen, the male gametophyte, plays a pivotal role in plant reproduction, being essential for successful fertilization and seed production in Angiosperms. Thus, understanding its biology is crucial for ensuring optimal crop yields. Carbonylation is an irreversible post-translational modification (PTM) characterized by the non-enzymatic addition of carbonyl groups to amino acid residues, leading to protein malfunction and cell death. Carbonylation leads to disruption of protein structure and function. On the other hand, S-acylation or palmitoylation is another PTM that involves the reversible attachment of palmitic acid to specific Cys residues of proteins by the action of protein S-acyltransferases (PATs). This PTM plays crucial roles response to biotic and abiotic stresses, hormonal signaling, cell polarization and expansion, and cytoskeleton organization [1].
Protein labeling techniques for in vivo studies have garnered considerable attention, with cycloaddition reactions emerging as promising methods due to their compatibility with diverse conditions. However, the cytotoxic effects of copper catalysts have limited their widespread application [2]. This study aimed to develop innovative protein labeling methods for assessing protein carbonylation and S-acylation in plant tissues.

Methods
For S-acylation studies, we developed a new method for incorporating fluorescence-labeled palmitic acid into proteins using an azide-alkyne cycloaddition reaction. This method was experimentally validated using an in vitro culture system for pollen. In a first approach, the synthetic lipid containing the azide functional group was added to the culture medium, either in the presence or not of a chemical PAT inhibitor [3], being incorporated to proteins by the enzymatic machinery of germinating pollen grains. Then, pollen was fixed and the fluorescent labeling reaction was performed and visualized by fluorescence microscopy. Alternatively, the fluorescent labeling was carried out exogenously. Then, copper was removed using size exclusion techniques, thus eliminating its cytotoxic effect. Finally, pollen was germinated in the presence of the palmitol-fluorochrome conjugate and the dynamics of the S-acylome was monitored by using time-lapse fluorescence microscopy.
For detecting carbonylated proteins, we developed two different methods. First, we carried out derivatization of carbonyl groups by DNPH (2,4-dinitrophenylhydrazine) [4] to hydrazone groups, which were further detected using an Alexa Fluor 488-conjugated antibody. Alternatively, we used the fluorescent probe BzCH (7-hydrazinyl-4-methyl-2H-1-benzopyran-2-one,), which is capable of binding specifically to carbonyl groups [5]. These two methods were further experimentally validated as above in the presence or absence of the ROS inducer Paraquat in the culture medium. Moreover, this method also allowed to quantify protein carbonylation by conducting derivatization assays followed by image processing and fluorescence quantification.

Results
Both fluorescent labeling approaches revealed the dynamics of S-acylated proteins in growing pollen tubes, showing a similar distribution pattern of S-acylated proteins. Thus, a high fluorescent signal was observed in the cytoplasm of the vegetative cell and, to a lesser extent, in the cytoplasm of the pollen tube. Fluorescence significantly decreased in the presence of the PAT inhibitor, suggesting that was mostly due to the activity of PAT enzymes. Moreover, the exogenous labeling of the synthetic lipid allowed the real-time monitoring of the S-acylome in a single growing pollen tube by using time-lapse microscopy.
On the other hand, carbonylated proteins were found to accumulate in the cytoplasm of the vegetative cell of non-germinated pollen grains, suggesting that this PTM may be a marker of loss of pollen viability. In addition, we observed a consistent accumulation pattern of carbonylated proteins at the apical region of the pollen tube. Interestingly, protein carbonylation levels significantly increased in the presence of Paraquat in the germination medium, suggesting that ROS-mediated oxidative stress may triggers carbonylation, leading to pollen tube growth arrest.

Conclusions
In this work we have developed a novel and reliable fluorescent labeling method to study S-acylation of plant proteins, overcoming limitations associated with cytotoxic catalysts during cycloaddition reactions. Moreover, we have set up two methods to localize carbonylated proteins in plant tissues by using fluorescent probes.

This research was funded by projects PID2020-113324GB-100 and TED2021-130015B-C22 from MICIIN/AEI, both of them cofinanced by the ERDF program of the European Union. Salvador Priego and Andrea Román were granted with a PhD fellowship from Junta de Andalucía and MICINN, respectively.

Keywords:

carbonylation, cycloaddition, pollen, S-acylation, fluorescent-probes

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Supersilent AlCoFeNiCux (x = 0.6 – 3.0) high-entropy alloys

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PS-02 (3), Lecture Theater 4, august 30, 2024, 14:00 - 16:00

BACKGROUND INCL. AIMS
In the search for the materials that possess a combination of excellent magnetic softness and vanishing magnetostriction, the ferromagnetic high-entropy alloy (HEA) system AlCoFeNiCux (x = 0.6–3.0) was investigated.

HEAs are expected to find applications as supersilent (inaudible to a human ear) materials for transformers, magnetocaloric coolers, and other “humming” electromagnetic machinery.

METHODS
The AlCoFeNiCux HEAs with the Cu content x = 0.6–3.0 were prepared from high purity elements (>99.99 wt.%) by arc melting under an argon atmosphere. Altogether, there were seven samples fabricated.

An evolution of the crystal structure and chemical composition was observed with increasing Cu content by XRD and SEM EDS characterization. The phases constitutions and their domain sizes were characterized by correlative SEM and TEM techniques, as HEAs usually have highly complex structures on different spatial scales that contribute to specific physical properties such as magnetic softness and magnetostriction.

The magnetic properties were investigated by a Quantum Design Magnetic Property Measurement System MPMS3 magnetometer. The magnetometer uses a superconducting magnet with variable magnetic field of ± 7 tesla and the temperature range of the measurements is from 1.8 K to 400 K.

Electrical resistivity was measured by a Quantum Design Physical Property Measurement System PPMS 9T by. The apparatus uses a superconducting magnet with a variable magnetic field of ±9 tesla and the temperature range of the measurements is between 1.9 K and 400 K.

RESULTS
XRD revealed the evolution of the samples’ crystal structure. At low Cu contents, x = 0.6 and 0.8, the predominant phase is bcc (≈85 wt.%) with the unit cell parameter a = 2.869 Å, and the second phase is fcc (≈15 wt.%) with a = 3.612 Å. On increasing the Cu content, the fraction of the fcc phase increases, while the bcc phase fraction decreases. Detailed observation of the fcc-phase diffraction peaks reveals that there are actually two fcc-phases with slightly different unit cell sizes, denoted as fcc(L)-large and fcc(S)-small.
For the two highest Cu contents (x = 2.5 and 3.0), the bcc phase is no longer visible in the XRD patterns and the structure is two-phase, composed of the fcc(L) and fcc(S) phases.

The development of the microstructure of all alloys was made visual by SEM BSE imaging. All alloys show inhomogeneous microstructure, where the brighter parts are enriched in the heaviest element Cu.

The TEM analysis of the AlCoFeNiCu2.5 alloy reveals that the three constituent phases are nanostructured on the 10 nm scale due to fine dispersion of the Guinier-Preston (GP) zones that are enriched in Cu. The GP zones were observed in all three phases except at the locations of the highest Cu enrichment. The concentrations of the chemical elements also show nanometer-scale variations. It is straightforward to anticipate this result to the entire series of the investigated AlCoFeNiCux (x = 0.6–3.0) HEAs, so that the alloys can be considered as nanostructured composite materials with a multiphase (up to three phases) microstructure.

Magnetic softness characterization was performed by determining the magnetization versus the magnetic field, M(H), hysteresis loops in the magnetic field sweep $\mu_0 H = \pm 7$ T. Rapid change of the M(H) curves in the close vicinity of $H = 0$ and small coercive fields Hc are evident for all samples, revealing magnetic softness.

Magnetostriction experiments were performed at RT in a static magnetic field range $-0.2\ \text{T} < \mu_0 H < 0.2\ \text{T}$ by measuring the samples' elongation (or contraction) with a strain gage. The magnetostriction curves $\lambda(H)$ at RT show the largest (positive) value $\lambda_s = 21\ \mu\text{m m}^{-1}$ for the lowest Cu content x = 0.6. The positive saturation magnetostriction decreases with increasing x and vanishes ($\lambda_s = 0$) at x = 2.0. For the two alloys with the highest Cu contents, x = 2.5 and 3.0, λ_s remains very close to zero. In practical terms, all AlCoFeNiCux alloys in the Cu content range $2.0 \le x \le 3.0$ can be considered as vanishing-magnetostriction HEAs, so that the precise Cu concentration within this range is unimportant.

Superior parameters were obtained for the composition AlCoFeNiCu2.0, which shows precisely zero magnetostriction, $\lambda_s = 0$, reasonably low coercivity $H_c \approx 650\ \text{A m}^{-1}$ and substantial saturation magnetic polarization of $J_s \approx 0.55$ T, which is about the same as that of the mumetal, a widely used commercial soft magnet.

CONCLUSIONS

Magnetic softness and vanishing magnetostriction originate from the choice of constituent elements (magnetic Co, Fe, and Ni and nonmagnetic Al and Cu) and the multiphase composite microstructure is nanostructured on the 10 nm scale.

Magnetic softness originates from the nanostructured character of the alloys that are random mixtures of the ferromagnetic Al–Co–Fe–Ni domains and nonmagnetic or weakly magnetic Cu-rich domains on the 10 nm scale, which makes the mechanism of exchange-averaging of magnetic anisotropy efficient.

Since vanishing magnetostriction is a prerequisite for the supersilence of the material in audio-frequency AC applications, such HEAs are expected to find application as supersilent magnetically soft materials in transformers, electromotors, generators, magnetocaloric coolers, and other "humming" electromagnetic machinery.

We found that the alloys in the high Cu content range x = 2.0–3.0 indeed possess the targeted combination.

Keywords:

High-entropy alloys, magnetostriction, supersilence

Reference:

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Development and characterization of a laser-driven cold-field emission source

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IM-08, Lecture Theater 2, august 28, 2024, 10:30 - 12:30

Background
Ultrafast transmission electron microscopy (UTEM) utilizing pulsed electron sources, has emerged as a crucial tool for probing ultrafast dynamics at the nanoscale. Recent breakthroughs in laser-driven Schottky electron emitters have significantly enhanced the spatial and temporal coherence of electron pulses, achieving temporal pulse widths below 200 fs and electron spot sizes as small as 1 nm [1]. The potential for further enhancement in pulse coherence is anticipated through the application of laser-driven cold-field emitters, especially when operated in a linear photoemission regime [2].
Methods
Here, we present the development and characterization of a long-term-stable laser-driven cold-field emitter source, designed for advanced ultrafast transmission electron microscopes and operating at 200 keV electron energy [3]. Based on a JEOL cold-field emitter gun (CFEG), the source utilizes a sharp single-crystalline (310) tungsten tip within a vacuum level of 10^-12 to 10^-11 mbar. For pulsed operation, the source is modified to allow for a direct optical line-of-sight onto the emitter through opposing optical entrance windows.
Results
In the initial implementations, we conducted a comprehensive characterization of the photoemission yield from the tip using a continuous-wave optical illumination at 3.59 and 3.06 eV photon energy focused to a 20 µm scale spot on the emitter apex (Fig. 1c, right inset). Notably, the employed photon energy is below the work function of tungsten so that linear photoemission is only feasible at the apex of the tip which exhibits a Schottky-reduced effective workfunction. Continuous field emission, following a Fowler-Nordheim description, is observed at large electric extraction fields without illumination (blue curve in Fig. 1b). Illumination at 1 mW optical power reveals an additional photoelectron contribution at low extraction field values, for which continuous field emission is absent (yellow and red curves in Fig. 1b). The onset of photocurrents is dependent on the photon energy of the illuminating light field. Above this threshold the current scales linearly with light intensity (Fig. 1c). Notably, under extreme high vacuum (XHV) conditions near the emitter, no significant drop in photoemission yield is observed over a 10-hour period (Fig. 1c, left inset).
In a subsequent step, the emitter is operated as part of the Regensburg JEOL F200 UTEM (Fig. 1a), using ultrashort laser pulses with a duration of 200 fs at a central wavelength of 345 nm. We systematically carried out an extensive pulse characterization, including the pulsed electron energy distribution, pulse duration, and the spatial attributes of electron brightness across varied excitation fields.
When operated at a state in which both, field- and photoemission can occur, the final kinetic energy of electron generate by the two emission processes are needly separated (Fig. 1d). Performing an electron-photon cross correlation we obtain 300 fs-electron pulse duration and energy width of about 300 meV.

Remarkably, our findings reveal that focal sizes of below 0.74 nm and a normalized peak brightness of 10^14 A/m²sr (Fig. 1e) can be achieved, surpassing the brightness of the laser-driven Schottky field emitter constructed in our lab by an order of magnitude. Currently, small electron focal spot sizes are limited to a low current-regime, due to the onset of spherical aberration at larger convergence angles. It should be possible to further improve the transversal beam properties at higher pulse charges utilizing a probe aberration corrector.

Conclusion

In conclusion, we demonstrate the successful development of a stable, laser-driven cold-field emitter source for ultrafast transmission electron microscopy applications. Our findings illustrate the potential to significantly enhance electron beam brightness and reduce energy spread of electron pulses, paving the way for further advancements in probing ultrafast dynamics at the nanoscale.

Keywords:

UTEM
Electron-pulse
Laser-driven
Cold-field-Emitter

Reference:

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[2] F. Houdellier et al., Ultramicroscopy 186, 128 (2018).
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Microchannel Plate-based Detector with High Pressure Operation up to 1 Pa for Scanning Electron Microscopy

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Poster Group 1

Background incl. aims
Microchannel Plates (MCP) are electron multipliers with a secondary electron amplifier mechanism. MCP detectors have many advantages such as small size, high temporal resolution, high gain, and very high detection efficiency for electron energies of the order of tens to hundreds of electron volts in scanning electron microscopy (SEM). Recently, almost all commercially available SEMs are operated in low vacuum conditions caused by flowing neutralization gas into the specimen chamber to suppress charging on the sample surface. However, MCPs cannot be utilized in low vacuum conditions because of ion feedback.
Ion feedback, a peculiar phenomenon of secondary electron multipliers, can cause discharges and degradation of signal-to-noise ratio. Ions generated by electron ionization are converted into electrons and multiplied by the secondary multiplier. The generation of ion feedback cannot be suppressed under low vacuum due to the presence of neutralization gas in SEM.
MCPs are powerful to detect secondary and backscattered electrons in SEM, but the challenge is stable operation under low vacuum conditions. Therefore, we have developed a novel detector structure that maintains the advantages of MCPs even under low vacuum conditions. In particular, its specific structure has proven to reduce ion feedback . Instead of using a bi-planar structure, as is the case in conventional MCP detectors, a triode structure is adopted. When a novel electric field configuration is applied to the electrodes, the triode structure can effectively suppress ion feedback even under low vacuum conditions.

Methods
The mechanism of ion feedback was investigated, when residual gas ions are generated by reactions between the residual gas and secondary electrons from the MCP channels. The ions (ionized residual gases) are accelerated by the electric field towards the MCP. If they can gain enough energy to release secondary electrons as they impinge on the channel walls, a second electron avalanche will be initiated. These “false” after-pulses would not only disturb the measurement, but could also ultimately lead to a permanent glow discharge. After-pulses appear several tens of nanoseconds after the "original" output signal, occur repeatedly and sometimes become even larger compared to the original signal. Considering the collision probability of electrons and gas molecules, ion feedback is likely to disturb the output signal, when MCPs are operated at a higher gain, or when increasing the input signal to the MCP.
It is found that residual gas ions causing ion feedback occur between MCP and anode, rather than inside the MCP channels. We concluded that controlling ions is more important than suppressing ion generation. Therefore, a triode-type detector structure was developed, in which a mesh anode was placed between MCPs and a dynode. It is confirmed that the dynode prevents ion feedback by capturing residual gas ions.

Results

First, we examined the effect of the triode structure for operation under low vacuum conditions by using a first prototype detector, which was composed of chevron stack MCPs (dimeter of 14.5 mm), a mesh anode and a metal dynode, in a vacuum chamber. While adjusting the degree of vacuum in the chamber, the detector was operated in two voltage modes: standard potential mode, i.e., MCP-in < MCP-out < anode = dynode, and novel potential mode, i.e., MCP- in < dynode < MCP-out < anode. As a result, when the gain of detector is low, there is no difference between the two potential modes in high pressure operation up to 1 Pa with or without input signals. When the gain becomes higher (105), ion feedback occurs at 10-3 Pa in standard potential mode, even without input signals, but does not occur in novel potential mode up to 1 Pa. Hence, it is confirmed that MCP detectors are capable of high-pressure operation by adapting the triode structure with novel potential mode.
The second prototype detector is under development with a very compact size to optimize it for SEM. The detector can be simplified by setting MCP-in and dynode at the same potential, and it fits into confined space by having an outer diameter of 35 mm, a height of 3 mm and a center hole diameter of 6mm. Our target specification of the detector is to maintain a gain of 5×106 up to 1 Pa without generating ion feedback signal.

Keywords:

MCP, triode detector, low vacuum

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Solving the crystallographic phase problem by linearizing dynamical electron diffraction

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

Background and aims
In contrast to kinematical diffraction intensities, which are insensitive to the phases of structure factors, dynamical diffraction intensities, as observed in most electron diffraction patterns, encode sums of phases of structure factor triplets and higher order multiplets, i.e. sets of structure factors for which the sum of the corresponding reciprocal lattice vectors vanishes. The scattering matrix S(kt)n,m = [exp(iTA(kt)]n,m, which is given by the matrix exponential of the product of the wavelength-scaled specimen thickness T and the structure factor matrix A(kt), the diagonal of which depends on the incident beam tilt kt, can be expanded to a sum of monomials in the structure factors, as shown in eqn. (1) [1] with the C-coefficients given by expression (2) and examples by expressions (4). Having diffraction data for multiple angles of incidence for a tilt range of several tens of mrad along kx and ky [2] allows the T- and kt-dependent C-coefficients (eqns. (3) & (4)) to be different enough, so that the individual monomials can be determined. For thicker specimen, the expansion order q would be very high, so a stacking of layers with a limited expansion order is proposed.

Methods
Large-angle rocking-beam electron diffraction (LARBED) [2] is a technique which can be used to acquire 2D rocking curve information from nearly arbitrarily small areas of the specimen for beam tilt angles of up to +/- 100 mrad in both tilt dimensions, and more, if the specimen is tilted as well. The diffracted intensities for every beam tilt are then extracted from these 4D datasets and the corresponding coefficients computed according to expression (2) [3]. In order to limit the number of terms in the linear set of equations, only coefficients of significant amplitude should then be kept. An alternative approach which also works for thicker specimen, is to consider the crystalline sample to consist of many identical layers that are stacked on top of each other, use a very limited expansion order (q = 1 is sufficient – in that case we call the approximation first-order expansion stacked Bloch wave (FOESBW) see eqn. (4)), but consider the structure factors in each layer to be independent from one another. This makes the complex scattering amplitude then a linear function in the structure factors, and a solution may then be found by solving this system of equations under the constraint that the structure factors in all of the layers are as close to each other as possible.

Results
Figure (a) shows a simulated LARBED pattern of GaN (110) (thickness 10 nm, acc. voltage 200 kV, max. g-vector 20 1/nm, as indicated by the white dash-dotted circle) on a logarithmic scale. The green dashed circle indicates the tilt range of the illumination during the LARBED simulation. Fig. (b) shows the central part of the diffraction pattern on a linear scale. The geometry of the representation of the LARBED dataset is illustrated by highlighting that the scale of the LARBED discs is 10 times larger than that of the distance between discs (descan = 0.9 [2]). Fig. (c) shows the structure of GaN in the (110) zone axis (1 x 2 unit cells are being displayed). Fig. (d) shows the original

crystal potential simulated using independent atom scattering factors. Fig. (e) shows the crystal potential resulting from all 194 structure factors that have been reconstructed using the FOESBW approach. Fig. (f) shows a bandwidth-limited potential resulting from a set of structure factors that was refined from the FOESBW-reconstruction using a simplex optimization of the full scattering matrix. The potential has been bandwidth limited to the resolution of the diffraction data (20 1/nm). All three potential maps are displayed using the same color map and -range. While these simulated results have also been presented in [3], we will also demonstrate the application to experimental data of various structures acquired using the Nion HERMES equipped with a Dectris ELA detector [4].

Keywords:

electron diffraction, crystallographic phase problem

Reference:

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Structural and spectroscopic studies of Cu-doped NiO thin films

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Poster Group 2

Background
Nickel oxide (NiO) is a wide-band gap p-type oxide semiconductor with good optical transparency and high chemical stability [1, 2]. These properties have been exploited for applications in photovoltaics and light-emitting diodes as a hole-transporting layer [1, 2]. Doping NiO with other metal elements, such as Cu, is an effective way to adjust its grain size, crystallinity, transmittance, and ultimately its conductivity for wider use in optoelectronic devices [2-4]. Polycrystalline Cu-doped NiO films have been fabricated with different techniques using mainly glass substrates. However, studies on single-crystal thin films are lacking, even though they are fundamental to understanding the interplay between structure and functionality of doped NiO films. In this work, we have employed electron microscopy methods in conjunction with photon spectroscopies to gain insight into the role of Cu concentration on the structural, optical, and electronic properties of NiO films grown by Molecular Beam Epitaxy (MBE) as a function of doping/alloying concentrations.

Methods
A series of Ni1-xCuxO thin films with five different Cu nominal concentrations (x = 0, 3, 6, 10, 24, and 42 at.%) were grown on (001)-oriented MgO substrates by MBE. The structural, optical, and electronic properties of the grown films were characterised by in-situ reflection high-energy electron diffraction (RHEED), X-ray diffraction (XRD), ultraviolet-visible spectroscopy (UV-Vis), energy dispersive x-ray spectroscopy (EDX), X-ray photoemission spectra (XPS), atomic force microscopy (AFM), scanning transmission electron microscopy (STEM), electron energy loss spectroscopy (EELS), and current-voltage (I-V) and hot probe measurements.

Results
The in-situ RHEED analysis of the grown films shows the presence of diffraction spots along modulated RHEED streaks for undoped and 3 at.% Cu-doped NiO (indicative of layer-by-layer growth) and distinctive transmission spots (indicative of 3D island growth) for higher Cu concentration doping, with segregation of the Cu metallic phase as the Cu content reaches 10 at.%. XRD results and diffractogram patterns from cross-sectional HAADF-STEM images show the structure of the grown films remains a single crystal cubic with [001] as the preferred growth orientation, with polycrystallinity developing as Cu content exceeds 10 at.%, with observation of the Cu metallic phase in 42% Cu:NiO films (Figure 1a–c). An increase in Cu concentration also results in increased surface roughness, as confirmed by AFM and cross-sectional HAADF-STEM images. The STEM-EDX maps show a uniform distribution of the Cu in 3 at.% Cu-doped NiO film, Cu ions are likely to be substituted for Ni ions, and a segregation of Cu grains as Cu concentration increases (Figure 1d-f), which is further confirmation of Cu-phase segregation. The correlation between EELS and XPS indicates the

existence of a mixture of oxidation states of Cu (Cu0, Cu+, and Cu2+) up to 10 at.% of Cu, with Cu0 becoming dominant as its concentration increases. The UV-vis absorption spectra show that an increase in Cu content results in a redshift of the absorption edges, leading to a decrease in the band gap of the grown films as Cu concentrations increase. The band gap measurements by EELS were correlated to UV-VIS measurements and were found to be in good agreement. The electrical measurements show the conductivity of NiO films increases from 2.8×10^-5 (Ω.cm)^-1 for un-doped NiO thin film and reaches the highest value of 4.1×10^-1 (Ω.cm)^-1 for NiO film with 42 at.% Cu.

Conclusion
Undoped NiO and low Cu-doped NiO films are grown as a single crystal film, and as Cu exceeds 10 at.%, it starts segregation and forms secondary phases within the NiO film. The Cu doping increases the surface roughness, as confirmed by RHEED, AFM, and STEM. The band gap of the grown films decreases as a function of Cu concentration. The characterization techniques indicate that at the lowest Cu concentration (3 at.%), Cu ions seem to be substituted for Ni ions, and as Cu concentrations increase, Cu likely starts segregating and forming Cu grains within the films. Cu phase segregation is very likely responsible for the conductivity transition from p-type to n-type properties of NiO film with increasing Cu concentrations. This result could open the door for new applications where Cu-doped NiO can be applied as p-type, n-type, or both layers in the same devices.

Keywords:

STEM, AFM, EELS, NiO, doping

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From DNA Nanotechnology to biomedical insight: Towards single-molecule spatial omics

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LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

Super-resolution fluorescence microscopy is a powerful tool for biophysical and biological research. The transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) can be leveraged for easy-to-implement multiplexed super-resolution imaging that achieves molecular-scale resolution across large fields of view. This seminar will introduce recent technical advancements in DNA-PAINT including approaches that achieve sub-10-nm spatial resolution and spectrally unlimited multiplexing in whole cells followed by recent developments in novel protein labeling probes such as Slow Off-rate Modified Aptamers (SOMAmers), that have the potential to facilitate DNA-barcoded labeling of much of the proteome within intact cellular environments. Applications of these new approaches will be discussed in cell surface receptor imaging and neuroscience. Visualization and quantification of cell surface receptors at thus far elusive spatial resolutions and levels of multiplexing yield fundamental insights into the molecular architecture of surface receptor interactions thus enabling the future development of more refined “pattern”-based therapeutics. A key approach in implementing these methods has been to leverage standard off-the-shelf fluorescence microscopy hardware as a tool for spatial omics, thus democratizing the ability to visualize most biomolecules and probe their network-wide interactions in single cells, tissues, and beyond with single-molecule-based "Localizomics".

Keywords:

Super-resolution microscopy, DNA Microscopy

Reference:

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Nature Methods (2018).

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Band gap shifts in Ga-doped ZnO using momentum-resolved EELS

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Poster Group 2

Band gap tuning is central to semiconductor research, and is typically done by doping, which in essence alters the material's band structure. Transparent conductive oxides (TCOs) are renowned for their high optical transmittance combined with low electrical resistivity, usually achieved via high doping (~10^{20} /cm^3) to fill the conduction band with charge carriers. This process leads to a widening of the optical band gap: an effect known as the Burstein-Moss (BM) shift (1-3). Conversely, a competing phenomenon known as band gap narrowing (BGN) is also present above a critical carrier density, due to band renormalization (4). While the BM shift and BGN have been studied in the optical limit ($q \rightarrow 0$) , reports on their momentum dependencies are lacking in literature. We use momentum-resolved electron energy loss spectroscopy (q-EELS) to study how the band gap signal in both doped and undoped ZnO evolves with transferred momentum. We show that doping induces a distinct modification of the momentum dependent EEL band gap signal. This result is discussed in terms of the competing effects of the BM shift and BGN.

The experiments were done on a monochromated FEI Titan G2 60-300 kV at 60 kV, equipped with a Gatan 965 GIF. q-EEL spectra were acquired by a nanoprobe with a convergence angle of $\alpha = 0.27$ mrad, with a corresponding diffraction-limited ~ 4 nm probe size. A rectangular spectrometer entrance slit was inserted in the diffraction plane selecting primarily for momentum transfers perpendicular to the energy dispersive direction. Post specimen lens rotation was set to allow for high-symmetry directions of the Brillouin zone to be admitted by the rectangular spectrometer selection slit, here the ΓM direction. Two samples were studied: the first is a thin film of Ga-doped ZnO (GZO) grown on a sapphire substrate using molecular beam epitaxy. This film displays a polycrystalline structure and a charge carrier concentration of $1 \cdot 10^{21}$ /cm^3, determined through Hall-effect measurements. The second sample is a purchased single crystal ZnO substrate with a purity level of at least 99.99%.

q-EEL spectra of the undoped and doped ZnO are shown in Fig. 1a. We track the band gap signal energy by a parabolic fit (5) and as a function of momentum transfer (see Fig. 1b). At the optical limit (within experimental momentum resolution), the band gap signals of ZnO and GZO is determined to be 3.22 eV and 3.74 eV, respectively. The difference in these values is 0.52 eV, clearly showing the effects of BGN and the BM shift. At $q > 0$, the GZO band gap follows a shallow dispersion curve, gradually approaching the curve of the non-doped ZnO, to coincide at $q \geq 0.65$ 1/Å . Hence, our results suggest that the GZO conduction band is unaffected by doping beyond this q-value (at least within experimental uncertainty). If assuming a simplistic model wherein we have an unaltered ZnO band structure with an elevated Fermi level, the conduction bands of GZO and ZnO would be expected to be the same at $k > 0.3$ 1/Å. This approach alone clearly does not agree with our experiments. We suggest that a deviation of the band topology of GZO from that of ZnO contributes significantly to the difference between that predicted by this simplistic approach and our experimental observations. The roles of the BM and BGN effects will be discussed for finite

momentum transfers, as will insights gained with respect to dopant-induced band structure modifications of the ZnO-GZO system.

We demonstrate and discuss how q-EELS can be used for investigating the influence of doping of the finite q response of ZnO. This approach holds promise for probing the electro-optical response of various technologically important materials, with potential applications in solar cells, transistors, light emitters, and beyond.

Figure 1: (a) Electron-energy loss edges of ZnO and GZO at q → 0, after background-subtraction. (b) Band gap signals for ZnO and GZO as a function of q, obtained by q-EELS and extracted by fitting as in (a).

Keywords:

q-EELS, Semiconductors, Momentum-Resolved

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Structure and mechanism of Zorya anti-phage defense system

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Zorya is a recently identified and widely distributed bacterial immune system, which protects against phage invasion. It consists of a predicted membrane-embedded complex (ZorAB) and soluble components that differ among Zorya subtypes, notably ZorC and ZorD, in type I Zorya systems. Here, we reveal the molecular basis of the Zorya defense system using cryo-electron microscopy, mutagenesis, fluorescence microscopy, proteomics, and functional studies. We demonstrate that ZorAB shares the stoichiometry of other 5:2 inner membrane ion-driven rotary motors. Additionally, ZorA5B2 features a dimeric ZorB peptidoglycan binding domain and a pentameric α-helical coiled-coil tail made of ZorA that projects approximately 700 Å into the cytoplasm. We further characterize the structure and function of the soluble Zorya components, ZorC and ZorD, and find that they harbour DNA binding and nuclease activity, respectively. Comprehensive functional and mutational analyses demonstrates that all Zorya components work in concert to protect bacterial cells against invading phages. We present evidence that ZorAB operates as an ion-driven motor that becomes activated and anchors to the cell wall upon sensing of cell envelope perturbations during phage invasion. Subsequently, ZorAB transfers the phage invasion signal through the ZorA cytoplasmic tail to the soluble effectors, which function to prevent phage propagation. In summary, our study elucidates the foundational mechanisms of Zorya function and reveals a novel triggering signal for the rapid activation of an anti-phage defense system.

Keywords:

bacterial defense-system, rotary-motor, ion-channel, cryo-EM

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Electron beam propagation impact on high-resolution quantitative chemical analysis of GaN/AlGaN 1 nm-thick quantum wells

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Background: High-resolution energy dispersive x-ray spectroscopy (HR-EDX) in STEM is a technique of choice for conducting chemical analyses of III-nitride materials quantum wells (QWs) used as UV sources [1]. When travelling through a crystalline material, the phenomenon of “cross-talk” – the interaction of the electron beam (EB) with surrounding atomic columns - can significantly affect the quantitative results. Although the phenomenon was widely studied [2], there is still a lack of experimental works showing the possibility to quantify the impact of these effects on chemical analyses. The present contribution aims to show how to correlate HR-EDX mappings with inelastic multislice simulations [3] to determine the aluminum content in such devices.
Methods: Two GaN QWs with respective widths of 1.5 and 10 nm (Figure 1a) are studied, both surrounded by Al0.1Ga0.9N barriers. Both structures are grown by plasma assisted molecular beam epitaxy (MBE), and the samples are prepared with a Ga+ focused ion beam scanning electron microscopy (FIB-SEM) based on the standard lift-out method. STEM-EDX analyses are conducted using a probe-corrected TFS Titan Themis microscope operating at 200 kV and equipped with a Super-X detector system. Spectroscopic high-resolution data are acquired through a multi-frame spectrum-imaging approach, with thousands of frames rapidly acquired at high-resolution and automatically realigned thereafter. EDX quantification analyses are conducted using the ζ factors approach [4], and considers the inhomogeneity of the sample in the x-rays absorption correction process. Finally, beam propagation and EDX intensity maps were simulated using the µSTEM software [3].
Results: Figure 1b highlights the significant difference of EDX Al K emission observed between the two structures studied, when the probe is positioned at the middle of the respective QWs. The simulation of the probe propagation (Figure 1c) reveals that, for the 1.5 nm width layer, the electron wave is quickly delocalized into the adjacent barriers even for thin samples (~30 nm), explaining the disparity in intensity measured between the two structures. Quantification of this signal using the ζ factor method [4] estimates the Al content inside the 1.5 nm width QW around 1 at.%, significantly impacting the structure’s band properties. To understand the effect of such beam broadening on EDX quantification, we compare the experimental results with a simulated HR-EDX profile of Al K obtained from a given theoretical structure (Figure 1d). The chemical properties of this hypothetical structure, considering interfaces chemical gradient and well aluminum content, were adjusted until achieving optimal agreement between experimental and simulated profiles (Figure 1e). If a significant chemical gradient at the interfaces is confirmed, the results reveal that most of the Al K emission detected within the QW can be attributed by the propagation of EB in the surrounding layers.
Conclusion: This study aims to present a clear methodology to detect the effect of beam broadening on HR-EDX quantitative analyses. The demonstration is done on a regular heterostructure and conventionally (FIB) prepared sample, showcasing its potential application on various systems. In this presentation, we will particularly focus on which conditions this comparison between experimental data and simulations can be carried out to quantitatively detect these slight changes in chemical

compositions at high spatial resolution. This work, performed on the Platform for NanoCharacterisation (PFNC) of CEA, was supported by the “Recherche Technologique de Base” Program of the French Ministry of Research.

Keywords:

HR-EDX, chemical quantification, e-beam propagation

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Structural remodeling of neural circuits through synthetic biological control of neuron-astrocyte interactions

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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

Background incl. aims
In the adult hippocampus, synaptic plasticity is essential for information processing, learning, and memory formation. Astrocytes, the most common type of glial cells, play pivotal roles in synapse formation, maintenance, and elimination. However, our understanding and manipulation of complex interactions between neurons and astrocytes are just the beginning.
Methods
Here, we present a synthetic approach for synapse elimination via engineering of direct neuro-glial interactions which result in the uptake of synaptic components by astrocytes. Trogocytosis is a process in which a cell takes up a portion of another cell's membrane and internalizes it. Induced synthetic trogocytosis, the uptake of ligand-labeled membrane fragments by receptor-expressing cells, has the property of unidirectional molecular transfer. We assessed potential structural changes induced by engineered neuron-astrocyte interactions using 3D correlative light and electron microscopy (CLEM) and array tomograhy.
Results
Our results provided clear evidence for the partial capture and elimination of presynaptic boutons and axons in ligand-expressing CA3 neurons by receptor-expressing astrocytes in the CA1 area. Notably, astrocytes mainly engulfed partial axonal debris or small vesicles at the initial stage of viral expression, while they later swallowed up larger presynaptic materials including mitochondria.
Conclusion
This approach will provide an insight into how the brain adaptively shapes neural circuits upon synapse elimination and offer a versatile means to structurally modulate cell-cell connections.

Keywords:

synthetic trogocytosis, neuro-glia interaction, neural-circuits

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EBSD analysis of twin boundaries of prismatic calcite in oyster shells

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Poster Group 2

Oyster shells are biocomposites consisting of calcium carbonate crystals with a percentage of organic matrix. The organic fraction in these shells plays a crucial role in stress distribution control, leading to a hierarchical structure with high self-organization and low internal energy. This microstructure, with its unique combination of high strength and fracture toughness, holds significant potential for the development of advanced biomimetic materials.
We studied two specimens from the order Ostreida, Pinna nobilis Linnaeus, 1758 (Mollusca, Ostreida, Pinnidae), which comes from the Mediterranean Sea, and Pteria penguin Röding, 1798 (Mollusca, Ostreida, Pterioidea) collected in Lapu Lapu, Cebu, Philippines. The electron backscatter diffraction (EBSD) technique was used to study the crystallographic relations observed between prisms in a columnar calcite prismatic (CCP) layer. EBSD analysis was performed using a FEI Versa 3D FEG scanning electron microscope (SEM) equipped with an Oxford Instruments Symmetry S2 CMOS-based EBSD detector. EBSD data were collected using Aztec 6.1 software from the polished cross-section. The system was operated in low vacuum (LV-SEM) mode at a pressure of 40 Pa and an accelerating voltage of 15 kV.
The obliquely oriented calcite prismatic grains to the predicted load's direction are observed in P. nobilis. EBSD results show that strictly defined low-energy boundaries, i.e. rotation angles, are preferred. The disorientation described by the 60° rotation about the c-axis is mainly observed. Two symmetrically equivalent orientation relationships correspond to it, and one of them is observed in this case. The twin boundary (0 1 -1 0) is present, positioned perpendicular to the growth line, enabling the prisms to curve. The other preferred disorientations are also generated by twin relationships, with mirror planes characterized by higher indices: (1 -5 4 0) for 38° and (4 7 -11 0) for 18°. These new, previously unknown orientation relationships are common in P. nobilis shells. The shell is ornamented with radial ribs that strengthen it while giving it the flexibility needed to tighten its seal against predators. The growth lines become flatter in the area outside the ribs with the shell thickening, which affects the change in the direction of the c-axis, which becomes more parallel to the normal direction (ND). Such a microstructure improves hardness and compressive strength, which reaches an outstanding value of 700 MPa. In the case of P. penguin, EBSD was combined with theoretical boundary energy analysis using the Gautam-Howe method, which revealed that prisms as basic structural units show good mutual fitting and a sequence of twins arising within them is used to relax internal stresses. Consequently, a low-energy structure with an exceptionally high compressive strength of 600 MPa is achieved.

1. K. Nalepka et al. Ribs of Pinna nobilis shell induce unexpected microstructural changes that provide unique mechanical properties, Materials Science and Engineering: A (2022) 829, 142163

Acknowledgements:

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Keywords:

EBSD, twin boundaries, mollusc shells

Reference:

1. K. Nalepka et al. Ribs of Pinna nobilis shell induce unexpected microstructural changes that provide unique mechanical properties, Materials Science and Engineering: A (2022) 829, 142163

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An analytical ion microscope for high-resolution imaging, nanoscale analytics and nanofabrication

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Poster Group 1

The Liquid Metal Alloy Ion Source (LMAIS) has been described as a high-impact technology offering new insights into the structure and function of nanomaterials [1]. Combining the high brightness of a Liquid Metal Ion Source (LMIS) with the capability of emitting light and heavy ions such as Silicon and Gold or Lithium and Bismuth simultaneously makes the LMAIS the ideal ion source for high-resolution imaging, nanofabrication, and nano-analytics (Fig. 1) [2]. The ion species are emitted simultaneously from a single source and separated in a downstream Wien filter. This mature source technology allows for high-resolution Secondary Electron (SE) imaging with exceptional surface details by using light primary ions as well as adjusting the required sputter yield and resolution for nanofabrication and sample modification by selecting the most suitable ion species from the LMAIS.
With a top-down FIB geometry and capability of fast ion toggling a novel workflow for 3D sample reconstruction with sample tilt becomes possible: heavy ions have an excellent depth resolution for sample delayering. The selection of various beam paths or looping strategies as known from many applications in nanofabrication minimize selective sputtering and redeposition while digging into the sample.
Intermittent imaging with light ions prevents further sputtering and allows 2D sample imaging for mapping the region of interest layer by layer. Obtained ion images are then stacked for 3D sample reconstruction [2].

By adding a specifically designed compact magnetic sector mass spectrometer, the ion microscope becomes a high-resolution analytical instrument [3].
Secondary Ion Mass Spectrometry (SIMS) is a robust and highly sensitive surface analysis method capable of detecting all elements ranging from hydrogen to uranium. It offers trace element identification, differentiation of isotopes, elemental imaging at the nanoscale, shallow depth profiling, and three-dimensional analysis.
By selecting the most suitable ion species from the LMAIS the SIMS system takes advantage of the LMAIS technology by selecting the most suitable primary ion species for the analysis.
The SIMS system is based on:
(i) specifically designed secondary ion extraction and transfer optics for highest extraction efficiency and transmission, resulting in excellent sensitivity.
(ii) a compact floating double focusing magnetic sector mass spectrometer allowing operation in the DC mode at high transmission and hence avoiding secondary ion losses due to duty cycles like in TOF systems.
(iii) a focal plane detection system allowing the detection of all masses in parallel (up to 400 m/z).

This contribution outlines the working principles and features of the focal plane magnetic SIMS detector combined with a LMAIS. By combining LMAIS technology with a stable stage and sensitive SIMS unit, this system offers a pathway for advanced nano-analytics, surpassing conventional methodologies for sample analysis.

Keywords:

FIB, SIMS, LMAIS, Microscopy, Ion

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Benzofuran-Tetrahydrodipyrazolopyridine Hybrids: Novel Compounds for Potential Lung Cancer Treatment

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Poster Group 2

Aims: Benzofuran and tetrahydrodipyrazolopyridine are heterocyclic compounds with diverse biological activities. Benzofuran derivatives have potent anticancer, antiviral, and antimicrobial properties [1]. Tetrahydrodipyrazolopyridines are used in various pharmaceuticals and exhibit anticancer, antiallergic, and antiherpetic properties [2, 3]. The hybridization of these two molecular frameworks presents an innovative approach to crafting novel compounds with significant biological activity. Lung cancer is one of the most common types of cancer worldwide and is a serious health problem affecting both men and women. Although current lung cancer treatment methods are effective against certain types of the disease, many patients may develop resistance or develop side effects. Therefore, the discovery and development of new molecules that are more effective, have fewer side effects and can prevent the development of resistance is an important need in lung cancer treatment [4].
Methods: In this study, benzofuran-tetrahydrodipyrazolopyridine hybrids were successfully synthesized with high efficiency via a room-temperature condensation reaction. The synthesis involved the benzofuran derivatives with an aldehyde functional group, ethyl acetoacetate, hydrazine, and ammonium acetate. The biological activity of the new molecules synthesized within the scope of the study was obtained by MTT test on A549 lung adenocarcinoma cells.
Results and Conclusions: The resulting hybrids were characterized using proton (1H) and carbon (13C) nuclear magnetic resonance (NMR) spectroscopy, which confirmed their structure and purity. As a result of the Mtt test, the IC50 values for the molecule coded DB-1 were determined as 5.26µm for 24 hours at the lowest concentration, while the 24 hour value for the other molecule coded DB-2 was determined as 15.58µm.

Keywords:

Benzofuran, tetrahydrodipyrazolopyridine, lung cancer, hybrid

Reference:

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Gallium liquid bridge evolution on varied substrates

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Poster Group 2

Room-temperature liquid metals (LMs) with high conductivity and deformability have received considerable attention in soft and stretchable electronics [1]. In recent years, there has been extraordinary progress in the fabrication of LM-based elastic circuits such as embedding LM particles in an elastomer, mixing LMPs with a solid conductive filler composite, doping LMPs in a polymer matrix, etc. [2]. The formation and separation of liquid bridges between LM particles correspond directly to the connection and disconnection processes of these components, respectively. Hence, unveiling the kinetics of liquid bridge formation and evolution is crucial for achieving controllable design of stretchable LM-based conductors in the assembly of electronic components, thus propelling the development of electronics with enhanced flexibility and adaptability.
Herein, we employed in-situ transmission electron microscopy (TEM) techniques to systematically investigate the wetting behavior between Gallium (Ga), a typical liquid metal, and various substrates, as well as the influence of surface Ga oxide layer thickness on the evolution of Ga liquid bridge formation. As a demonstration, Figure 1 illustrates the dynamic evolution process of Ga liquid bridges on Mo substrates. Our findings indicate that the presence of a thin oxide layer promotes the fluidity of gallium, thereby facilitating the formation of liquid bridges, with their evolution being influenced by interfacial wettability dynamics. Moreover, a delicate equilibrium between viscoelasticity and surface tension emerges with a slight increase in the thickness of the surface oxide layer, resulting in the formation of super-stretched liquid bridges. In contrast, an excessive thickness of the oxide layer constrains the formation of liquid bridges due to its high viscoelasticity.

Keywords:

Liquid metal, liquid bridge, Wettability

Reference:

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Multiplexed 3D imaging of single-cell organization and tissue morphology in the multicellular intestinal organoid

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Organisms develop within the physical context of their external environment. Molecular signals and physical properties or constraints provide external conditions with which the developing organism interacts and to which it responds. This dynamic reciprocal interaction results in and is required for morphogenesis and self-organization. Thus, a mechanistic understanding of emergent tissue-scale phenomena, such as tissue shape and patterning, require spatial quantification methods that can link single-cell properties and interactions to tissue-scale measurements. To address this need for a multiscale spatial analysis method, we developed scMultiplex, a bioimage analysis method that combines spinning disk confocal fluorescence microscopy, machine learning, parallelized image processing, and optimized imaging and staining protocols to quantify whole tissue shape, cell composition, and molecular expression in 3D. We use mouse small intestinal organoid development as a model multicellular system that self-organizes from a single cell into a complex 1,000+ cell structure. Specifically, the full single-cell composition of the fixed organoid is imaged and segmented in 3D, with molecular expression, cell type markers, and cell states identified with immunostaining. Cells are spatially linked across iterative rounds of staining and imaging to achieve multiplexing of ~10-20 protein markers. Organoid tissue shape features are also extracted from a reconstructed surface mesh using a machine learning approach. We thus apply scMultiplex to thousands of organoids to link cell type composition and spatial arrangement to tissue shape emergence and uncover mechanisms of intestinal morphogenesis. This approach can be applied to diverse multicellular systems to address fundamental questions in developmental and systems biology on emergence of higher-level organization from single-cell behavior.

Keywords:

multiscale, multiplexing, morphogenesis, self-organization, organoids

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Achieving methanol photo-oxidation to hydrogen and formaldehyde over lead-free halide perovskite

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Poster Group 2

Background
Hydrogen can be a source of clean energy, but its production heavily relies on fossil fuels [1]. Methanol can be a good storage vessel for hydrogen, as each molecule contains four hydrogen atoms. Industrially, methanol can be decomposed into H_2, CO and/or CO_2, but high temperature and pressure are required [2]. Alternatively, at milder conditions such as room temperature photo-oxidation, it can be split into hydrogen and formaldehyde, but a suitable catalyst is required. In this research project, I have been studying $Cs_2AgBiBr_6$ (CABB) as a type of lead-free halide perovskite photocatalyst, which has been found to possess the ability to dehydrogenate methanol to acquire H_2. Multiple electron microscopy techniques have been used to examine the structures of CABB before and after reactions. As CABB degrades in methanol easily, the aims of this project are to keep the structure of CABB stable in the reactions, and to enhance hydrogen production rates.
Methods
Synthesis of bulk CABB: CsBr, AgBr and $BiBr_3$ were mixed with HBr, heated to 110°C, and kept for 2 hours. After cooling, CABB was collected by using centrifuge and dried in vacuum oven.
Synthesis of CABB nanocubes [3]: Cesium acetate, bismuth acetate and silver acetate were dissolved in oleylamine, oleic acid and 1-octadecene. The mixture was heated under vacuum, and then filled with argon at a higher temperature, where bromotrimethylsilane was rapidly injected. Next, the mixture was quenched in water bath, centrifuged and dried.
Synthesis of CABB nanosheets [4]: Cs_2CO_3 was dissolved in oleic acid by heating in vacuum followed by argon to prepare Cs-oleate. Separately, $BiBr_3$, $AgNO_3$, octadecene, HBr, oleic acid, oleylamine were mixed and heated in vacuum. The mixture was cooled to room temperature, and Cs-oleate solution was injected into the mixture. After that, the mixture was then heated in argon. Finally it was quenched in water bath, centrifuged and dried.
Photocatalytic tests: For reactions in liquid methanol, CABB was put into liquid methanol with or without bromide additives, and irradiated with 300W Xe lamp. For reactions in gaseous methanol, CABB was placed in the reactor but not in direct contact with liquid methanol, and irradiated with 300W Xe lamp.
Characterisations: Quantification of hydrogen was performed on Agilent 7890B Gas Chromatograph. Powder X-Ray (XRD) data were collected by Bruker D8 Advance Eco, using Cu Kα1,2 as source. Transmission electron microscope (TEM) images were obtained on a JEOL JEM-2100 at 200kV. Scanning transmission electron microscope (STEM) images were obtained using JEOL ARM-200F operated at 200kV.
Results
Without additional support, all three forms of CABB (bulk, nanocubes, nanosheets) degrade quickly in methanol. Adding bromide additives allows bulk CABB to remain stable during the photoreaction, and it showed an overall H_2 production rate of 167 µmol h-1 g-1. However, for nanosize CABB the protection given by bromide additives turned out to be inadequate, and CABB still degraded during

the photoreaction. The H_2 generation rates were 76 µmol h-1 g-1 for CABB nanocubes, and 74 µmol h-1 g-1 for nanosheets. STEM images clearly showed their change in structures after reaction. Reaction with methanol vapour is another approach to keep CABB stable in the photoreaction, without the need of bromide additives. XRD results demonstrated that the structure of bulk CABB was retained during the reaction. An overall H_2 generation rate of 524 µmol h-1 g-1 was achieved. Nanosize CABB will be tested and characterised using this method soon.

Conclusion

Both bromide additive method and methanol vapour method could prevent the structural degradation of halide perovskite CABB, and the results showed that photo-oxidation of methanol using CABB as photocatalyst is feasible. However, both methods has been proved to be useful on bulk CABB only, while for CABB nanocubes and nanosheets more research is needed.

Keywords:

Microscopy, perovskite, photocatalyst, methanol oxidation

Reference:

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In-situ TEM ion irradiation studies of layered MAX phase materials

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Materials in the core of a nuclear reactor are exposed to severe environments, particularly in future Generation IV nuclear systems, which perform in environments with high temperatures and high fluxes of fast neutrons, causing hundreds of displacements per atom (dpa) as well as substantial helium (He) doping by nuclear transmutation. MAX phases have attracted attention for their potential in nuclear materials due to their demonstration of high stabilities [1]. This study investigates the impact of sequential irradiation with light and heavy ion irradiation of MAX phases, specifically comparing the irradiation induced changes in Ti_3SiC_2 and Ti_3AlC_2. Cross sections of both MAX phases were prepared using focused ion beam milling. These were irradiated inside the MIAMI transmission electron microscopy (TEM), in situ TEM irradiation facility [2], first with 75 keV He+ ions at a dose of 2.6 x 10^{16} ions/cm² up to 3 dpa, and then with 600 keV Ar^2+ ions with a total fluence of 9.2 x 10^{16} ions/cm² up to 50 dpa, at both room temperature and 350°C. TEM imaging and diffraction are used during in-situ ion irradiation for direct observation of the microstructural and unit cell evolution. In-situ TEM observations indicate that both materials retained their crystal structures for the full irradiation dose. There was no observation of significant amorphization, phase transformations, He bubbles, or decomposition. The helium irradiation-induced damage, manifested as point defects, increases with damage level (dpa), resulting in anisotropic changes in lattice parameters in both structures up to ~ 9% and at both room temperature and irradiated temperatures. Interestingly, a significant recovery towards the as-synthesized unit cell parameters was observed post-irradiation within a few days of ambient storage at room temperature. Subsequent irradiation then followed similar irradiation damage profiles as the original pristine MAX phase with lattice parameter changes up to ~ 8% without significant amorphization, followed by another room temperature structural recovery when the irradiation is stopped. This observation of exceptional irradiation stability combined with room temperature recovery provides new evidence of the potential of MAX phases in protective coatings for nuclear applications and for radiation sensing and detection.

Keywords:

In-situ radiation, MAX-phases, radiation tolerance.

Reference:

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Nanoscale tribology of hair fibres over large displacements

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Background incl. aims
Hair is a protein-based, filament-like biomaterial that grows from follicles beneath the surface of the skin. The evolution, structure and function of human hair is of great interest to anatomists and anthropologists, and the use of hair for social and cultural functions is well established. There have been many scientific studies regarding hair structure, the physicochemical properties of the hair cuticle surface, and hair damage mechanisms. Atomic force microscopy has played a crucial role for studies of friction and conditioners applied to single hair fibres.
Methods
This work reports the development of a method for measuring the tribological properties of two orthogonally crossed cylinders over a 2 mm displacement with nanoscale precision in loads and displacement. Specifically, a human hair fibre was used as the lower cylinder, while the upper cylinder was a 0.5 mm section of 75 µm diameter tungsten wire. Measurements were performed at a sliding velocity of 20 µm/s, the compressive normal load between the cylinders was 600 nN, and data were recorded every 2 nm.
Results
Topography and lateral load were recorded throughout the bidirectional measurement, with differential friction observed upon comparison of the ‘with cuticle’ and ‘against cuticle’ sliding directions (Fig. 1). To assess the influence of sliding velocity on the frictional behaviour a sequence of increasing velocities in the range 0.01-1.0 mm/s was programmed; the total displacement of this measurement covered 5 mm.
Conclusion
The mean friction coefficient did not vary significantly with sliding velocity, although occasional high lateral loads were observed at velocities in excess of 0.1 mm/s.

Keywords:

fibre, friction, hair, load, tribology

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Manufacture and calibration of high stiffness AFM cantilevers

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Poster Group 2

Background incl. aims
Atomic force microscopy (AFM) employs microfabricated cantilevers as sensing elements, which are used to measure surface topography and interaction forces. The flexible free end of a cantilever often presents either a pyramidal tip or a colloid probe particle. Force-displacement measurements have been applied to a wide variety of scientific and engineering disciplines, and across many industrial sectors. For many studies, the use of colloid probes or chemical functionalisation permits the selective study of a particular material/material interaction, often under non-ambient environments. Force-displacement measurements can provide information regarding sample mechanical properties, during tip/sample approach and contact, as well as adhesive properties, during the tip/sample separation. The spring constant is a measure of the cantilever stiffness, i.e. the resistance to bending. The spring constant of a rectangular cantilever can be estimated using Euler-Bernoulli beam theory. Once calibrated, the spring constant is used to convert normal (i.e. vertical) deflections into normal forces using Hooke's law.
Methods
The range of AFM cantilevers commercially manufactured means that spring constants in the approximate range 0.001 to 100 N/m are available. Deflections in the range 0.1-100 nm are typically measurable on the PSD, and hence forces can be measured in the picoNewton to microNewton range. Accurate control of the beam thickness during fabrication is particularly difficult to achieve, due to the nature of the etching process employed. The width and length of the beam are generally much more reliable and repeatable. Given the sensitivity of the spring constant to the beam thickness, typically proportional to the (thickness) cubed, accurate calibration is a necessity for accurate force-displacement measurements.
Results
We are currently calibrating 40 different designs of rectangular AFM cantilever, designed using Timoshenko beam theory, manufactured from Si. The various designs incorporate a range of widths, lengths, and thicknesses. These cantilevers are expected to exhibit spring constants in the range 100 to 10,000 N/m. This would afford researchers the opportunity to perform adhesion, indentation, and tribological testing with normal loads approaching 1 mN, whilst retaining the displacement resolution of the AFM.
Conclusion
We present the latest results of this project, including measured cantilever resonant frequencies and calculated spring constants, which are compared to analytic expressions and finite element models.

Keywords:

cantilever, load, microfabrication, spring constant

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Solvent-induced softening of polymethyl methacrylate surfaces

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Poster Group 2

Background incl. aims
Exposing plastics to solvents is known to alter their surface and sub-surface properties, depending on the extent and duration of exposure. Amorphous plastics are prone to penetration and diffusion by low molecular mass solvents, leading to physical deformation including swelling, cracking, and dissolution. Unwanted or unexpected solid/liquid interactions can irretrievably compromise the visual characteristics of an object were it to be cleaned using an inappropriate method. This is of particular importance to the heritage and conservation sector, who use solvent-based cleaning systems to preserve the appearance of culturally-important artworks over many decades. There is increasing interest in the applicability of nanocharacterisation techniques to inform remedial conservation strategies for plastic artworks, supporting the preservation of the original intent of the artists and designers.
Methods
Polymethyl methacrylate (PMMA) is an important plastic in modern art and design collections, having been used since the mid-20th Century for creating artworks but also aircraft cockpit hoods, automotive and architectural lighting, and even windows on deep-sea submersibles. According to Hildebrand solubility theory, PMMA does not dissolve in aliphatic alcohols such as ethanol.
Results
We report that nanomechanical mapping of PMMA surfaces exposed to liquid ethanol experienced a decrease in elastic modulus according to an approximately exponential relationship. Further, interrogated regions of the surface were observed to have their mechanical properties altered because of the compressive load applied during the measurement process (Fig. 1).
Conclusion
These findings, which are of use to heritage and conservation scientists, also suggest opportunities for developing novel micro- and nanofabrication strategies, whereby a surface which has been temporarily and reversibly softened can be manipulated topographically, for example using nanolithography, nanoscratching, or nanoindentation.

Keywords:

ethanol, modulus, PMMA, polymer, solvent

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In-situ oxidation and reduction study of Ni/NiO by open cell environmental TEM

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Poster Group 2

Background and Aim
Metallic NPs are widely used as a catalyst, in heterogeneous catalysis, with high reactivity and selectivity [1]. Ni/NiO nanoparticles (NPs) have attracted significant research attention as catalysts due to their low cost and wide applications that includes, CO oxidation, oxygen evolution, photocatalysis and water-gas shift reaction [2, 3]. Many catalytic reactions require the oxidation and reduction of metal/metal oxides where the dynamic interaction between catalyst and gas proceeds via intermediate products and adsorbed species to form a solid-gas interface. Atomic changes at this interface are linked directly to the catalytic processes [4]. This involves morphology changes, oxide formation, surface facets modification, as well as surface reconstruction. These dynamic structural changes play an important role in the catalytic activity of the NPs. Therefore, tracking these structural changes at the atomic scale under the reaction conditions is vital for the identification of intermediate structures and ultimately understanding the reaction mechanisms. Open-cell Environmental TEM/STEM (E(S)TEM) has the capability for in-situ studies of gas-solid reactions by allowing gases in the sample area without the need of special sample holders [5]. In this work we employed aberration-corrected E(S)TEM to investigate the evolution of NiO NP surfaces during reduction in vacuum and H2 environments and subsequent oxidation of Ni metal pristine surfaces in O2, H2O and CO2 + H2O vapour environments.
Method
Two types of Ni samples were investigated: thin films grown by Molecular Beam Epitaxy (MBE) and NPs synthetized by chemical methods. The MBE Ni films, 2 nm in thickness, were directly grown onto SiN membranes of heating MEMS chips (DENSsolutions) and afterwards exposed to air. Similarly, Ni NPs dispersed in ethanol were prepared for in-situ experiments by drop cast deposition of 5µl solution of NPs onto the chips membranes. DENSsolutions Wildfire MEMS heating system was used to form metallic NPs by annealing in vacuum and 1Pa H2 atmosphere. A set of samples were heated gradually (10°C per minute) from RT to 500°C to track the particles formation/evolution. To minimise beam exposure, TEM images and diffraction patterns were only acquired at every 100°C interval. For another set of samples, live movies were acquired during the reduction under 1Pa H2. In both cases, once obtained pristine metallic surfaces, the oxidation process under O2, H2O and CO2 + H2O vapor environments was followed by video recording (5fps) using OneView Camera.
Results
MBE thin films after exposure to air showed a partial natural oxidation. Time series HRTEM imaging and selected area electron diffraction showed NPs formation and full reduction to Ni metal at 300°C following by an increase of crystallinity at higher temperatures in both vacuum and 1 Pa H2

environments. Chemically grown Ni NPs showed a thin NiO shell (Figure 1a), fully reduced at 400°C under 1Pa H2 (Figure 1b). Video recording revealed NP migration and coalescence followed by facets formations and surface reconstruction to eventually gain clean metallic surfaces. Furthermore, introducing oxidising environment (1Pa O2 gas at 400°C) lead to an initial epitaxial oxide growth followed after1.8s by the growth of polycrystalline NiO grains. After 1hr of exposure to O2 the reaction ends with fully formed NiO Kirkendall voids structures (Figure1 c,d). In addition, subsurface atomic oxide layers dynamics were captured and oxidation rates along two main crystallographic direction [111], [200] were measured of (14.6 ±1.8 planes/s) and (11.2 ±1.2 planes/s) respectively with a combined total of 1.7±0.1 nm/s. This higher value than the rate reported in literature (0.5nm/s) [6] could be due to the continuum exposure to the electron beam that accelerate the reaction. Moreover, a comparison of oxidation of Ni NPs under water vapour vs water vapour plus CO2 shows that in both cases nanoparticles tend to coalesce and form irregular 2D clusters of few NiO NPs, however only in the case of CO2 and water vapour gas condition faceted surfaces are formed.

Conclusion

In this study, open-cell environmental TEM was used to investigate Ni/NiO thin films and nanoparticles reduction and oxidation under different environmental conditions (vacuum, H2, O2, H2O and H2O +CO2) from room temperature to 500°C.

The NPs formation and reduction were followed by time series HRTEM imaging and selected area electron diffraction with intermittent beam exposure to reduce beam driven effects until the formation of single crystal Ni nanoparticles with pristine metallic surfaces.

In addition, the dynamic behaviour of such surfaces formation and evolution was successfully followed by live video recording to determine the reduction and oxidation mechanism, rates, facets formation and interaction.

Keywords:

ETEM (Environmental Transmission Electron Microscopy)

Reference:

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[3] S Xia et al., Phys. Chem. Chemical Physics 22 (22) (2020) p. 12630.
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14753, 2019

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Crystalline analysis by W-SEM using a newly developed EBSD detector

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Poster Group 1

Background incl. aims
Understanding crystalline properties of material is essential for controlling its physical characteristics. EBSD method in SEM (SEM-EBSD) is a well-established technique for investigating the phase orientation and identification of single or polycrystalline materials. It is commonly used to determine crystalline properties in micro and nanotextures [1]. The development of SEM-EBSD significantly contributes to the analysis of microstructure, crystallography and physical properties in various fields, including materials science and mineralogy, such as metals, ceramics, and minerals. Thus, the SEM-EBSD method is widely recognized as a requisite tool in these fields now.
To obtain high-quality EBSD measurement results, large probe currents ranging from several 10 to 100 nA are generally required. Therefore, the SEM equipped with a Schottky-type field emission gun (FE-SEM) has been basically employed for EBSD measurements due to its ability to maintain high resolution even when large probe currents are applied. On the other hand, the crystal grains to be measured by EBSD are usually a few to several 10 of micrometers in size, which falls within the measurable range of the SEM with Tungsten thermal filament (W-SEM). However, there are few cases where W-SEM is installed together with the EBSD detector in laboratory, because the spatial resolution of Tungsten filament gun when using a large probe current is significantly inferior to that of field-emission guns [2].
Recently, CMOS has increasingly replaced CCD as the image sensor in EBSD detectors. This shift has made it possible to obtain fast and clear EBSD patterns with less probe current, resulting in more practical EBSD measurement by W-SEM. Here, we present a new EBSD detector from Bruker (e-Flash XS) in combination with JEOL's entry-level W-SEM. Although the body of e-Flash XS is significantly smaller than the conventional EBSD detectors, its CMOS image sensor provides high-definition EBSD patterns even at a small beam current, enabling fast analysis. The EDS detector is also included in this system, called ED-XS, which allows an elemental analysis during the EBSD measurement simultaneously. This report presents an overview of the e-Flash XS with ED-XS system and one of its applications.
Methods
The ED-XS system including an EDS (XFlash 630; Bruker Nano GmbH) and an EBSD detector (e-Flash XS; Bruker Nano GmbH) is installed on the entry model W-SEM, JSM-IT200 (JEOL, Ltd.). Details are described in the following "Results" section. Cross section of zirconia ceramic made with Cross Section Polisher (IP-19520CCP; JEOL, Ltd.) was used as a specimen. The crystal structure of cubic zirconia (ZrO_2, Fm-3m, No. 225) was used for EBSD pattern analysis. The acquisition parameters were an acceleration voltage of 20 kV, a probe current of 15 nA, a working distance (WD) of 7 mm, and a chamber vacuum of 10 Pa.
Results
Graphic (a) displays an external view of the complete ED-XS system with JSM-IT200. Simultaneous acquisition of EDS and EBSD is possible because the detectors are located in the same direction. The phosphor screen inside the sample chamber can be manually attached and detached. A specimen to be measured is mounted on a pin-type stub and placed in a designated holder with

the specimen surface tilted at 70 degrees. This holder allows high-resolution EBSD measurements with a short WD of less than 15 mm (minimum 6 mm) without tilting the stage.

The pattern quality map of the zirconia ceramic obtained by e-Flash XS is shown in graphic (b) at a magnification of 5,000. Although this ceramic is the non-conductive material, EBSD measurements can still be performed without a conductive coating using the low-vacuum feature. The pattern quality map reflects the shape of the grains and their boundaries based on the clarity value of the Kikuchi pattern image. Then, the crystal grain size of the zirconia ceramic measured in this study was found to be approximately 2-3 µm for the largest ones and several 100 nms for the smallest ones. Additionally, inverse pole figure (IPF) map indicates that the orientation of the individual grains are random, while kernel average misorientation (KAM) map demonstrates that there is little local plastic deformation in the ceramic. Moreover, simultaneous EDS elemental map revealed that Zr and O were mainly present thoughout the measurement area, with small amounts of Y and Hf also detected. No segregation of other elements was observed, indicating the absence of foreign materials.

Conclusion

In this report, we present a new EBSD detector e-Flash XS and ED-XS integrated system designed for the compact model of W-SEM. As described, this small detector can measure the properties of crystal grains as small as several 100 nm, even under low-vacuum conditions using W-SEM. Electron channeling contrast images are generally used for measuring crystal grains. However, it is important to note that the contrast of these images can be affected by changes in the acceleration voltage or angle of incidence of the electron beam. On the other hand, crystal grain measurement via EBSD can be performed independently for acceleration voltage and angle of incidence. In addition, it is possible to analyze distortion and deformation resulting from differences in crystallographic orientation. This system is optimal for all SEM users, especially W-SEM users, to enhance their crystallographic analysis.

Keywords:

EBSD
W-SEM
Crystal analysis

Reference:

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Atomic-resolution investigation of 2D hematene

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Poster Group 2

2D materials have attracted scientific research for decades, since they posses distinct physico-chemical properties compared to their 3D counterparts due to quantum confinement. These atomically thin structures such as graphene, hBN, etc. are typically based on so-called van der Waals systems. These 3D counterparts are defined by strong bonds oriented in-plane and solely weak bonding interaction between the layers.

However, 2D materials can also be created from a parent material with strong bonding interactions in all three directions. For these, it is significantly more complicated to cleave them into individual layers. After cleavage into atomically thin layer, the non-van der Waals 2D materials could offer a playground to explore the changes in properties induced by dimensionality restriction. Recently, a new member of these non-van der Waals 2D materials has been introduced: hematene, the 2D form of α-Fe_2O_3 [1].

The dimensional confinement of hematene structure is expected to introduce intrinsic strain to the lattice. This distortion of interatomic positions affects electronic configuration and therefore the magnetic, electronic and optical behavior of the system [2]. Here, we employ transmission electron microscopy and selected area electron diffraction to investigate the atomic structure and distortions of the lattice in thin hematene sheets with the aim to quantify the intrinsic strain to facilitate the physical description of the material.

Keywords:

Hematene, Iron oxides, 2D

Reference:

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An Improved Method for Growing Primary Neurons on Electron Microscopy Grids Co-Cultured with Astrocytes

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IM-11 (2), Lecture Theater 5, august 30, 2024, 10:30 - 12:30

With the increasing popularity of cryo-electron tomography (cryo-ET) in recent years, the quest to establish a method for growing primary neurons directly on electron microscopy grids (EM grids) has been ongoing. Here we describe a straightforward way to establish a mature neuronal network on EM grids, which includes formation of synaptic contacts. These synapses were thin enough to allow for direct visualization of small filaments such as SNARE proteins tethering the synaptic vesicle (SV) to the active zone plasma membrane on a Titan Krios without prior focused ion-beam milling.

Keywords:

primary neurons; astrocytes; cryo-EM; synapse

Reference:

Radecke, J., Seeger, R., Kádková, A., Laugks, U., Khosrozadeh, A., Goldie, K. N., Lučić, V., Sørensen, J. B., & Zuber, B. (2023). Morphofunctional changes at the active zone during synaptic vesicle exocytosis. EMBO Reports, 24(5). https://doi.org/10.15252/EMBR.202255719

Kumar, I.; Paudyal, A.; Kádková, A.; Stewart, M.; Sørensen, J.B.; Radecke, J. An Improved Method for Growing Primary Neurons on Electron Microscopy Grids Co-Cultured with Astrocytes. Int. J. Mol. Sci. 2023, 24, 15191. https://doi.org/10.3390/ijms242015191

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Microstructure and thermal stability of ultrafine-grained CuZn5 processed by HPT

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Poster Group 2

Background and aims:
High-pressure torsion (HPT) is widely known as an effective method for severe plastic deformation (SPD). This intense shear strain, combined with high pressure, not only results in notable grain refinement but also introduces a substantial density of dislocations, thereby enhancing mechanical strength [1].
Concurrently, ensuring the stability of ultrafine-grained (UFG) microstructures is crucial for their reliable performance in practical applications. Grain growth can compromise the enhanced strength of UFG materials, highlighting the importance of investigating microstructural stability for their commercial feasibility [2].
This concern is particularly notable in certain pure metals with low stacking fault energy such as fcc metals. In order to avoid grain growth, the grain boundary (GB) migration has to be limited. This can be done through the segregation of solute elements, which depends on the GB character. In this work, we use copper (Cu) as bulk material and add different amounts of Zn to examine the impact of varying Zn content on microstructural changes and thermal stability of GBs after HPT processing at room temperature.

Methods:
CuZn5 solid solutions were processed using HPT with a pressure of 4.5 GPa. The thermal behaviors of these materials were studied using differential scanning calorimetry (DSC). Scanning transmission electron microscopy (TEM) in combination with energy dispersive X-ray spectroscopy (EDX) was used to investigate the microstructure and composition of Cu-5at.%Zn (CuZn5), with a focus on GB segregation before and after annealing at different temperatures. Additionally, 4D-STEM (ACOM) was utilized to extract information on grain size distribution, grain orientation, and grain boundary types.

Results:
Annealing of CuZn5 up to 300°C by DSC shows a single exothermic peak at about 275°C. it should be mentioned that no grain growth or changes in the grain orientations occurred up to 200°C. High-angle annular dark-field (HAADF)-STEM imaging helps to understand the effect of Zn on the microstructure and GBs. From the 4D-STEM orientation mapping, the GBs can be classified into low-angle GBs, special coincidence site lattice (CSL) boundaries as well as high-angle general GBs. Together with STEM-EDX, it was found out that low energy boundaries such as Σ3 twin boundary is not enriched by Zn. On the other hand, Zn general GBs are segregated with Zn. This observation agrees well with the theory that solute segregation depends strongly on the GB energy. While special Σ-boundaries have low energy, they are less affected by solute segregation.
Conclusion:
CuZn5 maintains a single-phase structure even after annealing, suggesting that the improved thermal stability of the alloys compared to pure Cu is attributed to the presence of solute content. This is due

to the solute-drag effect [3], hindering the diffusion of GBs and contributing to the enhanced thermal stability of the alloys

Keywords:

Cu alloys; HPT; DSC; ACOM

Reference:

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Microstructural Influence on Sodium Filament Growth in All Solid-state Batteries

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PS-04 (3), Plenary, august 27, 2024, 10:30 - 12:30

Background incl. aims
All solid-state batteries (ASSBs) using metal anode (e.g. lithium and sodium) are projected to possess high energy and power density and avoid the fire risk of liquid electrolyte counterparts. However, few commercial ASSBs working at room temperature are reported due to sluggish kinetics and severe solid-solid interfacial problems. Among various interfacial problems, the dendritic growth potentially leading to cell failure cannot be yet avoided through the high elastic modulus of solid electrolytes (SEs) as initially expected for ASSBs. Apart from the mechanical property of grain boundaries (GBs), their electronic properties are also expected to be responsible for lithium filamentary dendrite growth and penetration in the SEs. Although this intergranular growth mechanism in inorganic SE based lithium ASSBs is well studied, there is still much to be learned for sodium ASSBs. Especially, anisotropic Na+ ion transport presented in grain bulk of the well-known Na superionic conductor (NaSICON) (e.g. Na3Zr2Si2PO14) even with 3D transport path, not to speak of Na-β''-alumina with layered crystal structure and 2D transport path. Its contribution to the overall Na+ ion transport and the sodium filament growth, for instance, at GBs is still unclear.
Methods & Results
Due to the outstanding stability to Na metal, we used polycrystalline Na-β''-alumina SE (Ionotec. Ltd, UK) as a model material to investigate the microstructural impact on the sodium filament growth. A transmission electron microscopy (TEM) suitable Cu | Na-β''-alumina | Au(Pt) multilayer system was prepared with a focused ion beam (FIB) (FEI Strata 400S) equipment. The electric bias was applied on this system through a scanning tunneling microscopy (STM) nanotip (ZEPtools Technology Company) as schematically illustrated in Figure 1a - b. Na+ ion transport can be prompted by the biasing from the Na-β''-alumina towards the Au/Pt layer and cathodic deposition of sodium occurred at the interface between Na-β''-alumina and the Au/Pt layer in a transmission electron microscope (ThermoFischer Scientific Themis Z). Time-series scanning transmission electron microscopy (STEM) imaging (Figure 1c – f) was used to record the morphological changes e.g. filament growth at the interface between the SE and electrode as well as at the GB. In addition, to build a direct correlation between the filament growth and microstructure, automated crystal orientation mapping (ACOM) by precession electron diffraction-assisted 4D STEM (Figure 1g) was conducted across the polycrystalline Na-β''-alumina specimen. It revealed randomly oriented crystals distributed in the polycrystalline Na-β''-alumina mostly connected by random HAGBs, but hardly any coincidence site lattice boundaries have been observed. Furthermore, since the Na+ ion transport can be different in the grains due to the distinctive orientation of the Na+ ion conduction planes and the external electric field, the GB behavior can also be different as the schematic illustration in Figure 1h for corresponding types. In this case, the filament growth occurred at the GB, which can block the ion transport due to the anisotropic character as shown in Figure 1g.

In addition, significant electron beam effects have been excluded by blank beam reference experiments and sodium filament formation has further been confirmed by post-mortem secondary ion mass spectrometry (SIMS) studies. Moreover, the observed microstructure has been used to simulate the anisotropic Na+ ion transport in the Na-β''-alumina. This helps to understand sodium filament formation and how a critical filament network might form leading to the failure of the battery.

Conclusion

In our study, the relationship between the microstructure and sodium filament growth as well as Na+ ion transport was explored through a crystal orientation analysis. Moreover, sodium filament growth occurred at random HAGBs. The anisotropic ion transport can contribute to the Na+ ion transport blockage of GBs. This blockage at GBs seems to facilitate formation of sodium filaments. Therefore, the microstructure including GB types and orientation should be taken into account for optimizing oxide based SE performance both in terms of sodium filament formation as well as overall ionic conductivity.

Keywords:

solid electrolytes, microstructure, filament growth

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Cryo-EM and ED are driving structural studies at the University of Warsaw

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Poster Group 2

Background incl. aims
In 2019 the University of Warsaw purchased and installed one of the first cryo-EM microscopes in the country - the 200kV Glacios equipped with a Falcon3EC camera and a phase plate solution. In the next few years the Cryomicroscopy and Electron Diffraction Core Facility has been established and started providing many local structural biologists and chemists with a direct access to this groundbreaking and Noble-winning cryo-EM technology. To date, there have been only two cryo-EM Core Facilities operating in Poland which provide services in all cryo-EM modalities.
Methods
This poster shows the current possibilities of our Core Facility and a range of services which are offered to our users. We follow the open-access policy and welcome users from both national and international academic institutions as well as industry.
Results
Our recent developments include benchmarking the Single Particle Analysis (SPA) reconstruction of GroEL with GroTAC peptide at the 2.45Å resolution level with local resolution reaching 2.2Å (PDB: 8S32) and the 2.27Å reconstruction of the AbiK bacterial polymerase (PDB:7R06). The results are further enhanced with the upgrade of the microscope to the micro-ED functionality allowing for a rapid structure determination of small molecules and/or proteins based on electron diffraction data. On top of that, we have recently completed a process of building up a €0.5M-worth IT infrastructure support for the cryo-EM data storage (up to 1.6PB dedicated storage space) and expanding on the data processing capabilities (utilising our 200 GPU-based computer cluster) which will be a unique set-up of its kind not only in Poland but also in Central Europe.
Conclusion
We highlight the importance of smaller cryo-EM Core Facilities such as ours to serve as a first point of contact for users, in particular for those who are new to the cryo-EM field and would like to explore different possibilities of getting high quality data prior to applying for the measurement time on high-end 300kV microscopes. We also show that being able to offer all cryo-EM modalities in one instrument (SPA, cryo-ET and micro-ED) significantly boosts a research potential and opens up new possibilities across many Life Science applications.

Keywords:

cryo-EM, SPA, micro-ED, cryo-ET

Reference:

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Observation of logic states of HfO2-based ferroelectric FETs using STEM-DPC

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Poster Group 2

Background incl. aims
In recent years, ferroelectric field-effect transistors (FeFETs) have become valuable alternatives to the flash technology for nonvolatile memory applications. Not only do they incorporate ultra-fast and energy-efficient switching as well as radiation hardness, but they also provide high thermal stability of their logic states. [1, 2]
The low-VT and high-VT states have been intensively studied and characterized, e.g., as described in [1], yet the understanding of the exact electrostatic field in the polarized ferroelectric (FE) gate stack is still subject of discussion. While various TCAD simulations aim to analyze these from a theoretical perspective [3], utilizing scanning transmission electron microscopy (STEM) can deliver more direct insights. Recent work [4] has shown the observation of the FE polarization in STEM utilizing the differential phase contrast (DPC) method. In addition, STEM-DPC analysis of FE devices would provide the opportunity to conduct failure analysis, not only of structural defects in single devices, but also of imperfections in the crystallographic phase and the resulting electrostatic field itself.

Methods
DPC is a STEM imaging method which can visualize local electrostatic and magnetic fields in specimens at high resolution. The fields are detected as a shift of the transmitted bright-field disk on a segmented annular or a pixelated detector resulting from the interaction of the local fields with the incident electron beam. Since the FE polarization in FE materials creates electrostatic fields, the DPC method is suitable for the investigation of local polarization effects in the thin FE layers of FeFETs.
In this study, HfO_2-based FeFET arrays were programmed in a striped pattern, as described in [2]. The FeFETs consisted of 10 nm FE HfO_2, a 1 nm SiO_2 interfacial layer, TiN and a top electrode made from polycrystalline Si. For investigation with the DPC method, TEM specimens of the programmed array were prepared by in-situ lift-out utilizing the focused ion beam (FIB). A convergent electron beam was employed to achieve high spatial resolution and thus investigate the FE polarization states in the FE material.

Results
The local charge carrier density was calculated from the DPC data and analyzed for the investigated FeFETs, respectively. The FeFETs programmed in low-VT and high-VT states showed opposing gradients of the charge carrier density in the FE layer. The direction of the electrostatic fields indicated by those gradients was found to be in accordance with the theoretical direction of the electrostatic fields due to the FE polarization of the material.

Conclusion

In future investigations, the use of DPC can provide deeper insights into the properties of FeFETs. Combining DPC with other techniques, such as precession electron diffraction (PED), could yield additional information about these devices. Employing PED would help reduce diffraction-related artifacts in DPC data. Furthermore, PED can identify grains of both FE and non-FE phases within the material. In addition, observing the in-situ switching behavior of FE devices would contribute significantly to understanding the electric behavior of FeFETs during operation.

Keywords:

DPC, STEM, FeFET, logic states

Reference:

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Novel, low-cost hardware for ‘STEM in SEM’ imaging

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Poster Group 1

Background
Scanning transmission mode imaging can offer better contrast and resolution than secondary electron imaging in nano, soft and biological materials[1]. The concept of ‘STEM in SEM’ is to replicate the improved image quality of scanning transmission electron microscopy (STEM) in lower-cost scanning electron microscopes (SEM). In the hardware discussed in this work, a conversion plate allows the transmitted electrons to produce a signal using the Everhart-Thornley Detector (ETD). This enables bright field STEM imaging to be added to most SEMs through using only an additional sample holder.

Although this is not a new concept [2], little work has been published on the optimisation of the design and operational parameters. A higher efficiency allows a lower probe current to be used. In the SEM, the spot size is limited by the probe current and the brightness of the source. Due to this the improved efficiency in ‘STEM in SEM’ results in improved resolution.

The schematic (a) shows the basic concept of the ‘STEM in SEM’ holder. A standard TEM grid is clamped between plates at the top of the holder. The electron beam passes through the sample and reaches a conversion plate. Here transmitted electrons generate secondary electrons within the plate which may be detected by the ETD. Furthermore, an aperture may be inserted between the sample and the conversion plate to control the acceptance angle of the detector.

Methods
The optimisation process consisted of a series of experiments analysing the effect of each parameter in isolation.

The efficiency of the conversion of transmitted electrons to secondary electrons was simulated using CASINO, a Monte Carlo method simulation program [3] . The modelling indicated an improved conversion yield when an oxide forming metal coats the surface. The model was validated by comparing the ETD signal produced when a range of coated glass substrates were scanned with a focussed electron beam. The increase in secondary electron yield due to a sputtered aluminium surface coating was verified experimentally. As secondary electrons only escape from within a few nanometres of the surface, only a coating of high-yield material is needed. This was implemented at a low cost by sputtering a gold-aluminium bilayer coating onto glass substrate.

Significant geometrical optimisations in both the angle of the conversion plate and the working distance were found. The image brightness was measured whilst varying the conversion plate angle. It was found that the efficiency of the plate has a significant peak at the optimised angle. It is thought that this is for two reasons. Firstly, as the angle increases, the transmitted electrons travel a greater distance within the material at a depth at which secondary electrons can escape. Secondly, the alignment of the emitted electrons to the ETD improves the detection efficiency.

One of the challenges with 'STEM in SEM' is the convolution of the desired transmitted signal with undesirable secondary electrons from the sample. The difference in detection efficiency of the ETD at different working distances was used to combat this. The ETD has poor detection efficiencies close to the polepiece. When using a sample working distance of 3mm and a converter plate working distance of 18mm, the signal was dominated by the transmission mode. The noise was further reduced by a reduction of the ETD cage bias from the 250-400V typically used to 50V. This limited the detected electrons to those emitted aligned closely to the detector.

The overall performance of the 'STEM in SEM 'converter was evaluated by imaging drop cast 40nm and 20nm nominal size silver nanoparticles on a holey carbon TEM grid. Ostwald ripening of the nanoparticles produced a broad distribution of particle sizes for resolution testing. The samples were imaged at 30kV using a tungsten filament SEM. The other microscope parameters were adjusted to suit the two imaging methods.

Results
The figure shows silver nanoparticles on a holey carbon film observed whilst using a tungsten filament SEM in (b) secondary electron mode at 30kX magnification and (c) using the STEM converter at 75kX magnification. The contrast was improved using the optimised 'STEM in SEM' converter when compared to secondary electron imaging. An improvement in contrast to noise ratio was seen in the STEM images. The improved contrast enables faster and easier imaging of the nanoparticles in the STEM mode. The smallest particles seen in secondary electron mode were 75nm in diameter. With the STEM converter, this was improved to 25nm.

Conclusion
The optimised 'STEM in SEM' converter presented here is a low-cost method to enable transmission mode imaging in the SEM. It has been shown this can be utilised to improve the image quality when imaging nanoparticles. Although, this does not represent new functionality compared to dedicated silicon STEM detectors, it is a much more accessible method of adding STEM capabilities to an SEM.

Keywords:

STEM, SEM, nanoparticles, low-voltage STEM

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Structural phase transition induced microstructure changes in V2O3 based hybrid magnetic heterostructures

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Poster Group 2

Vanadiumsesquioxide (V_2O_3) stands out in the realm of transition metal oxides for its distinctive and multifaceted phase transitions, offering a rich ground for exploration in the context of structural, electrical and magnetic properties [1, 2]. At room temperature, V_2O_3 is in a rhombohedral phase, exhibiting metallic properties and upon cooling to below ~150 K it undergoes a structural phase transition to an insulating monoclinic phase. Through the transition the material also changes from a paramagnetic state to a low temperature antiferromagnetic state and has been observed to induce an exchange bias into overlying magnetic layers [3].

In this work we present an investigation on the interplay between the microstructure of V_2O_3 thin films, deposited using reactive dc magnetron sputtering, and their structural properties during the phase transition. Specifically, we examine the changes in the microstructure during the phase coexistence region of the V_2O_3 layer and how these changes correlate with alterations in the magnetic properties of overlying magnetic layers in V_2O_3/Ni heterostructures [4].

Using scanning transmission electron microscopy (STEM) using high-angle annular dark-field (HAADF-STEM) imaging, energy dispersive x-ray analysis (STEM-EDX) and electron energy loss spectroscopy (EELS) we investigate the stoichiometric composition of the films and confirm their crystalline quality at the sub-nm lateral scale and observe an atomically flat interface between the Al_2O_3 substrate and the V_2O_3 layer, as well as a sharp interface between V_2O_3 and the overlying magnetic Ni layer.

The simultaneous presence of two phases in V_2O_3 during its structural phase transition was identified with temperature dependent high resolution x-ray diffraction measurements performed at the BM28 (XMaS) beamline at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. The measurements reveal an increase in surface roughness during the transition which is attributed to the coexistence of the two phases in different regions of the film. During the phase coexistence region we observe a concomitant increase in the coercivity of the magnetic layer correlated with the increased roughness of the V_2O_3 surface.

Keywords:

Phase transitions, STEM, Magnetism, Heterostructures

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In situ closed-cell microscopy study of Ti3C2Tz MXene

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Poster Group 2

Background incl. aims
MXenes are referred to as 2D transition metal carbides, nitrides, or carbonitrides and first reported in 2011. They are described by the general Mn+1XnTz (n=1,2 or 3) formula, where M is a transition metal, X stands for C or/and N and Tz represents the surface terminating species [1]. Owning to their structural and chemical diversity, MXenes enable the wide variety of properties, which makes them competitive in a wide range of applications including catalysis, energy storage, supercapacitors, sensors, spintronics, thermoelectrics and beyond [2].
Despite the immense progress in the field of MXene, a number of unresolved challenges remains, including a fundamental understanding of post synthesis stability and processing of MXenes at application relevant environments. In particularly, the detailed knowledge of the degradation mechanisms, in the environment(s) containing presence of water, is highly debated in the literature.
Previously, transmission electron microscopy (TEM) studies provided atomic level insights into the Ti3C2Tz structure and surfaces [3]. In situ TEM was applied to study Ti3C2Tz surface termination alternations [4]. Post synthesis in situ gas heating treatments of Ti3C2Tz was successfully demonstrated via environmental TEM studies [5].
The introduction of environmental closed-cell holders brings unique opportunities for the study of MXenes under real-world conditions closer to practical processing and/or application environments inside TEM.
In this study, we employed in situ closed-cell TEM to study the role of water on thermal stability of Ti3C2Tz MXene sheets during annealing in Ar or N2 atmospheres.

Methods
Ti3C2Tz MXene sheets were synthesized by the previously reported MILD method in which selective extraction of Al from Ti3AlC2 MAX phase and delamination of the layers were achieved using hydrochloric acid and lithium fluoride.
In situ experiments were performed in the Linköping double Cs corrected FEI Titan3 60-300, using a closed-cell gas heating system (Protochips, Atmosphere). During pretreatment, holder was pumped and purge with Ar to remove residual gasses from the holder tip. This was followed by continues Ar flow while annealing at 300°C for 60 min. In situ heating of Ti3C2Tz was accomplished by increasing the temperature from 600 °C to 1000 °C in steps of 100 °C and holding for 15 min at each step while continuously flowing Ar (inert) or N2 (no affinity for Ti3C2Tz) gasses at 760 torr.
After each heating step the temperature was lowered to 300 °C for acquisition of images and spectra. The structural and chemical evolution of Ti3C2Tz was tracked using high angle annular dark field STEM (HAADF-STEM) imaging and electron energy loss spectroscopy (EELS). A residual gas analyser (RGA) was used to monitor the status of the experimental and residual gases in the system.

Results

To explore the effect of water on thermal stability at atmospheric pressures, Ti3C2Tz was annealed to high temperatures while been kept at flowing Ar (99,9999% purity) or N2 (99,9999% purity) gasses at 760 torr.
The graph shows a series of HAADF-STEM images recorded during in situ heating of Ti3C2Tz during exposure to flowing N2 at 760 Torr. The morphology of Ti3C2Tz sheets remained largely unaffected up to 800 °C as observed by imaging and tracking Ti-L2,3 fine edge structure evolution. At 900 °C the morphology of the Ti3C2Tz changed entirely as it decomposed to titanium oxide nano particles. At 1000 °C the titanium oxide particles coarsened while the amorphous SiN window undergone the crystallization.
Independent of the applied atmosphere (Ar or N2), the decomposition of Ti3C2Tz resulted in the formation to titanium oxide nanoparticles at high temperatures. Water pressure decrease, achieved via extensive baking of the system, from ~3x10-7 torr to ~6x10-9 torr delayed titanium oxide particle formation and coarsening by more than 100 °C. The lower water level also suppressed SiN window crystallization at 1000 °C. Thus, the oxidation is suggested to occur from the residual water present in the system while the thermal activation offset for oxidation could be directly linked to the water pressure level.

Conclusions
The study uncovered the effect of water on thermal behaviour of Ti3C2Tz during annealing at Ar or N2 atmospheres. Residual water has the detrimental effect on the offset of the observed oxidation phenomena. Consequently, obtained results increase understanding of factors and conditions responsible for Ti3C2Tz environmental stability. As important, study highlights the importance of water level control for MXene gas reaction experiments using in situ closed-cell TEM and beyond.

Keywords:

MXene, insitu closed-cell, thermal stability

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Exploring Cell-Material Interactions at Focal Adhesion Points

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Poster Group 1

Background
In tissue engineering, electrospun fibers are pivotal scaffolds, shaping cell interactions through their mechanical strength and surface characteristics. Focal adhesion sites, multi-protein complexes, regulating cell adhesion dynamics, form mechanical links between intracellular actin bundles and the substrate. Widely used in bioengineering, PMMA fibers play a crucial role as an integral component of bone implants, serving as an adhesive platform for various cell types (1). The aim of the research is to investigate the distribution and structure of proteins paxillin and vinculin in focal adhesion (FA) sites using advanced microscopy techniques, particularly AiryScan confocal super-resolution microscopy and the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) cluster algorithm. Moreover, this research is performed on cells bind to polymer fibers commonly used in tissue scaffolds and compared to standard glass slides. Therefore, our results contribute to designing scaffold and understanding the interactions between cells and materials applied in/for tissue regeneration.
Methods
PMMA fibers were electrospuned using the EC-DIG apparatus with climate control. Studies involved human osteoblast-like cell line MG-63 on PMMA electrospun fibers. Cells were cultured under standard conditions, fixed, and permeabilized after 3 days. Actin filaments were visualized with Alexa Fluor 488 Phalloidin, and nuclear DNA was stained with DAPI. Focal adhesion proteins (vinculin and paxillin) were labeled with immunofluorescence. Confocal microscopy, specifically Zeiss LSM 900 with the high-resolution concept Airyscan, captured multicolor 3D microscopy images of cells connecting to PMMA fibers. Image analysis was performed by using ImageJ.
Results
AiryScan microscopy offered detailed insight into cell adhesion onto PMMA fibers, confirming the binding process. Additionally, DBSCAN cluster analysis revealed substrate-specific correlations in osteoblast behavior, providing a quantitative understanding of cell-PMMA interactions. Effective adhesion correlated with well-organized, larger focal adhesions characterized by increased protein accumulation. Interestingly, a shift in paxillin and vinculin signals was observed during cell attachment to both glass and polymer fibers. Focal adhesions on polymer fibers appeared smaller and elliptical but exhibited higher protein density compared to glass (2). These attributes, influenced by paxillin and vinculin, likely signify cell adhesion strength. This innovative cluster analysis uncovers variations in adhesion clusters, offering valuable insights for scaffold design refinement, diverse substrate adhesion evaluation, and enhanced cellular interactions in biomedical applications.
Conclusion
We introduce a new method for analyzing protein distribution in cell adhesion to polymer fibers, crucial for tissue engineering scaffolds. Our study reveals significant differences in adhesion complex distribution compared to glass, with a focus on vinculin and paxillin. By quantitatively comparing osteoblast adhesion on glass and polymer scaffolds, we provide the first detailed analysis in a model

incorporating polymer fibers, paving the way for understanding cell-material interactions in medical biomaterial research.

Acknowledgements
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Keywords:

adhesion, fibers, PMMA, paxillin, Airyscan

Reference:

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The effect of fixation-induced cell blebbing: how to minimize a loss of proteins from cells?

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LS-05, Lecture Theater 5, august 26, 2024, 14:00 - 15:00

In the course of the preparation of biological samples for various microscopy techniques, the first and the most essential step is a fixation. The efficiency of the fixation procedure is estimated by two main factors: the preservation of the cellular constituents and the suitability for further treatments like immunolabelling. However, till now the detailed mechanisms of the cell fixation are not fully understood and we rely mostly on empirical experiences. We concentrated on studying the fixation-induced cell blebbing, and searched for the way to minimize this deteriorating effect. By means of different microscopy methods, we quantitatively assessed the loss of cytoplasmic content during the rupture of blebs. With holography microscopy time-lapse experiments, we found that the key points are the formation and rupture of blebs, and we discuss the possible mechanism of blebs appearance. Importantly, it was shown that up to 30% of soluble proteins can be lost from cytoplasm after the blebs rupture. Based on these data, we tested a wide range of fixation mixtures applied during different time intervals to minimize the loss of cytoplasmic proteins. Taking into account different quantitative parameters, we determined the optimal procedures to fix the cells. Finally, we provide some recommendations on the fixation protocols which are suitable for future experiments.

Keywords:

formaldehyde fixation, blebbing, artefacts, microscopy

Reference:

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Platform for the in-situ measurement of magnetic transport properties in the transmission electron microscope

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Background incl. aims
In order to unveil the magneto-resistive fingerprint of Skyrmions, it is necessary to measure the material's magnetic field dependence of the Hall resistance while acquiring Lorentz TEM (L-TEM) images at the same time [1, 2]. We demonstrate a measurement platform allowing for in-situ magnetic transport measurements in a TEM [3] by utilizing a DENSsolutions biasing holder and a Hall sensor for quantification of the objective lens' magnetic field. We aim to correlate the Hall effect in skyrmionic materials with the magnetic field dependent occurrence of topologically protected magnetic phases such as the helical phase and Skyrmions [4].

Methods
In-situ L-TEM investigations were conducted using a FEI Themis-Z double-corrected microscope (300 kV, X-FEG) equipped with a fast Ceta-M camera. A DENSsolutions Lightning holder with spring contacts connected to electrical feedthroughs was used to measure the Hall voltage.
In Lorentz mode, the objective lens of the microscope was used to apply an external magnetic field perpendicular to the sample plane. The magnetic field of the objective lens was calibrated with an Asensor Technology analog Hall sensor fitted into the in-situ biasing holder, see Fig. 1 (a). A Co8Zn9Mn3 TEM lamella was cut using a Thermofisher G4 Hydra Plasma FIB. Subsequently, the lamella was positioned on a commercial chip and soldered to the underlying substrate by Pt deposition in the FIB. The DENSsolutions nano-chip was then further modified for the Hall measurements: In order to realise a four-point measurement geometry, insulating SiOx patches were deposited on top of the inner tracks. By writing W tracks over the SiOx, the conductive paths were bridged (cf. Fig. 1 (b)).

Results & Conclusions
The demonstrated in-situ setup allows for the simultaneous acquisition of L-TEM images and the measurement of the Hall voltage as function of the out-of-plane magnetic field, which we can control precisely by tuning the objective lens excitation. Fig. 1 (c) shows the Skyrmion phase in Co8Zn9Mn3 at 142 mT. Although the lamella is hosting Skyrmions, no signature of topological solitons is observable in the Hall voltage. The observed Skyrmions do not exhibit any distinctive characteristics in the magnetic transport measurements.

Graphic caption
Figure 1 (a) Magnetic field of the objective lens of a FEI Themis-Z double-corrected TEM as measured with an analog Hall sensor fitted into a DENSsolutions Lightning holder (inset). (b) SEM image of the

modified MEMS chip with a Co8Zn9Mn3 TEM lamella, showing the FIB deposited W tracks and SiOx rectangles. (c) L-TEM image of the Skyrmion phase in Co8Zn9Mn3 at 142 mT.

Keywords:

In-situ, magnetic transport, Lorentz TEM

Reference:

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5. S. Schneider gratefully acknowledges financial support through the Walter Benjamin Programme of the German Research Foundation (DFG) within project 458685885.

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The development of multimodal imaging with functional silica nanoparticle

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Poster Group 1

Background incl. aims
Organic silica nanoparticles (SiNPs) can be customized in various ways, such as the introduction of fluorescent dyes, addition of metal particles, and surface modification with some chemicals. It was shown that SiNPs containing fluorescent dyes with iron cores could be used for in vivo multimodal MRI and light microscopy imaging. We investigated the SiNPs with fluorescent dye is able to use as the tag for correlative light-electron microscopy, and developed novel SiNPs with heavy metals for correlative light-electron microscopy (CLEM), multicolor CLEM, and multimodal imaging with micro CT.

Methods
(1)We introduced SiNPs containing fluorescent dyes into Paramecium and performed CLEM observations.
(2)We made SiNPs contain green fluorecence dye with gold nano particles (green Au SiNPs) and SiNPs contain red fluorecense dye with iron nano particles (Red Fe SiNPs), deveoloped multicolor CLEM combined with the EDS mapping. After each of the produced particles was introduced into cultured cells, the cells were mixed and co-cultured, and fluorescence observation, electron microscope observation, and EDS mapping were performed.
(3)We performed multimodal imaging using light microscopy, µCT, and electron microscopy using the spleens of the mice intravenously injected with SiNPs contain fluorecence dye with gold nano particles .

Results
(1) The fluorescence of SiNPs introduced into Paramecium was maintained even after sample preparation for electron microscopy, and the fluorescence could be observed in ultra-thin sections. This result indicates that fluorescent dyes incorporated into SiNPs are resistant to chemical treatments for electron microscopy (Pre-fixation with glutaraldehyde, post-fixation with osmium tetroxide, dehydration with ethanol, embedding with epoxy resin, ultra-thin sectioning). In this Paramecium sample, we were able to observe SiNPs within the phagosomes by TEM in regions where fluorescence was observed by light microscopy. However, it was difficult to identify the region where the particles were introduced from the low-magnification electron microscope image, because the SiNPs have a weak contrast in the electron microscope image. Therefore, we carried gold particles on the surface of SiNPs containing fluorescent dyes to enhance the contrast in electron microscopic images. This particle had a very high contrast in electron microscope observation, and it was possible to identify its location even in very low-magnification observation with an electron microscope.
(2) As a result of EDS mapping, although iron could not be detected, particles with gold added and particles without gold added can be distinguish and this localization correlates with the fluorescence image.
(3) We succeeded in CLEM observation by introducing SiNPs at 1 µg/ml, and by introducing SiNPs at 5 µg/ml or more, it became possible to visualize SiNPs in tissues using µCT.

Conclusion
We succeed to perform multicolor CLEM by combining heavy metal-loaded fluorescent organic silica nanoparticles and EDS mapping. By increasing the introduction amount of SiNPs carrying gold particles, it is possible to visualize with µCT, and multimodal imaging of optical microscopy-µCT-electron microscopy becomes possible. It suggests that it is possible to overcome the barriers between devices by synthesizing new SiNPs that match the principle of the device to be measured.

Keywords:

Multicolor CLEM, EDS mapping, µCT

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Hybrid electrospun scaffolds for enhanced collagen formation in regeneration processes: electron and confocal microscopy analysis

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Poster Group 1

Background
Extracellular matrix (ECM) of bone tissue is a natural composite containing organic and inorganic components, that can be biomimicked using electrospun fibers. Addition of ceramic filler into the polymer fibers can enhance the mechanical properties as well as improve bioactivity. However, to obtain the best hybrid scaffold the uniform distribution of particles and reduction of aggregations are necessary [1]. Here, the careful design of composite fibers can trigger cellular adhesion, proliferation and tissue regeneration via enhance collagen formation in the in vitro cell culture [2]. Therefore, the goal of this work is to optimize and verified production of hybrid electrospun fibers for effective bone tissue scaffolds including visualization using advance electron and confocal microscopy analysis.
Methods
Blend and co-axial electrospinning were employed to manufacture bone scaffolds of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with incorporated titanium dioxide nanoparticles (TiO_2) using TechNOVA electrospinning device [3]. Morphology of obtained fibers was evaluated by scanning electron microscopy (SEM) with secondary electron detector (Merlin, Zeiss). Distribution and aggregation of TiO_2 within polymer fibers were studied by SEM imaging with back scattered detector (BSE), energy dispersive X-Ray spectroscopy (EDS, detector Brucker). Additionally similar analysis was also performed at the cross-sections prepared by focused ion beam (FIB, Crossbeam 350, Zeiss). In vitro cell culture study was performed with osteoblast cells up to 14 days. Cell proliferation and morphology in response to various scaffolds was verified after 1, 3 and 7 days of incubation. The collagen formation was confirmed by immunofluorescence staining and visualized by confocal laser scanning microscopy (CLSM, LSM 900, Zeiss).
Results
Over 20% increase in fiber diameter was observed when PHBV was enriched with TiO_2 nanoparticles, reaching 3.84 µm for sample prepared by co-axial process. Blend fibers had smooth surface with visible large particles aggregations, locally exceeding 5 μm^2, in majority covered by polymer. On the other hand, the fibers prepared using co-axial nozzle had core-shell morphology, with polymer fibers homogenously decorated with ceramic particles with significantly smaller aggregations up to 0.3 μm^2. In response to core-shell fibers cells exhibited significantly higher proliferation after 7 days with excellent spreading within the scaffold structure. Additionally, they produced vast network of collagen fibers as the important stage of ECM formation, which was not observed in such extent for any other tested scaffold.
Conclusion
Current study shows the importance of electrospinning strategy for single-step production of bioactive scaffolds supporting bone regeneration. For cellular guidance it is essential not only to provide composite morphology but also to ensure proper particles distribution and availability, which was achieved by co-axial electrospinning. The presence of ceramic nanoparticles, effectively integrated onto the surface of polymer fibers, enhances cells adhesion, ingrowth and promotes the formation of ECM, which are necessary in tissue regeneration processes.

Acknowledgements
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Keywords:

Organic-inorganic composites, bone, collagen staining

Reference:

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Self-assembly of amphiphilic dendrimers investigated by standard, cryo and liquid TEM

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Poster Group 1

The self-assembly of amphiphilic molecules represents a captivating avenue for the creation of innovative materials in drug delivery. In our investigation, we explore the assembling properties of amphiphilic poly(amidoamine) dendrimers, focusing on two distinct dendrimers: one terminated with tertiary amine groups N(CH3)2 denoted as dendrimer A, and another terminated with primary amine groups NH2 denoted as dendrimer B. Our study highlight the influence of surface chemical modifications on their size and packing dynamics, unveiling pathways towards finely tuned molecular architectures.
Complementary techniques of standard, Cryo and Liquid TEM are used to characterize the molecular assemblies.
Concerning dendrimer A, all the TEM technics are consistent with the formation of small nanomicelles ranging in size from 15 to 25 nm, as depicted in figures 1a and b. However, liquid TEM provides additional insights into the arrangement of dendrimers within these micelles. In the expanded view of a single micelle (fig. 1c), regularly spaced dark spots inside the micelle can be observed. The average distance between them is about 5 nm, which is comparable to the diameter of the volume containing the hydrophilic heads with tertiary amines. This supplementary information enriches our knowledge of the intricate assembly of dendrimer A within the micellar structure.
On the other hand, as seen in figures 2a and b, dendrimers B terminated with primary amine assemble in larger particles around 100 nm. Details on the assembly is clearly seen in the liquid TEM images (fig. 2b,c). The magnified image of a particle in fig. 2c corresponds to the aggregation of several micelles with size around (15- 25 nm) into a large particle of around 100 nm.
The Israelachvili assembly model's prediction1 aligns closely with the behavior of dendrimer A, illustrating its propensity to assemble into nanomicelle. However, the assembly process for dendrimer B unfolds uniquely, showcasing distinctions that necessitate a closer examination only made possible through liquid TEM. This sophisticated imaging technique unveils intricate structural nuances that had remained elusive until now, marking a significant advancement in our understanding of dendrimer assembly.
Conclusion : Our study reveals that dendrimer A exhibits consistent formation of small nanomicelles (fig. 3a) of around 20 nm, supported by various TEM techniques. Meanwhile, dendrimer B displays distinct assembly behavior (fig. 3b), forming larger particles around 100 nm. The intricate details of dendrimer A's micelle and the distinctive assembly process of dendrimer B emphasize the critical role of advanced imaging techniques such as liquid TEM in unraveling complex structural properties. This advancement significantly contributes to our comprehension of dendrimer assembly dynamics.

Keywords:

Nanomicelles- Cryo TEM- Liquid TEM

Reference:

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Microglial contactology–mapping the connection network of microglia from the micrometer to the nanometer range

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Poster Group 1

The dynamic development of microscopic techniques offers new opportunities for the comprehensive study of biological processes. However, the application of methods covering a wide resolution range requires the right questions to be asked and the definition of a precise target, without which it is difficult to obtain relevant results. In the following, I would like to demonstrate the application of microscopic modalities with different resolution capabilities in the field of neuroscience by highlighting examples from my own work.
Our research focuses on microglial cells which are the main immune cells of the brain. These cells are essential for the normal functioning of the brain and play an important role in maintaining brain homeostasis, proper development and function of neurons, the formation of their synaptic connections and the regulation of inflammatory processes. They also play a role in the control of cerebral blood flow. Carrying out these complex tasks requires continuous communication between the microglia and the other cells of the brain. However, our current understanding of the cell-cell interactions of microglia with neurons, other glial cells and blood vessel cells is rather incomplete.
We uses different microscopic methods, ranging from micrometre magnification to nanometre resolution, to reveal the connectivity of microglial cells. Starting from the lower resolution, we first investigated the location and heterogeneity of glial cells (microglia, astrocytes, oligodendrocytes) and neurons in the cerebral cortex using slide scanner microscope. With this method, we can obtain information on the distribution and quantity of the cells under investigation in a small but fast way for many samples. We then used a higher-resolution confocal laser scanning microscope to take a closer look at how many different cells a microglial cell interacts with at a given time point and how many different cells it interacts with. Then, to achieve nanometre resolution, we used scanning electron microscopy serial section tomography to verify that the putative contact sites visualised by diffraction-limited confocal microscopy are real direct contacts, where the plasma membranes of microglia and other cells are brought within nanometre proximity of each other. Furthermore, these images can be used to reveal the intracellular ultrastructure characteristic of the different contacts.
In order to study the dynamics and function of these connections, we use two-photon microscopy in vivo, in a departure from the mainly anatomical microscopy techniques used so far.Our studies will allow us to determine the lifetime, stability and signalling pathway involved in the connection of each contact.
By combining these different microscopic techniques, we can gain a complete view of both the anatomical structure and the functional role of microglial cells in their interactions with other cells. Our studies will be carried out in mouse, human adult and elderly samples, which will help us to understand the subcellular processes underlying ageing and related neurodegenerative neurodegenerative diseases such as Alzheimer's disease.
The project KDP-12-10/PALY-2022 was funded by the Ministry of Culture and Innovation with support from the National Research Development and Innovation Fund under the KDP-2021 grant scheme. The project was supported by the Richter Gedeon Talentum Foundation (1103 Budapest, Gyömrői u. 19-21.).

Keywords:

microglia, SEM, CLSM, somatic junction

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Insights on the disordered nature in amorphous-based anode materials from Electron Pair Distribution Function

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Poster Group 1

In the field of material science, the ability to determine structures at high spatial resolution is paramount for understanding their properties. While traditional X-ray and electron diffraction techniques have been successful in determining crystalline structures, the need for new techniques is evident. Conventional methods fall short in providing detailed structural information for disordered structures, such as those found in nanostructured and amorphous materials, making our research on the disordered nature of anode materials all the more significant.

The pair distribution function (PDF) method based on X-ray and neutron diffraction is widely employed for providing quantitative information in amorphous materials [1]. Nonetheless, understanding medium-range structural order in amorphous materials is non-trivial, where the position of atoms cannot be assigned by any equation based on translation vectors. Electron Diffraction (ED) related PDF (ePDF) in Transmission Electron Microscope has the advantage of investigating ordering locally. However, ePDF-based analyses may be affected by dynamical effects, altering the scattering intensities.

This work aims to expand the developed methodology to battery materials research by employing electron diffraction data to obtain PDF coupled with low-loss electron energy loss spectroscopy [2]. This will enable us to delve into the role of volume expansion during lithiation in silicon-carbon-based anode materials, which is a crucial aspect of the electrode's functioning.

Keywords:

S/TEM, ePDF, EELS, batteries

Reference:

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The nature of diffuse scattering in fcc metal alloys – investigation by 3D-ED and 4D-STEM

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PS-02 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background and aims:
Previous investigations of some fcc metal alloys have shown the presence of diffuse 'spots' between Bragg reflections at <111> and <112> zone axes. These have been attributed to various effects including thermal diffuse scattering, surface steps, stacking faults or nano twins, short range chemical ordering and even relrod spiking from HOLZ [1,2]. Such diffuse scattering may arises from the presence of local crystalline order/disorder in the alloy. Where it occurs, 3D 'shape' and strength of the diffuse scattering can reveal the origin of the disorder. For example, a linear defect in real space would give rise to a 'plane' of diffuse scattering while a planar defect would produce a diffuse 'rod' [3]. We hypotheses that the 1/3 {422} and 1/2 {311} diffuse 'spots' seen previously are in fact where the Ewald sphere intersects with a <111>* reciprocal lattice 'rod'. Here we use a combination of 3D-ED and scanning electron diffraction (SED), a variant of 4D-STEM, to reveal the nature of the diffuse scattering.

Method:
Two fcc alloys were investigated. The first was a single crystal Ni-base superalloy (CMSX-4) which has a γ matrix (A1 structure) and γ' precipitates ($L1_2$ structure). This specimen was taken from a <001> tensile sample and electropolished as a 3 mm disc. The second sample was an age-hardened AuPt24Pd alloy which was homogenised and then cooled at 5 K/min. This second alloy undergoes phase separation into two regions of chemical segregation: one rich in Au and Pd, and another rich in Pt. The alloy specimen was a TEM foil that was prepared by FIB lift out.

A FEI Tecnai Osiris 80-200 TEM was used to acquire selected area diffraction patterns and HR-TEM images of the specimens. A Thermo Fisher Spectra 300 TEM was used for the 3D-ED, SED and complementary STEM-EDX experiments.

3D-ED was performed using the continuous rotation electron diffraction (cRED) method. Here, the sample is rotated continuously so that a 'movie' with many hundreds of frames is acquired across a tilt range, in this case +/- 60 °. By extracting these movie frames, and considering each as a central section through 3D reciprocal space, a full diffraction 'tomogram' can be reconstructed. This technique has been used primarily for structural determination using the 3D distribution of Bragg reflections. However, here we consider the 3D distribution of diffuse intensity between the Bragg reflections.

The 3D reconstruction was achieved using a combination of python scripts (using the open-source packages hyperspy and pyXem) and the program PETS2 [4]; the 3D

visualisation was performed in either Vesta or Avizo. Strain maps (using SED) and associated elemental maps (EDX) were determined using hyperspy and pyXem.

Results:
Figure 1(a) shows a zero order Laue zone (ZOLZ) section (Fourier slice) taken from the reconstructed 3D reciprocal space of the Ni-base superalloy sample. The zone axis orientation is <110> and the weak Bragg reflections are from the ordered γ' precipitates. In this section, we can see clear evidence of lines of diffuse scattering in the <111>* direction, which is likely to originate from the γ phase. Moreover, these lines of diffuse scattering do not appear along the <111>* rows that pass through the origin as indicated by the dashed arrows in figure 1(a). Figure 1(b) shows a ZOLZ slice from the <111> zone axis. The diffuse scattering is now present as faint, relatively broad 'spots' of diffuse scattering at the 1/3 {422} position. Similar results were obtained using conventional selected area diffraction and SED. Figure 1(c) shows a FOLZ slice from another <110> axis, 60 degrees from that in (a). This illustrates how information can be obtained with this method that is not readily accessible through conventional 2D diffraction. Again diffuse 'lines' are seen in figure 1(c) between Bragg reflections. It is notable that these lines are shifted with respect to those in figure 1(a) as they were obtained from a higher section in reciprocal space.

Similar diffraction results, shown in Figure 1(d), are seen for the AuPt24Pd alloy. The strain and EDX maps (not shown here) indicate clear phase separation at the 10-20 nm scale in the <110> and <100> directions but these orientations rule out that this is the origin of the <111>* diffuse scattering seen in the diffraction patterns.

The results described above are only consistent with the 3D model of diffuse scattering illustrated in figure 1(e) in which rods of diffuse intensity run parallel to <111>*.

Given the nature of the diffuse scattering seen in these alloys it is likely that it is related to a change of local atomic structure in the {111} planes. The lack of diffuse scattering through the origin indicates that any atomic displacement related to the local structure in the {111} planes is transverse in nature – an analogy would be the 'streaking' seen in <110> patterns from fcc crystals arising from stacking faults with shear vectors 1/6 <112> in the {111} planes. However, here, given the rather broad nature of the diffuse scattering it is speculated that its origin may be highly localised chemical order within the {111} planes, leading to small atomic displacements in the plane because of the change in inter-atomic bonding between atom species. Such small, localised shears in the {111} plane would be expected to give rise to the <111>* diffuse rods seen.

Conclusions:
3D-ED has be used to determine the 3D structure of diffuse scattering in fcc metallic alloys. Through the analysis of Laue zone sections, taken from the 3D reconstruction (tomogram) of the diffraction tilt series, it has been shown that the diffuse scattering can be described using a series of <111>* diffuse rods whose origin is likely to be small transverse displacements (shears) in the {111} planes brought about by changes in local atomic structure [5].

Keywords:

Streaking, Diffuse Scattering, 3D-ED, SED

Reference:

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Microstructural assessment of mechanically alloyed low activation 9-Cr oxide-dispersion strengthened steels

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Poster Group 2

Background incl. aims
In the foreseeable future, it will become more and more necessary to boost energy efficiency to tackle challenges imposed by climate change. Therefore, higher process temperatures are fundamental. Oxide dispersion strengthened (ODS) steels can meet these requirements since they were developed as structural materials in extreme environments such as for example gas turbines, aerospace applications, and nuclear fusion reactors [1]. The latter application limits the use of acceptable elements to those exhibiting low activation [2].
ODS steels represent a class of advanced structural materials that possess compared to conventional type steels superior high-temperature strength and stability. The origin of these exceptional performance lies in their micro and nanostructure, which is characterized by a fine dispersion of nanoscale oxide particles in the steel matrix. These oxide nanoparticles hamper the motion of dislocations and grain boundaries by pinning. Typical oxide nanoparticles consist of Y_2O_3, Y-Ti oxides, Al_2O_3 or similar stable oxides [3].
Electron microscopy is used to characterize grain sizes as well as the size, number densities, structure and chemistry of oxide nanoparticles [3]. Additionally, unwanted nanoscale process remnants need to be monitored.
The aim was to study the mechanical performance as well as microstructure of the ODS steel to reduce the time required for mechanical alloying. When scaling to industrial-scale quantities this helps to reduce production costs.

Methods
Several kilograms of ODS material (Fe-9Cr-1W-0.2Ti+Y_2O_3) was processed by mechanical alloying of the powder under Ar atmosphere in a Simoloyer CM20 and after 5 h, 15 h, and 25 h milling time material was extracted. Afterwards, the material was HIPed at 1150°C and 100 MPa. Each material was tested for hardness and yield strength. Finally, the samples were analyzed by scanning and transmission electron microscopy using a Zeiss Merlin and a Thermofisher Talos F200X. Mainly, electron-backscatter diffraction (EBSD), energy-dispersive X-ray analysis (EDX) and electron energy-loss spectroscopy (EELS) were used.

Results
EBSD maps were employed to determine the grain sizes across different milling times using OIM Analysis. It was found that there is no significant variation in the grain sizes with milling time. In numbers, the average grain size evolved just slightly between 4.6 µm (5 h) and 4.8 µm (25 h) if evaluation with respect to number fraction was chosen.
Thus, TEM experiments were carried out to investigate the nanostructure of the materials and to look for trends depending on the milling time. STEM-EDX elemental mapping revealed that a trend that ODS particles split into two types at short milling times: Y-rich and Ti-rich, whereas at longer

milling times (> 15 h) Y-Ti oxides are observed. Average ODS particle sizes and particle number densities extracted from STEM-EDX elemental mappings are between 25 nm and 50 nm and in the order of 10^{20} particles per m^3 for all samples. The ODS particle size increases from 25 nm at 15 h to 50 nm at 25 h milling time whereas the number densities seem not to depend on the milling time. However, it was also found for all analyzed samples that the distribution of ODS particles is not homogeneous in all grains. In some sample regions, fine ODS particles are present inside grains whereas coarser particles are observed at grain boundaries. A meticulous analysis by EDX and EELS of single ODS particles at higher magnification revealed that some can have a complex chemical structure.
Additionally, the presence of Al-containing nanoparticles was noted, likely stemming from unintentional introduction during the production process. Moreover, nanometer-sized Ar bubbles, attributed to milling in an Ar atmosphere, were observed adhering to ODS particles. These bubbles exhibited an increasing number density with milling time, while maintaining a consistent average size.
Further analysis revealed a few micrometer-sized $M_{23}C_6$-type precipitates in some areas on the sample that can be attributed to excess carbon.
Tensile tests were conducted within the temperature range of 500°C and 800°C. The obtained yield strengths were about 350 MPa and 160 MPa for 500°C and 800°C, respectively. Hardness measurements exhibited no discernible correlation with milling time.

Conclusion
Electron microscopy revealed that a milling time between 5 h and 25 h does not change the microstructure significantly. ODS particle distribution varied, with finer particles within grains and coarser ones at boundaries. TEM revealed Y-rich and Ti-rich particles initially, transitioning to Y-Ti oxides with longer milling. ODS particle number densities showed no significant change with milling time. Some ODS particles had complex chemical structures. However, unintentionally introduced elements like Al or process-related elements like Ar must be closely monitored to ensure material quality and performance of the ODS steel.
Mechanical performance of the ODS material exceeds that of ODS-free material by roughly a factor of 3 at all temperatures.
Thus, the milling time could be reduced in a future industrial ODS steel production process.

Keywords:

ODS, TEM, SEM, mechanical properties

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Cholesteatoma removal efficiency evaluated by Variable Pressure Scanning Electron Microscopy

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Poster Group 1

Background incl. aims
Cholesteatomas are well-defined non-cancerous cystic lesion that results from the aberrant development of the keratinizing squamous epithelium within the middle ear. They can erode into the CNS and cause severe complications thus surgical removal is needed and is usually performed by manual dissection 1 however, with the possibility of recurrence. To improve the effectiveness of this technique the combined use of Mesna 5% (sodium 2-mercaptoethanesulfonate) has been introduced, this method is called Chemically Assisted Dissection (CADISS®). Our work aimed to evaluate the effectiveness of this combined surgical procedure during middle ear cholesteatoma removal, in terms of the absence of residual pathological tissue.
Methods
6 incus bones involved in cholesteatoma and removed during surgery were randomly divided into 2 groups: a) 3 subjected to CADISS-assisted dissection; b) 3 subjected to manual dissection. Samples were fixed in glutaraldehyde 2.5% in PBS (0.1M, pH 7.4) immediately upon recovery for at least 24 hours. Samples were then washed in PBS and underwent OsO4 post-fixation for 1 h. After washing samples were impregnated with tannic acid 1% for 30 min, then washed, dried on absorbent paper, and directly observed at Hitachi SU 3500 at 30 Pa and 10 kV operating conditions. Images were analyzed by the software Hitachi Map 3D advanced 8.2 (Digital Surf, France) to provide quantitative measurements of cholesteatoma tissue debris on the incus bone surface 2.
Results
Our results show that CADISS-assisted dissection provides a better outcome in terms of clean surface area concerning manual dissection. Data from software-aided BSE image analysis revealed that the clean area/ total surface area ratio is higher in the CADISS method samples (19.7±3.61) than in the manual dissection group referred to as the control group (4.57±1.66), the difference is statistically significant as revealed by t-Test results ($t= 20.91$ $P<0.001$).
The ability of Variable pressure SEM is that observation of samples in their native hydrated state is possible. The absence of dehydration steps in sample preparation allowed the observation of the samples as they came from the operatory room, without any artifact due to the preparation procedure. It is mandatory to use a procedure that does not modify sample surfaces if a comparison between two different surface cleaning procedures has to be performed.
Conclusion
The use of VP-SEM allowed sample observation without dehydration procedures, decreasing the risk of losing the pathological tissue while ossicle processing and allowing the comparison of the surgical technique's effectiveness. Our study also shows how still is important a morphological approach in establishing new surgical technique validity and how the application of innovative image analysis software can transform Scanning electron microscopy from a qualitative imaging modality into a quantitative technique.

Keywords:

Variable pressure scanning electron microscopy

Reference:

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3D Electron Diffraction Study on the Local Structure of a doped Metal-organic Framework TCNQ@ZIF-4

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Poster Group 2

Background incl. aims
Zeolitic Imidazolate Frameworks (ZIFs) become a more and more important sub-family of Metal-organic Frameworks (MOFs). ZIF-4 is one of the prototypical structures among ZIFs, which possesses the basic components of $M(im)^2$ (M = Co^{2+}/Zn^{2+} and im = imidazolate). Exclusive investigations have been applied on ZIF-4 such as mechanical properties[1] and thermal behaviors[2], however its conductivity is still missing.
Methods
7,7,8,8-tetracyanoquinododimethane (TCNQ) forms stable radical anions upon reduction, which can serve as an electron acceptor during the charge mobility.[3] Meanwhile, the transition metals, especially Co^{2+} are expected to donate electrons (i.e. to form Co^{3+}). The incorporation of TCNQ in the pores of ZIF-4 is expected to build up the pathway between the electron donor and acceptor to realize the mechanism of hoping transport[4]. A facial solvothermal method[5] was adopted to dope TCNQ@ZIF-4.
Results and Conclusion
Electron diffraction is revealing that the TCNQ has been successfully incorporated in the pores of frameworks. Further fine structures around the pore area urgently need to be resolved.

Keywords:

3D_Electron_Diffraction, Metal-organic Frameworks, local_Structure, Conductivity

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Structural study of perovskite-structure transition metal oxide thin film using Cs-corrected STEM: case of $LaVO_3/DyScO_3$

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Poster Group 1

Rare-earth transition metal perovskite oxides illustrate strong correlations between atomic structure and functional properties (1). When deposited as thin films, this enables manipulation of their electronic properties, for instance by tuning their composition or strain state. To understand these correlations, Cs-corrected scanning transmission electron microscopy (Cs-STEM) has become an important tool to measure the deposited lattices, not only to resolve and identify atomic columns, but also to locate their positions with a few picometer precision.
In this study, we focus on rare-earth vanadate films of composition $LaVO_3$ that are grown, at different temperatures, by pulsed laser deposition (PLD) on (110) $DyScO_3$ substrates. The epitaxial strain resulting from the lattice mismatch between the $DyScO_3$ substrate and $LaVO_3$ film is supposed to be around +0.5% (film under tension). However, in a series of thin films, a change in $LaVO_3$ unit cell volume is observed that depends on the deposition temperature. A double-corrected FEI/Thermo Fisher Scientific Titan Themis 60-300 is used to study the different samples in STEM mode, operated at 300 kV with beam currents down to 50 pA. STEM images are recorded as 90° rotation series, which are then processed using rigid and non-rigid alignment to maximize measurement precision (2). Both the film and the substrate adopt a Pbmn orthorhombic lattice. Depending on the TEM lamellae orientation, different characteristic orthorhombic distortions of the sample can be probed. Data treatment using the open-source python library Atomap (3) coupled with an in-house library allows measurements of the various distortions, i.e., oxygen octahedral rotations and A-site cation antipolar displacements.
Here, we investigate in depth how the different PLD growth parameters affect the $LaVO_3$ film structure. As suggested by X-ray diffraction measurements, key factors include growth temperature and O_2 partial pressure. With the Cs-STEM measurements, we are able to monitor their effects in real space. In particular, we have recently identified a novel structural organization in the $LaVO_3$ film called the “switching plane,” where the orthorhombic long axis direction switches 90° due to a competition between opposing energetic influences on the film’s orientation (4). We study the influence of growth temperature (and unit cell volume), and O_2 partial pressure on the switching plane formation and location, as characterized by spatially mapping the magnitude and direction of the orthorhombic distortions across the film thickness. Our latest results suggest that induced strain state and/or thermal energy play major roles in defining the presence of this novel structural formation.

Keywords:

Perovskite thin film, orthorhombic distortion

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Restructuring of silver catalysts after oxidation reactions – looking beneath the surface

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Poster Group 2

Introduction

The silver catalysed partial oxidation of methanol is one of the most used industrial processes to produce formaldehyde globally [1]. The industrial catalysts take the form of unsupported silver particles, but it is not possible to run under differential conditions with a particle bed setup at a lab scale. Therefore, we instead use an annular reactor that enables operating at low oxygen and methanol conversions, isothermal conditions and suppressing unwanted gas phase reactions [2]. We study the methanol to formaldehyde reaction and its sub-reactions including oxidation of CO and H_2, with and without water in the feed. At elevated temperatures, oxygen dissolves into the silver, the silver restructures, and pinholes form [3]. Electron microscopy techniques enable us to get a complete view of the restructuring that extends beneath the silver surface. We aim to understand the structural changes as a function of the gas mixture and couple this to the kinetic studies.

Methods

Silver cylinders (Ø 10 mm, 10-20 mm length) were machined from rods (Goodfellow) and placed inside a narrow quartz tube reactor that extended upstream to ensure low path length and linear flow. Fresh silver catalysts were exposed continuously as they were heated up and held for 12, 36 and 84 h at furnace setpoints in the range of 620-650 °C. Gas chromatography enabled quantification of all products except for H_2O. Microstructural characterisation of the catalysts was done after subsequent cooling. For comparison, also fresh silver catalysts and catalysts heated in inert gas and air were characterised. Scanning electron microscopy (SEM) enabled studying large areas of the catalyst surfaces. Focused ion beam (FIB)-SEM tomography, slice-and-view, provided three-dimensional views of the silver grains and enclosed cavities beneath the surface. Further, we prepared cross-sectional lamellae using FIB, collected scanning precession electron diffraction (SPED) data in the transmission electron microscope and did orientation mapping using pyxem [4].

Results

The kinetic studies show that the conversion of CO is enhanced by H_2, while it is completely inhibited if H_2O is added to the feed. Moreover, the methanol conversion and selectivity towards formaldehyde increases with time if H_2O is added. The microstructural characterisation reveals that the fresh silver is decorated by surface scratches and has a nanocrystalline grain structure in the near-surface region. Drastic changes are seen after exposure to the various reaction atmospheres at elevated temperatures. All heated catalysts display larger grains with twins. Faceted pinholes and sub-surface cavities (Ø$\lesssim$10 µm) appear after oxidation of H_2 and syngas (CO and H_2), also with H_2O in the feed. For several catalysts, smaller rounded cavities down to a few nanometres in diameter can also be seen. Most of the heated silver catalysts show surface faceting and preferential exposure of {111} surface planes. An exception is the catalyst after methanol oxidation, which displays rounded surface features and no distinct faceting.

Conclusions
Kinetic data obtained from an annular reactor setup with cylindrical silver catalysts show how the catalyst activity changes with time in various gas mixtures. Microstructural characterisation using primarily FIB-SEM and SPED discloses the silver catalyst restructuring that has occurred after the different oxidation reactions. Distinct changes were documented in terms of grain structure, surface faceting and (sub)-surface pores. By combining the results from the characterisation and the kinetic studies, we explain how the reaction mixture and resulting restructuring affect the catalyst activity.

Keywords:

Silver catalyst; FIB-SEM tomography; SPED

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Deep learning powered light optical microscopy for steel reseach

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Poster Group 2

Background incl. aims
Steel is by far the world's most important, multi-functional, and most adaptable material. The excellent mechanical properties of advanced steels are determined by their microstructure. The microstructure of advanced steels is a combination of different phases or constituents with complex substructures and its characterization is extremely challenging. The light optical microscopy (LOM) is a traditional, crucial, and dominantly utilized technique for investigation of steel microstructure up to now. The LOM has certain advantages over electron microscopes: extremely low-cost, low maintenance costs, fast and easy training of the staff, and easy accessibility. The biggest disadvantage of the LOM is limited spatial resolution. It makes this technique unsuitable for characterization of modern steels. This research aims to utilize advanced deep learning (DL) techniques to enhance the contrast and increase the amount of information in the LOM images of advanced steels.
Methods
Software-based transformation of low-quality images into their high(er)-quality equivalents is a challenging task. It is fundamentally impossible to create high-resolution images with more information than was stored in the original data without hallucinating the details. In this research, we propose that a proportion of the high-resolution information is already stored in the low-resolution images, however, it is hidden from human perception in the image blurriness, deformations and noise. A machine learning (ML) model can generalize about the general properties of the steel materials from their high-resolution images and therefore it can help to unlock more information from the LOM images beyond simple denoising and sharpening. This hypothesis was validated using a pilot proof of concept study concerning transforming LOM images into “pseudo” scanning electron microscopy (SEM) images using deep learning techniques trained on an image dataset of four types of steels. We tested different models and trained each of them to distill all important information from low-resolution images, to combine it with general knowledge of the investigated materials- to be generalized about by the model during training- and to generate high-resolution images. We started with a so-called U-Net neural network architecture [1] but eventually switched to a model based on Generative Adversarial Networks (GANs) [2]. This allowed us to achieve high precision and consistency in the generation process.
Results
Fig. 1 shows results of our pilot experiments. The first row displays as measured pre-processed data; the second row are transformed LOM images. As the labels indicate, the first column represents the U-net results, the rest are GAN results. We have marked some occurrences of several phases which may include: bainite (“B”), ferrite (“F”), pearlite (“P”) and secondary phases (“SP”), each example indicated by an arrow).
Conclusion
Even though the GAN transformation performs better than the standalone U-Net transformation, there is still room for further improvement. In future research, we intend to explore the efficacy of

employing diffusion models and vision transformers in the realm of image transformation. Specifically, our focus will be on enhancing the quality of images obtained through light optical microscopy to approximate the high-fidelity imagery characteristic of scanning electron microscopy.

Keywords:

correlative microscopy, deep learning, steels

Reference:

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High quality graphene TEM supports for high-resolution transmission electron microscopy

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Poster Group 2

Background incl. aims
Transmission electron microscopy (TEM) plays a critical role in the investigation of the atomic structure, morphology and chemical composition of nanomaterials. The field has significantly evolved during the last decade through the implementation of improved aberration correctors, novel detectors, more stable stages/holders and the implementation of many new low dose techniques. However, further progress in spatial resolution, contrast and potentially beam damage can still be achieved from improvements in sample preparation, particularly from the optimization of the TEM sample support.
The ideal sample support would be as thin and clean as possible in order to avoid interference with the sample signal, resulting in a background noise and a reduction of the contrast in the final (S)TEM image. Conventional grids consist of a fine metal mesh covered with an amorphous carbon layer (~20 nm), usually perforated with holes. For thin samples, this 20 nm is too thick to provide contrast, therefore a continuous support layer of 2 nm – 5 nm amorphous carbon is often used.
Graphene-based TEM grids have recently appeared as a promising alternative to the regular carbon coated TEM grids. Graphene, a monolayer of tightly bound carbon atoms, possesses exceptional properties. The monoatomic thickness of graphene minimizes the background signal, significantly improving signal to noise ratio which is crucial for high resolution imaging. Additionally, graphene's excellent electrical and thermal conductivity makes it an ideal candidate for dissipating excess heat and charge from the sample, mitigating electron beam induced damage.
However, to fully capitalize on the advantages of these new TEM grids, the graphene substrates need to be of exceptional quality. Therefore, we identify the current state of the art of the available graphene TEM substrates and discuss the existing problems with these grids and how these can be overcome.

Methods
Utilizing TEM and STEM, we evaluate key quality metrics of the currently available graphene TEM grids and discuss their relative importance. Based on these parameters, we compare the commercial grids with in-house made grids and discern their advantage in several advanced experiments.

Results
Unfortunately, depending on the transfer method and subsequent cleaning method, the graphene quality strongly fluctuates. Graphene grids are often plagued by cracks, folds, metal nanoparticles and polymer residues. Such impurities add background noise, introduce undesired obstructions in TEM images and degrade the conductive properties of the graphene. On the contrary, our in-house graphene grids alleviate some off these drawbacks, enabling previously unattainable experiments such as the visualization of surface ligands.

Conclusion

Current graphene TEM grids often lack the necessary quality for advanced experiments. Our in-house produced graphene TEM grids demonstrate significantly higher quality, enabling a set of previously unfeasible experiments.

Keywords:

Graphene TEM substrate

Reference:

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Application of secondary electron hyperspectral imaging to the analysis of pharmaceutical materials

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Poster Group 1

Background
The importance of pharmaceuticals to the health and wellbeing of society is clear, and it is of upmost importance to have reliable characterisation techniques applicable to the pharmaceutical manufacturing process. Pharmaceutical materials are often complex mixtures, containing multiple organic and possibly inorganic components. The stability and longevity of tablets can be increased through the addition of a coating, and this is evident in many commercially available pain relief medications. The effectiveness of the coating is linked to its surface coverage, and nanoscale characterisation is useful to determine this.
Secondary electron hyperspectral imaging (SEHI) is a recently developed novel electron microscopy technique that provides enhanced surface characterisation. Spectra sensitive to composition, chemical bonding and structure can be formed by collection of the secondary electrons that are emitted from a material following irradiation by an electron beam in the SEM. A variety of materials have been examined by SEHI, not limited to perovskites [1], polymer blends [2] and biomaterials [3], and various carbon materials [4]; however, the technique has not previously been applied to pharmaceutical materials.

Methods
SEM images and SEHI were collected using an FEI Helios G4 CX, a dual beam scanning electron microscope. The hyperspectral images were collected using a monochromated 1 kV accelerating voltage, a typical vacuum pressure of 10^-6 mbar, and a range of probe currents. Complimentary EDX spectroscopy has been undertaken using an Oxford Instruments 150 mm^2 EDX detector. An example commercially available pain relief tablet, Nurofen Plus, has been examined, in addition to reference materials ibuprofen, anatase and rutile. Samples have been prepared by both embedding in Field's metal and by FIB-prepared lamellae.

Results
Sample preparation is a particular challenge in this work, with the non-conductive pharmaceutical samples charging and causing anomalous features in the produced secondary electron spectra. Various sample preparation methods have been compared, each with advantages and disadvantages, with guidance from previous work with sample preparation of powder materials utilised [5]. The chosen commercially available pain relief tablet has a coating containing an inorganic pigment (TiO_2) which has led to the analysis of the different structures, anatase and rutile. The applicability of SEHI to the damage and integrity of the coatings has been examined, to assess this technique for the nanoscale characterisation of formulated pharmaceutical products.

Conclusions

In conclusion, this work will present the first analysis of pharmaceutical materials by secondary electron hyperspectral imaging. The advantages and challenges associated with this technique when applied to these materials will be detailed.

Keywords:

SEM; SEHI; pharmaceuticals

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Immunoexpression of estrogen receptor α in the ovary of mice after chronic exposure to arsenic(III)-oxide

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Poster Group 1

Background
Arsenic (As) is a naturally occurring metalloid found in soil and groundwater. As it can enter the food chain, As poses a serious environmental and health risk. In humans, the severity of symptoms of As poisoning depends on the dose and duration of exposure. Chronic exposure to low concentrations of As leads to dysfunction in virtually all body systems, including the impairment of the normal function of endocrine organs. Ovarian estrogens are the most important regulators of female fertility. Acting through specific receptors, estrogens regulate the growth and development of follicles, oocytes and granulosa cells in the ovaries as well as the function of ovulation. The aim of this study was therefore to determine the distribution of estrogen receptor α (ERα) in the ovaries of mice after administration of As(III)-oxide, which is considered to be the most toxic form of inorganic As.
Methods
Female mice from the Naval Medical Research Institute (NMRI, Bethesda, USA) aged approx. 6 months at the end of the experiment were used. Since birth, animals from the experimental group (n=6) drank water in which As(III)-oxide was dissolved at 10.6 mg/l, while the mice in the control group (n=6) drank tap water. For immunohistochemical localization of ERα in the ovaries of the mice, the sections were incubated with rabbit monoclonal anti-human ERα primary antibody (IR084, Dako, Agilent Technologies, Denmark), 1 h at room temperature and then visualized using EnVision FLEX, High pH (Link) system (K8000, Dako, Agilent Technologies, Denmark) for 30 min at room temperature. For negative control, primary anti-ERα antibody was omitted. After hematoxylin counterstaining, slides were analyzed with a Leica DMLB microscope (Leica Microsystems, Wetzlar, Germany).
Results
The control group is characterized by the preserved structure of the ovarian surface epithelium, under which there were numerous follicles in various stages of development as well as numerous corpora lutea and stromal cells. The results showed that the expression of the estrogen receptor was more pronounced in the experimental group than in the control group. A particularly positive response was observed in the corpora lutea, the secondary follicles, and the granulosa cells of the antral follicles, while immunostaining was absent in the stromal cells.
Conclusion
The present results show that chronic arsenic exposure affects the pattern and intensity of ERα-immunoexpression, suggesting involvement in the impairment of estrogen signaling with possible disruption of reproductive function.

Keywords:

Mice, arsenic(III)-oxide, ovaries, immunohistochemistry

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Structural recognition and stabilization of tyrosine hydroxylase by the J-domain protein DNAJC12

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Poster Group 1

Pathogenic variants of the J-domain protein and Hsp70 cochaperone DNAJC12 cause parkinsonism, which seems associated with a defective interaction of DNAJC12 with tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis. Here, we report the characterization of TH:DNAJC12 complex formation, showing that DNAJC12 binding stabilizes TH and delays its time-dependent aggregation in an Hsp70-independent manner, while maintaining TH activity and regulatory inhibition by dopamine. Interestingly, although DNAJC12 alone is less efficient than other DNAJs to activate Hsc70, the TH:DNAJC12 complex efficiently stimulates its ATPase activity. Cryoelectron microscopy reveals two DNAJC12 monomers bound per TH tetramer, each embracing one of the two regulatory domain dimers, leaving all active sites available for substrate and dopamine interaction. Biochemical data confirm the key role of the DNAJC12 last eight residues in TH binding, explaining the molecular disease mechanism of C-terminal truncated DNAJC12 variants.

Keywords:

DNAJC12, Hsp40, Hsp/Hsc70, tyrosine-hydroxylase, tyrosine-hydroxylase-deficiency

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STRUCTURAL CHARACTERIZATION OF FZD7, THE IMPORTANCE OF WATER NETWORK AND CHOLESTEROL FOR CLASS F GPCRS

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background
For more than 30 years, since the discovery of WNTs, WNT/β-catenin and planar cell polarity signaling has formed the basis for what we understand to be the primary output of the interaction between the protein ligands of the WNT family and their receptors known as FZDs (ten isoforms: FZD1-10). FZD7 is one of the best characterized receptors within the family and plays a critical role in many biological processes including migration of mesendoderm cells during development and renewal of intestinal stem cells in adults. Moreover, FZD7 has been highlighted for its involvement in tumor development predominantly in the gastrointestinal tract. This research aims to provide a better understanding of FZDs in general with a highlight on FZD7 by combining structural, computational, and pharmacological tools.

Methods
In this study, we apply a combination of conventional cryo-electron microscopy (cryo-EM) single particle analysis, MD simulations, and phylogenetic analysis to draw FZD family-wide conclusions on structural aspects and mechanisms of FZD activation. These data are complemented with pharmacological experiments employing genetically encoded biosensors to functionally validate our structural findings. This comprehensive approach provides us with insights into the function of FZD7 specifically, as well as FZDs in a broader context.

Results
We report the structure of inactive FZD7, without any stabilizing mutations, determined by cryo-EM. This allowed us to provide a direct comparison with the G protein bound FZD7 and to confirm previously identified residues involved in G protein binding mechanism.
We characterized a fluctuating water pocket in the core of the receptor important for FZD7 dynamics and used a phylogenetic analysis to define conserved residue defining the water pocket base that remains hermetic upon G protein binding unlike what is observed in Class A GPCRs. Molecular dynamics simulations were then used to investigate the temporal distribution of those water molecules and their importance for potential conformational changes in FZD7. Additionally, we discovered lipids that interact with the receptor core and a conserved cholesterol binding site. This site plays a pivotal role in the association of FZD7 with a transducer protein, Dishevelled (DVL), and in the initiation of downstream signaling and the formation of signalosomes.

Conclusion
We provided a high-resolution structure of FZD7 and defined functionally relevant features of FZDs dynamic and signaling.

Keywords:

GPCRs, FZDs, FZD7, Cryo-EM

Reference:

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Towards Quantitative analysis of electrostatic potential of monolayer WSe2 using electron ptychography

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Poster Group 2

4D-STEM has thrived thanks to the emergence of fast pixelated detectors in the last few years. The latter enables recording the rich information of the diffraction patterns in STEM [1]. This also makes it possible to map magnetic and electric properties at microscopic scales using methods like Center-of-Mass (CoM) and electron ptychography. Compared to conventional imaging techniques in STEM, electron ptychography enables imaging of the materials beyond the aberration-limited resolution with an optimal use of electron dose. The resolution achieved by this technique was demonstrated to be as small as 20 pm [2], and the ability to recover the diffraction pattern outside the detector plane allows for super-resolution imaging [3]. Quantitative ptychography, where the potential change over an atom can be directly related to the present chemical species combined with the effect of bonding, will have many applications, but has yet to be demonstrated in literature. Since, to the best of our knowledge, no publication directly compares experimental ptychographic reconstructed phase/potential maps or profiles with simulated data, quantitative ptychography appears still challenging.

In this presentation, 4D-STEM diffraction maps of WSe2 were simulated using abTEM[5]. Then the datasets were reconstructed using electron ptychography on the simulated diffraction maps using different reconstruction algorithms, namely ePIE and difference map (DM), implemented in the PtyPy[4]. The reconstructed diffraction pattern as well as the object function were compared using the ground truth of the input to the simulation, to better understand the strengths and weaknesses of both algorithms. The influence of padding was evaluated on the reconstructed data. Finally, this study allows comparison with reconstructions on experimentally obtained 4D-STEM maps on WSe2 or other 2D materials, intending to develop a method of quantifying the electrostatic potential.

The simulations for the 4D-STEM dataset were done on monolayer WSe2 with focused and defocused probes working at 80 kV with abTEM. The convergence semi-angle is 20 mrad. The diffraction patterns were sampled at 0.41 mrad/pixel and then cropped to a size of 256x256 or 512x512. This results in a collection angle of 51.7 mrad and 103.4 mrad at the edge. The dataset of diffraction patterns of size 256x256 was then fed to the ptychography reconstruction using ePIE and DM in the python package PtyPy: One with added padded zeros around the diffraction pattern up to doubled collecting angle and one without. An extra reconstruction using padded diffraction patterns up to 1024x1024 was done to see if it affected the recovered diffraction pattern. An analysis of a random diffraction pattern generated from the reconstructed probe and the reconstructed object function is shown in Fig. 1. While the reconstructions without padding show a good agreement with the original diffraction pattern, the reconstructions with padding fit the original pattern even better. However, for padded reconstruction, ePIE failed to recover the signals while DM smoothly transits and continues to fit outside the detector region. Further analysis of the power spectrum of the object

function exhibits a similar tendency that can be observed: until about 1.65 Å-1, padded ePIE reconstruction (orange dashed profile) matches the input simulation well, while DM matches up to a cutoff at 1.75 Å-1. The same analysis with extra padding up to 1024x1024 shows no significant signal improvement, implying that the effect of padding has a limited reach.

This work presented a possible method to quantitatively analyze the electrostatic potential of the monolayer WSe2. Padding in the reconstruction can improve the resolution, however, is limited. Moreover, the comparison between low-pass-filtered ground truth and reconstructions with a simulated finite-sized detector shows quantitative agreement. Care must therefore be taken in experiments since this finite-size constraint is always present.

Acknowledgments: This project received funding from the European Research Council under the European Union's H2020 Research and Innovation programme via the e-See project (Grant No. 758385). Y.L. thanks the Ecole Doctoral de Physique de UGA for the PhD scholarship. Experiments have been performed at the Nanocharacterisation platform PFNC in Minatec.

Keywords:

ptychography 2D material quantification

Reference:

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Functional and Structural Insights into the Modulation of the Chaperonin CCT by Small Molecules

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Poster Group 1

Chaperonins, also known as Hsp60s, are a family of chaperones whose structure consists of two juxtaposed oligomeric rings with a cavity in their interior, where the folding process takes place. CCT (Chaperonin Containing TCP-1) is the most complex of all chaperonins, and it participates in the folding of over 10% of newly synthesized proteins (1). There are many evidences that prove that CCT plays a crucial role in the development of different diseases. The first of these diseases is cancer, where CCT has shown to be upregulated, promoting tumor growth and mobility. Furthermore, CCT inhibition has been linked to tumor regression (2,3). On the other hand, CCT has been observed to be inhibited during neurodegenerative diseases such as Huntington disease, in accordance with the fact that CCT can inhibit the aggregation of polyQ proteins in vitro and in vivo (4,5). Although CCT has proven to play a critical role in the development of many diseases, there has been no relevant effort in the discovery of small molecules capable of modulating the activity of this proteins with a potential therapeutic effect, probably due to the structural complexity of the chaperonin. That is why in this work we screened the compounds included in the Prestwick chemical library using Differential Scanning Fluorimetry, from which three stabilizers and five destabilizers were identified. We then performed functional assays in order to characterize the effect that these small molecules have over CCT. ATPase assays have allowed us to identify two molecules that inhibit both activities of CCT, and another molecule that enhances them. Folding and binding assays are being currently carried out. We have optimized the vitrification conditions for CCT in the presence of small molecules diluted in DMSO, a solvent that prevents the proper distribution of the chaperonin in the cryo-electron microscopy grids. This has allowed us to carry out several preliminary 3D refinements: a ~5 Å resolution volume of CCT incubated with the inhibiting molecule Drug 2; a ~4 Å volume of CCT incubated with the activating molecule Drug 5; and a ~3.2 Å resolution volume (at the equatorial domains) of CCT incubated with HSF1A-biotin, a labelled derivative of an endogenous inhibitor of the chaperonin. Several protocols are now being used in order to identify extra densities which could be attributed to each of these small molecules bound to CCT. This structural information provides precise insight on the inhibiting mechanism of this small molecule, and supports its use as an inhibitor of the CCT chaperonin. These results may set the basis for the development of new drugs targeting CCT.

Keywords:

Therapeutic-target, molecular-chaperones, CCT, small-molecules, cryoEM

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Using super-resolution imaging to understand protein organization within Z-discs of striated muscle.

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LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

The Z-disc is a complex structure found at the ends of muscle sarcomeres in striated muscle. It is approximately 100nm in width and comprises over 30 different proteins, which play key structural and signaling roles. However, we have little understanding into how these proteins are organized. Electron microscopy approaches have so far been unable to resolve the positions of most of these proteins. Light microscopy approaches are hampered by low resolution, failure of antibodies to penetrate the dense Z-disc structure (Parker et al., 2023), and the large size and flexibility of antibodies is not well suited to super-resolution fluorescence microscopy approaches. Affimers, are small (~10kDa, 3-4 nm in size) non-antibody binding proteins, developed at Leeds, that overcome the challenges of imaging the organization of proteins within the Z-disc (Cordell et al., 2022; Tiede et al., 2017). Their small size means that they penetrate the Z-disc better than antibodies and that they only introduce a small linkage error, as fluorescent dye molecules conjugated to Affimers are close to the epitope of interest.
Here, we have used Affimers to begin to determine protein organization within the Z-disc, using dSTORM. We isolated Affimers against at >10 different Z-disc protein epitopes, targeting titin, α-actinin-2 (ACTN2), ZASP (LIM domain-binding protein 3), myotilin, capping protein, and others. To achieve this, we expressed and purified domains using E.coli from each of these proteins, using a 'BAP' tag (biotinylation site) to ensure that the proteins are biotinylated as they are expressed, and a HIS tag for purification. Purified proteins were used in a phage display library to isolate binders, which were then subcloned, an unique C-terminal cysteine added for subsequent conjugation of the purified Affimers to fluorophores via a maleimide linkage, and the Affimers expressed and purified from E.coli (Tiede et al., 2017). Purified, dye labelled (e.g. Alexa 647 for dSTORM and iPALM) Affimers were used to label myofibrils isolated from mouse or pig hearts and imaged using an Abbelight dSTORM microscope. Alternatively, Affimers were fused to mEos3, expressed and purified and used in labelling. The resulting image datasets (xyz localisation) were analysed using PERPL to gain a better understanding of their organisation with Z-discs (Curd et al., 2015). We have now successfully tested these Affimers in STED, dSTORM and in iPALM, and the results reveal that Affimers penetrate the Z-disc much better than antibodies, and the overall organisation of specific Z-disc proteins with high (~15nm or better), X,Y and Z resolution.

Keywords:

dSTORM, Affimers, super-resolution imaging,

Reference:

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Determination of Ti3C2Tx Mxene few layers stacks architecture using valence EELS and diffraction

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

The properties of two-dimensional (2D) materials generally depend on their architecture, e.g. the number of layers in a stack, the interlayer distance or the layer functionalization. Focusing on the most studied MXene compound to date, i.e. Ti3C2Tx 2D layers (where T-groups usually correspond to O, OH, F and/or Cl surface terminations inherited from the synthesis process), it has been shown that physical properties have a significant dependence on the number of layers in few-layer stacks. Beyond their thickness, MXene properties are also very sensitive to the interlayer distance which can be increased by intercalation of ions and/or molecules, or reduced by removing the interlayer intercalated water. In order to evidence the thickness/properties interplay in this rich family of 2D materials, one thus needs to precisely determine the architecture of a given MXene few-layer stack during TEM experiments. In this context, we show that valence EELS (VEELS) which corresponds to the excitation of the materials valence electrons, combined to density functional theory (DFT) simulations, provides a direct way to quantify (i) the number of layers in Ti3C2Tx few layer stacks for thicknesses up to ~10 layers, and (ii) the average inter-layer distance. [1]
Figure 1 shows a comparison between experimental VEEL spectra recorded on different Ti3C2Tx multilayers and the spectra simulated for different numbers of layers. To push the comparison quantitatively the mean squared error between experiments and simulations has been plotted, allowing to determine the exact number of layers in the thinnest samples. In addition, the position of the main peak, corresponding to the bulk plasmon, is closely correlated with the distance between sheets in the stack. Its shift can thus be used to estimate interlayer distance variations in a MXene multilayer with nanometer scale resolution
Finally, the thickness of a sample can also be obtained through diffraction measurements and intensity profiles STEM-HAADF analysis. Therefore in order to validate the thickness measurements obtained by the VEELS method, position averaged CBED (PACBED) patterns and STEM-HAADF images were recorded on the very same areas of the Ti3C2Tx MXenes. Different families of reflections exhibit different behavior of their pendellösung allowing to determine the sample thickness by comparison between experimental and simulated intensity ratios (Figure 2). PACBED and STEM-HAADF analysis confirm the outcomes of the VEEL spectra analysis.
TB, SC, VM and JP acknowledge the ANR through the MXENE-CAT project. JMZ is partially supported by DOE DE-SC0022060 and RLY was supported by Intel Corporation through an SRC project (Award #54071821)
Figure 1: (Left) Comparison between experimental VEEL spectra (black and grey curves) and DFT simulations considering thickness effects with the Kröger formula (color curves). Spectra were recorded on a FEI Themis Z microscope, equipped with a monochromator and operated at 80 kV. (Right) Mean squared error between experimental curves and simulations. It shows the very good precision on thickness determination for very thin samples.

Figure 2 : Pendellösung of the 100 and 110 reflections in the [001] zone axis and intensity ratio between the reflexions as a function of the thickness.

Keywords:

low-loss, thickness measurement, diffraction

Reference:

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Analysis of fire gilding on medieval jewellery using focused ion beam

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Poster Group 2

Fire gilding was a frequently used decorative method in the manufacture of medieval jewellery. Standard metallographic preparation of gilded samples often results in deformation of soft gilded layer resulting in an altered microstructure. For that reason, focused ion beam (FIB) milling is particularly well adapted since it allows to obtain damage free cross-section cuts to analyse fragile gilded layer as demonstrated by previous studies on hollow spherical pendants known as gombiky from the 10th century [1]. To compare the skills of the craftsmen in fire gilding in terms of thickness and porosity on other gombiky, more specimens from the same archaeological context were investigated. A characterisation of the gilding layer was also performed to determine the sublayer structure observed in previous studies [1] and to distinguish different metallic phases present in the gilding layer.
Microstructure was examined by scanning electron microscopy (SEM). Energy dispersive spectroscopy (EDS) and electron backscatter diffraction (EBSD) were used to determine chemical composition and crystallographic structure of gilded layers. Samples were then further investigated in more detail by transmission electron microscopy (TEM). Automated crystal orientation mapping (ACOM) was used for thorough examination of the sublayers.
The analysis revealed that gilded layer consists of several Au-Hg-Cu-Ag phases and Cu-Ag precipitates. Amorphous silicon residual from final polishing of gombiky after fire gilding process is also present in the microstructure of gilded layer. Furthermore, it is shown that there is a sublayer gold structure formed on interface between gilded layer and copper substrate. This sublayer is also visible around voids in gilded layer which are created by evaporation of mercury during fire gilding process.
The precise characterisation of gilding layers revealed different quality of the layers; therefore, confirming different level of skill and knowledge of craftsmen in fire gilding. The two-layer structure of the fire gilding layer was observed in all cases. Further information concerning the metallic phases present in the gilding were evidenced.

Keywords:

Fire gilding, Focused ion beam

Reference:

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Denoising 4D STEM datasets with PCA

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Poster Group 1

Background incl. aims
4D Scanning Transmission Electron Microscopy (4D STEM) provides crucial insights into materials structures by utilizing Differential Phase Contrast (DPC), ptychography, orientation and strain mapping. However, 4D STEM datasets often suffer from noise, hindering accurate analysis. It would be desired to denoise 4D STEM data by Principal Component Analysis (PCA) as it was successfully applied for EELS and EDX STEM datasets [1]. Additionally, PCA allows for accurate separation of the constituting compounds by clustering in the latent PCA factor space.

Methods
The sample used for this demonstration is organic solar cell blend DRCN5T:PC71BM solvent vapour annealed in chloroform. The 4D STEM dataset was obtained in micro-probe STEM (convergence angle ~1 mrad) with a Titan Themis microscope operating at 300kV. The data was collected using a OneView IS detector operating at frame rate of 400 fps (2.5 ms frame time) and probe current of about 5 pA.

We developed a Python-based PCA routine and integrated it into the temDM in-house developed 'Spectrum Imager' tool. This tool, originally designed for visualizing and denoising EELS and EDX spectrum-images [2], is presently adapted for 4D STEM datasets processing, including re-centring, clipping, viewing with virtual circular/ring apertures et cet. [3].

Results
When scanning the focussed beam across a sample, a shift of the diffraction pattern inevitably occurs, known as the pivot effect. While this effect can be minimized through accurate instrument tuning, it cannot be eliminated completely. We found that even a slight pivot effect dramatically influences PCA results, overriding any structural variations. In the considered example, the pivot shift within the scanned field of view was less than 2 pixels. Nevertheless, the data variation in the PCA factor space predominantly reflected the pivot variation rather than the structural changes in the sample (Fig.2, upper).

The diffraction shift was measured with the sub-pixel precision in each individual diffraction pattern using the centre-of-mass method. It important to note that simply centring the patterns is not an option as this would preclude any DPC measurements. Instead, we assumed that the pivot shift changes linearly along both x- and y- directions and calculated this by the least-square fit. The diffraction patterns were then shifted with the cubic-spline interpolation to account for the linear pivot. After the consequent PCA, the principal components clearly revealed the structural changes in the sample (Fig.2, lower).

Conclusion

4D STEM datasets can be successfully denoised by PCA, however it requires precise data pre-processing, particularly addressing the pivot shift. The sub-pixel interpolation is mandatory for the accurate subtraction of the pivot shift.

Figure caption
Impact of pivot correction on PCA results. The clusters are selected as hypercylinders in the latent PCA factor space. Without correction, instrumental pivot shift dominates in the PCA factor space. After correction, clustering in the factor space clearly reveals the structural compounds.

Keywords:

4D STEM, software, PCA, denoising

Reference:

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Nanotubes of (SmxY1-x)S-TaS2 based on Quaternary Misfit Layered Compounds (MLCs)

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Poster Group 2

Background including aims.
Misfit layered compounds (MLCs) have garnered considerable attention due to their fascinating chemistry and properties [1]. MLCs consist of two different layered oxides or chalcogenides that are stacked alternately along their c direction. The MLC stack is composed of metal chalcogenide (MX) which possesses a distorted rock salt structure and a transition metal dichalcogenide (TX2) which crystallizes in a hexagonal structure [1,2]. The properties of MLCs are determined by the chemical and structural interplay between MX and TX2. MLC-nanotubes (NTs) synthesized via the chemical vapor technique (CVT) offer potential applications in thermoelectrics due to the complementary properties of the two layered compounds [1]. Recently, a modified synthesis method of MLC-NTs has permitted the introduction of additional elements to form a quaternary compound starting from LaS-TaS2 [3,4]. Here, we present an in-depth electron microscopy analysis of the novel family of (SmxY1-x)S-TaS2 nanostructures [5]. In this novel family, the partial exchange of Sm(S) by Y(S) provides a pathway for the fine control of the MLC structure and its properties.

Methods
MLC-NTs made of (SmxY1-x)S-TaS2 were synthesized via the CVT technique [1-3] by varying the precursor proportions of Sm vs Y between x=0 to x=1. The samples will be designated by the Sm percentage, Sm20 corresponds to (Sm0.2Y0.8)S-TaS2 and similarly. To analyze these NTs, different TEM techniques (high-resolution (scanning)TEM (HR(S)TEM) imaging, selected area electron diffraction (SAED), electron energy loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDS)) were performed. These TEM studies were developed using two aberration corrected Thermo Fisher Scientific Titan microscopes. Raman spectroscopy has also been employed to study these NTs.

Results
The detailed analysis of the NTs by electron microscopy and different spectroscopies verifies the partial substitution of Sm by Y in the (Sm,Y)S subsystem and also reveals the structural changes when compared to the pure SmS- or YS-TaS2 MLC-NTs. These structural changes can be linked to the slight difference in the lattice parameters of SmS and YS. Figure (a) corresponds to the SAED pattern of Sm40 (Sm0.4Y0.6)S-TaS2 sample where the reflections marked by the green dotted circles represent the reflections from (Sm,Y)S and reflections marked by red dotted circles represent the TaS2 subsystems, respectively. The c-axis periodicity (1.13 nm) is indicated by yellow arrows. The c-axis is perpendicular to the tube axis, which is given by the purple double arrow. A high-angle annular dark-field STEM image of a Sm80 NT is shown in Figure (b) and illustrates the repetitive layer stacking of (Sm,Y)S and TaS2. The inset of (b) shows a low-magnification TEM image of the Sm80 NT. Figure (c) depicts a Raman spectrum acquired from an individual Sm80 NT, which was fitted using a set of

Lorentz functions. In a vibrational spectroscopy such as Raman, a Lorentz line shape is used to model pure vibrational modes, which only undergo homogeneous line broadening. Raman spectroscopy measurements of the whole set of samples reveal the tunability of the vibrational properties of these NTs. The study of the elemental composition of these NTs by EDS is shown exemplary for a NT of the Sm80 sample in Fig 1(d), which reveals an average atomic weight percentage of around 17 at. % Ta, 24 at. % Sm+Y, and 59 at. % S. The EDS results obtained from all the samples show that the substitution of Sm by Y is homogeneous and in-phase with structural changes in the lattice parameter. Further low-loss EELS studies and electric properties of these samples are in progress. These investigations will also provide a more complete understanding of these systems including their electronic/optoelectronic properties.

Keywords:

Inorganic-nanotubes, misfit-layered compounds-(MLC), structural analysis

Reference:

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Multicore@shell nanoparticles synthesized from a multicomponent target by gas aggregation cluster source

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Poster Group 2

In nanoscience, the fabrication of multifunctional nanomaterials with unique properties as well as their optimization is an ongoing challenge. The synthesis by gas cluster aggregation source (GAS) offers the opportunity to fabricate a wide range of nanoparticles in terms of particle size, morphology and chemical composition in a single preparation step.
In this work, nanoparticles from titanium oxide (TiOx) and gold (Au) are fabricated by GAS from a single multicomponent target.
The morphology of the resulting particles is analyzed by bright field transmission electron microscopy (TEM) as well as high angle annular dark field (HAADF) scanning TEM (STEM). The crystal structures are determined by selected area electron diffraction (SAED), high resolution TEM (HRTEM) as well as high resolution STEM (HRSTEM). Energy dispersive X-ray spectroscopy (EDX) is combined with STEM to gain local information on the chemistry of the particles. X-ray photoelectron spectroscopy (XPS) as well as X-ray diffraction (XRD) are applied to confirm and complement the results from TEM.
In TEM, the nanoparticles were observed to consist of several Au cores embedded in a TiOx matrix, resulting in a multicore@shell (Au@TiOx) morphology. The Au was present in its standard cubic crystal phase while TiOx was found to be in the amorphous state. Changes in the GAS source pressure were applied to alter the particle size and deposition rate. Upon heat treatment for 1 h at 500 °C in air, the TiOx crystallized to anatase TiO2 while the Au partially formed larger particles and partially diffused to the surface of the nanoparticles.
The preparation of Au@TiOx nanoparticles by GAS gives the possibility to prepare nanoparticles of complex morphology. Heat treatment of the material can be applied to tailor the crystal structure of the TiOx as well as the distribution of the Au. This approach can solve the issue of poor adhesion of Au on TiO2 since Au nanoparticles are mechanically fixed in a TiO2 matrix. The approach of GAS preparation of multicomponent nanoparticles can in principle be applied to a broad variety of materials and material combinations.

Keywords:

Multicomponent Nanoparticles, TiO2, Au, GAS

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Minimising the distortive effects of diffraction on magnetic STEM-DPC imaging of monocrystalline thin films

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Development of state of the art electric and magnetic devices requires high resolution characterisation techniques, one such being scanning transmission electron microscopy – differential phase contrast (STEM-DPC), which gives quantitative magnetic information while allowing concurrent acquisition of structural and chemical information in the same sample area. Magnetic STEM-DPC characterization relies on detecting minute shifts of the electron probe, often in the range of a couple of microradians. At highly diffracting conditions, the electron probe can get heavily distorted, making it difficult to extract the small shifts caused by the in-plane magnetic field.
In this work, we acquired STEM-DPC datasets using a 4D-STEM detector on a monocrystalline magnetic freestanding La0.7Sr0.3MnO3 (LSMO) thin-film. We acquired several tilt orientations at a temperature of -150.0˚C, giving a systematic overview of diffraction induced distortions on probe shape and magnetic signal. Fig. 1 shows two STEM-DPC images from low- and high-diffraction conditions together with probes and line profiles as indicated on the figure, clearly demonstrating the distortive effects of diffraction. Tilt series were done at increasing angles away from the zone axis and with a constant off-axis angle but rotating around the zone axis. By examining the random variations in the STEM-DPC signal and comparing to the difference in signal between magnetic domains, a quantitative value for the signal to noise ratio was obtained.
This presentation will show which considerations and mitigation measures should be used to optimise STEM-DPC imaging in heavily diffracting samples, and how different data processing algorithms handle the distortions in the electron probe.

Keywords:

TEM STEM-DPC magnetism functional method

Reference:

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Operando Environmental TEM observations of SOEC Ni-YSZ fuel electrode dynamics

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Poster Group 1

Power-to-X is emerging as a key player in the decarbonization of our energy infrastructure and at its core is electrolysis. The most efficient electrolysis technology is Solid Oxide Electrolysis Cells (SOEC) [1] however, large scale implementation has been halted by the concerns regarding loss of efficiency caused by degradation. The most important degradation process occurs in the Ni-Yttria stabilized zirconia (YSZ) fuel electrode. The driving factors are changes in Ni particle morphology and wetting angle, which are suggested to be correlated to the local SOEC operating conditions [2, 3]. Previous studies which have set out to investigate changes to the Ni morphology during SOEC conditions have only done so using post-mortem analysis where the sum of all the experimental parameters are observed. This makes it difficult to identify the correlation between the local test conditions and the morphological changes to the Ni particles.

To study the dynamic electrode processes occurring under standard SOEC operating conditions, we apply a newly developed operando electrochemical environmental transmission electron microscopy (ETEM) method [4, 5]. The method is adapted so that it allows us to follow the morphological changes of Ni particles in Ni-YSZ fuel electrodes, enabling us to map the degradation as a function of operating conditions. The aim of this project is to bring SOEC technology ahead by determining a so-called safe operational window which can limit, or possibly eliminate, the detrimental and irreversible degradation of Ni-YSZ fuel electrodes.

Sample preparation is carried out with a Zeiss FIB-SEM dual-beam which enables micromachining of bulk samples. The prepared samples are placed on a MEMS-chip from DENSsolutions which is capable of simultaneous heating and biasing. The finalized samples are imaged using a TITAN ETEM which allows the samples to be subjected to a reactive gas environment. Our method presents a novel way of studying the dynamic processes occurring in Ni-YSZ fuel electrodes as a function of real-life operating conditions. This will allow us to determine a safe operational window in which the most severe degradation can be avoided. This will ensure longer operating lifetimes of SOEC and make the technology more desirable in terms of large-scale implementation and ultimately advancing the decarbonization of our energy infrastructure.

Keywords:

Power-to-X, FIB-SEM, ETEM, Ni-wetting

Reference:

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Inflammatory mechanism in Diabetes – Ultrastructural investigations of endocrine pancreas using correlative electron microscopy (STEM)

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Poster Group 2

Patients suffering with type 1 diabetes show a major reduction of beta cells within their endocrine pancreas. To gain deeper insights into mechanisms of beta cells, correlative electron microscopy was the method of choice to understand the ultrastructure of insulin producing beta cells. The first step of this workflow was scanning electron microscopy employing the STEM (scanning transmission electron microscopy) detector. STEM images were generated with the benefit of great overviews on the one hand and giving information on cell interaction and arrangement within the entire organ at high resolution on the other hand. For an even more detailed investigation a suitable region of interest (ROI) in the pancreatic islets was preselected on STEM images. The sample was subsequently observed in the transmission
electron microscope (TEM) which was possible without any further processing. To gather more information on the respective healthy and diabetic phenotypes, we used a deep
learning-based approach to achieve high classification accuracy when discriminating between healthy and NOD samples of beta cells. We further explored the link between the number of granules in a sample and the healthy/NOD class it belongs to. Applying this insight to train neural networks that jointly learn both classification and counting tasks or rely on transfer learning strategies, performance on classification is improved. The additional counting tasks allow for a more robust representation, which is especially useful since only limited data are available. The findings strongly support the underlying hypothesis that early onset diabetes leads to a reduction in insulin-containing granules. Nevertheless, classification on its own already delivers strong results: There are additional factors other than the number of granules that play a major role in the decision process, such as changed appearances in the granules themselves or the surrounding tissue.

Keywords:

Scanning transmission electron microscopy; diabetes

Reference:

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810

Structure and Composition of High-Entropy-Alloy Nanoparticle synthesized by Pulsed Laser Ablation in Liquid

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Poster Group 2

Background incl. aims
Recently, synthesis of colloidal alloy nanoparticles (NPs) by pulsed laser ablation in liquid (LAL) was successfully transferred to high entropy alloy (HEA) systems. HEA NPs are particularly interesting for heterogenous catalysis and form a simple solid solution structure despite their high chemical complexity. In this work the influence of the target composition, solvent and laser pulse duration on the structure as well the chemical composition of these NPs is investigated. Two HEA systems are chosen: CoCrFeMnNi (Cantor) and AgAuCuPdPt (Noble metal). While in preliminary work crystalline and amorphous NPs with oxide-shells were found, it is unclear to what extent the excess of one element in the base target leads to elemental segregation not dominated by oxidation.
Methods
The nanoparticles were synthesized by LAL in a circulating solvent (Acetone vs. Ethanol) and NIR-laser pulse durations (10 ps vs. 10 ns pulse duration) and characterized in (S)TEM. For chemical analysis methods like Z-contrast imaging and nanoprobe elemental mapping were applied, while the structural analysis was performed using methods like TEM-high-resolution phase-contrast imaging (-HR imaging) and selected area electron diffraction (SAED).
Results
All Cantor alloy systems show a pulse energy dependency of the phase structure with fcc solid solution with ps-lasers and amorphous NPs with ns-lasers, while additional core-shell and multi-core structured NPs driven by Mn and Cr oxidation are also found. Further, oxide-core-multi-shell NPs were observed where localized target oxidation could provide nuclei for their formation. Interestingly, a predominant fcc-structure without pronounced elemental segregation was found upon enrichment of Mn or Cr in the system synthesized with the ps-laser. Further, NPs synthesized by the ns- laser maintained their amorphous structure upon enrichment of either Cr or Mn however additional crystalline oxides were detectable.
The noble metal systems showed no segregation into core-shell or multi-core structured NPs. Instead, two solid solution NP fractions appear, which are Ag-rich and Pt-rich. Contrary to the Cantor alloy systems and verified by HR-imaging the noble metal NPs retain their crystalline structure when synthesized with ns-lasers, although some hints on amorphous structures in Cu-rich and Ag-rich NPs based on SAED warrant further examination.
Conclusion
In conclusion, the Cantor alloy system shows the possibility of amorphous and crystalline solid solution NPs controlled by the laser pulse duration. Segregation into core-shell, multi-core or even oxide-core-multi-shell NPs is dominated by oxidation. The noble metal system displays de-mixing tendencies between Ag and Pt resulting in two solid solution NPs fractions. This could hint towards different cooling rates during particle formation offering insights into the HEA formation mechanism by LAL.

Keywords:

High-entropy-alloy, nanoparticle, pulsed-laser-ablation

Reference:

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Towards customized in situ TEM Chips for “device-like” geometries

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Poster Group 1

Functional nanostructures and thin films have applications within the next generation of memory storage, energy storage, quantum computers and sensor technology [1,2]. Their characteristic properties originate from the nano or even atomic scale, necessitating the combination of high-resolution techniques and imaging of functional properties to better understand their structure-property relationships. Scanning transmission electron microscopy (STEM) can be used to capture the structure, chemical composition, and functional properties of these materials.

For in situ STEM characterization, the material of interest must be transferred to an electron-transparent region of a TEM chip. For thin films, this means depositing it on the membrane of a commercial TEM chip, or alternatively, transferring it by FIB lift-out or similar techniques. However, for larger systems consisting of nano- or micro-sized structures relevant for prototyping of new device concepts, this quickly becomes challenging, as the field of view required for such geometries may be many times larger than what is feasibly prepared by plan-view FIB lift-out. An alternative approach would be to fabricate these “device-like” structures directly on the TEM membrane. However, performing advanced lithography steps on a fragile, pre-made, electron-transparent window involves a non-negligible probability of fracturing it. We aim to solve this by fabricating TEM membranes directly from the substrate, etching electron-transparent windows from its back side after processing the front side. Similar work has been performed on customizable TEM chips for nanowires [3], but here we aim to utilize this approach for specimens requiring a larger field of view. The outlook for a simple, reproducible, fabrication process is many, as the advantages stretch across customizable window sizes and tailored chip designs. Additionally, it may expand the type of material systems that can be studied in situ, as-grown and in-plane without the need FIB lift-out.

The first step in achieving a robust protocol for the fabrication of customizable TEM chips is to optimize the back-etch to release electron-transparent membranes. As proof of concept, back-etched TEM chips were fabricated for a simple ferromagnetic $Ni_{80}Fe_{20}$ thin film (Fig 1.). Starting with a double-side polished silicon wafer coated with 30 nm Si_3N_4, the etch-mask was fabricated by patterning the Si_3N_4 on the back side of the wafer by photolithography and inductively coupled plasma reactive-ion etching (ICP-RIE). A 20 nm $Ni_{80}Fe_{20}$ thin film was deposited on the front side by electron beam evaporation, and thereafter protected by AR-PC 5040 protective coating. The electron-transparent windows were released by immersing the wafer in a KOH bath, and consecutive removal of the protective coating. TEM characterization of the windows shows successful fabrication, with the $Ni_{80}Fe_{20}$ retaining its ferromagnetic properties. This provides further evidence that the sample preparation was successful and that the front side was sufficiently protected from the KOH etch.

The next steps will focus on fabricating $Ni_{80}Fe_{20}$ nanomagnet arrays by electron beam lithography on a similar Si/Si_3N_4 substrate with wires for in situ biasing. As such, this presentation will also show the next steps towards doing in operando studies of magnetic device concepts. [4]

Figure 1: a) Schematic of the TEM membrane fabrication steps. The insets show the cross-section of the wafer. b-d) Low-magnification STEM image, SAED pattern, and STEM-differential phase contrast (DPC) image of the back-etched $Ni_{80}Fe_{20}$ TEM window. Some particle contamination can be observed on the back-etched samples. e) EELS spectrum of the back-etched $Ni_{80}Fe_{20}$ thin film against a reference $Ni_{80}Fe_{20}$ specimen.

Keywords:

STEM, in-situ TEM chip, nanofabrication

Reference:

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Structure of refractory high entropy alloy and high entropy nitride thin films

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Poster Group 2

Refractory high entropy alloy and nitride films have attracted attention due to their versatile properties, such as satisfactory mechanical properties, excellent anti-oxidation performance, outstanding corrosion resistance, and vitally important thermostability, indicating high application potentials. In this work, microstructural characterization of VCrNbMoTaW (high entropy alloy - HEA) and (VCrNbMoTaW)N (high entropy nitride - HEN) thin films was carried out by scanning (SEM) and transmission electron microscopy (TEM). The thin films with thicknesses of ca. 5 µm were grown by magnetron sputtering from a multiple-elemental compound target. Cross-section TEM samples were prepared using focus ion beam. For the determination of the thin film composition, energy-dispersive X-ray (EDX) spectroscopy was used. For the crystallographic investigations, conventional TEM with selected area diffraction and high resolution TEM imaging (HRTEM) with fast Fourier transformation (diffractogram) were applied.
SEM-investigation of the surfaces of HEA and HEN thin films shows that the two materials exhibit different surface topographies with significantly different size of the growth features. As further TEM observation shows, they are columns with diameter on the surface much larger for HEA layer (more then 1 µm) than for HEN layer (up to 0.14 µm). The columns in HEA film are not always perpendicular to substrate surface and are elongated in <112> direction. The width of the grains increases in the growth direction and reaches a few hundred nm for the HEA films. EDX measurements on the surface in SEM show almost equal atomic concentrations of all 6 metallic elements in both layers: for HEA thin films, the individual elemental concentrations are around 16-17 %, and for HEN thin films all metal concentrations are between 9 and 11%. The measured N-concentration in HEN is ca. 42-43 at.%. EDX-maps of the surfaces in SEM (on large scale of a few µm) and in cross-section samples in TEM (on small scale of a few nm) of both HEA and HEN thin films show homogeneous distribution of all elements, also of nitrogen in HEN layer. It is found, that HEA thin films exhibit a body-centered cubic (BCC) structure with a lattice parameter of a=0.313 nm.
Incorporating Nitrogen in the 6-component HEA thin film leads to a change of the crystal structure of the HEN thin films. Figure 1 shows HRTEM images of a HEN thin film with evaluated (left hand side) and simulated (right hand side) diffractograms from areas 1-3 of the HRTEM images. The corresponding enlarged filtered point patterns of areas 1-3 are displayed in insertions of the HRTEM images. The evaluations indicate that the HEN thin film has a face-centered cubic (FCC) structure with a lattice parameter of a=0.425 nm. The areas 1-3 in Figure 1 exactly correspond to [111] (area 1), [001] (area 2) and [110] (area 3) zone axes orientations. As for the HEA thin films, we observe for the HEN thin film again a columnar growth, but with a much smaller columnar width of a few tens nm and extension in <110> direction. The length of columns in the HEN thin film is also much smaller (ca. a few hundred nm) in comparison with the HEA thin film (a few µm). As we can see in Figure 1, the width of the columns, which are limited by white dotted lines, increases in the growth direction. Moreover, the existence of 6 forbidden reflections (on the blue dashed circle) in diffractogram of [111]-zone axis orientation from area 1 in Figure 1 can be explained by diffraction from very small and misorientated grains of the HEN thin film. These reflections correspond to ¼ {422} of the first Laue zone. Due to the existence of very small and strained grains, we observe for the HEN thin film a

misorientation of sub-grains inside columns of up to 18 °. Different defects observed in both HEA and HEN thin films, such as dislocations and stacking faults were also analysed in this work. For the HEN thin film, we detect (111) stacking faults, which are typical for FCC structure.
Thus, incorporating of Nitrogen in high entropy alloy film leads to a change in the crystal structure from BCC to FCC, a decrease in grain the size with large misorientations and the introduction of many different defects.

Figure 1. HRTEM images of HEN thin films with evaluated (left) and simulated (right) diffractograms from areas 1-3 and corresponding enlarged filtered point patterns of areas 1-3 (insertions).

Keywords:

High entropy alloy

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Fast nanoparticle passage over cell membrane identified using 3D STED cross-sectional imaging

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Poster Group 1

Background

Inorganic nanosized particulate matter (nanoparticles - NPs, a.k.a. ultrafine particles) has for a long time been known to be harmful to the environment, which is supported by recent studies.[1] What raises even more concern is the inherent toxicity of many nanoparticles once they are taken up into the body via inhalation, digestion or skin abrasion,[1] from where they translocate and affect crucial organs, such as the heart, liver and even brain.[2] Air-borne nanoparticles are especially problematic, because their inhalation is difficult to avoid and because the lungs are a very thin barrier that can easily be damaged.

Currently, some of the most recognized ways of NP entry into cells are various forms of endocytosis, e.g. phagocytosis or pinocytosis that occur in several seconds to minutes. The option of direct nanoparticle entry into the cell via physical disruption of the plasma membrane is usually not considered, although simulations confirm this is possible.[3]

In the presented work, we have exposed living murine lung epithelial cells to titanium dioxide nanoparticles and employed 3D Stimulated Emission Depletion (3D-STED) microscopy to confirm that nanoparticles can pass the plasma membrane in less than a second, suggesting a faster mode of nanoparticle entry into the cell.

Methods

The presented measurements were performed using a custom-built laser scanning Abberior STED microscope with an UPLSAPO60XW 60x water immersion 1.2 NA objective. We recorded successive xz images at 30 nm lateral pixel size and 33 nm axial pixel size, the field of 7 µm and a 30 µs dwell time per pixel. The duration of the acquired time-lapse was 50 s with a time step of 1 s. For two-color fluorescent imaging, we used a 561 nm and 640 nm laser (both having 6 µW in sample plane) in combination with a 605/50 nm and 685/70 nm filter set, respectively. To increase the lateral and axial resolution, a 775 nm STED laser with a 3D STED mask was used at 46 mW.

LA-4 lung murine cells were seeded into an Ibidi µ-dish at confluency 30-40% to reach confluency of 60-80% on the day of the exposure. The medium used was Ham's F-12K medium supplemented with 15% FBS, 1% P/S, 1% NEAA, 2 × 10−3 M l-gln. Right before observation under the microscope, Live Cell Imaging Solution was added (30% of total media volume) to the cell culture media to ensure long-term cell survival although the cells were maintained at 37°C in a 5% CO2 humidified atmosphere in a custom-built stage-top incubator. The plasma membrane of cells was labelled with 1 µg/ml Cellmask Orange for the duration of the experiment..

The cells were exposed to titanium dioxide nanotubes, labelled with Alexa647 [4] at a cell/tissue normalized surface dose 10:1 (ratio between the surface of nanoparticles and the cell growth area). Nanoparticles were aerosolized onto the living cells, which were covered with a thin layer (200 nm-500 nm) of cell media. The thin layer of water was achieved by removing most of the cell media right before the exposure. Aerosolization was performed using Aeroneb Pro nebulizer that produces 4-6 µm sized drops.

Results

With a system simulating inhalation exposure in lungs, where titanium dioxide nanotubes (in red) were nebulized on top of living cells (in green) with only a thin layer of water on top, we were able to directly capture nanoparticles passing through the plasma membrane in matter of seconds. At time points exceeding 20 s, we can see nanoparticles also accumulating at the membrane, while still passing through it. We are able to see bare nanoparticles (just red color inside the cells) and membrane-coated nanoparticles (yellow color) inside and outside the cells. This fast entry of nanoparticles indicates that nanoparticles pass the membrane quickly, most probably caused by direct contact with the membrane. We propose the mechanism of entry to be nanoparticle wrapping – the direct interaction of the nanoparticle surface with membrane lipids.[5] This then also creates holes in the membrane through which other nanoparticles can pass the membrane bare.

Conclusions

By employing 3D-STED microscopy, we confirmed with 40 nm lateral and 500 nm axial resolution that nanoparticle pass over the cell membrane faster than in one second. This indicates that nanoparticles are able to enter living cells quicker than with the usual endocytotic biological uptake. This entry is quite possibly caused by direct interaction of NPs and cells, for example by nanoparticle wrapping into membrane lipids.[5]

Keywords:

live-cell microscopy, nanoparticle tracking, 3D-STED

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An examination of exit-wave reconstruction algorithms for low dose imaging at atomic-scale resolution

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Poster Group 2

Electron microscopy is a technology which is used in many modern research fields and in cases relying on heterogeneous materials the only viable solution for achieving atomic scale resolution. High-resolution electron microscopy utilizes a parallel, broad beam of high energy electrons to probe a sample and images at high spatial resolution are obtained by measuring the intensity of the electron wave function with a loss of its phase. By acquiring a series of images at different focus values, both the amplitude and phase of the electron wave function can be retrieved at the exit of the sample surface. The reconstructed exit wave provides the maximal amount of structural information, and it also enhances signal-to-noise ratios and therefore represents powerful phase imaging technique in low-dose-rate regime for suppressing beam-induced sample alterations for chemical and biological sciences. However, several schemes are employed to reconstruct the exit wave functions and are often employed without reference to their basic assumptions. In this study we provide a quantitative comparison of the most commonly used algorithms and discuss their pros and cons. Using simulation of HRTEM images in abTEM we compare how these algorithms perform in the regime of low dose imaging. Furthermore, we implement and present an algorithm for exit-wave reconstruction using abTEM and Python based on the paper by Oxley, et al. [1].
To compare different exit wave reconstruction algorithms on an equal basis, we simulate focal series of high-resolution transmission electron microscopy (HRTEM) images of model structures. Specifically, we use the multislice algorithm included in the abTEM package for HRTEM image simulation of structures modelled with the atomic simulation environment (ASE) package. Optical parameters incudes a primary electron energy of 300 keV, spherical aberration coefficient of -12 µm, an image pixel size of 0.1 Å and a focal range of -10 to 10 nm in steps of 1 nm. The focal series were reconstructed by using the three different algorithms [1-3] as implemented in commercial and open accessible codes and the reconstructions are then compared both qualitatively and quantitatively using a sum of square error (SSE) measure. Also, for reference, we implemented our own version of the algorithm by [1].
While the three algorithms overall perform similarly, we find differences reflecting a dose-dependence. We present data showing how an increase in the length of the focal series increases the quality of the reconstruction and results indicating an optimal tradeoff between dose and focal series length.
We investigated exit wave reconstruction algorithms implemented in Python codes and commercial packages. The comparison of the reconstruction the exit wave from a simulated focal series of HRTEM image shows differences depending on the overall image signal-to-noise levels. The present finding indicates that careful optimizing of the focal series image acquisition is needed to capture the finest structural features under low dose and dose-rate imaging conditions.

Keywords:

Exit-wave reconstruction, low-dose-rate, atomic-scale resolution

Reference:

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Examination of the Pathophysiology of Peripheral Olfactory Dysfunction and Alpha Melanocyte Stimulating Hormone Therapy

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Poster Group 1

Background incl. aims
Olfactory dysfunction occurs in various diseases such as upper respiratory tract infections, head traumas, and neurodegenerative disorders. Particularly, the impact of anosmia on human health and quality of life has become noteworthy, especially in light of the Covid-19 pandemic. Despite the widespread occurrence of olfactory disorders globally, there is insufficient knowledge regarding the pathophysiology and treatment of olfactory dysfunction. Peripheral olfactory dysfunction is characterized by damage to olfactory sensory neurons in the olfactory epithelium (OE). This study aimed to investigate the contribution of the olfactory bulb (OB) and subventricular zone (SVZ) to the recovery of olfactory dysfunction in rats with a model of peripheral olfactory disorder. Additionally, the study aimed to examine the therapeutic effect of Alpha-Melanocyte Stimulating Hormone (α-MSH) in the treatment of olfactory dysfunction.

Methods
Peripheral olfactory dysfunction was induced in rats by intranasal administration of zinc sulfate. Half of the rats with induced olfactory dysfunction were treated with α-MSH, while the other half received saline. Prior to the experiment, after the induction of olfactory dysfunction, and before euthanasia, rats underwent three rounds of olfactory tests (buried food test and odor discrimination test). After sacrifice on days 4 and 21, nasal and brain tissues were collected and examined using immunohistochemistry and immunofluorescence methods. Neurogenesis, inflammation, and growth factor markers were utilized to observe the pathophysiology of olfactory dysfunction and the effect of α-MSH treatment. For demonstrating neurogenesis in the OE, CK-14, ASCL1, GAP43, and OMP were examined. To illustrate neurogenesis in the brain, Nestin, ASCL1, DCX, and NeuN were labeled. Inflammation and growth factors were indicated by labeling TNF-α, IL-10, BDNF, and NGF in the OE, OB, and SVZ.

Results
In the group with induced olfactory dysfunction in the OE, statistically significant differences were observed in GAP43, BDNF, and NGF. In the group with induced olfactory dysfunction in the OB, TNF-α and IL-10 showed statistically significant differences. In the SVZ group treated with α-MSH, a statistical difference was detected in TNF-α immunoreactivities. The time taken to complete olfactory tests increased after the application of zinc sulfate to the rats. In olfactory tests conducted before euthanasia, the time to complete the tests decreased, approaching normal durations.

Conclusion

On the 4th day of olfactory dysfunction, a decrease in the number of immature neurons in the OE was observed. BDNF and NGF showed increases on different days during olfactory dysfunction. Additionally, it is suggested that growth factors in the OE may influence inflammation in the OB through the olfactory ensheathing cells (OEC). Based on these results, it was determined that peripheral olfactory dysfunction did not affect neurogenesis in the brain. However, it is believed that growth factors in the OE play a role in the pathophysiology of olfactory dysfunction in both the OE and OB. Furthermore, it was demonstrated that α-MSH was not effective in the applied dose and duration for the treatment of olfactory dysfunction.

Keywords:

Olfactory dysfunction, α-MSH, Growth factors

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Simultaneous Indexing of Spot and Kikuchi Patterns in Scanning Electron Microscopy (SEM)

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Poster Group 2

Background incl. aims
Introduced in 2011, Transmission Kikuchi Diffraction (TKD) overcame the challenge in characterizing isolated nanoparticles or ultrafine grains in thin film structure, which was a limiting factor in electron backscattered diffraction (EBSD) due to the small interaction volume and insufficient scatterings [1]. Traditionally, TKD relies on indexing the Kikuchi patterns of the forward-scattered electrons from an electron transparent sample, and it can be performed using a standard EBSD detector in either an off-axis or on-axis configuration with a spatial resolution down to a few nm in on-axis configuration [2]. However, the formation of Kikuchi pattern requires sufficient sample thickness, meaning very thin (e.g. 2D materials) or very light (e.g. organic) materials cannot be investigated via the traditional TKD method in SEM. Hence, a different approach has been developed recently to acquire and analyze the spot diffraction pattern in transmission mode in SEM instead, and it has successfully been used to index 2D materials such as graphene at nm resolution across mm range [3]. Since both spot patterns and Kikuchi patterns have been individually indexed in SEM, the natural next-step is to combine the two techniques so that the on-axis TKD configuration can be applied to a wider range of sample thickness. However, when acquiring both spot and Kikuchi patterns, the intense direct beam often drowns out the spot patterns, and the drastic difference in intensity between the spots and Kikuchi patterns (up to 10^3) makes the detection of both patterns with a conventional CCD detector challenging. Here we present our methods for acquiring both patterns without detector saturation and highlight the advantage by simultaneously indexing spot and Kikuchi patterns in a multi-layered Au thin film structure.

Methods
Two methods have been experimented to address the intensity issue: (1) By applying a filter to the CCD detector screen, the intensity near the optical axis can be reduced hence revealing the geometry of the spot patterns. (2) By acquiring diffraction patterns at different exposure time at each scanned location and combining/stacking the patterns via image fusion algorithm, the dynamic range can be greatly increased and both Kikuchi and spot patterns can be revealed.

Results
We will illustrate the simultaneous acquisition spot and Kikuchi patterns using both methodologies and provide a comprehensive analysis of their respective advantages and disadvantages on single and multi-layered thin film with various thicknesses. Finally, we will introduce and evaluate the two approaches for obtaining orientation mapping with both spot and Kikuchi diffraction patterns, as well as discussing their strengths and limitations concerning accuracy, throughput, and computation power requirement.

Conclusion

Both CCD detector filtering and multi-exposure image acquisition are valid strategies to acquire both spot and Kikuchi patterns without detector saturation in on-axis TKD configuration. The simultaneous indexing of both spot and Kikuchi patterns makes up for the thickness limitation of the individual spot/Kikuchi pattern indexing, and expands the range of samples investigated with a single technique. With this approach, we aim to expand TKD from an exit-surface characterization technique into a volume information extraction technique with the diffraction information of both spot and Kikuchi signals.

Keywords:

TKD, simultaneous indexing, detector saturation.

Reference:

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Physical determinants of YAP mechanotransduction in multicellular assemblies

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Poster Group 1

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Background incl. aims
Cells within tissues constantly receive biochemical and mechanical cues. Biochemical cues are evenly distributed across a monolayer due to the uniform spread of growth factors in the culture environment. Mechanical cues are highly localized, arising from cellular processes such as cell divisions, extrusions, and interactions with neighboring cells, involving pushing and pulling. These cues are sensed and interpreted by individual cells through a process called mechanotransduction. Depending on the input, cells make decisions that impact their micro-environment, creating a feedback loop regulating tissue homeostasis.1

The Hippo signaling pathway plays a crucial role in modulating cellular responses. Various upstream affectors have been shown to regulate it. One important constituent of the Hippo pathway is "Yes-associated protein 1" (YAP). When the Hippo pathway is on, YAP is phosphorylated and is permitted to enter the nucleus. When the Hippo pathway is off, YAP can shuttle into the nucleus and regulate gene transcription by interaction with DNA binding factors. 2

Nuclear compression, either independently or by disrupting the connection between the nucleoskeleton and the cytoskeleton, has been demonstrated to effectively regulate the localization of YAP. 3 YAP enters the cell nucleus through nuclear pores. The expansion of nuclear pores is linked to the shape of the nucleus. When the nucleus is elongated and stretched, the nuclear pores tend to widen. 4

The response of YAP to mechanical cues has been studied in the context of single cells or in multicellular contexts with high and low local density conditions. However, we found YAP expression and local cell density to be highly heterogeneous across the MDCK monolayer at the two seeding densities that we chose. Thus, we decided to characterize YAP activation across the MDCK monolayer in terms of local cell density.

Methods
Madin-Darby canine kidney (MDCK) cells are cultured at a high and low global cell density. When a propper monolayer is formed, cells are fixed and immunostained with YAP antibodies and Hoechst. The samples are imaged using a spinning-disc confocal microscope. Z-stacks are taken to reconstruct the cells in 3D. The nuclear stacks are segmented using Cellpose for extraction of nuclear shape properties. The focal plane is segmented in 2D to calculate the local cell density and YAP nuclear/cytoplasmic (n/c) ratio.

By manipulating e-cadherin expression using an e-cadherin KO MDCK cell line as well as altering actomyosin cytoskeleton integrity with Latrunculin A and ROCK-mediated myosin contractility with Y-27632, we aim to untangle their individual contributions from local cell density on YAP activation.

Results
YAP activation is determined by local cell density. A clear relationship can be observed between the YAP nuclear to cytoplasmic (n/c) ratio and local cell density. On average, YAP activation steadily decreases with an increasing number of neighboring cells. In contrast to the single-cell scenario, where less spread cells have larger nuclear volumes, cells with tall and high nuclei are found at lower local cell densities. These trends are preserved when performing perturbation experiments, highlighting the importance of local cell density and nuclear shape in the context of YAP activation and potentially also other processes.

Conclusion
By manipulating e-cadherin expression using an e-cadherin KO MDCK cell line as well as altering actomyosin cytoskeleton integrity with Latrunculin A and ROCK-mediated myosin contractility with Y-27632, we aim to untangle their individual contributions from local cell density on YAP activation.

Theme: Mechanotransduction in multicellular assemblies
Subtheme: Power of microscopy and 2D/3D image analysis

Keywords:

Mechanotransduction, YAP, 2D, 3D segmentation

Reference:

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Dual-color CLEM imaging for genetically encodable enzymatic fluorescence signal amplification method using APEX (FLEX)

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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

Background
Correlative Light and Electron Microscopy (CLEM) is a useful imaging approach that allows us to obtain two types of different imaging information from the same sample. Fluorescence microscopy (FM) provides multi-color information on different proteins of interest (POIs), while electron microscopy (EM) reveals the ultrastructure of cellular organelles. As a result, CLEM, which has become an essential tool in cell biology and neuroscience, enables a comprehensive understanding of POIs and their connections to intracellular structures or subcellular organelles1,2. For fluorescence-mediated visualization of intracellular regions of interest, fluorescent proteins (FPs) are commonly used. However, the fluorescence of intracellular FPs is often lost, especially during harsh EM sample preparation, such as fixation, dehydration, heating and resin embedding3. Especially for highly dynamic organelles such as mitochondria and lysosomes, the precise localization of these organelles can change as they are observed live in the light microscope and are actively moving even during the short delay time for fixation. To overcome this problem, we developed a fluorescent reporter that can effectively maintain its fluorescence signal under harsh EM sample processing conditions and developed a method to obtain distortion-free CLEM images using it.

Methods
Given the difficulty of maintaining the fluorescence signal during EM sample preparation with conventional fluorescent proteins, we use a two-colour CLEM technique that integrates small molecule probe-based signal amplification using genetically encoded peroxidases with mEosEM4, which retains fluorescence even in strong fixatives. In particular, based on our previous findings on peroxidase-based fluorescent substrates, we synthesised and applied to CLEM imaging a newly designed JF-induced probe based on the styryl-benzothiazolium phenol probe genfluor (JF), known for its fluorescent signal amplification by peroxidase5.

Results
As a results, we developed a fluorescent probe (JFT1) that exhibits a fluorescence-amplified signal when APEX2 is expressed. Unlike conventional fluorescent labels or fluorescent proteins that lose their fluorescence upon OsO4 pretreatment, JFT1 produces a well-retained and restricted fluorescence pattern in both OsO4 pretreated and OsO4 post-treated samples. The application of FLEX targeting to lysosomes in conjunction with mito-mEosEM facilitated the visualization of interactions between lysosomes and mitochondria. It also revealed distinct contacts between these two organelles in response to the lysosomal stressors bafilomycin and U18666A. Furthermore, the

interactions observed under the influence of bafilomycin and U18666A revealed distinct mechanisms of action as indicated by CLEM imaging. Taken together, we propose that our FLEX approach is a very useful way to target different APEX-POI combinations for fluorescence and high-resolution EM imaging.

Keywords:

CLEM. APEX, Proximity-labeling, Fluorescent probe

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Multimodal mechano-microscopy reveals mechanical phenotypes of breast cancer spheroids in three dimensions

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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

Background incl. aims

Cancer cell invasion relies on an equilibrium between cell deformability and the biophysical constraints imposed by the extracellular matrix (ECM), and multicellular spheroids are a powerful model to study biochemical and biophysical interactions between cancer cells during growth and progression. However, little is known about how the biomechanics of the three-dimensional (3D) tumour microenvironment (TME) control cancer cell behaviours. 3D TME models remain underutilised, because contemporary mechanical quantification tools are limited to surface measurements, and there is a lack of enabling technologies that can measure subcellular-scale elasticity and co-register it with the morphology and function of cells in a 3D microenvironment.

Here, we develop a multimodal mechano-microscopy system that integrates optical coherence microscopy-based elasticity imaging with confocal fluorescence microscopy. We use this multimodal microscope to quantify local mechanics of cancer cell spheroids in 3D TMEs.

Methods

Our multimodal mechano-microscopy setup integrates high-resolution interferometric detection of optical coherence microscopy (OCM), a high-resolution variant of optical coherence tomography (OCT), with compression elastography and confocal fluorescence microscopy.[1] The system utilises a supercontinuum laser, whose output was shaped to a spectral range of 650 nm to 950 nm. This spectrum corresponded to a full-width at half-maximum (FWHM) bandwidth of ~250 nm, providing a measured OCM axial resolution of 1.4 µm in air. The system was implemented as a Michelson interferometer in a dual-arm configuration with the same optics in the reference beam path to match optical dispersion. The sample arm beam was expanded to fill the entrance pupil of a 20X 0.75NA objective lens providing a measured lateral resolution of 0.5 µm. Scanning was achieved using a 2D galvanometer system. A spectrometer comprising a 2048-pixel line camera was used to detect the spectral interference at each xy location.

The integrated confocal fluorescence microscope comprised two laser lines at 405 nm and 488 nm for excitation and a photomultiplier tube as the detector. The confocal system used the same galvanometer scanning arm and optics as the OCM system to scan the focal point in the sample. The emitted fluorescence from the sample was focused into a multi-modal optical fibre, which also acted

as the confocal pinhole. The measured lateral resolution of the confocal imaging system was 0.5 µm, and the axial resolution ~8 µm.

Sample preparation is described elsewhere in more detail [1]. Briefly, MCF-7 and MDA-MB-231 cells were suspended in gelatin methacryloyl (GelMA) solution. The solution was pipetted into moulds placed on glass slides, and exposed to UV light for 15 or 60 s to produce soft and stiff gels, respectively. The cells were then cultured for 6-16 days to allow spheroid formation and growth. Prior to imaging, the cells were labelled with nuclear dye Hoechst 33342 and membrane dye CellMask Green for fluorescence microscopy.

For imaging, the gel sample with a thickness of ~400–500 µm and a 1 mm thick compliant silicone layer were compressed between the coverslip and a rigid optical window attached to an annular piezoelectric actuator (Fig 1a). Two B-scans were acquired at each y-location, one at the unloaded and the other at the loaded state. Local displacement was calculated from the phase difference between the two B-scans and strain was estimated as the gradient of the axial displacement with depth.[2] Strain in the layer was related to stress through knowledge of the stress–strain response of the compliant layer in contact with the sample. Confocal images were acquired directly after the mechano-microscopy measurement.

Results

To test the setup, Young's modulus of blank, soft and stiff gelatin methacryloyl (GelMA) samples was first measured using mechano-microscopy and was found to be in good agreement with AFM surface measurement (Fig 1b). The multimodal setup was then used to obtain volumetric maps of living breast cancer cell spheroids cultured in the gels. Fig 1c,d shows example en face and B-scan Young's modulus of a non-metastatic spheroid, and Fig 1e-g show an example of a volumetric map where (e) OCM intensity, (f) Young's modulus and (g) confocal fluorescence image of the nuclear (cyan) and membrane (red) fluorescent dyes.

By imaging both metastatic (MCF-7) and non-metastatic (MDA-MB-231) breast cancer cell spheroids cultured in both soft and stiff gels (n≥4 for each condition), we observe that non-metastatic cancer spheroids show no invasion while showing increased peripheral cell elasticity in both stiff and soft environments. Metastatic cancer spheroids, however, show ECM-mediated softening in a stiff microenvironment and, in a soft environment, initiate cell invasion with peripheral softening associated with early metastatic dissemination. This exemplar of live-cell 3D mechanotyping supports that invasion increases cell deformability in a 3D context, illustrating the power of multimodal mechano-microscopy for quantitative mechanobiology in situ.

Conclusion

We demonstrate a multimodal imaging system that integrates an OCM-based subcellular mechano-microscopy system with a multi-channel confocal fluorescence microscopy system. This multimodal mechano-microscopy presents a new opportunity for quantifying local Young's modulus in live 3D samples with subcellular resolution, directly revealing mechanical phenotypes in situ.

Keywords:

Multimodal microscopy, elastography, OCM

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Transmission electron microscopy studies of ferroelectric ZrO2 thin films

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Poster Group 1

INTRODUCTION
The discovery of ferroelectricity in zirconia and hafnia [1] based thin films revolutionized the research in the field of ferroelectrics and paved the way for their integration into real applications. However, the wake-up phenomenon associated with the presence of non-polar phases and/or defects in the films still hinders the reliability of the device operation. Therefore, new strategies for achieving ferroelectric single-phase orthorhombic zirconia and hafnia based thin films are welcomed.

EXPERIMENTAL/THEORETICAL STUDY
8-nm thick ZrO2 thin films were grown by ion-beam sputter deposition (IBSD) onto 0.7 wt% Nb-doped SrTiO3 substrates with (001), (011) and (111) orientations.
Cross-sectional TEM samples were made by first mechanical thinning followed by Ar+ ion milling on a Gatan PIPS machine at 4 kV acceleration voltage and 6 degrees beam incidence angle. TEM observations were performed using a probe-corrected analytical high-resolution JEMARM 200F electron microscope operated at 200 kV.
RESULTS AND DISCUSSIONS
We note the Figure inserted in the graphic area by Figure 1. Fig. 1(a), (b) show HRTEM images of a ZrO2/(011)Nb:STO thin film. The ZrO2/(011)Nb:STO structure with (111) and planes of the o-phase are indexed. The FFT pattern shown in Fig. 1(c) corresponds to an area delimited by the red square inside the ZrO2 layer in Fig. 1(a). It contains a well-defined pattern of spots, proving a high crystalline quality of the ZrO2 film in terms of grain size and preferential crystallographic orientation. The main FFT peaks were measured, indexed and assigned to the (13-1), (111) and (02-2) planes of the orthorhombic structure of ZrO2 in the [-211] zone axis orientation. [2]
CONCLUSIONS
The HRTEM investigations indicate that substrate orientation control proves to be an efficient way to manipulate the phase of the as-grown ZrO2 thin films. We also studied ZrO2 films grown on (001)Nb:STO and these second set of films showing mixed orthorhombic and monoclinic phases. Macroscopic ferroelectric measurements of the phase pure, orthorhombic (111)ZrO2 films showed a remnant polarization of ~14 µC/cm2 and a coercive field of ~1.4 MV/cm. Also, piezo force microscopy measurements showed that polar domains could be written/read and reversibly switched with a phase change of 180°.

Keywords:

HRTEM, Ferroelectric, Thin film

Reference:

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Characterization of the structure and chemistry of solid electrolyte

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Poster Group 1

Background incl. aims
Solid-state Li metal batteries (SSLMBs) have been of great interest for electric vehicles due to their high energy density and safety features. Garnet-type $Li_7La_3Zr_2O_{12}$ (LLZO) solid electrolyte (SE) is a promising candidate due to its high ionic conductivity and stability with Li metal. While its preparation and electrical properties have been extensively studied, research on air handleability and Li metal wettability has been relatively limited. Addressing Li_2CO_3 formation on LLZO surfaces is critical because the formed Li_2CO_3 hinders the electrical properties, creates poor interfaces, and reduces metal wettability [1]. We developed a Li_2CO_3-proof LLZO (AH-LLZO) SE that is stored and handled in humid air without compromising electrical properties and simultaneously shows excellent Li metal wettability.
Methods
To understand the air-handling ability of LLZO, we performed characterization of structure and chemistry using SEM, XPS and STEM-EELS.Two samples were prepared by a simple solid-state reaction. One sample (C-LLZO) has a known composition ($Li_7La_3Zr_2O_{12}$) with a large excess amount of Li to compensate for the expected Li loss during high temperature treatment, and the other sample (AH-LLZO) contains excess Al and the same large amount of excess Li.
Results
SEM-EDS analysis reveals a distribution of Al both in the bulk and at the grain boundary of AH-LLZO. Some Al segregates into the grain boundary, while some is doped into the bulk. Elemental profiles of the Al $L_{2,3}$- and La $N_{4,5}$-edge via STEM-EELS further confirm that the Al in present both regions. The grain shows an almost constant amount of Al, but a sharp increase at the grain boundary suggests an Al-rich environment, possibly $LiAl_5O_8$-related phases, which is the most stable Li-deficient Al-rich phase [2]. High-resolution XPS confirms the absence of carbon-related peaks at a depth of 100 nm from the AH-LLZO surface which is clearly distinguished from C-LLZO, which exhibits a strong carbonate peak. To confirm the detailed surface structure, we performed STEM-EELS analysis across LLZO grain boundary for two LLZO. Consistent with the XPS results, AH-LLZO does not have any carbonrelated peaks in the grain or at the grain boundary, indicating that there is no Li_2CO_3 propagation into the core via the grain boundary. In contrast, C-LLZO exhibits carbon signals inside the grain boundary, suggesting that Li_2CO_3 formation/propagation in the bulk through the grain boundary can be occurred [3]. The EELS peaks for the oxygen at the grain boundary are similar to the oxygen binding energy of Al-rich Li-Al-O-related compounds such as $LiAl_5O_8$. This Li-Al-O-related phase at both the surface and the grain boundary blocks against water or carbonate infiltration, leading to the suppression of the Li_2CO_3 formation/propagation.
Conclusion
The presence of excess Al and Li without charge/site balance in the LLZO causes a distinctive microstructure with Al-doped LLZO grains and Li-Al-O phases at the grain boundary/surface. In the Al-doped LLZO grain, the preferential occupation of Al at 96h sites can reduce the LiOH formation and then suppress Li_2CO_3 formation. The Li-Al-O-related phases on the surface and at the grain boundary, particularly the $LiAl_5O_8$-like phase, act as a hydrophobic barrier, blocking water adsorption.

Keywords:

Solid electrolyte, LLZO, STEM-EELS

Reference:

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Liquid Phase 3D Electron Diffraction Combination Provides New Possibilities for Polymorphism Studies

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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

Background incl. aims
Liquid Phase Transmission Electron Microscopy (LPTEM) is a powerful method for investigating in-situ fluidic reactions and materials under liquid conditions. Traditionally, this involves enclosing liquids within silicon nitride membranes, thus eliminating the need for cryogenic or dry samples. However, it comes with a downfall of low spectroscopical signal due to large liquid and membrane thickness. To address this limitation, we introduce a novel nanochannel system capable of nanofluidic regulation for LPTEM. With a SiNx membrane thickness of 25 nm and a sub-200 nm liquid height, this system offers high resolution and enables excellent spectroscopic data [1,2]. This opens the possibility of developing completely new techniques with LPTEM, contributing to its rapid expansion in various atomic-scale characterization methods.
By combining the nanochannel system with existing methodologies for atomic characterization, we can observe samples in-situ during liquid transformations. This eliminates the need to remove samples from their liquid environment, allowing for direct observation of transient intermediate phases during processes like crystallization or polymorphic form changes.
To ensure accurate crystal determination inside the nanochannel, we utilize 3D Electron Diffraction (3D ED). This method enables the spatial structure determination of nano-scaled crystals.
In this work, we aim to combine 3D ED with nanochannel chips from Insight Chips, seen in Figure b to study the crystallization of glycine from liquid and characterize any observed transformations. Glycine is commonly used as a model system for polymorphism as it exhibits different polymorphic states depending on its liquid medium. Previous research has demonstrated the utility of 3D ED in tracking the crystallization of glycine from aqueous solution [3].

Methods
In this study, we utilize a TEM holder from Figure a, which has four tubes connected to four O-ring sealed in/outlets on a chip. The chips in/outlets are sealed during fabrication with a nitride membrane, ensuring the nanochannels are clean and hydrophilic upon puncturing before use. After puncturing the membrane, a 50% saturated glycine solution in deionized water was drop-casted on the inlet and the solution entered the channels through capillary forces. Pure ethanol was subjected to the other side of the chip with the same drop-casting method. Crystals of glycine were observed under an optical microscope, in Figure b, as they have a different refractive index than water and give rise to different colors due to multilayer reflection. Thereafter, the presence of crystals was observed in a TEM, displayed in Figure c.
The 3D ED data from the crystals was collected on a Thermo Fisher Themis TEM with an acceleration voltage of 300 keV. 3D ED data was obtained using a selected area aperture on individual crystals in the channels, with crystal lengths ranging from 1.5 to 4.0 µm. All 3D ED data were collected at room temperature while rotating the chip continuously between –20° and +25° in α tilt, illustrated in Figure

d. The exposure time (0.2 s) and rotation speed (0.85 s^–1) were chosen so that individual diffraction frames were integrated over 0.17° of reciprocal space. The estimated dose rate was 2 e Å^–2 s^–1, with each collection taking on average 240 s, the total estimated dose per 3D ED data collection was 480 e Å^–2.
REDp was used to determine the unit cell and space group from 3D ED data. Data reduction and integration were performed with XDS. Structures were solved via dual space methods using SHELXT and refined with SHELXL in the Shelxle interface.

Results
The phase change precipitated chip was investigated using LPTEM. A total of 18 crystals were identified and analyzed by 3D ED, and out of these, 8 were identified as α-glycine and 4 were identified as β-glycine. The remaining 6 datasets did not provide conclusive results due to limited completeness or the crystal being polycrystalline. Due to low data completeness (24.2%), ab-initio structure determination was not possible. However, 3D ED data could still be used to refine the positions and isotropic atomic displacement of all non-H atoms. The presence of SiNx windows and concentrated glycine solution surrounding the crystal led to slightly increased background noise and diffuse scattering in TEM analysis. 3D ED data collected on crystals immersed in liquid were sufficient for structure determination. The results of crystallization in nanochannels and in-situ 3D ED experiments provided us with the opportunity to capture the early stages of the glycine crystallization process.
Although the overall crystallographic information was worse than the data collected on conventional TEM grids [3], the structure model could still be accurately determined.

Conclusion
The work carried out here demonstrates that the combination of LPTEM and 3D ED was successful in studying the structure of glycine crystals in liquid. 3D ED data from crystals of α-glycine and β-glycine polymorphs, both in and out of liquid were eligible for structure analyses. Both α- and β-glycine polymorphs were identified via imaging and crystallographic data. Liquid phase 3D ED is an advanced and challenging technique, but the findings and experimental capability established here raise the prospect of future works on studying structural responsiveness to external stimuli, like temperature or even chemical processes in solution using a combination of LPTEM and 3D ED.

Keywords:

3DED, LPTEM, Nanochannel, Crystallography, Polymorphism

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Charge density mapping of supported nanoparticle electrocatalysts by 4D STEM

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Poster Group 2

The negative impact of fossil fuel consumption on the environment, along with being a limited energy resource, makes fossil fuels unappealing from a standpoint of future-proof energy frameworks. Hydrogen fuel cells play an important role in such a system, mainly for energy storage and applications for transportation. Proton exchange membrane fuel cells are electrochemical devices which convert hydrogen and oxygen into water, generating electrical current. Platinum nanoparticles supported on carbon show very high activity towards the hydrogen oxidation reaction. On the other hand, the oxygen reduction reaction (ORR) proves to be more difficult in terms of finding a material with adequate performance, both in terms of activity as well as durability. The activity of electrocatalysts can be increased either by increasing their surface area, or by modifying the material composition such that the intrinsic activity is improved. However, the long term stability of fuel cells is hindered by degradation of the electrocatalyst during operation. One idea to tackle this issue is the use of catalyst supports which serve as more than just as a substrate. The metal-support interaction, in which the support influences the catalyst through strain or by changing the electronic structure, is a way of tuning performance of a nanocatalyst. At present, few methods are available that can directly characterize this interaction. A method for probing the catalyst-support interaction would be beneficial for accelerating the process of creating improved ORR catalysts. 4D scanning transmission electron microscopy (4D STEM) is a technique that can be utilized in order to locally probe and analyse the interaction between nanoparticles and their support. Namely, 4D STEM involves using a fast, pixelated electron detector which captures diffraction patterns at each probe position in a scan. The divergence of the center of mass (dCOM) of the electron probe is proportional to charge density. As such, charge redistribution occurring at the catalyst-support interface can be visualized. However, particle orientation and support thickness affect dCOM image contrast, and complicate the interpretation of dCOM directly as charge density. In the following research, a methodology for determining the charge density distribution of supported nanoparticle electrocatalysts is developed. As a model system, platinum supported on graphene was chosen for its simple composition and uniform thickness. After establishing the methodology, it is applied to platinum supported on titanium oxynitride and amorphous carbon, selected as a more complex catalyst-support system.

Keywords:

4D STEM, charge mapping, nanocatalyst

Reference:

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Correlative study of hematite-based photoanodes for solar water splitting by transmission electron and X-ray microscopies

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Poster Group 1

Background incl. aims
In the framework of hydrogen production as means for renewable energy storage, titanium-doped hematite (Ti:α-Fe_2O_3) nanorods have received renewed interest for potential use as a photoanode in photoelectrochemical cells, owing to their high theoretical photoelectrochemical activity, absorption spectrum compatible with sunlight, as well as the abundance of its constituents, allowing cost- and resource-efficient production of hydrogen by solar water splitting (SWS) [1].

In a previous work [2], a two-step synthesis for this material was developed. First, using $FeCl_3$ and $TiCl_3$ precursors, the deposition by aqueous chemical growth (ACG) of an intermediate phase, Ti-doped akageneite (Ti:β-FeOOH) on a glass/FTO substrate was carried out. This step is followed by the annealing of the intermediate phase in a controlled oxygen and nitrogen atmosphere to yield the final Ti-doped hematite photoelectrode structure.

Both synthesis steps are very sensitive to various parameters, which can change the outcome of the final iron oxide structure and its efficiency for the SWS process. Parameters such as the pH of the initial precursor solution, age of the $TiCl_3$ precursor, time and temperature of the thermohydrolysis process as well as of the annealing process, and annealing atmosphere, may all have an impact on the structure and the properties of the final material [3].

Herein, we study the as-obtained hematite nanostructures to characterize their morphology, crystalline structure, chemical composition and electronic properties, as a function of the various synthesis parameters. A correlative approach combining transmission electron microscopy (TEM), yielding images with high spatial resolution, with synchrotron-based scanning transmission X-ray microscopy (STXM) exhibiting chemical sensitivity, in the aim to extract morphological and structural features from the former, electronic structure and chemical coordination from the latter.

Furthermore, we relate the results obtained by these microscopy techniques with photoelectrochemical (PEC) results, to establish a structure-function relationship and thus to be able to optimize the synthesis of the photoanodes for yielding higher efficiency in the hydrogen production process.

Finally, in situ environmental TEM was used to study the annealing step under varying gas atmospheres to elucidate the transition mechanism as a function of gas composition, time and temperature.

Method

To characterize the Ti:α-Fe_2O_3 structures, a correlative approach combining TEM and STXM was implemented. Samples were recovered by scratching the surface of the hematite/FTO glass substrate, retrieving the hematite powder to be placed on either Cu grids or SiN membranes, both of which can be analyzed in TEM and STXM successively. In STXM, the samples were measured at the absorption edges of Ti L2,3 (450-470 eV), O K (520-560 eV) and Fe L2,3 (700-740 eV), to provide localized XAS spectra describing the chemical state and coordination of the atoms of interest.
In TEM, conventional and high-resolution images were acquired in classical TEM and in STEM (BF and HAADF) to obtain morphological and structural information, corroborated by electron diffraction data and EDX spectroscopy maps, the latter to be compared with the chemical information obtained by STXM. Also, in situ environmental TEM experiments were carried out using a sealed cell-based holder inside the microscope in which a controlled gas atmosphere and temperature can be applied to the sample, allowing to monitor changes in morphology and chemical composition in real time.
PEC measurements were carried out in a dedicated PEC cell, using the hematite-based photoanode as working electrode, a Pt foil as counter electrode and an Ag/AgCl reference electrode, under a back-side illumination of 1 sun (0.1 W/cm²).

Results

For the first step, it was found that using an aged $TiCl_3$ precursor leads to the formation of mixed phase compounds, composed of both hematite (α-Fe_2(III)O_3), i.e. only Fe3+ cations in the structure, and a reduced phase, identified as magnetite (Fe_3(II, III)O_4), containing both Fe2+ and Fe3+, in the final products. In contrast, samples prepared with a fresh $TiCl_3$ precursor, only pure hematite was obtained (as shown in Fig. 2), which is the active phase for PEC and therefore the targeted result.
A comparative study changing the composition of the annealing gas, using various ratios of O_2/N_2, ranging from 0% to 100% has shown that the partial reduction of the sample is independent from the gas composition, meaning that this phenomenon is independent of the annealing environment but rather inherent to the specific sample composition. In fact, STXM-XANES measurements on the aged precursor revealed a change in its oxidation state, from Ti(III) to Ti(IV). The Ti(IV) cations incorporated in the akaganeite structure causes the reduction of Fe(III) to Fe(II) by charge compensation, yielding a partially reduced hematite structure after annealing, independently of the gas atmosphere.
These results were confirmed by in situ TEM measurements, showcasing a similar mechanism for the annealing independent of gas composition for samples prepared with the aged precursor, confirming the hypothesis that the reduction behavior originates from precursor ageing.
PEC measurements revealed that the photocurrent Jph of the samples prepared with aged precursor were, on average, lower than samples prepared with a fresh precursor, which was linked to the presence of the reduced phased in the former samples.

Conclusion

In this work, we have showcased the impact of the precursor aging, which, despite the low concentration of the dopant (< 1 at. %), plays a tremendous role on the reduction of targeted hematite phase and thus the efficiency for the solar water splitting application.
Further work is also currently being carried out to understand the influence of other important parameters, particularly the pH of the precursor solution, for which preliminary results showed that it directly impacts the length of the nanorods, yielding to varying PEC efficiencies.

Keywords:

Correlative, TEM, STXM, photoelectrochemistry, hydrogen

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Entanglement in Bragg Scattering

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Poster Group 2

Whenever two quantum systems interact and their state changes (in a non-separable way), they become entangled. In essence, they can no longer be seen as two separate systems, but rather as a single, large system (until they decohere). Since electron microscopy deals with elementary particles as well as structures and processes on the atomic scale – i.e., quantum objects – entanglement is fundamental. In inelastic scattering (EELS), this has been embraced fully for quite some time by the use of the mixed dynamic form factor (MDFF) [1,2]. To our knowledge, the same cannot be said for the much more widespread (and theoretically simpler) case of elastic scattering.
The strange effects of entanglement occur also in Bragg diffraction, as we are dealing with the two interacting quantum systems of the probe beam and the crystal. Take the two-beam case as an example: Measuring in the diffraction plane and detecting the probe electron in the 0 or in the g diffracted beam results in a momentum transfer of either 0 or -g ħ to the scatterer. On the other hand, measuring in the image plane leaves the scatterer in a quantum superposition of two states with different momenta like Schrödinger's cat. This shows that a measurement on one part of an entangled system can have an effect on the other [3]. Therefore, it is critical to include entanglement in the theoretical description of the process to get complete and accurate predictions.
In this work, the joint quantum state of the probe beam and crystal system is described by its density matrix [4] which captures all aspects of the state. It allows to predict the evolution of the state and the outcome of measurements on parts of the system (the beam electron in the present context) by virtue of the reduced density matrix. In the 1-dimensional case, the N degrees of freedom of the scatterer (the positions of the N atoms) can be rewritten as N-1 relative coordinates of a rigid lattice and the position of the center of mass (CM). It is shown that the diagonal elements of the density matrix in a momentum basis correspond to the well-known scattering distribution from conventional kinematic scattering theory. The relative coordinates define the amplitude whereas the CM position imprints a lattice periodic phase factor. Apart from the diagonal terms, the reduced density matrix features off-diagonal elements, which give insight into correlation and interference effects. Among other things, these can be used for determining the decoherence properties of the electron beam.
A rigorous treatment of the entangled system "probe electron/crystal" leads to the standard (reciprocal space) description of Bragg diffraction as scattering on a static periodic Coulomb potential. But on top of that, it also contains additional information on the quantum system, that is accessible in other bases (such as in position space). This work sheds light onto the quantum mechanical measuring process in the context of electron microscopy and therefore contributes to a better understanding on a fundamental level. [5]

Keywords:

scattering, entanglement, interaction, quantum mechanics

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[5] SL acknowledges financial support by the Austrian Science Fund (FWF) under grant nr. I4309-N36. PS thanks Helmut Kohl for valuable comments

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Three-dimensional, correlative electron microscopy and immuno-labelling revealed new principles of the Golgi complex functioning

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Poster Group 2

Goal. The Golgi complex (GC) is one of the main organelles of the cell, it has a complex organization and provides transport of proteins and lipids, as well as their glycosylation. It was necessary to understand the principles of the GC operation.
Methods. The use of three-dimensional electron microscopy (EM), immune EM, and correlative light EM made it possible to abandon the use of the old radigma explaining Golgi function and provide the new one [1, 2].
Results. We have shown that membrane spherical vesicles in the area of endoplasmic reticulum (ER) exits sites are produced mostly with the help of a COPI/ARF machine. There are no completely isolated carriers between the ER and GC. COPI-dependent Golgi vesicles contain a sharply reduced content of retrograde and anterograde cargoes. At the same time, during their transport through the GC, the concentration of soluble, membrane and mega-cargoes increases, which cannot be explained using the maturation model [3, 4].
The role of cisternal perforations for intra-Golgi transport is shown [5].
It was found that the GC may be in a state of rest or transport. The non-transporting GC does not have a cis-most and trans-most cisternae attached to the poles of the medial GC. The beginning of transport leads to their attachment to the stack of medial cisternae in a GM130 and G97 dependent manner, respectively.
The size of the exit site from the GC depends on the amount of cargo being transported [3,4]. With the synchronous movement of a large number of cargo, the exit is carried out from the last two medial cisternae and TMC. When transporting a small amount of cargo, the last medial cisterna and TMC function as the Golgi exit site.
Membrane fusion is important for both moving through and exiting the GC. Post-Golgi carriers ferrying cargoes to the baso-lateral plasma membrane are not completely isolated and always pass through endosomes to change the set of their SNAREs.
The mapping of the main protein machines involved in intracellular transport in various compartments and during various functional states, as well as their changes in different phases of the cell cycle, are presented [2, 5].
Conclusion. The intracellular transport model based on the kiss-and-ran mechanism most fully corresponds to the data obtained.

Keywords:

Golgi; Electron microscopy, Transport, Correlative

Reference:

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Guided growth of 1D van der Waals Nanowires for Enhanced Optoelectronic Functionalities

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Poster Group 2

Background:
Nanomaterials, particularly those with anisotropic characteristics, have attracted significant interest in materials research due to their vast potential applications in diverse fields like optoelectronics, photocatalysis, solar cells, energy storage, sensors, and electronic devices. To unlock these possibilities, precise control over the structure, geometry, and surface arrangement is crucial. Our research group addresses this challenge by developing innovative techniques for "guided growth" of materials on surfaces.
1D van der Waals (vdW) materials, characterized by their chain-like structures connected by weak vdW forces, have emerged as promising candidates for various applications owing to their inherent anisotropic nature and intrinsic 1D dimensionality. We aim to leverage these unique properties, combined with our expertise in guided growth, to fabricate well-aligned nanowires of 1D vdW materials with superior electronic and optoelectronic functionalities. In this work, we present the successful growth of well-aligned Sb_2Se_3 and Sb_2S_3 nanowires on various substrates, including both covalent epitaxy substrates like sapphire and vdW epitaxy substrates like $ReSe_2$.
Method:
The nanowires were synthesized in our group's custom-built chemical vapor deposition (CVD) system. The structure and morphology of the nanowires were characterized using scanning electron microscopy (SEM). Compositional analysis was performed using energy-dispersive X-ray spectroscopy (EDS) and Raman spectroscopy. High-resolution transmission electron microscopy (HR-TEM) is planned to determine the crystal orientation and atomic structure. Additionally, the optical and electronic properties of the wires will be investigated through photoluminescence (PL) spectroscopy. Finally, photodetectors will be fabricated, and their optoelectronic properties will be characterized.
Results:
We successfully synthesized well-aligned nanowires of Sb_2S_3 and Sb_2Se_3 on C-plane sapphire substrates. SEM analysis confirmed the nanowire structure and revealed their oriented directions. EDS confirmed the chemical composition of the nanowires. Focused Ion Beam (FIB) milling is planned in the coming weeks to prepare cross-sectional lamellae for HR-TEM analysis, which will elucidate the epitaxial relationships and growth plane orientation. Following this, a photodetector will be fabricated to characterize the optoelectronic properties.
Furthermore, promising initial results have been obtained for guided growth of Sb_2Se_3 nanowires on $ReSe_2$ flakes, demonstrating successful vdW epitaxy. This mixed-dimensional heterostructure was characterized using Raman and PL spectroscopy. Similar to the previous case, a cross-sectional lamella will be prepared for characterization of the epitaxial relationships within this mixed-dimensional heterostructure using HR-TEM.
Conclusion:
This work demonstrates the successful synthesis of well-aligned Sb_2Se_3 and Sb_2S_3 nanowires on various substrates using our guided growth methods. Further analysis using HR-TEM and fabrication

of photodetectors are planned to fully characterize their properties. Additionally, initial results on Sb_2Se_3 nanowires grown on $ReSe_2$ flakes via vdW epitaxy are encouraging, signifying the potential for exploring novel mixed-dimensional heterostructures. This research paves the way for engineering next-generation optoelectronic devices utilizing precisely aligned 1D vdW nanowires

Keywords:

1D, guided growth, anisotropic

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Detailed TEM Studies of 1D Nanostructures based on Layered Cobalt Oxide

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Poster Group 1

Background incl. aims:
The oxide misfit layered compounds (MLC) are heterostructures with complex stacking usually formed by two ceramic oxide layers. These oxides exhibit remarkable structural and chemical complexity and show very interesting electronic, thermoelectric, and magnetic phenomena [1-5]. It is interesting to mention that due to the intrinsic asymmetry of MLCs, they tend to bend and shape into tubular structures in the form of 1D nanomaterials: nanotubes (NTs) and nano-scrolls (NS) [1-5]. There are two different synthesis routes of these nanomaterials, the starting material is either a mixture of the individual constitutive elements or directly is the corresponding bulk structure. Following the latter pathway, recently, we have developed novel synthesis method based on a crystal conversion process, which parts from a bulk structure with a different, related crystal structure [4,5]. Here, we will present the detailed electron microscopy analysis of nanotubes and scrolls based on layered CoO_2 that have been obtained using this method with two different starting compounds: $Sr_6Co_5O_{15}$ [4] and $Ca_3Co_2O_6$ [5].

Methods
The synthesis procedure starts from the mentioned bulk oxide materials with a quasi 1D crystal structure, which are treated in a hydrothermal process in basic environment. Under this environment, the structure becomes unstable, leading to the dissolution of Sr (Ca) ions and leaving behind cobalt oxide chains. These chains then bend to 1D tubular or scrolled nanostructures. To investigate these 1D systems, different TEM techniques (HR(S)TEM imaging, SAED, X-EDS and EELS) were performed using two aberration corrected Thermo Fisher Scientific Titan microscopes. Furthermore, the electronic/electrical properties of these nanostructures were investigated in-depth at the individual nano-object level by patterning with electron beam lithography.

Results
A large quantity of strontium deficient misfit $SrCoO_2$-CoO_2 (SCO) nanotubes is produced [4]. In these NTs, CoO_2 layers are stabilized by intercalation between $SrCoO_2$ layers (forming a misfit unit), possibly with the aid of Na ions. In comparison to a previous study on similar nanostructures [3], the NTs obtained by the crystal conversion process are Sr-deficient, yielding a structure predominantly made of CoO_2 layers, Figure (a)-(c). These NTs are semiconducting and possess an extremely high ampacity (10^9 A/cm^2), which is the highest reported ampacity value to date in any inorganic oxide-based material, Figure (d) [4]. The nanotubes also show a breakdown power per unit channel length of 38.3 W/cm, the highest among the regularly used interconnect materials [4].
Regarding the calcium cobaltite (CCO) system, a large quantity of stable and pure CoO_2 nano-scrolls have been produced for the first time and investigated in depth, see Figure (g)-(j) [5]. These NSs possess a narrow range of diameter and wall width suggesting that the CoO_2 stabilization occurs

under specific circumstances. These nanostructures are semiconducting with a high current-carrying capacity of 4 10^5 A/cm² and an extremely high breakdown voltage of up to 270 kV/cm.

Conclusion
These detailed structural and chemical investigations at the atomic level have demonstrated the existence of new 1D systems based on layered cobalt oxide, both as pure phase and in intercalated form in misfit layered compounds. Furthermore, we have shown that these 1D nanostructures are potential building blocks for high-power electronic applications, fulfilling the requirement suggested by ITRS.

Graphic
Figure: (a) Crystal structures of the Sr-deficient SCO-NTs with intercalated CoO_2 layers: (left) along the cross-sectional direction, and (right) along the a-axis. (b) HRTEM image of a CCO NT. Line profiles show two d-spacings at the NT border, which are attributed to intercalated CoO_2 (0.54 nm) and the SCO misfit structure (0.89 nm). (c) SAED pattern of the NT (inset (b)). Assignment of the different patterns corresponding to the $SrCoO_2$ and CoO_2 subsystems, respectively. (d) Comparison of the ampacity of SCO-NTs (with/without contact resistance) with other inorganic-based semiconductor nanowires/nanotubes (NWs/NTs). (e) Schematic representation of these 1D Nanostructures based on Oxide Misfit Layered Compounds illustrating their outstanding electrical and electronic characteristics. (f) Crystal structure of (left) $Ca_3Co_2O_6$ revealing the separation of Ca and Co columns and (right) schematic representation of a CoO_2 NT and a CoO_2 NS. (g) TEM image showing CoO_2 NS. (h) HRSTEM image of CoO_2 nano-scroll with lattice distances marked. (i) SAED pattern of the CoO_2 NS. (j) Id–Vd curve up to breakdown for the two-probe device (of a CCO NS/NT) shown in the inset.

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Keywords:

Ceramics, Oxide-misfit-layered-compounds, Nanotubes/Nano-scrolls, Electrical properties

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Microscopic characterization of graphene derivates in life science

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Background incl. aims
Graphene derivatives are becoming increasingly important materials in the field of biomedicine, particularly in neurosciences. Their ability to interact with cells and their potential applications in neuroapplications present an intriguing and revolutionary potential1. This study focuses on the microscopic characterization of various graphene derivatives with the aim of identifying the most suitable ones for neuroapplications, taking into account cell uptake and their biocompatibility. The objective of this study is to conduct microscopic characterization of graphene derivatives and evaluate their interaction with cellular structures. Specifically, we focus on their distribution within cells, cellular uptake, and biocompatibility.
Methods
Microscopic characterization of graphene derivatives was performed using several techniques, including the combination of scanning electron microscopy (SEM) with atomic force microscopy (AFM), transmission electron microscopy (TEM), high-resolution TEM (HR-TEM), time-lapse confocal microscopy and Raman spectroscopy. These methods allowed us to examine the morphology, structure, and interaction of derivatives with cellular structures.
Results
Our microscopic characterization revealed that different graphene derivatives exhibit distinct morphology and structure. Using SEM/AFM, we observed differences in particle size and shape. TEM and HR-TEM enabled detailed examination of the internal structure of derivatives. Time-lapse confocal microscopy will be employed to track the dynamic interaction of graphene derivatives with cellular structures over time, providing insights into their uptake kinetics, intracellular trafficking, and potential effects on cellular dynamics. Raman spectroscopy provided information about the chemical structure of derivatives. An important finding was that certain derivatives demonstrated higher cellular uptake and better biocompatibility than others.
Conclusion
Based on our results, microscopic characterization is crucial for selecting optimal graphene derivatives for neuroapplications. Certain derivatives exhibited significantly enhanced ability to interact with cells and higher biocompatibility, indicating their potential for use in neurosciences2. Further studies focusing on long-term effects and applications of these derivatives are necessary to assess their full therapeutic potential.

Keywords:

Graphene, cells, SEM/AFM, TEM, Raman

Reference:

1Nejabat M. et al. J Biomed Mater Res A. 105(8), 2355, 2017
2Su X. et al. Biosens Bioelectron. 92, 489, 2016
3Chaloupková et al., Analytical Methods, 15 (42), 5582-5588, 2023

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Microscopic characterization of graphene derivates in life science

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Poster Group 1

Background incl. aims
Graphene derivatives are becoming increasingly important materials in the field of biomedicine, particularly in neurosciences. Their ability to interact with cells and their potential applications in neuroapplications present an intriguing and revolutionary potential1. This study focuses on the microscopic characterization of various graphene derivatives with the aim of identifying the most suitable ones for neuroapplications, taking into account cell uptake and their biocompatibility. The objective of this study is to conduct microscopic characterization of graphene derivatives and evaluate their interaction with cellular structures. Specifically, we focus on their distribution within cells, cellular uptake, and biocompatibility.
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3Chaloupková et al., Analytical Methods, 15 (42), 5582-5588, 2023

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3D calibration for SEM and optical microscopy - First results with next generation 3D standards

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Poster Group 1

Marker based 3D standards, in the form of cascading step-slope pyramids, enable the calibration of all three scaling factors and all three coupling factors of measurement instruments using only a single sample and a single measurement [1]. They are suitable for automated calibration and therefore offer better convenience than conventional methods and are therefore increasingly applied [2]. With the introduction of a new scalable wafer-based manufacturing technology, the current application range for calibration of atomic force microscopy (AFM) and SEM can be expanded to optical 3D measurement instruments. First prototypes of these next generation 3D standards are applied for example calibrations of topographic 3D-SEM and confocal laser scanning microscopy (CLSM). The results show the easy and smooth application of the new standards as well as the high conformity of the calculated calibration parameter with conventional methods.
Currently used 3D standards are produced with FIB. Each standard is therefore a cost-intensive custom-made product that also requires time-consuming calibration. The maximum size of these standards is 80 µm with a marker diameter below 1 µm, which cannot be sufficiently resolved with optical 3D microscopes. Therefore, a wafer-based mask process for the fabrication of 3D standards was developed, allowing many structures to be fabricated reproducibly and the geometric dimensions to be adapted to the respective device to be calibrated. First results are prototypes with sizes of 400 µm and 1200 µm for use with optical microscopes, as well as standards with 80 µm size as replacement for AFM and SEM calibration [3]. Figure 1 (SEM and CLSM image with field of view about 400 µm) shows a first prototype, which was successfully used for the calibration of a CLSM (Olympus Lext OLS 4100). The calibration results were compared to the results from a conventional calibration which was carried out as a combination of a step height and a grid standard.
Compared to currently established traceability of FIB standards, which finally based on a reference measurement with a metrological large-range AFM (Met. LR-AFM), providing the required reference data for the wafer-based manufactured standards is easier and more efficient due to their better conformity. Therefore, a combination of traceable calibrated SEM and stylus profilometry was used for the measurement of the reference data [3].
For the coordinate measurement of the reference marks with subpixel methods, as well for the calibration parameter estimation with statistical methods, the dedicated software microCal was applied, which was already validated for this purpose [4].
The prototypes of the next level 3D standards can be used with the applied CLSM and the calibration software microCal without any problems and due to automatization very effective. All markers were measured with the required accuracy. In comparison with the more complex conventional CLSM calibration, the difference between lateral and height scale is below 4x10-4 and difference in lateral shearing is 1x10-4. In addition, a significant vertical shearing was determined, which is hardly possible to identify with conventional methods.
Ongoing work is on further accuracy aspects of the new calibration samples and the specification of the uncertainty budget for providing traceable reference data. Due to the promising results, it is

planned to make the new generation of 3D calibration samples commercially available in the near future.

Keywords:

3d metrology standards calibration

Reference:

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Exit of different cargoes from the Golgi and their post-Golgi trafficking

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Poster Group 2

Proteins and lipids are synthesized in the ER, delivered at the Golgi complex, then exit the Golgi complex and finally, delivered toward their final destination. Until now the information about the Golgi-to-plasmalemma transport (GPT) and the formation of Golgi-to-PM carriers (GPC) and their fate is controversial. For a long time, the vesicular model played a role of paradigm for all steps of intracellular transport. However, several years ago it was demonstrated that the transport from the Golgi complex to the plasma membrane (PM) is carried out by irregular GPCs [1,2]. Pathways for different cargoes are not well established. It is not clear whether there is a necessity for the fusion of the post-GC carriers with endosomes. Here, we examined mechanisms of GPT, namely, the three-dimensional structure of post-Golgi carriers, patterns of their exit from the Golgi complex, their transformation during their delivery to the baso-lateral PM and their dependency on the amount of cargo transported and fusion with endosomes.
All reagents, the cells and the cargo synchronization protocols were described in [3]. We studied conventional cargoes: PCI, PCI-GFP (PFP), tsVSVG, tsVSVG-GFP (VFP); ASGPR, albumin, and GFP-albumin (AFP) using STEM and TEM tomography, correlative light electron microscopy (CLEM), high pressure freezing (HPF), immuno-EM, focused ion beam (FIB-SEM).The GPC exit from GC depends on the amount of cargo moving through it. The temperature-sensitive glycoprotein G of vesicular stomatitis virus (VSVG) and procollagen-I (PCI) can also exit from the last two medial Golgi cisternae when a large amount of them is transported, whereas when a small amount of PCI is transported, it passes through the last medial cisterna and the trans-most cisterna (TMC) and then exits the TGN zone. Albumin exits the GC through TMC and TGN, where accumulative large vacuoles are formed. Albumin is enriched in the vacuole(s). Most of these vacuoles contain low concentration of VLDL. Smaller but distinct vacuoles are enriched in VLDL. This vacuole could be connected with the PM through thin tubule. Fusion of GPCs with endosomes and then their subsequent fission is necessary to remove resident Golgi proteins from GPCs and replace SNAREs in GPCs. Using advanced methods of the high-resolution 3D imaging (STEM and TEM tomography, FIB-SEM) after a cryo-immobilization procedure (HPF) has been shown that near the GC and during their passage to the PM, GPCs are always connected with at least one GC/TGN/endosome structure. The exchange of SNARE proteins ensures the subsequent fusion of GPCs with the PM. Thus, the kiss-and-run model is the most powerful model for the explanation of GPT.

Keywords:

Golgi, post-Golgi, transport, 3DEM, tomography

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FIB-induced nanorod formation in 2D layered crystals

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Poster Group 2

In this study, we present our findings of very unusual behavior shown by bulk crystals of 2D layered materials, such as transition-metal diselenides, when subjected to focused ion beam (FIB)-milling. During FIB preparation of lamellae for transmission electron microscopy (TEM) analysis, we encountered a very pronounced curtaining effect for this type of materials. This initially leads to thickness variations in the TEM lamella, and upon further FIB milling, to the formation of wire- or rod-like structures. The curtaining is observed on different length scales from the micro- to the nanometer range. Examples of such structures can be seen in figure 1.
Interestingly, the rod-like structures form, when the milling direction is perpendicular to the 2D layers. However, the formation of rods only occurs for some crystal orientations, whereas an in-plane rotation of the bulk crystal can lead to a homogeneous milling behavior without curtaining. Furthermore, the choice of the ion beam milling parameters seems to have an influence on the resulting structures.
We present further investigations of these wire- or rod-like structures for several 2D materials with different compositions. The goal is to expand our knowledge of the conditions that either lead to or prevent the formation of the nanorods and to gain a deeper understanding of the effect that causes these structures to form.

Keywords:

Focused ion beam
2D materials

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Sub-nanometer mapping of strain-induced band structure variations in different semiconductor device configurations

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Poster Group 1

Over the last three decades, heterostructured semiconductor nanowires (NWs) have been in the spotlight because of their potential as nanoengineered building blocks for a new generation of electronic, photonic and plasmonic, devices for quantum, sensing, and energy-harvesting applications [1]. The nanometer-scale lateral dimensions and resulting quasi one-dimensional morphology of the NWs facilitate the release of the inherent epitaxial strain, preserving high crystal quality even when interfacing highly mismatched materials. Therefore, the characterization of plastic and elastic strain relaxation mechanisms is key to understand and gain control over the optoelectronic properties of epitaxially grown planar structures. Here we propose a methodology to correlate these optoelectronic properties, namely the bandgap, with the structural properties, namely the strain, and prove its concept with ZnSe/ZnTe core-shell nanowires as radial p-n junctions, used as electro-optic detectors among other applications. The method is based on electron energy loss spectroscopy (EELS) to take advantage of sub-nanometric spatial resolutions.
To go through this correlation, we first evaluate the strain, by means of Geometrical Phase Analysis (GPA), in different ZnSe/ZnTe planar systems, which modulate their strain relaxation mechanisms and morphology depending on the substrate (sapphire) plane exposed, determining the final epitaxy of the system. Afterwards, the low-loss EELS regime is acquired, and our methodology is applied to extract and spatially map the bandgap energy, which can be linked with the previous strain measurements [2].
The proposed novel methodology for low-loss EELS analysis can get over limiting scenarios which are affected by parasitic signals such as Cherenkov radiation and surface modes. Until now, these contributions to the low-loss regime that hide valuable signals such as phonons, bandgap and plasmons, hampered more ambitious experimental setups and limited them to low refractive index materials or off-axis EELS [3]. Interestingly, our methodology allows the data cleaning and removal of parasitic modes in any acquisition condition and for materials with any dielectric description. In summary, the method uses unsupervised machine learning for spectrally mapping distinct regions and later applies a spectrum-wise theory-supported correction. These corrections based on classical electrodynamics enable the removal of the percentage of signal attributed to parasitic signals, therefore ensuring the remaining signal is only composed of intrinsic properties of the materials.
The present proof of concept focused on bandgap mapping, which is key knowledge in optoelectronics. However, the methodology is thought to be reproducible to any property if the adequate level of theory is applied. Importantly, this methodology and EELS high spatial resolution

proved its sensitivity by allowing us to distinguish a strain-induced direct to indirect bandgap transition at the interface between the ZnSe core and the ZnTe shell of one of the studied systems. In addition, although the original methodology was developed in the ZnSe/ZnTe heterojunction, preliminary results will be shown on Ge/SiGe quantum wells for holding spin qubits [4] and Ge/Si nanowires aiming towards topological quantum computing, which push the method to more challenging scenarios with bandgaps below 1eV.

Keywords:

EELS, bandgap, strain, methodology

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Investigating Bone Microstructure with ATUM-SEM: Implications for Pathological Conditions

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Poster Group 2

Background incl. Aims

Bone tissue's hierarchical structure is pivotal for various mechanical, biological, and chemical processes essential to health. However, the mechanical properties of bones are susceptible to deterioration, leading to an increased risk of fragility fractures associated with ageing, vitamin D deficiency, and bone density pathologies, such as osteoporosis [1]. The prevalence of these chronic illnesses, coupled with rising life expectancy, has become a significant public health concern. Additionally, the COVID-19 pandemic potentially exacerbates bone health issues, leading to decreased bone mass and strength in long-term hospitalised patients [2]. Bone fractures result in significant psycho-social and economic burdens, yet current understanding is limited to macro- and mesoscale levels. The challenge lies in comprehending the mechanobiological perspective at the multiscale level (between the mm and nm ranges). This knowledge gap, coupled with the influence of other intricate structural parameters, underscores the need for advancements in research tools and technical approaches.

Methods

Automated Tape Collecting Ultramicrotome Scanning Electron Microscopy (ATUM-SEM) has been introduced as an effective method for capturing high-quality images of hard tissues [3]. In this case study, the choice of using ATUM-SEM was driven by the need for a non-destructive approach that could enable the examination of the largest possible volume. The method was chosen for its flexibility, which allows cutting slices sections with a chosen thickness while preserving all of them for subsequent observations. Despite a thorough literature search, no suitable protocol was found to prepare human trabecular bone compatible with the ATUM-SEM-based approach. Consequently, it was determined that the best course of action was to utilise the same workflow previously used in the analysis of biological samples, with some adapted modifications made on a case-by-case basis. Trabecular bone samples from female and male patients diagnosed with different clinical conditions, namely osteoporotic, COVID-19, and a healthy control group, were evaluated. The specimens were processed by cutting them into macroscopic shapes appropriate for applying the ATUM-SEM approach, followed by careful cleaning and dehydration to improve their embedding in the resin. The resin embedding process was optimised for efficient sectioning, and a good trimming and polishing of the face of the specimen block was crucial for a stable section collection protocol. Slices, with thickness ranging from 300 nm to 400 nm, were collected onto Kapton tape using an Ultramicrotome. The imaging workflow that was utilised to reconstruct the acquired volume was managed using Atlas 5 Array tomography software and Fiji. Creating a 3D representation requires

accurate positioning of images in a virtual volume through registration. The alignment of the image stacks was accomplished using the TrakEM2 plug-in. Finally, Data visualisation is achieved using the 3D volume rendering present in Fiji.

Results

The ATUM-SEM data sets have yielded both qualitative and quantitative analyses, revealing distinctions in lacuna size and shape between osteoporotic and non-osteoporotic cases. Specifically, the osteoporotic and COVID-19 lacunae were larger, less stretched, and more rounded, with the average dimensions ranging from 6.12 µm x 12.27 µm x 10.8 µm and 7.17 µm x 20.96 µm x 6.9 µm, respectively. In the femoral head, lacuna volume exhibited internal variability, with osteoporotic and COVID-19 cases showing pronounced differences compared to healthy specimens (Figure 1). In the femoral head, the volume of the lacunae presents internal variability, ranging from 26% in healthy specimens to 14% in osteoporotic ones and 18% in COVID-19 patients. The volume difference is more pronounced when comparing the physiological state with the two pathological conditions. The volume of the osteoporotic lacuna is approximately 39% larger than the healthy sample, while the volume of the COVID-19 lacuna is around 53%. The complexity of the COVID-19 pathology requires further cellular-level studies to isolate its direct effects on bone microstructure and understand lacunar alterations among different variants, even if the possibility of bone micro-structural deterioration due to COVID-19 is proven [4]. The results of increased lacunar sphericity in osteoporotic subjects are consistent with other research works demonstrating that lacunar stretch is reduced in patients affected by this pathology [4]. Nevertheless, image segmentation and processing still need to be improved for quantitative analysis, demanding improvements in automated segmentation routines. While there are ongoing debates regarding the impact of age on bone microstructure, the study has identified age-related variations in the characteristics of canaliculi and lacunae.

Conclusion

This study highlights the importance of examining bone microstructure at finer levels to understand bone health and disease. ATUM-SEM provides high-resolution imaging and detailed analysis of bone microstructures, leading to better diagnosis and treatment of bone-related disorders. Exploring ATUM-SEM's clinical applications for early bone pathology detection shows promise and may lead to targeted therapies. These future directions in ATUM-SEM analysis of bone pathologies pave the way for enhanced research and clinical applications in the field, leading to a future where targeted interventions can mitigate the burden of bone-related disorders

Keywords:

Bone-microstructure, Osteoporosis, Lacunae-canaliculi-system, ATUM-SEM, 3D-imaging.

Reference:

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Automated Detection of Material Defects for High-throughput Electron Micrographs Analysis

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Poster Group 2

Background
Detailed analysis of material's microstructure is required for predicting material properties and fine-tuning manufacturing parameters to achieve the desired characteristics. This feedback process is particularly important when it comes to developing new production techniques such as metal additive manufacturing (AM).
Our goal is to provide a holistic solution that enables reliable materials characterization using a high - throughput approach in a scanning electron microscope (SEM) with automatic detection of characteristic microstructural features, especially defects such as cracks and pores. The scope of the detection framework that we cover includes all aspects of pre-processing the raw images from the microscope, defect identification and visualization of statistical results. In this way, it serves to overcome the current need for automation, providing researchers with a transparent and customizable process for their structural analysis. The customizable definitions of the parameters used in our algorithm also enable optimizations for applications in other fields of imaging, such as optical microscopy in biomedical research.
Methods
We acquired large-area secondary electron (SE) and backscattered electron (BSE) SEM images of an additively manufactured metal sample (316L steel) thereby varying dwell times from 10 microseconds to 100 nanoseconds and pixel size (i.e. magnification) from 391 to 49 nm. This allowed us to develop our image analysis algorithm to identify structural defects across a wide range of imaging conditions and systematically investigate the loss of precision in detection as image acquisition time was lowered (i.e. lower dwell time, larger pixel size). Once the micrographs were acquired, they were passed through a boundary detection procedure which isolates the relevant sample area. Filtering was then performed using multiple thresholding and convolution layers.
Results
Preliminary results have shown great promise in comparison to the manually labelled sections and flexibility to different imaging conditions. Performance metrics have also been compared with the most widely used classical tools in the field as well as with AI tools with which our software overlaps in scope.
The defects were characterized and selected according to user specifications in order to output statistical results and sample descriptors directly from the software interface. The robustness of the detection process has been verified using methods including comparisons with manually labelled sections and results obtained at highest dwell time and magnification. In this way, the development focuses on optimizing result consistency with shorter SEM image acquisition time, focusing on high-throughput analysis.
Conclusion
We have demonstrated in a systematic study the effect of SEM image acquisition parameters, such as dwell time and magnification, on the robustness of automatically detecting defects in AM 316L. This now enables the selection of optimised image acquisition parameters and a minimised data acquisition time (high throughput).

The scope of our developed approach is more comprehensive than any currently available tool can offer. The aim of this development is to make defect analysis more transparent and reliable by providing a unifying platform.

Keywords:

Electron Microscopy, High-throughput Defect Analysis

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Control of inter-particle distance between nanoparticles using DNA origami

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Poster Group 2

Background incl. aims
Metallic nanoparticle pairs, known as dimers, have been used to enhance the excitation rate of single quantum emitters. Among other parameters, the inter-particle distance (d) has crucial impact on the electro-magnetic field around the particles, leading to enhancement of signals. Thus, precisely controlling d is desired for achieving the optimized performance for the given applications. Usually, d is controlled by using linker molecules. In this study, we alternatively utilized the DNA strands to control d. Since this technique has several independent parameters, it ensures a high degree of customization.
Methods
The synthesis of dimers was adapted by Gür et. al. [1] and consists of 2 steps. In the first step, we functionalized the nanoparticles using using single strands of poly-thymine. In the second step, selected staples of the DNA origami were extended with pieces of single-stranded poly-adenine. The complementary strands connect via hydrogen bonding, forming the strand of length (L) (Fig. 1A). We analyzed multiple secondary electron (SE) images of these self-assembled gold dimers using different L from different samples in order to verify the reliability of the control of d. Based on geometric considerations (Fig. 1B), we expect an increase in the standard deviation σ of d. We therefore required large amount of dimers to obtain statistical significance. The detailed analysis of the SE images was achieved by using a novel analysis tool developed for this task. The tool uses the intensity thresholding technique to segregate the DNA origami from the silicon wafer, and then analyzes each origami individually. We use circle Hough transform (CHT) to detect the spherical particles, with a set of novel classification routines to assign one of 4 classes (monomer, dimer, single particle on wafer, agglomerate) to each particle (Fig. 2)
Results
We analyzed the distribution of d across multiple samples using our tool, measuring more than one thousand dimers per sample. While the general trend of σ increasing with L could be observed in the experimental data, the proposed effect of L on σ is smaller than expected (Fig. 3). The deviation between the simulation and experiment can be attributed to our model only taking sample geometry with few assumptions about the DNA strands into account. However, based on our observations, more complex effects of DNA bonding and the interactions between nanoparticles, DNA strands and DNA origami led to notable change in the distribution of d. Therefore, an accurate prediction of the distribution of d requires a more complex model.
Conclusions
We investigated multiple samples, in which the different inter-particle distances of gold dimers were achieved using different DNA strand length on the DNA origamies. Based on sample geometry, we expected a significant effect of the DNA strand length on the distribution of d, with longer strands resulting in a broader distribution. Our experiments confirm the existence of this trend, even though

the effect is smaller than our initial expectations. Our results show that complex interactions within the material system have significant effects on the distribution of d, making an accurate prediction of the distribution more complex than anticipated.

Keywords:

gold dimers, DNA origami

Reference:

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Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway

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Eukaryotic cells maintain cellular proteostasis through intricate protein quality control systems that orchestrate chaperone-mediated protein folding and protein degradation systems, preventing potential harm from accumulated misfolded proteins. The chaperone system attempts to refold the abnormal proteins and solubilize aggregated proteins; if unsuccessful, these aberrant proteins are removed through the protein degradation system.
In the ubiquitin-proteasome system (UPS), the degradation process is initiated by the recognition of ubiquitinated substrates by the 19S regulatory particle (RP), which is then followed by unfolding and translocation of substrates in the 20S catalytic particle (CP) that executes protein degradation. Aside from the canonical subunits, proteasome function is finely tuned by cofactors that contain ubiquitin-like (UBL) domains which are involved in recruiting substrates to the 26S proteasome.
On the other hand, Bag1 has been shown to interact with Hsc70/Hsp70 to modulate the chaperone activities. Through an ATP-driven conformational cycle, Hsp70 can recognize misfolded proteins, promote refolding, prevent protein aggregation, and resolubilize protein aggregates. Despite their many different roles, all members of the Hsp70 family contain two highly conserved structural domains: the substrate-binding domain (SBD) and the nucleotide-binding domain (NBD). The ADP/ATP switch is catalyzed by a group of cochaperones called nucleotide exchange factors (NEF), which bind to the NBD and favor ADP release from the active site and ATP re-uptake. Bag1 is one of such NEF and contains both UBL and BAG domains and interacts with the 26S proteasome through the UBL domain to degrade unfolded proteins. However, how cochaperone Bag1 bridges between protein folding and degradation systems, and how Bag1 enhances degradation of unfolded proteins remain unanswered.
In this work, using cryoelectron microscopy (cryoEM) and different biochemical and biophysical techniques, we have revealed that Bag1 plays a key role in Hsp70-mediated, proteasome-dependent protein degradation, not only by physically linking Hsp70 to the proteasome (through its subunit Rpn1), thus promoting protein delivery to the latter but also by inducing a series of conformational changes in the 19S that facilitate the client protein degradation.

Keywords:

Chaperone-mediated-degradation, Proteasome, Hsp70, Bag-1, CryoEM

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Analysis of epigenetic modifications with the LiveMIEL (Live-cell Microscopic Imaging of Epigenetic Landscapes) approach

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Poster Group 2

Background incl. aims
Epigenetic modifications are reversible covalent chemical modifications of DNA, histones, and microRNAs. These modifications act as recognition sites for chromatin-binding proteins that regulate the access to the genomic DNA during replication and transcription. A clear and self-consistent picture of these modifications in dynamics is crucial for our deeper understanding of epigenetic regulation of the genome. Reader domains play a key role in the interpretation of the histone code, which is an essential part of the epigenetic regulation, because they interact with histone modifications and recruit proteins with specific activities to a given chromatin locus.
Methods
The data was obtained using the LiveMIEL (Live-cell Microscopic Imaging of Epigenetic Landscapes) analysis [1], which exploits computer vision and machine learning approaches to classify cells according to specific patterns of epigenetic modifications. The concept of image processing and analysis included segmentation, extraction of characteristic features from segmented images, and clustering of the obtained data. For this purpose, we created a HEK293T cell line stably expressing the MPP8-Red sensor based on the MPP8 reader domain fused to the Katushka fluorescent protein. We also used the Cdt1-TagBFP2 and mVenus-hGeminin plasmids (components of the FUCCI-CA cell cycle indicator system). Then we collected fluorescent microscopy images and derived texture and 98 morphological features from each segmented nucleus.
Results
Here we present our data on the redistribution of histone modification H3K9me3, which is known to correlate with the suppression of gene transcription during the cell cycle and after treatment with several drug classes. After extracting information from all of the obtained images of each single nucleus, the data clustering analysis was performed. The optimal number of clusters (n=3) was then determined using the silhouette coefficient analysis. The results of LiveMIEL analysis obtained from the HEK293T-(MPP8-Katushka + FUCCI-CA) cells showed a good agreement with the similarly processed data from the cells' cycle phases (G1, S, G2, and M) with FUCCI-CA as a fluorescent indicator. We observed the most drastic changes in the H3K9me3 profile in the G2 and M phases compared to the S and G1 phases.
Furthermore, we demonstrated a statistically significant effect of small dosages (starting at 0.01 µM) of AT9283, OTX015, EPZ-6438, and RG2833 drugs with the LiveMIEL analysis. The impact of the studied drugs on the architecture of H3K9me3 in the nucleus was confirmed with the MTT assay.
Conclusion
The approaches and models developed in this project could be found useful for analysis of biological material. For instance, such material could be obtained from patients as a part in the newly-developing pipelines for personalized medicine. The other promising option is screening of the epigenetic effects of drugs at a high throughput and low cost.

This work was supported by Russian Science Foundation grant 24-74-10080.

Keywords:

epigenetics; histone modification; machine learning

Reference:

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The EXCITE² Network: a European infrastructure providing transnational access to leading-edge imaging facilities

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Poster Group 2

The EXCITE² Network is a leading European electron and X-ray imaging infrastructure for research into Earth materials. This network is revolutionizing Earth and environmental material science research by consolidating 19 research facilities in 12 European and associated partner countries into a unified infrastructure that will provide transnational access to advanced imaging technologies. This open access to our state-of-the-art microscopy facilities enables scientists to decipher complex processes within Earth materials across scales ranging from nanometres to hundreds of centimetres. Understanding these processes is critical to creating a sustainable, carbon-neutral society, as they govern various phenomena, including environmental toxicity and its impact on human health, critical metal extraction for renewable energies, geothermal energy extraction, subsurface energy storage, and the utilization of Earth materials for long-term storage of climate-altering gases.
EXCITE² is driving interdisciplinary collaboration and cross-fertilisation across academic institutions, scientific disciplines, and industry, propelling Europe towards a sustainable future. In addition to access to leading-edge imaging facilities, the network offers early career researchers a chance to team up with leading Earth-materials scientists, develop new collaborations, engage in cross-disciplinary initiatives, and execute progressive electron and X-ray imaging projects that contribute to the advancement of Earth-materials research. We connect with you, provide access to workshops and webinars, assist in preparing your proposals for facility access, and actively support your research goals. Moreover, the initiative offers dedicated training programmes to a new generation of researchers within the European open science landscape. EXCITE² is introducing innovative service developments, such as artificial intelligence and leading-edge imaging experiments, to increase the user's problem-solving capacity. As such, EXCITE² is paving the way towards a sustainable future, driving scientific excellence, and creating positive societal impact.
Access to EXCITE² can be requested by applying to our transnational access calls. Interested? Have a look on our website (https://excite-network.eu) – and apply for the next call!

Keywords:

EXCITE, Transnational access, Electron microscopy

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Structural Probing of Charging Mechanism in LiNiO2 at High Voltage using Microscopy and Spectroscopy

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Poster Group 1

Background
The demand of high-energy lithium-ion batteries boosts the interest in increasing Ni content in cathodes and raising charge cutoff voltage. LiNiO2 is a critical archetypal material for high energy density Li-ion batteries, forming the basis of Ni-rich cathodes in use today. Unfortunately, LiNiO2 suffers from a number of problems at high states of charge which have been linked with oxygen redox. Substantial efforts have been made to understand the structural transitions that take place when Li is extracted from LiNiO2, but there remains considerable debate over its structural mechanisms, in particular the extend of Ni oxidation and oxygen participation when charging at high cutoff voltage. [1,2]

Methods
We use polycrystalline LiNiO2 powder to make composite electrodes into Li coin cells. After charging and discharging to specific states of charge, we disassemble the cell for following characterizations. We use the annular dark-field scanning transmission electron microscopy (ADF-STEM) to directly examine structural changes at the atomic level. We employ combined neutron and synchrotron X-ray powder diffraction analysis that takes account of the stacking faults, and quantified the Ni vacancies formed when the material is charged across the voltage plateau. High resolution resonant inelastic X-ray scattering (RIXS) at the O K-edge is used to detect the molecular O2 that could be formed associated with O-redox. Ni L-edge and O K-edge X-ray absorption spectra are collected to probe the chemical state changing in LiNiO2. Chemical analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) is used to check if there is Ni dissolution into the electrolyte after cycling.

Results
The atomic-resolution showed the surface densification and cation mixing evolution near surface region of LiNiO2 over charging to high cut-off voltage (Fig. 1a). When charging acrossing the 4.2V plateatu vs. Li+/Li, the particles form core–shell nature, with a Ni-rich, Ni1.75O2 rocksalt-like shell approximately of 5 nm thickness at the top of charge. The refinement of neutron and synchrotron X-ray powder diffraction demonstrates 8% Ni vacancies form in the originally fully dense transition metal layer at 4.2 V (Fig. 1b). Chemical analysis by ICP shows that the Ni absent from the bulk does not leave the particles. While in principle there is sufficient Ni3+/4+ oxidation capacity to account for all the Li+ removed on charge without O2- oxidation, high resolution RIXS at the O K-edge confirms the presence of trapped molecular O2, on charging across the plateau, which is accommodated Ni vacancy clusters. Ni L-edge XAS measurements in fluorescence yield mode implies the average oxidation state of Ni of less than +4, coexisting with the presence of O-redox at the top of charge (Fig. 1c,d).

Conclusion

Our results reveal that the structural instability in LiNiO2 is more extensive than previously thought. As illustrated in Fig. 1e, beyond the 4.2 V vs. Li+/Li plateau, Ni accumulates at and near the surface of the particles, forming a Ni-rich shell approximately 5 nm thick; enhanced by loss of O2 from the surface. This shell has composition of Ni1.75O2 shell a Ni oxidation state of +2.3. The oxidation of O2− across the 4.2 V plateau forms O2 trapped in the particles and is accompanied by the formation of 8% Ni vacancies on the transition metal sites of previously fully dense transition metal layers. The overall Ni oxidation state of the particles measured by XAS in fluorescence yield mode after charging across the plateau to 4.3 V vs. Li+/Li is approximately +3.8; however, taking account of the shell thickness and the shell Ni oxidation state of +2.3, this indicates a Ni oxidation state in the core closer to +4 for compositions beyond the plateau.

Keywords:

LiNiO2, charging mechanism, electron microscopy

Reference:

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Advanced acquisition strategies for lab-based diffraction contrast tomography

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Poster Group 1

Background incl. aims
Non-destructive imaging of a polycrystalline material's 3D grain microstructure is key to a better understanding of the material performance and to validating computational models for predicting the material behavior. In addition, an integrated microstructural modeling approach, ensuring the handshake between modeling and experimentation, relies on adequate experimental statistics of 3D sample volumes that are representative in both size and shape.

Methods
In this study, we exploit lab-based diffraction contrast tomography (DCT) [1,2] to experimentally map the 3D grain structure. The introduction of three new advanced acquisition strategies for lab-based DCT – named Helical Phyllotaxis, Helical Phyllotaxis Raster, and Helical Phyllotaxis HART – enables grain mapping of longer, larger, as well as high-aspect ratio samples [3]. The implementation of these advanced acquisition strategies combines a golden angle rotation with vertical and horizontal translations to perform a seamless data collection that has a uniform sample illumination both angularly and spatially. Reconstruction of the corresponding data is equally seamless, simultaneously using all data to reconstruct the full illuminated volume without the need for stitching of data subsets or sample subvolumes.

Results
We will present and discuss different acquisition strategies with emphasis on how to approach a given acquisition problem inherent to the sample. Particular emphasis will be on the Helical Phyllotaxis HART (high-aspect ratio tomography for plate-like samples) strategy that enables investigations of a hitherto inaccessible class of sample geometries comprising industrially relevant materials like rolled metal sheets and electrical steels [4].

Conclusion
The advanced acquisition strategies take lab-based non-destructive 3D grain mapping to the next level of throughput, grain statistics and versatility. While the throughput warrants 4D studies of materials microstructural evolution, a representative sample volume is a prerequisite for successful model predictions, and the versatility enables studies of samples or components under more realistic in situ or in operando conditions.

Keywords:

Lab-based DCT, Advanced Acquisition

Reference:

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Thallium and arsenic incorporation in roméite group minerals

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Poster Group 2

Background incl. aims
Roméite group minerals (RGM) are oxides, which belong to the large pyrochlore supergroup of minerals. The general formula of the pyrochlore supergroup is $A_2B_2X_6Y$ (A = Na, Ca, Ag, Mn, Sr, Ba, Fe(II), Pb, Sn(II), Sb(III), Bi(III), Y, REE, Sc, U, Th, □ and H_2O, B = Ta, Nb, Ti, Sb(V), W, V(V), Sn(IV), Zr, Hf, Fe(III), Mg, Al, and Si, X = O, OH, or F and Y = (OH)–, F–, O2–, □, H_2O, or even large cations, such as K, Rb, Cs). The crystal structure of RGM is build-up of BO_6 octahedral framework that forms tunnels along [110] where 8-coordinated A cations and Y anions are hosted. In the RGM, Sb(V) is the dominant cation at the B site, but on the A-site, larger cations can be incorporated where also the ion-exchange can take place. Therefore, RGM are often used for the immobilization of the toxic metals, such as Pb and Sr and have been frequently reported as weathering products in mining wastes and smelting residues resulting from Sb mining activities.
Methods
High-resolution electron microscopy, both transmission (TEM) and scanning (SEM), including energy dispersive spectroscopy (EDS), energy electron loss spectroscopy (EELS), and selected area electron diffraction (SAED), are used to characterize Sb- and As-rich samples from mining waste dumps from the Sb-As-Cr Lojane deposit, North Macedonia, and Tl- and Sb-rich samples from weathered technosols of the central part of the Sb–As–Tl–Au Allchar deposit, North Macedonia. These techniques are supplemented by X-ray diffraction (XRD) and Raman spectroscopy.
Results
RGM from the Lojane deposit are observed as extremely thin (< 10 µm) weathering crusts encircling grains of stibnite, Sb_2S_3 and realgar, AsS. Larger homogenous grains up to 500 µm are formed by Sb-dominant variants of this oxide, which are also characterized by broad dehydration cracks, indicating that they were originally formed as gels. The analysed thin crusts and aggregates are most likely poorly crystalline to amorphous, As-dominant RGM-like phases, which appear darker grey in the SEM images, and nano- to microcrystalline, Sb-dominant RGM, which appear very light grey in the SEM images. The crystallographic position of arsenic in the RGM in our samples is not fully clear. In the RGM, As(V) may occupy the octahedrally coordinated A-position, but it strongly prefers tetrahedral coordination. The distorted cubic B-position in RGM is too large for the As(V) cation. These crystal-chemical preferences explain why the As is mostly in the X-ray amorphous phases. However, EDS point analyses clearly document that the RGM may incorporate considerable As.
In the mining waste dumps of Allchar deposit, Tl dissolved during weathering is besides other Tl-oxides, such as avicennite, Tl_2O_3 and amorphous Tl-Mn-oxides, reprecipitated as tiny spherulitic aggregates (up to 2 µm) of a Tl-Sb-oxide (a new mineral species). TEM-based SAED on Tl-Sb-oxide nano-particles confirmed that the Tl-Sb-oxide is crystalline, and chemical composition analysed by EDS-line and area scans confirmed a Tl:Sb ratio of 2.5. The cell parameters calculated from the SEAD pattern show good comparison with the members of pyrochlore-type structure.
Conclusion

Understanding the potential pathways for Tl and As incorporation in pyrochlore-type structures addresses not only the growing environmental concern over these two priority pollutants, but also contributes to the larger field of waste management.

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Keywords:

thallium, arsenic, mining wastes, nano-minerals

Reference:

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Impact of improved tracking method on structure determination of perovskites from 3D ED data

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Poster Group 2

Background incl. aims
Perovskites (ABX_3) and perovskite-related compounds are some of the most chemically diverse crystal structures with many possible substitutions at A and/or B sites, as demonstrated in oxide systems [1,2]. These materials can be produced using facile processing techniques and yield high performance devices, although they are characterized by large compositional and structural heterogeneities at multiple length scales. Even small structural changes can be induced by strain engineering and open a way to enabling new or deliberately modified material properties. XRD structure refinement requires great effort, especially in the case of accurate characterization of small structural distortions, oxygen positions, and octahedral rotations. Additionally, the contribution of several oriented domains makes structure refinement more complex.
Methods
3D electron diffraction (3D ED) technique [3,4], which may or may not be coupled with precession electron diffraction, allows not only to determine an average structural model, but also to refine it with good accuracy. Moreover, tracking methods, which have been developed in our group [5], can significantly improve the diffraction intensities and overall detailed structure description. They also provide support in answering questions about structural details.
Results
In the present work, we perform structure analysis of perovskites to demonstrate its improvement by dedicated data acquisition routines. By implementing dynamic theory in the refinement, 3D analysis of region of interest in the range of tens of nm and accounting for precession motion, it is possible to obtain reliable and accurate refinement of perovskite structures. Taking into account the selective contribution of differently oriented grains allows for a more accurate description of the structure.
Conclusions
Implementing the dedicated data acquisition strategy and improving the diffraction pattern analysis, pave the way for a new routine characterization of structure details in complex perovskite materials. This places 3D electron diffraction even closer to complementary scanning transmission electron microscopy (STEM) and convergent beam electron diffraction (CBED) techniques, which are usually a choice to map octahedral tilts or atomic vacancies at grain boundaries.

Acknowledgment
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Keywords:

3D ED, electron diffraction, perovskites

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Effects of melatonin and alpha-lipoic acid on collagen and VEGF expression in palatal wound healing

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Poster Group 1

Background incl. aims
Reactive oxygen species (ROS) play a crucial role in the normal physiology of wound healing. The balance between high and low levels of ROS is critical during the healing process. As regulators of oxidative stress, antioxidants are proposed as targets for new therapies to accelerate wound healing. Alpha-lipoic acid and melatonin are antioxidants that have been shown to be involved in wound healing in many different organs. In addition to protective effects against oxidative stress, they also exert anti-inflammatory effects. In this study, we aimed to evaluate the effects of alpha-lipoic acid and melatonin on rat palate wound healing and whether they have a synergistic effect when used together.
Methods
A total of 64 male and female adult Wistar rats were randomly divided into 4 main groups. All rats were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) injections before creating a 5 mm diameter wound using a punch biopsy in the central area of the hard palate. Experimental groups were treated with daily intraperitoneal injections of alpha-lipoic acid (60 mg/kg/day) or melatonin (30 mg/kg/day) or both of them until sacrification. Control group did not get any injection. 2 sub-groups were created for each group, according to sacrification days; 5 or 10 days after wound creation. Palatal biopsies were fixed with 10% neutral buffered formalin. After decalcification for 8 days in decalcification solution, samples were dehydrated with increasing grades of ethanol, cleared with toluene, and embedded in paraffin. 5 µm thick sections were taken with microtome. Masson trichrome stain was performed to evaluate the amount of collagen fibers in sections. VEGF expression was determined using the immunohistochemistry method. After taking photographs of the sections, Image J software was used to evaluate the results of the stainings. Statistical analyses were performed using IBM SPSS version 24.
Results
Collagen fiber density in the granulation tissue in the 5th day samples of the melatonin-treated group was significantly higher than that in the control group ($p<0.008$). On the 10th day, the collagen fiber density in the granulation tissue of the alpha-lipoic acid + melatonin group was significantly higher than that of the control group ($p<0.008$). In immunohistochemical staining using VEGF, positive immunoreactivity was detected in the epithelial areas of all group samples. Cytoplasmic and widespread VEGF expression was observed in all epithelial layers (except the stratum corneum-keratin layer), especially more prominently in the stratum granulosum. No specific and significant immunoreactivity was found in the granulation tissue or subepithelial connective tissue in the wound area. VEGF expression on the 5th day was significantly higher in the alpha-lipoic acid + melatonin group compared to the control group ($p<0.008$).
Conclusion
Growth factors and collagen production are one of the key factors that determine the wound healing. According to our results, the combinatory application of alpha-lipoic acid and melatonin

might be effective in palatal wound healing via collagen synthesis in the early period and via VEGF expression in the late period.

Keywords:

alpha-lipoic acid, melatonin, wound healing

Reference:

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TEMsuite – A Matlab-based software platform for TEM data analysis

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Poster Group 2

Background incl. aims
Transmission electron microscopy (TEM) can provide a wealth of information on structure and composition at the atomic level for many different kinds of samples. To obtain this information, numerous different imaging, diffraction or spectroscopic techniques are available, e.g., high-angle annular dark-field (HAADF) scanning (S)TEM, selected-area electron diffraction (SAED), electron energy-loss spectroscopy (EELS) or energy-dispersive X-ray spectroscopy (EDX) to name some of the more common ones. To perform an advanced data analysis, again a multitude of dedicated software exists, many of them being freely available, such as HyperSpy for multidimensional spectroscopic analysis [1] or PETS for analysis of diffraction data [2]. Despite the availability of these specialized software, most users, especially those that only occasionally perform TEM analysis, still use commercial, proprietary software to perform basic data treatment and the threshold to learn yet another, even more specialized software is high. With the aim to speed up my own data processing tasks, I have written a software platform based on Matlab that facilitates several basic and advanced data analysis processes and which I have made freely available [3].

Methods
The software is written in Matlab with a graphical user interface (GUI) made up of a main window, a figure window for display and several windows to control the data analysis (Figure A). The software follows a session-based approach that allows to load and process different TEM data types at the same time. The sessions can be stored and reloaded at a later point to continue the data analysis. The following list describes several of the tasks that TEMsuite allows to perform: Basic image processing (contrast settings, line scans, export images as .jpg or .tif, add a scale bar, …), alignment of images series and export to movie, FFT filtering and analysis of reflection spots in the FFT. The software possesses a comprehensive set of tools for diffraction analysis including an automatic peak detection, fitting and correlation as well as the calculation of radial or azimuthal profiles. EELS multidimensional data can be visualized and analyzed statistically (principal-component analysis (PCA), non-negative matrix factorization (NNMF)). Core-loss EELS data can be quantified using theoretical scattering cross sections and the background-subtracted spectra can be fitted to obtain chemical information of the studied specimens.

Results
Several publications contain results from data analyses conducted with TEMsuite, for example the study on quasi-van der Waals epitaxial growth of WS_2 on sapphire [4] or the investigation of iron/cobalt-oxide core-shell nanoparticles for catalytic applications [5]. Figure (B) shows the NNMF analysis of an EELS spectrum image obtained from the interface between sapphire substrate (left), WO_3 interface and WS2 and carbon protection layers. The analysis yielded the clear separation between the four contributions and the plasmon peak energies of the associated spectra correspond with expected values [4]. The chemical map (Fe: green, Co: blue, O: red) obtained by a spatially-

resolved EELS analysis of an iron/cobalt-oxide core-shell nanoparticle shows the clear formation of a Co-rich shell (top left in Figure C) [5]. The Co presence manifests itself as well in the intensity ratio of the O-K pre-peak and the following valley, as visualized in the spectrum and the corresponding map (top right) in Figure C.

Conclusion
I developed the TEMsuite software initially to speed up my own TEM data processing but improved its design and setup with the aim to obtain a version that can be used by other users. The software is thought as platform for basic and advanced TEM data analysis currently focusing on image, FFT, diffraction and mainly EELS analysis and it is planned to be further expanded to include more techniques throughout the following years. The software is freely available as source code [3].

Figure caption: (A) TEMsuite GUI with main window (top left) including the list of loaded image and spectroscopy data, figure window (right) with an example EELS-spectrum image quantification and the EELS window (bottom left) to control the EELS data analysis. (b) Example analysis of a quasi-van der Waals epitaxially grown monolayer of WS_2 by NNMF yielding four contributions of carbon protection layer, sapphire substrate, interface (WO_3) and WS_2 monolater. (C) Example analysis of an EELS spectrum image of a Fe/Co-oxide core-shell nanoparticle with chemical map (top left), comparison of O-K spectra from shell and core (bottom) and map of the intensity ratio between the O-K pre-peak at 532 eV and the following valley (top right).

Keywords:

Data analysis, software, TEM, EELS

Reference:

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NiTi shape memory alloy microstructure after high stress at elevated temperatures containing modulated M2 martensite

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Poster Group 2

NiTi shape memory alloy microstructure after high stress at elevated temperatures containing modulated M2 martensite
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Background
Nanocrystalline wires with shape memory NiTi were deformed in the martensitic state at low temperatures of -125 °C up to a stress of ~750 MPa. In the next step, the wires were heated well above the temperature Af while maintaining a constant stress of ~750 MPa. The wire was driven towards its thermomechanical stability limits under such a specific condition and finally transformed into plastically deformed austenite. Microstructure of nanocrystalline NiTi shape memory wire was analyzed in Transmission Electron Microscope (TEM) with the aim to reveal the microstructures of martensite variants and lattice defects evolving in the heated wire, and at the same time to reveal the mechanism of plastic deformation and highly constrained reverse martensitic transformation. Although the constrained NiTi martensite wire should transform back to austenite at elevated temperatures, the applied stress prevents this reverse transformation and plastic deformation of martensite occurs instead. However, the mechanism of plastic deformation of martensite under such elevated temperature and high stress conditions remains unclear.

Materials and Methods
The NiTi shape memory wire was commercially prepared by Fort Wayne Metals in cold work state with the following parameters FWM #5 Ti-50.5 at.% Ni, 42% CW, diameter 0.1 mm. Such wire was heat treated by the electric power pulse method with power density 160 W/mm3 and pulse time 15 ms. Final wire has a fully recrystallized microstructure with a mean grain size d = 250 nm and showing transformation temperatures Ms = 63 °C, Af = 93 °C.
Wire microstructure was investigated using TEM specifically FEI Tecnai TF20 X-twin equipped with a field emission gun operating at 200 keV. TEM lamellae were extract from the deformed NiTi wires by Focused Ion Beam (FIB) lift-out technique using a FEI Quanta 3D FIB-SEM microscope. TEM, HRTEM, SAED and nanobeam electron diffraction micrographs were acquired for a detailed understanding of the resulting martensitic microstructure. TEM diffraction patterns and HRTEM images were indexed using the CrysTBox [1] software. HRTEM images have been also processed by Geometric Phase Analysis (GPA), which is part of the software, and it was developed to analyze local strain fields by HRTEM, for example strain fields around dislocation cores. Nanobeam diffraction analysis of modulated martensite was also performed, to have local diffraction information.

Results
TEM analysis of the wires revealed that, when the martensite is heated under the stress towards its stability limits, so the microstructure contains mixed regular B19' and modulated M2 martensitic lattices with a high density of lattice defects, e.g., dislocations. Such a complex microstructure could

not be characterized by conventional TEM, therefore HRTEM and nanobeam electron diffraction were used.
TEM analysis showed deformation bands in the microstructure of the martensitic variants were found everywhere in the martensite microstructure. The microstructures of martensitic variants produced by kwinking consist of deformation bands in which the martensitic lattices are rotated with respect to the martensitic matrix sharing a common [010] zone axis. A large number of dislocations in the martensite microstructure were also found here. More detailed TEM analyzes showed that in addition to these commonly observed findings, abnormal diffraction patterns were also observed. Diffraction patterns (SAED, FFT of HRTEM and nanobeam diffraction) from various regions could not be indexed using the lattice parameters of the B19' monoclinic lattice, because of the additional diffraction points appeared halfway between the 000 and 001 points. The occurrence of such additional diffraction spots indicates that the martensite has a modulated lattice with a unit cell twice as long in the c-direction.
During heat treatment the oriented martensite heated at constant applied stress undergoes a B19' => M2 structure change and moreover the martensite plastically deforms before and during the transformation to austenite. The study revealed that the plastic deformation of martensite variants takes place via kwinking deformation involving coordinated dislocation slip, twinning and simultaneously created a long period modulated crystal structure. It is assumed that the modulated martensite structure arises from the B19' martensite structure through coordinated slip of partial dislocations with Burger's vector b = a/2 [100] on the (001) crystal planes. Coordinated slip was forced by kwinking deformation. The partial dislocations began to slide as the martensite was exposed to elevated temperatures.

Conclusions
To find out the deformation process of the martensitic wire, which was exposed to high stress under high temperature conditions and forced to its stability limit, we analyzed the microstructure of the wires annealed to different maximum temperatures by TEM. During this research we found that locally within the grain in some wires the diffraction patterns exhibit a modulated structure. From the diffraction patterns, it was found that the modulated structure has a unit cell twice as long in the c-direction. The formation of these areas within the grains will be due to the plastic deformation occurring in the wire under constant stress and elevated temperature, above the temperature Af.
It was revealed that the heated martensite wire plastically deformed by kwinking deformation and simultaneously transformed into a long-term modulated monoclinic structure with a high density of dislocation defects, before and while undergoing reverse martensitic transformation into plastically deformed austenite. Based on the results, it is suggested that the modulated martensite structure is formed through the coordinated slip of partial dislocations with Burger's vector b = a/2 [100] on (001) crystal planes, thereby achieving a large shear deformation of the crystal lattice, which is forced by kwinking deformation. The modulated structure will be considered as a local and temporary martensite structure that appears before it transforms to austenite under forced heating. During transformation martensite to austenite the modulated martensite generates high density of dislocation defects and a corresponding lattice strain in austenite microstructure.

Keywords
TEM, NiTi, Martensitic transformation, Shape memory alloys, Modulated martensite

References
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Keywords:

TEM, NiTi, SMA, Modulated martensite

Reference:

[1] M. Klinger. More features, more tools, more CrysTBox. Journal of Applied Crystallography, 50(4), 2017. doi:10.1107/S1600576717006793

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Influence of the deposition parameters on properties of gold plasmonic antennas

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Poster Group 2

The properties of plasmonic antennas depend on a variety of factors, including the antenna size, shape, and material. Although gold plasmonic antennas have been studied extensively over the last few years, the influence of parameters used for gold thin film deposition on the resulting plasmonic behaviour of fabricated antennas remains unexplored.
We deposited gold films with thicknesses of 20 nm, 30 nm, and 40 nm at various deposition rates by an ion-beam-assisted deposition. We analysed the structure of deposited films by X ray diffraction and transmission electron microscopy. We fabricated gold plasmonic antennas by FIB lithography [1, 2] and evaluated the ease of fabrication for each deposited layer used, based on the fabrication yield of three antenna types. Further on, we studied the plasmonic behaviour of fabricated antennas by EELS [3] and compared the plasmon resonance intensity and Q factors of individual antennas.
Our results [4] show, that for fabrication of plasmonic antennas by FIB lithography, the films deposited at 0.3 nm/s are optimal, as the fabrication yields of these layers are the highest. However, in terms of plasmonic properties, antennas fabricated from 20- and 30-nm thick layers deposited at 0.1 nm/s exhibit the highest plasmon resonance intensity and highest Q factors. In the case of 40 nm thick layers, the best plasmonic performance is achieved in antennas fabricated out of gold films deposited at 0.3 nm/s. Figure below shows the results for 30-nm-thick gold layer: fabrication yield (A), EEL spectra for two specific electron beam positions at the corners (B) and in the gap (C) of a bowtie antenna, and Q factros of the transverse dipole (TD) and longitudinal dipole (LD) mode (D).
This study sheds light on the interplay between gold thin film deposition parameters and the resultant plasmonic behaviour of antennas, offering valuable recommendations for optimizing the fabrication process of plasmonic devices.

Keywords:

gold films; plasmonic antennas; EELS

Reference:

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3D TEM and SEM-array tomography of Hailey-Hailey Disease human skin biopsies

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background:
ATP2C1 gene encodes for the secretory pathway Ca2+/Mn2+ pump ATPase type 1 (SPCA1) localizing at the Golgi apparatus. Mutations on the human ATP2C1 gene, causing decreased levels of the SPCA1 expression, have been identified as the cause of the Hailey–Hailey disease (HHD), a rare skin disorder. More than 200 unique mutations have been found, with any hot-spot on the ATP2C1 gene. The HHD patients present raw, blistered areas and lesions, particularly in skin folds where there is moisture and friction. No effective cure exists. Due to reduced expression of functional SPCA1 in HHD patients, altered Ca2+ homeostasis and responsiveness in HHD keratinocytes results in creating an early defect in differentiation process due to a reduced production of involucrin. Keratin expression was also delayed in acantholytic epidermal segments of HHD skin. This results in fragile skin layers. However, no deep investigation at ultrastructural level have been never performed.

Methods:
Advanced gene sequencing has been used to identify mutations on the ATP2C1 gene of HHD patients for this study.
Using volume Scanning Electron Microscopy (SEM)-array tomography we investigated the status of the cell-cell interaction in the epidermis of five different HHD patients. Epoxy resin embedded samples have been sectioned (300nm) using the Powertome-XL ultramicrotome (RMC Boeckler, USA) and serial sections were collected on Kapton tape with an automated ATUMtome collection, and then transferred on Si-wafer support for being imaged in the SEM Gemini 450 (Zeiss, Germany). Sequential images where then managed and over-imposed to generate a 3D model of the cell-to-cell interactions.
Furthermore, using 3D-Transmission Electron Microscopy -tomography we also investigated the alterations in the secretory pathway, with particular focus on the Golgi apparatus, in the keratinocytes (epidermis) and in fibroblasts (dermis). Semi-thin sections (250nm) cut with a UltraCut-UC6 microtome (Leica, Austria) were collected onto formvar-coated copper slot grids and imaged with a Talos L120C Transmission Electron Microscope (TEM) (Thermo Fisher Sc., USA) using motorized tilt-rotate specimen holders. Tilt series datasets were collected at 13500x magnification,

using automated data acquisition and image alignment routines as the grids housing the sections were serially tilted over a range of ~130° (±65°) using the microscope Tomo program. Tomograms computed from aligned 2D views were computationally registered and combined in 3D space. All of the above procedures - along with subsequent image segmentation, surface mesh computation/generation, 3-dimensional visualization and quantitative analysis - were performed using the IMOD software package maintained and distributed by the Boulder Laboratory for 3D Electron Microscopy of Cells at the University of Colorado (Boulder, CO, USA).

Results:
We discovered that interactions among keratinocytes are significantly disrupted, leading to the formation of 'empty spaces' between them. Keratinocytes maintain their tenuous connections primarily through few reamining desmosomes. The overall thickening of the epidermis primarily results from an increase in the number of layers of keratinocytes rather than an expansion of intercellular spaces.
At subcellular level, the Golgi apparatus looks highly swollen, blebbed, and pinched off to larger “bubbles”, rather than vesiculated like previously published in in vitro studies. The overall volume of the Golgi apparatus looks sensitively increased, due to the higher number of the cisternae as well as their volume.
Identification of the heterozygous mutations in the ATP2C1 gene of the 5 patients with HHD revelaed 2 novel mutations (c.2378_2381insTTGT, c.2599A>G), and 3 previously reported nonsense mutation (c.1327C/T, c.1402C>T, c.2554insT).

Conclusions:
We proved a workflow of investigating the overall ultrastructure organization of the human skin in the HHD. For the first time, we observed intracellular morphological organization in the Golgi apparatus of the HHD which is notably different form the previous investigated studies using cell line models. Using SEM array tomography, we also give e deeper view of the general disorganization of the cell-cell contact in the epidermis of the patients. Using combines SEM and TEM methodology we present and feasible approach to deeply investigate poorly studied pathologies, like rare genetic disease.

Keywords:

Hailey-Hailey_Disease; SEM-array-tomography; 3D-TEM- tomography; Golgi_apparatus.

Reference:

N/A

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Precise light and fluorescent microscopy guided sequential cryo FIB lift-out

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Background incl. aims
Cryo-TEM tomography is a well-established imaging technique for revealing intracellular ultrastructure at the level of individual molecules. However, the requirement for electron transparency limited what could be imaged. Cryo-FIB lamella made deep intracellular structures accessible by removing the excess material. This approach works very well for objects and structures that are abundant in target cells. When the region of interest (ROI) is not found in every single cell, but rather in one or a few cells within a relatively large multicellular organism, the thinking changes significantly. It is no longer effective to rely on a stochastic approach or "luck". An effective way of target identification and localizations can be provided by cryo-fluorescence imaging and image correlation. Thin samples up to a few micrometers of thickness are vitrifiable by plunge freezing into liquid ethane, directly on TEM grids, making the workflow relatively straightforward. Thicker ones require HPF to achieve vitrification, which results in bulk sample inside a metal carrier and requires cryo lift-out to get the ROI to the TEM. That not only increases the amount of effort required to manufacture every lamella, but also complicates the navigation and ROI localization. We aim to develop and demonstrate a workflow enabling cryo-TEM visualization of a specific subcellular ROI from a multicellular organism such as C. elegans.
Methods
In a nutshell, the proposed method consists of high-pressure freezing (HPF; Leica EM ICE) in transparent cryoprotectant that results in a vitrified frozen-hydrated sample attached to a suitable support, typically a TEM grid or a HPF carrier, preserved in a life-like condition. The sample is then imaged in cryo light and fluorescence microscope (Leica EM Cryo CLEM) to screen for ROIs and identifiable features and transferred to the cryo FIB-SEM machine (Tescan Amber Cryo). The ROIs are then marked and material around the intended lamella is removed with FIB. Lamella is lifted out using a nanomanipulator (Tescan cryo-nanomanipulator) and welded to a receptor grid with pre-milled slot of appropriate width and thinned down to <200nm through 2 or 3 steps of consequent fluorescent microscopy check and FIB milling.
Results
We were able to acquire a TEM tilt series on a selected part of cell nucleus containing a fluorescently labeled feature of interest. Since there were single digit nuclei expressing desired phenotype close to each other, multiple consecutive lamellae were lifted out from each processed worm and polished with a reasonable success rate.
Conclusion
The method demonstrated here enables cryo TEM processing of a fluorescently labeled feature from within large multicellular biological samples. It consists of targeted cryo FIB lift-out using Tescan cryo-nanomanipulator mounted in Tescan Amber Cryo FIB-SEM with multiple fluorescence checks performed using Leica EM Cryo CLEM during the preparation. This will provide researches with multiple high-resolution windows within context of larger volume mapped using cryo fluorescence microscopy. As a result, we can offset the miniscule volume of space visualizable by the cryo TEM

tomography which, until now, was mostly limited to abundant objects of interest. While the workflow by itself is relatively labor intensive, for rare objects and phenomena, it is much more practical than any untargeted approach.

Acknowledgement
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Keywords:

CLEM, CryoFIB lift-out, cryo ET

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Nanoscale structural and spectroscopic characterization of hard carbons for Na-ion batteries

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Poster Group 1

Background incl. aims
Sodium-ion batteries are one of the best candidates to replace lithium-ion batteries for stationary applications, as a cost-effective and sustainable alternative. [1] A key role in their development and integration is the use of renewable carbon sources. Here we are characterizing the structure and chemical composition of bio-derived hard carbons, used as anodes in sodium ion batteries. We aim at a fundamental understanding of parameters that may influence Na storage in these materials.
Methods
Several samples of hard carbon materials were investigated by means of scanning transmission electron microscopy (STEM), employing annular dark field (ADF), integrated differential phase contrast (iDPC) and electron energy loss spectroscopy (EELS), as well as by vibrational spectroscopy, namely, Fourier-transform infrared (FTIR) and Raman spectroscopy.
Raman and IR measurements were carried out on powder samples. TEM samples were prepared by dispersing hard carbon powders in ethanol and drop-casting solution onto a holey carbon film grid.
Results
Previously, bright field imaging in (S)TEM has been prefered for this type of structures.[2] We show that the use of iDPC in combination with ADF STEM imaging at low voltage (80kV) has paramount benefits for the investigation of hard carbons, while no significant beam damage is observed. In iDPC STEM, graphitic layers could be readily imaged, as demonstrated by Graphic A. Moreover, measurements of the d002-spacing are feasible, either directly within images or by analysing their FFT, where also the d100- and d110-spacing of graphite can be measured. In addition, we can observe nanosized pores, which are confirmed in particular by the correlation of iDPC and ADF images. EELS spectra reveal a fine structure of the C K edge that lies between the typical signatures of graphite and amorphous carbon. Both the d002-spacing and the nanoscale pores are supposed to be the key to Na storage capabilities of hard carbons.
FTIR measurements of hard carbons using a diamond ATR crystal face challenges in achieving total reflection condition due to the similarity of refractive indexes. Using Ge with much higher refractive index should be adapted instead for accurate spectra acquisition. FTIR and Raman measurements (exemplary spectra B and C in the Graphic, respectively) allow us to detect differences in crystallinity and surface groups of hard carbon samples with prospects of using Raman in-situ during the Na loading process.
Conclusions
The microscopic and spectroscopic methods chosen for hard carbon characterization have been tested and optimized to meet the specific needs of the material. Next, the characterization results will be compared to electrochemistry measurements to see if certain morphological or chemical characteristics are influencing the Na storage properties. [3]

Keywords:

Na-ion battery, iDPC, Raman, FTIR

Reference:

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All-Micro: a Network for Sustainable Innovation in Slovenia- Italy Area

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Poster Group 1

AllMicro, ALLiance to boost cross-border innovation through MICROscopy, project born from the core idea of an open and inclusive innovation process, allowing a free flow of knowledge and technological collaboration. This leads to the creation of technological novelty, which in turn opens up new markets and encourages entrepreneurship. Research and innovation not only improve products and services but also create investment opportunities, ultimately boosting competitiveness and generating employment [1]. Understanding cutting-edge methods in optical and electron microscopy is frequently confined to highly specialized organizations and individuals, posing a challenge for those seeking to utilize these tools to enhance their creativity and competitiveness on a global scale. Additionally, the significant expenses associated with equipment and ongoing technological advancements hinder the transition of these techniques from academic or fundamental research settings to practical applications in industries and services. This project establishes an international network focused on advanced optical and electron microscopy methods, connecting academic and research institutions with technology parks.
Technology parks Friuli Innovazione (FINN/TEC4I.FVG, Italy) and Primorski Tehnoloski Park (PTP, Slovenia) are vital hubs for fostering innovation, offering their facilities and training services to further bolster collaboration and skill development. They leverage extensive networks to identify potential partners from academia and the private sector, provide essential physical infrastructure and administrative support, cultivate vibrant innovation ecosystems to encourage knowledge exchange and idea generation, and support commercialization efforts through services like intellectual property management and access to funding [3]. Their crucial role is to identify and engage potential partners interested in exploring the opportunities presented by the new microscopy techniques.
In this joint project between Slovenia and Italy, the Universities of Trieste (Italy) and Nova Gorica (Slovenia), along with the research centers of the Istituto Officina dei Materiali (National Research Council, Italy) and the Nanocenter (Slovenia), will contribute to the network's goals. State-of-the-art facilities ranging from electron to optical to atomic force microscopy will be accessible within the target area. These facilities will be available for training and utilization across a broad spectrum of applications in materials science, biology, and various academic and industrial fields. This valuable expertise covers a wide range of knowledge areas and complements one another.
Here, the main objective is to share insights into the capabilities of optical and electron microscopy in driving technological progress and innovation, particularly within the designated region. This area, encompassing numerous small labs with limited resources, holds immense potential. By uniting these labs, a critical mass can be achieved, leading to a formidable presence for economic and innovative

development within the region. Additionally, this initiative aims to enhance the competitiveness of all businesses across the larger region by harnessing the collective strength of these united labs.
First, the project focuses on creating and maintaining the ALL-MICRO network to develop synergies among partners. It involves establishing project management infrastructure and conducting meetings for information exchange and collaboration. The next step is to outreach to stakeholders interested in advanced microscopy methodologies, facilitated by technology hub partners. Pilot projects are conducted to demonstrate the potential of advanced microscopy techniques and initiate synergistic partnerships. In this way, by forming a unified network that operates in harmony, spreading the word, and sharing information this project creates a positive atmosphere that encourages other labs to join in or fosters fruitful collaborations with companies. Moreover, the ALL-Micro network will organize and maintain a series of high-specialization courses aiming at the formation of a future class of experts able to take full advantage of advanced microscopy methodology in R&D.
In conclusion, the ALL-MICRO project not only presents a promising opportunity to bridge the gap between academia, industry, and research in advanced microscopy but also aligns with an environmental sustainability perspective. By establishing a cross-border network and leveraging expertise from academic institutions, research centers, and technology hubs in both Italy and Slovenia, the project aims to disseminate knowledge and drive innovation in microscopy techniques. Through collaborative efforts and strategic partnerships, it seeks to enhance regional competitiveness while promoting environmental sustainability. This objective necessitates the crossing of national borders to share resources and avoid duplication of costly and environmentally impactful instrumentation, ensuring a more sustainable approach to research and development. With structured management and active stakeholder participation, the project has the potential to deliver significant benefits and make a lasting impact on microscopy techniques and innovation in the region. With the success of this collaborative project, we are planning to extend the network partners and the target area to nearby countries such as Croatia, Austria, or Balkan countries. This will provide a useful precedent for potential similar approaches in regions different from ours.

Keywords:

Electron Microscopy, networking, All-Micro, Italy-Slovenia

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Investigating nitrogen doped nanocarbon materials as potential carbon dioxide adsorbers

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

Background
The climate crisis, propelled by the continuous rise of carbon dioxide emissions, has spurred research to find technical solutions for this problem. One possibility is to directly capture carbon dioxide from the atmosphere and sequester it inside suitable long-term storage. Unfortunately, the success of this method strongly depends on the efficiency of the adsorber material. Here, nanocarbon materials such as graphene and carbon nanotubes could come into play, as they offer several beneficial characteristics like high surface area and stability. However, the catch is their nonreactive nature. To enable chemical adsorption one could alter the chemical structure of the nanocarbons by inserting new elements through doping. Nitrogen is a promising candidate, as it can be exchanged with the carbon atoms inside the crystal lattice of the materials. It is known to be a crucial component in other carbon dioxide fixating mechanisms, for example in plants. One goal of this research is to find novel routes for nitrogen doping of these materials and try to understand and control the process of ion implantation. Furthermore, the nitrogen doped nanocarbons are investigated as carbon dioxide adsorbers.
Methods
Nitrogen doping is realised by different methods of ion bombardment. HRTEM is used to examine the effects of doping on the atomic level. Further investigation is conducted with STEM coupled with EDX, FIB-SEM coupled with ToF-SIMS, AFM and XPS. A self-built setup is established, dedicated to measure the carbon dioxide capture performance of the materials, a scheme of it is shown in part A of the figure.
Results
Various methods of ion bombardment were successfully used to dope graphene with nitrogen. This was verified by HRTEM, observing implanted nitrogen atoms as well as defects introduced by nitrogen ion bombardment. The same treatment is conducted with carbon nanotube films. These films are produced via filtration of carbon nanotube suspension in a controlled manner and various thicknesses, which are checked by AFM. FIB-SEM with ToF-SIMS as well as XPS verify that nitrogen is present in the upper 100 nm of the films that have been bombarded with nitrogen ions. Further investigation through STEM coupled with EDX give insight into the ion dosage dependent distribution of nitrogen in thicker carbon nanotube bundles after doping, as shown in part B of the figure. Additionally, HRTEM shows the deterioration of the carbon nanotube crystal structure after excessive ion bombardment, which can be seen in part C of the figure. Carbon dioxide capture performance of various carbon nanotube films has been measured with the dedicated setup. An increase in performance was observed after treatment with nitrogen ions.
Conclusion
Different ways of ion bombardment were used to implant nitrogen into the crystal structure of graphene and carbon nanotubes. This was verified by various microscopic and spectroscopic methods. The effects of the doping procedure were investigated on atomic as well as microscopic

level, giving insights in the doping mechanisms during ion bombardment. Furthermore, it was shown that nitrogen doping changes carbon nanotube films in a way, that improves their ability to sequester carbon dioxide from the atmosphere.

Keywords:

Nanocarbons, nitrogen doping, carbon capture

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Achieving High-Resolution Electron Nano-crystallography using HVEM and PED

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Poster Group 2

Background
To achieve higher resolution in TEM, a Cs corrector is typically employed as a hardware solution. Alternatively, software processing techniques like Maximum-likelihood (MAL), Exit-wave function reconstruction (EWFR), Iterative wave-function reconstruction (IWFR), and Phase extension offer an affordable solution without requiring expensive hardware. However, these methods necessitate acquiring multiple HREM images (3-20) for processing. HVEMs are beneficial for HREM due to their deeper sample penetration and reduced dynamical effects. PED further minimizes dynamical effects, enabling high-resolution diffraction data even for thicker samples analyzed with lower-voltage TEMs. This study employed a combined approach of HVEM, PED, and phase extension to achieve sub-Ångstrom resolution imaging of Si single crystal samples. Additionally, we conducted a comparative analysis of the results with those obtained using a conventional TEM equipped with a monochromator and a Cs corrector.
Methods
A Si single crystal was prepared using ion thinning and employed as a test specimen for high-resolution electron microscopy (HREM). High-resolution electron microscopy (HREM) imaging of the Si [211] zone axis was performed using a high-voltage electron microscope (HVEM). The high-resolution images used for comparative analysis were obtained using a conventional TEM equipped with a monochromator and a Cs corrector at an accelerating voltage of 200kV. An energy-filtered precession electron diffraction (EF-PED) pattern was obtained using an energy-filtered TEM equipped with a precession unit. The specimen thickness was estimated using the log-ratio method and information from the low-loss spectra. Image processing of the HREM data involved background noise reduction and phase extension using a combination of software programs (Digital Micrograph, CRISP, HREM filters, and VEC).
Results
A high-resolution (HR) image of the Si [211] projection with an atomic resolution of 1.2 Å was obtained using a high-voltage electron microscope (HVEM). Additionally, a precession electron diffraction (PED) pattern satisfying the quasi-kinematical condition was acquired with an enhanced spatial resolution of 0.63 Å using a beam precession system. By applying the phase extension technique to these combined data, Si atomic columns with an atomic spacing of 0.78 Å in the Si [211] projection were successfully resolved.
Conclusion
The HVEM and PED combination method has demonstrated its potential to achieve high resolution even in thick samples by leveraging HVEM's high penetration power and PED's ability to minimize dynamical effects. By employing this technique, the reduction in resolution caused by sample thickness in high-resolution imaging analysis using Cs-corrector can be partially compensated, which is expected to significantly aid in the accurate analysis and understanding of the structure and properties of nano-crystals.

Keywords:

HVEM, PED, Phase extension technigue

Reference:

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The mixed ionic-electronic conductors studied by advanced transmission electron microscopy

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Poster Group 1

A successful oxygen transport membrane (OTM) requires not only an outstanding permeation performance, but also chemical, electrical, thermal and mechanical stabilities under practical conditions. Among promising candidates, dual-phase OTMs (DP-OTMs) are attracting continuous attention, in which two ceramic phases are coupled to provide mixed ionic-electronic conductivity. In particularly, a fluorite-spinel composite CexGd1-xO2-δ-FeyCo3-yO4 (CGO-FCO) was reported to possess both a significant oxygen permeability as well as a high tolerance under exhaust gas conditions.[1] Thus, considerable efforts have been made to further push the overall performance of CGO-FCO. However, the bottleneck still lies in the inevitable phase interation and the complex grain boundary (GB) structures. In turn, modulating the phase interaction and GB properties offers the possibility of materials engineering, which requires however a precise understanding of the emerging phase and the GBs behaviors down to atomic scale.
In our work, dense pellets of CGO-FCO with different nominal compositions were prepared by solid state reactive sintering.[2] As a result of the phase interaction, an emerging phase with the structure of GdFeO3 (GFO) was often located between CGO phases. Further energy-dispersive X-ray spectroscopy (EDXS) and electron energy loss spectroscopy (EELS) studies revealed varying compositions of the GFO phase, and its formation reduces the Fe and Co segregation at the CGO GBs significantly.[3] Besides, both crystal and chemical structure at the CGO GBs were quantified down to the sub-nm scale, as they are directly related to the ionic conductivity of the membranes.[4] CGO GBs with different coherences were compared. For the Σ3[101] GB in Fig. 1 with a high coherence, only a single layer of Gd segregation can be noticed at the edge of each grain, while the non-solute segregations from Fe and Co are restricted between the two grains. Any variation of the Ce valence state is also largely limited. In contrast, for CGO GBs with a lower coherence, all the atomic-site specific lattice distortions, elemental distributions, and valence state variations were found to be much more significant.
In summary, GFO was identified as the emerging phase within CGO-FCO. Its tunable electronic properties further broaden the opportunity for membrane optimization through phase engineering. Besides, Simultaneous Gd enrichment and Ce depletion within the ending atomic layers of the adjacent CGO grains are generally observed, while Fe and Co are segregated into the CGO GBs with varying amounts and phases. The comparison between GBs with different coherencies indicates that the valence states of Ce, and the enriched Gd, Fe and Co are all sensitive to the local structural defects at the GB. A more coherent GB would thus be beneficial for an improved GB conductivity. Our findings constitute a crucial piece for the complete picture of structure-property relationship of CGO-FCO, which may also shed light on the understanding towards other oxide heterointerface phenomena.

Keywords:

ceramic membranes, GB-segregation, aberration-corrected TEM

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The reversible phase transition of Gd-doped ceria by in situ TEM

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Poster Group 1

Owing to the flexile valence switching between Ce4+ and Ce3+ cations and the facile formation of oxygen vacancies, ceria has been recognized as one of the best candidates for catalysts and solid electrolytes. The formation and migration of oxygen vacancy under external stimuli thus constitute the fundamental processes of the functional oxide materials and their associated devices, which are often linked with modification in the ceria structure and subsequent phase transitions. Typically, ceria crystallizes in a fluorite-type (F-type) cubic structure (space group Fm-3m, a≈5.42 Å). Transition from F-type to the so-called C-type (space group Ia-3) takes place when enough oxygen vacancies are introduced to the system, in Fig. 1. As a result, the cell parameter is doubled, the M site is six-coordinated to O, and the crystallographic positions are split. However, given the challenges in visualizing oxygen dynamics with adequate temporal and spatial resolution, the structural evolution of ceria in response to external stimuli remains rarely explored.[1] Questions pertaining to transition details, including the selection of external stimuli, the behaviors of oxygen vacancies, the feasibility of fine tuning as well as the underlying mechanism remain unsolved.[2]
For our study, the Gd-doped ceria (CGO) with high oxygen ion conductivity as well as giant electrostriction under external electric field is chosen. Dense pellets of Gd-doped ceria were prepared by solid state reactive sintering.[3] The phase transition of CGO is in situ probed down to sub-Å scale by transmission electron microscopy (TEM), where the electron beam (e-beam) is also serving conveniently as an external stimulus. Negative spherical aberration imaging (NCSI)[4] and integrated differential phase contrast (iDPC) imaging are employed, enabling high contrast for both light oxygen and heavy metal atoms, as well as facilitating the measurement of atomic positions with ultra-high precision. Fully manageable transitions are achieved through adjustments to the electron dose rate (EDR). Quantifying lattice distortions also allows a direct estimation of the local oxygen vacancy concentration. The electron energy loss spectroscopy (EELS) reveals varying Ce valence within the CGO. Finally, a collective rearrangement of the oxygen vacancies is proposed to account for our observations, and the simulated HRTEM images show a good agreement with the experimental results. Further numeric calculation would then be necessary to establish a solid theory.
In summary, the reversible phase transitions of CGO were explored in situ with picometer precision. Unique insights into the transition process are gained, such as anisotropic ordering of the oxygen vacancies, direct interpretation of the local oxygen vacancy concentration, and feasible modulation of the transition efficiency. These findings showcase great promise for diverse energy-related applications, including enhancing the performance of ceria-based catalysts and advancing next-generation memristors. The same principle could also be extended to other functional oxides.

Keywords:

Phase transition, in-situ TEM, NCSI

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In-Situ Manipulation of Growth Mechanisms in Ni-Seeded GaP Nanowires

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PS-01 (2), Lecture Theater 3, august 28, 2024, 14:00 - 16:00

III-V semiconductor nanowires (NWs) have emerged as a promising class of materials for next-generation nanoelectronics and photonics due to their unique electronic, optical, and mechanical properties.[1] The ability to grow III-V NWs with high precision and controllability has led to the development of numerous applications in the fields of electronics, photonics, and energy conversion.[2] Thus, precise control over the growth of III-V NWs has been a major topic that enables the creation of uniform and reproducible nanostructures with tailored properties.
So far, the bottom-up approach is widely used for synthesizing the semiconductor NWs, where an intrinsic or foreign metal (called as seed particle) is often used to promote the anisotropic one-dimensional growth. There has been abundant research focusing on the influence of seed material, preparation and dynamics on NW growth.[3] However, most of such research are carried out in a post-growth condition, where the NWs are cooled and transferred from the synthesis equipment for further analysis. Thus, there is a lack of understanding on the dynamic interplay between seed particles and NWs under the growth environment, such as the real-time crystal structure and composition of the seed particles and their impact on the corresponding NWs.
This conference contribution reports a detailed investigation on the growth of gallium phosphide (GaP) NWs using Ni as seed material. An aberration-corrected environmental transmission electron microscope (ETEM) integrated with a metal-organic chemical vapor deposition (MOCVD) system was used. Ni nanoparticles were transformed into Ni_5P_4 crystals by the supply of phosphine. After adding trimethylgallium (TMGa) to the precursor supply, the nucleation of GaP NWs was observed and captured by high-resolution TEM (HRTEM) movies. The phases involved in the nucleation and NW growth process were characterized via energy-dispersive X-ray spectroscopy (EDS) and power spectra obtained from the HRTEM images.
In the investigated range of partial pressures, two types of seed particles were observed: At low V/III ratios (defined as corrected partial pressure ratio between group V and group III supplies), NWs grew from two Ni-Ga phases at a similar growth rate, namely the solid NiGa phase, as well as the Ni_2Ga_3 phase. In contrast, at high V/III ratios, NWs grew from Ni_2P particles, where interdiffusion through Ni_2P contributes to multiple nucleation and leads to a much higher growth rate. The ability to manipulate the seed particle phases offers the potential for precise control over the growth of compositionally abrupt heterostructure NWs, where group III or V elements can be individually switched. Our research results represent a significant step forward in the replacement of Au as a conventional seed particle material, as well as in the ability to locally modify the composition, doping levels, and morphology within the same NW.

Keywords:

In situ TEM, semiconductor nanowires

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Vitamin A affects urothelial injury and regeneration in cyclophosphamide-induced cystitis

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Poster Group 1

Background
Vitamin A and its derivatives, the retinoids, are involved in tissue regeneration as they can modulate cell proliferation, differentiation, apoptosis (1) and the inflammatory response (2). A single injection of cyclophosphamide (CP) in rodents disrupts the blood-urine barrier of the urinary bladder by causing injury to the urothelium, which is followed by rapid regeneration (3–5). We used a mouse model for CP-induced urothelial injury, which is also a model for the inflammation of the urinary bladder known as cystitis. The aim of our study was to investigate the effects of a vitamin A-enriched diet on CP-induced cystitis. We analysed the proliferation and differentiation of urothelial cells as well as the transcriptome of the urinary bladder.
Methods
We randomly divided 80 female BALB/c mice (8 weeks old) into 8 groups of 10 mice each (permit No. U34401-4/2022/18). 4 groups received a vitamin A-enriched diet (containing 566081UI retinyl-acetate per kilogramme; VitA groups) 1 week before intraperitoneal (i.p.) injection of CP or saline (S) and the other 4 received a normal diet (N groups). Urinary bladders were collected 1 or 3 days after i.p. injection (VitA CP1d, N CP1d, VitA S1d, N S1d, VitA CP3d, N CP3d, VitA S3d, N S3d groups) and then dissected and prepared for RNA isolation followed by RNA sequencing (RNAseq) and for scanning electron microscopy (SEM). Half of each urinary bladder was embedded in paraffin for immunolabelling. The proliferation index was determined by quantification of Ki67-positive urothelial cells, while differentiation was determined by the percentage of uroplakins' (UPs) positive apical membrane length of superficial urothelial cells. We also determined the presence of highly differentiated superficial urothelial cells by immunolabelling of keratin 20 (Krt20). SEM was used to analyse the differentiation stage of the superficial urothelial cells by assessing the percentage of microridges (terminal differentiation stage) on the entire surface examined.
Data analysis was performed with ImageJ and statistically analysed with Excel and GraphPad Prism 8.01. Results are presented as mean values and standard deviations (mean ± SD) unless specified otherwise.
Results
The RNAseq results showed higher expression of genes involved in the cell cycle and PI3k-Akt signalling in the VitA CP1d group compared to the N CP1d group, indicating higher proliferation in this group. Genes involved in cell cycle (Bub1, Bub1a, Ccna2, Cdc25b, Cdk1, Knl1) and PI3k-Akt signalling (Itga3, Epha2, Spp1, Ereg, Areg and Il6) were significantly up-regulated, as revealed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis. The higher proliferation indices were subsequently confirmed in the urothelial cells of the VitA CP1d group (29.5% ± 20.5%) compared to the N CP1d group (19.6% ± 16.1%). In the VitA CP3d and N CP3d groups, the proliferation indices were 42.2%±17% and 38.4%±14.3%, respectively. In comparison, all groups with S injection (VitA S1d, N S1d, VitA S3d and N S3d group) had lower proliferation index of 1 to 2%.

All groups with S injection (VitA S1d, N S1d, VitA S3d and N S3d) had terminally differentiated superficial urothelial cells with positive UPs and Krt20 immunolabelling and microridges on their apical surface. One day after CP injection (VitA CP1d, N CP1d groups), the differentiation stage of superficial urothelial cells was significantly lower compared to the S injection groups (VitA S1d, N S1d), as indicated by the presence of microridges. The VitA CP1d group exhibited a slightly higher differentiation stage (13.9%±25.1% of microridges) than the N CP1d group (9.1%±13.6% of microridges). On the other hand, 3 days after CP injection, the VitA CP3d group showed a slightly lower UPs-positive apical surface length of superficial urothelial cells (76.6%±15.4%) than the N CP3d group (88.1%±13.5%), while no difference was observed in the presence of microridges (VitA CP3d - 6.1%±14.8% and N CP3d - 7.0%±10.3%). There was no difference in Krt20 positive superficial urothelial cells between the CP-treated groups (VitA CP1d, N CP1d, VitA CP3d and N CP3d).
Conclusion
Our results suggest that increased vitamin A intake before the onset of CP-induced cystitis influences urothelial injury and regeneration. A vitamin A-enriched diet resulted in upregulation of several genes involved in the cell cycle and PI3k-Akt signalling and increased proliferation of urothelial cells during the initial stages of regeneration (day 1). These events accelerated the formation of the initial blood-urine barrier. We were able to demonstrate that proliferation remained increased in groups with VitA-enriched diet also in the later phases of regeneration (day 3), while no difference was observed in the differentiation stage of the superficial urothelial cells. We can conclude that vitamin A influences the initial regeneration, but its effects are no longer observed in the later stages of regeneration.

Keywords:

Retinoids, cyclophosphamide, urothelium, acute cystitis

Reference:

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4D-STEM Optical Sectioning of Dopants in Diamond

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Poster Group 1

Due to improvements of aberration correctors in scanning transmission electron microscopes (STEM), larger convergent semi-angles can be used as more of the probe can be collected without introducing aberrations to the image. These larger convergent semi-angles lead to two notable improvements in the reconstructed image; an improved lateral resolution and a reduced depth of field [1]. The benefits of a better lateral resolution are immediately obvious, but by reducing the depth of field, new methods of imaging can be obtained, namely optical sectioning [2]. Optical sectioning involves collecting many micrographs of a sample with varying defocus which allows for investigation into the third dimension of the material instead of the two lateral dimensions as in conventional STEM. Four dimensional scanning transmission electron microscopy (4D-STEM) builds on conventional STEM by using superfast pixelated detectors to collect the entire diffraction pattern which allows us to reconstruct the sample using different methods such as conventional reconstructions like high angle annular dark field (HAADF) as well as more advanced methods, only available to 4D-STEM such as centre-of-mass (COM) imaging and ptychography. Here we present a body simulation work to investigate the efficacy of optical sectioning through a range of 4D-STEM data reconstruction techniques and a range of dopants within the sample.
Simulations are done using the ab initio simulation software plugin abTEM which utilizes the multislice algorithm [3]. Using this software we generate 4D-STEM datasets with a 200kV accelerating voltage and a convergence angle of 30mrad. For this work, the probe aberrations are set to zero to match ideal imaging conditions. The samples used were diamond crystal of 15nm thickness with different substitutional and interstitial dopants of masses ranging from 14Si to 82Pb. The location in the z direction of these dopants was also varied to investigate efficacy of the techniques dependent on the depth of the dopant within the sample.
The reconstruction techniques used here consist of HAADF which has been the standard for imaging heavy elements within materials since the contrast of HAADF reconstructions follows a power law of z^n where 1≤n≤2 [4]. Common 4D-STEM techniques are also implemented. COM which works by finding the average deflection of the probe by the sample in the diffraction plane, as well as its subsidiaries, integrated centre of mass (iCOM) and differentiated centre of mass (dCOM). Furthermore, the recently proposed technique of symmetry STEM (S-STEM) which involves investigating the symmetry of the diffraction pattern by rotating or reflecting the diffraction pattern and cross correlating it with the original diffraction pattern to reconstruct [5].
Using the techniques described we find that different imaging methods excel depending on the relative weight of the dopant compared to the bulk crystal. As expected, HAADF provides a simple and effective method for imaging heavier atoms regardless of position in the crystal. The comparison is less clear when investigating lighter atoms such as silicon. In these regimes methods such as iCOM and dCOM prove themselves to be powerful tools for finding the location of the atom within the structure. We find that S-STEM is less of an effective tool than the others described, but this may be a poor representation of the true case, since relaxation of the crystal lattice may lead to strain in the sample for which S-STEM has been suggested to be a useful tool for identifying point defects [5]. It still may be an unreliable tool for optical sectioning though since S-STEM is robust at reconstructing

atomic resolution images regardless of defocus as we have demonstrated with our results, as symmetry is independent of defocus.
The methods here result in a detailed analysis of how utilizing all the variables and tools available when imaging these materials using 4D-STEM can be leveraged to obtain the best results for optical sectioning. This work focuses on diamond as a bulk crystal within which dopants are placed so could be used as an effective framework for investigating the composition of colour centres such as nitrogen and silicon vacancy centres.
Figure 1: (a), (b) and (c) shows COM, dCOM and iCOM reconstructions of diamond respectively with germanium dopant within box drawn on (a). (c) and (e) show line profiles of (b) and (c) along the line drawn. (f) Schematic of diamond view down <100> zone axis with Cu dopant place on the top surface, middle and bottom surface of the crystal, respectively. (g) S-STEM reconstruction of the lattice shown in (f) with 180o rotation of the diffraction pattern, red line shows path of line profile shown in (h). (h) Intensity line profile of S-STEM reconstruction with different dopants/vacancies in the crystal.

Keywords:

4D-STEM, iCOM-STEM, Optical Sectioning, 3D-Imaging

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Correlative microscopy and spectroscopy of nanophotonic materials

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Poster Group 1

We present a comprehensive study of nanophotonic materials by correlative electron and optical microscopy and spectroscopy. We focus on a phase-changing material vanadium dioxide, which is relevant for active plasmonics and optical metasurfaces [1], and on lead-halide perovskite ($CsPbBr_3$) [2]. We performed a comprehensive analysis of a VO_2 nanoparticle and $CsPbBr_3$ nanocrystals using a combination of analytical transmission electron microscopy and optical methods like ellipsometry and transmission.

We have explored the metal-insulator transition (MIT) in the single vanadium dioxide nanoparticle caused by in-situ heating and we have identified the dielectric and the metallic phase of the nanoparticle by imaging, diffraction, electron energy loss spectroscopy, and optical transmission. Our results show that differences in high-resolution images and diffraction patterns obtained at high and low temperatures confirm that MIT is related to a modification of the crystal lattice. In low-loss EELS, the MIT is manifested by the emergence of the plasmon peak in the high-temperature metallic phase. Moreover, we have shown that the transition can be observed directly using imaging techniques such as STEM-ADF and DF-TEM with no need for in-situ spectroscopy. This finding allowed us to study the hysteresis of the MIT in vanadium dioxide with a high spatial resolution. We have observed that the transition from the dielectric to metallic phase is smooth and spans a rather large temperature range while the backward transition is abrupt.

Further, we analyzed $CsPbBr_3$ nanocrystals using correlative microscopy allowing the examination of structural, chemical, and optical properties from identical areas. Moreover, the use of 4D-STEM in a FIB/SEM allowed us to determine the crystallographic orientation of individual nanoparticles. Our results show that their stoichiometry is uniform and independent of their morphology. The photoluminescence peak emission energy is dependent on the dimensions of nanocrystals, with a blue shift up to 9 nm in wavelength with a decreasing size due to the confinement effect [2].

To conclude, our results provide a comprehensive analysis of the MIT in the single vanadium dioxide nanoparticle and pave the way to phase-changing devices made of vanadium dioxide. Further, we have shown that our $CsPbBr_3$ nanocrystals are of high quality, exhibiting bright and size-tunable PL emission [3].

Keywords:

vanadium dioxide; nanoparticles; EELS; 4D-STEM

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Birefringence analysis of hemozoin with surface plasmon microscopy towards malarial species distinction

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Poster Group 1

I. Introduction

Female Anopheles mosquitoes carrying the Plasmodium parasite are the primary vectors of malaria mostly prevalent in countries exhibiting tropical climate [1]. The parasite species Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria, Plasmodium ovale, and Plasmodium knowlesi are the five that can infect humans. Plasmodium falciparum is responsible for 70% of the infections, whereas Plasmodium vivax is responsible for 20% of them [1]. WHO reports that 249 million cases were recorded in 2022, of which 233 million (about 94%) were reported in Africa. Nearly half of all cases were reported in Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%). Malaria is expected to have caused 608,000 fatalities worldwide in 2022. Just four nations accounted for more than half of all deaths: Tanzania (4%), Niger (6%), the Democratic Republic of the Congo (12%), and Nigeria (31%). Eleven countries namely Burkina Faso, Cameroon, the Democratic Republic of the Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda, and Tanzania account for almost 70% of the world's malaria cases [2].

There exist many techniques for detection of malarial species. Conventional light microscopy involves examining blood smears with Giemsa stain has been the golden standard, while other techniques including rapid diagnostic test and polymerase chain reaction (PCR) have also been utilized. Most of these techniques require a long processing time and are not quantitative in general. Further, label free techniques are scarce in malarial detection.
This work is based on the anisotropic characterization of hemozoin utilizing surface plasmons. We explicitly studied the difference in the birefringence of whole blood with and without hemozoin.

II. Methods

Focused surface plasmon microscopy [3] is a technique that measures light-matter interaction at sub-wavelength level to investigate local optical properties of the sample at the metal-dielectric interface. It relies on optical near-field effects to map the refractive index profile of the samples with high spatial resolution. Numerous samples (i.e., thin films and liquids) have been probed (probe size:180 nm) by this technique to reveal circular absorption pattern at the exit pupil of the high numerical aperture microscope objective. By correlating the radius of the absorption pattern one can determine the refractive index of the sample. Further, anisotropic characteristics of the sample (if present) can be extracted from the elliptical absorption pattern to reveal the magnitude of birefringence and orientation of its fast axis respectively [4].
NLAF21 coverslips (refractive index: 1.78) were cleaned with ethanol, acetone, and isopropanol for 5 mins each. Then the coverslips were subjected to oxygen plasma for 5 mins. Then a chromium layer (~2 nm, r.i.: 3.316+i3.312) followed by gold layer (~ 47 nm, r.i.: 0.18377+i3.4313) were deposited by E-beam vapor deposition. The chromium layer was added for a better adhesion of the gold layer. The thickness of the subsequent layers was correlated with X-ray diffraction analysis. Artificial blood (~1

µl) was added on top of the substrate. Later various concentrations of hemozoin were mixed in the artificial blood and pipetted on top of the substrate.

III. Results

The reflected spatial frequency distribution at the exit pupil of the high numerical aperture objective lens was recorded. When artificial blood was used as sample, we observed a circular absorption pattern at the exit pupil plane indicating the absence of any anisotropic components. The refractive index was recorded from the radius of the circular absorption pattern with a semi-automated custom developed software. When hemozoin was mixed in artificial blood, we observed an elliptical absorption pattern at the exit pupil plane. The birefringence was extracted from these images with the developed software as stated before.

IV. Conclusion

In this proof-of-concept study, we conclude that refractive index and more so birefringence can be used as a label-free estimator differentiating healthy blood and malarial blood. Further this approach can be used to distinguish between various malarial species as reported previously based on the refractive index [5]. We believe the quantitative characterization of the hemozoin crystals from various plasmodium species could be used in future to easily distinguish the species.

Keywords:

Label-free
Birefringence
Malaria
Surface plasmon

Reference:

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In situ examination of oxygen vacancy dynamics in epitaxial LaCoO3 thin films

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Poster Group 2

Background incl. aims
Understanding the coupling effect between lattice strain and the energy required to create oxygen vacancies is crucial for the rational optimization of the catalytic performance of thin film perovskite electrocatalysts. However, contradictory results have been reported regarding the impact of lattice strain on both the oxygen vacancy content and formation energy, differing in terms of extent, direction, and mechanism. Theoretical studies have indicated that oxygen vacancy defects may either increase or decrease with increasing tensile strain. Additionally, the formation energy of oxygen vacancies has been observed to follow either a curved or nearly linear trend as the strain state transitions from compression to tension. These theoretical findings suggest that the energetics of oxygen vacancy formation could be highly complex and material dependent. Therefore, our study aimed to experimentally investigate the concentration and distribution of oxygen vacancies in epitaxial thin films of LaCoO3 (LCO), a highly active perovskite oxide material, for both the oxygen reduction reaction and the oxygen evolution reaction. We observed the formation behavior of oxygen vacancies at the atomic level in real time using in situ transmission electron microscopy (TEM) with an electron beam exposure function. This technique allowed us to study the dynamic behavior of oxygen vacancies under compressive and tensile lattice strains. Additionally, we utilized high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) images to directly analyze the local mapping and visualization of oxygen vacancies at the unit cell level in the LCO thin films.
Methods
On (001) LaAlO3 (LAO) and SrTiO3 (STO) substrates, LCO thin films were synthesized using the sol-gel technique. Cross-sectional TEM specimens were prepared using a focused ion beam (Quanta 3D FEG, FEI), and HAADF STEM images were acquired using TEM (Mono ARM 200F, JEOL) operating at 200 kV. The beam convergence semi-angle was set to 23.81 mrad, and the collection semi-angles ranged from 68 to 280 mrad. A consistent electron dose rate was maintained during electron beam irradiation. Wiener filtering, implemented via the HREM-Filters software package (HREM Research), was utilized to enhance image clarity.
Results
Atomic-scale HAADF STEM images depicting the initial conditions of the LCO thin films on the LAO and STO substrates are presented in Figs. 1 (a) and (f), respectively. A 15 × 15 nm region within the LCO thin film was monitored for changes in lattice parameters, encompassing 1,444 unit cells. HAADF STEM images were obtained from identical pristine regions of the LCO thin film at various time intervals as the electron beam irradiation time was increased. Figs. 1 (b–e) and (g–j) exhibit high-resolution HAADF STEM images selected at 20-minute intervals in the LCO thin film on the LAO and STO substrates, respectively. The time series of the HAADF STEM images for both LCO thin films revealed the appearance of dark strip patterns over time. The modulated contrast patterns observed in the HAADF STEM images of the LCO thin films are attributed to the ordering of oxygen vacancies, leading to localized lattice expansion and the formation of modulated dark stripe patterns. The onset time of the first dark-strip pattern differed between the two LCO thin films. Specifically, the first dark

strip pattern emerged after approximately 60 minutes of continuous electron beam exposure in the LCO thin film on the LAO substrate and after 20 minutes in the LCO thin film on the STO substrate. The red arrows indicate the first dark-strip pattern in Figs. 1 (d) and (g). Subsequently, dark-strip patterns emerged at various positions and continued to grow in the out-of-plane direction. The maximum growth of dark strip patterns in the two LCO thin films occurred after 80 minutes. Furthermore, the distribution of dark strip patterns was observed more frequently in the LCO thin films on STO substrates.

Conclusions

HAADF STEM images confirm uniform thickness and initial conditions of LCO thin films on LAO and STO substrates. With increasing electron beam irradiation time, distinctive dark strip patterns emerge, indicating oxygen vacancy ordering. Variations in pattern appearance and behavior are noted between substrates, with higher tensile strain accelerating pattern onset and distribution frequency. Quantitative analysis of lattice parameter changes corroborates these observations, confirming expanded unit cells associated with oxygen vacancies. Notably, films under higher tensile strain exhibit more uniformly distributed enlarged unit cells and significantly higher oxygen vacancy concentration. These findings support previous studies suggesting reduced formation energy of oxygen vacancies under tensile strain. Overall, this research provides valuable experimental insights into oxygen vacancy formation and distribution mechanisms in LCO thin films under strain, advancing our understanding and informing tailored material design for electrochemical applications.

Keywords:

Oxygen vacancies, Strain states

Reference:

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Oscillatory redox behavior of nickel catalysts observed by operando SEM

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Poster Group 2

Background

A catalytic cycle in heterogeneous catalysis involves the adsorption, reaction, and desorption of species on the surface of an active catalyst. This cycle repeats as long as the catalyst remains active and is inked to the concept of turnover frequency - defined by chemists to estimate the number of conversions per active site per unit of time. The cyclic behavior that occurs at the molecular or atomistic level involves catalyst restructuring and cannot be separated from the larger scale processes involving collective phenomena of heat and mass flow. Under reaction conditions, the coupling of processes dominating at different time and length scales can give rise to complex dynamics that are observable at both microscopic and macroscopic levels [1,2].
The aim of this work is to investigate how catalytic function emerges in the interplay between reactive species and catalyst. We employ operando scanning electron microscopy to study the simple model system of hydrogen oxidation. This reaction is known to exhibit oscillatory behavior characterized by periodic variations in the reaction rate [3]. This is primarily due to the dynamic and cyclic interplay between the adsorption of reactants, the formation of water, and the autocatalytic formation and reduction of nickel oxide.

Methods

The experiments were conducted using a FEI Quanta 600F Environmental Scanning Electron Microscope (ESEM) at pressures between 20 to 30 Pa. The instrument is attached to a home-built gas feeding system and equipped with a home-built laser heating stage. For reactivity studies, an electron ionization quadrupole mass spectrometer was used. Images were recorded using the gaseous secondary electron detector.

Results

As hydrogen and oxygen are adsorbed and react to form water, the surface periodically transitions between a highly active metallic state and a less active oxidized state. Changes in the oxidation state of the surface result in a change in the secondary electron yield and are detected as brightness variations. In the case of reactions carried out on polycrystalline nickel foils, a dependence of the reactivity on the grain orientation is observed, as well as spillover coupling at grain boundaries and a complex, spiral-like pattern of propagating reaction fronts on some of the grains. Superimposed on the grain dependent surface dynamics are collective oscillations associated with reaction induced variations in gas phase composition. By varying the oxygen flow or temperature, i.e., one of the key control parameters, we are able to systematically follow the transition of the system from a steady state to oscillatory, and ultimately chaotic behavior. Notably, bifurcations are observed, marking shifts in the system's behavior. Switching from a polycrystalline foil to single crystals removes coupling effects due to grains with different activity and shows the formation of intriguing spiral waves under otherwise steady state conditions. Our results reveal the complexity of simple reactions and provide valuable insights into the most relevant coupling mechanisms. It furthermore demonstrates that catalysis cannot be understood based on ex-situ studies.

Conclusion
On the polycrystalline Ni foil, oscillatory behavior between oxidized and reduced states correlates with significant changes in catalytic activity. The reactivity is influenced by the orientation of the surface grains. In contrast, the single crystal (100) Ni surface exhibits initial oscillations that stabilize into spiral-like reaction fronts. Unlike the transient patterns observed on the polycrystalline foil, these spirals are persistent. The stability is attributed to the absence of neighboring grain interactions which typically introduce complexities and destabilize reaction fronts on polycrystalline surfaces.

Keywords:

Operando SEM, Oscillations, Dynamic Catalyst

Reference:

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Structural Characterization of MOFs using Transmission Electron Microscopy and Software Innovation

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Poster Group 2

In recent years, Transmission Electron Microscopy (TEM) has emerged as an indispensable tool for crystallography1, offering unparalleled insights into the atomic structures of complex materials. This is particularly relevant for Metal-Organic Frameworks (MOFs), whose intricate architectures and diverse applications in areas like gas storage, catalysis, and drug delivery depend heavily on their precise atomic arrangements.
Our study employs a TEM-based approach, three-dimensional electron diffraction (3DED)2, to probe the crystal structures of targeted MOFs. While 3DED offers powerful capabilities, challenges such as crystal drift can hinder its efficiency. To overcome these obstacles, we developed LibraEDT, a software for electron diffraction experiments on the Zeiss Libra 120. LibraEDT introduces several key advancements, including automated crystal tracking to maintain sample alignment within the electron beam, thereby reducing beam damage and enhancing data quality. Additionally, LibraEDT facilitates extended data collection range, above 120º, with precision tracking, ensuring optimal results. The software interfaces seamlessly with ASI Timepix single electron detectors allowing for precise crystal screening, tracking and automatic diffraction screening on a predefined crystal set, all while minimizing radiation exposure.
We applied LibraEDT to the structural analysis of two distinct new MOFs. First, we investigated a MOF synthesized through an environmentally friendly mechanochemical process using water and biocompatible precursors. The structure, solved via 3DED, was dynamically refined to reveal the presence of water molecules within its channels, a key insight for understanding its properties. Second, we studied a novel heterometallic MOF exhibiting a complex and highly disordered structure. It shows an orthorhombic structure with a spacegroup of Cmmm and a cell volume above 11000 A^3, which can be advantageous for certain applications. The 3DED analysis revealed a unique tetranuclear metallic cluster composed of both titanium (IV) and calcium (II) centers, offering a potential platform for tailored functionality.3
This work was carried out with the support from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956099 (NanED – Electron Nanocrystallography – H2020-MSCAITN)

Keywords:

3D-ED, Crystallography, TEM, MOFs

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Transmission electron microscopy of dermal collagen using ethanolic phosphotungstic acid staining

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Poster Group 1

Background
For conventional transmission electron microscopy, biological samples are treated with heavy metal salt solutions to enhance contrast, either by staining ultrathin sections with lead citrate, uranyl acetate or lanthanoid salts, or by staining tissue blocks before embedding, using similar substances. However, we found that these substances are insufficient for imaging collagen fibrils and collagen VII anchoring fibrils. The latter are critical structures for a stable attachment of the epidermis to the underlying dermis, and accordingly the subject of studies concerning skin defects such as epidermolysis bullosa. Their structure and exact position can only be imaged by electron microscopy. Phosphotungstic acid (PTA) is known to interact with nucleic acids and positively charged proteins, and is widely used for light histologic staining procedures of connective tissue. It is also used in TEM, but mainly for negative staining. Only a few protocols for section or block staining are available in the recent literature. We tested PTA staining of collagen for TEM and were able to establish a block staining procedure that can be integrated in the dehydration steps.
Methods
Human and murine skin and in vitro skin equivalents were fixed in paraformaldehyde and glutaraldehyde, and postfixed in osmium tetroxide. Blocks were stained in 1% PTA in 70% ethanol, followed by further dehydration in ethanol, embedding in epoxy resin and ultrathin sectioning. Sections were analyzed in a Zeiss EM910 at 80kV.
Results
The combination of osmiumtetroxide and ethanolic PTA resulted in a versatile staining of all tissue components. We observed strong contrast of all intracellular compartments, well characterized chromatin, dark mitochondria, strong staining of ribosomes and very intensive staining of desmosomes and hemidesmosomes. Collagen fibrils, anchoring fibrils and other fibrous extracellular materials including the basal lamina were depicted in high quality.
Conclusion
Ethanolic PTA staining is a fast, easy and non-radioactive block staining method for structurally precise TEM tissue analysis. It provides good results for both, intracellular structures and extracellular fibres such as different types of collagen. This protocol may be an interesting option for various studies on cell and tissue microstructure since there is, for example, an increasing demand for reliable block staining techniques for 3D analysis of serially sectioned and scanned resin blocks.

Keywords:

TEM, staining, phosphotungstic acid, collagen,

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From design to « operando » experiments in the SEM: application to battery studies

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

In batteries, electrochemical reactions take place at the interfaces (electrodes-electrolyte, etc.), inducing more or less reversible change such as poor ion transport, dendrite formation, electrochemical degradation and chemo-mechanical degradation[1]. Among the new-generation systems under development, all-solid-state batteries (ASSBs) are no exception to this rule. Understanding the phenomena at interfaces that lead to premature battery ageing is therefore crucial to understanding and finding ways of improving the battery. Thanks to the emergence of in-situ and operando techniques, these complex, constantly evolving interfaces can now be studied by live, battery-scale observations using SEM[2].
To carry out this type of study, we have developed a simple electrochemical cell (Figure 1) that can be used to operate all-solid state lithium metal batteries inside the SEM. The use of lithium metal, which is an air-sensitive compound, led us to fabricate a sealed box enabling the battery to be transferred between the glovebox and the SEM (and vice versa). Morphological (secondary and backscattered electron imaging) and chemical (X-ray energy dispersive spectroscopy) changes at solid-state interfaces could thus be monitored in real time and during battery operation.
In this study, we focused on sulfide-based solid electrolyte (SE) particle size distribution and its impact on electrochemical performance. A batch of Argyrodite (Li6PS5Cl (LPSCl)) was sieved to obtain two batches of particles range (0,5-20µm and 50-150µm). The AASBs were prepared using the two particles size distribution while keeping the anode (lithium metal), cathode (active material: NMC) composite formation and the fabrication process constant.
From the two particles sized distribution, the one with the large particles have better electrochemical performance. From morphological point of view at the cathode composite electrode, the formation of a cathode electrolyte interface is visible in the different batteries but is more developed in the case of small sized particles. In the solid electrolyte, the type of cracks differs with relatively strait feature for small particles and sinuous one for large one. At the anodic interface, a lost in contact between the SE separator and the lithium is observed and increased with the cycling process with a higher impact for the small particles (Figure 2.a and b). By investigating further, the formation of dendrites with different morphologies are visible at the anodic interfaces (Figure2.c).
We categorized the observed changes into three modes: (i) electrical failure by the formation of lithium dendrites of different morphologies following the ES going up to short circuit in the case of small particles, (ii) mechanical failure by the formation of cracks in the electrolyte whose shape and propagation strongly depend on the distribution particles size and (iii) electrochemical failure with the formation of solid electrolyte interphases on the surface of the active material. The main obstacles for the use of lithium metal are related to the propagation of lithium dendrites and thus to the mechanical instability of solid electrolytes.

Keywords:

SEM, operando cycling, battery

Reference:

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High-Z Nanoparticle Tagging in Cryo-STEM for Localisation in Cryo-ET: Theory and Damage

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Poster Group 1

Background:
A key issue with the established method of cryo-electron tomography (Cryo-ET) often lies in the challenge of accurately locating complexes or proteins of interest within the crowded cellular environment of the generated tomograms. This project aims to use cryo-scanning transmission electron microscopy (STEM) to highlight regions of tomograms containing the protein of interest by tagging with a minimally-sized heavy atom nanoparticle for downstream processing. A fine balance must be achieved between resolution, signal-to-noise ratio (SNR), depth of focus, and damage to the lamella.

Methods:
A scan generator offers alternative (non-raster) methods of scanning the beam to offset damage and allow higher electron fluences to be used without compromising ice quality. Small raster and interleaved scans (< 200 nm) were compared in the same quantifoil hole of vitreous ice, using an equal overall electron fluence and flux.
Analysis of elastic scattering cross section theory yields an approximate minimal size of nanoparticles for detection in STEM of vitreous amorphous specimens. Single particle-like sample preparation was employed to freeze varying sizes of gold nanoparticles (0.8-4 nm) in different thicknesses of ice. These were subsequently exposed to STEM to assess a drop-off in SNR with increasing collection angles using an annular dark field detector.

Results:
Scanning in an alternative fashion using long dwell times demonstrated a significant reduction in mass loss. Raster scanning appeared to be marginally better than interleaved scanning when using shorter dwell times (20 µs), as evidenced by greater loss of intensity in the scanned areas, normalised to reference areas taken within the same hole of vitreous ice. However, using longer dwell times (250 µs or 500 µs) reversed this effect, showing raster scanning to be significantly more damaging than interleaved, melting the ice completely in thin samples for raster scanning, whilst maintaining the ice intact using an interleaved sequence.

Conclusions:
These findings provide valuable first steps toward optimizing cryo-STEM imaging for detecting nanoparticles and correlating these findings with in-situ Transmission Electron Microscopy (TEM).

Keywords:

STEM, Cryo-ET, Nanoparticle, ADF

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Combination of TEM, LM and micro-CT to image insects: the perspective of crop protection strategies

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Poster Group 2

Background
Invasive insects, in addition to several already established species, are a serious threat to crop production and an important hazard as vectors of pathogens of severe diseases. This is an increasing problem due to trans-continental trade, travel and climate change. Therefore, new strategies for crop protection and new approaches to minimize the adverse effects of pathogen spread are needed. A better understanding of insect functional ultrastructure during animal development and under stress conditions is crucial to facilitate formulation of new innovative solutions. Microscopic analyses enable comprehensive characterization of the structure of insect organs and localization of selected molecules at different scales, and in combination with biochemical analyses that unravel selected aspects at the molecular level, constitute a toolbox for the integration of structure and function and for an in-depth evaluation of the effects of xenobiotics. The first aim of our study was to establish a procedure in which micro-computed tomography, light and transmission electron microscopy will be used in combination to image insect digestive system in larvae and adults, spanning the range from molecular resolution to imaging of the whole organisms. Our second aim was to apply the method to characterize the midgut of two insect species that are important from the perspective of crop protection, spotted wing drosophila (Drosophila suzukii) and Colorado potato beetle (Leptinotarsa decemlineata).
Methods
The insect gut is positioned at the interface between the external and internal environment and it is a likely target of xenobiotics. As the midgut epithelium plays a central role in digestion, nutrient absorption and protection against toxins and pathogens, we have characterized the midgut functional ultrastructure in larvae and adults to get insight into changes during development and to evaluate the alterations of the gut epithelium due to exposure to selected xenobiotics - fungal lectins and protease inhibitors. Micro-CT imaging was performed on whole animals that were chemically fixed. Subsequent processing and segmentation of the micro-CT data was performed using Neoscan80 and Dragonfly software. Sections of the whole larvae or dissected gut samples were imaged by LM and TEM in different regions along the anterior-posterior axis, focused on the characterization of the luminal surface of the epithelium, the distribution of stem/progenitor cells and alterations of the epithelium architecture after exposure to selected xenobiotics.
Results and conclusions
The digestive system in insects consists of foregut, midgut and hindgut. The highly convoluted midgut is the longest part of the alimentary canal and occupies a large part of D. suzukii larva body volume (Figs. 1A, B). The midgut epithelium comprises several cell types (Fig. 1C), enterocytes bear numerous microvilli on the apical surface (Fig. 1D). Stem cells are abundant and appear as clusters of cells in the basal region of the epithelium (Fig. 1E). In the midgut of Colorado potato beetle larvae columnar enterocytes with dense apical microvilli prevail and numerous stem cells reside in clusters in the

basal part of the gut epithelium. Stem cells do not form septate junctions with neighbouring cells, while enterocytes display abundant intercellular junctions. Our current work is focused on the determination of the effects of entomotoxic fungal proteins on the digestive system by a combination of imaging methods and biochemical approaches to identify the target molecules and elucidate their mode of action.

Keywords:

Midgut, stem cells, insect larvae

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High-resolution structural analysis of cation-mixed (Fe,Mn,Ni)PS3 van der Waals single crystals

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Poster Group 2

Transition-metal (TM)-based van der Waals (vdW) materials ("2D – materials") recently received particular attention due to their tunable magnetic properties arising from unpaired magnetic moments due to the partially filled d-shells of the TM ions. The subclass of TM phosphorous trisulfides (TMPS3) has attracted significant interest due to its wide range of TM elements, resulting in diverse properties [1]. The structure of TMPS3 is defined by its vdW nature. Layers exhibit the monoclinic crystal structure, space group 3 C2/m, and are only bound by weak vdW forces [2], which allows for easy exfoliation and leads to 2D-like properties being observable in 3D crystals.
In this study, transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), electron energy loss spectroscopy (EELS), and continuous rotation electron diffraction (cRED) have been combined to study the cation-mixed (Fe,Mn,Ni)PS3 van der Waals single crystals with different Fe, Mn, and Ni concentrations produced in a bulk state by chemical vapor transport (CVT) [3].
The high-resolution (HR) TEM image (a) shows the cross-section of a (Fe,Mn,Ni)PS3 single crystalline sample, prepared by Cryo-Ultramicrotome. An amorphous oxide layer formed on the edge of the sample is highlighted with a yellow dashed line. It has a thickness of 5 nm. The magnified HRTEM image (b) shows the layered structure with a layer distance of 0.65 ± 0.02 nm. Bright spots (atomic columns) correspond to S and P. The darker lines are the planes of TM ions. Ultrasonically exfoliated layers are shown on TEM image (c) on which selected area electron diffraction (SAED) (d) and EDX mapping (e) have been performed. The lattice parameters a = 0.647 nm, b = 1.072 nm, c = 0.698 nm, $\alpha = \gamma = 90°$ and $\beta = 111.22°$ were estimated from the SAED pattern and slightly deviate from earlier reported FePS3, MnPS3 and NiPS3 mono-compounds [4,5].
Work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 405553726 – TRR 270, Z02"

Keywords:

3D ED, cRED, 2D-materials, TMPS3

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STEM-EELS and Image simulations of inelastic spin-scattering of magnons in confined geometries

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Background incl. aims
Application of magnons requires generation, manipulation, and their characterisation in high resolution in both energy and spatial scales. Magnons are commonly studied by inelastic neutron scattering techniques, time-resolved Kerr microscopy, and Brillouin light scattering. While these techniques probe the energy-momentum dispersion of magnons with high energy resolution, their spatial resolution is fundamentally limited to hundreds of nanometres.
Progress in electron beam monochromators in scanning transmission electron microscopy (STEM) has enabled the use of electron beams to study magnons with nanometer and sub-nanometer spatial resolution. Previous studies have successfully collected signals of scattered electrons through electron energy loss spectroscopy (EELS), demonstrating the detection of collective excitations like phonons [1]. The energy range of phonon modes, extending up to a few hundred meV in solid-state materials, aligns qualitatively with magnons, hence efforts aiming to detect magnons using STEM-EELS are ongoing.
Although the interaction strength between magnons and electrons is known to be three or four orders of magnitude weaker compared to the Coulomb interaction, experimental evidence has shown that magnetic ordering can be detected through Bragg reflections of electrons [2]. Furthermore, recent theoretical predictions suggest an EELS signal from thermal magnons in bulk specimens [2, 3], supporting the potential use of STEM-EELS for measuring magnons in confined geometries, including interfaces and surfaces.
Methods
In this study, we employ the second quantization of the Heisenberg Hamiltonian to calculate the dynamics of magnon modes in finite systems, allowing the inclusion of thin films, interfaces, and heterostructures between ferromagnetic (FM) and antiferromagnetic (AFM) layers, with different relative orientations between the electron beam and the Curie/Neel vector. We then employ the general formalism by van Hove [4] to calculate the double cross-section accounting for the spin-spin interaction of the electron probe with the system, as well as the effect of the system's vector potential on the charged probe's canonical momentum. In addition, by using the multislice method, we simulate high resolution STEM images due to the electron-magnon interaction.
Results
We present our result on simulations using Fe, NiO and YIG as prototypical examples of the effects captured by the method, Figure 1. By quantifying separately, the spin-based and charge-based interactions in STEM-EELS, we reveal distinct behaviours: charge-based interaction shows a linear dependence on beam momentum, while spin-based interaction follows an inverse relationship akin to phonons, suggesting potential magnon-related peak enhancement compared to phonon peaks at higher acceleration voltages. Additionally, charge-based interaction exhibits an inverse square

dependence with a scattering vector. Furthermore, the image simulations show a dependence with the magnetic moment's orientation relative to the electron beam.

Conclusion

Overall, our study advances our understanding of electron-matter interactions in EELS, offering insights for designing and interpreting experiments probing the magnetic properties of nanomaterials. The magnon EELS calculations suggest that these interactions would be dominant for higher beam acceleration and within the first Brillouin zone, while the image simulations point to the possibility of probing the local magnetic moment's orientations.

Keywords:

magnons, EELS, image simulations, STEM

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Microscopy of body surface sculpturing in terrestrial crustaceans

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Poster Group 1

Background
Terrestrial isopods (Oniscidea) are a successful group of terrestrial crustaceans, partly due to their finely structured exoskeletal cuticle. The cuticle is an elaborate extracellular matrix deposited by epidermal cells and consists of several layers. The epicuticle, the thinnest and outermost layer, forms remarkably diverse and complex structures, such as scales and other micrometre-sized ornaments that influence the surface properties of the cuticle and the interaction of isopods with their environment [1, 2]. However, not much is known about the formation of the epicuticle and these structures. The aim of this study was to develop a simple protocol to investigate dorsal epicuticle formation in terrestrial isopods during molt. For this purpose, a combination of scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) was applied to image the deposition of the new epicuticle during molt in the terrestrial isopod Porcellio scaber.
Methods
Tergites of P. scaber specimens in different stages of cuticle deposition during preparation for molt [3] were dissected, fixed in a mixture of paraformaldehyde and glutaraldehyde, and decalcified with EDTA. For SEM imaging, the samples were osmicated and embedded in paraffin. At this stage, the old cuticle was cut off with a razor blade to expose the ecdysial space for observation. Paraffin was then removed, and samples were air-dried in hexamethyldisilazane and sputter-coated with platinum. Prepared samples were imaged using a JSM-7500 field emission scanning electron microscope (JEOL). For CLSM imaging, the decalcified samples were incubated in methyl green, a far-red emitting fluorescent DNA dye, to visualize cell nuclei [4]. Subsequently, the samples were cleared and mounted in ProLong™ Glass Antifade Mountant (Invitrogen). Series of optical sections were acquired using a Stellaris 8 confocal microscope (Leica).
Results and conclusions
Using the described procedure, we were able to expose the ecdysial space and the surface of the epidermis and observe the new epicuticle in different stages of its formation with SEM from a perspective never seen before. In the late stage of cuticle deposition, we observed the fully sculptured new epicuticle with numerous scales and sensory tricorns (Fig. 1A), whereas in the early stage, the surface of the underlying epidermis was exposed, showing stubby apical cell membrane protrusions and only the outlines of the edges of new scales. Using CLSM, we were able to observe cuticle autofluorescence in relation to DNA-bound methyl green fluorescence and thus determine the position of epidermal cells in relation to the position of emerging epicuticular structures (Fig. 1B). In this way, we established that each scale is likely deposited by a single epidermal cell. The presented protocol allowed us to observe the complex architecture of the epicuticle during its deposition and to gain information about the mechanism of epicuticular sculpturing by the epidermal cells that secrete it. In the future, we plan to extend this protocol by analysing the changes in epidermal cell ultrastructure during epicuticle formation using transmission electron microscopy.

Keywords:

arthropods, cuticle, SEM, CLSM

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Toxic buffers used in microbial sample preservation for SEM can be replaced by PBS

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Poster Group 1

The structural preservation of uncultivated, oxygen-sensitive microorganisms for imaging via scanning electron microscopy (SEM), often relies on toxic buffers like cacodylate. As such, studies on certain archaea named Candidatus Altiarchaeum which can dominate deep biosphere ecosystems by forming biofilms using hook-shaped cell appendages (hami), currently heavily rely on cacodylate buffers. However, the twelve principles of green chemistry state that researchers should strive to minimize the use of toxic substances and ideally replace them entirely with non-toxic alternatives. Following these principles, we here tested the influence of alternative, less toxic buffers (phosphate-buffered saline (PBS) and PHEM (PIPES-HEPES-EGTA-MgCl2)) for the preservation of Ca. Altiarchaeum hamiconexum for SEM and compared them with preservation using cacodylate for subsequent imaging.

Fixation of Ca. Altiarchaeum hamiconexum was conducted immediately after the collection of the biofilm from a cold sulfidic spring located in Regensburg (Bavaria). Samples were preserved using 4% formaldehyde final concentration in cacodylate, PHEM or PBS, respectively. For SEM preparation, 2.5% glutaraldehyde in cacodylate, PHEM or PBS, were used, after which samples were rinsed four times with the corresponding buffer and post fixated with 1% osmium tetroxide in cacodylate, PHEM or PBS. A dehydration gradient was then applied with 10%, 20%, 40%, 60%, 80% and 100% acetone. Quality of preservation was examined using SEM after critical point drying and sputter coating with platinum/palladium. The so prepared archaeal cells were analyzed in comparative manner by experienced scientists used to investigate Ca. Altiarchaeum hamiconexum ultrastructure prepared with cacodylate buffer.

The preservation with cacodylate and PBS revealed no noticeable differences. The hami displayed a high degree of preservation and no visible difference in the thickness and length of those structures. However, all cells, independent of the preservation buffer used, showed deformations. The samples preserved in PHEM exhibited the least preservation of the hami, with many cells showing complete absence of appendages.

To reduce risks for researchers and the environment, the use of toxic substances in laboratory conditions should be avoided if less dangerous alternatives exist. In this study, no visible differences were observed for the quality of hami preservation between toxic buffer cacodylate and PBS. PBS can effectively replace cacodylate for preserving the ultrastructure of hami of Ca. Altiarchaeum hamiconexum for the purposes of high-resolution microscopy.

Keywords:

Sanple preparation, Microbiology, Buffer replacement

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Leveraging AutoScript for Cross-Platform Deep Learning Solutions in Electron Microscopy

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Poster Group 2

Python's widespread adoption is supported by a wide community that has developed an extensive toolkit of open-source libraries. Electron microscopy vendors have introduced Application Programming Interfaces (APIs) enabling users to design sequences for comprehensive control over electron optics, detectors, sample positioning, and data acquisition. [1,2,3]
These Python-accessible libraries facilitate classical image processing tasks, including object detection, drift correction, and feature tracking, which have been pillars of electron microscopy scripting for over twenty years. Applications range from Focused Ion Beam (FIB)/Scanning Electron Microscopy (SEM) defect analysis and Transmission Electron Microscopy (TEM) lamella preparation to semiconductor metrology, with TEM image processing and data analysis being particularly prominent.
The adoption of closed-loop image processing in electron microscopy is driven by the increasing volume of multimodal data generated by these instruments. Direct data offloading is inefficient and risks post-processing error identification, leading to valuable time losses.
Recent advancements in machine learning and deep learning have significantly enhanced their performance and integration speed, facilitating their inclusion in closed-loop automation sequences for complex tasks such as object detection, classification, and feature extraction.
ThermoFisher Scientific's AutoScript, a cross-platform Python-based API, streamlines and refines electron microscopy workflows by harnessing deep learning techniques. This paper highlights two applications:
1. SEM Feature Analysis: Utilizing a Thermo Scientific Helios microscope, this experiment automates the creation of binary masks for feature analysis on aluminum powder samples. Instance segmentation enables feature separation, counting, and property analysis (e.g., size and shape), allowing real-time experimental optimization based on material properties identified by deep learning algorithms.
2. TEM Atom Detection: Demonstrated with a Thermo Scientific Talos F200, this application automates the detection of atom positions and diameters in HR-STEM images of SrTiO3. Neural networks facilitate rapid atomic structure predictions and interface identification, optimizing imaging parameters on-the-fly.
These cases exemplify the transformative impact of cross-platform, Python-based APIs in electron microscopy, showcasing their potential to enhance efficiency, accuracy, and the scope of achievable experiments through automation and advanced data analysis.

Keywords:

EM Automation, Deep Learning, AI

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Exploring Role of Voltage-Gated Ion Channels in LGI1 Autoantibody-Induced Epilepsy: Insights from a Mouse Model

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Poster Group 1

Epilepsy is a debilitating condition affecting 0.5% to 1.0% of the world's population (1), but its underlying etiology often remains unknown, hence most anti-epilepsy therapies aim for reduction in seizure symptoms. Nevertheless, recent studies suggest that up to 1 to 7 out of 20 individuals with new onset seizures may have an autoimmune cause (2). Although rare, the most common epilepsy-associated autoantibodies target different proteins in the brain, such as N-methyl-d-aspartate receptor (NMDAR), leucine-rich glioma-inactivated protein 1 (LGI1), and glutamic acid decarboxylase 65 (GAD65) (3).
LGI1 is a soluble secreted protein which links Adam22/23 proteins across the synaptic cleft. LGI1 is also abundant in the axon initial segments of in the hippocampus, in particular, in the dentate gyrus (4). Anti-LGI1 autoantibodies have been found in both cerebral spinal fluid and serum of acquired epilepsy patients with symptoms of limbic encephalitis with amnesia and seizures. The specific mechanisms how any autoantibody leads to epileptic seizures is unknown. However, previous studies showed human LGI1 autoantibodies co-precipitated with voltage-gated (VG) potassium channel complexes in rodent tissue (5). Since VG-ion channels help maintain homeostasis in neuronal excitability, we hypothesize that dysregulation of these channels may be triggering LGI1 autoantibody-induced neuropathophysiology. In this study, our aim is to detect a possible mechanism for seizure onset after LGI1 autoantibody exposure. To do this, we are investigating if changes in either the distribution or numbers of VG-ion channels occur when mice are exposed to LGI1 autoantibodies.
Methods:
In mice injected with monoclonal LGI1-antibody into the hippocampus, epileptic seizures were induced, while no symptoms were observed in mice injected with control serum only. Ultrastructural observation of immunolabeled freeze-fractured replicas with colloidal gold antibodies quantified Cav2.1 calcium channels and Kv1.1 potassium channels in axon initial segments and active zones (AZ) of the dentate gyrus granule cell axons (mossy fibers).
Results:
LGI1-autoantibody-injected animals showed that Cav2.1 was slightly reduced in AZ, compared to control animals, while Kv1.1 density was low in control groups. This suggests that neither Cav2.1 and Kv1.1 could be causing mossy fiber hyperactivity at synapses. However, many other VG-ion channels remain that can be investigated using these same methods. Importantly, we observed a significant reduction in synaptic vesicle numbers in mossy fiber boutons in LGI1-autoantibody-injected mice with epilepsy symptoms compared to asymptomatic controls. The synaptic vesicle depletion has implications for presynaptic hyper-excitability and altered neurotransmitter release, and could serve as a marker for affected AZs in epilepsy.
Conclusions:

These results suggest that our mouse model will be useful in investigating if alteration of VG-ion channels trigger hyperexcitability of neurons and contribute to epilepsy in LGI1 autoimmune encephalitis. In the future, these investigations may have useful implications for helping to find triggers of epilepsy with unknown etiologies.

Keywords:

Epilepsy, LGI1, VG-ion-channels, SDS-freeze-fracture-immunolabeling

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Ultrastructural analysis of liver injury and regeneration after microcystin-LR intoxication in whitefish

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Poster Group 2

Microcystin-LR (MC-LR) is a cyanobacterial hepatotoxin that is produced in large quantities during algal blooms. It poses a health threat to aquatic organisms in particular, but also to non-aquatic animals because it contaminates drinking water. MC-LR is an extremely dangerous toxin, as the LD50 for intraperitoneal administration in mice is only 25–150 µg/kg body weight (1). MC-LR acts on mature hepatocytes expressing organic anion transporting polypeptides, which are responsible for the uptake of the toxin, but not on cells of the bile duct system (2). Our previous study has shown that the high dose of MC-LR (100 µg/kg body weight) caused severe injury of hepatocytes at the beginning of the post-exposure period, while the low dose (10 µg/kg body weight) caused less, probably reversible, hepatocytes damage and its effects were observed later in the post-exposure period in whitefish (3). Transmission electron microscopy (TEM) showed that the main targets for the cytotoxic effects of MC-LR were endoplasmic reticulum, cytoskeleton, and chromatin. The high dose of toxin induced massive hepatocyte death within 24–48 hours after administration. Histological examinations showed that the liver regenerated after this damage. Unfortunately, the samples for ultrastructural examinations were not collected during the regeneration phase, so that the details of this process remain unknown. The aim of the present study was to characterize the processes of liver injury and regeneration after single administration of a high dose of MC-LR to whitefish. For TEM-like imaging of large sample areas, we used highly sensitive, high resolution detection of backscattered electrons (BSE) in a field-emission scanning electron microscope (4).
Juvenile whitefish (29.9±1.6 g) were anesthetized in MS-222 solution and received an intraperitoneal injection of MC-LR solution (100 µg/kg body weight) or phosphate buffer saline (control). Randomly selected fish were euthanized by immersion in overdosed MS-222 solution 1, 2, 6, 9 and 14 days after administration of the toxin or saline. Liver samples were immediately fixed in a mixture of 1 % paraformaldehyde and 2.5 % glutaraldehyde in 0.2 M phosphate buffer for 2 hours at 4°C, then washed and post-fixed in 2 % osmium tetroxide for 2 hours. After dehydration, the samples were embedded in Epon 812. Ultrathin sections were cut using the PT3D PowerTome ultramicrotome with ASH2 (Boeckeler Instruments, USA), placed on silicon wafers, and contrasted with uranyl acetate and lead citrate. Sections were imaged using a SenseBSD backscatter electrons detector in SEM Gemini 450 at 1.4 kV, controlled by Atlas 5 software (Carl Zeiss, Germany).
The liver parenchyma of the control fish consisted of polygonal hepatocytes arranged in cords running along the capillary blood vessels. The vascular domains of hepatocytes were covered by very long, irregular microvilli. Hepatocytes were characterized by a euchromatin-rich nucleus, long, parallel cisterns of granular endoplasmic reticulum, a well-developed smooth endoplasmic reticulum and numerous mitochondria, usually with electron-dense matrix. Lysosomes were mostly located close to the lateral domain. The glycogen particles and lipid droplets were infrequently noted. In the samples taken 24 hours after the toxin administration, hepatocytes had lost their polygonal shape and were oval or irregular. Oval cells usually had a low electron density, while irregular cells had a moderate or high electron density. Microvilli were absent both on the vascular domain and on the

cell membrane creating the bile canaliculi. The endoplasmic reticulum was present in the form of large, differently shaped vesicles that filled the entire cytoplasm. The nuclear envelope was usually dilated. Mitochondria remained unchanged in majority of cells. The lipid droplets with different diameters were frequently observed. Some hepatocyte nuclei showed chromatin clamping. The next day, 48 hours after MC-LR administration, the majority of hepatocytes, usually of high or moderate electron density, showed prominent condensation and marginalization of chromatin. Mitochondria in these cells were swollen with electron lucent matrix or condensed with electron dark matrix. Oval, electron lucent necrotic cells and typical, round, electron-dense apoptotic cells were also observed. Some areas of the liver parenchyma contained cell debris. Despite the severe damage of hepatocytes, the capillaries retained their continuity, but cell debris was sometimes found in the blood vessels. Single macrophages were also found. On day 6 after exposure, the liver parenchyma contained numerous phagocytes located between cell debris and remnants of apoptotic cells. The cells of the bile ducts formed short cords with numerous intercellular canaliculi. The apical parts of these cells were filled with keratin filaments. The cords of epithelial cells originating the bile ducts were much more numerous on the day 9 after intoxication. The cells forming the cords were rich in cytoplasm, which contained well-developed rough endoplasmic reticulum and Golgi apparatus as well as numerous myelin-like figures. Mitoses were observed in these cells. The liver parenchyma on day 14 after administration of the toxin consisted mainly of immature hepatocytes with well-developed endoplasmic reticulum, numerous mitochondria and glycogen particles. Hepatocytes were poorly equipped with microvilli, both in the vascular domain and in the bile canaliculi. Like the epithelial cells of the bile ducts, hepatocytes showed an accumulation of keratin filaments under the cell membrane of the biliary pool. Macrophages were observed between hepatocytes.
In conclusion, three phases can be distinguished in the course of changes in liver induced by a single administration of the sublethal dose of MC-LR: damage of hepatocytes, infiltration of macrophages and phagocytosis of cellular debris, and proliferation of progenitor cells located in the bile duct system and their differentiation into hepatocytes. The neighbouring hepatocytes differ in their response to MC-LR and take the pathway to apoptotic death or necrosis. Macrophages remain in the regenerating liver for a long time, suggesting their role in the control of transformation of liver progenitor cells into hepatocytes. Liver regeneration after damage of the majority of hepatocytes occurs via the proliferation and differentiation of progenitor cells located in the bile duct system. Our results have also showed that the highly sensitive detection of BSE is an extremely powerful technique for study of tissue ultrastructure.
Funded by the Minister of Science under the Regional Initiative of Excellence Program and National Science Centre of Poland (2016/21/B/NZ9/03566).

Keywords:

whitefish, toxin, liver, ultrastructure

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This version of the EMC 2024 Abstract Book is without any graphics

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Effect of chloroquine and naringin on the relationship between ER stress and mitochondria in trophoblasts

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Poster Group 1

Background incl. aims
The placenta is an interface that transports nutrients, oxygen, and waste products between the mother and the fetus. Trophoblast cells in the fetal part of the placenta differentiate for the development of a healthy placenta and the continuity of pregnancy and perform functions such as invasion and secretion. It is known that autophagy plays an essential role in these functions. Autophagy is a process responsible for degrading damaged structures to maintain cellular homeostasis. Suppression of autophagy, oxidative stress, and apoptosis in trophoblasts can lead to pathologies such as implantation failures, spontaneous miscarriages, and preeclampsia. Naringin (Nar) is a bioflavonoid with anti-apoptotic, anti-inflammatory, and antioxidant properties (Bharti ve ark., 2014). Previous studies have shown that Nar stimulates ER stress-mediated apoptosis in colon and cervical cancer cells and inhibits mitochondria and ER stress-mediated apoptotic pathways in endothelial cells. This study aims to investigate the effect of Nar on endoplasmic reticulum (ER) stress (Grp78 and PERK), mitochondria dynamics (Fis1 and MFN2), and the ER-mitochondria relationship after hydrogen peroxide (H2O2)-induced oxidative stress and the inhibition of autophagy (chloroquine; CQ) in trophoblast cells.
Methods
IC50 analysis was performed using the data obtained after the CCK8 experiment to determine the most appropriate H2O2, CQ, and Nar doses to be applied to the cells. Seven experimental groups were constructed: Control (C), H2O2, CQ, Nar, H2O2+Nar, CQ+Nar, and H2O2+CQ+Nar. To examine both ER stress and mt dynamic protein levels in cells and the relationship between ER and mitochondria, Grp78 and Fis1 and p-Perk and MFN2 proteins were double labeled by immunofluorescence (IF) method. In addition, apoptosis was evaluated by Caspase-3 protein labeling using IF, and the ER-mitochondria relationship was assessed by electron microscopy.
Results
As a result of IF, while Grp78 expression increased in CQ-treated trophoblasts, no change was observed in Fis1+ mt fission. There was a significant increase in Grp78 and Fis1 protein levels in the Nar-applied groups. Also, the p-Perk level decreased significantly in the CQ+Nar group, while MFN2+ mt fusion increased dramatically in the Nar-applied groups. Moreover, it was observed that the autophagy inhibition in trophoblasts did not show any change in the mt dynamic balance, but Nar had the opposite effect. In addition, Caspase-3 labeling showed a significant increase in apoptosis in the CQ group compared to the C and Nar groups. Nar decreased Caspase-3-mediated apoptosis combined with CQ but was ineffective when administered with H2O2.
Conclusion
ER stress (GRP78) and apoptosis increased by inhibiting autophagy (CQ), but apoptosis decreased after CQ and Nar application, although GRP78 increased. This decrease may be due to mt fission. Increased ER stress may reduce Caspase3-mediated apoptosis by triggering fission in the mitochondria, which is closely related. In addition, Nar applied to trophoblasts with inhibited autophagy caused a severe decrease in the level of p-PERK, an important protein in the ER-mitochondria relationship, and this decrease affected the level of apoptosis. Still, p-PERK did not

show any effect on mt dynamics. In conclusion, it has been observed that ER stress and mt dynamic-induced cell death may occur in pathological conditions due to autophagy in trophoblasts, and Nar may be effective in these mechanisms. The experiments regarding the study are still being conducted; ER stress mechanism and mitophagy affecting mt dynamics will be investigated by immunolabeling ATF6 and IRE1, Pink and Parkin.

Keywords:

Trophoblast, naringin, autophagy, ER, mitochondria

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Understanding field evaporation sequences by in-situ correlative microscopy and simulation

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Poster Group 1

Background
Atom Probe Tomography (APT) provides detailed information on a material's microstructure and its chemical composition. This technique enables access to the 3D position and chemical nature of each atom in a sample. The process involves evaporating the atoms using an electric field and reconstructing a 3D model of a needle-shaped specimen. However, the resolution of APT can be degraded for heterogeneous materials by artifacts potentially associated with the reconstruction methods. Furthermore, the geometric model of the hemisphere on a truncated cone used for APT reconstructions relies on assumptions about variations in the tip curvature along the evaporation sequences. These fluctuations depend on variations in the evaporation field, which are governed by the distribution of areas of higher or lower evaporation field in the sample. This is for instance illustrated in Figure 1.
Tracking the shape of the APT tip during evaporation can provide valuable information about the tip radius, which can help improve the reconstruction algorithm, mapping the electric field around the tip can also indicate areas of higher or lower electric field. In-situ correlative electron microscopy, achieved by performing APT experiments in a STEM can consequently help understanding the evaporation sequences.

Methods
4D-STEM [1] experiments were carried out on a polarized Al-Fe APT tip subjected to 1 kV. A CheeTah Timepix3 pixelated detector was used to record a diffraction pattern for each pixel as the tip was scanned. To mitigate dynamic diffraction effects, a precession angle of 0.7 degrees was used.
Data analysis was performed using the Libertem [2] software. For simulating the field evaporation process, we utilized the Robin-Rolland [3] algorithm that calculates the electric charge on each atom and predicts evaporation events by identifying atoms that reach a critical evaporation field threshold.

Results
Our experiments showed variations in electrostatic fields around a polarized Al-Fe tip, subjected to a voltage of 1 kV. This variation is shown in Figure 2. The analysis shows a constant electrostatic field inside the tip, indicated by the blue color. A high field was observed near the apex of the tip, indicating a high probability of evaporation, confirmed by the results of simulation, represented in Figure 3.

Conclusion
In this study, we have developed an approach that makes it possible to follow the shape of an APT tip during evaporation, by performing APT experiments in a (S)TEM. In situ correlative microscopy was achieved by developing a sample holder capable of applying a voltage of up to 8 kV. In addition, 4D-STEM experiments made it possible to visualize the electrostatic field on a polarized APT tip. These results could help improve the accuracy and 3D reconstruction of atom probe data. The integration

of numerical modeling can be used to predict material behavior during APT analysis and to confirm experimental results.

Keywords:

APT, (S)TEM, In-situ correlative microscopy

Reference:

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FTIR-Imaging of a Tintoretto-Fresco

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Poster Group 1

Background incl. aims
Molecular spectroscopy tools are employed to investigate the vibrational properties of a wide variety of samples. The chemical composition and physical properties of materials are stored in the molecular bonds and can be read from the vibrational spectrum. Fourier transform infrared (FTIR) spectroscopy has long been utilized to investigate materials present in artwork. This study of Tintoretto's work at the Scuola Grande di San Rocco (Venice, Italy) aims to understand the composition of its pictorial layers.
Methods
The cross-section of the Tintoretto painting sample was embedded in resin to assess the five layers. The sample was analyzed in several regions using a Thermo Scientific Nicolet RaptIR FTIR microscope in corroboration with scanning electron microscopy with energy-dispersive x-ray spectroscopy. All mappings were performed in ATR mode with a Germanium micro-ATR.
Results
The cross-section of the painting revealed the existence of a lipidic preparation layer and finishing layer. The preparation layer contains traces of proteins, pointing to the use of animal glue. The sandwiched layers contain different pigments and filler materials. The study confirmed assumptions and knowledge on the use of materials and techniques in the 16th century. In contrast, semiconductor nanostructures are state-of-the-art technology of the 21st century.
Conclusion
All materials that were expected to be found in the paint sample were successfully identified using the Nicolet RaptIR FTIR Microscope, combined with the multicomponent search function of OMNIC Paradigm Software. The power of spectroscopic techniques is pivotal in the creation, conservation, consolidation, and understanding of materials across all scientific fields.

Keywords:

FTIR, Imaging, Art&Conservation, Spectroscopy, ATR

Reference:

[1] Bravo, B.; Izzo, F.C.; Zendri, E.; Balliana, E. „Analysis of a Tintoretto stratigraphy with micro-FTIR spectroscopy," Thermo Fisher Scientific, Application Note:56373, 2023

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Raman-Imaging of the Strain-Distribution in Semiconductors

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Poster Group 1

Background incl. aims
Molecular spectroscopy tools are employed to investigate the vibrational properties of a wide variety of samples. The chemical composition and physical properties of materials are stored in the molecular bonds and can be read from the vibrational spectrum. This poster is dedicated to the influence of a buried stressor on the strain-dependent site control of InGaAs quantum dots. Raman spectroscopy is highly sensitive to subtle changes in the crystal lattice structure. Under strain, its molecular bonds shift slightly, altering the vibrational frequencies of the lattice. The resulting shifts in Raman peaks contain information about the amount and type of strain present.
Methods
The quantum dot sample was analyzed with a Thermo Fisher Scientific DXR3xi Raman imaging microscope and a 532 nm laser. The results are correlated with atomic force microscopy assessments of the sample height profile.
Results
InGaAs quantum dots grow at sites exhibiting a considerably larger strain than their surrounding. Tuning of the buried stressor size results in the precise control of the distribution, emission, and density of the quantum dots. The overlay of Raman strain mappings with atomic force microscopy height profiles allows for a deeper understanding of the physical properties. In combination with computational simulations, the results forecast the design of new devices with distinct emission patterns.
Conclusion
Besides the chemical composition, Raman spectroscopy is powerful in assessing the physical properties such as the strain in semiconductors. The power of spectroscopic techniques is pivotal in the creation, conservation, consolidation, and understanding of materials across all scientific fields.

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[2] Limame, I.; Heisinger, F. et al., “Epitaxial growth and characterization of multi-layer site-controlled InGaAs quantum dots based on the buried stressor method,” Applied Physics Letters, vol. 124, no. 6, Feb. 2024, doi: 10.1063/5.0187074.

Keywords:

Raman, Spectroscopy, Semiconductor, Strain, AFM

Reference:

[1] Ries, M; Heisinger, F., “Using Raman spectroscopy to determine the strain in semiconductor samples,” Thermo Fisher Scientific, Application Note: 1023, 2024
[2] Limame, I.; Heisinger, F. et al., “Epitaxial growth and characterization of multi-layer site-controlled InGaAs quantum dots based on the buried stressor method,” Applied Physics Letters, vol. 124, no. 6, Feb. 2024, doi: 10.1063/5.0187074.

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Towards electron beam shaping in an Ultrafast Transmission Electron Microscope

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Poster Group 2

Background incl. aims
In 1933, Ernst Ruska developed the first Transmission Electron Microscope (TEM) which became the primary tool for studying matter. The picometer De Broglie wavelength and the numerous signals generated by an electron allow us to probe many properties of a sample at the atomic scale. Indeed, by analyzing the amplitude, phase, deflected angle and the energy of the electron beam after its interaction with the sample we can fully reconstruct its chemical composition or its magnetic/electric properties. However, unlike conventional optics, where the amplitude, phase and polarization of a light beam can be fully controlled using e.g. a Spatial Light Modulator (SLM), the electron beam cannot be easily manipulated in a TEM, making electrons insensitive to certain fundamental properties such as chirality. The development of Ultrafast Transmission Electron Microscopes (UTEM), whose working principle is illustrated on figure 1, has opened new possibilities. In 2020 it has been proposed to shape the electron beam through inelastic electron-light interactions in Photon-Induced Near field Electron Microscopy (PINEM) experiments [1] as sketched in figure 1. The PINEM interaction, based on a pump-probe experiment, relies on energy exchanges between an ultrashort electron pulse and a strong optical near-field induced on a sample by a femtosecond laser pulse. One of the main properties of this interaction is to imprint the laser beam phase on the wavefunction describing the electron after the interaction. Two years later, a first practical demonstration [2] illustrated the capability of this technique to generate Gaussian/Hermite-Gaussian electron beams.
The aim of our work is to develop an experimental set up for electron beam arbitrary shaping on a brand-new UTEM developed at CEMES [3]. This new 300 keV UTEM based on a modified Cold Field-Emission Electron Gun (CFEG) has been equipped with two sample stages that will enable us to use the shaped electron beam to probe a sample of interest.
Methods
This work is divided in three mains parts. First, we will develop the optical set up to spatially shape the laser beam by means of a Spatial Light Modulator (SLM) and fully characterize (amplitude and phase) the laser beam before its injection into the Ultrafast TEM (UTEM). In order to use the full potential of the SLM, we will implement various phase retrieval algorithms depending on the needs i.e. control over the intensity/phase distribution or both. In the second part of the project, we will perform Electron Energy Gain Spectroscopy (EEGS) experiments on our UTEM with a standard spatial shape of a laser beam i.e. a gaussian beam. The last step is to add the laser beam shaper set up in order to use the properties of the inelastic electron-light interaction in PINEM experiments to shape the electron beam.
Results
For the time being, the optical set up already enables us to generate any desired spatial distribution of a laser beam such as vortex, Laguerre-Gaussian beams or more generally any intensity and phase distribution – characterized thanks to a Mach Zehnder interferometer - in excellent agreement with the numerical simulations. Moreover, our new 300 keV UTEM based on a modified CFEG is operational and allows us to perform PINEM experiments. The electron beam shaping experiments

are currently under active development. In parallel, we have developed numerical simulations which combine diffraction theory and electrodynamical simulations using the Green Dyadic Method (pyGDM (python package) [4]) to anticipate our future experiments. These calculations allow us to compute the electron wave function after its interaction with the shaped laser beam and its subsequent propagation in the TEM column.

Conclusion

The double sample stage available on our UTEM will allow us to use the properties of the inelastic electron-light interaction in PINEM experiments to shape the electron beam on the first sample stage in order to probe a sample of interest on the second stage. This poster presentation will mainly be focused on the optical part of the experiment i.e. the generation of shaped laser beams with arbitrary amplitude and phase distributions and also on the numerical simulations of the electron beam shaping experiments that will be realized on our brand new UTEM.

Keywords:

UTEM
Electron-beam shaping
PINEM
SLM

Reference:

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Deconvolution of SBF-SEM images improves quality of data in volume electron microscopy

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Introduction
The serial block face scanning electron microscopy (SBF-SEM) is a state-of-the-art method for volume imaging of biological objects. Despite many advantages, it suffers from an unavoidable trade-off between acquisition time and image quality: faster acquisition gives noisier, and consequently less resolved images. This problem is known in light microscopy and solved there by applying different image processing methods such as denoising and deconvolution with classical and AI algorithms. We transfer this concept to electron microscopy by designing SBF-SEM point spread function (PSF) and using it for deconvolving the images with the aim of getting less noisy images with higher resolution.
Methods
First, we model 3D SBF-SEM PSF with Monte Carlo simulations of the electron penetration and scattering in an EPON resin with CASINO software for beam energies ranging from 1kV to 15kV. Second, we image the sample with FEI Quanta 250 FEG scanning electron microscope equipped with a 3View2.XP in situ ultramicrotome using the beam energy within simulated values. Finally, we deconvolve acquired images with Huygens Professional software (SVI, the Netherlands) using simulated PSF and custom-made script with 3D CMLE algorithm and corresponding rescaling of SBF-SEM PSF to light microscopy range.
Results
The simulated SBF-SEM PSF has a characteristic pear-shaped form with additional difference to light microscopy, that it only exists in the lower half-space due to the nature of electron scattering in the sample. We applied simulated SBF-SEM PSF in deconvolution of biological specimens. Deconvolved images have higher quality and consequently reveal finer structures, for example allowing for an improved definition of z-lines in skeletal muscle of a mouse, see picture. As the quality of the image is significantly improved after deconvolution, developed methodology can be applied for improving images with lower beam energy and higher imaging speed.
Conclusions
Transfer of the 3D deconvolution concept from light microscopy to SBF-SEM allows for improvement in resolution and speed of imaging in volume electron microscopy in life sciences.

Keywords:

SBF-SEM, deconvolution, PSF

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In Situ SEM at Elevated Temperature for Materials Science

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Poster Group 2

Understanding the relationships between microstructure and properties under various conditions is essential for further material development. SEM is suitable for studying not only microstructure but also changes in these materials under different and variable conditions. In the case of elevated temperatures, phenomena associated with diffusion, phase transformations, grain boundaries and precipitation are particularly significant in materials science [1]. Several limitations of in situ experiments in SEM are related to the vacuum environment, which acts as an electrical and thermal insulator. The thermal insulating property of vacuum reduces the risk of damage to microscope components during heating experiments but also complicates sample heating and cooling and temperature measurement. Vacuum prevents both sample oxidation and equipment oxidation during experiments at high temperatures. The development of specific detectors has been necessary to carry out in situ studies under extreme conditions, such as high pressure and temperature [2]. The aim of these experiments was to verify the possibilities of observing phenomena characteristic of materials science using in situ SEM at elevated temperatures. In addition to the phenomena themselves, the aim was to verify the visualization of structure by thermal etching [3]. Pilot experiments were conducted, including melting of the AlMgSi1 alloy, dissolution of secondary phases and visualization of primary austenite grains of TRIP steel, dissolution of intermetallic phases and melting of the AlSi9Cu3(Fe) alloy, phase transformation of the Ti6Al4V alloy, precipitation of phases during artificial aging of CuBe2 and AlMgSi0.5 alloys, and thermal etching of ZrO_2 ceramic for grain visualization.
The samples underwent a complete conventional metallographic procedure, including cutting on the precision metallographic cutter, followed by mounting in resin, and successive grinding and polishing steps. Grinding was performed using SiC papers with grit sizes of 220, 500, 1200, 2000, and 4000, while polishing utilized diamond pastes with abrasive sizes of 3 µm, 1 µm, and 0.25 µm, concluding with chemical-mechanical polishing using the colloidal suspension with SiO_2 particles. Before heating, the samples were chemically or color etched to reveal the real structure. Some samples underwent imaging in the SEM using the LOM Zeiss Axio Observer 7 before heating for correlative imaging. In situ heating experiments took place in the SEM Thermofisher Scientific Quattro S, utilizing the Newtec Scientific FurnaSEM 1300 stage for heating up to 1200 °C. The sample was restrained to a 12 mm diameter by a platinum pad, ensuring uniform heating. To enhance thermal conductivity and dissipate charge, samples were affixed to the platinum pad using a carbon suspension. Subsequently, a thermal shield with a 4 mm diameter aperture was installed above the sample to mitigate thermal stress on the microscope chamber components. In addition to the ETD detector, samples were also imaged during heating using the BSE detector Crytur Karmen, equipped for in situ experiments at elevated temperatures. The scintillation crystal of the Karmen detector is coated with a thin layer of aluminum, 100 nm thick, designed to prevent penetration of external light signals into the photomultiplier, such as light emitted by the heated sample [4].
During the melting of the AlMgSi1 alloy, an Al_2O_3 pad was inserted between the sample and the platinum pad. Upon reaching a temperature of 700 °C, microdendrites were observed at the edges of

the sample, indicating localized melting. However, this temperature is approximately 150 °C higher than the theoretical melting temperature of the alloy. In the case of TRIP steel, reaching a heating temperature of 900 °C made the boundaries of primary austenite grains visible. With prolonged exposure at this temperature, secondary phases began to dissolve, gradually increasing the contrast of the grains. Further experiments were carried out using a carbon suspension, and it was verified with an optical pyrometer that the heating temperature and the sample surface temperature could differ by approximately 50 °C. For the AlSi9Cu3(Fe) alloy, at a heating temperature of 550 °C, the dissolution of $CuAl_2$ was observed. During exposure at a heating temperature of 900 °C for the Ti6Al4V alloy, it was possible to observe the transformation of the globular α phase with an HCP lattice to the β phase with a BCC lattice. While a transformation to the α phase was expected during cooling, there was no observed change in morphology. Artificial aging of the CuBe2 alloy occurred at a temperature of 315 °C (Fig. 1). Precipitation of the γ phase likely began before reaching the selected temperature. During exposure, the precipitated particles slightly increased in size. Apart from precipitation, the contrast at the boundaries of some grains began to increase, resulting in their expansion. In the case of artificial aging of the AlMgSi0.5 alloy at a heating temperature of 180 °C, no structural changes were observed. The feasibility of in situ thermal etching was confirmed on ZrO_2 ceramic. The sample was imaged at room temperature and then continuously during exposure to heat. During heating, unstable behavior was observed due to sample charging, the evaporation of water from the carbon suspension used to adhere it to the platinum pad, and thermal expansion. Upon reaching a heating temperature of 750 °C, the contrast of grains in both detectors began to increase slightly. There was no sample charging. Already at a heating temperature of 900 °C, the contrast of grains substantially increased, remaining largely unchanged throughout the exposure period for both detectors.

The in situ SEM experiments at elevated temperatures were successfully conducted, demonstrating the potential applications of this method in materials science. During the experiments, partial insights were gained, and practical procedures were developed, leading to greater validity of the results. The window for pyrometer measurements allowed for further refinement of surface temperature measurements. Direct temperature measurement on the sample may lead to further precision. The carbon suspension for high temperatures improved heat conduction and charge dissipation from the sample. Under specific conditions, samples can also be melted. Thanks to special metallographic preparation, it was possible to observe the real structure already at room temperature; however, further experiments are needed to verify the introduction of artifacts from the preparation process.

Keywords:

in-situ SEM, correlative-microscopy, heat-treatment, thermal-etching

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Quantitative Super-Resolution Methods for Cryo-CLEM

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Poster Group 2

Background
Cryogenic ultrastructural imaging techniques such as cryo-electron tomography have produced a revolution in how the structures of biological systems are investigated. This has been done by enabling the determination of structures of protein complexes immersed in a complex biological matrix within vitrified cells and model organisms. However, so far successes have mostly been limited to highly abundant complexes or to structures that are relatively unambiguous and easy to identify through electron microscopy.

To realize the full potential of this revolution, we need to be able to pinpoint lower abundance species and obtain functional annotations on the state of objects of interest. This would then be correlated to ultrastructural information to build a complete picture of the structure-function relationships underpinning biological processes. Fluorescence imaging at cryogenic conditions has the potential to meet these demands. However, wide-field images acquired at low numeric aperture (NA) using an air immersion objective have a low resolving power and cannot provide accurate enough three-dimensional (3D) localization to enable the assignment of functional annotations to individual objects of interest or target sample debulking to ensure the preservation of the structures of interest. It is therefore necessary to develop super-resolved cryo-fluorescence workflows capable of fulfilling this role and enabling new biological discoveries.

Here, we will present the current state of our development of super-resolution solid immersion lens stochastic optical reconstruction microscopy (superSIL-STORM) [1]. The superSIL lens can go to larger magnifications than regular air objective lenses, with the advantage over traditional oil immersion lenses that they are cryo compatible. We will show the application of superSIL-STORM to various protein clusters in E. coli cells and discuss the advantages and limitations of the technique. We will then further discuss its future applicability to cryogenic correlative light and electron microscopy (cryo-CLEM) workflows.

Methods
Our SIL assemblies were made in-house using nanolenses (Hyper Hemispheric Ball lens, Cubic Zirconia, 1.0 mm diameter x 0.73 mm thickness, λ\4 flatness - Knight Optical, LBB2018-C), stainless steel foil (FE22-FL-000120) and cryo-compatible adhesive (Loctite Stycast, 2850 FT) [1,2].

We used 100 nm TetraSpeckTM microspheres as fiducial markers. For plunge-freezing of the samples we used an FEI Vitrobot Mark V, using manual blotting. SuperSIL assemblies were glow discharged before use. A 1% PEI solution was applied to the substrates before adding the cells and then the fiducial markers.

For fluorescence imaging an in-house built super-resolution cryo-STORM setup was used, as described in [1]. STORM analysis was conducted using standard STORM software: the preinstalled ImageJ plugin for ThunderSTORM, the source code from https://github.com/ZhuangLab/storm-analysis for DAOSTORM. For ThunderSTORM, an initial Lowered Gaussian filter was applied before a maximum likelihood Gaussian fit searched for the single molecule PSFs. For DAOSTORM the value for "model" was set to "2D". Drift correction of the sample was done through in-house constructed algorithms [1].

Results
To demonstrate the capabilities and limitations of the superSIL-STORM technique, we study two different protein clusters in E. coli cells.

We assess the ability of single-colour superSIL-STORM to analyse protein clustering in VNp-LZ-mNeonGreen expressing E. coli. VNp-LZ is a short recombinant peptide which is introduced in bacteria to favour the formation of internal vesicles and has been shown by EM to cluster around them. Our superSIL-STORM analysis reveals clustering of the protein around empty spaces within the cells, which are likely to correspond to the internal vesicles shown previously [3]. Through further data analysis, we estimate most of the protein clusters in 100 nm large patches, with an overall distribution of nearest neighbours ranging between 25 nm - 200 nm. Future studies combining these results with EM work for cryo-CLEM will help to reveal the nature of these clusters.

Next, we assess proteins in E. coli cells that are involved in the outer membrane protein (OMP) secretion pathway: the BAM complex. Various mechanisms for how the BAM complex translocates proteins across the outer membrane have been suggested before, but more information is needed for a complete understanding of how this is done. We look at clustering of the major BAM complex subunit BamA with either the periplasmic chaperone SurA or the client OmpA. Overall, we find strong co-clustering of BamA with OmpA, but not of BamA with SurA. The first indicates an agreement with previous findings that OMPs are turned over in patches, away from the original OMP insertion sites. The latter indicates that the co-location of BamA with the chaperone is transient in nature and may depend on a variety of factors, including the metabolic state of the imaged cell.

Conclusions
We have developed workflows for the use of superSIL-STORM. We present here the basic principles of this technique and we show its application to study membrane protein complexes in E. coli. Further work is taking place to integrate this technique in cryo-CLEM workflows.

Keywords:

STORM, superSIL, membrane proteins, cryo-CLEM

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Characterization of a multiphase nucleus of spheroidal graphite cast irons by transmission electron microscopy techniques

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Poster Group 2

Background incl. aims
Spheroidal graphite cast irons are obtained by submitting a cast iron melt to a spheroidizing treatment with a Fe-Si-Mg alloy, followed by so-called post inoculation with a Fe-Si alloy. These additions contain other elements which are mainly strong deoxidizers and/or desulfurizers. It is well established that these two treatments interact to give the final particles acting as graphite nuclei [1]. Accordingly, many of the observations made on graphite nuclei reported the presence of oxides, sulfides and/or oxysulfides [1], though nitrides have been reported from times to times [2]. The fact that the nuclei appear multiphase in most of the reported examples complicates the analysis of the sequence of phase transformations leading to the nuclei. Microstructural, chemical and crystallographic characterization on a multiphase nucleus of a spheroidal graphite in a cast iron manufactured by thin-wall casting is the aim of this study. To describe many phases constituting it, scanning transmission electron microscopy (STEM) coupled with energy dispersive X-ray spectroscopy (EDS) and electron diffraction analyses are carried out. The nucleus contains two parts, one multi-phase rounded "head" encapsulated in an Al-Mg-Si nitride and an elongated "tail" consisting of the same nitride. The central part of the head seems to be related to the inoculant added to the cast iron melt. From this, the precipitation process leading to the final graphite nucleus is inferred.

Methods
A cast iron, i.e. mainly a Fe-C-Si alloy (final composition: 3.8 C, 1.9 Si, bal. Fe, wt.%) was spheroidized with a Fe-Si-Mg alloy. A commercial Fe-Si inoculant was added to the melt at the time of pouring in a thin-wall casting. Because of the high cooling rate, the material contained metastable carbides which were eliminated by a heat-treatment at 950 °C for 15 minutes.
A sample cut perpendicularly to the casting surface was prepared by standard metallographic methods. The microstructure consisted in graphite nodules within an iron-rich matrix. Focused ion beam (FIB)-lift out technique was used to prepare a thin foil of a selected nodule in a FEI NanoLab HELIOS 600i FIB/SEM. A transmission electron microscope JEOL cold-FEG JEM-ARM200F equipped with a probe Cs corrector and a SDD CENTURIO-X EDS detector was used for characterization. Selected area electron diffraction (SAED) and nano-beam diffraction (NBD) were employed to record diffraction patterns.

Results
Bright field (BF) STEM image of a thin diametric section of a graphite nodule prepared by FIB shows the presence of a nucleus in the middle of the nodule. This nucleus appears composed of two parts: a round head loosely connected to an elongated tail. High-angle annular dark-field (HAADF) STEM

images evidence a grey contrast of both parts, with bright areas in the head which are indicative of the presence of at least one element much heavier than carbon in the nucleus. Also, the head presents different grey contrasts indicating a multi-phase nature with a fan-like faceted precipitate. To determine the chemical nature associated with these various contrasts, STEM-EDS mapping was carried out. The elongated tail having an almost uniform grey contrast appears mostly composed of Al, Mg, Si and N, with however a few isolated small bright spots rich in iron. In contrast, the rounded head exhibited at least four different phases: 1) an inner facetted center rich in Fe; 2) a shell rich in S and Mg surrounding the center; 3) the fan-like precipitate mainly composed of Ti and developing outwards from this shell; and 4) a structure having the same composition as the elongated tail. Finally, SAED and NBD were performed on different parts of the nucleus after appropriate rotation of the sample for each of the phases. The Al-Mg-Si-N phase present in both the elongated tail and the outer area of the head has been indexed as trigonal nitride $A_2Mg_5Si_5N_{12}$ according to Solberg and Onsoien [3]. Concerning the other phases in the rounded head, the center could be indexed as bcc-Fe [4] and the fan-like precipitate as cubic carbo-nitride $Ti_2(C,N)$ [5]. Unfortunately, the shell rich in S and Mg could hardly be characterized.
Observing the spatial distribution of the phases and considering their chemical composition and crystallographic structures, we may suppose a formation sequence of the nucleus as follows: bcc-Fe is the center of nucleus on which a shell rich in S and Mg precipitates; then, the Ti carbo-nitride fan-like structure develops from this shell; and finally (Al,Mg,Si) nitride precipitates from all around this carbo-nitride and appears to be the actual substrate for graphite nucleation. It is conceivable that the tail developed together with the graphite nodule during the graphitizing heat-treatment.

Conclusion
Transmission and analytical electron microscopy allowed determining the multi-phase nature of a graphite nucleus in a spheroidal graphite cast irons. This nucleus was composed of two different parts:

- A rounded multi-phase head organized as follows: 1) an inner facetted center rich in bcc-Fe; 2) a shell rich in S and Mg surrounding this center; 3) then a fan-like fcc Ti carbo-nitride developing from this shell; and finally 4) an external trigonal nitride rich in Al, Mg and Si around the aggregate.
- An elongated tail having the same composition as the outer nitride in the head.

In the literature, it is generally considered that the inoculant fully dissolves in the cast iron melt to lead to precipitation of oxides, sulfides and nitrides. In contrast to previous studies, this study shows that the center of the rounded head which was indexed as bcc-Fe could be a remaining of the Fe-Si inoculant.

Keywords:

Electron-diffraction spheroidal-graphite cast-iron nucleus STEM-EDS

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Biorestoration Potential of Carbonatogenic Bacteria on Lithotypes: SEM and AFM characterization

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Poster Group 2

Background incl. aims.

Microbially-induced calcium carbonate precipitation (MICCP), a bio-geochemical process, is a promising technology for eco-friendly applications particularly in the restoration of ornamental limestones.
This study explores the efficacy of the biorestoration treatment using Lysinbacillus fusiformis 3.20 strain as a carrier for the MICCP on three different lithotypes: calcarenite (MC), travertine (TR), and marble (MA). Despite sharing similar mineral compositions, these lithologies originate from different depositional settings, resulting in different petrophysical properties and microstructural features. To understand the biomineralization of the three different lithotypes the surface alterations and nanostructural changes were characterized by SEM (Scanning Electron Microscopy) and AFM (Atomic Force Microscopy).

Methods.

Small bricks of the three lithologies were treated spraying the bacterium culture twice a day for seven days at 28°C. Nanocaracterization of the surfaces of the three lithotypes was performed pre- and post-treatment using the Zeiss Auriga (SEM) operating at 1.5 keV and Bruker's Dimension Icon (AFM) using RTESPA-300 probes in tapping mode.

Results.

SEM micrographs revealed significant differences between the untreated and treated samples, particularly on the surfaces of the MC and TR stones. The treated samples showed the presence of calcified bacterial cells and newly formed crystals. In the MC samples, the crystals presented rhombohedral shapes, while the TR samples showed less homogenous aggregates. However, the treatment showed no significant effect on the MA samples, as no visible crystals or bacterial cells were observed after treatment. AFM analysis confirmed these results, showing the presence of bacteria and new crystals on the MC and TR samples post-treatment, with the bacterium being able to deposit calcium carbonate on the pore edges and covering the surfaces.

Conclusion.

The results obtained in controlled laboratory conditions emphasize that for the same treatment, the morphological variations of the bioprecipitation changes are strongly dependent on the lithology. Thus, for a future in situ application of this technique for stone conservation and restoration, the treatment will be optimized depending taking into account the differences of the lithologies.

Keywords:

Biorestoration, SEM, AFM, biomineralization

Reference:

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Numerical study on high orbital angular momentum vortex electron beams in hafnium dioxide

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Poster Group 2

Background incl. aims
Electron vortex beams (EVB) are electron beams that have a quantized orbital angular momentum (OAM) along their propagation axis. These beams have found applications in electron magnetic circular dichroism (EMCD) and beam focusing, and have attracted new attention with the development of programmable phase plates [1, 2]. In this study we aim to improve our understanding of the behavior of vortex beams and their OAM in complex electronic materials.
Methods
We present a numerical study of vortex electron beams modeled as Bessel beams [3, 4]. The commonly used multislice algorithm is utilized to propagate the beam through crystalline material.
Results
We compare our results with previous work [3, 4] for OAM with quantum number l=1 and make predictions for higher angular momentum beams in new materials. The results indicate a reduction in the expectation value of the OAM with propagation depth inside the crystal potential, which depends on the initial value of l. We present simulation results for HfO_2 systematically investigating different beam shaping parameters. Furthermore, we investigate the numerical limits of our method.
Conclusion
Our method is capable of reproducing previously published work for low OAM. It shows physically conclusive results for higher angular momentum vortex beams.

Keywords:

electron vortex beam; multislice simulation

Reference:

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Free electron spectroscopy seen through the prism of quantum optics

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Poster Group 2

Background incl. aims
Photon Induced Near-field Electron Microscopy (PINEM) denotes the modulations of free electrons by a sample – typically an optical cavity – pumped by a laser source. This effect has been theoretically predicted and experimentally demonstrated in an ultrafast transmission electron microscope (UTEM) more than a decade ago [1]. Following these seminal developments, PINEM has been used to reconstruct the temporal dynamics of nano-optical systems with an attosecond temporal resolution [2].
Due to recent experimental developments in integrated photonics [3], the problem of PINEM in the low occupation regime – i.e. when the cavity is populated by a weak number of photons - has drawn an increasing theoretical interest. Indeed, in this situation, the classical description of the electromagnetic field falls short to describe the electron-cavity interaction, and a full quantum description becomes required. Several works have already pioneered this problem [4,5] and predicted strong quantum mechanical effects in PINEM. The goal of our work is to pursue this effort and provide an alternative and intuitive approach to this problem by using tools borrowed from the field of quantum optics.
Methods
The goal of our study is thus to determine the electron-cavity state before and after inelastic interaction. To do so, following a standard quantum optics description, we describe the cavity state as a quantum superposition of number (or Fock) states. We then apply perturbation theory to calculate the electron Wigner function after interaction with the cavity state. The latter is the central object of our theory and represents - roughly speaking - a probability distribution of the electron in the phase space (energy-time). It constitutes a powerful visualization tool enabling us to understand the mechanisms of electron-light interaction at the quantum scale, as shown on figure 1.
Results
In this contribution, we will introduce our approach and demonstrate its efficiency on several problems.
In a first part, we will illustrate the influence of the cavity quantum optical state on the energy and temporal structure of the electron beam during the PINEM interaction. For example, after interaction with a number state – i.e. when the initial number of photons is perfectly known – we will show that the electron-cavity coupled state becomes a coherent superposition of entangled (electron-photon) energy-number states. On the other hand, if the electron interacts with a coherent state – i.e. a Poissonian superposition of number states – one would only obtain correlations between the electron energy states and the photon number states.
In a second part, we will show that a PINEM measurement in the quantum regime is deeply connected to the standard quantum limit to the temporal resolution in a UTEM. Indeed, the Heinsberg uncertainty principle governs the inelastic interaction between the electron and the cavity, making impossible to exactly know the number of photons and the temporal dynamics of the field in the cavity at the same time. We will show that this uncertainty relation has a dramatic influence on the quantum path interferences otherwise observed in the classical regime of PINEM. In that regard,

the Wigner function appears to be an ideal tool to visualize the effect of the uncertainty principle in phase-space.
In a third part, we will tackle the question of the quantum to classical transition in free electron scattering and show that the Wigner function elegantly highlight the transition between the stimulated and spontaneous scattering regimes in electron energy-loss spectroscopy (EELS).
Finally, in a fourth part, we will introduce to PINEM a central basis of quantum optics: the displaced number state (DNS). This basis will allow us to shade a new light on the interaction between electron and a coherent state of light. In particular, or calculation demonstrate that in this configuration, the final electron-cavity state is a coherent superposition of DNS entangled with similar electron states than the one predicted by the classical model. This creates a bridge between the classical and the quantum description of PINEM.
Conclusion
In this work, we present a complete phases-space description of inelastic fast electron-light interaction, in which the central object – the Wigner function – provides a powerful visualization tool. Using this approach, we tackle important problems in the field of EELS and PINEM, such as the quantum to classical transition, or the distinction between stimulated and spontaneous processes. In that respect, our work demonstrates the central role played by the number-phase uncertainty relation. Beyond these fundamental questions, our approach will find application in the description of future experiments in the field of EELS and PINEM, such as the probing of non-linearity in EELS or the realization of atom-cavity coupling in a TEM.

Keywords:

UTEM
Theory
Quantum-Optics
Light-matter interaction

Reference:

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Correlation between manufacturing parameters, properties and microstructure in 2D-C/SiC composites

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Poster Group 1

Background incl. aims
C/SiC composites are non-oxide Ceramic Matrix Composites (CMCs) highly sought-after for high-temperature applications in the aerospace industry. These composites consist of a silicon carbide matrix and carbon fibers, which contribute to their excellent mechanical performances.
Although there is common awareness of the influence of raw materials selection - as well as of manufacturing and processing methods - on the thermo-mechanical properties of C/SiC composites, the impact of manufacturing parameters on microstructures is not widely documented in the literature. This is particularly true for C/SiCs produced by means of Liquid Silicon Infiltration (LSI).
Additionally, for many aerospace applications, exposure to oxidative environments is often involved, which can cause material recession due to the rapid oxidation of carbon. Such oxidation can determine a change in the microstructure and a consequent degradation of the mechanical properties. An in-depth knowledge of the microstructure of such composites can help foresee their evolution in oxidative environments and therefore their potential failure in use.
This study focuses on IsiComp® and OxyComp®, two C/SiC composites produced through LSI. Due to the selection of processing parameters, the two composites show different microstructures. IsiComp® has a laminate structure, in which fiber bundles are wrapped by the SiC matrix. In OxyComp® the SiC matrix penetrates within the fiber bundles and the ceramic matrix is highly cross-linked, almost resembling the microstructure of a pure ceramic. Despite its peculiar microstructure, OxyComp® has good mechanical properties and shows a pseudo-ductile behavior under stress, which is unusual, considering that ceramics retain a brittle behavior under mechanical stress.
In this frame, this research aims to quantify microstructural changes due to varied manufacturing parameters and their impact on microstructures, and therefore, on the thermo-mechanical properties of the composites. Another aim is to delineate the property and microstructure evolution during oxidation in aerospace-like environments - including vapor-rich combustion-type atmospheres. Understanding these relationships is crucial to predicting the behavior of C/SiCs under operating conditions and selecting the most suitable material for certain applications.
Methods
Samples are produced using vacuum bagging techniques, then pyrolyzed and converted into a CMC via LSI. Physical, thermal, and mechanical properties (bending) were tested before and after exposure to oxidative environments according to standard procedures.
Oxidation experiments were conducted in a vertical furnace equipped with a water vapor-gas mixing system and a Quadrupole Mass Spectrometer (QMS) for the in-situ analysis. The structural evolution is quantitatively described using 2D electron microscopy images and 3D images from X-ray tomography. Image analysis is conducted using quasi-automated tools provided by Fiji and Wolfram Mathematica.
Results

As for IsiComp®, the molding pressure strongly affects the size of residual pores in the pyrolyzed preform and the thickness of the ceramic channels in the CMC. The higher the molding pressure, the higher is number of thin (typically less than 30 µm) vertical fractures in the pyrolyzed preform, and no large pores are observed. This enhances silicon infiltration, leading to a homogeneous LSI microstructure. Circular isolated pores resulting from gas generated during the resin polymerization can be eliminated by adjusting the curing cycle parameters.
Surprisingly enough, despite processing parameters having a strong impact on the texture of the pyrolyzed preform, no major effect can be detected in terms of thermal and mechanical properties of the CMC after LSI.
Under oxidative conditions, ISiComp® performs similarly to 2D-C/SiCs described in the literature.
As for OxyComp®, the fiber-to-matrix ratio within pyrolyzed preforms can be tuned by changing certain processing parameters, which eventually affect the pervasiveness of silicon penetration during LSI. The size distribution of SiSiC channels in the CMC reflects the intensity of silicon penetration within fiber bundles. The higher the fraction of thin channels (<7.5 µm-thick), the greater the residual deformation under a fixed mechanical stress. Thermal properties are less affected by the extent of silicon penetration within fiber bundles, as they are more influenced by the continuity of ceramic matrix in a specific direction.
Thanks to its microstructure OxyComp® performs very well under oxidative conditions.
For both composites, it was observed that prolonged exposure at relatively low temperatures causes intra-bundle fiber oxidation, which undermines both material integrity and mechanical properties more strongly than short-term exposure at higher temperatures does.
Conclusion
This study investigates the relationship among manufacturing/processing parameters, microstructures, and thermo-mechanical properties of IsiComp and OxyComp, two C/SiC composites developed by Petroceramics S.p.A.
The manufacturing pressure significantly influences the microstructure of ISiComp®, but no major effects were observed in terms of mechanical and thermal properties measured. It can be supposed that the observed differences in the pyrolyzed preform are attenuated in the CMC, which indicates the final material is highly reproducible despite any difference in the processing parameters.
Conversely, variations of the microstructure of OxyComp® strongly affect its mechanical behavior (bending) but have a weaker impact on the thermal conductivity. The effect of differences in the texture of OxyCOmp® on the oxidation resistance is still under investigation.
The microstructural description derived from 2D-SEM images is consistent with the one obtained from 3D X-ray tomography images.
The pervasive presence of SiC matrix within the fiber bundle makes OxyComp® better oxidation-resistant than ISiComp®, both in air and in a H2O-vapor-saturated atmosphere.
OxyComp shows superior oxidation resistance compared to IsiComp, mostly due to thin intra-bundle channels which slow down the oxidation progression. For both composites, it was observed that prolonged oxidation at relatively low temperatures has a major impact on the mechanical properties of the composite in use.

Keywords:

CMCs, microstructural description, thermo-mechanical properties

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Analytical TEM of materials for gas sensing

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Poster Group 2

Background incl. aims
Metal-oxide semiconductor (MOX) layers are frequently used as gas sensors since they can detect different types of gases, such as CO, CO2, CH4, NO2, etc. and also due to their increased sensitivity, quick response times and reduced fabrication costs. The main challenge for this type of materials is to improve their selectivity [1].
Morphology and structure play an important role during the interaction between the target gas and the sensing layer since porosity, grain size, grain faceting, crystal phase and elemental composition can influence the sensor signal. All these properties depend on the synthesis route.
Our study is focused on the synthesis and determination of the structure, morphology and electrical properties of different MOX systems, such as NiO (p-type semiconductor), SnO2 and Gd2O3 (n-type semiconductors).
Methods
NiO was synthesized by hydrothermal route followed by calcination at 400 °C and 500 °C [2,3], while SnO2 (doped with different concentrations of Gd: 5%, 10% and 20%) and pure Gd2O3 were synthesized by co-precipitation [4].
The morphology and the structure of these samples were investigated using analytical TEM techniques: CTEM, SAED, HRTEM and STEM-EDS. These results were correlated with X-ray Diffraction (XRD), Electron Paramagnetic Resonance Spectroscopy (EPR) and X-ray Photoelectron Spectroscopy (XPS) data as well as with the electrical response of the fully characterized materials to CO and CO2 target gases.
Results
p-type semiconductors (NiO)
Increasing the calcination temperature induced visible morphological changes, as suggested by XRD patterns and observed by TEM imaging. One notable difference consists in the fact that the calcination temperature increased the nanoparticle size from ~9 nm to ~20 nm, with the formation of intragrain structural defects (e.g. twin boundaries), as revealed by HRTEM imaging (see graphic, [2]).
The electric response was tested in the CO atmosphere. Considering the observed morphological differences, a decrease in the sensor response was expected, but the opposite effect was observed [2].
To understand this phenomenon, an in-depth analysis was performed, using spectroscopic techniques: STEM-EDS, EPR and XPS. The EPR investigations revealed the formation of metallic Ni clusters in the sample calcined at 500 °C and the analytical TEM techniques (SAED, CTEM and STEM-EDS) confirmed their presence. The formation of these metallic clusters, corroborated with the morpho-structural properties, could explain the different sensing properties of the samples calcined at different temperatures [3].
n-type semiconductors (SnO2 doped with Gd, Gd2O3)

The increase in the dopant concentration had a notable effect on the morphology and the structure of the samples. The TEM analyses correlated with the Rietveld refinement of XRD patterns revealed the formation of SnO2-Gd2O3 nanocomposites. The presence of the two crystallographic phases was analyzed using different analytical TEM techniques: CTEM, SAED and STEM-EDS, which confirmed the formation of the cubic Gd2O3 secondary phase. The electric response was tested in the CO2 atmosphere, at different values of relative humidity, to determine the sensing mechanism of the SnO2-Gd2O3 nanocomposites [4].

Conclusions

Analytical TEM techniques correlated with XRD, EPR, XPS and electrical measurements offered valuable information for understanding the sensing mechanism of metal-oxide semiconductor materials in the presence of different gases, i.e. NiO in the presence of CO and SnO2/Gd2O3 exposed to CO2.

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Keywords:

Analytical TEM
Metal-oxide semiconductors
Gas-sensing

Reference:

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Three-dimensional ultrastructure of ovine pinealocytes

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Poster Group 2

The pineal gland via secretion of melatonin plays an important role in the regulation of processes that occur in the daily and seasonal rhythms. Significant differences between species have been described in ultrastructure of the pineal gland in mammals. Mammalian pinealocytes differ mainly in the structure of endoplasmic reticulum and mitochondria as well as in the presence of clear and granular vesicles, lipid droplets, vacuoles, dense bodies and synaptic ribbons. In addition, depending on the species, both capillary vessels with a continuous wall and those with pores were found. The organization of the pineal parenchyma is very complex due to the presence of numerous cell processes. Based on classical two-dimensional transmission electron microscopy, mammalian pinealocytes have been described as cells with the main process running towards the capillary vessels and forming the bulbous ending close to or inside the perivascular space, and with some small, short processes. The improvement of backscattered electron imaging and the development of volume electron microscopy open the way for a more reliable and precise characterization of the pineal gland structure.
The pineal glands of domestic sheep aged 9 months were prepared according to the modified protocol of Deerinck et al. (1). For high-resolution imaging of the large sample areas, ultrathin sections were cut with the PT3D PowerTome ultramicrotome with ASH2 (Boeckeler Instruments, USA) and placed on silicon wafers. Some sections were additionally contrasted with uranyl acetate and lead citrate. The sections were imaged using the SenseBSD backscatter electron detector in SEM Gemini 450 at 1.4 kV, controlled by Atlas 5 software (Carl Zeiss, Germany). Serial block face imaging was performed with the 3View 2XP and the OnPoint detector (Gatan, USA) operating in SEM Gemini 450 with the focal charge compensation system (Carl Zeiss, Germany). Stacks of 1000 - 2000 images (15,000 x 15,000 pixels, pixel size 5 nm, section thickness 50 nm) were segmented manually and automatically using Microscopy Image Browser software. The models were visualized with Amira 3D (Thermo Fisher Scientific, USA).
The imaging of ultrathin sections on silicon wafers allowed ultrastructural visualization of large sample areas (1 x 2 mm) with a resolution 5 nm/pixel. The pineal gland capsule and the connective tissue trabeculae running from the capsule into the gland were completely separated from the parenchyma by the basement membrane. They contained blood vessels and nerve fibers. The parenchyma comprised pinealocytes and glial cells, mainly astrocytes. The cell bodies of pinealocytes formed small groups. Similar groups formed cell bodies of astrocytes. The large areas of parenchyma between the cell bodies were filled with the cellular processes, which in most cases contained intermediate filaments. Processes of pinealocytes, which distinguished by the presence of microtubules, were much less frequently found in these areas. There were also round or oval structures ranging in size from 0.2 to 5 µm filled with fine granular material of low electron density, the origin and character of which cannot be recognized on 2D images. The capillary vessels running through the parenchyma were surrounded by a thick layer of collagen fibers separated from the rest of the parenchyma by the continuous basement membrane. From the outside, this membrane was covered by ending of astrocytes.

The three-dimensional reconstruction of the pineal parenchyma revealed that pinealocytes of the sheep consisted of an irregularly shaped cell body and two types of processes: “large” processes and “small” processes. The surface of the cell body formed conspicuous invaginations to adapt to the numerous processes of other cells running in its vicinity. The shape of the cell body and the localization of the nucleus within it differed greatly between pinealocytes. The cell body comprised the Golgi apparatus, which consisted of 3 – 6 dictyosomes, a very dense network of tubules and cisternae of endoplasmic reticulum, numerous elongated mitochondria and a few lipid droplets. The number of “large” processes growing out of the cell body varied between 3 and 5. These processes often divided dichotomously into secondary processes, which form further branches. The “large” processes consisted of alternating wider and narrower parts. The wider parts, with a size of 1 – 3 µm, comprised large mitochondria, tubules of endoplasmic reticulum, vesicles and microtubules. Narrower parts, with a size of 0.2 - 1 µm, contained only a few tubules of endoplasmic reticulum and microtubules. The “small” processes grew out of the cell body in a number of 3 - 10. They form branches and contain tubules of endoplasmic reticulum, vesicles, microtubules and sometimes small mitochondria. The pinealocyte processes did not form bulbous endings.
The results obtained indicate that the descriptions of ovine pinealocytes based on transmission electron microscopy (2) should be revised. These cells have a few “large” processes of similar size that form branches, instead of the main process. The processes of ovine pinealocytes did form neither bulbous endings nor any ending close to perivascular spaces. It appears that the wider parts of pinealocyte processes play an important role in melatonin synthesis and secretion, increasing the area in which a pinealocyte realises the hormone. Our morphological observations are consistent with previous physiological data showing that melatonin concentration in cerebrospinal fluid is about 20-fold higher than in blood and that it is higher in the pineal recess than in the ventral part of the third ventricle (3). The differences in melatonin concentrations suggest that pineal hormone is released from the ovine pineal gland to the third ventricle in greater quantities than to the blood. Taken together, the morphological and biochemical data seem to indicate that the ovine pinealocytes realize melatonin into the extracellular space around the cell body and the cell processes, from where this lipophilic hormone enters the third ventricle and the intrapineal blood vessels, which are separated from the parenchyma by the blood-brain barrier. Comparison of the results of 3D studies on rat (4) and ovine pinealocytes shows that the differences between the species may be even more pronounced and important than previously thought based on 2D data.
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Keywords:

pinealocyte, pineal, ultrastructure, vEM, SBF-SEM,

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Mass microscopy allows for high throughput, high spatial resolution mass spectrometry imaging

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Poster Group 1

Background:

Mass spectrometry imaging (MSI) is an untargeted technique that maps the spatial localizations of 100s to 1,000s of molecules on complex surfaces.[1] MSI has shown great potential in tissue diagnostics for personalized medicine,[2] yet suffers from low throughput. For instance, state-of-the-art commercial matrix-assisted laser desorption/ionization (MALDI) MSI instruments measure up to 50 pixels/s.[3] Thus, acquiring images consisting of millions to billions of pixels can require days of measuring time, which would be impractical for clinical diagnostics. The low throughput of MSI is due to the way images are acquired, namely in microprobe-mode, in which a focused ionizing beam scans a surface and acquires pixels sequentially. This approach is especially time-consuming at high spatial resolution, as every linear decrease in pixel size is accompanied by a quadratic increase in the amount of pixels to be scanned. An alternative approach that bypasses this nonlinear relationship is mass microscopy. Here, a larger area is ionized simultaneously, the resulting ion image is pushed into the gas phase, analyzed by time-of-flight (TOF) mass spectrometry, and magnified onto a pixel-based spatially sensitive detector. Each pixel of this detector records its own mass spectrum allowing for the parallel acquisition of tens of thousands of mass spectra. Yet in the past, mass microscopy has not been a practical approach due to the lack of spatially-sensitive detectors with sufficiently short time resolution.
Here, we show that combining mass microscopy with a Timepix3 based detector with 1.56 ns time resolution turns mass microscopy into a viable approach that simultaneously allows for high throughput and high lateral resolution. As a first proof-of-concept, we published secondary ion mass spectrometry (SIMS) images at acquisition speeds up to 600,000 pixels /s with 2.5 µm lateral resolving power.[4] At this speed, we could record a gigapixel image within 34 minutes. Still, SIMS is an ionization technique primarily suited for elements and small molecules. Thus, we developed a laser setup that allows performing MALDI fast mass microscopy. We show early proof-of-concept images of TEM grids and tissues acquired at up to 5,230 pixels/s. Furthermore, we optimized our detector with a novel scintillator screen that enhances sensitivity and mass resolution.

Methods:

A TRIFT II mass spectrometer (Physical Electronics, Chanhassen, USA) equipped with a C60 ion gun (Ionoptika, Chandler's Ford, UK) was modified by adding a TPX3CAM (Amsterdam Scientific Instruments, Amsterdam, The Netherlands), an optical setup with a homogenized laser beam (Explorer One, Spectra-Physics, Stahnsdorf, Germany), and a Cry 60 scintillator (Crytur, Turnov, Czech Republic). First tests were conducted on TEM grids (Agar Scientific, Stansted, UK) placed on top of dried sodium acetate, crystal violet, and sprayed 2,5-Dihydroxybenzoic acid (DHB) films containing cetrimonium chloride. Later, a mouse brain section was measured using DHB matrix. All samples were coated with gold to improve surface conductivity. Data processing was performed with custom software.

Results:

We performed MALDI mass microscopy on TEM grids at intentionally slow speeds of 2,000 pixels/s with a spatial resolving power of at least 4 µm. This resolving power is comparable with state-of-the-art instruments that operate at or below acquisition speeds of 50 pixels/s and independent of matrix crystal size. Encouraged by these results, we imaged a mouse brain at 5,230 pixels/s, detecting lipids up to 1,000 Da. Yet, this image suffers from poor sensitivity in the higher mass range and from artefacts, which we attribute to surface charge effects. Thus, we explore charge-compensation methods, including gold coating on the surface and adding conductive additives to the matrix. Meanwhile, the implementation of the novel scintillator screen enhanced detector sensitivity tenfold, and in combination with optimized data processing improved our time resolution from 290 to <45 ns.

Conclusions:

The advent of the Timepix3 turns mass microscopy into a viable approach for high throughput, high spatial resolution SIMS MSI. The novel laser setup extends the applicability of mass microscopy to MALDI MSI and will in future allow for fast mapping of lipids, peptides, and even proteins. Meanwhile, the novel scintillator screen enhances detector sensitivity and allows for improvements in mass resolution. In the future, we strive for even higher mass resolution and throughput. For MALDI mass microscopy, it will be essential to develop better methods that prevent surface charging.

Keywords:

Mass spectrometry
High throughput
Detectors

Reference:

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A high-throughput compositional study of nanocrystals using a machine learning-assisted algorithm for STEM hyperspectral datasets

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Poster Group 2

Background incl. aims

In nanomaterials research, the analysis of heterogeneous ensembles of nanocrystals presents a significant challenge, often requiring the extraction of information from diverse classes of particles or phases, in a process that is often prone to operator bias and poor statistics. Lead Halide Perovskite (LHP) nanocrystals (NCs) are a promising class of materials for optoelectronic applications that feature a broad compositional flexibility and are often the subject of extensive studies that cover a broad parameter space for the synthesis or modification of the basic structures. Here we focus on CsPbCl3 NCs where the halide is replaced through exposure to increasing amounts of iodine and using STEM-EDX to track ex situ the change in halide [1]. Our primary objective is to extract compositional information at the single-particle scale while minimising the electron dose onto the sample, a critical consideration to preserve sample integrity and minimise carbon contamination. The analysis of nanocrystals poses unique challenges due to their heterogeneous nature and varying chemical compositions. To address this gap, our research seeks to leverage the capabilities of TEM hyperspectral imaging coupled with machine learning algorithms. The primary objective is twofold: first, to develop a robust framework capable of extracting detailed compositional information from individual nanocrystals within a large ensemble, and second, to enable high-throughput analysis.

Methods
The proposed methodology entails the integration of a machine learning-assisted algorithm (Segment every grain – SEG [2]) within HyperSpy [3] , a versatile environment designed for the analysis of hyperspectral datasets. SEG can reliably identify features in STEM micrographs, and the algorithm is designed to process datasets with a low signal-to-noise ratio (SNR), mitigating the risk of sample damage or alteration.
The algorithm is designed around modular Python scripts to construct adaptable analysis pipelines. These scripts facilitate seamless integration with existing workflows, allowing researchers to tailor the analysis to specific datasets or scientific inquiries. The modular nature of the scripts enhances flexibility and scalability for samples that produce a very low signal due to their limited thickness or beam sensitivity.

Results
Preliminary results demonstrate the efficacy of the proposed approach in extracting detailed compositional information from a population of nanocrystals in sets of STEM hyperspectral datasets. A halide replacement effect through a jump-the-gap mechanism is observable as the nanocrystal population generally splits into the two populations, with some outlier values that can be studied in detail in further work. A study of the lateral size distribution is carried out across all the nanocrystals

of different chemical compositions of multiple samples, tracking the morphology through chemical conversion. Furthermore, a frame-by-frame analysis is employed to study the effect of the electron dose damage on the chemical composition of the nanocrystals, finding similar behaviour for different halide species.

Conclusion
The integration of machine learning with STEM hyperspectral datasets represents a significant advancement in nanocrystal analysis. By combining state-of-the-art algorithms with open-source platforms and modular scripting, researchers can unlock new insights into the complex world of nanomaterials through high-throughput data processing algorithms. This flexible algorithm can be customised and developed towards automated in-line analysis in the future.

Keywords:

electron microscopy, STEM-EDX, machine learning

Reference:

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Simulating iDPC tomography of CeO2 nanoparticles with experimentally realistic parameters and conditions

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Poster Group 1

Background incl. aims
Electron Tomography (ET) is a 3D analysis technique that produces a 3D reconstruction from a tilt series of microscopy images at 1-5° increments. Electron tomography can be used to characterise the 3D structure of nanoparticles at atomic resolution from an input of 40-150 images, depending on the specific reconstruction algorithm and parameters. Atomic electron tomography has been possible with HAADF-STEM for over a decade (Scott et al., 2012) . However, a limitation of the technique is that prolonged exposure to the electron beam can cause damage or changes to samples, prohibiting an accurate reconstruction.
Integrated Differential Phase Contrast (iDPC) imaging is a technique which has contrast proportional to atomic electrostatic potential captured using a segmented detector. iDPC provides a better signal-to-noise ratio than HAADF at similar electron doses due to use of the bright-field disk and allows for atomic-resolution imaging with lower electron doses. iDPC additionally enables simultaneous imaging of light and heavy atoms, due to its image contrast being proportional to Z rather than Z^2 as in HAADF (Lazić et al., 2016).
Atom and defect positions at the surface of nanoparticles are crucial to catalytic performance. Accurate characterisation of 3D structure can therefore explain trends in catalytic activity/selectivity and assist in the rational design of catalysts. iDPC provides an excellent technique to image light atoms and their vacancies, even alongside atoms of higher atomic number. This is particularly valuable in the imaging of metal oxides, where surface termination and surface defect density can greatly impact catalytic properties.
We aim to demonstrate that an iDPC atomic resolution ET reconstruction is able to provide the locations of oxygen atoms, in addition to metal atoms within a nanoparticle. To investigate this, we simulate tilt series of iDPC and HAADF images, and generate tomographic reconstructions of a CeO_2 nanoparticle. CeO_2 was chosen due to its prevalence in catalysis and its requirement of a flux lower than $7x10^5$ $e^-nm^{-2}s^{-1}$ to avoid beam damage (Johnston-Peck et al., 2016).
With these reconstructions, we assess the ability of iDPC tomography to distinguish between oxygen-terminated and cerium-terminated surfaces, and to detect surface oxygen vacancies. Additionally, we demonstrate that iDPC will produce accurate tomographic reconstructions when applied at lower doses, resulting in reduced beam damage, compared to HAADF tomography.
Method
Atomic models of octahedral ceria nanoparticles of lengths of 3 nm and 7 nm are built using the Python package Atomic Simulation Environment (ASE), using an additional Python package (Wulffpack) to adjust particle truncation based on surface energies.
iDPC-STEM and HAADF-STEM images of these models are simulated using the PRISM algorithm within the Python package abTEM (Madsen & Susi, 2020). We define set values of semiangle cutoff (17.5 mrad for iDPC, 30 mrad for HAADF), accelerating voltage (200 kV), PRISM interpolation factor (4) and collection angle (13-56 mrad for iDPC, 56-200 mrad for HAADF). A total of 151 images are simulated with tilt angles ranging from ±75°. The effects of finite electron dose (via Poisson noise) and partial spatial coherence (via Gaussian noise) are added in post-processing.

3D reconstruction is performed using the ASTRA toolbox, applying the ASTRA-SIRT algorithm. TVM and GENFIRE reconstruction algorithms are also compared when using reduced numbers of projections. Reconstructions are formed with 1, 3 or 5° increments between each image. Determination of atom intensities in the resulting reconstructions are performed through ImageJ.

Results

Oxygen atoms were clearly distinguishable in the tomographic reconstructions of the iDPC images (without the addition of noise). The SIRT iDPC reconstruction had a Ce:O intensity ratio of 170:60 compared to 135:5 for HAADF. The iDPC reconstruction additionally displayed a 177.6% intensity increase when comparing the locations of surface oxygens to vacancies, enabling clear differentiation between oxygen atoms and vacancies. This visible difference is demonstrated in the attached reconstruction slice graphic.

Partial spatial coherence (source size 0.2 – 0.4 eV), and finite electron doses (50 – 50000 e^{-} /Å²) were applied to successfully demonstrate the impact of these conditions on the quality of tomographic reconstructions. This has been quantified by measuring atom intensities in the reconstruction and assessed qualitatively by comparing the ability to visually distinguish individual oxygen atoms and vacancies without further processing. We plan to additionally assess aberrations (such as defocus).

The SIRT iDPC reconstruction with a dose per image of 5000 e^{-} /Å² and a source size of 0.3 eV had a 256.50% intensity increase of the locations of surface oxygens compared to vacancies, showing vacancy detection is still feasible under low-dose experimental conditions. This reconstruction also displayed a Ce:O intensity ratio of 104:44, compared to 23:5 for a HAADF reconstruction under the same conditions. This HAADF reconstruction contained no visible oxygens, as expected.

These simulations confirm the resolution limits of different acquisition conditions for iDPC tomography, enabling experimental application of optimum conditions, and a known error margin to operate within.

Conclusions

Simulating iDPC and HAADF-STEM tomography has successfully confirmed parameters and requirements for atomic resolution iDPC tomography, enabling future experimental applications. By confirming the lower dose requirement for iDPC tomography, we have demonstrated the ability to create tomographic reconstructions of beam-sensitive samples which could not be captured with HAADF tomography.

We have additionally confirmed the ability of iDPC-STEM tomography to distinguish between vacancies and oxygen and cerium atoms within a 3D reconstruction of a CeO_2 nanoparticle. This demonstrates the potential of iDPC-STEM tomography as a technique for analysis of the surface termination of metal oxides, detection of oxygen vacancies and support-nanoparticle interface analysis.

Keywords:

Tomography, Python, iDPC, Simulation, Low-dose

Reference:

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Microvessel and mitochondria changes in a mouse model of Alzheimer's disease

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Poster Group 2

Background and aim: Reduced oxygen availability is prevalent in several conditions affecting the brain including Alzheimer's disease [1, 2]. The mitochondria are main consumers of oxygen for ATP production, and the mitochondrial network adapts to changes in substrate availability and metabolic needs of the cell with dynamic shape transitions. Fusion of mitochondria enables transfer/sharing of organelle components, thereby improving energy conversion efficiency, and fission makes removal of damaged mitochondria possible. The cristae of the inner mitochondrial membrane, where oxidative phosphorylation takes place, are also remodelled in adaptation to energy substrate availability. The close relation between mitochondria morphology and function makes analyses of this organelle valuable in the search of an improved understanding of the subcellular effects of decreased tissue oxygenation in vivo. This is central in the elucidation of Alzheimer's disease pathogenesis and identification of novel therapeutic targets. We aim to investigate the effect of insufficient oxygen availability on the structure and function of mitochondria in a mouse model of Alzheimer's disease.
Methods: At the microscale, brains undergo MR scanning to examine the correlation between non-invasive detection of structural modelling and subsequent histological observations. Capillaries from the hippocampal strata oriens, -radiatum, and -lacunosum moleculare in CA1 are analysed using stereology to assess vascular changes. Based on these findings, a specific stratum is chosen for nanoscale analysis of mitochondria using vEM. The total volume and volume distribution of mitochondria are acquired, and crista related parameters evaluated in 3D applying our newly developed method [3]. Mitochondrial respiration is assessed with Seahorse assays on tissue punches and isolated mitochondria obtained from the hippocampus.
Results and Conclusion: Analyses are ongoing and hopefully results can be presented at EMC2024.

Keywords:

cross-scale microscopy, microvessels, mitochondria, Seahorse

Reference:

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Cryo-EM Reveals RECQ5's Regulatory Role in RNAPII-Mediated Transcription

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Poster Group 1

Background

In eukaryotic nuclei, the synthesis of mRNA is carried out by RNA polymerase II (RNAPII), a crucial enzyme in transcriptional processes. RECQ5 helicase, a general elongation factor, associates with RNAPII and controls its movement along genes. Despite its significance, the precise mechanism through which RECQ5 regulates RNAPII movement remained unknown.

Methods

We employed a combination of cryo-electron microscopy methods to investigate the interaction between RNAPII and RECQ5. These methods included single-particle analysis (SPA), correlative light-electron microscopy (CLEM), and subtomogram averaging (STA).

Results

Here, we present the details of the interaction between RECQ5 and RNAPII determined by cryo-electron microscopy. SPA reconstruction with a resolution of 3 Å revealed near-atomic level details of the interaction between the RECQ5 helix and RNAPII DNA. Additionally, cryo-EM imaging showed that the RNAPII with RECQ5 complex formed large, spherical objects resembling condensates. CLEM confirmed the presence of both fluorescently labeled RNAPII and RECQ5 within these objects. STA revealed the organization of condensates, further enhancing our understanding of their functional assembly. The subtomogram averaging model of RNAPII with RECQ5 reached a resolution of 7 Å, confirming the observed interaction between the RECQ5 helix and RNAPII DNA across analytical methods.

Conclusions

Our study emphasizes RECQ5's crucial role in modulating transcriptional processes by using the brake-helix to regulate RNAPII movement along genes. Additionally, we discovered that condensates contain tens to hundreds of RNAPII molecules with RECQ5. The study provides valuable insights into how transcriptional machinery functions in eukaryotic nuclei.

Keywords:

RNApolymeraseII, Tomography, Clem, SPA

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Surface tension of Au-catalysed GaAs-nanowires

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Poster Group 2

Droplets are held together by the surface tension, which is the force from the surface molecules attracting each other. It is not only the phenomenon explaining droplet formation, but it is also an important factor, when studying vapor-liquid-solid growth, which is a commonly used growth mechanism for nanowires. Today however, growth models and predictions are based on approximate values of the surface tension, lacking in reports of empirical values e.g., for Au-catalyzed GaAs-nanowires.
An external electric field has previously been used to deform the catalyst droplet on AuSi eutectic droplets on silicon nanowires and the balance of field and surface tension can be used to measure the surface tension. Here, we use a similar procedure growing Au-catalyzed GaAs nanowires on microfabricated Si-cantilevers, which opens up for many types of in-situ experiments, deforming the catalyst in an arsine atmosphere using a unique ETEM with a purpose-built gas injection system. Image analysis and 3D simulations (Fig. 1a) using COMSOL Multiphysics are used to compare the droplet shape e.g., the aspect ratio of the droplet at various fields and temperatures (Fig. 1b) reporting an empirical value of the surface tension for the first time and with improved analysis also an updated value on the AuSi surface tension reported earlier.
In conclusion, an empirical value of the surface tension of the AuGa eutectic of GaAs nanowires is reported using locally heated Si-cantilevers in an ETEM. This will be compared with theoretical predictions.

Keywords:

GaAs-nanowires, catalyst, surface tension, MEMS

Reference:

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TEM observations of threading dislocations in gallium nitride under external stimuli

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Poster Group 2

Background incl. aims
Gallium nitride becomes increasingly utilized in power electronics and currently competes with silicon carbide for the lead in many power applications. Each of the two materials faces different challenges. In GaN, for example, the effect of threading dislocations in GaN-on-Si epitaxial layers is still an open issue. Despite great effort in research of this material, it is questionable whether the high concentrations of dislocations deteriorate device operation [1]. Additionally, dislocation motion at elevated temperature, under local electric field or passing current could be significant in delayed device failure. Localization, identification and operando observation of dislocations is a highly complex task, requiring a correlative multimodal approach [2]. Here, we present initial attempts which include operando TEM observations of threading dislocations.

Methods
We localize and identify dislocations by means of electron channeling contrast in SEM, conductive AFM and wet etching. Subsequently, dislocation dynamics is observed by TEM on FIB-prepared cross-sectional lamellae on MEMS chips.

Results
We inspect the effect of heat, electrical current and electric field on the dislocation motion. The dislocations are stable at elevated temperatures, and they start to move/unpin if extreme current densities over tens of kA/cm2 are passing through (i.e. much higher than common current densities in GaN devices,). We will also present our first attempts to observe the motion initiated by an extreme electric field, generated by a sharp tip close to the lamella surface.

Conclusion
In this contribution, we demonstrate a correlative approach to dislocation identification, combining SEM, TEM, wet etching and probe microscopy. We show the results of our TEM observations of dislocation motion under external stimuli (heat, electric current and large electric field). The dislocations seem to be immobile until only very extreme conditions are met.

Acknowledgements
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Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the national granting authorities. Neither the European Union nor the national granting authorities can be held responsible for them.

Keywords:

Gallium nitride, dislocations, in-situ TEM

Reference:

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Deep Learning Style Transfer for Elastic Image Registration of Visually Distinct Correlative Microscopy Images

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Background
Correlative microscopy involves using multiple imaging techniques on the same sample to enhance the overall understanding of the data by correlating the information gathered from each method. [1] While Correlative Light and Electron Microscopy (CLEM) is an established method in life sciences, its application in materials science remains underutilized yet equally crucial. [2] The primary challenge in materials science correlative microscopy lies in co-localizing and registering (aligning) microstructure images, a task complicated by possible microstructure complexity compared to cellular images. In our previous work, we described a method developed to generate a large dataset of light optical microscope (LOM) and scanning electron microscopy (SEM) images for training a deep learning (DL) model aimed at microstructure image enhancement. [3] To achieve automatic alignment of distinct images, we found it necessary to include Confocal Laser Scanning Microscope (CLSM) images, which serve as a visual intermediary. Despite the Keyence VK-X1100 CLSM being fully automated, software limitations precluded the use of its automation features. This resulted in a bottleneck, as the raw images from mapping could not be saved, forcing reliance on semi-automatic imaging. In addition, too small and unalterable overlap between images hindering further processing. Initially, we trained a deep learning model built on Generative Adversarial Networks (GAN) to enhance LOM images. However, improving this model requires additional data, which is time-consuming to obtain. Therefore, we suggest using the trained model as a style transfer filter to eliminate the need for CLSM images. This approach enables the simulation of SEM appearance from LOM images, thereby assisting the elastic image transformation tool (bUnwarpJ [4]) in identifying common features across images, facilitating their alignment.

Methods
We used pre-aligned images from the aforementioned dataset to effectively demonstrate the success ratio of image registration with the bUnwarpJ ImageJ tool, comparing its performance on both the original and style transferred images. This dataset consists of LOM and SEM-CBS micrographs of TRIP steel (512x512 px, 20x20 µm field of view), which were initially aligned using a workflow detailed in our previous work.[3] We employed 252 image pairs to evaluate our hypothesis. The investigation began with elastic registration on the original LOM (source) and SEM-CBS (target) images, establishing a baseline success rate. Subsequently, a GAN model was introduced to transform LOM images into CBS-like counterparts. This transformation aimed to narrow the visual disparity between LOM and CBS images, facilitating improved registration accuracy by the bUnwarpJ tool. The transformations devised for the predicted images were then applied to the original LOM images to achieve the desired alignment. Given the absence of a robust metric for registration (aligning) quality, evaluations were subjectively categorized into four tiers: poor, bad, decent, good.

Results

Elastic registration from raw LOM to CBS images was notably unsuccessful across the majority of cases (poor: 160, bad: 90, decent: 1, good: 1). This failure is immediately apparent, with the algorithm causing significant distortion and deformation of the source LOM images. In contrast, the application of GAN to transform LOM into CBS-like images resulted in a marked improvement in registration convergence across all examined instances (poor: 0, bad: 0, decent: 8, good: 244), as evidenced by the provided examples. However, the overall quality of this image registration remains a matter of contention. The inherent low quality of LOM images limits the fidelity of their transformation into CBS-like counterparts, leading to inaccuracies particularly in the depiction of grain boundaries. These inaccuracies result in mismatches when aligning to the target CBS image boundaries. Conversely, while CLSM serves as a comparable intermediary and faces similar challenges, it benefits from a precise physics-based acquisition method, which mitigates issues associated with generative model-induced artifacts. However, this approach requires a two-step alignment process: first from LOM to CLSM and then from SEM-CBS to CLSM. This sequential alignment process inherently doubles the potential for error. Furthermore, as previously highlighted, CLSM mapping represents a significant time-consuming bottleneck.

Conclusion

This study demonstrates the value of style transfer in facilitating the alignment of a large volume of corresponding images from different microscopy techniques. Utilizing LOM and SEM microstructure images of TRIP steel (20×20 µm field of view) as a case study, we illustrate the method's potential to bridge the visual modality gap between disparate imaging techniques. Initially, this approach requires the manual creation or clever generation of a dataset of aligned image pairs to train a deep learning model. Once established, this model significantly simplifies the alignment challenges for subsequent and future datasets of a similar nature. Although, utilization of a less complex U-Net model—typically employed for segmentation—could reduce the size of the required training dataset, our work leveraged a more sophisticated GAN model, necessitating a larger dataset. This work underscores the potential for enhancing image registration through preprocessing with GAN-based transformations, effectively addressing modality differences in correlative microscopy.

Keywords:

Correlative-microscopy, image-registration, deep-learning, style-transfer

Reference:

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Image Registration Based Navigation of Region of Interests in Volume Correlative Light and Electron Microscopy

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Background and Purpose:
Volume correlative light and electron microscopy (vCLEM) has the potential to provide both function and ultrastructure of biological specimens by combining light and electron microscopy. One attractive approach for vCLEM is to prepare samples for electron microscopy (EM) in a way that preserves fluorescence even after they've been embedded in resin (in-resin fluorescence preservation). This allows for navigation to a ROI using fluorescence and EM images acquired at various points through the imaging workflow, including the final ultrathin sections used for transmission electron microscopy (TEM) imaging.
Image registration of light microscopy (LM) and EM images at various scales is required throughout this process. Current trends in image registration rely on methods that warp one image based on user-defined corresponding landmarks. While these methods yield high accuracy, they require significant user time and effort in selecting corresponding landmarks. Recent advancements have introduced automated methods that leverage Laplacian-of-Gaussian filtering or point cloud mapping. While these methods enhance both efficiency and reproducibility, their applications have primarily been limited to cell CLEM, leaving their efficacy in the realm of tissue CLEM unverified. Moreover, achieving ROI navigation through image registration across multiple scales remains a challenge, as existing tools lack the capability to visualize this process effectively.
Here, we aim to bridge this gap by proposing an efficient ROI navigation workflow that leverages new image registration techniques.

Methods:
We use image datasets encompassing mouse brain tissues. The mice are labelled with intrabodies specifically targeting inhibitory synapses, alongside cytoplasmic markers that comprehensively labelled the entire neurons. They are anesthetized, perfused, and brain vibratome sections are collected. Then, cell nuclei are labelled with DAPI and blood vessels with tomato lectin LEL. Next, the tissues are high-pressure frozen, freeze-substituted, and resin embedded. Following the acquisition of a confocal image of the resin block, it is sectioned into ultrathin slices (200 nm) using an ultramicrotome, and fluorescence images collected of some of those sections. Finally, TEM images of these ultrathin sections are obtained.
Our workflow facilitates the acquisition of EM images corresponding to ROIs identified using LM images. It encompasses two key components:

1. Semi-automated image registration algorithm: This algorithm aims to achieve the registration of images acquired through diverse microscopy techniques. Its core functionality consists of two primary processes: (a) object segmentation and (b) image registration. In (a) object segmentation, the objects that appear commonly in both images are segmented either automatically or with a degree of user interaction. During (b) image registration, images are warped to match objects in (a)

across imaging modalities. Image registration metrics such as mutual information are calculated to assess the quality of the warping. Arranged in descending order of value, a user is presented with the overlayed images corresponding to the warping yielding the highest metric value. With the help of the user, the successful registration parameters are determined. In scenarios where one image has greater dimensions than the other, the larger image undergoes a raster scan. During this process, the image value registration metric is computed at each scan position. Notably, if the input consists of an image stack, additional scanning additionally occurs along the depth axis.

2. ROI navigation viewer: This viewer facilitates the visualization of image stack and section image registration results, ultimately guiding navigation towards ROI acquisition via high-resolution EM. The viewer leverages the Napari framework for its implementation and has multiple advantages.

(a) Three-dimensional visualization
This function accepts the image stack and warping parameters to display the three-dimensional positioning of the ROI and ultrathin section fluorescence (and TEM) images relative to the entire low-magnification confocal microscopy image stack. This comprehensive view allows for at-a-glance comprehension of the spatial relationships between multiple images and datasets. Additionally, the depth information from the ROI surface serves as a guide for selecting ultrathin sections.

(b) Overlaying an ROI on an ultramicrotome stereomicroscope image
This function accepts an ultramicrotome stereomicroscope image, a transmitted DIC image, and a confocal microscope image of a tissue block and overlays the ROI location onto the ultramicrotome image to facilitate the precise trimming of the tissue block containing the ROI.
Notably, the transmitted DIC image serves as an intermediary to achieve accurate superposition onto the ultramicrotome image.

(c) Overlaying ROI on low-magnification EM image
This function accepts block confocal microscopy image of a tissue block and its ultrathin section EM image, and displays the location of the ROI overlayed on the low-magnification ultrathin section EM image to facilitate acquisition of high-magnification EM images of the ROI.

Results:
We assessed the efficacy of the proposed workflow using image datasets encompassing brain tissues from two mice. The image acquisition procedure is detailed within the background section. The key distinction between the two datasets is the presence or absence of blood vessel staining (Datasets 1 and 2, respectively).

For the Dataset 1, we succeeded in navigation of the entire block image (2 mm x 2 mm x 0.1 mm, pixel size 0.57 µm in the X and Y, 1 µm in Z) to the single nuclei (roughly size of 5 x 5 µm2, pixel size: 7 nm x 7 nm). For the Dataset 2, we applied Functions 1 through 3 sequentially and succeeded in navigation of the entire block image (600 x 600 x 35 μm^3, pixel size: 0.63 µm in X and Y, 1 µm in Z) to the single axon (roughly size of 1 x 20 µm2, pixel size: 3.4 nm x 3.4 nm).

Conclusion:
While vCLEM offers unparalleled access to three-dimensional information regarding both cellular function and ultrastructure, navigating the ROI for EM image acquisition has been laborious. Our segmentation-based image registration algorithm facilitates seamless navigation across modalities

and scales. Coupled with our interactive viewer capable of visualizing registration results and guiding ROI navigation from LM to EM imaging, this integrated approach enables efficient ROI navigation.

Keywords:

Image registration, Automation, Visualization

Reference:

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Microstructural Characterization of Electron Beam Welded Joints between EHEA and Austenitic Stainless Steel

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Poster Group 2

Background incl. aims
Eutectic high-entropy alloys (EHEAs) have been around for a decade but only recently has become a hot topic in a field of metal alloys . As a subclass of high-entropy alloys (HEAs) enriched with a variety of advantages of eutectic alloys, EHEAs possess superior mechanical properties opening a broad spectrum of possible applications in industry [1]. These alloys are best characterized by the absence of the conventional „strength ductility trade-off“ and remarkable stability over a wide temperature range due to their high-entropy state. Combined with excellent castability, mitigating segregation and shrinkage, they appear to be an ideal candidate for demanding design scenarios such as exposure to elevated temperatures or mechanical stresses commonly encountered in the energy industry [2]. Therefore, they can be seen as a possible competitor for austenitic stainless steels, which possess high ductility with lower tensile strength values and are used for such applications nowadays [3,4]. In this regard , examining the behavior of these specific materials when welded together is prudent, as it can provide valuable insights into their compatibility and suitability for coexistence in industrial applications or utilization in tandem within various industrial sectors. Motivated by the imperative to harness the full potential of advanced materials, our study focuses on the characterization of heterogeneous weld joints between AlCoCrFeNi2.1 EHEA and austenitic steel EN 1.4571, achieved through electron beam welding. The primary objectives of this study are to conduct a comprehensive investigation into the microstructural characteristics of the weld interface and constituent materials. Through a multifaceted approach encompassing various analytical techniques, tests, and image analysis methodologies, our aim is to elucidate as much information as possible, thereby enhancing our understanding of the welding process and the resultant material properties.
Methods
The electron beam welding process was employed to fabricate weld joints between EHEA (AlCoCrFeNi2.1) and austenitic stainless steel (EN 1.4571). Three sets of samples were prepared, each subjected to different welding parameters: beam currents of 13 mA, 17 mA, and 25 mA, with corresponding welding speeds of 10 mm/s, 20 mm/s, 30 mm/s respectively. Prior to welding, samples underwent traditional metallographic procedure. The final preparation step involved electro polishing with parameters meticulously adjusted for optimal results. The microstructure of the weld joints and base materials was examined using multiple microscopy techniques. Light optical microscopy provided a macroscopic view of the weld interface, while confocal scanning laser microscopy offered high-resolution imaging of surface features. Additionally, scanning electron microscopy (SEM) was employed to investigate microstructural details at higher magnifications. On the SEM, Energy Dispersive X-ray Spectroscopy (EDS) was employed for elemental analysis of the weld zones. EDS mapping was conducted to assess the spatial distribution of alloying elements and identify any potential elemental segregation. Furthermore, Electron Backscatter Diffraction (EBSD) was utilized for phase identification and crystallographic analysis of the welds and base materials. Apart from microscopic analysis, mechanical properties of the weld joints were assessed through

tensile testing and nanoindentation for hardness evaluation. Tensile tests were conducted to measure the mechanical strength and ductility of the weld joints. On top of that, convolutional neural networks (CNNs) were employed for image analysis to estimate the fractions of BCC and FCC phases present in the microstructure.

Results

All observations were conducted in the same manner to achieve comparable results. The fusion area was examined at three levels: top, middle, and bottom. The top area was defined as 1 mm below the surface of the weld, while the bottom area was 1 mm above the root of the weld, with the middle area positioned equidistantly between these top and bottom regions. Summarizing the results, in all scenarios, EDX line scans along the axis of the weld showed minimal fluctuations in chemical composition. Conversely, significant chemical composition changes were observed when examining lines across the weld, particularly at the fusion zone boundaries, while the elemental levels remained stable after the transition. This phenomenon was consistent at every level of observation, with increasing fluctuations in the middle and bottom areas. It is also noteworthy that carbon nitrides from EN 1.4571 were observed across various regions of the fusion zone, contributing to the complexity of the microstructure and hindering the diffusion of chromium due to carbon and nitrogen binding. From a phase perspective, the entire fusion area exhibited a face-centered cubic (FCC) dendritic structure with interdendritic space formed by body-centered cubic (BCC) precipitates, resembling the EHEA-like phase map, which consists of FCC and BCC lamellas, while EN 1.4571, consistent with its inherent properties, maintained an FCC phase. Moreover, BCC stripes were predominantly observed at the interface between EHEA and the fusion zone, likely as a product of segregation. Conversely, the transition between EN 1.4571 and the fusion zone primarily consisted of an FCC phase with smaller BCC precipitates appearing deeper into the fusion zone. This observation is further supported by the grain orientation map, where grains adjacent to EN 1.4571 maintained larger sizes comparable to the base material matrix on this side of the weld, while also exhibiting almost identical orientation to the nearest grains of the base material.

As one delves deeper into the fusion zone, the relative grain size diminishes, and their orientation becomes more random, coinciding with the appearance of precipitates. These precipitates, besides exhibiting a BCC phase, displayed an inner structure that was analyzed, albeit yielding no further discoveries.

Conclusion

Optimal parameters for welding EHEA together with EN1.4571, as well as metallographic preparation of such welds, were established. The microstructure was subsequently observed and analyzed using a spectrum of different techniques, offering profound insights into both the welding process and the resultant structure. Our observations unveiled a finely nuanced and complex microstructure within the fusion zone, adorned with small BCC precipitates ranging from a few to tens of micrometres. Additionally, EBSD analysis underscored the pivotal role of the weld interface in shaping the evolution of the microstructure. Continued analysis, including the exploration of AI-powered image analysis techniques for fusion zone estimation, is warranted to deepen our understanding and uncover further intricacies.

Keywords:

EHEA, austenitic-stainless-steel, electron-beam-welding, microstructure-analysis, ML

Reference:

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The authors acknowledge that the reserch was funded from the Lumina Quaeruntur fellowship established by the Academy Council of the Czech Academy of Sciences, award for prospective researchers recieved by Dr. Mikmeková.

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Mechanisms of microglial responses to injury related focal ATP events

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Poster Group 1

Microglia, the main immunocompetent cells in the central nervous system, rapidly respond to disturbances in their microenvironment, largely relying on purinergic (ATP, ADP) signaling through P2Y12 receptors. However, while the effects of exogenous ATP on microglial activity have been extensively tested in previous studies, the mechanisms involved in microglial response to endogenous, focal ATP events in the brain tissue are unclear. To visualize changes in the extracellular ATP levels, we introduced a sensitive fluorescent ATP sensor (GRAB-ATP) to cultured neurons and astrocytes and prepared acute brain slices from mice expressing the sensor in VGlut1+ neurons. We observed both spontaneous and injury-related ATP events in astrocytes and neurons in vitro, and detected significant extracellular ATP increase after slice preparation or secondary injury, even hours after slice cutting. ATP accumulation due to blockade of ecto-ATPase activity indicated an ongoing ATP release from the damaged tissue. Interestingly, we also observed a highly dynamic, focal, flashing ATP activity, persisting for hours ex vivo. Importantly, microglia processes were recruited to complex, nano- and micromolar ATP events in a P2Y12R and CX3CR1 dependent way, leading to marked morphological transformation of the cells. Microglial response to spontaneous- and injury-related ATP signals also occurs in vivo as revealed by in vivo two-photon microscopy. We suggest that focal, submilimolar ATP events represent a major modulator of microglial states and are likely to alter inflammatory responses in different CNS disorders.

Keywords:

ATP, microglia, acute brain slice

Reference:

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Complementary vEM approaches for ultrastructural changes during the development of D. melanogaster germline intercellular bridges

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Poster Group 1

Background and aims
The fruit fly Drosophila melanogaster is an excellent model for studying biological processes, and many questions in cell and developmental biology have been answered using this system. Incomplete cytokinesis is an essential process studied in many cell types which allows cells to form stable intercellular bridges that connect them to each other. Although intercellular bridges are found in somatic and germ cells throughout the animal kingdom, the most well-studied are those within the developing egg chamber. Many essential pathways and processes are required to allow the egg chamber to develop into a mature fly egg. The egg chamber consists of a cluster of 16 interconnected germ cells surrounded by a layer of somatic cells. Following each of the four germ cell divisions that create the germ cell cluster, the midbody is re-organized to form a stable intercellular bridge or ring canal. These ring canals undergo significant growth, which allows cytoplasmatic contents and nutrients to be transferred from supporting nurse cells to the growing oocyte. Although fluorescence and electron microscopy-based studies have been used to study the role of ring canal proteins during oogenesis, a complete ultrastructural characterization of these structures is lacking.
Methods
Electron Microscopy is a powerful tool for imaging cellular structures at the nanoscale, providing valuable insight into the ultrastructure of many cell and tissue types. Volume microscopy techniques such as Focused Ion Beam, Scanning Block Face, and Array Tomography have considerably advanced the potential applications of SEM-based approaches. To learn more about ultrastructural changes in the ring canals throughout oogenesis, we have used a combination of complementary vEM approaches – FIB-SEM to monitor the early stages of oogenesis and AT-SEM to follow the structures in later stages of oogenesis. We have aligned and segmented the stacks of images to analyze the structure of the ring canals during the development of the egg chamber.
Results
By using a complementary set of vEM-based approaches, we were able to visualize ultrastructural changes in the germline ring canals throughout oogenesis. We segmented the large data set collected using Focused Ion Beam SEM to generate a complete 3D model of the germarium and an early-stage egg chamber. The combination of AT-SEM and FIB-SEM allowed us to gain insight into previously unappreciated aspects of ring canal structure and function. In addition, we have characterized the microvilli meshwork surrounding the ring canal structures (Figure). These structures have been proposed to be essential to anchor the ring canals within the nurse cell membranes, but such a detailed three-dimensional view of their orientation has not been produced.
Conclusions
This combination of complementary vEM approaches allowed us to capitalize on the strengths and overcome the limitations of each individual approach. This modern framework could be used to answer biological questions in other tissues or organisms that face similar technical challenges; for example, in older egg chambers, the ring canals are very small structures within a large sample

volume. Our work illustrates the potential scientific insight that can be provided by a high-content dataset, which could potentially become a valuable resource for further analysis by additional groups in this field. Although the focus was on ultrastructural changes in the germline RCs, our dataset contains valuable details of additional cell types and structures.

Keywords:

EM, 3d, ArrayTomo, segmentation, Drosophila

Reference:

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Kline A, Curry T, Lewellyn L. The Misshapen kinase regulates the size and stability of the germline ring canals in the Drosophila egg chamber. Dev Biol. 2018 Aug 15;440(2):99-112

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Investigation on photocorrosion of TiO2 during photoelectrochemistry process by electron microscopy together with operando ICP-MS

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Poster Group 1

Background incl. aims
With the growing environmental deterioration and ever-increasing energy demand, exploring a renewable and clean energy for human development has been regarded as a promising research subject. Hydrogen is one of the most desirable energy carriers. Fujishima and Honda discovered that hydrogen can be generated from water splitting on a TiO_2 photoelectrode under UV light illumination.[1] TiO_2 is considered to be stable since its redox potential has been calculated to be more positive/negative relative to the water oxidation/reduction potential. This means that photogenerated charge carriers would participate in driving the desired water splitting reactions rather than oxidizing or reducing semiconductor photoelectrodes.[2] Nevertheless, it is reported that there is degradation of performance and alterations in surface morphology of TiO_2 under UV light irradiation. Understanding the dynamic catalyst behaviour during electrochemical application plays a key role in developing improved catalysts.
Methods
We applied scanning electron microscopy (SEM), aberration-corrected transmission electron microscopy (TEM), X-ray diffraction (XRD), x-ray photoelectron spectroscopy (XPS) and Raman spectroscopy together with operando inductively coupled plasma mass spectrometry (ICP-MS) coupled with illuminated scanning flow cell to investigate photoelectrochemical (PEC) corrosion pathways of TiO_2 semiconductor photoelectrodes. TiO_2 nanotube array was utilized as our model system to conduct the quantitative study in understanding the effect of the PEC conditions on its photostability. PEC measurements were performed on a Reference600 potentiostat (Gamry) with a three-electrode PEC cell in 0.1 M of $HClO_4$ solution as the electrolyte. The electrolyte was pumped through the PEC cell into ICP-MS for time-resolved analysis of the amount of dissolved titanium ions.
Results
The XRD pattern indicates that all peaks agree well with the tetragonal rutile phase (SG, $P4_2/mnm$; JCPDS No. 21-1276) which is further confirmed by Raman spectrum. X-ray photoelectron spectroscopy (XPS) analysis was performed to study the chemical components and the states of Ti and O in the TiO_2 nanocomposites. The XPS spectra reveal that the dominant elements are Ti, O of the TiO_2 composites. The peaks located at about 458 and 464 eV are corresponding to Ti 2p3/2 and Ti 2p1/2, respectively. SEM (Figure a, b) show that the prepared TiO_2 exhibits nanotube morphology with a thickness of 2.1 µm and an average diameter of 150 ± 20 nm. TiO_2 nanotube grows along with the direction [001] as demonstrated from TEM images (Figure c,d) and high-resolution TEM (HRTEM) image confirms that the surface is (110) facet. From operando ICP-MS setup (Figure f), we can measure the dissolution of TiO_2 during PEC process. Through this method, the operation parameters of PEC on photoelectrodes can be controlled to elucidate sufficiently by quantifying dissolved metal ions of photoelectrode surface in the electrolyte during the whole PEC process in real time.
Conclusions
In summary, our work demonstrates that electron microscopy characterization together with operando ICP-MS plays an important role in investigating the intrinsic mechanism of the improved

PEC performance and photostability of TiO_2. The technique will be strongly needed in our future research and offers significant guidance in (photo)electrochemistry field.
Keywords
TiO_2, electron microscopy, photocorrosion, photoelectrochemistry
Graphic
Figure 1. SEM images (a, b), TEM images (c, d), HRTEM image (e) and operando ICP-MS setup (f).

Keywords:

TiO_2, electron microscopy, photocorrosion, photoelectrochemistry

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Advancing the real-time regime for virus self-assembly processes using liquid-electron microscopy

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IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

Background
Determining the exquisite features of flexible biological assemblies remains a challenge in field. Although it is often neglected in high-resolution interpretations, dynamic information holds the secret to how molecules perform their essential duties. For small polymers, NMR studies paired with molecular dynamic (MD) simulations are a powerful combination. Microfluidic imaging techniques based on liquid-electron microscopy (liquid-EM) principles can fill this gap for larger macromolecules and soft polymer assemblies, pushing us closer to seeing live events in the sub-nanometer range.

Methods
Liquid-EM experiments were performed using a Thermo Fisher Scientific Talos F200C transmission electron microscope (TEM), operating at 200 kV under low-dose conditions. Non-infectious and de-identified SARS CoV-2 sub-viral assemblies were examined using a variety of specimen enclosures including ultra-thin Silicon Nitride (SiN) and carbon-based enclosures. Multi-framed movies and images were recorded over different timeframes and magnification ranges, and the output data was analyzed.

Results
Non-infectious SARS CoV-2 components were enriched from de-identified COVID patient serum and verified using SDS-PAGE and Western blot analysis. The enriched samples showed that Spike protein, Spike protein fragments, and Nucleocapsid (N) protein were present. Protein fractions were prepared in HEPES buffer solution (pH, 7.5) and placed in microchip enclosures using the thin-film hybrid approach. Liquid specimens were hermetically sealed using autoloader clips employed for automated cryo-EM preparation. Images of enriched SARS CoV-2 proteins and RNA in solution showed self-assembly processes happening within 72-hours at room temperature. The small black components in the background of the images were N proteins along with Spike proteins and other unidentified fragments. These individual items (~5 nm) were smaller in comparison to the larger sub-viral particles (~100 nm or greater) that assembled over time. The final complexes formed at the 72-hour time point are consistent in size and shape with full RNA-nucleocapsid particles that comprised the SARS CoV-2 virus. We posit these particles have high contrast with respect to the surrounding liquid due to their RNA content. Slices through a putative 8.25-Å structure of the larger assemblies showed internal features of the protein and RNA subunits. Formed particles were also be treated with over-the-counter substances to visualize disassembly steps (DiCecco et al, J. Vis. Exp, 2022).

Conclusion
The testing of alternative substrates and enclosures helped improve knowledge of biological processes in liquid droplets. We visualized processes involving sub-viral particles having features consistent nascent SARS-CoV-2 assemblies. Spike remnants on the particle surfaces were often visibly

although some particles lacked these features. White bubbling in the samples ensured the presence of the liquid layer and samples could be preserved up to 6 months. Chemical mapping experiments confirmed the presence of phosphate in the particles that were also coated with calcium. While these experiments are an excellent start to monitoring assembly pathways, additional work is needed to understand how building blocks form larger units, and to test how chemical reagents may affect these processes.

Keywords:

Liquid-EM, SARS-CoV-2, self-assembly, spike, nucleocapsid

Reference:

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Effects of corticosterone and amyloid beta on the corticosteroid receptors in organotypic brain slice cultures

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Poster Group 1

Background
Stress is accepted as an important risk factor for Alzheimer's disease (AD) (1,2).High levels of cortisol, which is the stress hormone, in the cerebrospinal fluid (CSF), plasma and serum of Alzheimer's patients and the decrease in hippocampus volume and declarative memory disorders in patients with major depression and chronic corticosteroid treatment give rise to thought for a possible relationship between AD and stress (2,3,4). This suggests that the increase in glucocorticoid levels, which occurs as a result of stress, may participate in the formation of pathological mechanisms seen in AD, and raises the question of whether stress triggers the pathways that cause neurodegeneration or not.
Methods
In the study; in organotypic brain slice cultures, which allows to obtain results closest to in vivo models by protecting tissue architecture and microenvironment, circadian rhythm and stress models with corticosterone application and Alzheimer-like model with amyloid beta 1-42 (Aβ1-42) peptide application were generated. The effects of these treatments on glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) expressions, which are corticosteroid receptors, were investigated at both mRNA and protein levels. Regional localizations of these proteins in the brain were also examined by immunofluorescence method.
Results
As a result of our study, we found that GR and MR expression levels and their localizations in the hippocampus region changed both in circadian rhythm and stress models created by corticosterone applications and in AD-like models created by amyloid beta 1-42 application, and we determined that these changes differ depending on the dose. When we evaluate the findings of corticosterone and Aβ1- 42 applications we obtained in our study together, we observed that GR protein level, and GR localization in the hippocampus, especially in the stratum pyramidalis region, were similarly affected.
Conclusion:
Our findings point out that the increase in corticosterone caused by stress may be involved in the formation of pathological mechanisms seen in AD.

Keywords:

Alzheimer's disease,corticosterone,organotypic brain slice cultures

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Morphological investigation of periodic structures created by focused ion gallium beam

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Poster Group 2

Background incl. aims
Surface periodic structures on solids by ion-beam irradiation were first observed in the early second half of the 20th century. The initial observation, in parallel with the investigation of ion techniques, was followed by several other experimental works and theoretical descriptions based on ion-material interaction. Some developed models in this field can predict the morphological properties of the periodic structures as a function of some parameters of the ionic irradiation. In our experimental work, periodic structures were created on silicon surface by a low-energy focused ion beam at different incidence angles. The created morphology of periodic structures was compared to the predictions of the most cited Bradley-Harper (BH) model. Based on the experimental work, one of our goals is to explain the deviation from the BH model. In addition, the material structural and optical properties of the periodic patterns were also investigated by some spectroscopic measurement techniques. These measurements were used to determine the amorphization of the structured surface layer. Moreover, the reflectances of the structured surfaces were determined as a function of the applied ion incidence angle. Another goal of our work was to achieve a controlled modification of the reflection on Si surfaces by low-energy ion irradiation.

Methods
Ion irradiation was performed on polished Si (111) by focused 2 keV gallium ion beam (FIB) at incident angles between 60° and 80°. The morphology of the irradiated Si surfaces was investigated by scanning electron microscopy (SEM) and atomic force microscopy (AFM). To determine the wavelength of the structured periodic patterns, the power spectral density (PSD) function calculated from a 2-dimensional Fast Fourier Transformation was used. The position of the first peak of the PSD function was used to determine the characteristic periodicity of the structured surface. Spectroscopic elllipsometer (SE) and reflectometer were used to study the material structure of the structured Si layers.

Results
Regular ripple formation was created at ion incident angles between 60° and 72.5°. The wavelength of the ripple patterns was measured between 38 nm and 60 nm. The trend of the measured wavelengths showed exponential growth in the mentioned range. The regular periodic structure broke up above 72.5° ion incidence angle and turned into a smooth surface up to 80°. The wave vector of the ripple formation was parallel to the incident ion beam. The rotation of ripples did not appear in the range of 60° and 80° ion incidence. This observation is not consistent with the predictions of the BH model. The amorphised layer thicknesses of the strutured surface were determined between 3.8 and 7.9 nm in the range of 60° and 80° ion incidence by SE. Furthermore, the reflectances of structured surface was measured in the range of 300 nm and 900 nm wavelength

of light. According to the measured results, a linear connection was observed between the reflectance and ion incident angle for 460 nm wavelength of light.

Conclusion
Periodic structures were created by low-energy focused ion beam on Si (111) in the range of given angles. The characteristic sizes of the formed structures could be controlled within a few of tens of nm by changing the ion incident angles. Based on the meaured morphology results, explaination was given for deviation from the prediction of the BH modell. The amorphization of irradiated surfaces was modified with different ion incidence angles. Furthermore, the reflectances of Si surfaces was fine-tuned in 460 nm wavelength of light by low-energy ion irraidation.

Keywords:

ripple formation, ion bombardment, silicon

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Cellulose-based Nanocomposites for Sensor Applications: Characterization and Performance Evaluation

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Poster Group 2

Background including aims: Cellulose, an abundant and renewable natural polymer, offers extensive opportunities for diverse applications in materials science due to its favorable properties [1,2]. In this context, nanocomposite materials combining cellulose or its derivatives with nanoparticles such as Fe_3O_4, Au, Ag, and TiO_2, emerge as versatile platforms for sensor development. For instance, the characteristics of nanocellulose-water interactions can be exploited for the fabrication of flexible humidity sensors [3]. This study aims to create nanocomposite sensors specialized for detecting humidity and volatile organic compounds (VOCs) exploiting the synergistic effects of nanoparticles within the fibrillated nanocellulose or cellulose acetate matrix. Additionally, we investigate the potential of these nanocomposites for UV photodetection in flexible sensor systems.
Methods: We utilize different approaches for synthesizing fibrillated nanocellulose, cellulose acetate, nanoparticles of Fe_3O_4, Au, Ag, or TiO_2, as well as the related nanocomposites, aiming to integrate nanoparticles into flexible cellulose matrices [4]. Advanced electron microscopy techniques including conventional and low-dose Transmission Electron Microscopy (TEM), high- and low-vacuum Scanning Electron Microscopy (SEM), Focused Ion Beam (FIB) sectioning and Energy-Dispersive X-ray Spectroscopy (EDX) mapping are employed for precise characterization of nanocomposite morphology, structure and nanoparticle distribution. Humidity and VOC sensing experiments, along with UV photodetection tests, are conducted in the custom-built sensing system to evaluate sensor performance.
Results: TEM analysis reveals the precise morphology and dispersion of nanoparticles within the cellulose matrix at the nanoscale, highlighting the homogeneity crucial for sensor efficiency. SEM results, including cross-sectional profiling, provide detailed microstructural insights and, in combination with EDX, elucidate the spatial distribution of nanoparticles. Our nanocomposite-based sensors demonstrate notable sensitivity, selectivity, and response time towards varying humidity levels and diverse VOCs, as well as promising UV detection capabilities [5].
Conclusion: This study advances nanocomposite-based sensor technology by exploring the complex relationship between nanoparticle composition, nanoparticle distribution within cellulose matrix, and sensor performance. The findings underscore the potential of these multifunctional nanocomposites for diverse sensor applications, including humidity, VOC, and UV detection in flexible sensor platforms, thus paving the way for next-generation sensing technologies.

Keywords:

Nanocellulose, Metallic Nanoparticles, Nanocomposites, Sensors

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Functionalization of Single-Walled Carbon Nanotubes Analyzed by Spatially-Resolved EELS

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Poster Group 2

Background incl. aims
Surface functionalization of 1D and 2D nanomaterials is a perfect way for controlling their properties [1-5]. Very detailed structural and chemical composition analyses, at the atomic scale, of such surface modifications are required in order to determine their impact on the electronic/optoelectronic properties. Transmission electron microscopy (TEM) and in particular, spatially-resolved electron energy loss spectroscopy (SR-EELS) developed in an aberration-corrected TEM, is the most powerful technique to get this information. Indeed, having access to a close to 1 angstrom electron probe, the atomic configuration and concentration of the different species of these functionalized nanomaterials can be obtained [1-5]. In this contribution, we report an in-depth study of the atomic configuration of covalent and non-covalent functionalized (pi-stacked and endohedral) single-walled (SW) C-NTs via SR-EELS [5].

Methods
These studies have been developed via spatially-resolved EELS performed using a liquid-nitrogen holder (−170 °C) and Thermo Fisher Scientific Titan Low-Base microscope, working at 80 kV. This microscope is equipped with a Cs probe corrector, a monochromator and an ultra-bright XFEG electron source. The HRTEM studies have been carried out in a Thermo Fisher Scientific Titan Cubed microscope (equipped with a Cs image corrector), under the same cryogenic conditions (−170 °C) and at 80kV.

Results
We have investigated different systems of functionalized NTs (covalent and non-covalent (endohedral and pi-stacked)), for getting local information about these different hybrid configurations, studying the chemical environment and bonding of the NTs and the organic moieties [2-5]. Figure 1 illustrates some of these results, in particular the case of endohedral functionalization: iron-phthalocyanine (Fe-Pc) moieties in single-walled nanotubes [5]. Figure 1 (a) corresponds to a HRTEM image of one of these individual filled single-walled C-NTs. Figure 1 (b) displays an atomic-sketch, showing the supramolecular order of the Fe-Pc within a SWNT and Fig. 1 (c) shows an atomic-sketch of one of these Fe-Pc molecules (a Fe atom is surrounded by 4 pyrrolic-like subunits). As it is well known, these NTs tend to be organized in bundles. Figures 1 (d) and (e), which correspond to of STEM (BF and HAADF) micrographs, show one these bundles of SW-CNTs. A 24x12 EELS spectrum-image (SPIM) has been recorded in the green marked area on one of these NT. (f) EEL spectra extracted from the squared regions marked in Fig. 1(e). They correspond to four EEL spectra each of them. C-K, N-K and Fe-$L_{2,3}$ edges are clearly seen. From these spectra, nitrogen and iron elemental maps can be obtained, see Fig. 1 (g) and (h). These studies reveal the supramolecular organization of

the organic moieties (in this present case showed in this figure, iron-phthalocyanines) used for the functionalization of the NTs [5].

Conclusion
In summary, these works provide very rich information about these hybrid and complex nanomaterials, opening fascinating perspectives for optoelectronic applications of such nano-systems. All these aspects will be discussed in this contribution.

Graphic - Figure: (a) HRTEM micrograph of a SW-CNT filled with iron-phthalocyanine (Fe-Pc) moieties. (b) Atomic-sketch showing the supramolecular order of these Fe-Pc entities within a SW-NT. (c) Atomic-sketch of a Fe-Pc (a Fe atom is surrounded by 4 pyrrolic-like subunits. (d)-(e) BF- and HAADF-STEM images of a bundle of filled SWNT. A SPIM-EELS has been recorded in the green marked area. (f) EEL spectra extracted from the squared regions marked in Fig. 1(e). They correspond to 4 EEL spectra each of them. C-K, N-K and Fe-L2,3 edges are clearly seen. (g)-(h) N and Fe elemental maps. [5]

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Keywords:

Functionalization, Nanotubes, Cryo-TEM, STEM-EELS, HR(S)TEM

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Electron beam manipulation with auto-ponderomotive potentials for interaction-free measurements

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Poster Group 1

Background incl. aims

In many electron microscopy applications, beam damage is a serious issue, often preventing true single particle imaging with atomic resolution to date. When one and the same electron interacts with the sample multiple times, the extracted information can be increased for the same amount of damage [1, 2, 3]. This is at the core of what is known in quantum mechanics as interaction-lean or also interaction-free or measurement (IFM). This concept has been demonstrated to work well with light [6]. We aim at realizing this approach with electrons.

The IFM concept was introduced by Elitzur and Vaidman [1]. Recently, a first demonstration experiment with electrons in an interferometer scheme but without multipassing was reported [4]. To increase the scheme's efficiency substantially, a resonator structure is needed to enable repeated electron-sample interrogation [5]. A quantum electron microscope can be set up in different ways [5], all of which are technically highly demanding. We focus on auto-ponderomotive structures acting as electron guide, beam splitter and resonator. We demonstrated electron beam guiding and splitting [6, 7], as well as 7 round trips in an auto-ponderomotive electron resonator [8]. The current state of the experiment will be reported.

Methods

Auto-ponderomotive structures rely on electrodes applied with alternating DC voltages. In the frame of the swift electrons, an oscillatory potential results forming transverse guiding and beam splitting potentials. We experimentally demonstrated electron beam guiding as well as electron beam splitting in a conventional Philips XL30 SEM at energies up to 9.5 keV and 1.7 eV respectively. Our auto-ponderomotive structures were aligned towards the electron beam with a 5D stage. The electron distribution after the auto-ponderomotive structures (guiding and splitting) was imaged with a micro-channel plate detector via fluorescence detected by a camera (Fig. 1).

Furthermore, we designed a 87.95 mm long resonator, consisting of a central auto-ponderomotive guiding structure [6] and two switchable electron mirrors acting as "barn doors" at the entrance and exit of the resonator. We laser-triggered electron bunches of a nanometric tungsten tip and used deflectors and electron optical elements to steer them into our resonator. The electron barn doors could be opened and closed within nanoseconds at a variable delay towards the laser triggering event. Varying the delay of the outcoupling mirror towards the delay-line detector resulted in detection of up to 7 round trips (Fig.2).

Results

We show successful electron beam guiding as well as electron beam splitting of up to 1.7 keV. In a separate setup, we could demonstrate up to 7 round trips in our auto-ponderomotive resonator. All three modalities create new possibilities for enhanced electron beam control needed for an interaction-free measurement with electrons in a future quantum electron microscope.

Conclusion

We demonstrated controlled electron beam guiding and resonated 50 eV electrons for up to 7 round trips. In addition, we show an electron beam splitting auto-ponderomotive layout for 1.7 keV electrons and electron guiding up to 9.5 keV.

Keywords:

Interaction-free-measurement, quantum-electron-microscope, electron-beam-splitter, electron-resonator, auto-ponderomotive-potentials

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Microstructure and mechanical properties of AlCu thin films in a wide range of composition

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Poster Group 2

Background incl. aims
Copper is a common additive in bulk aluminum alloys, where its effect on phase composition and strength of the alloys is well documented. Thin films, however, often contain non-equilibrium phases, which may change significantly the mechanical properties of the material. AlCu thin films in a wide range of composition have been less discussed in the literature, as the existing papers are mainly concentrating on the cases of small alloying concentration. In this work, we aimed to study systematically the microstructure and mechanical properties, as well as to understand their correlations in the case of AlCu thin films having compositions widely changing.
Methods
Thin films were deposited by dual DC magnetron sputtering of Al and Cu targets. Applying the micro-combinatorial technique [1] 15 Al1-xCux films with different compositions ($0 \leq x \leq 1$) were deposited within a single experiment (under the same vacuum conditions) on a single Si substrate. Depth sensing indentation (DSI) measurements were conducted to reveal the hardness and the deformation mechanism of the layers. Cross-sectional lamellas were prepared from each layer by the focused ion beam (FIB) technique, and then investigated by transmission electron microscopy (TEM) techniques, including: bright field, dark field and HRTEM imaging, selected area electron diffraction (SAED), TEM energy dispersive spectroscopy (EDS), scanning TEM (STEM).
Results
Layers near to the equiatomic compositions (Al59Cu41, Al48Cu52, Al39Cu61) are built up from crystalline columns of ~30-40 nm in diameter. At these compositions the SAED measurements show the presence of the Al4Cu9 crystalline phase, which differs from that predicted by the equilibrium phase diagram. Lamellas prepared from the indentation imprints also indicate the presence of deformation bands, suggesting an amorphous like deformation behavior. This is coupled with an exceptional hardness of ~ 16 GPa [2] and a step-like indentation curve during the loading stage of the indentation.
The pure Al and Cu layers also possess small grain-size (~300-400 nm) structure, coupled with an increased hardness compared to their bulk counterparts. Furthermore, indentation process taken on these ultrafine-grained thin film do not show the size effect often observed in the case of bulk materials. The absence of the indentation size effect may be the consequence of the deformation mechanism as intensive grain boundary sliding (GBS) is revealed in these layers by both surface SEM and cross-sectional TEM images.
Conclusion
Composition-dependent mechanical and structural properties of AlCu alloy thin films were determined over the entire alloying concentration range. The decreasing grain size and the appearance of non-equilibrium phases are in good agreement with the increasing alloying concentration - increasing hardness trend. The presence of GBS suggests a mitigation of the classical dislocation-driven plastic deformation mechanism, which is compatible with the absence of an indentation size effect.

Keywords:

AlCu thin films, TEM, nanoindentation

Reference:

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Enhanced Visible Light Photocatalytic Activity of Cu-Doped ZnO ALD Films Deposited on Porous Substrates

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Poster Group 2

Background incl. aims
Industrial development contributes significantly to environmental pollution, especially from organic pollutants, which underlines the urgent need for global wastewater recycling. Over the last two decades, extensive research has been conducted on the photocatalytic degradation of pollutants in wastewater [1]. This process begins with the absorption of light in the semiconductor. This generates free electrons and holes that react with oxygen and water on the semiconductor surface to produce chemically reactive products capable of degrading pollutant molecules into less harmful compounds [2].
ZnO, a wide bandgap semiconductor photocatalyst, facilitates photocatalysis under UV light exposure. Current research focuses on tailoring the energy bandgap and improving visible light absorption, which is often achieved by doping the semiconductor with transition metal atoms [3]. Copper is a promising candidate for doping ZnO as it is able to replace Zn in the matrix, creating native point defects such as oxygen vacancies, which significantly affect the material properties [4]. The effectiveness of photocatalysis is closely linked to the active surface area of the catalyst, which has led to a strong interest in the use of powders or colloidal suspensions of nanoparticles. This interest stems from their remarkable surface area-to-volume ratio, which enhances the availability of surface states that serve as reaction sites. However, the separation of nanoparticles from solution poses a major challenge. The development of highly efficient photocatalytic materials for water treatment must therefore be optimized to achieve practical benefits.
Both high surface area and high optical efficiency are essential to achieve increased photocatalytic activity in metal oxide films. Polycrystalline thin films deposited on porous substrates are promising candidates in this context, as they serve as effective photocatalysts without the need for post-treatment. They also facilitate complete recovery after the experiments. In order to take advantage of Cu-doped thin ZnO films for visible-light photocatalysis, different fabrication techniques have been used. In addition, atomic layer deposition (ALD) proves to be a promising technique for the growth of semiconductors or insulating thin films as it allows precise control over the film thickness and deposition on different substrates. [5].
Our goal is to find a photocatalyst that exhibits enhanced photocatalytic activity under UV and simulated sunlight irradiation. We achieve this by manipulating two parameters: the band gap of the ZnO films by doping with Cu atoms and the active surface area of the sample.

Methods
In this study, we have used the ALD technique to synthesize thin ZnO films and Cu-doped ZnO films on flat or porous silicon wafers. All pure ZnO samples were grown in a Beneq TSF 200 system at 180 °C using diethylzinc (DEZ, $Zn(C_2H_5)_2$) and distilled water (H_2O) as zinc and oxygen precursors, respectively. The source of copper in the ALD-grown ZnO samples doped with Cu was copper (II)

acetate ($Cu(OAc)_2$), evaporated at 180 °C from a hot source into the reaction chamber with nitrogen as carrier gas. The influence of the Cu dopants on the surface morphology and topology of the samples was investigated by scanning electron microscopy (SEM), the penetration depth of the ALD films into the pores was determined with focused ion beam (FIB), the crystallinity was investigated by grazing incidence x-ray diffraction (GIXRD). In addition, x-ray photoelectron spectroscopy (XPS) was used to analyze the chemical state of the Cu atoms and secondary ion mass spectrometry (SIMS) to determine the distribution of Cu atoms in the films. Finally, the photocatalytic activity was investigated by measuring the degradation rate of methylene blue (MB) in an aqueous solution under a UV and simulated sunlight irradiation.

Results

The ALD synthesis of ZnO, in which diethylzinc (DEZ) and water (H_2O) are used as precursors, usually leads to polycrystalline films. Size, shape and crystal orientation of the ZnO grains are influenced by deposition parameters such as temperature, pulsing and purging times of the precursors and substrate type. Our analysis starts with microscopic SEM images of undoped and Cu-doped ZnO samples on flat and porous substrates. The different surface morphologies observed in our samples correspond to variations in grain orientations within the films, as shown by the XRD patterns of the ALD-grown films. The penetration depth of pure ZnO and Cu-doped ZnO films on porous substrates was determined by examining the cross-section of the film profile within the pores using FIB.
The oxidation state of the copper was determined by XPS analysis around the Cu 2p core levels. The XPS analysis revealed two sharp and symmetrical peaks without satellite lines, demonstrating the presence of Cu+ states in the Cu-doped sample. In addition, the SIMS measurements determine the in-depth distribution of Cu dopants. The concentrations of Zn and Cu appear to be consistent throughout the film, with a sharp drop at the interface between the ZnO film and the Si substrate. Finally, we investigated the photocatalytic activity of pure and Cu-doped ZnO films grown on flat and porous substrates under UV light and simulated sunlight. For this purpose, we immersed the samples in an aqueous solution of methylene blue (MB) and monitored the degradation rate of MB. First, we investigated the photocatalytic activity of pure ZnO films grown on both flat and porous substrates under UV light. The porous sample showed many times higher photocatalytic activity than the flat sample. We then investigated the photocatalytic activity of Cu-doped ZnO films on flat and porous substrates under simulated sunlight irradiation. The increased surface-to-volume ratio improves the availability of surface sites, which significantly enhances the photocatalytic activity of the porous sample.

Conclusion

In summary, we have investigated the structural and photocatalytic properties of flat and porous ZnO films doped in situ with Cu during the ALD process. Our results shows that ZnO films synthesized on porous substrates exhibit better photocatalytic performance under UV light than those grown on flat substrates. In addition, the Cu-doped compared to the Cu-doped films on flat substrates. By doping the ZnO ALD films with Cu atoms, we successfully shifted the photocatalytic activity from UV to visible light. In addition, by increasing the surface-to-volume ratio, we have significantly increased the photocatalytic activity.

Keywords:

ZnO, thin films, photocatalysis, ALD

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Examining dislocation and stacking faults within Hybrid MBE-Grown (110)-oriented RuO2 films

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Poster Group 1

Background

Thanks to its high electrical conductivity and exceptional thermal and chemical stability, rutile ruthenium dioxide (RuO2) has attracted significant attention across a range of applications in energy storage and microelectronics [1]. Research indicates that RuO2 behaves as a Dirac nodal line semi-metal with flat band surface states, and it theoretically demonstrates a novel crystal Hall effect [2], thus reasserting itself as a quantum material [3]. Particularly intriguing is the rutile phase of ruthenium dioxide (RuO2), which epitaxially oriented along the (110) direction on rutile titanium dioxide (TiO2) substrates, displays remarkable anisotropic behavior, possibly originating from a 2.2% tensile strain along the [1$\bar{1}$0] direction and a 4.9% compressive strain along the [001] direction. This anisotropy, stemming from the interaction between crystallographic orientations and epitaxial strain, provides fertile ground for both fundamental research and technological exploration [4,5].
Transmission electron microscopy (TEM) researchers to probe the atomic-scale structure and defects at interfaces with unprecedented precision. In this context, the interface between RuO2 and TiO2 in (110)-oriented films has garnered considerable attention.
In this work, we characterize (110)-oriented RuO2 films on TiO2 substrates using TEM techniques. Specifically, we focus on the detection and analysis of dislocations and stacking faults within the RuO2 film, as well as the variations in defect density along different in-plane directions.

Methods
An 11 nm Hybrid MBE-grown rutile film of RuO2 oriented along (110) on rutile TiO2 (110) substrate was used in this research. Transmission electron microscopy (TEM) samples were prepared using a Focused Ion Beam Scanning Electron Microscope (FIB-SEM) equipped with a Ga ion gun (Thermo FEI Helios 5 FIB). A tungsten (W) and carbon (C) film were deposited on the area of interest before FIB milling to protect the surface from ion damage. A double-corrected TFS Spectra Ultra HRTEM/STEM (operated at 300 keV) is used to acquire high annular angle dark field (HAADF) micrographs and Energy-dispersive X-ray spectroscopy (EDS).

Results
Figure 1 displays High-Angle Annular Dark Field (HAADF) micrographs across the RuO2/TiO2(110) interface along [001] and [1$\bar{1}$0] zone axes. The accompanying EDS elemental maps in Fig. 1 revealed the presence of a Ru-oxide film on the surface of TiO2. Inverse FFT techniques were used to unveil defects during atomic-scale investigations. Interestingly, it can be observed from Fig. 1(b) and (c) that there are no defects at the RuO2/TiO2(110) interface along the [001] zone axis. However, various defects become apparent at the interface when changing the zone axis from [001] to [1$\bar{1}$0].
Severe lattice distortions were observed at the interface between RuO2/TiO2 (110) along [1$\bar{1}$0] zone axis. In the corresponding enlarged lattice, dislocation loop, stacking faults, and dense dislocation

arrays were observed. All were caused by strain induced lattice distortion in [1$\bar{1}$0] direction. An exploration as the origin on these defects and their confinement to the tensile strain zone axis is underway.

Conclusion

Aberration-corrected scanning transmission electron microscopy (STEM) operating at 300 keV was employed to analyze the atomic-scale interface of RuO_2 and TiO_2 (110) along both the [001] and [1$\bar{1}$0] zone axes. Utilizing the inverse FFT method, various dislocations and defects were identified. Specifically, dislocation arrays and dislocation loops were observed within the RuO_2 film along the [1$\bar{1}$0] zone axis. Furthermore, stacking faults were observed near the RuO_2/TiO_2 interface along the [1$\bar{1}$0] zone axis, potentially attributed to strain effects.

Acknowledgements
The authors would like to thank US AFOSR for financial support of this work, and the Canadian center for Electron Microscopy (CCEM) for their technical support.

Keywords:

STEM, RuO_2, TiO_2, Dislocations

Reference:

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On the accuracy of atomic-resolution DPC-STEM measurements

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Poster Group 2

Background
The study of prevalent defects and their impact on functional properties is a critical issue for the successful implementation of 2D materials in next generation devices. However, the direct quantitative characterization of a specific defect is a challenging task, requiring atomic resolution measurements that are sensitive to the electronic structure of the material. The use of differential phase contrast (DPC) in scanning transmission electron microscopy (STEM) is particularly promising for this purpose, leveraging the natural coupling between the electron probe and the atomic-scale electric fields within a sample, which leads to a redistribution of intensity at the diffraction plane. For thin specimens, the centre of mass (CoM) of the diffraction disc is then directly related to the phase gradient, allowing for the mapping of electrostatic forces between atoms of the material structure, a capability which has already been demonstrated in the study of several 2D materials [1–4].

CoM-based measurements can be performed with pixelated detectors, capable of recording the entire diffraction disc, or with segmented detectors that integrate the intensity of the disk over independent quadrants. Pixelated detectors offer the greatest accuracy, but the faster processing times associated with segmented detectors allow for the generation of DPC-STEM images in real time, making it easier to identify interesting features and to correct electron lens aberrations. Additionally, series acquisitions and larger fields of view become more manageable, greatly aiding in the observation of easily damaged 2D materials. These benefits, along with the fact that segmented detectors are still the most accessible option, make it worthwhile to explore methodologies to manage their reduced accuracy. For this reason, it is essential to understand the precise effect of instrumental parameters on segmented-detector DPC STEM observations, such that their influence may be accounted for when analysing the results.

Methods
In this work, the influence of key instrumental parameters – probe convergence angle, defocus and detector orientation – on the retrieval of atomic-scale electrostatic field and potential configurations obtained from COM-based images is analysed, by determining the qualitative and quantitative changes induced by each of the parameters. This analysis is based on extensive multislice simulations of DPC- and COM-STEM images, in which the impact of each parameter under study is evaluated independently. Due to the complications of directly interpreting and comparing the vectorial electrostatic field images, these are analysed by representing the vector field in scattergrams [5] – polar graphs where the COM vectors associated with the pixels of the original image are plotted as points (Figure 1). This representation allows us to readily evaluate and compare the maximum magnitude of the measured field configurations in every direction, as well as to more easily detect qualitative changes. The scalar electrostatic potential images are directly compared through relative

difference maps that show the increase or decrease in magnitude at every pixel of the imaged region, allowing for a more spatially detailed analysis.

Results
Analysis of the influence of convergence semi-angle shows how an increased angle leads to higher susceptibility to the aberrations studied, despite the improved resolution. In the case of defocus, a maximum relative change above 30% is determined in the electrostatic field images for an angle of 30 mrad, at the limit of image interpretability, whereas the impact grows at a slower rate with increasing defocus for angle of 21 mrad. Additionally, overfocus primarily affects quantification, while underfocus results in more qualitative changes to the electrostatic configurations, albeit with a reduced quantitative impact. Two-fold astigmatism has a more pronounced impact in the field configurations, with maximum relative magnitude changes higher than 60% and stronger qualitative effects. The influence of this asymmetric aberration is shown to have a resonance effect with the orientation of the segmented detector, varying the identified magnitude of quantitative and qualitative impacts.
The electrostatic potential images show the same trend as that identified in the field configurations; however, the average relative changes identified are much lower, demonstrating an improvement in image quality due to the integration applied.

Conclusions
Following the procedure described, the relative quantitative and qualitative changes in electrostatic field and potential images due to the influence of key experimental parameters are identified. Differences in impact between imaging at over- and underfocus are determined and the more complex impact of asymmetric aberrations is analysed by considering the influence of two-fold astigmatism, identifiying a resonance effect with the orientation of the segmented detector.

Keywords:

DPC-STEM; multislice image simulations; aberrations

Reference:

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Analysis of photon bunching in coherent cathodoluminescence

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Poster Group 2

Background
Cathodoluminescence (CL) microscopy, utilizing an electron beam of an electron microscope, is a powerful approach for accessing nanoscale optical information of materials and devices. Furthermore, the time correlation measurement of CL by the Hanbury-Brown=Twiss (HBT) interferometer enables lifetime analysis without pulsing the electron beam by utilizing the photon bunching features [1]. This CL-HBT microscopy has been applied in various ways, such as lifetime measurements of semiconductors and dielectrics [2] and evaluations of the Purcell effect [3]. The origin of the bunching in CL has been investigated through a semiclassical description [4] and descriptions starting from quantum master equation [5] and is understood to be due to the random time modulation of excitation event by the fast electrons. The CL process can be classified into two classes depending on their excitation mechanisms, namely coherent CL (CCL) and incoherent CL (ICL). In the CCL process, fast electrons directly generate photons, e.g., transition radiation or Cherenkov radiation. On the other hand, in the ICL, photons are indirectly excited through mediator particles, such as bulk plasmons or secondary electrons, thus involving relaxation processes. Examples of the ICL are emission from defects and semiconductors. It is theoretically expected that the photon bunching can be observed even in CCL as far as emission events of two or more photons are included. However, most discussions on photon bunching using CL-HBT focused on ICL, and few studies have been reported regarding CCL. In this study, we experimentally confirmed the bunching phenomenon in CCL. By formulating the correlation function, we propose a method to extract the photon statistics in individual excitation events, excluding the bunching enhancement effect due to the excitation timing modulation.
Methods
Considering the semiclassical model, where n-photons are excited by a single electron, split by a half-beam splitter, and then detected by two single-photon detectors, the correlation function can be formulated. The analytical formula includes the parameters of electron beam current, correlation time and inherent correlation factor which reflects the fluctuation of the number of photons excited by a single electron. In CCL, it is expected that the photon statistics follow the Poisson distribution, and the inherent correlation factor should equal to unity. We performed this statistical analysis for localized surface plasmon (LSP) radiation, which is one of the typical examples of CCL, using a silver nanosphere with a diameter of approximately 200 nm, as schematically shown in Fig.1 (a).
We utilized a scanning transmission electron microscope (STEM) equipped with a CL detection unit and an HBT interferometer [3]. The light emitted from the sample was guided out of the STEM column by a parabolic mirror to the HBT measurement system. We performed the measurement at room temperature at an accelerating voltage of 160 kV.
Results
From the HBT measurement of the LSP radiation, we observed photon bunching, as shown in Fig. 1(b). The correlation time is approximately 400 ps, which reflects only the temporal resolution of our instrument, indicating that the lifetime of LSP is extremely short and cannot be resolved. We can observe a trend where the bunching height decreases with an increase in the electron beam current.

Figure 1(c) shows an inversely proportional dependence of the correlation function on the electron beam current; such a tendency is described from our analytical formula. On the other hand, from Fig.1 (d), the values of the inherent correlation factors are approximately 1 for all the beam currents. This suggests that the photon statistics of LSP radiation follow the Poisson distribution, which is consistent with the theoretical predictions. Additionally, the independence of the inherent correlation factor on the electron beam current indicates that the pure photon statistics by a single electron excitation is effectively extracted by removing the effect of the time modulation of the excitation event by fast electrons.

Conclusions

Through the analysis of the correlation function obtained from the HBT measurement of CL, we successfully extracted the coefficient which reflects the fluctuation of the number of photons excited by a single electron. We believe that this method is useful to investigate the elementary processes of photon generation by fast electrons.

Keywords:

Cathodoluminescence, Photon statistics

Reference:

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The structural basis for energy extraction from air by Mycobacterium smegmatis

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background
Mycobacterium smegmatis is a heterotrophic, obligately aerobic soil-dwelling bacterium that requires organic carbon for growth. Due to fierce competition for resources in the soil, the bacterium often experiences deprivation of both oxygen and organic carbon sources. To cope with this, M. smegmatis has evolved to persist through metabolic flexibility, upregulating enzymes, such as the high-affinity [NiFe] hydrogenase Huc, that enable it to utilise electron donors and acceptors other than organic carbon and molecular oxygen. Huc provides electrons from H_2 to the cytochrome bcc-aa3 oxidase super complex via the quinone pool, which results in the generation of the proton motive force. The molecular details underpinning hydrogen oxidation in M. smegmatis were unknown. We aimed to characterise how Huc oxidises hydrogen and how the electrons liberated from this process are transferred to the respiratory chain.
Methods
In this work, we purified Huc natively from an M. smegmatis strain and visualised it by cryo-electron microscopy (cryo-EM). We used a wide range of techniques to characterise the enzymatic capabilities of this unique enzyme. Utilising an H_2 sensing electrode and gas chromatography, we quantitated the kinetics of hydrogen oxidation by Huc and the specificity of Huc to different electron acceptor molecules. Further molecular dynamics simulations and mass spectrometry enabled us to identify the specific electron acceptor substrate of Huc. To characterise how this enzyme differs from other low-affinity hydrogenases, we employed spectroscopic and electrochemical techniques.
Results
Our cryo-EM maps of Huc revealed an impressive enzymatic structure with electron density maps at a maximum resolution of 1.52 Å. Uniquely, Huc is an octamer of the large and small enzymatic subunits, with an additional novel membrane-associated central stalk. We showed that this stalk, called HucM, is critical for transporting the electron acceptor molecule menaquinone directly from the bacterial membrane to the enzyme. This long-range quinone transport is a unique feature of Huc. Further, we demonstrated for the first time a very high-affinity purified enzyme capable of oxidising atmospheric H_2 in isolation from the respiratory chain. In addition, we demonstrated that, unlike many other [NiFe] hydrogenases, Huc is insensitive to inhibition by oxygen.
Conclusions
This work represents the first molecular characterisation of a member of a novel family of hydrogenases capable of scavenging H_2 at atmospheric concentrations. This discovery will provide insights for the investigation and development of hydrogenase-based fuel cells that can operate at atmospheric levels of oxygen and use very small amounts of hydrogen to produce energy.

Keywords:

CryoEM, microbiology, enzymes, metabolism

Reference:

Grinter, R., A. Kropp, H. Venugopal, M. Senger, J. Badley, P. R. Cabotaje, R. Jia, Z. Duan, P. Huang, S. T. Stripp, C. K. Barlow, M. Belousoff, H. S. Shafaat, G. M. Cook, R. B. Schittenhelm, K. A. Vincent, S. Khalid, G. Berggren and C. Greening (2023). "Structural basis for bacterial energy extraction from atmospheric hydrogen." Nature 615(7952): 541-547.

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Simple and efficient three-dimensional reconstruction method of plant cells using transmission electron microscope

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Poster Group 2

Serial section transmission electron microscopy (ssTEM) is a vital technique in Volume Electron Microscopy (vEM) for unveiling fine structures of cells and tissues in three dimensions (3D). While the ssTEM provides superior X and Y resolution compared to other vEM, it is a challenge to acquire wide field of view images. Conventional mesh grids, for instance, often obscure the desired field of view with mesh bars. Even single-hole grids without bars, the support membrane remains susceptible to tearing and wrinkling, risking the loss of information. To address these challenges, we employed SiN Window Chip [1] for ssTEM. SiN Window Chip has no bars, ensuring uninterrupted observation without any missing information. In addition, its support membrane, made of high-strength silicon nitride film, minimizes the risk of tearing, distinguishing it from single-hole grids. We collected approximately 50 sections, each 70 nm in thickness, onto each of the five SiN Window Chips, resulting in a total of about 250 serial sections. The serial section images of a carrot leaf cell were obtained with a sample holder capable of simultaneously mounting multiple SiN Window Chips. For data acquisition, we used a new 120-kV transmission electron microscope (JEM-120i) equipped with a pole piece that provides high contrast. We attempted to undertake a segmentation analysis of organelles from the 177 TEM images.
Segmentation for annotating regions of interest continues to rely largely on manual methods. This task requires extensive time and effort, a challenge commonly encountered in other vEM as well. To streamline this task, we used convolutional neural networks (CNN) [2], a subtype of deep learning. Only a small number of images were required as training data; chloroplasts were manually segmented from just five images of 512x512 pixels (18.4 nm/pixel). Subsequently, a model was trained using deep learning based on these training data, and chloroplasts were inferred from all TEM images. The entire process, from model training to inference, took only an hour. In contrast, if performed manually, this process would take several weeks. As a result of 3D reconstruction of a cell body and chloroplasts, we gained insights into their shape, distribution, and volume.
Performing 3D reconstruction based on the inference results obtained through deep learning enables streamlined analysis of the shape and distribution of the target tissue. Additionally, the trained model can be applied to different serial section data. We successfully employed the model to other fields of view obtained from the same specimen. Therefore, we anticipate that this method can also reduce the time required for training data creation.

Keywords:

ssTEM
vEM
CNN
Deep Learning

Reference:

[1] Y. Konyuba et al., Microscopy, 67, 367-370 (2018)

[2] K. Konishi et al., Microscopy, 70, 526-535 (2021)

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Time-resolved TEM observation of CeO2 surface with electrostatic sub-framing system

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Poster Group 1

Time-resolved high speed TEM imaging technique is of great importance for in-situ observations such as heating/cooling, biasing, stressing, and liquid-cell experiments. Recently camera's frame rate is getting faster, but to dynamically visualize a structural change process of materials happen at a high speed, it is necessary to record TEM micrographs with a higher frame rate in the order of sub-millisecond.
Electrostatic type deflector can deflect the electron beam in tens of nanoseconds to microseconds. To using this property, there is a possibility to realize a high-speed imaging technique. In this study, we demonstrate a result of feasibility test for this system, electrostatic sub-flaming system developed by IDES Inc. This method improves temporal resolution by effectively increasing the frame rate while maintaining the same performance of the existing camera.
Fig. 1 shows schematic illustration of experimental setup. Electrostatic electron beam deflectors were installed beneath a projector lens cross-over in TEM column with a CMOS camera. The deflector itself acts as an aperture, limiting the area of the camera's sensor to which the electron beam is exposed, thus producing a small TEM image (sub-framed TEM image). By electrostatically deflecting the sub-framed TEM image at high speed in front of the camera sensor during exposure time, the images are laid out like 5x5 or 7x7 tiles. By doing this, each sub-framed image having a different time stamp. The difference between the timestamps of each sub-frame image is the time resolution.
Using this system, we observed time-series changes in (111) surface facet structure of CeO2 nanoparticles [1]. The data was recorded in 7x7 sub-frames. Since 49 sub-frames are recorded in one frame (CMOS Camera's exposure time is set to 40 ms), the exposure time per single sub-frame image is ~0.82 ms. The frame rate increased 25 fps to 1,225 fps. The 1st (0 ms) and 6th (4.08 ms) sub-frame TEM images are shown in Fig. 2. The atomic columns indicated by the arrows can be clearly seen in the 1st image, but are obscured in the 6th image. Motion of the atomic column of CeO2 (111) surface ware successfully observed in atomic level with a time scale of 4 ms by enhancing an effective frame rate of camera acquisition.

Keywords:

High-speed-imaging, High frame-rate, Sub-framed imaging

Reference:

[1] CeO2 Nano particles specimen: Courtesy of Johnson Matthey

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Continuous-flow LHe-cooling TEM sample holder with high stability at sub-10K and in situ biasing functionality

Penghan Lu[1], Denys Sutter[2], Dominik Biscette[2], András Kovács[1], Yan Lu[1], Joseph V. Vas[1], Michael Faley[1], Thibaud Denneulin[1], Damian Bucher[2], Amelia Estry[2], Johan Chang[2], Rafal E. Dunin-Borkowski[1]
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IM-04 (2), Lecture Theater 1, august 27, 2024, 14:00 - 16:00

Background incl. aims
Cryogenic transmission electron microscopy (cryo-TEM) has been significantly advanced in the past decade for imaging macromolecular protein complexes and cellular structures with close-to-atomic resolution in three dimensions [1]. Beyond life sciences, cryo-TEM has also enabled observation of otherwise inaccessible information in weakly bonded and reactive materials that typically degrade under electron irradiation or environmental exposure [2]. Furthermore, many of the exotic properties in quantum materials, such as, superconductivity, charge density waves, quantum hall effect, and topological behaviours, only manifest at extremely low temperatures [3]. Historically, a few top-entry TEM stages were developed allowing specimen temperature cooled down to even 1.5 K [4]. Those designs were seldom followed up because of the preference towards more flexible and versatile side-entry specimen holders, especially to introduce different kinds of external stimuli for in situ and operando studies. On the other hand, currently existed liquid helium (LHe) cooling side-entry holders are usually very difficult, if not impossible, to reach and maintain a long-time stability of the ultimately low temperature (close to LHe temperature, 4.2 K). This is firstly limited by their considerable mechanical and thermal instability, and, in addition, their base temperature hold time is typically short because of the low latent heat of LHe and the limited cryogen capacity of the dewar attached to the back end of the holder.
Methods
In this context, a new miniaturized continuous-flow LHe cryostat was designed for in situ cryo-TEM applications. This was initially created for X-ray diffraction experiments in pulsed magnetic fields and later adapted to TEM side-entry holders by condenZero, a spin-off company from the University of Zurich, with further test and optimization in cooperation with the Ernst Ruska-Centre (ER-C) in Research Centre Juelich. The full setup is illustrated in the enclosed figure. A purpose-designed vibration damping stage, consisting of a few active and passive damping units, is of vital importance to this system. It suppresses significantly the external vibrations from the floor and environment as well as from the boiling of cryogen during transfer. The LHe transfer line is developed to maximise its flexibility and thermal efficiency, which further minimises the vibration as well as the cooling loss during transfer. A gas He outlet port is spared on the transfer line to support He recovery in order to save the consumption of the valuable He resource. The transfer line, at its end, is docked to the specimen holder with a bellow connected in between for the final stage vibration isolation. The holder can host either standard 3mm TEM grids or MEMS chips for in situ and operando cryogenic measurements. At least 10 electrical feedthrough is integrated to this holder, with 4 for the built-in Cernox temperature sensor and 6 for electrical biasing or heating. The tip of the holder, where the specimen sits, is fully surrounded (except two holes of 3 mm in the top and bottom for electron beam transmission) by a thermal shield cooled at the same temperature as the specimen to avoid contamination build-up when the sample is cooled but also to suppress the heat radiation from the objective pole piece and other components that are at room temperature. This system is currently

installed on one of our image Cs-corrected microscopes, Titan HOLO, which is optimised for off-axis electron holography imaging (double biprisms with additional transfer lenses) as well as in situ and operando measurements (ultrawide pole piece gap). This microscope is recently upgraded with an ultrafast beam chopper and an event-driven delay-line detector, which would eventually allow time-resolved cryogenic biasing measurements.

Results

A typical cool-down curve is shown in the enclosed figure. Starting from room temperature, a base temperature of 5.2 K, measured by the Cernox sensor very close to the specimen position, was reached within one minute and kept stable with temperature fluctuation of +/- 2.5 mK over hours. Because of the continuous flow design as well as the highly efficient cryogenics that currently consumes only 2-3 liter LHe per hour, the ultimately low temperature can easily last for more than 24 hours with the 100 liter LHe dewar. We have tested this system with a few applications, including magnetic solitons, superconductors, dielectrics and soft matters, by measuring their defocused Lorentz imaging, off-axis electron holography or electron diffraction. As an example, which is shown in the enclosed figure, the high stability of this system at ultimately low temperature allows us to quantitatively map the magnetic states of Bloch skyrmions in B20-FeGe at 5.2 K with 200 mT out-of-plane magnetic fields using off-axis electron holography. Furthermore, we also tested the in situ biasing and heating capabilities based on different MEMS chips on this holder. With four-probe measurements, we can measure the electrical resistivity drop of superconducting structure across its transition temperature and can also heat up very locally the specimen from cryogenic to elevated temperature (above room temperature).

Conclusion

We have developed a new continuous-flow LHe-cooling holder for in situ and operando cryogenic TEM applications. It is featured by very swift cool-down and ultra-long cryo-hold time at ultimately low temperature with minimal thermal fluctuation as well as in situ electrical biasing and local heating functions. Further optimisation on this system will also be discussed during the presentation.

Keywords:

Liquid-helium, continuous-flow cryostat, cryo biasing

Reference:

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Revealing the origin of green light emission in Cs4PbBr6 by cathodoluminescence

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PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

Background
Metal halide perovskite is expected as next-generation material for the application for solar cells and light-emitting devices due to their excellent optical properties. Among them, Cs_4PbBr_6 has obtained significant attention as a luminescent material for its high-efficiency green emission and ease of synthesis. However, the mechanism of this green luminescence from Cs_4PbBr_6 has not been understood so far since the bandgap energy of Cs_4PbBr_6 lies in the ultraviolet range, which suggests that the green emission originates from intermediate states formed by impurities or defects within the host phase [1].
Previous discussions have proposed that $CsPbBr_3$ nanocrystals embedded in the Cs_4PbBr_6 host phase are the primary candidates for the green light source [1]. However, conventional photoluminescence measurements cannot spatially resolve such nanocrystals, and thus definitive evidence has been lacking. Recently, cathodoluminescence (CL) studies taking advantage of nanoscale spatial resolution achievable by electron excitation have reported the presence of microcrystals dispersed within the host phase [2]. However, detailed information of these microcrystals as well as their luminescence mechanism remains unclear, necessitating further elucidation of local optical properties.
In this study, we evaluated the nano-scale optical properties of Cs_4PbBr_6 powder samples using CL with scanning transmission electron microscopy (STEM). CL spectral and lifetime mapping analysis with a temperature dependence validated the inclusion of $CsPbBr_3$ nanoparticles within the Cs_4PbBr_6 host phase.

Methods
CL Measurement: Light emitted from the sample by electron irradiation was collimated by a parabolic mirror inside the microscope and directed toward the spectrometer for the CL spectrum measurement and a Hanbury Brown-Twiss (HBT) interferometer for the emission lifetime measurement. By the HBT measurement, one can measure emission lifetime without pulsing the electron beam. In these measurements, the light detection and electron beam scanning were synchronized, enabling CL spectral and lifetime mapping.
Sample Preparation: Cs_4PbBr_6 powder samples, were synthesized via a solution method. CsBr and $PbBr_2$ precursors were prepared and held in dimethyl sulfoxide (DMSO) for one hour. The precipitate, grown by the anti-solvent method, was washed with DMSO to obtain a powdered sample. X-ray diffraction (XRD) analysis confirmed only peaks corresponding to Cs_4PbBr_6 in the crystal structure.

Results
The secondary electron (SE) image of the measured powder is presented in Fig.(a). The CL image revealed the existence of bright spots with high luminescence intensity within the particle (Fig.(b)). Furthermore, the area-integrated spectrum showed peaks at emission wavelengths of 375nm,

520nm, and 700nm (referred to as Peak1, Peak2, and Broad Peak, respectively). According to previous studies, Peak1 is attributed to the optical transition of Pb ions originating from Cs_4PbBr_6 [3]. Broad peak can be attributed to emission derived from some other defects according to a radio-luminescence study by X-ray [4]. Peak2 is the green emission that we discuss in this study.
In the CL spectral mapping on the same measurement area that was conducted at low temperature (120K), the emission intensity of Peak1 exhibited a homogeneous distribution across the entire area, whereas Peak2 showed high intensity specifically in the bright spot regions (Fig.(c-d)). CL spectra were extracted from the region with single bright spot (Spot1), and regions without bright spots (Matrix), shown in Fig.(e). In all regions, the intensities of Peak1 and Broad Peak remained approximately the same, while the intensity of Peak2 changed depending on the presence of spots inside the signal extraction area in CL mapping (Fig.(d)). Particularly noteworthy is the significantly lower intensity of Peak2 in the Matrix region. On the basis of these findings, it is considered that nanoparticles forming the bright spots emit green light within the host phase of Cs_4PbBr_6.
To further investigate the optical properties of the green light emission, CL measurements were both performed at room temperature and low temperature (120K). It revealed that the emission wavelength is longer and the emission lifetime shorter at 120K than at room temperature. These trends are opposite to typical semiconductor materials but are consistent with $CsPbBr_3$ nanoparticles without a bulk host material [5]. This suggests that $CsPbBr_3$ nanoparticles surrounded by Cs_4PbBr_6 host phase are the origin of the highly effective green light emission.

Conclusions
In this study, we conducted nanoscale evaluation of the optical properties of Cs_4PbBr_6 using STEM-CL. By the spectral mapping, the presence of nanoparticles emitting green light within the Cs_4PbBr_6 host phase was observed. Furthermore, by measuring the spectra and lifetime under varying temperatures, it was confirmed that the characteristics of the green emission have the same tendency as bare $CsPbBr_3$ nanoparticles without a matrix. Thus, it is suggested that the highly effective green emission originates from $CsPbBr_3$ nanocrystals precipitated within the host Cs_4PbBr_6 material.

Keywords:

Cathodoluminescence
STEM
Perovskite
Lifetime

Reference:

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In-situ HAADF-STEM observation of phase transformation in microencapsulated Al-Cu-Si alloy

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Poster Group 1

Background incl. aims
The increasing relevance of latent heat storage through phase change materials (PCMs) in advanced thermal energy storage technologies is underscored by challenges such as deformation during thermal cycling. This issue is typically mitigated by encapsulating PCMs to create microencapsulated PCMs (MEPCMs) with high thermal conductivity, rigid structures, and improved durability.1 Despite these enhancements, hysteresis during solidification remains a concern. Recent innovations include a core-shell structure of Sn@α-Al2O3 MEPCM that lowers the energy barrier for nucleation, effectively reducing hysteresis through the formation of localized Sn nanoparticles on an α-Al2O3 nanoparticle.2 Expanding on this, we used an Al-Cu-Si alloy for a similar MEPCM structure, achieving notable hysteresis suppression. Unlike Sn, Al-Cu-Si does not volatilize, avoiding the formation of oxide byproducts over repeated thermal cycles. These MEPCMs were calcined at 1000°C in an O2 atmosphere, developing a dense α-Al2O3 shell. However, no localized nanoparticles were detected, prompting further in-situ HAADF-STEM studies to explore hysteresis mechanisms in Al-Cu-Si@α-Al2O3 MEPCM.
Methods
Al-Cu-Si alloy (m.p. = 529 °C, ϕ ≈ 38 µm, 99.95% purity, Hikari Materials Industry Co., Ltd, Japan) was encapsulated with α-Al2O3 nanoparticles (ϕ ≈ 100-200 nm, 99.99% purity, Kojundo Chemical Laboratory Co., Ltd., Japan) using a dry synthesis method, as described in our previous study.2 The encapsulation was facilitated by a rapidly spinning rotor equipped with blades, which promoted the adherence of α-Al2O3 nanoparticles to the Al-Cu-Si microparticles. Sintering at 1000°C for 6 hours in an O2 atmosphere helped form a dense α-Al2O3 shell. For in-situ S/TEM sample preparation, a 6 × 6 µm lamella of the MEPCM was processed using FIB milling (JEM-9320 FIB, JEOL) followed by a standard lift-off procedure. A lamella of the raw Al-Cu-Si alloy was also prepared for comparative analysis. The lamella was placed on a MEMS-based microheater (FEI) with a silicon nitride heating element capable of reaching temperatures up to 1000 °C (Fig. 1). The lamella was positioned over the electron-transmissible window, and the holey film was removed by FIB milling to eliminate non-sample-derived Si X-rays. To secure and enable heating of the lamella, a 300 nm thick tungsten (W) film was deposited at the contact area between the lamella and the heating pad using FIB. S/TEM observations were performed using a Titan3 G2 60-300 (FEI) double aberration-corrected microscope, operated at 300 kV. In-situ heating observation involved acquiring a series of HAADF images with a convergence angle of 17.9 mrad. The camera length was set to 115 mm for a HAADF collection range of 51–200 mrad. The series were taken with a pixel size of 512 × 512 and a dwell time of 2 µs/pixel, resulting in an image frame rate of 787 ms/frame. The probe current was set to 44 pA. The MEMS microheater's temperature was controlled using a closed-loop feedback system to prevent temperature undershoot or overshoot, starting from room temperature and increasing to a maximum of 500 °C at a rate of 5 °C min-1, similar to the heating rates used in differential scanning calorimetry (DSC) for latent heat measurements. A series of HAADF images were taken during each temperature ramp. The temperature was maintained at each 100 °C increment until no further changes were observed, and EDX analyses were performed at each stage. The FEI heating holder's

slight curvature over the microheater's window slightly compromised EDX signal counts for the 4-quadrant Super-X windowless detectors. EDX maps were acquired with a resolution of 256 × 256 pixels, a dwell time of 20 µs, and a total of 250 – 350 frames per map.

Results

The target observation area focused on the core-shell interface structure consisting of α-Al2O3 and the Al-Cu-Si alloy. Compared to the raw Al-Cu-Si, the calcined MEPCM exhibits coarsening of intermetallic Cu2Al and Si. In-situ heating observation of the raw substrate revealed initial dendritic Si structures becoming denser and increasingly aligned with the Cu2Al structure during heating from 100 to 500 °C. This suggests a possible higher melting temperature for Cu-Si formations, which remain solid even at the eutectic melting point of 529 °C. This compound is confirmed in the MEPCM calcined at 1000 °C, where it appears as dispersed nanoparticles in the primary Al matrix. During the temperature ramp from 100 to 300 °C, the applied voltage increased linearly while a notable increase in current is observed, likely due to the thermal lattice expansion of Cu2Al, which leads to fluctuations in electrical conductivity. This was validated by rigid registration of the acquired frames, confirming an approximate size increase of 100 nm in Cu2Al.

This behavior persists until 300 °C. Thereafter, during the ramp to 400 °C, the current value nearly stabilizes, indicating that the Cu2Al phase is reaching its thermal stability limit and beginning to dissolve into the Al matrix. A continued ramp to 500 °C results in complete phase dissolution. This dissolution involves the diffusion of Cu and Al atoms out of the Cu2Al phase and into the surrounding matrix, driven by the gradient in chemical potential caused by temperature changes and the concentration gradients of Cu and Al in the different phases. Interestingly, during the complete phase dissolution, some phases from the Cu2Al adhere to the α-Al2O3 surface. Although α-Al2O3 acts as an inert barrier, surface defects and partial charges may provide sites for adsorption or chemical interactions. If the lattice constants of the dissolute phases are close enough to those of the α-Al2O3, epitaxial growth or nucleation could occur. Atomic resolution HAADF observations in the area where adhesion occurred confirm the presence of seed crystals.

Conclusions

The in-situ HAADF-STEM observations have provided valuable insights into the thermal expansion and dissolution behaviors of Cu2Al within Al-Cu-Si@α-Al2O3 MEPCM during heating. A critical observation from this study is the adhesion of dissolute phases from Cu2Al to the α-Al2O3 surface during phase transformation. This adhesion suggests a potential for epitaxial growth or nucleation at the interface, which could effectively induce nucleation in the Al-Cu-Si alloy during solidification, thereby suppressing hysteresis.

Keywords:

In-situ, MEMS, HAADF, PCM, alloy

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Microstructural characterization of Zr/ZrN and ZrN/ZrCN hard coatings using TEM

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The nitride, carbide, and carbonitride of transition metal elements like Zr, W, Ti, and so on are commonly used to create hard coatings. Zirconium-based hard coatings are ideally suited for use in tribology, biomedical, and electronics applications due to their superior resistance to wear, corrosion, and erosion, as well as their high thermal stability, hardness, and biocompatibility.

We produced homogenous, robust, and mechanically well-performing Zr-based nanostructured hard coatings. Zr/ZrN and ZrN/ZrCN multilayers were deposited onto stainless steel 316L substrates by means of magnetron sputter deposition technique. Each individual layer's thickness ranged from 250 to 500 nm based on the coating's composition. The deposition parameters were adjusted to create two unique coating thicknesses of 2 and 3 µm.

Cross-sectional TEM lamellae were prepared and examined with a transmission electron microscope. To clarify the differences in phase and elemental constitution at the microscale, x-ray mapping data and selected area diffraction data was obtained from multilayers. The adherence was also evaluated by taking images and energy dispersive spectroscopy was used to examine the coating-substrate and layer-to-layer interfaces.

There was uniform, compact, and dense columnar growth of all the coating grains at the substrate/coatings interface, and no evidence of decohesion was seen. The ZrN/ZrCN coatings were found to have denser, finer, and more compact grains than the Zr/ZrN coatings. ZrN/ZrCN coatings outperformed Zr/ZrN coatings in terms of hardness, modulus, and H/E values. This suggests that ZrN/ZrCN coatings may be able to achieve better wear resistance and fracture toughness. The study's developed coatings are anticipated to be used in tribology due to their superior hardness and H/E properties.

Keywords:

Hard coatings, TEM, Magnetron deposition

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SEM Insights: Sample Temperature Evolution during EBSD

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Poster Group 2

Background incl. aims
Electron Backscatter Diffraction (EBSD), with application in geology, ceramics, semiconductor, and metals research, enables a comprehensive analysis of the microstructure in materials. By mapping crystallographic orientation it aids in understanding phenomena, such as character of grain boundaries, etc., for the investigation of crystal/grain growth, phase transformations, as well as the detailed analysis of material deformations and strain.
Despite its widespread use, still many challenges remain, such as the effect of sample surface roughness, sample preparation artifacts, sample drift during data acquisition, image distortions due to the sample positioning and drift, effect of surface contaminations, as well as simply the complexities in EBSD data interpretation. This is stimulating ongoing research and requests further improvements.
Furthermore, potential effects of the electron beam itself, particularly beam-induced heating, remain an unexplored concern . Such effects could affect the microstructure of examined samples or lead to inaccuracies in assumed temperature conditions during experiments, in particular in in-situ heating experiments. Here, we aim to investigate these effects to better understand their impact on samples and discuss potential associated drawbacks.
Methods
In-situ heating holders based on micro-electro-mechanical systems (MEMS) were initially developed for stable and accurate transmission electron microscopy (TEM) investigations, but have since also found application in in-situ SEM experiments. Equipped with a four-point measurement system on their heating spiral, these holders provide real-time temperature feedback based on the measured resistivity of the heating spiral material, enabling precise temperature control during experiments as well as a direct feedback on additional sample heating effects.
With this approach, in our study we want to investigate the effects of beam exposure on sample temperature of various materials, as well as significant data acquisition parameters (i.e. SEM acceleration voltage, beam current, dwell times) as they occur during EBSD measurements of bulk-like samples. By utilizing COMSOL, a finite element analysis tool, we simulate the Joule Heating of the MEMS device to predict the temperature distribution in our sample caused by the Electron Beam, thereby improving our understanding of the complex electron beam-sample interaction.
Results
Our study provides insights into the evolution of sample temperature under EBSD-relevant microscope conditions. We observed an increase in the sample temperature of approximately 40K in dependency of the acceleration voltage used for an Iron Sample. These findings we compare with our COMSOL modeling results.
Thereby, we will emphasize the significance of temperature effects, particularly in critical cases like beam-sensitive samples, where beam-induced sample heating could affect interpretation of microstructural analysis results.
Additionally, we highlight the effects of beam-induced heat during in-situ heating experiments in SEM as well as the importance of incorporating an active temperature control feedback loop in such setups.

Conclusion
Here we present a novel approach to real-time temperature monitoring of samples, particularly under EBSD-relevant measurement conditions. This will provide important insights into the dynamic thermal behavior of specimens during examination in a scanning electron microscope

Keywords:

SEM, EBSD, Beam Heating

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Development of chopped scan control for beam blanking

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Poster Group 1

Beam blanking is a technique in electron microscopy whereby the electron beam is interrupted from reaching the sample at high speeds or frequencies. This is required for a range of experiments, including lock-in amplification of minute signals, close control of electron dose/fluence, or capture of fast events with high temporal resolution or in synchronization with external clocks. Beam blanking is typically achieved by deflecting the beam from the optical axis with added blanking plates into a purposely designed blanking aperture. However, this approach requires installation of additional hardware into the microscope column. Development of an alternative approach is presented here, where the beam is deflected using the normal scan control coils into a vacuum or sacrificial point in the sample plane. This removes the need of additional components in the microscope column and places the added complexity of beam blanking into the scan controller only.

Whilst blanking with the scan coils can be thought of as adding a second blanking pattern on top of the regular scan pattern, such a hardware configuration does not provide means to choose the position of the vacuum or sacrificial beam position, or to synchronize the two patterns, therefore the normal scan controller must take on the task of beam blanking in addition to beam scanning. This enables the user to freely define a position for blanking, which may be from a pre-scanned image, or from preplanned coordinates. Generation of both patterns from the same controller also enables the freedom to choose if blanking and scanning are synchronised to the same clock or are free running. Use of the scan controller in this fashion requires complex scan algorithms, as established scan patterns in electron microscopy already include advanced flyback strategies at end of line and end of frame.

Attached Figure presents such a scan pattern with blanking – a very small 3x3 pixel scan is shown here to simplify presentation, but resolution in this scan mode is only limited by the scan resolution of 16-bit corresponding to 65,536 x 65,536 pixels. A 1 µs acquisition time was used, with a line start wait of 10 ns, a beam return time of 320 ns, without any pre-scan, pixel settling or additional holding time. Digital frame, line and pixel trigger outputs typically used for detector or camera synchronization are shown here to guide the presentation and were set to a duration of 200 ns. A blanking reference frequency of 10MHz was selected for this example in order to give a visible blanking duration comparable to the pixel acquisition time. The blanking position was set to 0,0 (top left) and the image scan was set to begin at 32768,32768 (middle) of the scan space. Note in the analog scan outputs for column and row signals how the beam is encoded to jump between the blanking pixel and the image pixels, which gives a chopped appearance to the analog traces.

Practical application of this chopped scan mode for beam blanking will be shown in TEM and SEM, including the limitations to be expected from using the scan coils for such fast beam motion. It will be shown that hysteresis and amplifier bandwidth limitations restrict the maximum blanking speed possible to a range of approximatively 100kHz, which could be overcome with further development of distortion removal algorithms, however all this is achieved without modifications to the microscope column.

Keywords:

Scan control, Beam blanking

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Analysis of the cause of imperfect adhesion of cataphoretic coating using SEM and EDS method

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Poster Group 2

The automotive industry is one of the most overloaded and stressed in the point of view materials selection, characterization, application and their corrosion prevention and surface protection. Cataphoretic coating is one of the used and technologically most advanced method of anticorrosive surface treatment processes and it is usually used as surface treatment of metal products. The process itself is a set of several steps of pre-treatment, paint application and curing and although each sub-process is rigorously controlled (including production of parts and their storage), it can occur that the final product of the cataphoretic coating process are parts with a defective coating. Consequently, the anti-corrosion protection can be reduced even due to relatively small defects and thereby increasing, among other things, the safety risk. In this contribution, case study of the cause analysis of a non-conforming cross-cut test of cataphoretic coating surface on metal part is shown, where it is necessary to use the methods of scanning electron microscopy (SEM) and scanning electron microscopy with energy dispersed X-ray spectroscopy (EDS).

The metal part with a non-conforming cross-cut test of cataphoretic coating surface were examined by the SEM and EDS method. SEM analyses were taken with scanning electron microscope Quanta FEG 250 (FEI) at the regime pressure of the low vacuum mode and high vacuum mode. Further, EDS analysis were measured with TEAM™ Software Suite, coupled with the Octane Plus System, as well at the regime pressure of the low vacuum mode and high vacuum mode.

A metal part with a cataphoretic coating on the surface was examined in a place with a non-conforming cross-cut test. In this place (of the peeled coating on the metal part), elements that may originate from the zinc-phosphate layer were locally detected. Therefore, the peeled off coating from its underside was further analysed. Differently sized crystals were found here, and their composition may correspond to the zinc-phosphate layer.

Analyzing a metal part with a cataphoretic coating on the surface with with a non-conforming cross-cut test by using SEM and EDS methods, it was proven that the zinc-phosphate layer in the pre-treatment was applied, but did not adhere to the surface of the part as it should have. It is necessary to further focus on the individual steps of pre-treatment. There may have been insufficient degreasing at the beginning of the pre-treatment process. Or the cause is the poor quality of the zinc-phosphate solution.

Keywords:

Cataphoretic coating, EDS, SEM

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TEM investigation of the defect structure in epitaxial corundum thin films

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Poster Group 2

Metal oxides M_2O_3 thin films have extensively been studied as they offer a broad range of functionalities. Sapphire Al_2O_3 is the most common substrate for the epitaxial growth of several M_2O_3 since it shares the same corundum lattice (R-3c) with several M_2O_3 systems. Examples include Cr_2O_3, a magnetoelectric antiferromagnet with applications in memory devices, and V_2O_3, a popular material to study strong electron correlations. α-Fe2O3 and α-Ga2O3 also present the same corundum structure and have potential applications in catalysis and magnetic devices as well as power electronics, respectively.
A good understanding of the defect formed during the film epitaxial growth is crucial for both fundamental studies and devices. Examples of such defects present in M_2O_3 thin films grown on c-cut sapphire include twinning, columnar growth, misfit dislocations, screw dislocations and oxygen vacancies. In this work, the microstructure of Cr_2O_3 and V_2O_3 thin films are studied systematically. Our results indicate for these two M_2O_3 systems significantly different defect mechanisms.

All the thin films are grown by oxygen-assisted molecular beam epitaxy (MBE) on c-cut sapphire substrates at high temperature. V_2O_3 samples are grown at 700°C and Cr_2O_3 samples at 800°C. Base pressure of the system is 10E-10 Torr, while during the deposition the O_2 partial pressure is measured with a residual gas analyzer and kept constant at 2E-6 Torr. Bilayer structures have also been grown with V_2O_3 on a Cr_2O_3 buffer layer. The epitaxial growth is monitored in-situ in the MBE chamber by reflection high-energy electron diffraction (RHEED). X-ray diffraction (XRD) ϕ-scans are performed ex-situ after growth, and the twinned fraction of the film was estimated.
For transmission electron microscopy (TEM), cross-sectional lamellae are prepared by focused ion beam using DualBeam Nova 600 NanoLab and imaged by means of a cold-FEG JEOL ARM200F microscope equipped with a STEM-Cs corrector, operated at 200kV. Geometric phase analysis (GPA) of STEM images is also performed using the Digital Micrograph plugin developed by Du (Ernst Ruska-Center for Microscopy).

TEM images confirm the epitaxial growth of all the thin films. GPA strain maps highlight the strong lattice deformation at the interface linked to the presence of misfit dislocations, which appear periodically with an average distance of 9.5±1.5 nm at the V_2O_3/Al_2O_3 interface, when imaged along the <-2110> zone axis. This is comparable to the value expected when the lattice mismatch is fully compensated by incorporation of misfit dislocations, i.e. 10.2 nm for a 4.2% in-plane lattice mismatch. In contrast, strain maps of Cr_2O_3/Al_2O_3 films present strong deformations across the entire thickness of the film, despite having a very similar in-plane mismatch with the substrate (4.4%). In addition to misfit dislocations, the sample contains columnar domains and twinning. Columnar domains are best imaged with HRTEM and bright field STEM, although the grain boundary is not visible in the high-resolution STEM images. A similar bright field contrast is present in the V_2O_3 thin films as well, albeit less dense and with irregular shape. The presence of columnar domains in Cr_2O_3 thin films has been reported previously, and their origin was explained by an additional strain

relaxation mechanism beside the incorporation of dislocations. Secondly, twinning can be observed in the XRD ϕ-scan of the planes {10-110}, as a second set of 3 peaks appears while rotating the sample by 360° introducing an apparent sixfold symmetry instead of the trigonal symmetry of the corundum structure. However, XRD is unable to detect small, twinned fractions of the film which can be observed by TEM and are always present. Increased twinning is observed for samples grown with a higher Cr-flux rate, indicating that the precise growth parameters affect the formation of the twins. On the other hand, V_2O_3 does not present any twinning when grown directly on sapphire. Grown on a twinned Cr_2O_3 buffer, V_2O_3 shows the ability to recover to the untwinned structure despite the underlying twinned structure. This result is in contrast with other works where twinning in V_2O_3 and Cr_2O_3 appeared to be comparable. The absence of columnar and twinned domains in V_2O_3 thin films indicates a different strain relaxation mechanism in both M_2O_3 thin films.

Cross sectional TEM analysis of corundum M_2O_3 thin films grown on sapphire substrates indicates an intrinsic difference in the defect structure formed in Cr_2O_3 and V_2O_3 thin films. The former present misfit dislocations, columnar and twinned domains in agreement with previous studies, while the latter does not present any twinning and compensates the lattice mismatch only by incorporation of misfit dislocations.

Keywords:

Epitaxy
Thin-Films
Defects
Corundum
MBE

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Automated Alignment Strategies for Integrating Cryo 3D-SIM and SXT Data

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IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

Background incl. aims
The correlation between Cryo 3D Structured Illumination Microscopy (3D-SIM) and Soft X-ray Tomography (SXT) has become a complementary tool for the comprehensive exploration of complex biological systems. The challenge in this correlative microscopy lies in aligning datasets acquired through different imaging modalities and reconstruction procedures. Cryo 3D-SIM and SXT data, while offering complementary insights into cellular structures, present inherent discrepancies due to their distinct imaging techniques. To address this challenge, our study aims to develop novel algorithms for automated alignment, with the goal of improving the integration of cryo 3D-SIM and SXT data. We aim to enhance the accuracy and efficiency of the correlative process by overcoming imaging and reconstruction differences, thereby facilitating comprehensive investigations into intricate cellular structures and their function.

Methods
Our study employs a multi-faceted approach to address the challenge of aligning datasets acquired through different imaging modalities and reconstruction procedures. Firstly, we utilize feature-based matching, leveraging distinctive features within both cryo 3D-SIM and SXT datasets. This involves conducting feature size range-based matching to establish correspondences between images, ensuring accurate alignment by identifying common structural landmarks present in both datasets. Subsequently, our alignment strategy is divided into two sequential processes. Initially, we perform a coarse 2D alignment, aligning a Soft X-ray mosaic view with a 2D projection of the 3D fluorescence data. Following this, we refine the alignment further through a fine iterative 3D angular and shift search, ensuring optimal spatial correspondence between the two 3D datasets. Additionally, considering the sparsity of SXT 3D data in the Fourier domain, we implement a novel 3D correlation process. This involves tomographic 3D-correlation based on 2D-correlation backprojection, focusing solely on effective data through tomographic backprojection reconstruction of 2D projection correlations. These methods collectively constitute our approach to automated alignment, aimed at enhancing the integration of cryo 3D-SIM and SXT data for cellular structures.

Results
We conducted extensive testing using experimental data to evaluate both 2D and 3D alignment approaches. For the 2D alignment approach, we utilized samples from the Huh-7.5 Human Hepatocellular Carcinoma Cell Line, where the signal emitted by mitotracker green was employed for alignment purposes. Despite the low resolution of the fluorescent signal, our approach consistently demonstrated good alignment across all cases. In contrast, for both 2D and 3D alignment approaches, we utilized experimental data from the work of Groen et al. [Groen21], which involved primary cardiac mouse fibroblasts and NIH-3T3 cells treated with TPR-Hsp90-AuNC with green emission (alexa-488). The manually aligned performed in [Groen21] has served as the benchmark for comparison with our automated results. Our findings reveal that the automated alignment results

exhibit a high degree of agreement with the manually aligned data, highlighting the reliability and accuracy of our methodology.

Conclusion
These results underscore the effectiveness of our approach in achieving precise alignment of both 2D and 3D datasets. The success of this automated alignment process relies on size range filters rather than specific landmarks, allowing for the effective fiducialization of various cellular structures, such as lipid droplets or mithochondrias. Additionally, our utilization of a 3D correlation process based on 2D correlations effectively addresses Fourier artifacts arising from the tomographic reconstruction process. Furthermore, the agreement between automated and manual alignment results further validates the robustness of our methodology, showcasing its potential for advancing correlative microscopy techniques.

Keywords:

Correlative microscopy, Soft X-ray Tomography

Reference:

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Reconstruction of Angstrom resolution exit-waves by the application of drift-corrected phase-shifting off-axis electron holography

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Poster Group 2

Background

Off-axis electron holography is a phase retrieval technique which enables access to the full complex-valued exit-wave of thin samples. The potential distribution at interfaces obtained from the measured phase information is highly relevant for in-situ experiments. Combining off-axis holography with the capabilities of an environmental TEM offers the ability for exit-wave reconstruction under external bias and in catalysis relevant gases and material systems. However, the conventional holography Fourier-reconstruction suffers from a trade-off between spatial and phase resolution caused by the fringe spacing and visibility. To tackle the open fundamentally questions in catalysis research, e.g. the atomic structure of the electrolyte-solid interface, the identification of active reaction sites and the influence of surface faceting, atomic resolution is highly desired. Therefore, state-of-the-art phase retrieval techniques must be adapted to the particular requirements of in-situ studies. Phase-shifting electron holography bypasses the spatial resolution limit by real-space evaluation of hologram series. However, to reach atomic resolution in reconstructed hologram-series, special care is needed to correct sample and biprism drift.

Methods

Phase-shifting holography acquires a series of holograms formed by tilted incident waves. This results in a shift of the hologram fringes, that are modulated by the potential of the specimen. If specimen and biprism drift are carefully corrected, the cosine intensity dependency of the hologram series can be used for linear fits of the local amplitude and phase of the exit wave. This obviates the use of the low-pass aperture which is necessary for the conventional reconstruction of off-axis holograms in the Fourier domain. The upper bound of the spatial resolution is thus only limited by the performance characteristics of the instrument, while the low-frequency information is also retained.

Results

Previous implementations of phase-shifting holography have been limited by the independent drift of biprism and sample and allowing for medium spatial resolution. We improve the reconstruction process by introducing a drift correction scheme and demonstrate exit wave reconstruction on platinum. The reconstructed exit-waves show reliable phase information at the 1 Å information limit of the used Titan 80-300 kV environmental transmission electron microscope. Simultaneously, the omission of the trade-off between fringe spacing and visibility leads to phase resolutions up to $2\pi/452$ rad at moderate birpism voltages of 250 V (fringe spacing 1 Å). The obtained phase and

amplitude information is validated at a thin Pt sample due to the excellent matching to frozen-lattice multi-slice image simulations.

Conclusions

In conclusion, we demonstrate the successful method improvement of the phase-shifting holography reconstruction process by introducing novel drift correction of the mixed signals of biprism and sample drifts. The reconstructed exit-waves of a thin platinum sample show spatial resolution up to the 1 Å information limit of the microscope simultaneously with a phase resolution up to 2π/452 rad. The exit-waves are in excellent agreement with multi-slice frozen lattice image simulations and preserve the high- and low-frequency information. The published method is applicable in any TEM equipped with a single electron biprism and thus allows to achieve high resolution off-axis holography in various instruments including those for in-situ applications. A software implementation for the acquisition, calibration and reconstruction is provided. The combination of environmental TEM and high-resolution phase-shifting electron holography grants access to the platinum-water interface at the atomic scale in ongoing studies.

Keywords:

atomic-scale, off-axis-holography, exit-wave reconstruction, ETEM

Reference:

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The effects of TiC network microstructure on the mechanical properties of spark plasma sintered Al2O3

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Poster Group 1

Background incl. aims
Al_2O_3-based composite materials are widely used for cutting tool applications due to their high strength, hardness and wear resistance. Despite these ideal mechanical properties, having low fracture toughness values and brittleness present problems during application. This study aimed to improve both the hardness and fracture toughness of Al_2O_3 simultaneously by taking advantage of different mechanisms with TiC network structures. Hardness was aimed to be increased by introducing the TiC secondary phase with high hardness throughout the Al_2O_3 matrix. On the other hand, fracture toughness was aimed to be improved by promoting crack deflection mechanisms by the formed network structure.
Methods
Al_2O_3 ceramics with TiC network microstructures were produced by the spark plasma sintering (SPS) technique by coating the Al_2O_3granules with 2-10 vol.% TiC powders, utilizing a simple dry coating method. The sintering process was carried out at a minimum of 1400°C dependent on the TiC content. The effects of various amounts of TiC addition and differing sintering temperatures on the microstructural and mechanical properties of Al_2O_3 were observed by scanning electron microscopy techniques as well as mechanical testing.
Results
Network structures of TiC were formed successfully around the Al2O3 matrix during the SPS process, resulting in simultaneous improvement of hardness and fracture toughness. Present TiC and Al2O3 phases were confirmed by the X-ray diffraction analysis. The fracture toughness of Al2O3 was improved by crack deflection and bridging mechanisms induced by the introduction of the network structure, as observed by the microstructural investigations. In addition, Vickers microhardness values of the samples were found to be increased with increasing volume fraction of TiC.
Conclusion
As a conclusion, in this study, mechanical properties of the Al2O3 ceramics were successfully improved by the formation of TiC network around Al2O3 granules. Electron microscopy studies revealed the formation of the continuous network around the matrix and microstructural features such as grain size, and porosity as well as toughening mechanisms.
This study was supported by Eskişehir Technical University Scientific Research Projects Commission under grant no 23ADP195

Keywords:

Al_2O_3, TiC, Network, SPS, Properties

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VitroJet: ice thickness control and measurement for time-efficient single particle structure determination3052

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Poster Group 2

Background incl. aims
Single particle cryo-electron microscopy (cryo-EM has emerged as a prevalent technique for structural biology due to its capacity to visualize of individual macromolecules, which is applied across a variety of diverse projects. However, the current practice of thickness screening and hole selection for data acquisition within the electron microscope consumes significant beam time. We aim to control and measure ice thickness during sample preparation, to predictably achieve high resolution.
Methods
The VitroJet is utilized for cryo-EM sample preparation, leveraging its automation to enhance productivity and increase accessibility for novice users. Pin-print sample deposition and jet vitrification combined provide a reproducible framework for generating high-quality grids.
Results
Multiple case studies employing the VitroJet showcase its benefits across various sample categories: membrane proteins, nucleosomes, fatty acid synthase, tick-borne virus, lipid nanoparticles, tobacco mosaic virus, and bacteriophages. By adjusting pin-printing velocity and standoff distance from pin to grid, we effectively regulated the median ice thickness, achieving a standard deviation below ± 11 nm for thicknesses up to 75 nm. The integrated optical camera enables precise measurement of ice thickness in individual holes with an accuracy below ± 20 nm within the 0 - 70 nm range. Data collection at 30 nm to 70 nm ice confirms the importance of ice thickness for time-efficient structure determination, with 3.7 times fewer particles needed to attain a resolution 3 Å.
Conclusion
Users in several labs worldwide have validated the versatility of the VitroJet across various sample types and workflows. The capability to control and measure ice thickness empowers users to optimize layer thickness on-the-fly during grid preparation, without the need of cryo-EM. Furthermore, the estimation of ice thickness facilitates targeted selection of holes for data collection based on optical imaging. This underscores how the VitroJet accelerates the entire workflow and enhances the efficiency of utilizing microscope infrastructure for data collection.

Keywords:

Sample preparation, reproducibility, productivity, versatility

Reference:

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Direct observation of charge modulation in nanoprecipitates by 4D STEM

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Poster Group 2

Background incl. aims
Charge modulations induced by structural, compositional and electronic variation in a solid can be investigated using various analytical techniques, including scanning tunnelling microscopy/spectroscopy, Raman spectroscopy, and recently available four-dimensional scanning transmission electron microscopy (4D-STEM), depending on the nature of the modulation. We explore the interplay between charge density modulation and structural distortion in nanoscale moiré structures, $MgZn_2$ nanoprecipitates imbedded in Al matrix using 4D-STEM.
Methods
A series of diffraction patterns by 4D-STEM offers a wide range of information, which includes local electric fields and charge density, in addition to atomic configurations. We integrated intensities at different scattering angles and directions to extract various types of images. Template matching was employed to the real space, depth-sectioned STEM images to locate the accurate atomic positions, which facilitated the mapping of local strain distributions within the imbedded nanostructures. Density functional theory (DFT) calculations are performed to find the origin of the charge modulation and multislice simulations are further employed to compare the experimental results with the theoretical prediction.
Results
We present center of mass (COM) images derived from the in-plane diffraction patterns of the MgZn2/Al heterostructure. Interestingly, the images clearly reveal charge density modulations which are highly dependent on the depth of the probe position in the specimen. Moreover, strain mapping within the Al matrix is intricately linked to charge modulation, suggesting the potential influence of structural distortion on the charge modulation. The periodicity and the location of the modulation are further analyzed to determine the correlation with the moiré supercell structures.
Conclusions
A series of multislice simulations generated numerous COM images, encompassing single atoms to stacks of multi-layers, at different probe depths. We propose that the primary contribution to the modulated charge distribution originates predominantly from the interface area between the $MgZn_2$/Al heterostructure. Furthermore, we demonstrate how manipulation of COM images provides more detailed insights into the structural and compositional variations within materials.

Keywords:

4D-STEM, Charge, Nanoprecipitates

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In-situ TEM annealing of vanadium oxide thin films

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Poster Group 2

The multitude of vanadium oxide phases are traditionally grouped into two families: Magnéli phases (VnO2n-1 where 3 ≤ n ≤ 9) and Wadsley phases (VnO2n+1 where 1 ≤ n ≤ 6). These groups are characterized by the oxidation states V3+/V4+ and V4+/V5+, respectively, separated by the purely V4+ phase, i.e. VO_2.
Many of these vanadium oxides present a metal-to-insulator transition (MIT) upon cooling coupled with structural and occasionally magnetic transitions. Of all the VOx phases, VO_2 has attracted the greatest attention in terms of technological applications, because the electrical transition occurs close to room temperature at 68°C. The semiconducting monoclinic phase (P21/c) is most stable at room temperature and transitions into the metallic rutile phase (P42/mnm) above the transition temperature. The transition is accompanied by a change in resistivity up to 5 orders of magnitude in single crystals as well as in optical properties. This abrupt change has triggered a broad range of applications: e.g. thermal sensors, electrical switches, smart windows, optical limiting, modulators, bolometers, and Mott field-effect transistors.
The growth of VO_2 thin films requires a higher oxygen partial pressure than what is typical for the growth by means of MBE. Therefore, crystalline VO_2 thin films have been obtained by growing amorphous VOx and to convert to VO_2 through a subsequent annealing process. Very little is known about the structural transition from the amorphous to the crystalline phase, especially how the VO_2 phase is established, as well as the structural transition underwent during the MIT.
However, an in-situ transmission electron microscopy (TEM) experiment using an environmental cell experiment provides the possibility to live monitoring of the oxidation and crystallization process of the film by mimicking the annealing process in a furnace-like environment. The structural and chemical information collected during the in-situ annealing can shed light on the complicated mechanism involved in VO_2 films as a function of the temperature and the annealing environment.

VOx thin films are deposited directly on the MEMS chips of a closed-cell gas reaction system (Atmosphere, Protochips) by oxygen-assisted molecular beam epitaxy (MBE) at room temperature. The film is directly in contact with the electron transparent membrane of the chip which consists of 30 nm of amorphous Si_3N_4. The vanadium flux in the MBE is calibrated to 0.1 Å/s with a quartz crystal microbalance, and the total thickness of the sample is estimated to around 40 nm. Base pressure of the system is 1E-10 Torr, while during the deposition O_2 partial pressure is measured with a residual gas analyzer and kept constant at 2E-6 Torr. The sample is then mounted on the TEM sample holder and inserted into a cold-FEG JEOL ARM200F microscope equipped with a STEM-Cs corrector. The MEMS chip is heated up to 500°C in Ar or Ar/H_2 mixtures at 760 Torr with a 0.1 sccm flow. Base pressure of the closed-cell is 1 Torr. The sample is imaged both in TEM and STEM mode, with bright field (BF) and high-angle annular dark field (HAADF) detectors. Electron energy loss spectroscopy (EELS) is performed with a Gatan Tridiem detector in STEM mode to evaluate the local oxidation state with the V L edges and O K edge.

The as-grown sample is amorphous and dominated by the V3+ stoichiometry. As temperature increases in the closed cell, the first transformation occurs between 290-310°C for both Ar and Ar/H_2 mixtures. Several grains of a few hundred nanometers in size form, with irregular shape and areas showing different HAADF contrast. Grain boundaries are decorated with dark regions linked to V-deficiency. These areas gradually disappear as the temperature approaches 420°C, where the main crystallization takes place. The morphology of the annealed film shows a great variability with the different gas mixtures applied, while the EELS spectra generally confirm the oxidation of V3+ to V4+ and/or V5+. The residual O_2 in the closed-cell gas reaction system is expected to be responsible of the oxidation of the film, and the H_2 insertion is intended to counterbalance and to avoid the sample being further oxidized to V_2O_5. The fully polycrystalline film is stable until about 500°C when it transforms into V_2O_5, which very often forms elongated grains and leaves holes behind between these grains. Electron beam can additionally influence the annealing as it induces local heating of the film, with consequences on the final morphology and oxidation state.

The annealing of vanadium oxide thin films on amorphous substrates has been investigated in several reported works, with the goal of obtaining VO_2 polycrystalline films. The outcome is often complex, as the films frequently include additional phases alongside the desired monoclinic VO_2 phase, due to under or over-oxidation. The presence of multiple crystalline and amorphous phases, which has a detrimental effect on the electrical transition, complicates the thin film characterization by the most common techniques (e.g. x-ray diffraction, scanning probe microscopies, and x-ray photoemission). A closed-cell experiment in the TEM provides the possibility to mimic the annealing environment and to observe the structural changes as a function of the temperature and the environment. These results can enhance the understanding of the annealing process by combining structural and chemical information at the nanometric scale. In particular, a detailed nano-scale understanding of the oxidation/reduction routes in vanadium oxide films on amorphous substrates can be gained.

Keywords:

In-situ TEM
Thin film
Oxidation

Reference:

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Annular silicon drift detector for advanced EDS on SEM applications

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Poster Group 1

Background incl. aims
Energy dispersive X-ray spectroscopy in an SEM can be challenging in certain cases: if no sample preparation (cutting, polishing) is possible or allowed to generate a flat sample surface (which would be ideal for analysis). Furthermore, samples having low electrical conductivity or high electron beam sensitivity can only be investigated using low beam currents, which in turn results in a low X-ray signal. Under such conditions, trace elements cannot be analyzed using EDS within reasonable measurement times.
Methods
We present a solution for maximizing X-ray signal collection: an annular EDS detector, inserted between the pole-piece and the sample. High count rates can be reached even at low beam currents as the four separate X-ray detector segments cover a large solid angle (up to 1.1sr). Due to the high count rates, element distribution mapping (including trace elements) is orders or magnitudes faster than using conventional EDS detection geometry. This configuration is also ideal for the analysis of samples with high topography as shadowing effects are minimized by X-ray detection from all spatial directions.
Results
We present various examples benefiting from the annular acquisition geometry. Li-ion battery components (anode and cathode particles) or black mass material can be mapped with high spatial resolution without the need of any meticulous sample preparation. Contaminants and inhomogeneities in the submicron range can be visualized within the micrometer-sized particles. We will demonstrate that the annular EDS detector is capable of identifying and mapping contaminants in the range of only a few tens of ppm within a few minutes of measurement time. These findings will be compared to a classic trace element detection method (X-ray fluorescence).
Conclusion
The annular EDS detector in an SEM is a proven tool for challenging elemental analysis, such as unprepared samples with high topography and low conductivity (for example battery components: anode, cathode particles, black mass). Due to its maximized collection solid angle, this type of detector yields significantly higher count rates compared to conventional EDS detectors and thus considerably reduces the necessary electron dose or measurement time. EDS using annular detectors is an analytical method capable of detecting and mapping contaminants in the sensitivity range of X-ray fluorescence methods but with the advantage of having high spatial resolution.

Keywords:

EDS, hyperspectral imaging, battery

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Automatic signal clasification of the Low-Loss Region in Electron Energy Loss Spectroscopy

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Poster Group 2

Localised Surface Plasmon Resonance (LSPR) is a non-propagating electron-density wave that is confined at the surface of a metallic nanoparticle. They can enhance the electromagnetic radiation, concentrating it into sub-wavelength volumes. Its resonance can be tuned by changing the surrounding medium and its geometry. This unique property opens a wide range of applications across various fields of applied research.
Electron Energy Loss Spectroscopy (EELS) within a Scanning Transmission Electron Microscope (STEM) has revealed remarkable capabilities in the analysis of plasmons at nanometric scale, as this technique achieves sub-angstrom spatial resolution and can excite the complete range of LSPR modes supported by the nanostructure. By employing EELS, the plasmonic properties can be correlated with geometric or structural characteristics, enabling a more comprehensive understanding of the plasmonic response.
In this study, based on the analysis of Silicon/Gold nanopillars samples, we demonstrate that clustering techniques can be used for detecting LSPRs in EELS. We propose a novel combination of unsupervised machine learning strategies that detect LSPRs in EELS spectrum images. To demonstrate the effectivity of this methodology, we studied Si/Au nanopillars. The detection of LSPRs is done by reducing the dimensionality of the data, clustering this low-dimensional space, and recuperate the spatial space. We demonstrate that using this methodology, it is possible to recover the LSPRs, among distinct spectra in a large EELS dataset, and easily make a plasmonic spatial map without the need for prior knowledge or labelling of the data.

Keywords:

EELS, Plasmons, Nanowires

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Is iron a hidden culprit in Alzheimer's disease?

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Poster Group 1

Background incl. aims
Up to 80% of dementia cases globally are down to Alzheimer's disease (AD), a major neurodegenerative disorder[1]. The disease was discovered in 1906 by Alois Alzheimer[2], and in spite of great research efforts, therapeutic progress has been slow. This is probably because AD is characterized by several factors, namely amyloid beta (Aβ) deposits, neurofibrillary tangles (NFTs), and neuronal inflammation, with an unclear connection between these factors. It has been shown previously that iron is dysregulated in AD pathology [3]. Here we used an organotypic tissue culture model for studying the contribution of iron overload to the disease.
Methods
We established an organotypic tissue culture model of AD, for which we cultivate either human or porcine brain tissue and inject Aβ monomers into the tissue, which triggers aggregation of Aβ and the deposition of the aggregates in the tissue. In order to study the contribution of excess iron in this model we injected ferric citrate into the tissue.
Results
A careful analysis of the shape of the deposits in vitro showed that iron changes the shape of the deposits. Furthermore, we reveal that combined iron overload and Aβ injection exacerbated neuronal loss, enhancing the toxicity of Aβ on its own.
Conclusion
The advantage of our organotypic brain tissue culture model is that the natural organization of the cells within the tissue is preserved. This technique will eventually also open the possibility of reducing animal experiments. This integrated approach will elucidate the complex mechanisms underlying neuronal degeneration in AD, paving the way for targeted therapeutic interventions.
Grant sponsors : Austrian Science Fund FWF, grants P29370, P32058; MEFO Graz.

Keywords:

Azheimer's disease, Amyloid beta, iron

Reference:

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Cryo-FIB milling and Cryo-electron tomography of tissue biopsies: BBQ method

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LS-08, Lecture Theater 4, august 28, 2024, 10:30 - 11:30

Cryo-electron tomography (CET) allows for unique insights into molecular structures in situ down to near atomic resolution. To access thicker cellular areas, thin samples of less than 250nm thickness are required and cryo-lamellae can be obtained by cryo-focused ion beam (cryo-FIB) milling. Specialized cells in tissue display unique molecular architectures that are not easily reproduced in isolated cell culture. While cryo-lamellae can be routinely prepared for cells in culture, there are only a few published examples of tissue CET due to the technical challenges associated with obtaining cryo-lamellae of tissue samples. To explore the full potential of CET for cell and developmental biology, better methods for cryo-lamellae preparation of tissues are required. Here we show a new workflow for cryo-FIB milling specifically for tissue samples. The workflow uses standard sample preparation equipment and on grid milling on a Gallium FIB and allows to routinely obtain cryo-lamellae on high-pressure frozen samples. The technique can be combined with automation and allows for the generation of tissue cryo-lamellae in a timely manner. Examples for several tissues obtained from mice and zebrafish are presented.

Keywords:

tomography, biopsy, cryo-ET, cryo-FIB, HPF

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Investigation of the effects of production conditions and additives on the microstructure of PVDF-HFP membranes

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Poster Group 1

Background incl. Aims
The Intergovernmental Panel on Climate Change (IPCC) reports on the global increase in average temperature and the escalating levels of CO2 emissions underscore the urgent need for sustainable energy solutions. Therefore, lithium-ion batteries (LIBs) have emerged as a key technology for energy storage, renewable energy systems and particularly in electric vehicles to reduce CO2 emissions. LIBs consist of a cell with a electrolyte containing membrane between anode and cathode. Besides the improvement of electrodes, membranes are key and active components that strongly affect battery performance due to its essential role to modulate both the ionic conductivity and effective lithium migration across the membrane's polymeric barrier PVDF's polymeric and chemical structure ensures the long-term durability of membranes compared to conventional PP and PE membranes; however, its limited adjustability of surface chemistry and micro-porosity restrict wettability and lithium-ion mobility. In the literature, it was shown that different processing conditions and inorganic particle additives may help to overcome these limitations. Metal-organic framework (MOF) particles, formed by metal ions surrounded by organic ligands, can be a promising additive for PVDF-HFP matrix due to its high surface area, adjustable pore size, and high thermal and chemical stability properties. This study aims to determine the effects of processing conditions as well as MOF addition on the microstructure and properties of the PVDF-HFP membranes.
Methods
The thermal-induced phase separation (TIPS) method was chosen to produce porous membranes. In this method, PVDF-HFP polymer, either with or without additives, was mixed with Dimethylformamide (DMF) solvent using a heated magnetic stirrer to obtain a homogeneous mixture. Then the final solution was subjected to degassing under vacuum to remove air bubbles. Subsequently, the solution was poured into a smooth glass surface and casted using a Dr. Blade equipment with three different polymer solid ratios and three different casting thicknesses, then subjected to heat treatment to remove the DMF solvent from the casted mixture. Produced membranes were analyzed by using SEM, XRD, TGA, DSC and FTIR analysis methods.
Results
Qualitative X-Ray diffraction analysis (XRD) of the samples revealed that they consisted of α and β PVDF phases. Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted to determine the quantitative phase ratios, and the effects of solid ratio and casting thickness on the beta phase ratio were examined. The calculations from the obtained analyses showed that the β phase ratio changes slightly depending on the solid ratio and casting thickness, consistent with the XRD results. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were also performed to calculate the crystallinity and estimate thermal stability. When the surface and cross-sectional microstructures of the produced samples were examined by scanning electron microscopy-secondary electron (SEM-SE) images, it was determined homogeneously distributed spongelike porous structure were successfully obtained. In the light of SEM images, the average pore diameter and average dry thickness of the samples were measured. Also, porosity and electrolyte uptake values were also measured depending on the processing parameters as well as additive ratios. With the addition of

MOF to the membranes' phase ratio remains similar, but DSC results showed an increase in the melting temperature. According to TGA results, changes in the decomposition temperature depending on the percentage of the additive were also observed.

Conclusions

This study shows that PVDF-HFP stable membranes can be successfully produced via TIPS methodology and reveals the effects of processing conditions and MOF additives on the properties important for the lithium-ion battery applications such as thickness, pore morphology, present phases and their amounts, crystallinity, porosity and electrolyte uptake.

This Study was supported by Eskisehir Technical University Scientific Research Commission under grant no 22ADP416.

Keywords:

Lithium-ion batteries, PVDF-HFP, Membrane, Morphology

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In-situ observation and analysis of Ni-based catalysts for dry reforming of methane

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Poster Group 2

Background incl. aims

Global warming is getting a tremendous issue because of the large release of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and so forth. Catalyst is one of promising solutions for global warming to reduce the greenhouse gases. Dry reforming of methane (DRM, $CH_4 + CO_2 \rightarrow 2H_2 + 2CO$) is a powerful reaction utilizing the two greenhouse gases. Ni-based catalysts are useful owing to their high catalytic activities and lower cost compared with noble metals. However, they are easily deactivated via carbon formation during RDM reactions, which is referred to as coking by generation from side reactions. While improvements in catalytic activities have been reported so far [1-3], coking and catalytic deactivation have been not fully studied yet. In this work, structural and chemical changes of Ni nanoparticles during DRM process were investigated using in-situ scanning transmission electron microscopy (STEM) with electron energy-loss spectroscopy (EELS) analysis.

Methods

Ni nanoparticles supported Al_2O_3 catalyst (Ni/Al_2O_3) powders were prepared with different mass ratios of 5 and 10 wt% through the impregnation method. Al_2O_3 powders were dissolved with $Ni(NO_3)_2{\cdot}6H_2O$ into ethanol. After the remaining ethanol was evaporated at 80 °C, the powders were heated in H_2 gas at 600 °C for 6 h to reduce the oxidized Ni. The prepared Ni/Al_2O_3 catalysts were observed in the DRM mixture gas conditions (50% CO_2 and 50% CH_4) with pressure of 0.3 ~ 10 Pa and then, were gradually heated at 150 - 450 °C. In comparison, they were also observed in pure CH_4 gas.

For in-situ observations, our previously developed gas environment heating specimen holder system was used [4]. The Ni/Al_2O_3 powders were dispersed on a Si-based heater chip (E-chip, Protochips, USA), which was arranged in the specimen room at the tip of the specimen holder connecting to the heater controller outside of an electron microscope column. The chip was sandwiched between the two orifice plates to create a differential pumping effect leading to a higher environmental pressure in the specimen room. The powders were observed through a double aberration-corrected electron microscope (JEM-ARM200F, JEOL, Japan) operating at an accelerating voltage of 200 kV. In addition, ex-situ experiments were conducted compared with in-situ ones to examine electron irradiation damages.

Results

The TEM and STEM observations of the prepared Ni/Al_2O_3 powders show the Ni nanoparticles with the size of 5 to 50 nm were uniformly dispersed on the Al_2O_3 support powders. After the ex-situ experiment in the DRM gas (10 Pa) at 450°C for 6 h, the powders were covered with thick carbon films due to coking. During in-situ observation at 550°C after ~15 min of elevating temperature, the similar carbon films were also observed on the Ni nanoparticles. This comparison demonstrated that the in-situ observation had little electron irradiation effects on the structures and reaction phenomena. The carbon deposition was observed even at 350°C in the DRM gas, although the amount of carbon was much less than that in the pure CH_4 gas condition.

Next, the chemical bonding and valence state of the Ni nanoparticles on the Al_2O_3 supports were measured under the DRM conditions by in-situ EELS. Before the DRM gas flowing, the Ni nanoparticles were mainly metallic Ni. But after the gas flowing, most of the nanoparticles changed

from Ni to oxidated Ni depending on temperature. Some Ni nanoparticles were oxidized at over 450°C and then reduced at over 550°C in the DRM gas. In addition, structural changes of the Ni nanoparticles were also observed with the oxidation and reduction reactions.

Conclusions

We observed the Ni nanoparticles on the Al_2O_3 supports through in-situ STEM and EELS to elucidate actual phenomena occurring on the Ni/Al_2O_3 catalysts under the DRM conditions. Carbon deposition on the Ni nanoparticles was observed even the in-situ observations. Furthermore, the change in the chemical bonding and valence state of the Ni nanoparticles were also measured on some Al_2O_3 supports depending on elevating temperature through in-situ EELS.

Keywords:

in-situ observation, catalytic materials, nanoparticles

Reference:

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Combining synchrotron-based microCT with plasma FIB-SEM for targeted Volume EM

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Background
Although offering the highest resolution for volume EM, conventional FIB-SEM imaging has a limited field of view, provides only surface-level information, and is unable to access information non-destructively from within the sample volume to pinpoint areas of interest. Hence, integrating an additional imaging modality capable of non-destructive volume imaging becomes necessary to accurately locate and target regions of interest within complex multilayered specimens. Micro-Computed Tomography (MicroCT) offers a larger 3D overview but at lower resolution lacks many subcellular details. For volume EM, the combination of the two methods offers precise targeting of regions of interest that can be pre-identified in the microCT volume.

Methods
We used the new microCT beamline on the 3 GeV Australian Synchrotron, which has an X-ray energy range from 8 to 40 keV. We imaged Epon resin embedded tissue blocks processed for slice and view imaging by FIB-SEM. We initially tested both monochromatic and white beams as well as different detector distances, but found no major differences, so choose to continue with the white beam to take advantage of the much shorter imaging times. Blocks were subsequently pre-trimmed by ultramicrotomy, transfer to a Helios Hydra plasma FIB-SEM and block cross-sections were aligned with the microCT volume. Final trimming was done using oxygen plasma milling on the plasma FIB before starting the slice and view run for volume EM.

Results
We here show examples where we targeted specific brain regions in zebrafish embryos by microCT and volume EM and the correlation of the two volumes.

Conclusion
Synchrotron based microCT is substantially faster than microCT on lab-based instruments. As plasma FIB allows milling of larger volumes more rapidly than conventional FIB-SEM it is possible to perform the final fine targeting on the FIB-SEM. In summary, the combination of synchrotron microCT and plasma FIB-SEM offers particular advantages for targeted volume EM.

Keywords:

synchrotron, brain, vEM, zebrafish, CLEM

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Innovative Nanoanalytical Approaches for Lithium Metal Interface Analysis

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Poster Group 1

Background

Lithium metal anodes are important for next generation high-energy batteries, enhancing energy density in terms of both volume and mass while remaining cost effective. They are already implemented in lithium-sulfur batteries nowadays. However Interfacial instability reactions during operation still represent a challenge for the commercialization of solid-state batteries and require further research.

Challenges in Characterization
The analytical characterization of lithium metal interfaces with high lateral resolution is essential for understanding chemical reactions. These interfaces are highly reactive in ambient air and in particular sensitive to electron and ion beam interactions at room temperature. Employing an argon filled glove box prevents Lithium from reacting with moisture and oxygen. Nevertheless, the reaction with the residual nitrogen content in argon-flooded glove boxes is often overlooked. Additionally, conventional electron microscope air locks are typically vented with nitrogen and are therefore another nitrogen contamination source when samples were transferred between microscopes.

Methods

We deposited lithium layers and other thin test layers by thermal evaporation in a vacuum glove box and flooded afterwards with pure argon gas. With this approach we achieved an oxygen-, water- and nitrogen-free environment in the glove box. Our workflow also involves nitrogen free transfer between the scanning electron microscopes (SEM) and transmission electron microscopes (TEM) under argon atmosphere as well as cryogenic electron microscopy. Chemical characterization of the interfaces with high spatial resolution is achieved by focused ion beam secondary ion mass spectroscopy (FIB-SIMS), energy dispersive x-ray spectroscopy (EDS) and electron energy loss spectroscopy (EELS).

Results and Conclusion

We demonstrate the preservation of metallic lithium via the analysis chain. We also show the possibility of depositing additional thin test layers within the glove box. The methodology and workflows shown in this work give rise to an understanding of Li metal / coating interfaces and can be adapted to study other thin coatings or reaction products sensitive to ambient conditions.

Keywords:

Li-battery
cryo-/electron-microscopy
inert-transfer
nitrogen-free

Lithium-deposition

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Mapping of phase separation of supramolecular protein assemblies by live-cell holotomography microscopy

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Poster Group 1

Supramolecular protein assemblies (SMPAs) have demonstrated to be a great tool for studying the biophysical properties of human cells. Liquid-liquid phase separation is a driving mechanism in the assembly of various intracellular structures. An oligomerizing biomimetic system, known as Corelets, has helped to map phase separation within the cell subregions by means of protein condensation. Corelets are photosensitive phase-separating photoinduced oligomers of self-interacting proteins. Previous studies on protein condensation by localized oligomerization showed that sequestering protein ligands to slowly diffusing nucleation centers move the cell into different regions of the phase diagram, resulting in localized phase separation. The real-time oxidative-induced stress in human cells has also remained an enigma in cellular biology. A previous work on the redox state of the cell showed that supramolecular protein assemblies have a self-assembly interface sensitive to the exposure to a thiol-specific oxidizing reagent. We worked with a system of self-associating proteins built onto a ferritin core, genetically encoded for the expression of supramolecular protein assemblies based on a fusion construct between citrine, a yellow fluorescent protein variant and the heavy chain human ferritin. Here, we use a novel 3D label-free non-invasive fluorescent live-cell imaging method, which allows performing high-precision in-vivo holotomographic microscopy of subcellular structures, coupled with cryo-electronic tomography and image processing to evaluate real-space structure of solid and liquid localized phase separation and of SMPA oxidation within the cell nucleus; which allows us to use the refractive index as a method to map the emergence and prevalence of the Corelets in the cells. Our work has allowed the three-dimensional visualization of phase separating condensates in mammalian cells.

Keywords:

Protein condensation, label-free, refractive index

Reference:

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Differential axon terminal spread and synapse numbers amongst afferent neurons onto a locust's movement detector

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

Background incl. aims
The locust giant movement detector neurons, LGMD1 and LGMD2, faithfully signal impending collisions by summing up information about changes in light levels across the compound eye. These changes in light levels are transmitted by trans-medullary afferent neurons that correspond to the thousands of facets of the locust's eye. It is known that the afferent neurons preserve anatomy, so each afferent transmits signals from a defined facet and reaches a defined area on the LGMDs dendritic tree, but anatomical studies of afferent neurons are very rare, with the exception of two full length – reconstructions from a fourth larval instar (reference). In order to detect differences in afferent anatomy, spread of their terminal arbour along the LGMD, and synapse numbers between neighbouring afferents and between the LGMD1 and LGMD2, we are 3D - reconstructing the terminal arbours of afferents of both the LGMD1 and LGMD2.
Methods
Tissue taken from adult locusts is chemically fixed, contrasted and embedded and either one of two 3D Scanning electron microscope methods are employed : either the tissue is serially sectioned within the chamber of a scanning electron microscope and the block face scanned (serial block face SEM), or series of sections are produced using an automated tape collection ultramicrotome (ATUMTome SEM), which are later scanned using Zeiss Atlas software in a Zeiss Gemini SEM.
Results
Surprisingly, in the LGMD2, we found two types of trans-medullary afferent neurons: first, second, other neurons that were rarer had a significantly larger spread of neurites and significantly more synapses among. Retinotopy appeared to be preserved for both neuron types.
Conclusion
The fact that some afferent neurons exhibit larger spread of arbours and more synapses onto the LGMD2 is surprising and has consequences for signal transduction. We assume that for the larger neurons, signal strength is increased at the cost of resolution.
Sponsored by the Austrian Science Fund FWF, grant 32058

Keywords:

LGMD, SBEM, ATUM Tome SEM

Reference:

Wernitznig S et al., J Comp Neurol 2022, 530:518

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Advanced approaches for the analysis of micro- to nano-quartz particles using SEM, ESEM, FIB-SEM, SBF-SEM

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Poster Group 2

Background incl. aims
Quartz micro- to nano-particles are used in a wide variety of industries, especially in the semiconductor industry, optics, as a filler for epoxy resins, etc.. During the preparation of these particles, it is essential to know and control one of the crucial parameters - the particle size distribution (PSD). The production process itself significantly affects the PSD as well as other morphological characteristics. The shape, texture, and size distribution of the particles have a significant impact on the material's mechanical strength, density, electrical properties, and thermal properties. Various methods are used to evaluate PSD. This paper discusses laser diffraction (LD), and microscopy methods: Scanning Electron Microscopy (SEM), Environmental Scanning Electron Microscopy (ESEM), Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and Serial Block-Face Scanning Electron Microscopy (SBF-SEM). The applications, sample preparation requirements, advantages and accuracy of PSD analysis for the above methods are compared and discussed. The study focuses on ESEM as an interesting, useful and cost-effective technique with many advantages for the study of quartz micro- to nano-particles.

Methods
Quartz powder was first measured using LD. For SEM and ESEM imaging, a non-commercial ESEM AQUASEM II was used. The samples were observed in vacuum and in water vapor at 200 Pa, at 2 magnifications to cover all particle sizes, and using three different types of detectors to detect backscattered and secondary electrons. The particles embedded in the resin were observed using high-resolution ESEM QUANTA 650 FEG at a water vapor pressure of 200 Pa equipped with a Gaseous Secondary Electron Detector. Sub-nanometer particles were observed with high magnification and under pressure of 200 Pa. A procedure has been developed for particle analysis using the Mountains SEM 9 Expert software. Next, the particles embedded in the resin were analyzed by FIB-SEM Tescan Amber. From the resin cube, layers with a thickness of 100 nm were successively sputtered with a Ga+ beam. The last method was SBF-SEM, where layers were sequentially cut from a 1x1x1 mm resin cube using a ConnectomX ultramicrotome in a Tescan Clara UHR SEM microscope.

Results
The result of particle analysis by LD is a plot of the cumulative distribution function of particle sizes. The size parameter is the equivalent spherical diameter. Comparisons were made between SEM/ESEM analyses of the particles, as well as between the powders themselves and their embedding in resin. Our results confirm that the ESEM provides accurate shape and size characterization of quartz particles at vapor pressures in the order of hundreds of Pa and working distances in the low mm range, regardless of the type of detector used. These conclusions were confirmed by the analysis of smaller particles (0.5 - 2 µm). ESEM has also enabled the identification and analysis of particles tens of nm in size. Images acquired with FIB-SEM were processed with 2 software. ORS Dragonfly provided an advanced view of the incorporation of particles embedded in

resin. The second, IP-SDK, allowed precise distribution and annotation of individual particles, which allowed accurate statistical evaluation of their distribution, sphericity, volume, aspect ratio, etc. Images obtained using the SBF-SEM method showed that during the cutting of each layer, the particles were disintegrating, breaking off, and being pulled out, preventing proper particle analysis.

Conclusions
LD is very fast, efficient and widespread method of particle analysis over a large size range. Its disadvantage is the impossibility to know the real morphology of the particles. SEM and ESEM are able to image not only the powder particles but also the particles embedded in the resin, making it possible to fully control the production and use of these particles in other products, which is their great advantage. The ability of SEM and ESEM to image the real morphology and shape of the particles also allows quantification of other morphological parameters, and provide information on the exact shape of the particles. ESEM is a full-fledged and innovative particle analysis tool, and brings a number of advantages, such as a significant reduction in sample charging, thus avoiding the formation of image artifacts that distort the resulting statistics. The potential risk of microscope damage due to contamination of released particles is also significantly reduced. It allows imaging of non-conducting particles without prior modification, and another important advantage is the ability to reveal structural details of even sub-micrometer sized particles. FIB-SEM is one of the most modern, and very advanced tools that allows the measurement of 3D morphological properties of particles, in particular their spatial distribution. However, it is crucial to choose the correct particle labelling procedures, as the accuracy of the resulting distribution depends on this. ORS Dragonfly is a great imaging tool, while IP-SDK provides better statistical evaluation capabilities. SBF- SEM proved to be unsuitable for this type of sample due to the high hardness of the resin.

Keywords:

particle-size distribution, ESEM, SBF-SEM, FIB-SEM

Reference:

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M. Olbert, V. Neděla, J. Jirák, J. Hudec, Size and shape analysis of micro- to nano-particles of quartz powders using advanced electron microscopy and laser diffraction methods, Powder Technology. 433 (2024), https://doi.org/10.1016/j.powtec.2023.119250.

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Enhanced sensitivity in label-free live-cell imaging using multi-pass stimulated Raman scattering microscopy

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Poster Group 1

Background incl. aims
Stimulated Raman scattering (SRS) microscopy is a label-free imaging technique used for measuring chemical concentrations with high spatial resolution. The chemical sensitivity of SRS microscopy is limited by photostress or photodamage in sensitive specimens or by the timescales of fast dynamics. To increase the signal-to-noise and thus the sensitivity of SRS microscopy in live samples, we implement a multi-pass imaging protocol.

Methods
Two femtosecond near-infrared laser beams—a pump beam and a Stokes beam—are used to drive SRS. Pulses from both beams are chirped to gain spectral resolution in the measurements [1]. A Pockels cell is used to rotate the polarization of the Stokes pulses, first to initiate and then to end the multi-pass process. An intensity modulation on the pump beam results in a time-varying intensity gain in the multi-passed Stokes beam due to SRS, which is detected with a lock-in amplifier.

Results
We demonstrate an enhanced signal-to-noise ratio in SRS measurements at constant optical dose relative to equivalent single-pass measurements in both spectroscopy and imaging, with signal-to-noise increases currently of about 6 dB. We further characterize the performance of the microscope as a function of the number of interactions between the Stokes pulse and the sample and numerically study the tradeoffs and optimal operating conditions for multi-pass imaging. Using this microscope, we image live biological targets including plant roots and nematodes and use the chemical specificity of SRS to differentiate important biomolecules with subcellular spatial resolution.

Conclusion
Multi-pass protocols enable higher signal-to-noise measurements and thus increased sensitivity or imaging speed, as we demonstrate here for label-free live-cell imaging in an SRS microscope. Multi-pass techniques can be used to increase the sensitivity of many dose-limited measurements up to fundamental quantum limits [2, 3]. We show that multi-passing overcomes the shot-noise-limited signal-to-noise ratio of a standard single-pass experiment conducted with the same total optical dose. This increased sensitivity could enable new measurements of the spatial distribution of low-concentration or weakly scattering molecules in dose-sensitive biological specimens without the need for disruptive labels.

Keywords:

Label-free, stimulated Raman scattering

Reference:

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Resonant inelastic light scattering micro-spectroscopy to probe inter-moiré miniband excitations in twisted 2D semiconductors

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Poster Group 2

Background incl. aims:
Resonant inelastic light scattering (RILS) is a powerful technique to study collective charge excitations in semiconductor quantum nanostructures including quantum wells and nanowires [1]. The application of this technique to probe collective behavior of electrons and holes in novel two-dimensional (2D) materials has not been explored yet. The moiré superlattice potential in twisted 2D semiconductors, e.g. WSe_2, leads to carrier localization and emergence of (flat) moiré bands in the electronic band structure, which host interesting correlation physics [2]. Although the signatures of such flat bands have been shown in recent studies including scanning tunneling spectroscopy (STS) [3] and angle resolved photoemission spectroscopy (ARPES) [4] , the observation of electronic transitions between them proving the emergence of these bands, especially near the K-point of the electronic structure, have remained elusive. In this work, we used RILS micro-spectroscopy to probe the inter-moiré band excitations (IMBE) in twisted bilayers of WSe_2 with a twist angle of ~3° and ~8°.
Methods:
The twisted WSe_2 bilayers were mechanically exfoliated from bulk crystals of WSe_2 using a dry deterministic transfer technique based on viscoelastic stamps. The bilayers were also encapsulated in multilayers of hBN to protect them from environment and to provide atomically flat interfaces. The multilayer stack was built upon a ~300nm silicon dioxide layer thermally grown on p-doped silicon substrate. After preparation, the samples were annealed in vacuum and then mounted inside a refrigerator and cooled down to 4K using liquid Helium. Different regions of the sample were identified using a white LED source and Köhler illumination in combination with piezoelectric x-y-z stages and imaged using a CMOS camera. The twisted bilayer regions of the sample were optically excited using a laser beam from a frequency tunable Ti : Sa laser (linewidth = 50 KHz), cleaned up using a monochromator (1nm bandpass) and coupled to the sample through a large numerical aperture (NA = 0.82) objective lens which is compatible with low temperatures. The excitation energies were chosen near the direct optical bandgap at the K-point pre-determined using photoluminescence spectroscopy. The laser spot size on the sample was ~2µm. The emitted and scattered light from the sample was collected using the same objective and guided to a triple-spectrometer operated in a subtractive mode to reject any stray light. The RILS spectra were recorded using a liquid nitrogen cooled CCD camera.

Results:
Our results show the signatures of transitions between the 1st and 2nd moiré bands for the twist angle of ~8° and between the 1st moiré band and 2nd, 3rd and higher moiré bands for a twist of ~3° [5] We further compare our results with the RILS spectra of natural bilayers of WSe_2 where the absence of IMBE as a consequence of absence of a moiré superlattice further consolidates our results. The IMBE transition energies are in excellent agreement with those expected from theoretical ab-initio calculations in spite of broadening resulting from twist angle disorder and non-vertical transitions [5].
Conclusions:
Our results quantify the IMBE energies at the K-point of the electronic structure, where the states relevant for correlation physics are hosted. They further establish low temperature RILS as a powerful non-invasive technique for probing collective charge excitations in twisted 2D semiconductors [5].

Keywords:

2D materials, RILS, moiré minibands

Reference:

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Investigation of earth-abundant photovoltaic material Zn3P2 nanostructures using electron microscopy

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Poster Group 2

Background incl. aims
Earth-abundant semiconductor material Zn_3P_2 is a promising alternative for photovoltaic applications. Unfortunately, there are not many suitable substrates for Zn_3P_2 nanostructure growth as it is difficult to find a lattice parameter and thermal expansion coefficient matching material. Therefore, growth of defect-free Zn_3P_2 nanostructures has been proven to be challenging as it tends to form defects during growth which results in poor crystal quality thus is detrimental for photovoltaic applications.[1][2] To minimize the number of defects, a new method of Zn_3P_2 growth is needed. Selected Area Epitaxy (SAE) and Vapour-Liquid-Solid (VLS) have been shown to be effective techniques in producing different morphologies of Zn_3P_2: nanowires, nanopyramids and thin films.[3] Both techniques reduce the interfacial area which is key in limiting defect formation. In both VLS and SAE indium has been utilized as a seed particle or the substrate (InP). By exchanging In for more earth-abundant Sn seed particles for VLS and InP substrate for Si, it would be possible to completely remove In from the growth process. It is also important to investigate high throughput methods of producing Zn_3P_2 nanostructures for possible future industrial scalability.
Methods
Metal organic vapour phase epitaxy (MOVPE) is an appealing method for growth of SAE and VLS Zn_3P_2. MOVPE is an epitaxy method that has a high through-put and is widely used in industry for manufacturing compound semiconductor devices. InP substrates have been used in our experiments with a silicon oxide (SiO_2) mask that was patterned by EBL to try and identify preliminary growth parameters for SAE growth. Growth times, temperature and precursor flows have been varied to try and find optimal conditions for Zn_3P_2 growth with minimal parasitic nucleation on the SiO_2 mask. For SAE patterning, displacement Talbot lithography (DTL) which is a lithography technique that uses collimated monochromatic light to create a periodic pattern on the substrate, was used. Moving away from EBL to DTL is a key step for scalability of Zn_3P_2 growth, as DTL can pattern much larger areas quicker. Si substrates were used for DTL dose and pattern testing. Sn seed particles on InP substrate for VLS growth were used. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to image and investigate the morphology and growth selectivity of the resulting Zn_3P_2 structures. Scanning TEM (STEM) together with x-ray energy dispersive spectroscopy (XEDS) was used to image and acquire compositional data of the nanowires.

Results
SAE Zn_3P_2 pyramidal structures by MOVPE on InP substrate were successfully grown (Fig.1a). By optimizing the precursor flows and temperature we were able to minimize most of the parasitic nucleation on the SiO_2 mask layer. DTL was used to pattern hexagonal pattern of holes on SiO_2 on Si substrates, which have diameter of 180nm-200nm and pitch of 500nm (Figure 1b). Sn seeded Zn_3P_2 nanowires were grown and then transferred to a TEM copper grid (Fig. 1 c-d). Additional compositional analysis of the nanowires was performed using XEDS coupled with STEM.

Conclusion
We have shown that growth of SAE Zn_3P_2 on InP substrate is possible using MOVPE. By optimizing the growth conditions parasitic growth is minimized on the mask. Si substrates were patterned by using DTL and desired size and pitch of the nanoholes was achieved. Nanowires were successfully grown by using Sn instead of In seed particles. We hope to exchange InP for Si thus removing any scarce elements from the production and enabling full scalability of Zn_3P_2 nanostructures. Future experiments will focus on realizing SAE Zn_3P_2 growth on the patterned Si substrates and exploring Si substrates for Sn seeded VLS nanowire growth.

Keywords:

Zn3P2 Earth-Abundant SAE VLS SEM

Reference:

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Engineering electron-electron interaction for advanced quantum metrology in electron microscopy

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Poster Group 2

Background incl. aims:
Electron microscopy (EM) is a fundamental instrument for our comprehension of nanoscale materials and biological samples, providing unparalleled resolution and structural information.
Standard electron microscopy uses beams where a single electron at a time forms the image. However, the recent demonstration of interacting electrons in Ultrafast TEM experiments [1,2] has opened up the possibility of a more complex electron beam characterized by strong electron-electron, mainly Coulomb, interaction.
In fact, electron-electron repulsion may significantly impact the spatial arrangement of beam electrons, leading to collective phenomena such as space charge, beam dispersion, and beam instability. While this is considered detrimental to image resolution and contrast, harnessing this new degree of freedom may be a new tool for electron microscopy and metrology.
In particular, electron-electron interactions can be represented as a non-linear Schrödinger equation and could bring quantum advantage in metrology.
This could be important in increasing the accuracy of measurements or reducing dose demand in imaging delicate matter (e.g., proteins). In fact, quantum discrimination theory [3] has helped us to ascertain the minimum number of electrons needed to discriminate between two particles, which depends on the superposition integral of the wave function corresponding to the imaging of the two particles. Unfortunately, unitary evolution of electrons in a microscope, even in the presence of beam shaping, cannot overcome such limitations. Conversely, the non-unitary wave evolution in multiple electron beams with "self-interaction" could potentially overcome this limitation.
Here, we aim to explore the feasibility of this new TEM metrology concept through quantitative wave simulations.

Methods:
We are using here a simplified mean field Hartree scheme [5] connected to multislice propagation of the electron wave function. We employ a modified multislice code [4], implemented in Python, to simulate the effects of electron-electron interactions alongside propagation. At each slice, the electron density is used to self-consistently calculate the self-interaction Coulomb potential.
In our simulation we used a Laguerre-Gauss (LG) multi-electron beam with an orbital angular momentum (OAM) of 10. The nm sized beam traverses here a cumulative distance of 280 nm.
For comparison we also investigated the behavior of a single-electron LG beam.

Results:
Our findings (as shown in the figures below) reveal that by increasing electron density (number of electrons per bunch) the gold atom becomes more visible, and that upon self interaction and

propagation the overlap integral [1], starts to decrease. This suggests that particle discrimination could be improved for interacting electron beam, if the electron density is sufficiently large.
In other words two initially identical multi-electron wave functions, where one of them is scattered by a single gold atom, progressively differentiate with propagation distance. The nontrivial evolution of the wave can be also interpreted in terms of vortex beam instability.

Conclusion:
In this preliminary study, we showcased with a relatively simple example how electron-electron interactions, generally considered detrimental to imaging and analysis, could potentially be exploited to enhance the discrimination between electron wave functions.

Figure Caption:
Fig.1 The amplitude (a,b) and phase (c,d) of the wavefront of a single electron LG beam (OAM=10) in the final () slice with and without gold, respectively. In e) the overlap integral as a function of propagation distance (slice number, with each slice comprising 14 nm) remains constant at a value of 1, denoting unitarian propagation.
The amplitude (f,g) and phase (h,i) of the wavefront of a multi-electron LG beam (OAM=10) in the final () slice with and without gold, respectively. In l) the overlap integral begins to exhibit a different trend, decreasing in value. This is attributed to the non-unitarity, arising from electron-electron interactions.

Keywords:

Electron microscopy, multislice, electron interactions,

Reference:

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Human Tumor Microbiome Detection using Correlative Light and Electron Microscopy

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Poster Group 2

In recent years, bacteria have shown to be present of human tumors, but their role and advantageous to the tumors or to the bacteria themselves is still mostly unclear. Characterization of the tumor microbiome is challenging because of its low biomass. In order to confirm the presence of bacteria in human tumors, various methodologies were conducted including immunohistochemistry by using antibodies against bacterial lipopolysaccharide and lipoteichoic acid to detect Gram-negative and Gram-positive bacteria, respectively. However verifying the presence of bacteria inside cancer cells is challenging, due to the small size of the bacteria and its sparsity in the tumor tissue. We used Correlative Light-Electron Microscopy (CLEM) that localize specific cellular components using fluorescence labeling and microscopy and visualize in high resolution details the cell ultrastructure by electron microscopy. Fluorescent labeling has been used to identify bacteria over a large area of interest in a sample, and particularly beneficial in samples exhibiting a sparse number of targets or events. In this work we were able to validate the presence of bacteria inside cancer cells of human breast tumor. Combined fluorescence staining of bacteria and transmission electron microscopy imaging of the same cells clearly demonstrated the intracellular localization of bacteria in tumors [1].

Keywords:

CLEM, Light and electron microscopy

Reference:

D. Nejman, I. Livyatan, G. Fuks, et al., The human tumor microbiome is composed of tumor type-specific intracellular bacteria, Science, 368 (6494) (2020), pp. 973-980

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Mean inner potential measurement by correlated EFTEM and phase-shifting holography

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Poster Group 2

Scientific Background
The mean inner potential (MIP) is an intrinsic material property describing the volume average of the atomic electrostatic potentials in a specimen. Accordingly, it depends on the atomic composition and structure and is sensitive to impurities. Typical values for the MIP lie on the order of 10-30V. However, literature values for common materials can vary by several volts, while values for less-common materials are often unavailable. Since the projected MIP across a homogeneous sample should be constant, the typically used off-axis holography approach involves reconstruction of the gradient of the projected MIP from a sample with a known wedge angle created by cleaving along lattice planes.
This approach has several limitations. Cleaving samples is only practical for single-crystalline, ideal samples. Alternatively, FIB-milling can be used to create the wedge profile, but this results in local deviations from the desired wedge angle as well as significant surface damage. Therefore, the reconstructed phase must be correlated to the local thickness of the material. It is imperative that the local thickness of the sample is known with a high spatial resolution, which then can be matched to the reconstructed phase at similar length-scale. The latter is particularly problematic, since the common Fourier sideband phase reconstruction introduces a low-pass envelope that degrades the spatial resolution of the phase map. We aim to improve the precision of the MIP measurement by correlating EFTEM thickness and off-axis holography phase with high fidelity using phase-shifting holography [1].
Methods
We have recently demonstrated the capability of phase-shifting holography with systematic beam tilt to reconstruct the complex exit-wave of a sample up to Angstrom resolution [2]. Here, we make use of the biprism drift in order to reconstruct the projected potential at lower magnifications, which allows us to reach a spatial resolution up to the pixel size of the camera device while still maintaining a large field of view. We demonstrate this method on a FIB-milled wedge from a lattice-matched heteroepitaxial AlAs-GaAs multilayer sample [3].
Wedge-shaped samples from AlAs-GaAs multiayers are prepared by Ga+ focused ion-beam milling and low-kV cleaning using stage and milling pattern rotations to optimize the wedge angle. The local thickness is mapped by EFTEM using the log-ratio technique. In order to reduce the uncertainty from the mean-free-path prediction, the crystalline part of the thickness wedge is measured by convergent beam electron diffraction (CBED). With the assumption that the FIB-damaged volume outside the sample edge is of homogeneous thickness, we thus calibrate the EFTEM thickness gradient at the sample tilt of the CBED two-beam condition. We then record series of off-axis holograms in Lorentz-TEM mode with a biprism voltage of 200V at zone-axis, two-beam and weakly diffracting tilt

conditions. The random drift of the biprism position is used in place of a systematic beam tilt, in order to generate a phase-shifting series. The holography data is then aligned and a narrow band of high visibility of the fringes in the vacuum region is selected in order to improve the cosine fit for phase-shifting reconstruction.

Results

Taking into account differences in magnification and lens rotation from the projection system, we are able to correlate local projected potential and local thickness with sub-nanometre precision (see Fig.1). Collecting all the data pairs and performing a linear regression results in an excellent fit to the linear phase prediction at kinematical conditions. However, results for different sample tilts also highlight the need for a dynamical scattering correction. The method thus is well-suited for homogeneous samples and uniform diffraction conditions.

Conclusions

Phase-shifting is a powerful tool to improve the correlation between off-axis holography and other TEM methods. Results at suitably large tilts off zone axis are in good argeement with literature, although MIP values appear to be systematically slightly larger than previously reported. Particular care must be taken in order to minimize or, barring that, account for the different influence of preparation damage and dynamical diffraction.

Caption

Fig.1: Measurements from AlAs-GaAs heteroepitaxial multilayers near the surface to the FIB-Pt layer, for MIP measurement. EFTEM t/λ thickness map (a), single-hologram phase reconstruction with an aperture of 1/3rd of the carrier frequency (b), and phase-shifting phase reconstruction from a subset of 90 individual holograms (c). Insets show magnified views of the amplitude of the reconstructed wave at the interface to the nanocrystalline FIB-Pt, with the approximate position of the surface marked by the dashed lines. All measurements were taken at weakly-diffracting sample tilt.

Keywords:

Off-axis Holography, Mean Inner Potential

Reference:

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Atomic-scale imaging of local structure of layered Cu-Te phases

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Poster Group 2

Background incl. aims
Copper-based chalcogenide materials exhibit distinct properties, making them promising candidates for a range of applications [1]. Among these materials, Cu-Te compounds have attracted significant interest due to their versatility in fields like thermoelectricity, memory technology, photothermal therapy, photovoltaics, and batteries. The properties of these alloys are closely linked to their local structure, making accurate description essential for optimizing device performance and advancing material development. The local structure of Cu-Te compounds remains a topic of ongoing discussion [2], especially concerning materials in their low-dimensional form, such as thin films.

This study addresses the controversial aspects of the local structure of Cu-Te phases prepared by thin-film solid-state reaction. Using Cs-corrected scanning transmission electron microscopy (STEM) and atomic-resolution electron energy loss spectroscopy (EELS), the atomic positions of metal and chalcogen atoms in Cu-Te structures are directly visualized. In addition, the investigation revealed the formation of new Cu-Te phase. A crystal structure model for this phase was proposed based on atomic-scale images.

Methods
Atomic-scale investigations are carried out via direct imaging in real space utilizing iDPC, ABF, HAADF and EELS methods. Cu-Te structures are synthesized from metal Cu layer and Sb_2Te_3 epitaxial thin films by thermal heating.

Results
The microstructural investigation of the Cu/Sb2Te3 system unveiled a reaction between the Cu layer and Sb2Te3 upon heating. This reaction led to the formation of van der Waals bonded layered Cu-Te structures, comprising double and triple layers of Te, as depicted in Fig 1. Contrary to existing literature, a detailed analysis of the atomic positions of Cu and quantification of interatomic distances revealed the crystallization of a Cu-rich trigonal Cu1.75Te phase (P-3m1) as the predominant double-layered Cu-Te structure.

Furthermore, a new metastable Cu-rich trigonal phase of Cu1.75Te (P3m1) was found, for which a structural model has been proposed. The phase features a triple-layered structure, resembling the Cu1.75Te double-layered phase. Notably, this metastable phase exhibits vacancies on Cu sites. A detailed analysis of the transition regions suggests that the triple-layered Cu1.75Te structure can easily transformed into the double-layer phase through rearrangements of Cu atoms. Crystal defects, including stacking faults and dislocations, were also observed within the crystal structures. Due to its resemblance to layered Sb_2Te_3 structure, a triple-layered structure was also fabricated as a thin film

through solid-state epitaxy of Cu and Sb_2Te_3 layers, using magnetron sputtering at room temperature.

Conclusion
The findings from this study offer valuable insights into the local structure and preferred phases of Cu-Te, which can be relevant during the cycling of Cu-based bulk thermoelectric materials [3]. This knowledge lays the groundwork for theoretical investigations into their properties and facilitates the design of novel layered materials.

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Fig. 1. (a) Atomic-resolution ABF-STEM image showing van der Waals bonded layered Cu1.75Te structures consisting of double (2Te) and triple layers (3Te) of Te. (b) High-resolution HAADF-STEM image and (c) corresponding atomic-resolution EELS maps of Cu (red colour) and Te (green colour).

Keywords:

Thin films, Nanoscale analysis, structure

Reference:

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Cryo-EM structure of the HD6 defensin helical assembly

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Poster Group 1

Defensins are small antimicrobial proteins produced by animals, plants, and fungi. HD6 is a human defensin secreted into the small intestine. Unlike other defensins, HD6 is not directly bactericidal but rather self assembles into «nanonets» [1] thought to physically trap bacteria and prevent them from penetrating the intestinal epithelium. The existing model for the nanonet filament structure, a spiral of HD6 dimers proposed based on an HD6 crystal structure, does not fully explain the long persistence length (microns) of HD6 filaments in vitro [2]. To resolve this apparent discrepancy, we determined structures of HD6 nanonet filaments using SPA cryo-EM.
HD6 filaments were produced in phosphate buffer according to a previous protocol [2]. The filaments demonstrated polymorphous behavior, so data were collected at different protein concentrations to favor different polymorphs. Specifically, low concentrations yielded predominantly thin filaments ~40 Å in diameter. Higher concentrations yielded thicker filaments ~80 Å in diameter. Cryo-EM data were collected on a Talos Arctica (Thermo Fisher Scientific) operated at 200 kV. EER movies were collected using EPU software in AFIS mode on a Falcon 4i direct detector at a nominal magnification of 190kx, corresponding to a physical pixel size of 0.73 Å. Image processing was performed using cryoSPARC v4 [3].
HD6 thin filaments were found to consist of stacked pairs of dimers spiraling along the helix axis with apparent D2 symmetry. Strong densities were observed along the axis and were interpreted as phosphate ions, each coordinated by multiple copies of a histidine, H5, in HD6. Mutation of H5 abolished filamentation. Once maps of sufficient resolution were obtained, a symmetry break became apparent: the two spirals of dimers in the thin filaments were found to be related by two-fold symmetry perpendicular to the helix axis, but not by true two-fold symmetry along the axis as would have been expected for D2. The repeating unit of the thin filament consists of two stacked HD6 dimers, with one protomer out of the four displaying a loop in a slightly different conformation than in the other three molecules. The thick filaments, in turn, formed by wrapping two additional HD6 dimer spirals around the thin filament without coordination of additional phosphate. The outer spirals adopt D2 symmetry and show helical rise and twist that differ from the inner filament.
The robust multiple micron length HD6 filaments are explained by the fact that two or more HD6 dimers constitute each level of the supramolecular assembly. Moreover, phosphate plays a structural role in stabilizing the filaments. HD6 helical polymorphs arise from an optional wrapping of additional stacked-dimer spirals around the phosphate-coordinating inner filament, and a symmetry mismatch exists between inner and outer spirals.

Keywords:

Cryo-EM, SPA, Defensins, HD6

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Temporal analysis of bone development in chick femur bone using label-free imaging

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Poster Group 1

Background incl. aims:
Long bones such as femur develop via the process of endochondral ossification (EO). During EO, chondrocytes proliferate, undergo hypertrophy and subsequent cell death with the replacement of hyaline cartilage with bone. The constructed cartilaginous matrix is invaded by blood vessels, osteoblasts, osteoclasts, and bone marrow cells before it develops into bone. Currently, the gold standard techniques to study organ development are micro computed tomography, histochemical assays, or through fluorescence microscopy using dyes or endogenously expressed fluorescent proteins protein. However, all these approaches are invasive, and can require fixation or lysis of tissue, therefore are unsuitable for in-vivo studies on humans for monitoring disease progression or therapeutic benefit.
However, multiphoton microscopy utilising non-ionising, benign radiation (NIR light) offers non-invasive, label-free structural and chemical details, without damage in contrast to histological approaches and X-ray scans. Additionally, label-free microscopy facilitates high sensitivity and resolution. In this work, we report an application of multiphoton microscopy with the techniques of Second Harmonic Generation (SHG), Coherent Anti-Stokes Raman Scattering (CARS) and 2-Photon Excited Auto-Fluorescence (2PaF) to monitor bone development. We establish the methodology using the chick model to provide proof-of-concept of its capability for potential use with patients.
Methods:
The femurs of chick embryos were isolated at different growth phases, i.e., at day 11, 14, and 18. The bones were fixed, embedded in paraffin wax, and sectioned. The sectioned slices were imaged with histological methods to establish the ground truth. The parallel sections were imaged with SHG, CARS and 2PaF. SHG images collagen fibres while CARS is used to image distribution of lipids at their vibrational frequency of 2845cm-1 and 2PaF images autofluorescence at 520 nm (primarily FAD and NADH). CARS was obtained in both backward and forward scattering geometries. Subsequently, label-free imaging data was analysed using image analysis using Fiji software. SHG images were analysed using CT-FIRE software and statistical analysis were performed.
Results:
Histological staining (Alcian Blue and Sirius Red) revealed a clear demarcation between the early cartilaginous femur and the surrounding tissue. Proteoglycans were visible within the cartilage region and intense collagen staining in the tissue surrounding the femur.
SHG images of chick femur bones (n=6) at Day 11, 14 and 18 highlighting collagen fibres were analysed using “CT-FIRE” to extract collagen fibre details. It was observed that the length of collagen fibres steadily increased throughout the developmental process from Day 11 to Day 18, unlike the fibre width that remained similar for early developmental stages before sudden increase at day 18. However, there were no observed differences in fibre straightness throughout the developmental process. 2PaF images provided context for cells and tissue areas while CARS images showed the distribution of lipids and protein structures and provided a readout of changes on development from

Day 11 to 18. Further analysis is in progress to develop imaging based multimodal signatures for bone development.

Conclusions:

Our work shows that non-invasive and non-destructive, structurally, and chemically selective multiphoton label-free imaging techniques can be advantageous for monitoring bone formation and can track its development. Additionally, the label-free techniques explored here allow objective and quantitative analysis of the developing bone since signals are retrieved only from molecules and structures in their native state providing intrinsic contrast and are dependent on their amounts.

Keywords:

Bone development, Multiphoton-microscopy, label-free imaging,

Reference:

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Study of nanolaser optical and structural properties at the nanometer scale

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Poster Group 2

Background incl. aims
Since their first demonstration by Huang et al in 2001[1], semiconductor nanolasers have attracted a lot of interest especially for their application in optoelectronic devices. They have the advantages to be cost-effective, easy to fabricate and of a micron size. Since 2001, various semiconductors and geometries demonstrated lasing properties, for example ZnO[1] or GaN [2] nanowires act as nanolasers.
Lasing could be induced by optical pumping, usually it is characterized by a drastic reduction of the emission spectrum width, increase of the light coherence evidenced by interferences of laser diffracted light. The most significant lasing properties of nanolasers are the value of the lasing threshold, the emission wavelength (see Figure 1) and the reduction of the carrier lifetime. Moreover, nanolasers emission wavelength above the lasing threshold are linked to the laser cavity's resonance modes, which in our case is the nanowire itself. Thus, the shape of the nanowire and the laser characteristics are closely related. Due the nanoscale variation, electron microscopy is a suitable technique to examine its nanoscopic characteristics. Therefore, it is essential to link the lasing properties with the nanolaser shape and the local luminescence.
Methods
We particularly focused on the use of cathodoluminescence in a scanning electron microscope (SEM) and a scanning transmission electron microscope (STEM) to characterize the optical properties of GaN nanowires. In both microscopes, the focalised electron beam locally creates charge carriers in the semiconductor, in a pear of interaction of either hundreds of nanometers (SEM) or tens of nanometers (STEM). We studied the luminescence due to carriers recombination. Using a spectrometer, we can obtain a spectrally resolved map of the sample emission. In time-resolved electron microscopy, we are able to measure the charge carrier lifetime thank to the decay curve of the luminescence after an electron pulse (30 ps in SEM and 300 fs in STEM). . In this project we measure the lasing threshold and the cavity mode of single nanolasers and correlate the results to their nanoscale geometry and optical properties using cathodoluminescence and time-resolved cathodoluminescence measured both in STEM and SEM.
Results
We investigated the lasing properties using micro-photoluminescence UV[4] of several nanolasers and observed strong heterogeneity in the lasing mode and lasing threshold. As expected, we found that the side-pumped nanolaser had a lower energy threshold than the vertically pumped ones[5], and we observe strong variation with the nanolaser radius. We observe the same nanolaser in cathodoluminescence and obtain spectrally and spatially resolved intensity maps, that show strong intensity and spectral variation along the nanowire. Moreover, time-resolved cathodoluminescence showed that the CL decay time varies from 90 ps to 130 ps when the nanolaser radius varies from 200nm to 500nm.
Conclusion

Finally, one of the main actual challenge on the nanolaser is to link the nano-laser shape to the lasing luminescence. In this project, we combine electron microscopy to study the cavity properties with both photoluminescence and cathodoluminescence, permitting to study respectively the macroscopic and microscopic luminescence properties.

Keywords:

Nanolasers
Time-resolved_Cathodoluminescence
Photoluminescence
Cathodoluminescence
III-N_Semiconductors

Reference:

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New strategies of TEM sample preparation for the mitigation of carbon contamination

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Poster Group 1

Transmission electron microscopy (TEM) measurements can be impaired by carbon contamination, which is caused by volatile organic components that get reduced by the electron probe and form a layer of amorphous carbon on the sample's surface. These contaminants often originate from the sample itself or are introduced during the TEM sample preparation process, e.g., when a sample is dispersed in a solvent for applying it to a TEM grid. In our work, we quantify the amount of accumulated contamination depending on the electron beam exposure time by electron energy loss spectroscopy (EELS) thickness measurements with a focus on different sample preparation parameters and mitigation strategies. A better understanding of the impact of these factors helps us to develop new sample pre-treatment strategies and thus drastically reduce carbon contamination in order to acquire clean TEM data.
TEM analysis was performed with a Thermo Scientific Talos F200X transmission electron microscope and a Gatan Continuum S spectrometer. Accumulated contamination was quantified by EELS thickness measurements by applying the log-ratio method [1]. The approach of contaminant removal in a self-built heatable vacuum sample cleaning station was compared regarding its efficiency to other established cleaning procedures.
Our experiments show that the amount of accumulated carbon contamination strongly depends on many factors that were investigated in our work: We have compared the amount of contamination caused by different solvents that are commonly used during the sample preparation or during the synthesis of a sample (fig. 1). Also, the impact of sample preparation parameters, i.e. the drying time of the sample, the duration a specimen remains in the microscope and the impact of the solvent's purity, was studied. In addition, we have investigated the efficiency of different established procedures for mitigating the formation of contamination. These strategies include established cleaning methods, that are compared to our novel approach of a designated sample cleaning station. Our results show in depth how the sample cleaning station performs compared to other mitigation strategies and can be integrated in the sample preparation process in order to achieve clean TEM measurements. The different methods were compared regarding their impact on the accumulated contamination (fig. 2), but also on their abrasiveness and influence on a sample's properties.
Carbon contamination can be reduced by careful consideration of the sample preparation parameters. Our results give insight in how to mitigate the deposition of contamination using established and new strategies, and can be extended to other challenging specimens, allowing to obtain high-quality, clean TEM measurements of samples that are prone to contamination.

Keywords:

Sample preparation; carbon contamination

Reference:

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Localisation of nanoparticles in whole cells using correlative cryo soft x-ray tomography and fluorescent microscopy

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IM-13 (2), Lecture Theater 5, august 27, 2024, 14:00 - 16:00

Background incl. aims
In recent years nanoparticles have emerged as important players in modern medicine, with clinical applications ranging from contrast agents in imaging to carriers for drug and gene delivery into tumors. Nanoparticle surfaces can be easily functionalized to target specific disease sites, and their small size and specific material characteristics facilitate their delivery and detection. Current nanoparticles include metals and and other inorganic-based compounds, as well as polymer, lipid, or bioinspired nanoparticles. These are being developed to diagnose and treat a variety of different diseases ranging from cancer to inflammation. In order to assess both the efficacy and the effect of nanoparticle delivery on cells a number of questions arise such as; does the nanoparticle reach the cytoplasm and nucleus of cells where it might exert therapeutic effects on intracellular molecules?, what subcellular compartments does the nanoparticle enter following delivery to cells?, or does nanoparticle uptake influence cell structure? These questions can be addressed by examination of cell structure.
Methods
Cryo-soft X-ray tomography (cryo-SXT) was used to deliver 3D ultrastructural volumes of intact cells without chemical fixation or staining, to reveal nanoparticle uptake for nanomedicine. Initially, integrated cryo fluorescence was used to screen an entire EM grid to reveal the location of suitable cells for tomography. Low magnification/large field of view 2D x-ray mosaics were then acquired over large areas of the grid before acquiring a tilt series from ±60° on selected targets. Data from both modalities were then overlaid to provide the location of nanoparticles in the context of whole cell ultrastructure.
Results
Cryo-SXT volumes combined with fluorescent light images showing the 3D distribution of organic and inorganic nanoparticles ranging in diameter from around 50 nm to 200 nm, in the context of the cellular landscape and surrounding organelles.
Conclusion.
Results prove the utility of lab-based cryo-SXT/FM for nanoparticle localisation in whole fully hydrated cells. The recent availability of the compact soft x-ray microscope will accelerate the further development of novel workflows and biological imaging applications that can benefit from this technique, including integration with electron microscopy.

Keywords:

cryo SXT/FM, correlative microscopy, nanomedicine

Reference:

We acknowledge funding from the European Union's Horizon 2020 Research and Innovation programme (No. 101120151, project CLEXM and No. 101017116, project CoCID) as well as the Irish Research Council (No. EBPPG-2020-278).

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Sample preparation for correlative light, soft X-ray tomography, and cryo FIB-SEM imaging of biological cells

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Poster Group 2

In this collaborative endeavor between SiriusXT and King's College London (KCL), we present our efforts to refine high-pressure freezing (HPF) protocols tailored to facilitate correlative imaging workflows integrating cryo-fluorescence microscopy, lab-based soft X-ray cryo-tomography (SXT), and cryoFIB-SEM for high-resolution three-dimensional (3D) imaging of biological cells. This refined protocol is followed by correlative light fluorescence, SXT, and FIB-SEM studies of biological cells, aiming to compare sample quality vitrified by plunge freezing and high-pressure freezing. Our motivation stems from the persistent uncertainty surrounding the efficacy of plunge freezing in adequately vitrifying thicker cellular components for high-resolution imaging purposes.
The primary objective of our study is to develop strategies to regulate ice thickness in high-pressure frozen samples, making them suitable for correlative imaging by light, SXT, and cryo-FIB-SEM techniques. Subsequently, we employ correlative imaging to investigate regions of interest, initially utilizing light fluorescence and soft X-ray tomography, followed by identification of regions of interest and further imaging of these regions using cryo FIB-SEM. We also compare the quality of frozen samples between high-pressure and plunge-frozen specimens. For our study we use two distinct biological organisms: the nannochloropsis microalgae and single-cell flagellate eukaryotes of the Euglena genus.
Optimal ice thickness for direct imaging by SXT without the need to thin the sample typically falls below fifteen micrometers, a threshold often exceeded by the conventional HPF "waffle" method, resulting in ice thicknesses around 20-25 micrometers. However, through modifications to the freezing procedure - such as liquid wicking and removal of planchettes spacer - we demonstrate the achievement of HPF ice thickness as low as 5-15 micrometers across significant fractions of the grid area.
This improvement eliminates the need to thin high-pressure frozen samples for SXT imaging. Using the lab-based SXT-100 for rapid 3D imaging of large areas with resolutions of 50-60 nm full-pitch, this approach enhances the throughput of cryo FIB-SEM imaging of high-pressure frozen samples by streamlining the process of identifying regions of interest for higher resolution imaging, albeit at a significantly slower imaging pace by cryo FIB-SEM.

Keywords:

SXT, cryoFIB-SEM, correlative microscopy, tomography

Reference:

We acknowledge funding from the European Union's Horizon

2020 Research and Innovation programme (No. 101120151, project CLEXM and No. 101017116, project CoCID) as well as the Irish Research Council (No. EBPPG-2020-278).

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Improved conventional TEM sample preparation exploiting the birefringence of materials

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Poster Group 2

Background incl. aims
Specimens for quality transmission electron microscopy (TEM) analyses must fulfil a range of requirements, which demand high precision during the prior preparation process. The exploitation of thickness-dependent interference colors occurring in birefringent materials can be used for an optimized procedure for conventional TEM specimen preparation. Birefringent materials display thickness-dependent interference colors in transmitted polarized light, which is analyzed by a second polarizer. The polarized light passing through a birefringent crystal splits into two components based on the refractive indices, resulting in an optical path difference called retardation. Recombining both components with the analyzer leads to annihilation of specific wavelengths through destructive interference, revealing observable interference colors. This phenomenon was first described by Auguste Michel-Lévy at the end of the 19th century along with a color chart of perpendicularly aligned polarizers for various birefringence values.
Methods
Our study leverages this phenomenon to optimize mechanical thinning in conventional sample preparation for transmission electron microscopy (TEM) [1]. For our experiments we employ two prominent semiconductor substrate materials from industry and research exhibiting birefringence: silicon carbide (4H-SiC) and sapphire (Al_2O_3). Figure 1 (a) displays the section of the Michel-Lévy chart containing the orange dotted 0.008-birefringence line of Al_2O_3 and the cyan dotted 0.056-birefringence line of 4H-SiC. We discuss our findings on dimpled and wedge-polished two-materials specimens, which are composed from a two-layer stack combining 4H-SiC and Al_2O_3 in one specimen. Top-view polarization microscopy images of the dimpled Al_2O_3/4H-SiC sample and the wedge-polished Al_2O_3/4H-SiC sample can be seen in Figure 1 (b) and 1 (c), respectively. For materials with small birefringence like Al_2O_3, where interference colors vanish at smaller thicknesses, we propose a complementary color chart for a parallel polarizer orientation, supplementing the Michel-Lévy chart for crossed polarizers.
Results
The thickness of the thinnest sample region can be estimated visually, with its color directly corresponding to the thickness on a color chart. During thinning, simple geometrical models that assume the specimen shape in dimple and wedge geometry can help to determine the thickness at the thinnest specimen position. A more complex RGB analysis of the occurring colors allows for measuring the required lateral fringes position with high accuracy. In all cases the achieved accuracy of thickness monitoring, validated by scanning electron microscopy (SEM), significantly exceeds the measurement capabilities of built-in mechanical gauges in thinning instruments.
Conclusion
Our proposed method facilitates a highly accurate determination of specimen thickness to avoid damage or breaking during mechanical thinning and reduces ion-milling times. The benefits of the approach are shown on sapphire and silicon carbide cross-section samples. The presented method is equally suitable for assessing specimen thickness during dimpling and wedge-polishing, and is

particularly useful at thicknesses below 20 µm, where the accuracy of mechanical techniques is insufficient. It is precise enough to be employed for a visual thickness estimation during the thinning process, but can be additionally optimized by analyzing the RGB spectrum of the occurring interference colors. It is applicable to all materials if additional birefringent materials serve as a thickness reference during polishing.

Keywords:

Preparation, Birefringence, Dimpling, Wedge Polishing

Reference:

A. Brozyniak, K. Stadlmann, P. Kürnsteiner, H. Groiss, Micron 177 (2024) 103580

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TEM studies of polarization nanodomains in (BaTiO3/SrTiO3)10 superlattices on silicon

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Poster Group 1

Over the last decade, it has been recognized that different topological structures formed in ferroelectrics significantly influence the physical properties of the material system. Ferroelectric oxides deposited on a semiconductor substrate are of particular interest for applications in nanoelectronics or photonics. A particularly attractive candidate of ferroelectric materials is $BaTiO_3$. We investigated the crystalline structure, defects and the polarization patterns in $(BaTiO_3/SrTiO_3)_{10}$ superlattices epitaxially grown by molecular beam epitaxy (MBE) on a silicon substrate. A thin epitaxial $SrTiO_3$ template directly grown on the substrate is mainly used to compensate for the lattice mismatch between $BaTiO_3$ and Si.
To determine the spontaneous polarization within the $(BaTiO_3/SrTiO_3)_{10}$ superlattice, the in-plane and out-of-plane strain components of the lattice were determined using Geometrical Phase Analysis (GPA) and using a direct peak finding method. Furthermore, a more refined peak finding algorithm was used to analyze the displacements of the Ti atoms relative to the center of their respective unit cells in real space. Therefore, high-resolution HAADF-STEM images, which show a direct visualization of the atom columns in a crystal, were recorded.
The analyses resulted in two-dimensional displacement maps of the Ti atoms, in which the areas of the $BaTiO_3$ layers revealed periodic, wave-like polarization patterns. These patterns are also partially continued in the $SrTiO_3$ layer. The two-dimensional strain maps show a strong modulation of the out-of-plane strain component and a periodic variation of the in-plane strain component in the region of the $BaTiO_3$ layers. The combination of the displacement maps and the strain maps shows a significant correlation between the two.
These investigations of $(BaTiO_3/SrTiO_3)_{10}$ superlattices contribute to the understanding of two-dimensional oxide ferroelectrics for the future integration of topological polar nanostructures in devices.

Keywords:

$BaTiO_3$, ferroelectrics, HAADF-STEM

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Charge Particle Optics Simulation Utilizing Hamiltonian Mechanics Perturbation Expansion and Boundary Elements Field Computation

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims
Advanced charge particle optics (CPO) requires fast and accurate computational tools for calculating (relativistic) particle trajectories including aberrations that, ideally, handle both arbitrary electrics and magnetic field sources, handle arbitrarily bend optical axis, allow incorporation of symmetries (e.g., rotational or mirror), incorporate optimization of design parameters such as pole piece diameter or pole distances. Ideally such tools should also be available under open source licenses in order to facilitate widespread use as well as distributed and sustainable development. Despite the enormous level of development and usefulness of commercial (e.g. Simion, EOD, Comsol) and open source packages, they often lack a subset of the above functionalities, somewhat hampering a wide spread development and use of advanced CPO for, e.g., Transmission Electron Microscopy, Secondary Ion Mass Spectroscopy, Photo Electron Spectroscopy, in academia, industry, and also teaching. The CPO software development described below intends to address that need.
Methods
Here we report on the development of an open source computational CPO framework incorporating the following principles to allow for an accurate, fast and flexible trajectory calculation: (A) We use boundary element method (BEM) computation of electric and magnetic fields, yielding smooth and accurate potentials, fields and higher-order derivatives at optical axis at arbitrary sampling, while reducing the meshing effort to surfaces (e.g., electrodes and pole pieces) of the CPO device. Herein, single layer representations of both electric and magnetic scalar potential are most efficient, while Green's representation with Calderon preconditioning allows stable single step solution of magnetic field distributions in the presence of high μr materials. (B) We employ semianalytical hierarchical solution of perturbation series of Hamiltonian equations of motion around an optical axis[1] in order to provide computationally effective, fast and accurate built-up of aberrations along particle trajectories. While not implemented yet the Hamiltonian perturbation expansion also facilitates straight forward extension to curved axis and the eikonal representation of aberrations. (C) We integrate the fast field and particle trajectory computation with non-linear optimization routines facilitating automatic optimization of design parameters (e.g., multipole sizes, pole piece gap) with respect to certain target functionalities. This tool chain is written in Python and makes use of advanced open source libraries (namely OpenCascade for CAD, gmsh for meshing, BEMPP for BEM field computation, sympy for semianalytic Hamiltonian mechanics perturbation expansion including automatic code generation, scipy for solving equations of motion, nlopt for geometry optimization) in a modular way, which are partly adapted to the specifics of CPO. Notably, BEMPP was extended by parallel just-in-time compiled numba and opencl kernels for field derivative computations on optical axis as required for computation of paraxial trajectories and aberrations.
Results
We demonstrate and discuss the above tool chain with the help several electrostatic and magnetostatic CPO / building blocks of CPO, notably electrostatic einzellens, electrostatic quadrupole – round aperture assembly (see Fig. 1) and magnetostatic quadrupole, touching implementation

(e.g., CAD import, defining boundary conditions, vector potential gauge), numerical (e.g., mesh size, precision of paraxial solution) and CPO (e.g., chromatic and geometric aberrations) aspects.
Conclusion
A modular combination of adapted BEM field computations and semianalytical perturbation series expansion of Hamiltonian equations of motion admits a computationally efficient modeling of CPO utilizing a combination of powerful and freely available open source software packages. Further development aims at incorporation of curved optical axis and general enhancement of functionality and user friendliness in order to support development of advanced CPO across the community.
Fig. 1: CPO of electrostatic quadrupole – aperture assembly (building block of low-voltage aberration corrector[2]): Computer aided design (CAD) modeling, meshing, and BEM field computation including axial multipoles. The octupole utilized for spherical aberration correction has been multiplied by 10 for visualization purposes.

Keywords:

Charge Particle Optics, Boundary Elements

Reference:

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Carbon nanoribbon formation by in-situ TEM manipulation of a C59N dithiolane derivative encapsulated into SWNTs

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Background
The synthesis of new kinds of functionalized carbon nanostructures continues to attract a growing amount of interest within solid-state physics research, due to the wide range of potential applications that it can yield [1]. Within this field, electron irradiation within an electron microscope is a great tool for in-situ studies on these structures, being able to characterize their formation and the changes they can undergo as they are happening at the nanoscale [2].
Within the subject of carbon nanostructures, single-wall carbon nanotubes (SWCNTs) have been a cornerstone in the field, not only as a malleable material but also as a medium for the fabrication of other materials. Within this new realm of materials, carbon 'peapods' (fullerenes and fullerene-based molecules encapsulated inside of single-walled nanotubes [3]) continue to increase interest regarding the controlled formation of nanostructures. A good case of this nanostructure formation is graphene nanoribbons (GNRs) templated by encapsulated functionalized fullerenes, which form the nanoribbons upon heating or beam irradiation [4]. Additionally, doping has been one of the main preferred methods for carbon nanostructure modification, with carbon being a relatively easy-to-dope material, improving the electrical properties of 1D carbon nanomaterials.
In this sense, functionalized C59N using a dithiolane (DT) moiety containing sulfur and oxygen [5] is a very promising material that may give rise to the creation of fine GNRs within SWNTs under beam irradiation, passivated with a combination of S, O, H and maybe N, possibly with some form of doping within their structure. To study these GNRs, previous in-situ studies on similar, non-doped samples offer micrographs before and after the GNR has been formed, but no intermediate states.
This study aims to get a fuller picture of the formation of GNR by acquiring micrographs not just before and after, but throughout the whole GNR formation process, and to delve into the structure these GNRs have.

Methods
In this work, these derivatives (C59N-DT) have been introduced into SWNTs and irradiated in situ within a TEM while acquiring high-resolution TEM videos at 80 kV, to have enough control over the dose to be able to modify the C59N-DT@SWCNTs when the beam was focused while having enough control over it not to damage the sample before the in situ studies.
Additionally, to discern the edge passivation on these nanoribbons, DFT calculations have been performed to discern the energetic viability of different GNR edges, modifying the geometry of the carbon edge, the different elements passivating it, and their relative concentration. The formation enthalpy of each edge configuration has been estimated as a way to discern which are the most

energetically favorable structures and, in turn, which ones are the least likely to prevail in these samples.

Results
Figure 1 shows the functionalized fullerene used for these studies (top left), an image series detailing the formation process of the nanoribbon (top right), and a formation enthalpy comparison for different mixed passivating edges (bottom).
The results of these TEM image series, as well as micrograph integrations from crucial moments in the in-situ study, point out to the formation of a first intermediate structure with the C59N-DT starting to fuse to each other and, later on, passivated, sometimes twisted GNRs.
Likewise, preliminary calculations seem to show that stoichiometrically favorable configurations, such as S-O co-passivated edges, as well as O-passivated edges are more thermodynamically stable than previously proposed S-passivated edges [3].

Conclusion
These results point to a successful way to obtain GNRs from a doped C59N-DT and show their formation in situ. DFT calculations show that the edges of these GNRs are probably made of S-O co-passivated edges.

Keywords:

Graphene nanoribbons, in-situ TEM, DFT

Reference:

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Chemical and morphological stability study of copper oxide nanocubes in controlled and non-controlled atmospheres

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Poster Group 2

Background
Cu-based formulations are high-prospect catalysts for their remarkable performance in environmental reactions and their role as a substitute for expensive noble metals. Depending on the reaction of interest, Cu-based catalysts are exposed to different oxidizing/reducing environments, in a temperature regime that may span over 200-800ºC1. Temperature-programmed experiments under H2 or O2 atmospheres have shown that copper phases are susceptible to release or capture oxygen, changing its oxidation state from Cu0 to Cu2+. These variations are concomitant with structural, chemical, and morphological modifications 2.
In relation with the gas-catalyst interaction, each exposed crystallographic facet may exhibit a singular ability to generate/annihilate oxygen vacancies3, and, therefore, a specific reactivity. The diverse and irregular surface of Cu-based nanoparticles hinder a direct relationship between the observed performance and their surface termination. The use of nanoparticles with controlled morphology and well-defined facets4, such as nanocubes, is proving to be a promising approach to address this complexity because it provides particles with homogeneous and explicit facets, facilitating the investigation of the H2/O2 interaction with copper phases and its chemical and structural consequences. To this end, we propose to synthesize CuOx nanocubes and characterize them by means of HR-TEM and EELS after application of different reducing/oxidizing thermal treatments. It will be paid careful attention to aspects such as their oxidation state, crystallographic homogeneity and stability.
Methods
The structural, chemical and morphological evolution of copper oxide nanocubes synthesized in our laboratory using very dilute solutions of Cu(NO3)2.3H2O, NaOH and ascorbic acid5 have been investigated after exposing the catalysts to oxidizing/reducing atmospheres at different temperatures. We have employed a FEI Talos F200X electron microscope operating at 200kV, capable of both transmission and scanning/transmission (TEM/STEM) modes and equipped with a Gatan Continuum Electron Energy Loss Spectrometer (EELS). The Cu-L2,3 white line EELS signals have been the primary focus of this study towards the determination of the oxidation state, processed using DigitalMicrograph software. More specifically the spatial distribution of CuOx phases have been determined with the Multiple Lineal Least Squares (MLLS) method. High quality Cu0, Cu1+ and Cu2+ references have been previously registered to perform the fitting. The EELS data have been complemented with the analysis of HR-TEM images, extracting the copper phase maps from the Fourier periodicities.
Results
STEM-HAADF images of the as prepared samples unveil the success of the synthesis, with particles exhibiting a cubic morphology spanning over 30 and 60 nm in size. The crystallographic analysis carried out by HR-TEM allowed us to confirm the presence of Cu2O phase (Figure a-b). In addition, a

zoomed image of the {100} faces reveals a rough surface assembled by low-dimension facets (Figure c). Aberration-corrected electron microscopy characterization will be used to better define this type of nanofacets.
To confirm the oxidation state of these systems, we conducted EELS analysis. The Cu-L2,3 EELS signal confirmed the presence of Cu2O, in agreement with the results obtained by HR-TEM. Prior to assessing the potential impact of different gas atmospheres on the initial state, we evaluated its stability over time. Thus, the morphology and Cu-L2,3 EELS features have been tracked after 1, 2 and 3 weeks (Figure d, e and f respectively). The evolution shows a progressive deterioration of the cubes, with areas exhibiting undefined morphologies which increase over time. These regions displayed Cu2+, indicating a transformation to CuO. Consequently, the samples are prone to capture O2 from air, triggering the destruction of the cubes. In contrast, when oxidation treatment was controlled in a muffle oven at 300ºC, the sample exhibited a hollow structure while preserving its morphology.
The pristine Cu2O nanocubes were also submitted to H2 at 100 and 150ºC. The reduction at 100ºC slightly altered the morphology, forming small nanoparticles on the surface of the nanocubes. However, at 150ºC, the HAADF images indicated sintering of the cubes, resulting in large (over 500 nm) aggregates, hindering a proper characterization.
An in-depth characterization is required to obtain the fine details ascribe to these thermal treatments. The implementation of Machine Learning routine to process HR-TEM images will provide a spatial distribution of each phase which will help determining the crystallography of the surface at the nanoscale.
Conclusion
In summary, the structural analysis highlights the morphological changes of Cu2O nanocubes under different oxidation conditions, with uncontrolled atmospheres leading to progressive oxidation while controlled treatments maintain morphology with a hollow structure. Further investigations are necessary to fully elucidate these phenomena. Additionally, observations in H2 atmosphere indicate a temperature-dependent morphological changes. Efforts to enhance accuracy in copper oxidation state studies through advanced methodologies are underway, promising deeper insights into nanomaterial behaviour.

Keywords:

Copper oxide, nanocubes, EELS, HR-TEM

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Comparison of the FMT assay with the Cell Painting approach in healthy patient derived fibroblasts.

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Poster Group 1

IPF, a fatal chronic disease of the lung, is caused by an inflammatory response that results in aberrant fibroblast activation, the deposition of excessive extracellular matrix, and progressive fibrotic remodelling of the lungs1. Though the exact pathophysiological mechanisms of IPF remain unknown, myofibroblasts are considered to play a major role in the pathology of IPF. Transforming growth factor β (TGF-β1), a well-established fibrogenic mediator, induces FMT. In cells undergoing FMT, increased expression of α-smooth muscle actin (αSMA) is observed. In vitro, increased αSMA expression positively correlates with contraction of myofibroblast populated collagen gels, indicating that αSMA is a strong marker of myofibroblast differentiation, and thus a relevant readout for lung fibrosis.

A validated, robust TGF-β1-induced FMT cell-based imaging assay in normal human lung fibroblasts (NHLF) with αSMA expression as a readout exists in-house to support multiple R&I IPF projects. As an inhibitor control, the highly specific Alk5 inhibitor SB-525334 is used to block TGF-β1 signalling2.

Cell Painting is a non-target hight content imaging assay to identify morphological profiles of different cell types with diverse compound treatments3. In this study the compounds used for the FMT assay have been simultaneously analysed via cell painting and the data has been compared. The Cell Painting results correlate well with the FMT data and could potentially be used as an alternative to the FMT assay. Especially while the assay is one day shorter.

Additionally, three patient-derived IPF cell lines have been tested with 27 compounds to see if it is possible to reverse the diseased phenotype to the healthy one, with or without TGF-β1 treatment. All three donors showed a different response profile when compared to each other, which made it difficult to distinguish if there are compounds being especially responsive to TGF-β1 treatment or to diseased cells, but not healthy ones. This needs further investigation.

Keywords:

alphaSMA, FMT, Cell Painting, HTS

Reference:

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2 Disease modelling of pulmonary fibrosis using human pluripotent stem cell-derived alveolar organoids, CellPress, Takahiro Suezawa, Shimpei Gotoh et al., PMID: 34798066 PMCID: PMC8693665 DOI: 10.1016/j.stemcr.2021.10.015

3 Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes, Nature Portfolio, Mark-Anthony Bray, Anne E Carpenter et al., PMID: 27560178 PMCID: PMC5223290 DOI: 10.1038/nprot.2016.105

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Robustness evaluation of electric field measurements via template matching in 4D-STEM

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Poster Group 2

Background incl. aims
Wave-based Scanning Probe Microscopy (SPM) techniques may provide a wealth of information even down to length scales significantly below the probe's extent. Tapping into it is possible thanks to two-dimensional detectors, which enable access to phase and/or absorption contrasts through the beam's interference patterns. The data analysis may then range from complex, stochastic approaches to simple and deterministic ones. One can take phase retrieval in coherent diffraction imaging [1] and center-of-mass (CoM) in field mapping [2-3] via four-dimensional scanning transmission electron microscopy (4D-STEM) as examples. However, with the dataset's enhanced dimensionality (compared to e.g. differential phase contrast imaging with a segmented detector) comes a broader range of field determination methods from which to choose.
Methods
In this presentation, we address the question of reliability in 4D-STEM electric field mapping by way of a comparison between the conventional CoM method on one hand, and several template matching (TM) approaches on the other hand. Our focus being on methodology, we use data acquired on a well-known silicon p-n junction [2-3] for simplicity. The imaged area is a 128-pixels-side square [see Figure 1(a)], with a step size of 5.4 nm, our detector is a 256x256 pixels Merlin camera based on medipix technology from Quantum Detectors, and the TEM is operated at 200 kV. The lamella's large thickness (326 nm of active material) and the electron beam's semi-convergence angle (992 µrad) allow us to preserve both signal-over-noise ratio and spatial resolution. Most importantly, the small probe size leads to a rigid, lateral shift of the electron beam without intensity redistributions; one may therefore expect good performances from TM.
As a first step, a common reference position (on the detector) for all compared methods is evaluated by averaging the beam's center position over 5120 pixels more than 100 nm away from the junction. We then run a standard CoM analysis along with TM and extract the junction's electric field from the corresponding results, after subtraction of the reference position. It must be noted that the TM algorithm we use (from the liberTEM [4] module) possesses sub-pixel accuracy. Denoting R the estimated beam radius on the detector and r the radial coordinate from the center, the tested, rotation-symmetric templates are: (i) a flat disk with radius R, (ii) the same disk multiplied with r/R, (iii) template (i) with a negative annulus around it, (iv) template (ii) with a negative annulus around it. A final template (v) is created from an actual data frame or the average of several of them.
Results
As can be seen on Figure 1, all templates yield distinct results, and none match the output of CoM. The significant spread in extracted field values suggests high sensitivity to the template's shape, and therefore to user input. That is why we now focus on the template resembling data the most: an acquired detector frame. Since the template (v) was created with a square cropping mask in order to exclude a Kikuchi band from the pattern, we first check whether a disk-shaped cropping mask (with a radius clearly above the beam's) produces different results. Not only is it the case, but we also find

out that slightly increasing the mask's radius strongly affects the template matching output, even though the Kikuchi band was still rejected (only background/weak beam tails were included).
This implies that a correlation-based shift determination places too much weight on absolute intensity levels, failing to accurately locate the pattern's edges. In order to alleviate this, we propose to perform template matching using not a raw detector frame, but the corresponding map (still in detector space) of the norm of its gradient [5]. Our goal consists in evaluating the robustness of this approach versus its two free parameters: the smoothing applied before gradient computation, and the threshold applied on the gradient's norm before TM.
Conclusions
With this study, we hope to improve the understanding of TM's limitations and/or biases in the context of quantitative field mapping. In addition to performing a comparison with CoM, we investigate the performance of two pixelated detectors, using direct detection or CMOS with a scintillator respectively. Finally, we develop an analysis which is guided as much as possible by the data themselves, with the objective to restrict the amount of arbitrariness as much as possible.
Acknowledgements
This project received funding from the European Research Council under the European Union's H2020 Research and Innovation programme via the e-See project (Grant No. 758385). Experiments have been performed at the Nanocharacterisation platform PFNC in Minatec, Grenoble as well as at TEM facility JEOL NEOARM, co-financed by the European Union under the European Regional Development Fund (ERDF, contract n° RA0023813).

Keywords:

4D-STEM, CoM, template matching

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Molecular insights into the biogenesis of box H/ACA snoRNPs

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Poster Group 1

Molecular insights into the biogenesis of box H/ACA snoRNPs
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Box H/ACA snoRNPs are protein-RNA complexes composed of NOP10, GAR1, and the catalytically active DKC1. The latter is known to interact with small non-coding RNAs containing the Box H/ACA motif. Guided by the RNA, the complex finds its target RNAs and converts the nuclear base uridine to pseudouridine, resulting in increased stability of the target RNA due to an additional hydrogen bond. Pseudouridines are vital for various cellular functions, such as protein translation, as they undergo frequent post-transcriptional modifications in ribosomal RNAs.
However, it is still poorly understood how the Box H/ACA snoRNP complexes and their individual protein precursors are assembled. SHQ1, a chaperone-like protein, is proposed to prevent DKC1 from binding to nonspecific ribonuceotides during its assembly in the cytosol and subsequently guide the complex to the nucleus, where it interacts with the AAA-ATPase complex RuvBL1/2. These ATPases are known to interact with PIH1D1 and RPAP3 proteins to form the R2TP complex, which is described to play a role in the assembly of multiple macromolecular complexes. Thus, the R2TP complex could be responsible for sequestering SHQ1 from DKC1, allowing DKC1 to interact with Box H/ACA snoRNAs and ultimately assemble the mature snoRNP.
The aim of this work is to elucidate the maturation of the Box H/ACA snoRNP by characterizing its precursor complexes composed of SHQ1, DKC1, and RuvBL1/2. This was achieved using size exclusion chromatography, dynamic light scattering, differential scanning fluorimetry, surface plasmon resonance, mass photometry, cross-linking mass spectrometry, and cryo-EM.
We were able to confirm the interaction between RuvBL1/2 and DKC1 or SHQ1. Additionally, we purified and stabilized the SHQ1:RuvBL1/2 complex and identified multiple complex species. Revealing the interaction site between the AAA ATPases and SHQ1 helped to reconstruct an initial 3D model of the complex's structure processing the movies collected in Cryo-EM experiments.
The biochemical and biophysical analysis of the interaction between R1R2 and SHQ1 allowed us to identify an additional electron density below the unique and regulartory domain II of the hexameric R1R2 complexes. However, our Cryo-EM reconstruction shows high flexibility for the complex, which is why mild cross-linking using GraFix might to be a valid option to determine a high resolution 3D structure.

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Keywords:

snoRNPs, PAQosome, Cryo-EM, SPR, Chaperones

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Structural Analysis of COPI Pathway in Chlamydomonas reinhardtii

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Poster Group 2

Recent advancements in FIB-milling throughput allow production of large tomography datasets from lamellae. Combined with improved processing of tomography data this has made it possible to produce sub-nanometer resolution for large protein complexes abundant in the cell. Despite these advances, in-lamella studies still mostly determine the consensus protein structure. In this study we aimed to correlate structural variability of the target protein with its in situ biological context to structurally describe a biological pathway. In particular we focused on the COPI pathway. Using a large tomography dataset from lamellae prepared by high-throughput plasma-FIB-milling we applied subtomogram averaging to determine the structure of the COPI complex. Next, we used image classification to analyze the biological cues involved in the pathway (e.g. binding of small proteins, cargo sorting, etc.). Further we correlated the results of image classification with different levels of biological context: from local lattice architecture to interactions at the organelle level, to describe different stages of COPI pathway. All in all, we showcase the potential of structural studies of biological pathways in situ.

Keywords:

cryoET, cryo-FIB-milling, COPI, membrane trafficking

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Elimination of HCV replication machinery early after antiviral treatment with DAA monitored by multimodal microscopy

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Poster Group 2

Background incl. aims
Hepatitis C virus (HCV) infection in cell culture constitutes an excellent model of persistent infection whereby the virus takes control of the infected cell without killing it. This strong interference with host cell homeostasis is manifested by a profound remodeling of the host endomembrane system as well as with a strong induction of virtually all stress response pathways in the cells. The availability of specific direct-acting antiviral (DAA) drugs against HCV provides a unique opportunity to revert this process and to define the ultrastructural events that follow viral replication blockade short after antiviral treatment.
Methods
Using confocal immunofluorescence and transmission electron microscopy (TEM) as well as the correlation of cryo-fluorescence microscopy and cryo-soft X-ray tomography (cryo-FM-SXT), we monitored the HCV replication machinery removal after antiviral treatment with DAA of a surrogate cell culture model of viral replication.
Results
To assess the impact of antiviral treatment of HCV-replicating cells, we treated cells bearing an HCV subgenomic replicon with a DAA combination of sofosbuvir, a polymerase inhibitor targeting NS5B, and daclatasvir, an NS5A-targeting antiviral. Analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment, concomitant with the reversion to baseline expression of HCV-induced stress markers, such as ATF3. A general survey of control cells and HCV replicons using correlative cryo-FM-SXT indicates that HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase is located in pleomorphic, high-absorption contrast organelles in DAA-treated cells. Three-dimensional reconstruction of these cells suggest that these organelles are spatially organized in layers proximal to the cell nuclei in areas with reduced mitochondrial content. TEM and cryo-FM-SXT studies confirmed the rapid elimination of the viral machinery, and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments. These and results by others suggest that HCV replication compartment is constantly recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in a transient activation of the endo-lysosomal system to achieve rapid viral machinery removal.
Conclusions
Overall, these TEM and correlative cryo-FM-SXT studies suggest that HCV replication machinery removal after DAA treatment entails transient proliferation of endo-lysosomes and MVB, but not that of double-membrane autophagosomes. Moreover, live fluorescence confocal microscopy indicates that NS5A remnants co-localize with an acidic compartment labeled with lysotracker green. Given

that a fraction of NS5A is found in endo-lysosomes also before antiviral treatment it is reasonable to propose that HCV replicase compartment size is balanced by a constant flux through lysosomal/MVB compartments.

Keywords:

HCV, DAA, cryo-FM-SXT, TEM, recycling-machinery

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In-vivo DAB cytochemistry and high-pressure freezing to determine the source of the human cytomegalovirus envelope

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Poster Group 1

Background/aims:

The human cytomegalovirus (HCMV), an enveloped DNA virus, significantly alters host cell morphology during infection. Formation of infectious virus progeny requires two critical envelopment processes: primary and secondary envelopment. Secondary envelopment occurs in a specialized juxtanuclear region known as the cytoplasmic viral assembly complex (cVAC), where capsids bud into the lumen of cellular vesicles and by this acquire the final virion envelope. The identity of these vesicles is debated, with both trans-Golgi and endosomal vesicles proposed as potential sources (1-3, and see abstract by T. Bergner, L. Cortez Rayas, J. von Einem, C. Read).

To address this, we adapted the protocol by Ellinger et al. 2010 (4) for cytochemical labelling of endocytic membranes for visualization by electron microscopy (EM). The protocol uses wheat germ agglutinin (WGA) conjugated to horseradish peroxidase (HRP) for labelling and diaminobenzidine (DAB) cytochemistry that is performed in living cells. WGA specifically binds to N-acetyl-glucosamine and sialic acid, abundant in the plasma membrane, whereby it is endocytosed in large quantities. The peroxidase-catalyzed DAB reaction results in a specific labelling of the endocytic compartment. Performing this reaction in vivo allows cryo-immobilization through high-pressure freezing. The combination with freezing within milliseconds allows capturing snapshot of the dynamic membrane system, making this approach suitable for pseudo-dynamic EM studies. Moreover, high-pressure freezing provides excellent structural preservation, allowing differentiated visualization of the endocytic compartment, HCMV capsids in different maturation stages, and their interaction during the secondary envelopment process in EM. This is a prerequisite for detailed 3D EM analysis.

Methods:

The study was performed with human foreskin fibroblasts infected with HCMV for 5 days, following the adapted protocol of Ellinger et al. 2010 (4). For pulse-chase experiments, WGA-HRP was added to the living cells and incubated for specific pulse times (e.g., 60 or 10 minutes). After the pulse, WGA-HRP was removed, and the samples were incubated for an additional chase period of 30 minutes. In vivo DAB cytochemistry was then performed by applying DAB to induce the formation of an insoluble reaction product at intracellular membranes, visible as a dark precipitate in EM. Samples were immediately cryo-immobilized through high-pressure freezing, freeze-substituted, and embedded in epoxy resin. Transmission electron microscopy (TEM) was used for quantitative analysis of 10 infected cells. Capsids were categorized as budding or enveloped and further categorized as either WGA-labelled or not labelled. 3D visualization of the endocytic compartment was achieved using STEM tomography.

Results:

TEM analysis revealed that the WGA-HRP precipitate is clearly visible and located along the intraluminal face of various intracellular membrane compartments, including vesicles, endosomes, multivesicular bodies, and the trans-side of the Golgi apparatus. Examination of the cVAC showed numerous capsids associated with WGA-labelled membranes. Notably, within 90 minutes of WGA-HRP pulse-chase, about 90% of budding capsids and 50% of enveloped capsids were WGA-labelled. This indicates rapid plasma membrane endocytosis, translocation to the cVAC and completion of the secondary envelopment process. Reducing the pulse-chase time to 30 minutes still resulted in WGA-labelled membranes that were used for secondary envelopment, suggesting that this process is even faster. STEM tomography further unambiguously identified capsid budding at the trans-side of Golgi cisternae.

Conclusion:

This study demonstrated that combining peroxidase-catalyzed cytochemistry with high-pressure freezing and freeze substitution ensures optimal structural preservation and specific labelling of the endocytic compartment suitable for EM studies. With this approach, we showed that endocytosed membranes are the primary source of the HCMV envelope. Additionally, the findings highlight the rapidity of secondary envelopment.

Keywords:

herpesvirus, cytochemistry, dynamics, TEM, STEM

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Electron energy loss spectroscopy for differentiating of minerals polymorphs

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Poster Group 1

Background incl. aims
Nanoscale phase identification in transmission electron microscopy TEM involves selected area or nanobeam electron diffraction combined with electron energy loss spectroscopy (EELS) or energy dispersive X-ray (EDX) analysis. Scanning TEM (STEM) extends this versatility by applying 4D-STEM and precession electron diffraction (PED) for orientational analysis. Recently, $CaCO_3$ polymorphs, calcite and aragonite, were characterized by STEM-EELS in the valence region (< 50 eV, VEELS) and the obtained results were verified by density functional theory (DFT) [1]. It was demonstrated that the near edge fine structures of spectra in the VEELS range closely relate to the electronic structure of materials and their crystal symmetries [1]. Moreover, VEELS is rather independent on crystallographic orientation which makes it a versatile technique even for nanoscale phase identification and mapping of 2D-materials [2].

In this study we applied Cs-corrected STEM and EELS mapping to visualize the distribution of (i) the $ZrSiO_4$ polymorphs zircon and reidite within the natural sample and distribution and structural relation of (ii) the coexisting SiO_2 polymorphs coesite and stishovite in synthetic high-pressure run products.

Methods
TEM foils of approx. 15 x 10 µm were prepared by a lift-out method using a focused ion beam (FIB). Phase distributions were visualized by applying HAADF imaging as well as the EELS and PED methods. Samples containing zircon and reidite were collected from the 70.3 Ma Kara impact structure crater-infill suevite breccias. Coexisting SiO_2 phases were synthesized in high-pressure multi-anvil experiments.

Results
Low magnification HAADF image (see Graphic section) shows reidite lamellae having various apparent thickness inside zircon matrix. Both phases were identified using SAED and high-resolution imaging. VEELS spectra of zircon and reidite were collected in the blue and green points, respectively. The difference (~ 2.3 eV) between position of the main peak at ~25 eV in zircon and at ~27 eV in reidite as well as other small features marked with the grey dashed lines were used to map the distribution of both phases. Corresponding EDX maps of zirconium, oxygen and silicon demonstrate uniform chemical elemental distribution in both phases. The phase distribution was also imaged using the PED data collected using a DigiSTAR device and the ASTAR software (NanoMEGAS SPRL, Belgium). In order to test applicability of the method, additional experiments were carried out to visualize distribution of stishovite and coesite (polymorphs of SiO_2), and kyanite (Al_2SiO_5) in a few FIB-prepared TEM specimens.

Conclusion
Our data demonstrate that phases having identical chemical composition may have distinct spectra in both low-loss and core-loss regions. This difference can be used to map their distribution in TEM specimens, which can be done at a very high spatial resolution, precision and acquisition speed. This allows minimizing the electron dose and thus the electron irradiation damage, hence, the presented EELS technique could also be applied to a variety of electron beam-sensitive materials. Moreover, a procession time for EELS mapping is much shorter comparing with e.g. PED. Thus, this approach has a great potential for phase mapping with unsurpassed spatial resolution for a series of geoscience applications.

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Keywords:

Zircon, reidite, EELS, phase mapping

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Transport of Intensity Phase Retrieval in the Presence of Intensity Variations and Unknown Boundary Conditions

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IM-03 (4), Plenary, august 29, 2024, 14:00 - 15:00

Background incl. aims
The so-called Transport of Intensity Equation (TIE) phase retrieval technique is widely applied in light, x-ray and electron optics to reconstruct, e.g., refractive indices or electric and magnetic fields in solids [1,2]. The TIE method reconstructs the phase from two or three mutually slightly defocused microscopy images by solving an elliptic partial differential equation – the TIE. Here, we present a largely improved TIE reconstruction algorithm, which properly considers intensity variations as well as unknown boundary conditions in a finite difference implementation of the Transport of Intensity partial differential equation. [3] That largely removes reconstruction artifacts encountered in state-of-the-art Poisson solvers of the TIE, hence significantly increasing the applicability of the technique. Notwithstanding, its widespread proliferation the TIE method in its predominantly employed form suffers from a number of fundamental challenges that often introduce reconstruction artifacts and hence degrade the information obtained from the phase: (I) Intensity variations of the wave functions are frequently either neglected or considered in an incomplete way, e.g., by equating the phase with the scalar "potential" pertaining to irrotational (particle) currents. (II) The boundary conditions (BCs) required to solve the TIE are often not known and strongly deviate from homogeneous Dirichlet, Neumann, or periodic Bcs, used in TIE solvers. (III) The reconstruction of small spatial frequencies in the phase is mildly (i.e., algebraically) ill-conditioned and, hence, requires regularization to suppress error amplification. Indeed, the above three challenges are intertwined in practical applications, because, e.g., erroneous long-range phase variations due to erroneous BCs or neglected amplitude variations are suppressed by regularization at the expense of introducing a regularization error. Here, we report on a TIE reconstruction algorithm that properly considers intensity variations, allows retrieving the correct BCs through a variational scheme, and can be additionally regularized, if necessary.

Methods
We numerically solve the TIE using a finite difference scheme: the equidistant x and y sampling points of the 2D image intensity are interlaced so that the phase and intensity at the sampling points may be represented as vectors. Subsequently, the directional derivatives along x and y are approximated by the left- and right-sided difference to the nearest neighbors, which can be written as a multiplication with bidiagonal matrices. With these building blocks, the whole TIE may be written as a system of linear equations, which can be inverted numerically to yield the phase. In order to suppress error amplification, the linear equation system may be additionally regularized by a Tikhonov scheme. In order to incorporate arbitrary inhomogeneous Dirichlet BCs, the discrete phase image is embedded in a frame comprising one pixel. The values of the phase on this frame (i.e., the unknown Dirichlet BCs) are retrieved through a variational scheme that minimizes the difference between the experimental defocused image intensities and the numerically defocused intensities obtained from the TIE reconstruction. To facilitate sufficiently fast convergence only a small number of Fourier coefficients of the BC function are retrieved in practice. The values on this frame are then

assigned to Dirichlet BCs. The above system of equations is solved in a numerically efficient way by exploiting sparse representations and sparse equation solvers.

Results

We demonstrate the improved performance of the TIE reconstruction algorithm at a set of simulated and experimental image intensities arising from magnetic structures investigated in TEM, focusing on Landau domain pattern in geometrically confined permalloy thin films. They consist of magnetic vortices and antivortices and are suited to demonstrate the main features of the enhanced TIE reconstruction (Fig. 1), namely consideration of intensity variations (Figs. 1a-c), automatic determination of nontrivial BCs (Figs. 1d-f), and its application on an experimental example (Figs. 1g-i).

Conclusion

We have demonstrated that solving the TIE with the help of a finite difference scheme incorporating a variation of the boundary conditions largely mitigates reconstruction artifacts encountered with the previously used Poisson reconstruction schemes. The reconstruction algorithm reduces or even removes the need for additional regularization and mostly improves the reconstruction of long-range variations of the phase. That opens new avenues for TIE reconstructions, where intensity variations cannot be avoided, and long-range fields are important (e.g., vector field electron tomography). Further improvements of the algorithm hinge on further optimization of the optimization algorithm employed for determining the boundary conditions, faster sparse matrix solvers, and transition from a finite difference to a finite elements scheme (that allow adaptive meshing), amongst others.

Fig. 1. TIE reconstructions for specific cases of magnetic Landau domain patterns. a) Simulated amplitude variation of a vortex configuration at the position of the vortex core. b,c) Magnetic induction reconstructed using conventional (Poisson) (b) and improved TIE (c) algorithm including the amplitude variations (a). d) Simulated magnetic induction of Antivortex pattern. e,f) Magnetic induction reconstructed of (d) using Poisson algorithm (e) and improved TIE (f) algorithm in the presence of unknown boundary conditions. g) Lorentz TEM image of a permalloy film with vortex-antivortex pair taken at an underfocus of 1mm. The scalebar corresponds to 1.5 µm. h,i) Magnetic induction reconstructed of (g) using Poisson algorithm (h) and improved TIE (i) algorithm.

Keywords:

Holography, Phase Retrieval, Magnetic Imaging

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Ultrastructural and chemical analysis of human Locus coeruleus using correlative microscopy and mass spectrometry

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Poster Group 1

Background and aims: Among the characteristic hallmarks observed in the human brain during aging is the neuronal accumulation of neuromelanin (NM) pigment and iron in substantia nigra (SN) and locus coeruleus (LC), the two brain areas mainly targeted in Alzheimers and Parkinsons disease (PD). The neuromelanin pigment accumulates inside the catecholaminergic neurons of SN and LC and there particularly within specific organelles (with an average size of 1.5 µm), called neuromelanin organelles. Within these organelles, neuromelanin clusters with with lipid and protein-based partitions of less than 100 nm each
Several spectroscopic studies investigated the role of neuromelanin pigments and metal dyshomeostasis in the SN during aging [1] and a found a correlation of both with disease progression. However, conducting chemical analysis on LC proves to be challenging due to the small size of the tissue. It would necessitate a substantial number of specimens which are difficult to collect. Moreover, only few studies attempted high lateral resolution subcellular (re)distribution analyses of iron storage in brain tissue, e.g. for SN [2] in order to find out where exactly the metal is stored on a subcellular basis.
Therefore, the aim of this work is to develop a workflow to study the ultrastructural metal distributions in NM organelles of LC on individual human tissue sections using novel high resolution analytical approaches based on secondary ion mass spectrometry (SIMS) performed on Focused Ion Beam instruments (FIB-SIMS)..o Chemical analysis were corroborated by CLEM (Abbrev.) to correlate the finding with the cellular and molecular changes that take place in relation to PD.
Methods: A total of 11 human LC tissues were collected from elderly (n=9) and PD (n=2) specimen (60-80 years old) and were either formalin fixed paraffin embedded (FFPE), Epon-embedded or just as fresh frozen. Tissue sections were investigated by light, fluorescence and electron microscopy for high resolution imaging and immunohistochemistry, respectively. The standard chemical analysis was performed by analytical electron microscopy and mass spectrometric imaging (MSI), using the CAMECA NanoSIMS 50 with the ability for isotopic identification and highest sensitivity and mass resolution and a lateral resolution down to 50-100 nm. Sub organellar distribution of metals in NM organelles was addressed on selected samples using FIB-SIMS with lateral resolution < 20 nm for SIMS and < 1nm for secondary electron (SE) imaging developed at LIST. A combined “TEM like”

ultrastructural investigation together with SIMS is possible on the so-called npSCOPE, an in house novel cryoFIB-SIMS platform with SE, SIMS and scanning transmission ion microscopy (STIM) detectors [2]. Taking the advantage of the cryo-chamber of the npSCOPE, it can also be used to perform respective analyses on frozen-hydrated brain samples and thereby minimizing preparation artefacts.
Results: A semi-quantitative SIMS approach was implemented here where a single tissue slice was imaged first using CLEM to identify the region of interest in LC tissues of both elderly control and PD subjects. Quantitative EDX maps and qualitative SIMS maps were acquired for regions of interest. Counts/ pixel ratios were calculated to get a semi-quantitative data set that shows different signal abundances for SIMS. Respective data on healthy elderly specimen showed that the melanic moiety of the NM organelle is composed by a mixture of eumelanin/ pheomelanin units with a sulfur/nitrogen ratio of (XXX), as identified by the sulphur signal which derives from the benzothiazines of the pheomelanin part of the NM. NM organells showed increased signal for iron, calcium, and aluminium, in comparison to the lipid moiety of the NM organelles or the cytoplasmic surrounding areas.
Interestingly, copper and zinc signal were at or below the detection limit, which is totally contrast to the isolated NM analysed using the Electron Paramagnetic resonance spectroscopy (EPR) that showed an actual accumulation of these two elements [1].
The higher spatial resolution of LIST's FIB-SIMS instrument in comparison to the nano-SIMS could more precisely identify the localization of these metals and non-metals in different sub-organellar compartments of the NM organelle.
In addition, PO2 signal which is an indicator of phosphorus rich areas like myelin sheath, nucleus and lipids was also week in the lipid moiety and lipid bodies of the NM organelle, which might strongly agree with the fact that the lipid portion of NM is mainly composed of dolichols, and this is to be proved by lipidomic profiling. Another method of molecular profiling is done by IHC, in order to identify the differential expression of the markers related to inflammation, PD and iron storage proteins, and in order to identify the regions that showed, by our elemental analysis, to be rich in iron but are not a NM organelle, who could be glial cells or ferritin rich areas.

Conclusion: We analysed the sub-cellular localization of certain marker elements for subcellular structures (PO for myelin), NM organelle compartments (based on Cl, S, CN, O signal distribution) in addition to the metal loading into these compartments in elderly controls and PD patients using routine and novel high-resolution chemical and structural imaging techniques in the NM in addition to its molecular proteomic and lipidomic profiling. Continued work will be done to identify more the significant differences in LC tissues of PD patients compared to healthy controls at the molecular and elemental levels, with investigations on fresh frozen samples for a better close to native analysis of PD pathophysiology also including molecular MSI,also done at high resolution, like MALDI and TOF-SIMS.

Keywords:

Correlative electron microscopy, SIMS, Parkinson

Reference:

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Atomic-scale structure and defect evolution in Σ5 [001] tilt grain boundaries in copper

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Poster Group 2

Background: Grain boundaries (GBs) are material imperfections that have a strong influence on material properties. They can act as preferred diffusion pathways for solutes and hence impact the transport properties of polycrystalline materials. In many cases, it is assumed that the GB adopts an idealized structure that can be described by the structural unit model in high-angle tilt boundaries that is often used to establish structure-property correlations. However, the relation between the atomic structure of GBs, and possible deviations from the ideal structure, and their transport properties often remains unexplored.
Methods: We fabricated a Cu bicrystal via a modified Bridgman method. The seed single crystals are aligned with their [001] axes being parallel, corresponding to the growth direction, and are misoriented by an angle of 36.9° to form a symmetric tilt grain boundary. Electron backscattered diffraction is then used to characterize the global GB structure, followed by aberration-corrected scanning transmission electron microscopy to study the atomic structure of a series of near-Σ5 (310) [001] symmetric tilt GB segments of the bicrystal.
Results: In the near Σ 5(310)[001] symmetric tilt grain boundaries, the kite-type structural unit is frequently observed throughout the GB regardless of the deviation from the desired misorientation. However, various GB defects are formed at the GB to accommodate deviations from the exact GB misorientation or inclination. We observe disconnections, asymmetric nanofacet segments and an array of secondary edge dislocations that appear periodically between the normal kite-type structural units. Furthermore, a beam-induced GB phase transformation is observed, characterized by the change of the structural unit as well as the re-shuffling of the atoms at the GB.
Conclusions: The variations in GB structure and the formation of GB defects present an intriguing picture, particularly when considering their potential influence on how solutes diffuse along the interface. These aspects are rarely explored and our atomic scale investigation of the evolution of GB structure lays out the stepping stone towards understanding their role on the kinetic properties of interfaces.

Keywords:

Grain boundary, STEM, fcc, copper

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Characterization of Precipitates in Petroleum Steels by Using Precession Electron Diffraction Technique

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Poster Group 2

Background incl. Aims
Steel has wide variety of grades and properties and not every type of steel applicable for all applications. Due to that reason, correct type of steel needs to be selected for a specific application. For example, for the petroleum and natural gas pipeline applications where pressurized fluid is transported for long distances, American Petroleum Institute (API) X70, X80 or X100 steels are used due to their properties such as high strength, fracture toughness, weldability, corrosion resistance and deformability. API steels are ferritic steels and to obtain the desired properties like high strength, this type of steels is micro alloyed with elements such as Nb, Ti and V with precipitation hardening. Properties of API steels, are modified by the thermo-mechanical rolling by tailoring the microstructure, in addition to the micro alloying. During production and rolling processes several precipitates with different compositions can be formed. Therefore, it is important to determine which precipitate is present in the produced steels, because mechanical properties of material may change with the type of the precipitate. This study aims the characterization of precipitate particle found in the petroleum steel by both conventional and novel precession electron diffraction methods in order to confirm which phase is formed during production.

Methods
In this study, thermomechanically rolled API-X70 steel sample was used. Samples are characterized by conventional TEM and STEM imaging techniques and in addition Precession Electron Diffraction (PED) technique is applied to characterize present phases as well as orientation relationship between the different phases. TEM sample was prepared with conventional mechanical thinning followed by an Ar-ion beam milling. Electron transparent sample is examined by using 200 keV field emission TEM (JEOL-JEM2100F) equipped with STEM high angle annular dark field (HAADF) detector (Fischione-Model 3000) and energy dispersive X-ray (EDX) spectrometer (JEOL-JED2300T). The TEM phase and orientation mapping results are obtained with nano sized probe (at NBD alpha 5 and 0.5 nm spot size) that is scanned in a user specified area over the sample up to 0.7° of precession angle and obtained diffraction patterns are analyzed with ASTAR™ V2 via template matching algorithm.

Results
Microstructure of the samples were investigated by the TEM and STEM imaging methods and these images revealed that steel matrix contains high atomic number element containing precipitate particles according to its contrast with respect to matrix. The sizes of the precipitate particles were ranging from 20 to 80 nm. Chemical composition of the precipitates was tried to be analyzed with EDX, however due to the thickness of the sample, x-ray signals were collected both from precipitate and over or underlaying matrix phase. Because of that reason exact chemical composition cannot be differentiated from the elements present in the matrix. In order to determine the type of the precipitate, phase identification was done by using the precession electron diffraction method by using the crystal structure differences between candidate precipitate phases. For this purpose, phase identification was conducted with calculation and template matching of diffraction patterns of all the

possible precipitate phases with experimental diffraction patterns. With this method, phase identification with high reliability was conducted with the additional X-ray spectrum obtained from the precipitate particle.

Conclusions

In this study, precipitate particle present in the API-X70 steel was successfully identified by using different TEM/STEM techniques, which cannot be identified by the EBSD due to the size of the precipitates. For the identification of the both X-ray spectrum as well as phase maps, obtained by the precession electron diffraction method, were used as a complimentary technique to each other in order to eliminate limitations of these methods.

Keywords:

PED, Steel, Precipitate, Phase identification

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Interpretable evaluation of STEM images of nanostructures via homology analysis

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Poster Group 2

Background incl. aims
Recently, a mathematical framework called persistent homology (PH) has made it possible to quantify materials structural information at a wide range of scales, from the atomic to the nanoscale. PH quantitatively expresses the hole structures of data in terms of their number and scale. Particularly, it is highly suited for discerning the subtle order within inhomogeneous structures, such as amorphous [1] and glass materials [2]. Until now, PH analysis has predominantly focused on three-dimensional atomic arrangements; however, it is also feasible on two-dimensional image data. Here, we applied PH analysis for two-dimensional TEM images, representing a highly useful approach in structural analysis.
In our previous works [3,4], the homological feature known as 'Betti number' was applied for structures of self-assembled Pt/CeO_2 nanocomposites, which were captured by scanning TEM (STEM). The N-th Betti number corresponds to the number of N-dimensional holes, such as connected components, rings (0- and 1-dimensional holes, respectively), and so forth. This homological feature could successfully quantify CeO_2 phase connectivity and further, relationship with the oxygen ion conductivity.
To explore more effective descriptor for the nanostructures, we apply one of the most used PH methods, Persistent Diagram (PD). The key concept in PD lies in tracking the scale required for the appearance (birth, b) and disappearance (death, d) of the N-dimensional holes by continuous deformation of object which called ‘filtration’. Consequently, it includes information on the shapes of the N-dimensional holes, unlike the Betti number. We aim to demonstrate its effectiveness for nano-structural analysis and extract important homological feature for classifying the Pt/CeO_2 nanostructures. To ensure both quantitativity and interpretability, we employed a consistent approach that directly extract interpretable features from PDs.

Method
Firstly, Pt/CeO_2 nanocomposites were synthesized by the annealing of the Pt_5Ce alloy. The 12 nanocomposites with various nanostructures were prepared by changing annealing temperature (500, 600, and 700℃) and syngas ratio (CO:O2 = 0:1, 1:1, 2:1, and 3:1). The nanostructures were characterized through STEM (JEM-2100F, JEOL, Japan) operating at an acceleration voltage of 200 kV. Then, the obtained images were binarized and noise-removed with the OpenCV library in Python. The sequential procedures related to PD acquisition, vectorization for Principal Component Analysis (PCA) were conducted by using data analysis software “Homcloud” [5]. Finally, we used random forest method to find the most important descriptor for classifying Pt/CeO_2 nanostructures.

Result
The binarized STEM images clearly show the self-assembled Pt/CeO_2 nanocomposites that consist of Pt (white) and CeO_2 (black) phases. We focused on the CeO_2 phase for PH analysis to examine its relationship with oxygen ion conductivity. The nanostructures changed from a maze-like to a striped

appearance as the annealing temperature increased. Their 0th and 1st PDs also changed depending on the structural changes.
To clarify the relationship between the structural changes and distribution changes of b-d points in PDs, we extracted five interpretable features, each based on the understanding of individual quadrants in the 0th and 1st PD. The three features, the average width and total length of the striped CeO_2 phases, and the number of CeO_2 phases can be obtained from the 0th PDs. Their trends toward annealing temperature coincide with those from the actual STEM images. The number of ring and gulf-like structures can be obtained from the 1st PDs, focusing on negative and positive b region, respectively. These quantification with PDs could capture trends more clearly compared with conventional observation.
Furthermore, we conducted PCA with vectorized PDs to extract their critical information, which emphasizes the difference between homological features in structures. By the first and second principal components in the 0th and 1st PDs, the 12 nanostructures were relatively well categorized. Through PD reconstruction using the first principal components in the 0th and 1st PDs, we identified a critical region in the PDs: the region in the 1st PDs with d value smaller than characteristic size. In this critical region, d value corresponds to the size of the CeO_2 gulf-like phases. This result suggests that the number of small gulf-like phases is effective as a simple interpretable feature to differentiate Pt/CeO_2 nanostructures. Finally, we applied all interpretable features extracted from PDs thus far to a random forest classification and evaluated their importance. As a result, two key descriptors emerged: the width of the CeO_2 phase and the number of small gulfs. Remarkably, in the scatter plot of the two descriptors, the 12 nanostructures could be classified effectively. In this manner, using a few simple interpretable descriptors, we can more easily discuss and quantitatively evaluate the structural differences arising from variation in synthesis conditions, compared to PCA.

Conclusion
This study investigated the effectiveness of PH analysis in analyzing the STEM images of the nanostructures. Firstly, five interpretable features could be extracted directly from the 0th (the average width and total length of the striped CeO_2 phases, and the number of CeO_2 phases) and 1st (the number of ring and gulf-like structures of CeO_2 phase) PDs. Regarding the gulf-like structure, the PCA results suggest that the number of smaller structures than the characteristic size could particularly differentiate the 12 nanostructures. Finally, we showed that key descriptors can classify nanostructures through the random forest classification, enabling us to interpret their differences easily.

Keywords:

persistent homology, interpretable machine learning

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Strategies for Multimodal Image Data Transformation to a Common Format for Cloud Integration and Visualization

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Poster Group 1

Due to their inherent complexity, biological systems require extensive imaging to achieve statistically significant results. Manual operation of microscopy equipment can be laborious and hinder the acquisition of large datasets, impacting reproducibility. To overcome these challenges, the integration of bioimage analysis with computer-controlled microscopes has led to the development of “smart microscopy”. This approach merges computer-controlled imaging systems, image analysis, and machine learning to execute automated imaging workflows that result in large and complex data sets.
At the Centre for Cellular Imaging of Gothenburg University, we are developing and delivering smart microscopy solutions to our scientific community through open-access services for academia and industry alike. However, with increasing automation comes a significant challenge: the sheer volume of resulting imaging datasets. These datasets can span several terabytes, can be generated in a matter of hours, often consist of “multiscale” and “multimodal” datasets, and in some cases, are stored in proprietary file formats. Understandably, this poses formidable challenges for tasks such as visualization, image analysis, data management and sharing with the community.
To address these challenges, we're employing a multifaceted approach that incorporates cutting-edge techniques and tools. This includes utilizing next-generation file formats like ome-zarr,[1] leveraging advanced visualization software such as napari,[2] and integrating collaborative image analysis platforms like webknossos.[3] This comprehensive strategy not only accelerates insight extraction from microscopy datasets but also ensures efficient resource utilization in the era of data-driven life sciences.
In this contribution, we focus on our efforts to generate open-access software tools that standardize the transformation of complex and proprietary file formats across a wide range of microscope solutions, including high content screening, light-sheet microscopy, and electron microscopy (both scanning and transmission). Of particular interest is our focus on datasets for correlative array tomography, including those obtained from the ZEISS MultiSEM,[4] and strategies for multimodal imaging with other technologies such as nanoSIMS[5]. Utilizing the emerging community standard ome-zarr provides us with access to a chunked, cloud-compatible format that enables rapid visualization and facilitates the analysis of large image datasets.

Keywords:

image analysis, data management, correlative

Reference:

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Phase-controlled formation of NixPy catalyst using environmental TEM for potential application in CO2 reduction

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Poster Group 2

Background:
The urgent need to mitigate climate change has driven intensive research into efficient strategies for reducing CO2 emission and potentially removing them from the atmosphere. Carbon capture and storage represents a promising approach, together with conversion of CO2 produced in industrial processes and from the environment into useful products and fuels via CO2 reduction reactions. Earth-abundant transition metal phosphides, i.e. nickel-phosphide (NixPy) catalysts, offer several advantages, including, cost- effectiveness, switchable selectivity for a range of reaction products, phase-dependent catalytic activity and potential for facilitating CO2 transformation into value-added products for renewable energy [1–3]. However, the development of this system as a tunable catalyst for CO2 reduction reactions is in its early stages, requiring a comprehensive understanding of synthesis processes to attain control over phases, size, shape/facets, and catalytic activities. Structural evolution that occurs during reactions are still largely unknown, and the relationship between facet structure and product selectivity remains poorly understood. This study aims to investigate the phase-controlled formation dynamics of nickel phosphide nanoparticles and their stabilities at higher temperatures using dedicated in-situ transmission electron microscopy (TEM) coupled with chemical vapor deposition (CVD) system.
Method:
The experiments were conducted using environmental transmission electron microscopy (ETEM) combined with a customized gas handling system. Size-selected nickel nanoparticles (10-30nm) were deposited onto micro-electro-mechanical system (MEMS) heating chips by an aerosol-phase fabrication method. These nanoparticles were then exposed to PH3 gas inside the TEM at appropriate temperatures up to 700°C to transform them into nickel-phosphides. Phase transformation of the nanoparticles was induced by changing the reaction parameters such as reaction temperature and PH3 gas flow inside TEM. The dynamic processes were followed by high-speed high-resolution imaging (up to 300 fps) during gas exposure. Structural information was obtained using power spectra of acquired high-resolution images, while composition was monitored by energy dispersive X-ray spectroscopy. The gas composition inside the microscope was monitored by an integrated residual gas analyzer.
Result:
Our Preliminary results focus on the formation dynamics of phase-controlled nickel phosphide nanoparticles. We investigated the role of reaction parameters, such as phosphene gas flow and heating temperature, on the phase transformation of nanoparticles. Two distinct phases were achieved with varying PH3 flow and temperature. Lower PH3 flow (0.3 sccm) at 300°C resulted in Ni2P phase formation, while higher PH3 concentration at elevated temperatures led to Ni5P4 phase formation. Intermediate experimental conditions, such as higher PH3 flow at lower temperature, resulted in a mixture of Ni2P and Ni5P4 phase particles. All the phases showed high thermal stability. These findings suggest that higher PH3 flow at elevated temperatures favors the incorporation of more phosphorus into the nickel phosphide nanoparticles. According to literature, both phases Ni2P

and Ni5P4 are interesting for catalytic activity for hydrogen evolution reaction [4] and CO2 reduction [2], respectively.

Conclusion:

This work demonstrates an in-situ solid-gas phase strategy for synthesizing nickel phosphide nanoparticles with controlled phase by varying PH3 gas flows and reaction temperatures. Such insights are critical for designing phase-engineered NixPy catalyst for sustainable CO2 conversion technologies, offering significant implications for addressing global climate challenges. The next step in this work would be to investigate these phases for CO2 reduction and to monitor the structure evaluations occur during the catalytic reactions.

Keywords:

Nickel phosphide, Phase engineering, In-situ

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Evaluation of inflammation and free fatty acid metabolism as biomarkers in female patients

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Poster Group 1

Background incl.aims: Ovulatory and tubaperitoneal factors constitute an important part of the female infertility factor. At the beginning of the reproductive period, there are approximately 400 thousand primordial follicles in the human ovary. Follicular fluid is the environment in which the oocyte resides throughout oogenesis and is important in oocyte development (1). Follicular fluid is aspirated with the oocyte during oocyte retrieval. By analyzing the follicular fluid, the relationship between the oocyte and surrounding cells can also be evaluated. The most common risk factors for infertility are smoking, body mass index being less than 18.5 kg/m2 or more than 25 kg/m2, excessive exercise or no exercise, alcohol consumption, caffeine consumption, and stress. IL-8 is known as a cytokine growth factor that contributes to the pathogenesis of endometriosis by promoting endometrial cell attachment, invasion, cell growth, proliferation, and immune protection in the endometrium (2). Although IL-8 has been shown to have an effect on many areas such as female infertility, endometriosis and Polycystic Ovary Syndrome, no study has been found showing its relationship with different parameters on oocyte quality. The aim of this study was to investigate the relationship between free fatty acids in follicular fluid, body mass index and oocyte reserve quality. It was aimed to evaluate the effects of data on the relationship between free fatty acid level in follicular fluid, IL-8, lipid droplet count, body mass index and peripheral blood smear in female infertility samples.
Methods: A total of 60 people who applied to the in vitro fertilization clinic for various reasons (male factor, female factor, unexplained infertility) were included in the study. In our study, a sample of 60 patients diagnosed with infertility was created. These patients will be evaluated in two groups according to body mass index. Body mass index of less than or more than 25 kg/m2 was evaluated as group 1 and group 2. Various exclusion criteria were applied to determine the patient groups in order not to affect the study design. In our study, free fatty acid (FABP) and IL-8 parameters in follicular fluid taken for routine treatment from women applying assisted reproductive techniques were evaluated by ELISA (Enzyme-Linked ImmunoSorbent Assay) method. In blood samples, peripheral smears were used to determine the distribution of shaped elements of blood and also to examine whether there was a correlation between them and body mass index. At the same time, Oil red staining of neutral lipids and lipid droplet morphology were identified by light microscopy. All data were analyzed by statistically.
Results: When the differences between the groups were examined, it was determined that FABP and IL-8 levels were higher in the group with high body mass index (group 2). The concentrations of follicular IL-8 and FABP were significantly higher in the group 2 and were positively correlated with the levels of fatty acids. At the same time, when blood smear samples were examined in this group, it was determined that the number of leukocytes and the number of lipid droplets was higher. Accordingly, a semiquantitative histopathological damage score was made and lipid droplet distribution and blood smear between groups was determined. It was observed that over body mass index and cellular degeneration caused infertility, inflammation and increased oxidative damage.

Conclusions: Follicular fluid and blood smear samples taken from women who have used assisted reproductive techniques will become more important in the diagnosis and course of the disease. By evaluating several parameters together, it is aimed to obtain faster and more reliable results in assisted reproductive techniques. IL8 and FABP affects female fertility at both serum level and light microscopic levels.

Keywords:

Follicular, fatty acid, Infertility, Microscopy.

Reference:

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The Healing Effect Of Ferulic Acid in Monosodium Glutamate-Induced Liver Injury

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Poster Group 1

Background incl. aims

Monosodium glutamate (MSG), a flavor enhancer in prepared foods, can lead to harmful effects in different organs and systems. Based on the literature data, monosodium glutamate is known to cause liver injury by generation of reactive oxygen species leading to damage of lipids, proteins, and DNA by the formation of free radicals (1). Ferulic acid, as a potent antioxidant, is mentioned to ameliorate the harmful effect of biomolecules by suppressing the production of reactive species and the oxidative stress. In this experimental study, MSG-induced liver injury putative healing effect of ferulic acid were assessed at histochemical, transmission electron microscopical and biochemical levels.

Methods

In this study, male Wistar albino rats (7 weeks old, 160-200g) were used and kept in a laboratory environment (Acıbadem University Experimental Animal Laboratory) with a temperature of 22±2°C and a standard light/dark (12/12 hours) cycle throughout the experimental period. Rats were randomly divided into 5 groups (n:8/group). Experimental groups were fed by a standard rat chow and tap water. Control group (Gr 1) was treated with 1 ml of distilled water by gavage every day during the experiment for 38 days. Rats in the DMSO+FA group (Gr 2) were given 1 ml of dimethyl sulfoxide (DMSO) for 28 days and FA solution (25mg/kg, dissolved in DMSO) by gavage in the last 10 days of the experiment. To create an MSG-induced liver injury model, MSG solution (600 mg/kg, dissolved in DMSO) was orally administered to the rats (MSG group-Gr 3) for 28 days (2). MSG+DMSO group (Gr 4) was treated by MSG solution (600 mg/kg, dissolved in DMSO) by gavage for 28 days and 1 ml of DMSO was given by gavage on the last 10 days of the experiment. MSG solution (600 mg/kg) for 28 days and then FA solution (25 mg/kg) on the last 10 days of the experiment were administered by gavage to the rats in the treatment group (MSG+FA-Gr 5) (3). At the end of the experiment the rats were sacrificed under anesthesia. Liver tissues samples were processed for light and transmission electron microscopical evaluations. Paraffin sections were stained with Hematoxylin-eosin (H&E), Masson's trichrome stains and Periodic acid-Schiff reaction (PAS). Histopathological scoring of the liver tissue was performed according to; vacuolization in hepatocyte cytoplasm, sinusoidal dilatation, leukocyte cell infiltration and distribution and amount of connective tissue in the parenchyme, as well as glycogen content within the hepatocytes. Oxidative stress markers, as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), total antioxidant capacity (TAS), total oxidant capacity (TOS) and oxidative stress index (OSI) values were measured for biochemical assesments (4).

Results

The light microscopical examinations revealed normal morphology of liver parenchyme in control and DMSO+FA groups. Steatosis and vacuolization in hepatocytes, sinusoidal dilatation, leukocyte infiltration, increased connective tissue in the parenchyme and decreased amount of glycogen in hepatocyte cytoplasm were detected in MSG and MSG+DMSO groups. Based on histopathological scoring, liver tissue injury was significantly reduced in MSG+FA group, compared to MSG and MSG+DMSO groups. Transmission electron microscopy revealed the similar ultrastructural data as in light microscopy to point out a prominent recovery in MSG+FA group. In MSG and MSG+DMSO groups, an increase in MDA, TOS and OSI levels were detected whilst GSH, TAS and SOD were decreased. Deteriorated biochemical data has been reversed and ameliorated in MSG+FA group, as the treatment group.

Conclusion

Based on the current biochemical and histopathological data, we could suggest that administration of ferulic acid to rats in this experimental model of MSG-induced liver injury contribute significantly to ameliorate/heal the hepatic damage by regulating the formation of reactive oxygen species.

Keywords:

MSG, Ferulic Acid,Liver, Microscopy.

Reference:

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In situ growth and phase engineering of manganese arsenide nanostructures in environmental TEM

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Poster Group 1

Background incl. aims
For magnetic materials, in situ synthesis offers an additional advantage by enabling the exploration of the magneto-structural relationship. Understanding magneto-structural phase transitions is crucial for the development of materials such as those used for magnetocaloric applications. One such material is MnAs, in bulk known for the ferromagnetic transition around 315K coupled with a structural transition from hexagonal (α-MnAs) to orthorhombic (β-MnAs). However, experimental studies of MnAs nanoparticles have shown discrepancies where the ferromagnetic transition occurs even in the absence of the structural transition.

Nanomaterial synthesis is usually done ex-situ, which can sometimes be tedious and time-inefficient, especially when exploring new or metastable materials. An environmental TEM (ETEM) specifically designed for exploring new material systems, allows for simultaneous exploration of synthesis parameters while observing the changing nanostructures with atomic resolution. Parameters can be fine-tuned, and even metastable material systems and crystal structures have been synthesized this way, showing promise for exploring not only new material systems but also those that have previously been discarded.

In this study, we focus on transition metal pnictides, particularly MnAs, due to their promising applications in spintronics, magnetic refrigeration, and data storage devices. During the preliminary work, the goal is to explore the diffusion behaviour of manganese, how to control the growth rate of MnAs and explore the various structural phase transitions, laying the groundwork for a deeper understanding of magnetic transition metal pnictides. In the long term, we would like to integrate Lorentz imaging techniques to also explore the magnetic properties. This way, material growth could be paired with atomic resolution imaging, chemical analysis, and even information about magnetic domains, and pave the way for a new approach to fundamental studies and phase engineering of magnetic nanomaterials.

Methods
MnO_2 nanoparticles were prepared via spark ablation and sputtered directly onto a MEMS chip for further work in the Hitachi HF-3300S 300kV ETEM. In the ETEM, arsine (AsH_3) was used as the precursor gas to facilitate the growth of MnAs nanostructures. Various parameters, including temperature, gas flow, and electron beam effects, were explored to map the parameter space of this material system and optimize the stability of both MnO_2 and MnAs. For analysis, HRTEM, FFT, SE and Z-contrast in STEM mode, and both TEM- and STEM-EDX were performed during the experiments.

Results
It was observed that AsH_3 diluted with H_2 during electron beam irradiation causes MnAs to form outside of the MnO_2 particles. Together with the oxide reducing in size and eventually disappearing,

this might indicate that the manganese atoms diffuse out of the oxide particles to crystallize into MnAs. This effect seems to be correlated with electron dose and is not observed in STEM mode. The fact that MnAs only form under a strong electron beam can be advantageous in the exploration of the parameter space, as no changes were observed for the non-exposed oxide particles. However, using manganese oxide particles instead of pure manganese also introduces some limitations. At lower temperatures, around 200°C, the stability of the MnO_2 nanoparticles under the electron beam was compromised, even without any AsH_3 supplied. However, at higher temperatures, around 400°C, the MnO_2 particles were observed to be more stable and thus preferable for control of the diffusion of manganese into MnAs.

Conclusion

MnAs was successfully synthesized from MnO_2-particles under AsH_3 atmosphere using an in situ ETEM due to what seems like an electron beam-activated process. These preliminary findings demonstrate the feasibility of synthesizing stable MnAs nanostructures in the ETEM environment and underscore the potential of this approach for exploring transition metal pnictides and similar material systems. The results show that this method is promising for future studies of magneto-structural relationships in transition metal pnictides, especially if magnetic imaging techniques are well integrated into the system. Additionally, the results are laying the foundation for further investigations into phase engineering and combinations with semiconducting III-V nanowires, such as GaAs and InAs, which already have been greatly explored in this system.

Keywords:

ETEM MnAs in-situ

Reference:

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Advanced image analysis techniques to support and streamline cell manufacturing

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Poster Group 2

Background
To achieve a high standard of cell manufacturing, it is imperative to maintain unwavering diligence and meticulous attention to detail throughout every phase of product development. Imaging of cells can play a vital role in research & development; nonetheless innovation is needed to bring the quantification and analysis of the imaging data produced to a standard suitable for cell manufacturing.

Methods
Visually monitoring cells at various stages of their development is an essential part of assessing their purity, function, and overall health. At bit.bio, we utilise bespoke image analysis and machine learning techniques to capture and monitor trends from imaging data that provide insights into cellular behaviours. This includes a combination of open-access and commercially available software, as well as bespoke and custom-made tools. The latter includes the use of Python packages to analyse microscopy-derived data that provides users with metrics to better understand imaging data. We have also developed and trained machine learning models that can process imaging data to provide consistent readouts on cell quality and purity.

Results
We have been able to successfully implement custom and highly-sophisticated image analysis tools that have enabled us to enhance our data mining capabilities from imaging data. These have played a pivotal role in streamlining our cell manufacturing capabilities by providing quality control readouts and supporting key decision-making steps in validating cell culturing and growth protocols.

Conclusions
Advances and developments in image processing methodologies at bit.bio have allowed us to accelerate our analysis workflows to produce a consistently high level of quantification of imaging data. The combination of custom scripts and packages with machine learning tools means that we have a comprehensive understanding of our imaging data, and this knowledge can be applied to make informed decisions on optimal culturing conditions, and provide assurances on cell quality to customers.

Keywords:

cell; machine learning; analysis; manufacturing

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Ultra-low voltage SEM observation for battery materials

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Poster Group 1

Background incl. aims
The scanning electron microscope (SEM) observation at an ultra-low accelerating voltage can visualize strictly superficial surface of the sample with a little electron beam irradiation damage, since the electron scattering volume into the sample is extremely small. However, interpretation of the captured image becomes complicated because the voltage contrast (VC) depending on emitted electron yield changes its behavior under the ultra-low accelerating voltage conditions [1]. Previously, the VC between binders and active materials of lithium-ion battery (LIB) anodes under ultra-low accelerating voltage conditions below 50 V were studied. As the result, we reported that the VC between the binder and the active material was dramatically changed among the ultra-low accelerating voltage conditions [2]. Especially, particle-like unique contrast, which was thought to reflect the binder structure, was observed at 20 V. Although this VC was presumed to be formed by the surface potential at the specific accelerating voltage, the formation mechanism was unknown. In this study, a simplified experiment simulating the binder on the LIB anode was performed to elucidate the binder VC formation mechanism.
Methods
Styrene-butadiene-rubber (SBR) particles: the primary material of the binder, were dispersed in water. The solution with the dispersed particles were dropped onto an osmium (Os) coated Si substrate. The solution was dried at the room temperature. The SBR particles were observed at accelerating voltages below 50 V using a Hitachi SU8700 field emission SEM [3]. The correlative observation with SEM and atomic force microscope (AFM) was performed using a Hitachi AFM5300E for surface potential measurements after electron beam irradiation. The “Air protection” sample holder was used to retain the sample in vacuum at the transfer between SEM and AFM.
Results
The graphic shows the observation results of the SBR particles, representing the “binder”, on the Os-coated Si substrate at the same area captured at accelerating voltages from 10 to 50 V. The SBR showed darker signal (indicated by the red arrow in image (e)) compared to the surrounding Si substrate at 50 V. As the accelerating voltage gradually decreased to 30 V, the contrast between the SBR and the substrate was almost disappeared. When the accelerating voltage decreased to 20 V, the SBR was observed with particle-like contrast. At the accelerating voltage of 10 V, mirror phenomenon, in which the primary electrons bounced off before impacting the sample surface, occurred. This result showed that the VC of the SBR on the Si substrate showed same manner of the binder on the active material in the previous work in spite of different substrate material. To clarify the principle of the VC formation mechanism, correlative AFM observations of the SEM observed area were also executed to measure the residual surface potential. As the result, the residual surface potential of the SBR, the “binder”, was confirmed to be varied by the accelerating voltage.
Conclusion

VC formation mechanism of the binder was investigated with the simplified specimen. The surface appearances of the “binder” were observed with the SEM and the surface potentials were also measured with the AFM correlatively, and SEM accelerating voltage dependency of the residual surface potential was found.

Keywords:

Ultra-low-voltage observation,Voltage contrast, Correlative observation

Reference:

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Structural Study of Polyphasic Mixtures Using 3d Electron Diffraction:A Case Study of Oxyresveratrol

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IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

Background
Oxyresveratrol is a naturally occurring compound found in Artocarpus Lakoocha which is known for its antioxidant and anti-inflammatory properties. We try to understand more about this compound by studying its crystal structure in the pure anhydrous form. This a crucial information for understanding how the crystal structure can be modified to address one of the problems with this natural product; its low solubility and bioactivity. The only known crystal structure of oxyresveratrol was reported by Deng et al. in a dihydrate form however the anhydrate form has not been reported. So far, there has been no success in growing single crystals of the anhydrate form large enough for structure determination using Single Crystal XRay Diffraction (SCXRD). An initial attempt to solve the anhydrate crystal structure using Powder XRay Diffraction (PXRD) was unsuccessful due to overlapping peaks and the presence of more than one phase. The PXRD pattern of the reported hydrated phase also did not fit all the peaks of the PXRD pattern of our sample. 3D electron diffraction (3DED) is a technique for structure solution of single crystals below 1µm and ideal for identifying phase mixtures. We apply 3DED for crystal structure determination and to better investigate all the possible phases that were present in the sample.

Method
3D ED data collection was performed in the Zeiss Libra 120kV Transmission Electron Microscope (TEM) at both room temperature and at -180˚C. Sample for the cooling experiment were put in water and plunged frozen in liquid ethane by applying a technique commonly used in cryo Electron Microscopy (cryoEM) to prevent sample dehydration. This method is used to identify possible hydrates in our sample whose crystal structure collapses in the high vacuum condition present in the TEM. Experimental PXRD was performed using a STOE Stadi P equipped with Cu-Kα1 radiation.
Results
In the room temperature experiment, we identified a new anhydrate oxyresveratrol crystal structure which has not been reported. It crystallizes non-centrosymmetric space group Pc and refined using dynamical refinement. Additionally, in the cryo experiment, a new polymorph of the dihydrate form of oxyresveratrol with the same space group as the anhydrous phase was found different from the reported hydrated phase which was P-1. The simulated PXRD pattern of the two new structures were compared with the experimental PXRD. We observed the presence of both phases of the new structures and a minor phase of the form 1 hydrated phase and fully fitted the patterns using Rietveld refinement.
Conclusion
The successful structural determination of these two new polymorphs demonstrates the possibility of directly determining structures from polyphasic mixtures by combining the power of both 3D ED and PXRD. This will improve efficiency and broaden the scope of phase identification which plays a crucial role in pharmaceutical development and within the field of crystal engineering.

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Keywords:

3delectron diffraction polymorphism crystal structure

Reference:

Reference

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Neuroprotective Effects of Bromelain in Peripheral Nerve Injuries: A Rat Sciatic Nerve Crush Injury Model

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Poster Group 1

Background: An aqueous, coarse extract called bromelain is obtained from the fruit and stems of the pineapple. Bromelain is a substance that can be easily absorbed into the body without losing its proteolytic activity and without showing any significant side effects. In vitro and in vivo studies have demonstrated various fibrinolytic, antiedema, antithrombotic, and anti-inflammatory activities of bromelain. Experimental studies have demonstrated the properties of bromelain, such as accelerating healing effect in tendon and muscle injuries, faster healing rate in burn wounds, and reducing post-surgical edema. This present study aims to investigate the neuroprotective effects of bromelain on peripheral nerve injuries, specifically through a rat sciatic nerve crush injury model.The study focused on the ability of bromelain to improve nerve regeneration and functional recovery after injury through histological and electron microscopic evaluations.

Materials and Methods: A controlled experimental study was conducted on 36 adult male Sprague-Dawley rats, divided into four groups: Sham, Control (Nerve injury), and two Treatment groups receiving oral bromelain at doses of 25 mg/kg and 50 mg/kg respectively. The sciatic nerve crush injury was induced using a standardized protocol. The intervention groups received their respective doses of bromelain daily for six weeks post-injury. Evaluation of neuroprotective effects was assessed through walking track analysis for functional recovery, hot plate tests for nociception, nerve conduction studies and histological examinations including light and transmission electron microscopy. Sciatic nerve samples taken from all the experiemental groups were fixed in 10% buffered formalin solution. Masson's trichrome stain was applied to paraffin section to reveal myelinated axon morphology. Tisue samples from sciatic nerve, fixed in 2.5% glutaraldehyde solution were processsed for transmission electron microscopy.

Results: The bromelain-treated groups exhibited significant improvements in functional recovery assessed by the Sciatic Functional Index (SFI) compared to the control group. The 50 mg/kg bromelain group showed the most pronounced improvement ($p<0.05$). Nociceptive testing indicated a reduction in pain sensitivity in bromelain-treated groups. Electrophysiological studies revealed enhanced nerve conduction velocities in treatment groups ($p<0.05$) with histochemical and ultrastructural analysis confirming accelerated nerve regeneration and reduced scar tissue formation. Statistically significant differences were observed between treatment groups and the control group with the higher bromelain dose demonstrating superior outcomes.

Conclusion: Bromelain administration in post-sciatic nerve crush injury in rats significantly enhances nerve regeneration, functional recovery, and reduces pain sensitivity. The study highlights bromelain's potential as a beneficial therapeutic agent in the management of peripheral nerve injuries with dose-dependent effectiveness. Further research is warranted to elucidate the underlying mechanisms and explore the clinical applicability of bromelain in peripheral nerve injury treatment.

Keywords:

Bromelain, nerve injury, histopathology

Reference:

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Establishment of 30mm diameter milling and curtaining effect reduction by large area planar surface milling

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Poster Group 1

Background incl. aims
The planar surface milling method is effective for sputtering a specimen surface by irradiating an Ar ion beam at an adjusted irradiation angle while the specimen is continuously rotated. This method is used for final finishing of mirror polished specimens by mechanical polishing for SEM observation and EBSD analysis. And the sputtering area is about 5 mm in diameter using the conventional planer surface milling method. However, there is an increasing demand for final finishing by planer surface milling over a larger area, such as a diameter of over 10 mm, where mechanical polishing is generally used. The followings are some of the issues with conventional planar surface milling.
Problem 1: Ion irradiation is performed from one direction at the periphery of the ion irradiation area. This causes a curtaining effect, making it difficult to obtain a flat surface over a large area.
Problem 2: Differences in etching rates are caused by differences in specimen material and crystal orientation, resulting in unevenness on the ion-irradiated surface1).
In this study, we examined methods to resolve these issues.
Methods
Specimen for this test is rolled aluminum foil (100 µm thickness) without mirror polishing or other pretreatments. This is because this specimen has uniform scratches in one direction generated during rolling process, and these scratches were evaluated as polishing scratches.
The Ar ion beam processing system IB-19530CP/IB-10500HMS (JEOL Ltd.), and IB-11550LSRH (JEOL Ltd.) as holders for planer surface milling were used for this study. By combining these units, the stage rotating and stage swinging during planer surface milling can be executed simultaneously. This enables milling over a large area. We named this method to large area planar surface milling. In this method, the center of rotation and the ion beam center are eccentrically aligned during the swing of the specimen stage, allowing ion beam irradiation of the outer periphery from various directions. Therefore, it can reduce the curtaining effect at the outer area of the ion irradiation area. In addition, the tilt angle of the planar surface milling holder is adjusted to make the ion beam irradiation angle lower to 10° or less, which can suppress the unevenness of the ion irradiated surface2).
Experiment 1: Verification of reducing the curtaining effect
Specimens were prepared by the planar milling method using a specimen rotation motion and by the large area planar milling method which combines the specimen rotation with the stage swing motion. Subsequently, the quality of surface was compared. Specimens were processed under the following conditions: an acceleration voltage of 10 kV, a processing time of 1 hour, and an ion beam irradiation angle of 5°. In the large area plane milling method, the stage swing angle was set to ±30°.
Experiment 2: Verification of unevenness reduction
Specimens were prepared using the large area planar surface milling method at ion irradiation angles of 5° and 2°, and the quality of surface was compared. Specimens were processed under the following conditions: an acceleration voltage of 10 kV, a stage swing angle of ±30°, and a processing time depended on irradiation angle of ion beam : 12 hours at an ion beam irradiation angle of 5° and 24 hours at an ion beam irradiation angle of 2°.

An FE-SEM (JSM-IT800 (JEOL Ltd.)) and an AFM (JSPM-5200 (JEOL Ltd.)) were used to observe each processed surface.

Results

1. comparison of the conventional method and large area planar surface milling method

The conventional method produces a flat surface in the vicinity of the milling center. However, striped structures can be seen on the surface about 5 mm away from the milling center due to the curtaining effect caused by the limited direction of ion beam irradiation. On the other hand, the large area planar surface milling method reduced the curtaining effect even at a distance of about 5 mm away from the milling center. (Fig. 1 A).

2. effect of irradiation angle on large area planar surface milling:

Unevenness due to differences in etching rate, such as crystal orientation, could be reduced by changing the ion beam incidence angle from 5° to 2°. In addition, the average surface roughness of the center of the processed area was measured using AFM. The average surface roughness was Ra: 86.7 nm at an ion beam irradiation angle of 5°, and Ra: 24.1 nm at an ion beam irradiation angle of 2°. This indicates that unevenness reduction was achieved even in the center of the processed area. The processing at an ion beam incidence angle of 2° extends the ion irradiation range, and thus reduces the curtaining effect over a wide area of 30 mm diameter (Fig. 1 B).

Conclusions

We examined the reduction of unevenness and increase of the milling area by the planar surface milling. Using a new method that combines a large area planar surface milling method with an extremely low-irradiation angle ion beam, the unevenness caused by etching rate differences in materials or crystal orientation was reduced and a large range of specimen processing could be performed. This method is expected to be applied to specimens that are not easy to mechanically polish or as an alternative technique to buff polishing, because it can produce a fine surface with reduced unevenness over a wide area exceeding 20 mm in diameter.

In this presentation, application examples will be presented in addition to the above results.

Keywords:

Ion etching, Specimen preparation method

Reference:

1 R. Gago, et al., Applied Physics Letter, 78, 3316-3318(2001).
2 A.W. Barnard, et al, Microscopy and microanalysis, 12, 1318-1319 (2006).

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Characterization of casting inclusions in superalloys by BSE and EDS

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Poster Group 2

Background incl. aims
Superalloys exhibit remarkable properties such as exceptional mechanical strength, resistance to thermal creep deformation, and robustness against corrosion and oxidation, allowing them to operate efficiently in extreme environments. Their compositions primarily include nickel, cobalt, iron and chromium, whit addition of tungsten, molybdenum, tantalum, niobium, titanium, and aluminum. Innovations in the chemical development of superalloys have enabled the precise tailoring of their properties, ensuring they meet the specific demands of their applications. These materials are crucial in applications demanding high performance at elevated temperatures, such as in aerospace turbine engines and marine engineering.
Superalloys are typically processed by investment casting (also known as lost-wax casting) under vacuum conditions to ensure the highest quality of the casting. During casting, different ceramic materials are used for crucible in which an alloy is melted, for cup in which a molten material is transported, for shell in which casting is performed, or for ceramic slurry used for coating a wax model. All of this is made of different size ceramic particles which during operation could chip and make inclusion in the cast part. For that reason, this work is performed to identify inclusions, and thus identify critical place in casting procedure.
Methods
The casting inclusion are observed at different cross sections of superalloy casted parts which were metallographically prepared and afterwards preliminary examined on Light Microscope Leitz Orthoplan, and further studied in backscatter electrons (BSE) mode on scanning electron microscope JOEL JSM-6460LV equipped with EDS system INCA Oxford Instruments, at 20 kV.
Results
It is found that ceramics inclusions are primarily present in the surface of cast part, thus making a critical place for fracture in operation. In backscatter electron imaging mode it is straightforward to distinguish between the most common inclusions, like: ZrO_2 from crucible, with distinctive white appearance (Fig. 1); Al_2O_3 from cup, with dark gray appearance (Fig. 2); or $ZrSiO_4$ inclusion, originating from ceramic slurry, which could be also simply identified due to the gray shade (Fig. 3). On figure 4, the presence of multiple different inclusions could be observed, also. All observed inclusions are also positively identified by quantitative EDS point or area analysis.
Conclusion
At the end, it could be summarized that backscatter electron (BSE) analysis is a fast and convenient method to identify casting inclusions in superalloys, and thus to identify an origin of inclusion and casting error. Further EDS analysis positively confirms identification of inclusions, and gives additional data about oxidation or other impurities present.

Keywords:

Superalloys, casting inclusions, BSE, EDS

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Thin Film Phase Plates for Cryo TEM: Fabrication, and Characterization Using Electron Holography

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Poster Group 2

BACKGROUND

Phase plates for transmission electron microscopy (TEM) have been a research field since 2001 and are of continuing interest to enhance contrast and increase resolution when imaging biological samples [1]. Unfortunately, biological samples are weak-phase objects in the electron microscope, providing minimal inherent contrast. This is in part because our detectors are only sensitive to variations in electron intensity and not phase. There are different approaches to overcome these limitations, including heavy metal staining (mass-thickness contrast), defocus phase contrast, and phase plate induced phase contrast.

We utilize phase plates to induce phase contrast. The most promising phase plates for soft-matter imaging are thin-film-based. These phase plates suffer from several limitations that plague their widespread implementation. These challenges include contamination, ease of installation and use, lack of reproducibility, and lack of full understanding and control of the induced phase shift [2]. We aim to develop, design, fabricate, implement and use these phase plate devices to reliably improve the contrast when imaging biological samples while staying close to focus, and that are easy to install, align, and use continuously.

METHODS

In this work we are developing thin film based phase plate devices, operating in Zernike and Hilbert mode respectively, to enhance phase contrast at minimal defocus. We are utilizing cleanroom technology to fabricate these devices to be reproducible and with known parameters. We are employing established CMOS thin film processes including dry and wet chemical etching and thin film deposition to create our devices. These phase plates, when installed in the back-focal plane of our TEMs, introduce a phase shift of $\Delta\phi = \pi/2$ (pi-half), or π (pi) respectively, to the scattered beam. The phase shift produced by a thin film is governed by the mean inner potential of the film, the film thickness and the accelerating voltage of the electron microscope. Using the cleanroom to fabricate these devices gives us both knowledge and control of the induced phase shift [3].

We are also employing the electron holography technique to measure the phase shift induced by our thin films. For that a bi-prism, in our case a biased gold wire, is placed in the selected area diffraction (SAD) plane of our TEM. With this we can make the part of the beam going through our material interfere with a reference part going through vacuum. We are thus creating a hologram in the image plane that contains the phase information of the beam going through the material relative to vacuum. After mathematical procedures to reconstruct and unwrap the phase information we can map the phase shift induced by our thin film material [4, 5].

RESULTS

We are able to utilize off-axis electron holography to reliably characterize the phase shift induced by the thin film material of our devices. Employing the accelerating voltage, the known film thickness, and the measured phase shift, we can determine the mean inner potential for the material. With the mean inner potential in hand we can now produce phase plate devices with the correct thickness to induce the target phase shift. With these devices we are able to optimize the contrast from a variety of weak-phase objects yielding TEM images with both higher resolution and higher contrast.

CONCLUSIONS

With the aforementioned approaches and techniques we are able to build a reliable device that improves the contrast when we image bio samples while keeping the defocus value very close to focus. The device can be reliably aligned in diffraction mode when mounted in the back-focal plane. We can address the most common drawbacks of material-based phase plates: we can make usage simple by installing them in the back-focal plane and alignment by aligning at the material edge visible in diffraction mode. Via employing electron holography we can also track the phase shift induced by our devices, which in turn improves the interpretability of phase contrast images.

Keywords:

Phase Plates, Electron Holography, TEM

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PFIB and SEM engineering of luminescent centres in hBN

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Poster Group 1

Hexagonal Boron Nitride (hBN) is a wide-band gap two-dimensional material, which has attracted much attention due to its ability to host luminescent centres with remarkable stability and single-photon in nature. This work explores the generation and characterization of luminescent centers in hBN on a SiO2/Si substrate using irradiation with a Scanning Electron Microscope (SEM) and Focused Ion Beam (FIB). We have investigated the impact of irradiation parameters on the optical and morphological properties as well as reproducibility of the luminescent centers using Photoluminescence (PL) spectroscopy, Atomic Force Microscopy (AFM), and SEM imaging. Our findings demonstrate the successful engineering of VB- centres, consistent with existing literature. We highlight the crucial role of material processing, such as the growth and annealing conditions of hBN and irradiation-specific parameters. This optimized methodology holds promise for generating B centres in hBN in a reproducible manner, providing a robust benchmark for future research in quantum technologies and other related fields.

Figure: a) Post PL map (left) and corresponding spectra from hBN flake treated with PFIB oxygen beam at different beam energies and doses. B) Post PL map (left) and corresponding spectra from hBN flake treated with the electron beam at 15 keV energy and different doses.

Keywords:

Irradiation Engineering; FIB; SEM

Reference:

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Non-toxic clearing and labeling with fluorescent REAfinityTM antibodies for enhanced 3D visualization of tissues

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Poster Group 1

Background
Generation of a 3D cellular topography of biological specimens is often essential to understand cellular and molecular processes. Especially, spatial visualization of heterogenous protein expression in solid tumor samples is considered important for advanced phenotyping and potential diagnostic applications. However, for a long time, biologists were limited to the visualization of thin tissue sections, which provides limited cellular context. Furthermore, sectioning is time-consuming and often prone to artifacts, like ripping and folding. While clearing methods in combination with light-sheet fluorescence microscopy have emerged as powerful tools for 3D reconstruction of tissues, most clearing techniques involve toxic reagents which require special safety provisions and generate extra effort for sample preparation as well as imaging. To address these shortcomings, we developed a clearing workflow based on non-toxic reagents that allows for efficient 3D imaging biological samples in combination with directly labeled REAfinityTM antibodies.
Methods
Briefly, samples were fixated with paraformaldehyde and permeabilized to facilitate delipidation and antibody-conjugate penetration during subsequent immunostaining. Whole-mount immunostaining was performed using REAfinityTM antibodies coupled to Vio® dye variants. Duration of the immunostaining depends on the tissue size and tissue type, ranging from 2 - 7 days. After staining, tissues were dehydrated using an ascending ethanol series. Subsequently, refractive index matching was performed; a process referred to as tissue clearing renders opaque tissues transparent and thereby improves optical light penetration depth. For blood-rich organs and tissues a carbamide peroxide based depigmentation module was applied to remove residual blood to facilitate increased light penetration during imaging. In addition, tertiary butanol and pH adjustment during dehydration and clearing process can be used to ensure preservation of endogenous fluorescent proteins. Imaging of large samples was performed using the UltraMicroscope Blaze light-sheet imaging platform. To eliminate noise, intensity inhomogeneities, striping artifacts and to enable deconvolution of imaging data, a postprocessing pipeline based on MACS® iQ 3D Large Volume software was applied.
Results
Our workflow enables 3D imaging of various biological samples, including mouse brain, lymph nodes, lung, intestine, liver, spleen, kidney, stem cell-derived organoids, xenografts, patient-derived tumor samples and tumor spheroids. To enable the effective clearing for blood-rich organs and tissues, an efficient depigmentation module was integrated to increase light transmission. Additionally, the protocol can be also modified to ensure preservation of endogenous fluorescence. For optimal 3D imaging, we combined the clearing procedure with whole-mount immunostaining of mouse tissues, human tumor samples and organoids using Miltenyi Biotec's REAfinityTM antibodies coupled to newly developed Vio® dye variants optimized for application in light-sheet microscopy. REAfinityTM antibodies are recombinant antibodies engineered to provide high specificity, purity, and superior lot-to-lot consistency compared to hybridoma-derived antibodies, whereas Vio® dyes are a new generation of fluorescent dyes characterized by remarkable brightness, photostability and highly

resistant against organic solvents during clearing procedure. UltraMicroscope Blaze light-sheet imaging platform enabled fast high resolution imaging of large samples. Our data indicates highly efficient labeling of the vasculature, different neuronal subtypes in mouse brains, as well as bright staining of neural progenitors and proliferative cells in cerebral organoids. Furthermore, carcinoma cells and lymphocytes were visualized in solid tumors and mouse lymph nodes, respectively. After data acquisition, a profound postprocessing pipeline based on the MACS® iQ 3D Large Volume software enables the elimination of noise, intensity inhomogeneities, striping artifacts and features deconvolution of imaging data, which ensures ideal preparation of 3D data for subsequent data visualization and quantification.

Conclusion

We present a robust, fast, flexible and non-toxic clearing procedure that allows for efficient 3D imaging of a wide range of biological samples. Optional modules for depigmentation of blood-rich organs and preservation of endogenous fluorescence can be applied in order to extend the procedure to applications with further requirements. By combining the advantages of REAfinityTM antibodies and Vio® dyes, we developed staining reagents that significantly reduce staining duration and are thus ideally suited for whole-mount tissue labeling, multiplexing, and imaging with light-sheet microscopy using the UltraMicroscope Blaze. Taken together, we developed an efficient clearing procedure combined with highly specific antibody conjugates and a data postprocessing pipeline for optimal 3D visualization of tissue and whole organs, paving the way for a better understanding of tissue structures and disease mechanisms. Extension of the clearing procedure for additional applications and constant development of further antibody conjugates coupled to additional Vio® dye variants will further increase marker coverage and the range of applications.

Keywords:

Tissue Clearing, 3D-IF, Ultramicroscopy, Lightsheet

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Deformation of steel chips due to machining

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Poster Group 2

Background incl. aims
During machining of materials, high temperatures and high pressures can occur. So, the materials are exposed to high plastic strain, high strain rates as well as high temperatures and heating rates. In order to be able to describe this, materials are examined to see how they change.
Chips that were removed by turning are very small in contrast to the remaining workpiece. They heat up so quickly that they reach more than half their melting temperature in just a few milliseconds. This in turn can significantly change the strength of the metal [1] and its resistance to the formation of chips. That´s why chips were chosen to be characterized concerning their microstructure.
Precise predictions through simulations are desired and the material model for the simulations must be examined [2]. Therefore, it is essential to compare simulations with experiments and observe actual changes in the microstructure of the chips and gain knowledge of the material behavior. To this end, microstructural investigations on chips were carried out using transmission electron microscopy (TEM).

Methods
For investigating the microstructure in TEM, electron transparent Focused Ion Beam (FIB) lamellae were prepared (Fig. 1). Chemical analyses were carried out by EDX mapping. Information for determining the grain size depending on the distance to the surface (depth) was obtained by bright field and corresponding dark field images.

Results
Several chips were investigated regarding the change of the microstructure from the surface into the depth of the material. Different cutting speeds and different final cooling rates led to a change in grain size over the depth from the surface. The EDX mappings showed an oxide layer on the surface of each sample of the chip.

Conclusion
Microstructure characterizations are important for investigating materials and improving the associated simulations. The present investigations on the steel chips pointed out a clear diffence between the change in microstructure caused by different cutting speeds and different cooling rates.

Keywords:

steel chips, microstructure, TEM, simulation

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Characterization of Cu doped zeolite by MicroED and electron ptychography

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Poster Group 2

Background incl. aims
Selective catalytic reduction (SCR) of NOx by ammonia has been widely applied to the removal of pollutants from diesel gas engines, and Cu doped small pore zeolites are known to exhibit excellent NOx conversion activity and N_2 selectivity. In particular, Cu doped CHA type zeolites (Cu-CHA) have been widely used in automobiles. One of the requirements for practical SCR catalysts is hydrothermal stability to withstand the harsh environment of over 700°C water vapor, and Cu-CHA is known to suffer hydrothermal degradation[1]. The origin of this degradation is proposed to be atomic-scale structural changes such as degradation of the CHA framework and local coordination environment changes of Cu atoms[2]. However, there are few studies of direct observation of this degradation reaction at the atomic level. The reason is that zeolite is an extremely electron sensitive material, so in widely used TEM technique it is difficult to observe zeolites in atomic resolution due to the poor electron beam efficiency. In this study, direct observation of Cu-CHA was performed using microcrystal electron diffraction (MicroED), which allows to analyze crystal structure at low electron doses, and electron ptychography[3], which utilizes 4D-STEM technology to achieve high electron beam efficiency.

Methods
Zeolite sample preparation: Cu-CHA was synthesized by the method described in the paper[3] (Sample A). The synthesized sample was kept at 800°C for 5 hours under 10%-water vapor/air circulation to test its hydrothermal durability (Sample B). Two types of samples were dispersed on carbon TEM grids for MicroED and TEM observation.
Preliminary observation by Micro ED: Sample A was analyzed by a Micro ED system (XtaLAB Synergy-ED, Rigaku/JEOL). The acceleration voltage was 200 kV, and diffraction patterns were obtained from -50 to 50 degrees in 0.5 degree increments. Structural analysis from the set of the diffraction patterns was performed by CrysAlis (Rigaku/JEOL).
Electron ptychography observation: For the two types of samples (Sample A and B) were heated on a hot plate at 200°C in the air for 20 minutes just before TEM observation for removing water and contaminants. For the ptychography observation, an aberration-corrected TEM (JEM-ARM200F, JEOL) equipped with a high-speed pixelated STEM detector (4Dcanvas™, JEOL) was used. The acceleration voltage was 200 kV. The convergence angle was 13.5 mrad, the probe current was 0.059 pA (49 e- per point), real-space pixels were 512×512, and diffraction patterns were acquired with 264×66 pixels and binned to 66×66 by post-processing. The frame time was fixed at 7500 fps.

Results
Fig. (a) shows the crystal structure of Cu-CHA ([100] orientation) determined from MicroED result. The reliability factor R in this analysis is 0.16 (<0.2), this suggests that the determined structure provides the highest reproducibility of the obtained set of the diffraction patterns. The atoms are shown as displacement ellipsoids in Fig.(a), and the Cu atom positions are predicted to be spread out.

Fig. (b) shows the phase images obtained by electron ptychography after the hydrothermal durability test. It suggests that the Cu site is changed after the hydrothermal durability test, while no structural changes in the CHA framework were observed.

Conclusion

To investigate the origin of hydrothermal degregation of Cu doped CHA type zeolite, which is useful as an SCR catalyst, MicroED analysis and electron ptychography observations were performed. The results suggest that the Cu site is changed without modifying the structure of the CHA framework after the hydrothermal degradation.

Keywords:

Electron ptychography, low-dose imaging

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From volume electron microscopy datasets to segmentation models, feature quantification, and data reuse

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Poster Group 2

Advancements in microscopy techniques have led to the generation of ever-increasing amount of data, demanding efficient analysis methods. Image segmentation tools, particularly those powered by Artificial Intelligence (AI), have become critical components for processing this data. However, segmentation is just the first step in unlocking the wealth of information hidden within these complex datasets. Quantitative analysis methodologies are essential for extracting meaningful biological insights.

Here delves into the power of AI-driven segmentation for quantitative analysis in electron microscopy. We demonstrate the segmentation of a U2OS cell FIB-SEM dataset [1] using a newly developed tools within Microscopy Image Browser (MIB) [2]. Our approach leverages the "segment-anything" model [3] for ground truth generation of training labels alongside a newly developed 2.5D deep learning workflow for image segmentation in DeepMIB tool [4] of MIB. This powerful combination allows for efficient generation of accurate models for various cellular organelles, including mitochondria, endoplasmic reticulum (ER), Golgi apparatus, nuclear envelope, lysosomes, and peroxisomes [5]. These models serve as the foundation for in-depth quantitative analysis.

Moving beyond segmentation, we explore potential analysis pathways. A typical pipeline might begin with volumetric analysis of organelles, providing valuable information about their size and distribution. This analysis can be further extended by developing custom methods to quantify specific features relevant to cell biology research. Examples include measuring the density of nuclear pores, quantifying the sheet-to-tubule ratio of the ER, or analyzing the contact points between ER and mitochondria.

In addition, we showcase the immense potential of large-volume electron microscopy data, extending its utility beyond the scope of the original research project. Sharing these datasets responsibly is crucial for unlocking their full potential and enabling broader scientific exploration. By fostering responsible data sharing practices [5], we can collectively extract even deeper insights from these rich datasets, leading to significant advancements in our understanding of cellular biology.

Keywords:

volume microscopy deep learning FIB-SEM

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Temporal characterization of femtosecond electron pulses inside ultrafast scanning electron microscope

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Poster Group 2

Background incl. aims
In recent years, ultrafast electron microscopy has been proved to be a promising method for examination of the dynamics of the most fundamental processes. The visualization of processes with high temporal and spatial resolution can be achieved with laser pump and electron probe experiments using single electron pulses from a nanoemitter to avoid temporal spread of the pulse by Coulomb forces. In order to perform pump-probe experiments, the exact knowledge of electron pulse duration is required. Only few techniques have been developed to perform the temporal characterization of ultrashort electron pulses with low electron density. One of the approaches, often used in ultrafast transmission electron microscopes (UTEMs), is based on electron-light cross-correlation utilizing the inelastic scattering of electrons induced by the interaction with optical near field around a nanostructure [1]. Such scheme is limited by the fact that the electron microscope has to be equipped with a high-resolution spectrometer, which allows to spectrally filter the inelastically scattered electrons. In this contribution we demonstrate the use of a different method for electron pulse characterization, which is based on scattering of electrons mediated by ponderomotive potential of an optical standing wave. This method is suitable for ultrafast scanning electron microscopes (USEM), for which the experimental determination of the length of the electron pulse is still challenging to this day.
Methods
Free electrons are scattered by an optical standing wave created by two counter-propagating pulsed optical beams of the same frequency in vacuum. This effect is driven by the ponderomotive potential of the optical field and is used as a technique to characterize duration of electron pulses [2,3]. Electrons traveling through the oscillating electromagnetic field of generated standing wave experience ponderomotive force proportional to the gradient of light intensity and are deflected from their original direction of motion. Electron pulse duration is then reconstructed from a time trace, which is given by the measured electron scattered signal taken at different electron pulse-laser pulse time delays, and which is a direct cross-correlation of electron and laser pulse. Although the described method is known, up until now it has not yet been used to characterize electron pulse durations for wide range of electron energies in a commercial scanning electron microscope (SEM).
Results
In our experimental setup, we generate low density electron pulses by a laser-triggered emission from a Schottky emitter in Verios 5 XHR SEM (energy range 1 to 30 keV) by illuminating the apex of the emitter tip with 257.5 nm laser pulses. Inside the SEM chamber, we implement a compact optical setup for the generation of optical grating with two counterpropagating laser pulses with central wavelength of 1030 nm and duration of 220 fs. The scattered electron signal is detected by a hybrid-pixel detector Timepix3. We characterize electron pulse durations for electrons in energy range from 5.5 keV to 30 keV. For the optimal setting with the electron emission regime with less than one electron per pulse, the electron pulse duration at the sample location spans from 0.4 ps full-width-half-maximum (FWHM) for 30 keV electrons to 2.7 ps FWHM for 5.5 keV electrons.
Conclusions

In the past, the development of methods for direct measurement of the temporal characterization of electron pulses has been focused primarily on UTEMs. Electron pulse durations in USEMs were only estimated on the basis of theoretical calculations. We describe the implementation of all-optical method for direct temporal characterization of femtosecond electron pulses in a commercial SEM.

Keywords:

ultrafast electron microscopy, electron pulses

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Optimizing optical STEM detection for faster acquisition speeds in scanning electron microscopy

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Poster Group 1

Background

Optimization of acquisition speed is important for large-scale and volume electron microscopy (EM) experiments of tissues and cells, as these are characterized by long acquisition times due to the low inherent throughput of electron microscopes. Developments in scanning electron microscopy (SEM) techniques have increased acquisition speed by orders of magnitude through the use of more sensitive detectors, electrostatic and magnetic immersion fields, and multibeam scanning electron microscopes (mSEM), in which the sample is scanned in parallel with an array of beams (1,2). Optical scanning transmission electron microscopy (OSTEM) offers a way to discriminate the signals from the individual beamlets in mSEM (3). In OSTEM, ultrathin biological sections are placed on a thin film-coated scintillator substrate, which converts transmitted electrons into photons. These photons are collected by a high NA optical objective and projected onto a multipixel photon counter (Figure 1A).

We have recently shown that OSTEM performs similar to backscatter electron detection (BSD) and SE detection on ultrathin biological samples (3), in terms of contrast, image resolution and signal-to-noise ratio (SNR). At short dwell times, OSTEM outperforms BSD and SE detection in SNR. However, BSD in combination with electrostatic immersion still outperforms OSTEM. Moreover, the SNR of OSTEM stagnates for moderate to high beam currents, suggesting a potential saturation point in the detection scheme. In general, signal generation and collection in OSTEM has not been thoroughly investigated and as a result, optimization of the OSTEM detector layout has not yet been performed.

To better understand signal generation and collection in OSTEM, a physics model is required that describes electron scattering in the substrate, its conversion to light, and the subsequent collection of this light signal. This model then must be verified with experimental results to be used to optimize the substrate design. We aim to develop this OSTEM signal generation model and use it to obtain a rational substrate design optimized for detection efficiency and acquisition speed.

Methods

In OSTEM, ultrathin biological sections are placed on a cerium-doped single-crystal yttrium aluminum garnet (ce:YAG) scintillator, coated with a thin (~30 nm) conductive Molybdenum coating. OSTEM is implemented in a Verios 460 (FEI Company) equipped with a SECOM integrated fluorescence microscope (Delmic) without the emission filters (Figure 1A). In an alternative setup, photons are directly detected by a multipixel photon counter array placed underneath the scintillator (Figure 1B). The photon output and SNR are evaluated by focusing the electron beam on the empty substrate surface or a biological sample respectively, and recording the photon intensity with the MPPC or a CCD camera, for a given beam current, dwell time and landing energy. The SNR is computed by averaging the spectral SNR over the full frequency space of every electron micrograph.

Electron scattering is modeled with Monte-Carlo simulations in CASINO (4) and NEBULA (5), by computing the electron energy loss per voxel. The material properties of ce:YAG and molybdenum are taken from literature. We assume that energy conversion to photons fully relies on low energy loss events (<50eV). Different possible saturation mechanisms are modeled by limiting the photon output as a function of the input energy per voxel. Additionally, the loss of photons in the detection is taken into account by a transmission coefficient that depends on the thin-film coating composition.

Results

The photon output of the empty substrate demonstrated a sublinear relationship with the beam current (Figure 1C), suggesting a partial saturation effect. MPPC saturation was excluded as the main cause, since validation experiments with a neutral density filter and a CMOS camera showed similar sublinear relationships. The photon output does increase linearly at higher beam energies (Figure 1D), indicating the partial saturation is prevented in a larger electron interaction volume. Initial modelling with CASINO software showed proportionally more scattering events in the thin-film coating than in the scintillator (Figure 1E), although the majority of secondary electron generation takes place in the scintillator. However, CASINO does not accurately model low-energy secondary electrons. To better understand the signal generation and simulate the energy deposition at low energy (0-50eV), for which most of the energy transfer to the active scintillator dopant is expected, we intend to use NEBULA to calculate the energy loss per voxel as a function of the electron beam energy and current. NEBULA has been developed to provide fast and accurate simulations of low energy electron-matter interactions with first-principle physical models (5).

Conclusion

Optical scanning transmission electron microscopy (OSTEM) is an alternative SEM detection technique for imaging thin biological samples, and can be used for electron detection in multibeam scanning electron microscopy. Partial saturation of the scintillator and energy loss in the thin-film coating of the scintillator are hypothesized to limit the signal generation in OSTEM. In this work, a first attempt is made to model energy conversion from a focused electron beam to a photon signal. With a physically valid model, different substrate combinations can be tested. Subsequent optimization of the OSTEM substrate may increase acquisition speeds in single-beam and multi-beam scanning electron microscopes.

Caption
Figure 1: Optimization of optical STEM detection. A: Optical STEM detection with optical detection path. B: Optical STEM detection with direction detection of photons. C: Photon intensity on detector as a function of beam current, demonstrating a sublinear relationship. D: Photon intensity as a function of beam energy, demonstrating a linear relationship for higher energies. E: CASINO simulation of interaction volume of a 4keV beam in ce:YAG (>0z) with 30nm molybdenum (-30z to 0z). Blue: primary electrons, red: backscattered electrons, green: secondary electrons.

Keywords:

SEM, OSTEM, Scintillation, Simulations

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HR-EBSD Analysis of High-Entropy Alloys: Understanding the Role of Alloying Elements in Mechanical Performance

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Poster Group 2

High-entropy alloys, like $Cr_{33}Co_{33}Ni_{33}$, boast remarkable mechanical strength attributed to solid solution strengthening. Research underscores the pivotal role of plastic deformation in these alloys, governed by the interplay of slip and twinning mechanisms, alongside the emergence of nanometric lamellas of HCP phase during advanced deformation stages. Grasping these deformation intricacies is imperative for elucidating the alloys' mechanical and microstructural characteristics. Yet, in-depth investigations into $Cr_{30}Co_{30}Ni_{30}Pd_{10}$, $Cr_{30}Co_{30}Ni_{30}V_{10}$ alloys remain limited. A meticulous characterization of these materials holds promise for unlocking valuable insights pertinent to their engineering applications.

Methods

Samples of $Cr_{30}Co_{30}Ni_{30}Pd_{10}$, $Cr_{30}Co_{30}Ni_{30}V_{10}$ alloys underwent interrupted tensile tests at various deformation levels, employing a deformation rate of $1X10^{-3}s^{-1}$. Following each deformation stage, high-resolution Electron Backscatter Diffraction (HR-EBSD) analyses were conducted using a FEG-SEM ThermoScientific Apreo 2 high-performance, equipped with an Instruments Symmetry 2 EBSD detector. This detector boasts indexing speeds exceeding 5700 patterns per second, coupled with a high-sensitivity CMOS camera. Data analysis was performed using CrossCourt software, enabling comprehensive assessments including simple orientation measurements, quantitative evaluations of elastic strain fields and stress, and precise measurement of residual stress within the sample. These analyses were conducted with a remarkable sensitivity of 1 part in 10000 and a spatial resolution of 100 nm.

Results

EBSD analyses facilitated the comprehensive mapping of microstructural evolution in both the $Cr_{30}Co_{30}Ni_{30}Pd_{10}$, $Cr_{30}Co_{30}Ni_{30}V_{10}$ alloys during tensile testing. Through the utilization of KAM (Kernel Average Misorientation) and GOS (Grain Orientation Spread) mapping techniques, the distribution of deformation across the microstructure was effectively delineated, alongside the discernment of deformation twin formations during the deformation step. Furthermore, it was discerned that the introduction of V facilitated mechanical deformation through twinning mechanisms, while the incorporation of Pd tended to increase alloy brittleness. This brittleness was notably evidenced by low KAM and GOS indices immediately preceding fracture in the Pd-containing alloy.

Conclusions

The employment of HR-EBSD emerged as pivotal in thoroughly scrutinizing these findings. Its capacity to furnish intricate microstructural insights empowered a profound comprehension of deformation

mechanisms and the nuanced impact of alloy constituents on mechanical performance. This underscores the indispensable role of advanced characterization methodologies in unraveling the intricate behaviors exhibited by materials under mechanical stress, thereby enriching our understanding of material science and engineering.

Keywords:

HR-EBSD, Microstructural Characterization, Mechanical Twinning,

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SEM-Cathodoluminescence Imaging and Spectroscopy - Applications in Archeology and Life Science

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Scanning electron microscopes (SEMs) are widely used as an imaging technique for any solid materials found on Earth. Newly synthesized materials, frozen biological samples, and geological and ancient materials can all be studied by SEMs. SEM-based spectroscopies, such as energy dispersive X-ray spectroscopy (EDS) and cathodoluminescence (CL), are applied to study the composition and structure of various samples. SEM-CL imaging and spectroscopy detect the emitted photons in the visual range. The luminescence results from band transitions can be related to different material properties, such as band gap, crystallinity, impurities, and defects in the structure.
We have been using SEM-CL as an imaging and spectroscopy tool in different fields, including less-studied ones such as archeology and life science. CL spectral measurements of $CaCO_3$ samples were performed as part of research in archeology, and the CL imaging of cholesterol crystals was implemented as part of a complete workflow to study their 3D structure.
Materials such as calcium carbonates ($CaCO_3$) found in archeological excavations can be studied with SEM-CL as their intrinsic defects and dopants create luminescent centers that can be detected. The various forms of $CaCO_3$ have unique defects that can be revealed by detecting the luminescence of the crystals. SEM-CL was used to study $CaCO_3$ in different forms: calcite and aragonite, which nucleate through geogenic and biogenic pathways, and pyrogenic lime plaster. The structural variations in $CaCO_3$ from various origins were measured by SEM-CL and correlated with radiocarbon dating (1-3). Using SEM-CL and complementing it with other analytical techniques, we formed a database of $CaCO_3$ samples from different sources. This database is of great importance in identifying the form of $CaCO_3$ when sorting materials from archeological excavations and increasing the accuracy of age determination by carbon dating.
SEM-CL of organic materials is quite challenging as their luminescence decay very fast under the irradiation of the electron beam. Cholesterol crystals are CL active and stable enough to be detected and imaged. Cryo SEM-CL was used as a first step in a correlative workflow that was developed to study atherosclerotic lesions in three dimensions at the nanometer scale. The cholesterol crystals and lipid droplets, both luminescence active, were identified by CL imaging. Following the CL imaging, the crystals were identified using SEM and moved to the FIB-SEM for volume imaging, all done under cryo conditions. The results of this workflow show that thin crystals appear to be associated with intracellular or extracellular lipid droplets and multilamellar bodies. The large crystals are independently positioned in the tissue and are not associated with specific cellular components (4).
In summary, SEM-CL studies of $CaCO_3$ samples and cholesterol crystals revealed the opportunity to use this method to explore materials and systems in fields such as life science or archeology.

Keywords:

Cathodoluminescence, SEM, Cryo, $CaCO_3$, Cholesterol

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Detection of quasicrystalline symmetries in electron diffraction data

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Poster Group 2

Introduction
Under certain alloying conditions, quasicrystals have been demonstrated to appear as nano-precipitates in aluminium alloys [1]. In addition to these exotic structures, crystalline approximant phases with similar compositions may appear in the very same systems. The approximants accommodate local icosahedral coordination, giving rise to pseudo-fivefold symmetric diffraction patterns when viewed along given irrational crystal directions [2]. In principle, the distinction between quasicrystalline and approximant diffraction patterns is sharply defined. However, in dealing with experimental data – especially from nano-sized precipitates – the difference is not necessarily obvious. In the following, we utilize scanning precession electron diffraction (SPED) in mapping out fivefold and pseudo-fivefold symmetries in selected material systems and discuss the designation of quasicrystalline precipitates based on diffraction data alone.
Methods
Four model systems were selected for the investigations: A powder of quasicrystalline AlCuFe, a modified 6082 aluminium alloy containing dispersoids of the α-AlFeSi approximant phase, an AlMgCuAg alloy, and a sample of this same alloy which was rapidly solidified though melt-spinning. A JEOL JEM-2100F transmission electron microscope equipped with a NanoMegas precession system and a Quantum detectors Merlin direct electron detector was used in the acquisition of the diffraction data. Data analysis was carried out using the PyXem python library [3].
Results and discussion
The quasicrystalline AlCuFe powder contained particles with large grains with a seeming lack of defects, producing dense fivefold symmetric diffraction patterns. The melt-spun AlMgCuAg was found to contain ~100 nm sized quasicrystalline particles, without any immediately obvious orientation relationships to the Al matrix. Diffraction patterns obtained from these particles were less dense than those from the large-grained AlCuFe sample, although a diffraction tilt-series confirmed their quasicrystallinity. Several different phases were present in the heat treated AlMgCuAg samples, some of which exhibited approximate fivefold rotation symmetries. The particles in question were sparsely distributed in the sample, and ~5 nm in diameter.

Figure 1 (a) shows a precession electron diffraction (PED) pattern obtained through SPED from a particle of interest in the heat treated AlMgCuAg alloy. The diffraction pattern exhibits approximate fivefold rotational symmetry, and sets of reflections fall onto rings with relative spacings which are roughly multiples of the golden mean $\tau=(1+\sqrt{5})/2$. The same diffraction pattern is shown in (b), represented in polar coordinates. Here, the first-order ring is clearly discernible.

Figures 1 (c) and (d) show electron diffraction patterns from particles found in the melt-spun AlMgCuAg sample and quasicrystalline AlCuFe particles, respectively. Both patterns exhibit a striking tenfold rotational symmetry, though it is noted that the patterns obtained from the – comparatively small – particles present in the melt-spun AlMgCuAg are considerably less dense than those acquired from the large-grained quasicrystalline AlCuFe powder.

The classification of presumably quasicrystalline nano-precipitates in aluminium solely by employment of (S)PED is non-trivial. However, we were able to designate these small particles as quasicrystals up to some tolerance. SPED allows for simultaneously sufficient spatial resolution and field of view to record the sparsely distributed ~5 nm precipitates in the AlCuMgAg sample and show that they are essentially quasicrystalline. Especially, as may be seen in Figure 1 (a), the quasicrystallinity of these particles dominate in the low-resolution domain of the diffraction pattern, hinting at long-range icosahedral ordering.

Complementary diffraction techniques, such as combining SPED with rotation electron diffraction, may help to further improve the accuracy of quasicrystal identification in complex sample systems. As such, it is possible to envision a fast and accurate routine for the mapping of quasicrystalline precipitates over relatively large projected regions – greatly aiding the study of the nucleation and growth of these exotic phases in metallic matrices.

Keywords:

Electron diffraction, quasicrystals, aluminium alloys

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2D and 3D Oxidation State Mapping in FeO/Fe3O4 Nanocubes Using the Fe-M2,3 EELS Edge

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Poster Group 1

Background
EELS in the STEM is capable of oxidation state quantification by analyzing the fine structure of the elemental ionization edges. The exact analysis procedure has been1 and continues to be2 a major focus of EELS research. In the last decade, oxidation state mapping at the atomic level 3 has become common and advances in data analysis have enabled 3D oxidation state mapping through tomographic reconstruction of EELS oxidation maps tilt-series4.
To date, most methods of oxidation state analysis involve ionization edges with onset energies above 100 eV. For example, the standard method for iron oxidation state analysis is based on the fine structure of the Fe-L2,3 ionization edges at ~708 eV. However, the small ionization cross-section of these ionization edges presents serious experimental challenges for beam-sensitive samples. For instance, beam damage and contamination issues limited the EELS tilt series in 4 to just half of the angles needed to obtain a full tomograph.
In this context, we explore the new possibilities for oxidation state analysis offered by the new generation of hybrid-pixel direct electron detectors5. Thanks to their almost-perfect DQE and zero read-out noise, using the standard oxidation state analysis methods, the same results can be obtained with a lower electron dose. Less obviously, we show that these detectors' high speed, large dynamic range and small point spread function facilitate EELS analysis using ionization edges that lie below 100 eV. This comes with mainly two advantages with respect to edges situated at higher energies: a much higher signal-to-noise ratio for the same electron dose, and the simultaneous acquisition of the full low-loss spectrum needed for accurate absolute quantification.
To demonstrate the novel oxidation state analysis method, we perform EELS oxidation state tomography on the FeO/Fe3O4 core-shell nanoparticles from 4 by analyzing the Fe-M2,3 ionization edges at ~54 eV.

Methods
FeO/Fe3O4 core-shell nanoparticle EELS spectrum images were acquired on an FEI TITAN Themis 300 S/TEM with a dwell time of 1 ms and a pixel size ranging from 0.2 nm for single maps to 0.4 for tomography studies. EELS maps were taken at 9 different angles ranging from -70 to 70 degrees for these tomographic studies.
Spectrum images were then analyzed using a combination of SVD decomposition of the four-dimensional dataset, blind source separation, and curve fitting EELS quantification.

Results

Figure 1 shows the EEL Fe-M2,3 spectra and maps corresponding to Fe3+ (in blue) and Fe2+ (in green) extracted from the dataset by blind source separation, which has proven successful. Furthermore, the 3D reconstruction of both oxidation states has been achieved, showing a differentiation between the core and the shell of the particle, and with minimal damage to the sample. A frame from this reconstruction can also be seen in Figure 1.

Conclusion

The combination of direct electron detection and the extension of oxidation state analysis to low-energy ionization edges has proven successful in performing EELS oxidation state tomography in beam-sensitive FeO/Fe3O4 core-shell nanoparticles, with a much lower electron dose than standard methods, notably minimizing sample damage and increasing the acquisition speed. This method sets a new standard for EELS analysis of suitable beam-sensitive materials.

Acknowledgments

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Keywords:

EELS, oxidation states, tomography

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Ultrafast Microbeam Electron Diffraction at 15-keV

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Poster Group 2

Ultrafast electron diffraction (UED) facilitates the time-resolved investigation of structural dynamics on an ultrafast timescale. In a UED experiment, a pump laser pulse triggers electronic and structural dynamics inside an investigated sample, which is diffractively probed by an ultrashort electron pulse at a variable time delay. Repeating the experiment with different time delays between the laser excitation and electron probing allows for a tracing of the dynamics with femtosecond resolution [1]. The performance of a UED instrument is largely determined by the electron source. Current dedicated setups are predominantly employing planar photocathodes [1]. In this contribution, we present a newly developed ultrafast electron diffraction setup based on a high-coherence electron microscope gun using a nanotip photoemitter [2]. Due to the small emission area of the tip, the electron source generates a low-emittance and high-peak-brightness photoelectron beam for ultrafast microbeam diffraction.
The setup is based on a ZrO/W(100) Schottky-type field emitter, as shown in graphic a). We trigger photoemission from the emitter's front facet by illumination with femtosecond laser pulses (515 nm center wavelength, duration about 200 fs) generated at a maximum 610 kHz repetition rate. The electron pulses are accelerated to a kinetic energy of 15 keV into the sample chamber hosting the sample mounted on a 3-axis manipulator. The diffraction pattern is recorded by a phosphor-screen microchannel-plate assembly and a CMOS camera. For quantitative beam characterization, we use a Faraday cup with fA-resolution and a knife-edge at the sample position to measure the beam current and emittance, respectively. By a measurement of the transient electric field effect, we characterize the temporal overlap at the sample plane and the overall current-dependent time resolution of the instrument [3]. To demonstrate high spatio-temporal UED performance, we carry out ultrafast microdiffraction on a free-standing 1T-tantalum disulfide film (1T-TaS_2).
The emitter operates in the linear photoemission regime with approximately one electron per pulse at the typical laser powers applied in the experiment. Using knife-edge scans, we determine a root-mean-squared (rms) beam size at the sample of 2.54(8) µm and an rms divergence of 0.27(1) mrad, leading to an energy-normalized emittance of 167(7) nm mrad. Measurements of the transient electric field effect yield upper limits to the full-width-at-half-maximum (FWHM) electron pulse duration of 1.02(3) ps and 2.10(3) ps for photoemission fluences of 0.05 mJ/cm² and 0.24 mJ/cm², respectively. The difference in pulse duration follows from increased stochastic and space charge broadening for higher pulse charges.
The small electron beam diameter at the sample position allows us to acquire a scanning electron diffraction dataset of a free-standing 1T-TaS_2 film. Using this 4D-Dataset (x, y, kx, ky), we can map the intensities of a selected diffraction spot in real-space as shown for the (01) spot in graphic b). We attribute the local maxima in the real-space image to a wrinkled structure of the flake. 1T-TaS_2 is subject to a charge-density-wave phase (CDW) accompanied with a periodic lattice distortion. At room temperature, the CDW structure is nearly commensurate (NC) to the underlying lattice (locally commensurate with a nearly regular array of discommensurations). However, upon heating above

353 K, the CDW becomes incommensurate (IC) to the underlying lattice with wave vectors aligned to the host lattice. This structural phase transition can be laser-driven, which was extensively studied in the past, allowing us to use it as a benchmark UED experiment for our setup [4,5]. We observe the NC-IC phase transition in 1T-TaS_2 with an intensity decrease of the NC diffraction spots and a rise of IC spot intensities after laser pumping, as plotted in graphic c). We measure the reduction in NC peak intensity with a FWHM time resolution of 1.09(7) ps. Combining the emittance, beam current, and pulse length, we can calculate a peak brightness of $3.0(3)\times10^{11}$ A/m²/sr for our electron gun.
We developed an ultrafast electron microdiffraction setup with enhanced spatial and momentum resolution at an energy of 15 keV. The low-emittance source allows us to conduct high-resolution spatial scans of crystalline samples and carry out time-resolved studies of the dynamics in inhomogeneous structures and for small sample dimensions. Furthermore, the setup is ideally suited for in-situ measurements under ultrahigh vacuum and/or cryogenic conditions.

Keywords:

ultrafast, Schottky-emitter, photoemission, 1T-tantalum-disulfide(1T-TaS_2), laser

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Contribution of residual gas and surface contaminants to contamination growth on irradiated samples

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Poster Group 2

Background incl. aims

Exposing a sample to an electron beam leads to growth of amorphous carbon contamination [1] deposited due to beam induced polymerization of hydrocarbons. This contamination is a challenge in electron microscopy because it can obscure fine details of the specimen, distort the signal obtained in elemental analysis and artificially modify surface reactions during reactive in situ experiments.
The sources of hydrocarbons are the residual gas in the instrument chamber and the sample surface itself, where the latter is often assumed to have the bigger impact on contamination growth [1].
This work is designed to quantify the contribution of these sources of hydrocarbons on the contamination growth using a suitable model [2] and is based on measurements from dedicated experiments. Similarly to other works [3], the contamination process is assumed to be a time-dependent reaction diffusion process driven by the electron beam. A source and a sink term are introduced to describe the supply of hydrocarbons from the residual gas in the microscope chamber and the removal of mobile hydrocarbons through polymerization by the electron beam. The contamination formation predicted by the model is compared with experimental results to determine the differently contributing parameter of the process.
This study analyzes the direct contribution of contaminants from the residual gas to polymerization by the electron beam and the indirect contribution by reestablishing an equilibrium density of surface contaminants. In addition, the contribution of surface contaminants to contamination growth by diffusion into the irradiated area is analyzed. It is intended to find from the specific process parameters not only insights regarding the supply of contaminants, their type and size, but also methods to reduce the contamination growth on illuminated sample surfaces.

Methods

To quantify the contamination growth, a thin amorphous carbon substrate (thickness < 10 nm) is illuminated homogeneously with a defocused electron beam in a FEI DualBeam Strata 400S. This is done for distinct time steps up to 20 min resulting in the growth of contamination rings.
The thickness and shape of the contamination rings is obtained through comparison of MC simulations with high-angle-annular-dark-field (HAADF) images taken in between 5 minutes of illumination. Both, irradiating and imaging are performed at primary electron energy of 20 keV and a current of 120 pA. The illuminated area is chosen to be sufficiently large (radius ~ 700 nm) to separate the individual parameters describing the contamination process.
The theoretical model used describes the change of the relative surface density of contaminants $n = N/N_0$, where $N_0 = N(t=0)$ is the density of contaminants prior to illumination [2].
The diffusion into the irradiated area is described by a diffusion constant D while the contribution of the residual gas in the microscope chamber is modeled by an adsorption frequency η that tends to reestablish the equilibrium surface density of contaminants. The removal of contaminants through polymerization into immobile contamination is described by a reaction cross section σ.

To obtain the dependency of these parameters on the residual gas condition, contamination growth experiments at different pressures are performed while evacuating the microscope chamber and also by using a cold trap. Mass spectrometer measurements are conducted to analyze the components of the residual gas in the microscope chamber, which could contribute to the supply of contaminants. Furthermore, contamination growth measurements are performed after removing contaminants from the sample surface by in situ plasma cleaning.

Results

The parameters of the model are obtained by fitting the simulated contamination shape and thickness to the experimental data.

The reaction cross section σ = 2.22e-20 m² and the diffusion constant D = 1.15e-15 m²/s don't change significantly between measurements with different residual gas pressures of the microscope chamber. The initial density of contaminants on the sample surface slightly increases from N_0 = 1.25e20 1/m² to N_0 = 1.60e20 1/m² with higher pressure indicating the adsorption of hydrocarbons from the ambient gas to the sample surface.

Figure 1 a) shows the concentration of small hydrocarbons in the residual gas of the microscope chamber as a function of the evacuation time. It can be seen that the partial pressure of these components decreases with a similar rate as the total pressure in the vacuum chamber. Figure 1 b) shows the adsorption frequency of the contaminants onto the sample surface with respect to the pressure inside the instrument chamber. Thus, lower pressure leads to a decrease of the adsorption frequency and a lower buildup of carbon contamination on the sample surface. Anyway, the value of η smaller than 0.001 Hz is very low compared with the other frequencies governing this process.

Experiments with in-chamber plasma cleaning of the sample indicate a substantially reduced contamination growth. Measurements before and after cleaning for 100 s show a decrease in the initial density of contaminants from 7e19 1/m² to 2.2e19 1/m² pointing out the effective removal of contaminants from the sample surface. Simultaneously, the diffusion constant D increases by 28%, while the reaction cross section decreases by 83%.

Conclusion

Comparing the adsorption frequency η to the other parameters leads to the conclusion that even though the residual gas has a perceivable impact on the contamination growth, its contribution remains low in comparison to that of the contaminants present on the sample surface prior to illumination. Besides the direct add to the irradiated area, the supply of contaminants from the residual gas to the whole sample surface to an equilibrium density must be considered for longer experimental durations. However, these contributions can be reduced by lowering the chamber pressure.

The alterations in the diffusion constant and reaction cross section after plasma cleaning imply the presence of more mobile and smaller contaminants [4]. This suggests that contaminants adsorbed from residual gas might be smaller than those removed from the sample surface, being probable for the molecules shown in Figure a).

The effectiveness of in situ plasma cleaning to remove several of the present mobile contaminants is shown by a lower carbon contamination.

Keywords:

Contamination,surface diffusion,electron microscopy

Reference:

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Measurement of electrostatic fields in Ge-doped AlGaN structures by off-axis electron holography

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Poster Group 2

Background incl. Aims
AlGaN LEDs have been greatly researched these last years for the development of ultraviolet emitters for many applications including disinfection [1]. For that purpose, we need AlGaN alloys with high Al content and high active dopant concentrations. In this presentation, we will discuss the potential of Ge as an alternative n-type dopant instead of Si for AlGaN structures with different Al contents. Indeed, silicon is a widely used n-type dopant but it contributes to edge-type dislocation climb and induces tensile stress. The interest of Ge is that it introduces less strain than silicon in the AlGaN lattice. Moreover, the distribution and activity of the dopants and the location of the electrical junction are very important parameters in the design and optimisation of LED structures. These properties can be measured by off-axis electron holography, which records both the amplitude and the electron phase from an interference pattern. As the change in phase of the electron beam is directly linked to the electrostatic potential, this allows us to measure the potentials across the structures with a nanometer resolution.
Methods
In this study, we examined 6 samples consisting of 675-nm-thick Ge-doped AlGaN grown by plasma-assisted molecular beam epitaxy (PAMBE) on 1µm-thick AlN-on-sapphire templates [2]. The first and the second samples were grown with an Al mole fraction of 12% and varying Ge concentrations (the temperature of Ge cell was 928°C and 1011°C respectively). The third and the fourth samples were grown with 36% Al and varying Ge concentrations (the temperature of Ge cell was 928°C and 840°C respectively). The fifth and the sixth samples were grown with 64% and 66% of aluminum respectively, with a temperature of 1011°C for the Ge cell. Lamellas for transmission electron microscopy (TEM) studies were prepared by in-situ lift-out focused ion beam (FIB) milling in an FEI Strata 400. Each lamella contained areas with different thicknesses to obtain information about the samples such as the inactive thickness and to find the optimum experimental conditions. Electron holography and electron dispersive x-ray (EDX) spectroscopy were then performed using a double-corrected FEI Titan Ultimate TEM operating at 200 kV equipped with a Gatan One View 4k camera.
Results
Using electron holography, we were able to map the electrostatic potential in the specimens. This requires a good sample preparation and optimized experimental parameters during the TEM experiments [3]. The measurement conditions are particularly demanding in the case of AlGaN, since dynamical diffraction is a problem and wide bandgap materials have a tendency to charge during examination. Additionally, the total potential measured in electron holography depends on strong polarisation fields, the mean inner potential (MIP) of our material and additional contributions from dopants. To get reliable measurements, we first deposit a thin carbon layer on the specimen to provide a conductive channel to evacuate the charge. Then, the MIP in the AlGaN is calculated from DFT simulations, which provides values of 15,9 V for bulk AlN and 16,9 V for bulk GaN [4]. Using a linear interpolation, we can estimate the MIP of Al0,12Ga0,88N (16,8 V), Al0,36Ga0,64N (16,5 V) and Al0,66Ga0,34N (16,2 V), so that the MIP contribution can be removed from the total measured

phase. Figure 1(a) shows a FIB lamella with several steps of different thicknesses, prepared from the sample with 36% Al and TGe=928°C. In order to measure accurately the thicknesses of these steps, we use the Convergent Beam Electron Diffraction (CBED) method [5]. Figure 1(b) shows an experimental CBED pattern compared to 1(c) a simulation for a 374nm thick specimen using the JEMS software. Figure 1(d) shows an amplitude image of this specimen. The amplitude image has homogenous contrast, suggesting reduced diffraction contrast, which means that the corresponding phase image can be correctly interpreted. Figures 1(e) and 1(f) show the corresponding phase image before and after carbon coating respectively. The comparison of the images shows the relevance of carbon deposition to reduce charging of the specimen. From Figure 1(f), we can deduce the phase difference across the AlGaN layer (3.2+/-0.1rad), as shown in the profile in Figure 1(g). Finally, it is possible to determine and compare the electrostatic potentials across the AlGaN layer using $\Delta\Phi 0(x,y)=CE.V(x,y).t(x,y)$ where CE = 7,289 × 10^−3 rad/V/nm for 200kV electrons. Thus, a step in electrostatic potential of 1.17+/-0.02 V has been measured in the AlGaN layer.

Conclusion

By optimising the experimental conditions, we will be able to show the effect of Al content on the activity of the dopants in the AlGaN layer. With this study, we also show that the off-axis electron holography is a useful method to measure the electrostatic potential with a nanometer resolution, and thus can be used to optimize the structures grown for the development of UV LEDs. We will couple holography with EDX to determine the dopant concentrations that provide the highest carrier concentrations.

This research was performed on the Platform for Nanocharacterisation (PFNC) at the CEA-LETI of Grenoble and was funded by the French National Research Agency (Grant ANR-22-CE09-0024).

Keywords:

Electron holography, AlGaN, Ge doping

Reference:

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PolSpec – broadband cost-effective hyperspectral imaging

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IM-05 (1), Lecture Theater 3, august 26, 2024, 10:30 - 12:30

Background incl. aims
Spectrally-resolved imaging is implemented in almost every optical imaging modality – from endoscopy through microscopy, tomography, and remote sensing. It is typically used to provide spectroscopic contrast, e.g., between different species or states of molecules or different “colour” labels, e.g., for pathology, spatial proteomics, etc., as well as diverse applications outside biomedicine. Increasingly, it is used with machine learning, e.g., to increase performance or automated identification/classification tasks. Spectrally resolved imaging can be classified as multispectral imaging, where detected photons are assigned to a relatively small number of discrete spectral bins, or hyperspectral imaging where photons are allocated to part of a continuous a wavelength range. Spectrally resolved imaging techniques based on filters that reject “out-of-band” photons are inefficient and techniques that acquire the full (x-y-λ) hyperspectral image data cube, e.g., using spectrographs, tend to be slow since at least one dimension requires sequentially acquisition. Where photons are “sorted” into multiple spectral bins for wide—field imaging, e.g., using (cascades of) dichroic beam splitters, multispectral imaging can be fast and efficient at the cost of complexity and limited flexibility. Here we present a new low-cost approach to flexible wide-field hyperspectral imaging using polarization optics instead of dielectric coatings or dispersive devices, including a demonstration of single-shot full-field hyperspectral imaging.
Methods
Our approach we describe as “PolSpec” is to use a Lyot filter to provide continuously varying loss modulation across the desired spectral range to generate orthogonal “spectral modulation vectors” (SMV) that can represent specific spectral components, as indicated in figure 1(a). If the modulation functions are contrived to be sinusoidal and cosinusoidal, then these SMV correspond to spectral phasors, but other orthogonal (or near orthogonal) modulation functions can be used. Direct acquisition of spectral phasor images (as opposed to lossy calculation of spectral phasors from hyperspectral image data) has recently been demonstrated [1,2] utilising specific (fixed) sinusoidal spectral filters with sequential image acquisition [1] or using a cascade of image splitters for single-shot acquisition [2]. We have implemented single-shot full-field hyperspectral imaging using SMV with $\cos(\theta)/\sin^2(\theta)$ spectral modulation with the configuration depicted in figure 1(b) for a system component cost of <€2500 using low-cost polymer films to provide the required polarisation and retardance components. The spectral range and discrimination of this approach can be easily tuned using different retarders (typically half-wave plates) or can be made electronically tuneable using a liquid crystal retarder (LCR). Single-shot operation can be achieved using a polarisation-resolving camera (“Polarsens™”) [3].
Results
Figure 1(c) shows a demonstration of single-shot full-field hyperspectral imaging using the system depicted in figure 1 to image a colour test chart displayed on a computer screen. The SMV plot clearly separates the different spectral components and by filtering in the SMV plane, we can generate colour images in object space.
Conclusion

We have demonstrated a flexible approach to full-field “hyperspectral” imaging using SMV and implemented a singe-shot version with low-cost polarisation optics components and a Polarsens™ camera. Most implementations of PolSpec begin with a polariser that will present up to 50% loss, e.g., when imaging fluorescence. In addition, the Polarsens™ camera also presents a 50% loss. For fluorescence imaging, it is desirable to use a cooled camera with which PolSpec can be implemented using a polarisation image splitter. If the retardance required for the SMV is provided using an LCR, the full SMV data set can be acquired in two (rapid) sequential photon efficient polarisation-resolved camera acquisitions. Many other PolSpec configurations are possible, including ~lossless polarisation-resolved hyperspectral imaging with some increased system complexity.

Keywords:

Spectrally resolved imaging, hyperspectral, polarization

Reference:

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[2] Wang et al, Cell Reports Methods 3 (2023) 100441; https://doi.org/10.1016/j.crmeth.2023.100441
[3] https://www.sony-semicon.com/en/technology/industry/polarsens.html

1001

Dynamics of an industrial Cu/ZnO catalyst revealed by operando TEM

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Introduction
Cu/ZnO catalysts have been used since the mid-1960s for both the water-gas shift (WGS) reaction and methanol synthesis. The key to understanding the activation, performance and deactivation of this catalyst system seems to lie in the delicate interplay between the formation of a CuZn alloy phase and the wetting of Cu nanoparticles by ZnOx after partial reduction through strong metal-support interactions (SMSI) [2,3]. The highly dynamic nature of the catalyst requires the use of in situ techniques. Using operando transmission electron microscopy (TEM), we aim to provide unprecedented insight into the morphological and structural evolution of an industrial catalyst during reductive activation and in CO2 hydrogenation conditions. These results are compared to model Cu/ZnO thin film heterostructure and Cu2O nanocube catalysts.

Materials and Methods
The catalyst (Cu/ZnO/Al2O3) was synthesized by calcination of a zincian malachite precursor with a Cu:Zn ratio of 70:30 and 3 mol % Al, following a protocol published previously. A sample was mounted in a DENS Climate environmental TEM holder, which was connected to our homebuilt gas feed setup. Selected area electron diffraction (SAED) temperature series were corrected for astigmatism [5], enabling us to track small lattice parameter changes and phase fractions from Rietveld refinement with the fast time resolution of SAED.

Results
The calcined sample is first activated (pH2 = 79 mbar, 10% H2, 4K.min-1) during a 2-step process involving the segregation of CuO NPs from a decomposing (Cu,Zn) carbonate phase from 110°C to 200°C, then reduction and sintering of these NPs from CuII to Cu0. A CuI intermediate is detected as well between 200°C and 250°C, with all 3 oxidation states co-existing at these temperatures until only Cu0 remains after 2h at 250°C. The final morphology consists of a network of Cu metallic nanoparticles linked by wurtzite ZnO. This was confirmed by in situ STEM-EELS measurements. A ZnOx overlayer, is sporadically observed after the reduction is complete after 2h at 250°C. Cooling the sample to 50°C then reveals a much fuller coverage, indicating that the overlayer is not stable at high temperatures and forms reversibly.

Observations in MeOH synthesis conditions (H2:CO2:He = 3:1:0.5) and reverse water gas shift (rWGS) conditions (H2:CO2:He = 1:1:0.5), shed tremendous insight into how Cu wetting by ZnOx is mediated by temperature as well as H2 and CO2 partial pressures through a delicate balance of Cu-Zn alloy formation and reoxidation on Cu NPs surfaces [5]. rWGS products (CO and H2O) were detected alongside these observations.

Increasing the temperature to 400°C showed a sudden increase of the Cu lattice parameter above the thermal expansion baseline, indicating CuZn alloy formation. Remarkably the lattice parameters then levels off over 30 minutes. This corresponds to a delayed increase of the water content in the reactor, suggesting that water plays a critical role in the reoxidation of Zn in the catalyst, and demonstrating the transient nature of CuZn alloys in this system.

Conclusion
In summary, extensive operando TEM experimental capabilities were leveraged to shed unprecedented insight into the morphological and structural evolution of Cu/ZnO catalysts during CO2 hydrogenation. Coupling mass spectrometry, electron diffraction and imaging yields a comprehensive description of the activation and reaction process. Altogether our results suggest that a frustrated phase transition between CuZn alloy and Cu-ZnO surface states, rather than one of the two being more active, could be key to the high performance of CZA catalysts for methanol synthesis.

Keywords:

Operando; catalysis; SAED; diffraction; EELS

Reference:

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1002

Structural and Electrical Characterization of $Hf_{0.5}Zr_{0.5}O_2$ Thin Films Crystallized by Rapid Thermal Annealing

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Poster Group 2

Background incl. aims
High-k dielectric thin films such as HfO_2, ZrO_2 and $Hf_{0.5}Zr_{0.5}O_2$ for semiconducting memory devices was selected as model systems. Metastable orthorhombic phase of $Hf_{0.5}Zr_{0.5}O_2$ (HZO) has ferroelectricity and has been the potential materials to next generation nanoscale electronic devices based on its superior physical scalability. In order to increase the ferroelectricity of HZO, understanding the basic generation mechanism of ferroelectric crystalline phases is crucial and is investigated structurally and electrically in this study.

Methods
10 nm thick $Hf_{0.5}Zr_{0.5}O_2$ films were deposited using atomic layer deposition (ALD) on 200 nm thick TiN electrodes as the top and bottom was crystallized by post-metal rapid thermal annealing (RTA) in the temperature range from 400 to 900℃. The crystalline phase of HZO was determined with high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) in TEM (JEOL, ARM-200F). The capacitance-voltage (C-V) characteristics were obtained by Agilent E4980A. Electrostatic Force Microscopy (EFM) and piezoresponse force microscopy (PFM) imaging was performed using NX-10 (Parks systems) on the HZO surface without the top electrode.

Results
From GI-XRD analysis results, the HZO samples annealed at 600 and 700 ℃ showed the higher orthorhombic phase and was consistent with the C-V measurement (Figure 1). The orthorhombic phase is confirmed with HR-STEM. The amplitude and phase images of EFM clearly showed the presence of ferroelectricity at the 600 ℃ RTA-treated sample. The C-V measurement showed that the dielectric constant was 8.8e-18 F/μm for a thickness of 10 nm and an area of $3.14e^4$ μm^2.

Conclusion
We investigated the basic generation mechanism of ferroelectric hafnia crystalline phases as a function of RTA temperature. We found that the higher ferroelectricity was shown from the samples annealed at 600 and 700 ℃ and that orthorhombic phases were dominant on the sample annealed at 600 ℃.

Keywords:

$Hf_{0.5}Zr_{0.5}O_2$, Ferroelectricity, HR-STEM, EFM

Reference:

[1] Geun Taek Yu et al., New Physics: Sae Mulli, Vol. 71, No. 11, November 2021, pp. 890~900

[2] This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education （NRF-2022R1F1A1063776）

1003

Investigating the photoelectronic properties of MoS2 thin layer with ITO nanoparticles using photoluminescence and cathodoluminescence

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Poster Group 2

Background
The development of sustainable, renewable energy requires novel solutions that enabling energy storage and conversion, for example for smoothing unstable/variable energy sources.
In order to do so, we can design nanostructures that can trap charge, utilising metal oxide (MO), such as Sn-doped In2O3 nanocrystals (ITO NCs). In ITO, aliovalent substitutional doping can effectively modulate charge carrier density. (1) The bandgap for ITO NCs, crucial for band engineering, is between 3.5 eV and 4.3 eV. (2) (3)
Molybdenum disulphide (MoS2) is a transition metal dichalcogenide (TMDC) semiconductor with an indirect bandgap of 1.3 eV in bulk form, while a direct bandgap of 1.8 eV when in a monolayer. (4)
In this work we use a combination of optical and electron spectroscopy to locally analyse the photodoping phenomenon, a light-driven charge accumulation of electrons, on ITO NCs when coupled with MoS2 monolayer, that act as a sacrificial hole scavenger, deposited on a SiO2 substrate. The innovation of this device lies in the dual role of converting solar energy and storing the resulting charges.

Methods
Photoluminescence (PL) denotes the emission of light from a material after the absorption of photons (electromagnetic radiation). The light emission occurs following photoexcitation, wherein incident photons promote electrons to higher energy states. The Micro-PL was measured using an inVia Renishaw Raman microscope with an excitation laser at 488nm.
Cathodoluminescence (CL) is an optical and electromagnetic phenomenon wherein electrons, typically in a scanning electron microscope, induce the emission of photons, often characterised by wavelengths within the visible spectrum.
CL data were acquired in an Attolight Allalin 4027 Chronos SEM-CL. The spectra were acquired with an iHR320 spectrometer) and an Andor 1024 pixel charge-coupled device. All the measurements were performed at room temperature under high vacuum (<10−7 10 80 100 mbar) with an acceleration voltage of 3 kv. Data were analysed using Lumispy / Hyperspy.

Results
By studying local PL and CL in correlation to the presence of specific MoS2 morphologies, such as monolayer, folded layers and other localised alterations, particularly in the vicinity of the ITO NCs, we aim to understand the local charge dynamics and the interplay between the MoS2 and the NCs.
Our observations reveal a notable reduction in emission intensity in areas surrounding and within the ITO-rich region, which we ascribe to successful charge transfer. The extent of the process is studied in 2D, and results from PL and CL are compared, providing information on different lengthscales and response to different excitation intensities.

Conclusion
Devices incorporating Indium Tin Oxide (ITO) nanocrystals (NCs) and MoS2, where photodoping occurs, present promising solutions for direct solar-to-charge conversion and storage. The parallel use of cathodoluminescence (CL) and photoluminescence (PL) enables a comprehensive evaluation of these properties at sub-micron lateral resolution.

Keywords:

Photodoping, Photoluminescence, Cathodoluminescence, ITO, MoS2

Reference:

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Electron tomography of radiation sensitive nanocomposites for additive manufacturing

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Poster Group 2

Additive manufacturing (AM) techniques impose an advantageous methodology for the fabrication of devices, providing the ability to reproduce complex geometries, unattainable by traditional manufacturing technologies. The continuous growth of these techniques promotes the development of new materials meeting the requirements of the fabrication methods while providing enhanced properties, where the development of nanocomposites is an effective strategy [1]. Understanding characteristics such as the 3D distribution of nanoadditives within the polymeric matrix is crucial for the potential functionality of the nanocomposites. Transmission electron microscopy (TEM) can play an essential role here, among which electron tomography (ET) outstands, as it provides relevant information at the nanometer and atomic scale in 3D to understand and correlate the material structure and its properties [2].
ET consists in acquiring series of projections from the sample in different orientations over a large angular amplitude, so-called tilt series. The tilt range and the tilt increment have a direct impact on the resolution of the final 3D reconstruction. However, when studying specimens that are electron-beam-sensitive, such as polymer nanocomposites, there is a restriction on the number of projections that can be acquired. Therefore, despite the numerous advantages rendered by ET, its implementation to polymeric materials remains largely under-exploited. One of the major reasons is that polymers are prone to damage under the electron beam resulting in the ease degradation during TEM-ET measurements, either by chain scission or cross-linking [3]. Consequently, the resolution limit achievable depends, among other parameters, on the total electron dose to which they can be exposed before being degraded [4]. To this end, the development of low dose TEM approaches and methodologies for imaging vulnerable materials is crucial.
Additionally, specimen preparation to electron-transparency represents a challenge when working with polymeric materials since the performance of TEM analyses requires from clean and thin specimens. The use of focused ion beam (FIB) instrument allows to obtain specimens with tailored geometry, such as needle-shape specimens, of interest for ET due to the possibility of doing full tilt range experiments [5]. However, FIB conditions need to be optimized as well in order to avoid damaging the polymer.
In this work, we demonstrate that low dose ET of polymers is feasible, which is extremely important in the development of AM nanocomposites. More specifically, this work relates to the study of the 3D distribution of metal nanoparticles and 2D metal dichalcogenides (TMDs) throughout acrylic resins used for stereolithography (SLA) using ET (figure 1). For that, FIB nanoneedles of the nanocomposites have been successfully fabricated and the electron-induced damage in the SLA acrylic resin has been studied to design optimal low dose ET experiments.

Keywords:

Low electron dose tomography, polymers

Reference:

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Hyperspectral CT allows for non-destructive elemental imaging in museum specimen

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IM-05 (3), Lecture Theater 3, august 27, 2024, 10:30 - 12:30

Background incl. aims
Fluid preserved animal specimens in the collections of natural history museums around the world constitute an invaluable archive of past and present animal diversity. Many researchers rely on these collections for e.g. anatomical, taxonomical and genetic studies. In recent years, non-invasive imaging techniques such as x-ray computed tomography (CT) and magnetic resonance imaging (MRI) are increasingly being used to extract anatomical information from preserved specimens without harm. Although conventional CT allows for precise measurement of radiodensity (often corresponding to physical density) and MRI can be weighted to highlight contents of interest (e.g. water, fat, bone, etc.), these techniques generally do not allow for high resolution element specific mapping i.e. only showing contrast agents or foreign objects made of a certain element.
In museum specimens, sometimes foreign objects have been introduced perimortem or postmortem e.g. projectile residues from the collection process or objects used for mounting the specimen. These foreign objects often consist of heavy metals and thus severely impact imaging and related measurements such as the estimation of bone mineral content. Also, they may constitute a health issue for collection staff. Therefore, a non-destructive method to identify elemental contents deep within museum specimens is desirable. Here we aimed to use hyperspectral micro-CT imaging to non-destructively determine the elemental content of a prized platypus specimens in the vast collection of fluid preserved mammals at the Natural History Museum of Denmark. This was the Dana platypus from The Carlsberg Foundation's Oceanographical Expedition round the World 1928-30 (a.k.a. the fourth Dana expedition). This specimen contained large centimeter-sized nodules with an unknown content.
Methods
The neurocranium and part of the bill of the Dana platypus was imaged using a UniTOM XL Spectral system (TESCAN GROUP, Brno, Czech Republic) equipped with a hyperspectral detector (channel size of 1 keV) with the following parameters: x-ray tube voltage = 160 kVp, x-ray tube current = 94 µA, x-ray tube power = 20 W, integration time = 83 ms, field-of-view = 76.4 × 76.4 × 27.1 mm3, spatial resolution = 0.108197 mm, acquisition time = ~10 h. K-edge subtraction to highlight the lead (Pb) signal (K-edge at 88.0045 keV) was performed using 1 channels width and 3 channels separation (91 keV – 85 keV).
Results

Different elements have different x-ray attenuation signatures in function of the x-ray photon energy. Hyperspectral CT clearly detected a K-edge at 88 keV in regions of interest placed within the foreign objects of the Dana platypus, which was not observed within bone voxels. This allowed for K-edge subtraction (integrating and subtracting channels on either side of a K-edge to produce element specific images) and the precise mapping of Pb in the neurocranium of the Dana platypus. In addition to confirming the presence of large concentrations of Pb in the foreign objects within the Dana platypus, hyperspectral imaging also showed that the widely distributed small dense particles within the fur of the specimen were not corroded lead residues nor another heavy element material e.g. arsenic or mercury residues from previous preservation methods.

Conclusion

In addition to widely used CT imaging and increasingly used MRI for museum specimen imaging, hyperspectral CT adds another dimension by providing element specific information. In this case demonstrating the content of heavily corroded lead shotgun pellets inside a >100 years old platypus specimen.

Keywords:

X-ray computed tomography, lead, non-invasive

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FIB-SEM/microtoming prepared Cross Section of a Stone Wool Fiber enabling (S)TEM investigation

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Poster Group 1

Stone Wool Fibers (SWFs) are aluminosilicate fibers widely used for building and housing insulation. Rocks are melted in a high-temperature furnace (~1500°C) and spun into elongated and cylindrical amorphous fibers approximately 5 µm in diameter and 2 mm in length [1]. ROCKWOOL is a world leading producer of mineral wool fibers used for building and housing insulation. ROCKWOOL continuously transitions to a greener production. This transition potentially influences the redox chemistry and thus the atomic scale network, which consequently could influence the fire resilient properties of SWFs. Our goal is to create a thin (approx. 40-140 nm) cross section of a single stone wool fiber to be able to obtain sub-nanometer scale structural and compositional information using (scanning) transmission electron microscopy ((S)TEM) based techniques. Only by exploring and understanding the structure of SWFs on all length scales (micro, nano, and atomic), will we fully be able to understand the mechanisms behind the fire protective properties of SWFs.
Current strategies used by ROCKWOOL to prepare cross sectional samples include mechanical polishing of SWFs embedded in an epoxy matrix. However, this technique is not suitable for STEM-based investigations due to the large sample thickness and the high likelihood of surface contamination caused by polishing. Thus, a new approach for thin sectioning optimal for (S)TEM analysis is needed. A major obstacle to obtaining the sought-after cross-section cross section is the non-conductive nature of the SWFs. Here, we present the current state of our exploration using focused ion beam scanning electron microscopy (FIB-SEM) and testing of using ultra-microtoming of fibers cast into an epoxy matrix for the preparation of cross sections of SWFs for further (S)TEM investigations. While bombarding the fiber with ions in the milling process, high precision is obtained by continuously monitoring and guiding the process by scanning the electron beam across the area of interest [2,3]. The non-conductive nature of the fibers will lead to build up of charge on the fiber surface and may cause deflection of the beam that otherwise enables the high-resolution imaging, which ensures a precise milling process. The cross-sectional lamella is prepared using a FEI VERSA 3D FIB-SEM system equipped with a Gallium ion source or a Leica EM UC7 ultra-microtome with a diamond knife. In our approach, the fibers for FIB-SEM are carefully placed in/on carbon paste that is added to a silicon wafer. Hereafter, the fibers are coated with 600 nm gold (Au) to shield the fiber throughout the milling process. Preliminary results have shown that optimal conditions for the milling include a low I-beam current of a maximum of 5 nA to avoid damage to the fiber upon sputtering.
Furthermore, the tilt angle used to properly clean and mill the thin section of a fiber is further under improvement. Currently, tilt angles of 50.5° and 53.5° are used as the FIB column is located at 52°. In general, the aim is to estimate the right trade-off between acquiring optimal imaging and milling without too much charge build up on the specimen [4]. A test of using ultra-microtoming for thin sectioning is investigated simultaneously to acquire the best possible cross section. This technique also ensures that no damage to the structure of the fiber is created as with FIB-SEM might lead to. A thin section of approx. ~40 nm was acquired with microtoming and initially investigated in STEM

mode using a TESCAN Clara scanning electron microscope. Both techniques show promising initial results on our way to creating a thin cross section of fiber. However, both techniques also show some obstacles to overcome in the process. For FIB, damage upon sputtering should preferably be avoided and strategies to minimize charging need to be optimized. For microtoming, a major current obstacle is to optimize the mechanical properties of the epoxy to match those of the fibres to optimize the cutting process. The process of acquiring this cross section for TEM investigations is still in the primary stages, focusing on practicing the lift-out process of the cross section and mapping out the charging effects.

Keywords:

Stone Wool, aluminosilicates, FIB-SEM, TEM

Reference:

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[4] D. C. Joy and C. S. Joy, Micron, 1996, 27(3-4), 247-263

1007

The importance of an open camera system demonstrated with wide-ranging applications of MerlinEM detector

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Poster Group 1

Background inc. aims
The MerlinEM detector has gained widespread acceptance in the transmission electron microscopy (TEM) community due to its versatility and adaptable integration with various systems. The detector provides hybrid pixel electron counting based on Medipix3 technology, bringing in high detective quantum efficiency, rapid readout speed, high dynamic range, and radiation hardness.
Methods
Enabling direct access to data and camera operation is essential in exploring new applications and improving established techniques. The MerlinEM system provides this, together with live 4D scanning transmission electron microscopy (4D-STEM) capabilities (Fig. 1). Additionally, Quantum Detectors recently redesigned the retractable version of the MerlinEM platform, MerlinEM RDP, which now fits more microscopes and will support future generations of hybrid pixel detectors (Fig. 2).
Results
Open data formats and remote operation capabilities were essential in exploring new applications and improving established techniques, such as imaging electromagnetic fields [1] and ptychographic imaging [2]. Rapid data streaming to third-party software has been utilised for live ptychography [2] and integrated centre of mass imaging [3], while collaborations with commercial partners like NanoMegas (scanning precession electron diffraction toolkit), CEOS and Université Paris-Saclay (spectrometer systems) enhanced the capabilities of TEM instrumentation.
Conclusions
The openness of the detectors has fostered community collaboration, exemplified by projects like LiberTEM [4], pyXem [5] and others. These initiatives facilitate sharing data processing routines, accelerating the development of innovative methods for extracting sample information and addressing a wider range of specimens more efficiently.

Keywords:

4D-STEM, electron counting, direct detection

Reference:

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1008

2D and 3D EELS Analyses on the Low-Energy Core-Loss Edges in Beam-sensitive Ryugu Asteroid Samples

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background
The Hayabusa2 space mission from the Japan Aerospace Exploration Agency returned materials from the Ryugu C-type asteroid to Earth in 2020. Due to its lack of atmosphere and low mass, Ryugu is less altered than other space bodies in the Solar System, preserving information about its primordial stages. One of its most interesting features is its phyllosilicate fibrous matrix, which can feature absorbed water and organic matter, as evidenced by remote infrared measurements[1] and laboratory analyses[2].
This matrix contains two families of phyllosilicates with different, albeit similar, chemical compositions and crystallographic structures: saponites (with a higher Si stoichiometry) and serpentines (with the presence of Al), intimately mixed at the nanoscale in a fibrous matrix and surrounded by various other systems such as sulfurs, and organic matter.
Until recently, the high sensitivity of the material to the electron beam[3] made it very difficult to perform electron microscopy analysis at the resolution required to address some intriguing questions, such as the presence of organic matter in the interlayers and the precise morphology of the intermixing of the different phyllosilicate families. Fortunately, the advent of direct electron detection, along with other instrumental and data analysis advances, has begun to expand the scope of analyses that can be performed in such beam-sensitive systems. Exciting recent developments include vibrational EELS[4] and 4D-STEM [5] analyses on Ryugu samples.
Here, we bring together several state-of-the-art techniques to achieve EELS analysis of FIB lamellas at unprecedented spatial resolution and EELS analytical tomography of nanopillars of these beam-sensitive samples. In particular, we utilize a hybrid-pixel direct electron detector for EELS acquisition, which offers almost perfect DQE, high-speed acquisition, and a wide dynamic range. This enables the development of a highly sensitive EELS analysis approach for all elements of interest in the sample. In particular, we acquire EELS low-loss spectrum images (SIs), instead of the usual core-loss and corresponding low-loss SIs. Since most of the elements present in these samples (Fe, Al, Mg, Si and S) showcase a major EELS edge in the 25- 200 eV spectral range, we can perform EELS quantification with a single, fast SI acquisition. This approach offers the potential of a higher signal-to-noise ratio in comparison to the more conventional ionization edges at higher energies, at the expense of a more convoluted spectral analysis.
Methods
FIB lamellas from the Ryugu asteroid were fabricated from Ryugu grains and, subsequently, nanopillars for tomography were created also using FIB. EELS spectrum images were acquired on an FEI TITAN Themis 300 S/TEM, in muti-frame mode (developed in-house), with a dwell time varying between 0.5 and 2 ms and a pixel size ranging from 1 to 2nm. For the tomographic tilt series, EELS spectrum images were acquired at 9 angles ranging from -70 to 70 degrees.

The resulting spectrum images were then analyzed using SVD decomposition of the three- and four-dimensional datasets, blind source separation and curve-fitting EELS quantification.

Results
Figure 1 illustrates the successful EELS elemental mapping of a FIB lamella using curve fitting with enough spatial resolution that allows us to discern the different types of fibers in the phyllosilicate matrix and the characterization of Fe-rich minerals at the nanoscale. This allows to separate the two main phases in the phyllosilicate matrix (saponite and serpentine) by means of EELS analytical tomography.

Conclusion
We present a new method for the EELS analysis of astromineral samples at the nanoscale that, thanks to its dose efficiency, enables unprecedented spatial resolution and EELS analytical tomography on beam-sensitive samples. The method can be extended to other samples presenting EELS ionization edges at low energies. Results show that this technique can provide at least as much information as other microscopy-based techniques like EDX, but with a higher spatial resolution and a fraction of the dose.
Our findings not only provide deeper insights into the structure and chemistry of samples from Ryugu but also show the potential of this methodology for broader application to beam-sensitive astrominerals and geomaterials, such as biominerals.

Keywords:

Ryugu, EELS, Tomography,

Reference:

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Setting up fringe-free imaging for SPA on Talos Arctica and Titan Krios microscopes

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[1]EM Unit, Department of Chemical Research Support, Weizmann institute of science, Rehovot, Israel
IM-11 (1), Lecture Theater 5, august 29, 2024, 10:30 - 12:30

Background:
Single particle cryo-EM requires large image data sets, while instrument time and sample availability are in many cases limiting factors. Increasing the data collection efficiency is therefore highly advantageous. One of the methods for increasing imaging efficiency is by reducing the diameter of the beam, such that more images can be taken from suitable sample areas on the TEM grid. In normal TEM imaging mode, the diameter of the beam is limited by appearance of Fresnel fringes from the C2 aperture. Fringe-free illumination reduces Fresnel fringes at the beam edges by simultaneously imaging the sample and C2 aperture in focus. The beam diameter can therefore be reduced significantly, allowing the collection of a larger number of images per-hole and higher throughput.

Methods and Results:
Installation of fringe-free illumination is commercially available from Thermo Fisher Scientific (TFS) and includes mechanical adjustment of the stage's eucentric height. However, purchasing and implementing the TFS product is not always possible or is simply unnecessary. Instead, the microscope can be aligned for fringe-free imaging by the local facility, and the alignment stored in a separate file. We have implemented this method on our Titan Krios G3 (3-condenser lens system) and Talos Arctica (2-condenser lens system), both resulting in equally high-resolution structures as in the normal alignment mode. On the Talos Arctica, fringe-free alignment using a 20 um condenser aperture results in a parallel illumination beam diameter of about 750 nm. The use of a small condenser aperture and imaging far from the original eucentric focus introduces several challenges in alignment and automated acquisition, however these are solvable.

Conclusions:
Fringe-free illumination increases SPA imaging throughput and can be implemented by experienced microscopists in both 2 and 3-condenser lens EMs. I will present the procedure for aligning both microscope types, as well as a script for quickly switching between the two alignment modes before starting EPU sessions.

Keywords:

single particle cryo-EM, fringe-free illumination

1010

Imaging ultrafast spin dynamics at the nanometer scale

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Poster Group 2

Background incl. aims
Understanding magnetic dynamics is not only important to fundamental physics but also critical to the development of future energy-efficient spintronic devices. Detection of ultrafast magnetic dynamics with high temporal-spatial resolution remains a great challenge. In recent years, the UEM (ultrafast electron microscope) has been rather active in ultrafast magnetism[1-4]. Based on a commercial TEM platform, UEM inherits the capability of high space resolution (nanometer scale) and versatile imaging modes. Moreover, the pulse duration of the photo-emitted electron can reach lower than 1 ps in the premium condition, which is compatible with most of the scenarios for the spin dynamics.
However, it is noteworthy that all these experiments rely on topological magnetic structures. Such limitation originates from the imaging mechanism of the Fresnel mode in the Lorentz TEM, which is the beam-deflecting effect driven by the transverse Lorentz force between the electron beam and local spin state. In short, the Lorentz image contrast in Fresnel mode relies on the in-plane spatial gradient of magnetization other than the magnetization itself. This means the Lorentz UEM could only image the ultrafast perturbations of the topological magnetic structure, and rule out other critical magnetic dynamics, such as ultrafast demagnetization, spin waves etc, which usually do not relate to a static topological structure.

Method and result
Here, we report a novel method of Lorentz ultrafast electron microscope (UEM), which can directly image the spin wave in real space [5]. Unlike traditional methods, our Lorentz contrast does not rely on topological magnetic structure. We introduce a structured-light pulse (transient optical grating (TOG)) onto the ferromagnetic sample, which can induce transient spatial magnetic gradient and then create time-resolved magnetic contrast under Lorentz mode. Then, the propagating spin waves, which are driven by the magnetic-elastic coupling, become ‘visible’ in the UEM.

Conclusion
In summary, we have for the first time directly imaged spin waves in the UEM via structured light, which opens a new door to detect and manipulate ultrafast magnetic dynamics with high temporal-spatial resolution.

Keywords:

UEM, Lorentz mode, spin waves

Reference:

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[5] G. Cao, S. Jiang, J. Åkerman, and J. Weissenrieder, " Femtosecond laser driven precessing magnetic gratings," Nanoscale 13, 3746–3756 (2021)

1011

Sequential tilting 4D-STEM for reliable electric field mapping across junctions

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Background incl. aims
Momentum-resolved scanning transmission electron microscopy (MRSTEM) is an increasingly popular technique to map nanometer range electric fields [1]. Such measurements are of particular importance for device structures, for example pn-junctions [2], in order to understand functional properties of devices at the relevant length scales. To measure electric fields, MRSTEM determines the momentum transfer from an electric field to an electron beam via the deflection of the electron beam. However, relating the momentum transfer to the electric field is significantly complicated under dynamic diffraction conditions. Therefore, strategies are required to reliably determine electric fields from MRSTEM measurements under dynamic diffraction conditions. One such approach is tilting the incident beam relative to the sample in order to probe different diffraction conditions [3]. In this contribution, we explore how tilt patterns can be optimized and how MRSTEM based electric field mapping can be improved.

Methods
To get full control over the beam tilt, we use a custom developed beam tilt procedure. Here, we first calibrate the beam tilt and de-tilt coils of our microscope to allow us setting arbitrary beam tilts in STEM mode. In a second step, we calibrate the beam shift pivot points to obtain negligible beam shift at the sample for different beam tilts. These alignments enable us to repeatedly scan over the same sample region with different beam tilts, generating the data required for detailed MRSTEM analysis. We apply this acquisition technique to high quality lattice-matched AlAs/GaAs multi layers. These samples have the advantage that the mapped momentum transfer originates almost exclusively from a change of the mean inner potential across the materials interface, making assessment of the MRSTEM results easier.

Results
To analyze the effects of beam tilt on MRSTEM measurements, we scan across the AlAs/GaAs interface as shown in Fig. 1 a). We apply the scan patterns shown in Fig. 1 b), both consisting of 61 beam tilts, by sequentially scanning across the interface 61 times and collecting MRSTEM data for each tilt. Fig. 1 c) compares the measured electric fields for the annular and the full circle tilt patterns. The full circle pattern results in a sharp peak at the interface and an electric field close to zero away from the interface, as expected for AlAs/GaAs layers, while the electric field measured with the annular pattern shows stronger deviations from the expected behavior. In addition, we also have access to diffraction patterns of individual beam tilts. Fig. 1 d) shows the electric fields mapped with the beam tilts marked in Fig. 1 b). The strong differences for different beam tilts demonstrate that having access to this information is essential to improve MRSTEM measurements.

Conclusion
Our results show that full control over beam tilts and the capability to create arbitrary tilt patterns has a huge potential to improve the quality and reliability of MRSTEM measurements. Particularly, the sequential acquisition of the data, giving access to individual beam tilts, is very beneficial for post-acquisition data examination and thus being fully consistent with the spirit of 4D-STEM of using as much information encoded in the data as possible.

Fig. 1: a) shows an annular dark field STEM image of the investigated AlAs/GaAs junction. The green line indicates the region scanned for field mapping. b) shows schematics of annular and full circle beam tilt patterns in reciprocal space, both consisting of 61 individual beam tilts with a maximum tilt angle of 7 mrad and a beam semi-convergence angle of 1 mrad. c) shows the electric field profiles obtained for the tilt patterns in b) scanned along the line indicated in a). d) shows electric field profiles of the same scan but for individual beam tilts. The corresponding beam tilts are indicated by colored circles in b).

Keywords:

4D-STEM, momentum-resolved STEM, PED, heterojunctions

Reference:

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1012

A validation methodology for size and shape measurement of nanoplastics by transmission electron microscopy

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Poster Group 2

Background incl. aims
The spread of plastics in the environment has led to the formation of microplastics (MPs) and even smaller nanoplastics (NPs) through processes such as aging, degradation, and fragmentation. While significant research has focused on quantification and understanding the potential harmful effects of larger MPs [1], characterizing NPs proves to be challenging, especially within complex matrices [2]. A comprehensive physicochemical characterization is however imperative, especially for evaluating their potential toxicological impacts. In this study, we establish a foundational framework for such measurements by validating a transmission electron microscopy (TEM)-based approach, in the context of the European project PlasticTrace [3]. This validation encompasses all analysis steps, from sample preparation to image analysis, focusing on reference materials composed of polystyrene nanoplastics. By establishing the full measurement uncertainty balance, we aim to determine the accuracy, precision and reliability of NP size and shape characterization by TEM.
Methods
The method is validated supporting on the Nanosphere size standards 3060A (60 ± 4 nm), 3200A (202 ± 4 nm) and 3500A (510 ± 7 nm), purchased from Thermo Fisher Scientific. They are part of a series of polystyrene micro/nanospheres with certified mean diameters traceable to the Standard Meter through the National Institute of Standards and Technology (NIST). The sample preparation consists of optimally diluting the colloidal sample suspension and bringing it on an Alcian blue pre-treated TEM grid. For each material, 15 different TEM specimens are prepared and imaged on 5 different days (3 per day). For each specimen, 10 images are recorded systematically and randomly over the grid surface. Each series of 10 images is analyzed using the ParticleSizer software in ImageJ. The intermediate precision of the quantitative TEM measurement is evaluated using a top-down approach [4] combining the uncertainty related to repeatability (within day) and uncertainty related to day-to-day variations (between day) obtained by ANOVA analysis. Adding the uncertainty related to calibration of the microscope and to trueness, allows to estimate the total combined and expanded uncertainty for the mean, mode and percentiles of parameters including the minimum and maximum Feret diameter (Fmin & Fmax), the equivalent circle diameter (ECD), the maximum inscribed circle diameter (MICD) and the aspect ratio (AR).
Results
Material stability was pertained throughout the validation study and homogeneous distribution of particles on the grid was achieved for all materials. Material 3060A is polydisperse with particle sizes ranging from 10 nm to 70 nm. Materials 3200A and 3500A are more uniform in size, however, a small fraction of particles (<2%) have a significantly larger or smaller diameter. STEM-EDX was performed to verify that all particles have the same elemental composition and thus belong to the polystyrene sample. The intermediate precision obtained is similar for all size parameters. It ranges from 1.1-6.7%, 0.7-1.5% and 0.3-0.7% for materials 3060A, 3200A and 3500A, respectively. The highest values correspond to the d10 percentile and the lowest values to the d75 or d90 perecentiles due to left skewedness of the size histograms (see graphic). The intermediate precision is highest for material 3060A due to the higher degree of polydispersity. For the AR, the intermediate precision is below 1%

for all materials and measurands. The main source of uncertainty is related to the trueness uncertainty for all materials. To assess the accuracy of our approach, a comparison with the certified size values (see Methods section) is required [5]. Specific information on how the certified diameter was obtained is lacking, however, we assume it corresponds to the mean ECD of the material. The values and expanded uncertainties we obtained for the mean ECD are 51±4 nm, 198±7 nm and 518±13 nm, for the three materials respectively. Based on a comparison with the certified values, we conclude that the two largest materials are accurately measured, however the smallest material presents a significant difference with respect to the reference value. Since the mode of the distribution is closer to the reference value of 60 nm, a difference in analysis algorithm e.g. leading to exclusion of a fraction of the smaller particles, might be at the origin of this discrepancy. We see indeed that for material 3060A, the outcome of the validation study is less robust against variations in image analysis settings. However, to make a final conclusion on the trueness of our approach, more detailed information on the certification of the materials is required.

Conclusion

This study establishes a robust methodology for the validation of TEM-based measurements on reference materials of polystyrene nanoplastics. By evaluating the measurement uncertainties associated with size and shape analysis, considerable precision is achieved, however, ensuring trueness can be challenging and requires detailed information on the reported certified diameters. These findings underscore the importance of ongoing refinement and harmonization in analysis algorithms to enhance the accuracy of nanoplastic characterization.

Keywords:

Nanoplastics, TEM, validation study, metrology

Reference:

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1013

TEM 3D Spectroscopic Imaging for Catalytic Activity Evaluation

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Poster Group 2

Changes in the shape of nano catalyst particles have a significant impact on catalytic activity. Basically, the smaller the particle, the more desirable it is for the surface volume increasing of the small particle to be rough, even down to the atomic level. Through previous of research[1,2], we have tried to increase the reaction volume of nanoparticles and to have a form that remains stable during catalytic reactions. In the beginning, the surface roughness was controlled in spherical particles, but then changed to a shape with multiple faces, induced so-called high-index crystal planes, and induced stress in a core-shell shape etc
However, now, from the point of view of economic feasibility and performance improvement, it is necessary to form holes inside the frame structure through the surface structure having three or more components, to create several frames and place clusters of three or four atoms on top. Maximization of catalytic activity is occurring through the training method.

In the field of analytical science, especially high spatial resolution transmission electron microscopy, these changes in nanoparticles present very challenging issues. Based on counting the shape or number of sides, now it is necessary to identify more than 10 sides, quantify the overall stress of the particle, and detect the components of three or four atoms. It is necessary to obtain three-dimensional information rather than the two-dimensional information of the past, and to measure performance. Due to the advancement of technology, a method has become necessary to connect which structural factors are important to the performance mechanism.
From this point of view, we present an analytical science approach protocol with the results obtained by applying the evaluation technology described in the previous section to particles that have secured excellent catalytic performance by synthesizing atomic-level clusters in a Ir-Ru based hetero-frame nanostructure.
First, we synthesized Ir2P/Ru2P particles and performed transmission electron microscopy crystal structure analysis and surface analysis spectroscopic mapping to reveal the particle formation mechanism for proper performance evaluation. 3D reconstruction of spectroscopic images was actually a very difficult and tedious task, and the results were difficult to draw conclusions about. In the end, machine learning was implemented and better results were obtained, but there are still many things to be solved.
The further work is to establish a method to evaluate the total volume of sites that affected catalytic activity through spectroscopic images through full-scale machine learning.

Keywords:

3D imaging, Catalysis, Machine Learning

Reference:

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This version of the EMC 2024 Abstract Book is without any graphics

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Large-volume cryoEM sample preparation for the investigation of the plant-microbiome interaction

Janine Liedtke[1], PhD. Jana Moravcová[2], PhD. Ing. Pavel Křepelka[2], Marije van Son[3], PhD Jirka Nováček[2], Dr. Vasilis Kokkoris[3], Prof. Dr. Thomas S. Shimizu[4], Prof. Dr. Toby Kiers[3], Prof. Dr. Ariane Briegel[1]
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Poster Group 1

Background incl. aims
In recent years, cryo-electron tomography (cryoET) has proven to be a powerful tool for studying the organization and complexity of cellular ultrastructures in their near-natural state. Nevertheless, its applicability was primarily restricted to single cells and monolayers due to the limited capabilities of volume vitrification techniques. Advances in cryo-electron microscopy (cryoEM) aim to overcome these limitations, which has led to the development of methods that can vitrify larger volumes. Despite these advances, further thinning of samples remained a challenge until focused plasma ion beam (PFIB) microscopy and the lift-out technique were introduced. By combining these improved technologies, we have succeeded in pushing the boundaries of sample preparation for cryoET. In particular, we set out to develop and optimize methods for the preparation of large-volume samples, focusing on samples from fungi and plants to facilitate the study of plant-microbiome interactions at the cellular, and ultrastructural level, and we present our results in this context.

Methods
Samples were collected from an arbuscular mycorrhizal (AMF) fiber network, known for its symbiotic relationship with approximately 80% of plant species. Additionally, samples of plant roots were obtained from both Arabidopsis thaliana seedlings and Ri T-DNA-transformed plant root organs cultivated on a standard MSR medium. Cryogenic preservation by high-pressure freezing (HPF) was performed in 3 mm planchettes and the waffle method on grids. Using hexadecane and phosphate buffer solution as filler to prevent the formation of gas bubbles. Prior to vitrification, all samples were fluorescence stained with Nile Red and Calcofluor to enhance visualization of lipid-rich structures and cell walls, respectively. Following, grids were evaluated and processed in Arctis cryo-PFIB, while planchettes were processed in Helios Hydra V a PFIB dual beam scanning electron microscope. Trenches were prepared, enabling both 3D large-volume imaging and preparation for cryo-lift-out on planchettes. Lamellae were prepared on grids and from lift-out samples and subsequently transferred to Titan Krios for data collection.

Results
This study demonstrates the successful vitrification of AMF, enabling the visualization of the cellular organization in a near-native state. Fluorescence staining with Nile Red and Calcofluor white facilitated the differentiation of cellular structures, including the distinction between filled and empty AMF hypha. In Addition, 3D volume images of both, the AMF network and plant root tissue, were generated, providing insights into their respective structures. In particular, plant organs provided better results compared to other plant tissue used in this study. Furthermore, the used methodology

enabled the imaging of plant tissue in a near-native state without additional fixation steps after HPF, which is a remarkable advance over previous approaches. The combination of methods offers a promising approach for studying plant-microbe interactions on a structural level and provides valuable insights into complex biological systems.

Conclusion
The combination of methods used for large-volume sample preparation in cryoET represents a significant advance in the study of plant-microbiome interactions at the structural level. The inclusion of fluorescent staining in the sample preparation facilitated the identification of areas of interest on the PFIB and enabled the precise alignment for lamella milling of different cellular structures. The acquisition of 3D volume images of both AMF networks and plant organs provides comprehensive insights into their structures and interactions. In particular, our approach demonstrates the enhanced vitrification quality of plant organs over other plant tissues. Furthermore, the ability to image plant tissue in a near-native state without additional fixation steps preserves tissue integrity and ensures accurate visualization of cellular ultrastructure. Overall, our methodology offers a promising opportunity to improve our understanding of plant-microbiome interactions. By elucidating complex dynamics at the cellular level, our work contributes to the broader knowledge of complex biological systems. Further refinement and application of our methodology have the potential to unravel the mechanisms underlying the interactions between plants and microbiomes.

Keywords:

large-volume, plant tissue, host-microbe, mycorrhiza

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The Development of Japanese ceramic ware (pottery and porcelain) Technology as Revealed by EPMA

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Poster Group 1

Introduction

The quality of Japanese ceramics ware has improved dramatically since the importation of advanced ceramics, especially percaline technology from the continent in the 17th century. In particular, Old Imari, (Ko-Imari), made after the 17th century using highly refined continental techniques, was loved by celebrities not only in Japan but around the world [1]. Of course, even before the 17th century, ancient Japanese pottery techniques had developed and many potteries were produced.
This report describes an observation and analysis of the development of medieval ceramics in Japan before and after the high level of ceramic ware technology was introduced.

Methods

The samples for analysis were pieces collected from the Chita kiln site [2] of Paleo-Tokoname, old pottery ware (early Sengoku period (late 800s)), the Komizo kiln site of early Arita porcelain ware (early 1600s), and the Kakiemon kiln site (late 1600s) [3] where ceramic porcelain technology was established. Thin sections were created by gluing a small piece of pottery onto a glass slide, and then grinding it. The thin sections were observed and analysed with an electron probe microanalyser (JEOL JXA-iHP200F).

Results

The backscattered electron (compositional) image (BEI) and the panchromatic cathodoluminescence (CL) image and Si mapping analysis results are shown in Figure 1. The upper part of the BEI shows the glazed part of the ceramic surface. The glazed width of the paleo Tokoname piece (Very initial period) (a) is thin and contains many impurities. Also, the Komizo piece (early period) (b) contains many impurities in the glaze, whereas the Kakiemon piece (established period) (c) contains fewer impurities. The panchromatic cathodoluminescence (CL) images show strong luminescence in the glazed areas of the Tokoname piece and the Komizo piece. On the contrary, no CL luminescence is observed in the Kakiemon piece glaze part. Also, the base of Tokoname piece shows stronger CL luminescence than the Komizo and Kakiemon pieces. Si mapping results show that the impurities in the glaze of the Tokoname piece and Komizo piece include Si-particles (Single arrows in the figure), indicating that many quartz fragments are still present. On the other hand, in the Kakiemon piece, these mineral particles are not found.
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Conclusion

The BEI and CL images and Si maps show that the glaze width of the Tokoname piece is thin and contains many impurities, indicating that the glaze firing technique is immature. Normally, pottery are made from low-glassy materials, while porcelain is made from high-glassy materials. Strong CL luminescence in the base of Tokoname piece indicates a high content of crystalline quartz fragments. The glaze width of the Komizo piece is well present, on the other hand Si and CL strong luminescence is observed in the glaze area, suggesting that early Arita ware glaze still contains unmelted quartz. In

the Kakiemon, glaze width is sufficient and these mineral particles are not found. These results suggest that the Kakiemon ware was produced at higher temperatures with better quality glaze than the Tokoname ware and Komizo ware was due to the development of pottery techniques. In other words, Japanese ceramics technology has improved at a very fast pace in just 100 years since the importation of pottery technology developed from the continent in the early 1600s.

Figure 1. BEI, CL images and Map analysis results. (a) Paleo Tokoname piece. (b) Komizo piece. (c) Kakiemon piece. Si grains with strong CL luminescence are identified in the Tokoname and Kamizo glaze parts (single arrows).

Keywords:

Old Imari, Map Analysis, CL

Reference:

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Relative thickness study of TEM samples

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Poster Group 1

Sample preparation methods have an important role in transmission electron microscopy (TEM). New materials (ceramics, thin films, composites, etc.) that are now present have a specific property and have to be precisely treated and usually post-treated. This poses ever-new challenges, constant adjustments, and improvements in advanced TEM preparation techniques like focused ion beam sample preparation, conventional sample preparation, and other TEM preparation methods.
In this paragraph, we compared two nowadays most frequently used TEM sample preparation techniques; conventional sample preparation and focused ion beam sample preparation. TEM samples were, in the last stage of preparation, final-treated using NanoMill® (model 1040, Fischione Instruments, Inc.) to achieve the best results for further TEM/STEM analysis. The study was created for a discussion about using different approaches to achieve the best result for the TEM sample preparation.
The first TEM sample was prepared using FEI Helios NanoLab NL650 dual-beam Focused Ion Beam (FIB). The sample was finally treated (thinned and cleaned) with NanoMill under specific conditions for FIB-type samples. The second TEM sample was conventionally prepared. The sample was thinned, dimpled down to transparent thickness (Dimple grinder, Gatan Inc.), and ion-milled using PIPS (Precision Ion Polishing System, Gatan Inc.) to achieve perforation. In the last stage, the sample was finally treated with NanoMill under specific conditions for conventional-type sample preparation.
We want to expose how important is to choose the proper technique and appropriate conditions for TEM sample preparation. Relative thickness study distinctly revealed improvement in sample quality that affects the final STEM investigations.

Keywords:

TEM, NanoMill, FIB, conventional TEM

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IN-SITU SYNTHESIS OF FexPy NANOPARTICLES

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Poster Group 2

Background incl. aims
Iron phosphide is an earth abundant material with several applications, such as an electrocatalyst for the hydrogen evolution reaction (HER) and the oxygen evolution reaction (OER). Water-splitting as a way of producing hydrogen gas is essential to explore due to its low carbon footprint, and using earth abundant catalysts such as iron phosphide is preferred due to being inexpensive, active, and electrochemically stable (1). Therefore, it is of interest to explore methods to manufacture iron phosphide and aim to achieve perfect crystallinity in combination with tuning the different crystalline phases, since they exhibit different catalytic activity. With an environmental transmission electron microscope (ETEM) it is possible to reveal the mechanism of the gas synthesis at an atomic scale in-situ.
Methods
The iron nanoparticles used are created by using spark ablation in a spark discharge generator and deposited on MEMS chips allowing for heating. The ETEM, Hitachi HF-3300S, interfaced with a metal organic chemical vapor deposition (MOCVD) system is the instrument used and the gases induced are PH_3 and H_2. Energy dispersive X-ray (EDX) spectroscopy is used for elemental information and enabled by the SDD X-MaxN 80T detector. Post-experiment data analysis is performed by utilizing FFT spectra obtained from the high-resolution TEM (HRTEM) images and videos from the Gatan OneView IS camera.
Results
By tuning gas flows and temperature synthesis of different iron phosphide phases were observed directly, currently the phases found are Fe_2P and FeP. The transformation into iron phosphide is nearly instantaneous when PH_3 enters the system, however by using dilution with H_2 it is possible to somewhat slow down the process. It also appears that using pure versus oxidized iron nanoparticles still seems to produce iron phosphide. Moreover, there have been observations of a transformation from FeP to Fe_2P by increasing temperature. Fe_2P has been proven to be very stable in various gaseous and temperature conditions, as well as in air.
Conclusions
Different phases of iron phosphide have been synthesized in the ETEM and dynamics of phase transformations have been investigated. The transition between phases has been observed, as well, indicating that it might be possible to realize phase engineering on iron phosphides even after phosphorus has entered the particles. Our future work will aim towards studying the transformation to iron phosphide further, exploring and characterizing the possible phases and achieving selection of crystal structure in a more controlled manner. By finding a reliable way of creating phases it can pave the way for comparing different phases and for example their catalytic activities in a fair way.

Keywords:

ETEM, Iron Phosphide, nanoparticles, in-situ

Reference:

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Metal-Organic Chemical Vapor Deposition in a Transmission Electron Microscope

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Poster Group 1

Background incl. aims
Using environmental transmission electron microscopy, researchers can observe - with high resolution and in real time - transformations and reactions under realistic conditions. Such studies benefit from precise control, monitoring, and variability of ambient gas, potentially including multiple independently controlled species to which a sample is exposed at the same time. This represents a challenge for ETEM analysis and has required the design of systems that integrate the capabilities of advanced crystal growth instrumentation with the specific requirements of TEM to allow elevated sample pressure and temperature, as well as a better understanding how the environment influences the analysis and vice versa. To address these challenges, we developed an ETEM system based on a Hitachi HF3300S instrument, which is integrated with a metal-organic chemical vapor deposition (MOCVD) system designed for real-time investigation of crystal growth processes of semiconductor nanostructures[1].
Here, we demonstrate how the design of the ETEM-MOCVD instrumentation enables independent control of the gas composition and flow that reaches the heated sample region, as well as rapid adjustments of the gas supply. We focus on the implemented solutions used for controlling gas concentration and pressures in the system and at the sample region. Solutions for calibration and monitoring partial pressures as well as total pressure in the microscope and at the sample are presented and evaluated, together with pressure correction factors of the pressure gauges for nitrogen (N_2), arsine (AsH_3), and phosphine (PH_3). In addition, we explore the influence that the temperature and gas supply to the microscope itself have on imaging and analysis, and conversely, how the imaging via electron beam influences the experiment. Effects of temperature and gas environment on spatial resolution are quantified, and we also demonstrate and quantify how X-ray energy-dispersive spectroscopy (EDS) analysis is influenced by the local reactive sample environment.

Methods
The ETEM is based on an image corrected Hitachi HF-3300S 300 kV TEM, with additional ion pump and differential pressure aperture. The gas handling system is custom built but based on industrial standard mass flow controllers, pressure controllers and vapor concentration measurement units (HORIBA). Nine different gases are controlled via the gas handling system, primarily for III-V semiconductor growth: trimethylgallium (TMGa), trimethylindium (TMIn), trimethylaluminum (TMAl), trimethylantimony (TMSb), AsH_3, PH_3, nitrogen, hydrogen, and oxygen. Other gases are possible by either replacing any of the existing sources, or by using external gas delivery and leak valves. Heating is done using MEMS heating chips.

Results
The design of the gas handling system and particularly the ability to dilute the precursor species with additional high flow of H_2 allows for a very wide range of precursor flows and partial pressures to be

achievable, spanning over four orders of magnitude (approximately 4 × 10-6 to 0.1 Pa of TMGa, or 7 × 10−5 to 2 Pa of AsH_3). These partial pressures are comparable to conventional MOCVD growth of binary semiconducting nanostructures.
Since the microscope is of an open design with local gas injection close to the heated area of the holder, a local higher pressure at the holder compared to the column pressure gauge location is expected. Using a MEMS heating chip as a pulsed Pirani gauge, we correlate pressure at the holder to the microscope column pressure. Depending on gas delivery method (via gas injection holder or side port injector) and holder tilt, pressure at the MEMS chip was found to be 1.6 to 2.8 times higher than column pressure gauge. If using additional lid on gas injection holder, pressure at the MEMS chip was found to be 200 times higher than the column pressure.
Achievable spatial resolution during gas exposure on a heated MEMS chip was estimated using Young's fringes. Measured resolution is slightly worse than the 0.86 Å achievable under optimized conditions[2], mainly due to stability of the holder and MEMS chip. Elevated temperature and gas exposure had a very minor effect on resolution.
Increasing temperature on the SiNx based MEMS chips, the thermal radiation of the chip increases. Such thermal radiation affects the EDS measurement mainly by increasing the strobe peak intensity, but at high enough chip temperature also energy shift of strobe peak and elemental peaks.

Conclusion
We present a system that merges an environmental TEM with an MOCVD setup for real-time study of crystal growth. The open holder design allows for a wide sample tilt range, quick gas switching, and various analysis techniques. The gas handling system replicates standard MOCVD conditions and is flexible for different gas pressures, flows, and precursor types. We found no significant effect of heating or gas pressure on spatial resolution, and minimal interaction of the electron beam with the gas.

Keywords:

ETEM, in-situ, MOCVD, semiconductor

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Quantitative Elemental Mapping Of Bimetallic Nanoparticles From Atomic Scale STEM-HAADF Images

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Poster Group 2

Background incl. aims

Alloying of metal at the nanoscale is often used to enhance nanoparticles' physical/chemical properties and engineer new ones leading to multimetallic nanoparticles (NPs) with different chemical structures [1]. Access to composition is paramount for a fundamental understanding of nanoalloys' chemical and physical properties. In transmission electron microscopy (TEM), particle composition can be accessed by spectroscopic techniques such as energy-dispersive X-ray spectroscopy (EDX). However, EDX requires a set-up with a high-brightness electron source and wide-angle detection to collect as much signal as possible. Even then, if we work at atomic resolution, the quantification of the composition of atomic columns is possible but limited as there is a lot of noise as compared to signal [2]. A promising alternative for quantitative composition determination is atomic-resolution high-angle annular dark field scanning TEM (HAADF-STEM) with strong compositional sensitivity [3]. In this contribution, we propose an innovative method to quantify the composition of individual atomic columns in bimetallic NPs from their intensities in 2D HAADF-STEM images using deep learning.

Methods

In this work, we studied CuAu NPs between 5 and 10 nm synthesized via pulsed laser deposition (PLD). The HAADF STEM images were acquired on a double-corrected cold FEG JEOL ARM 200F. Elemental composition from individual columns was retrieved from the corresponding HAADF signal with regression-based deep learning, U-Net [4]. The network was trained on simulated images, obtained with a multislice algorithm, and their corresponding elemental maps. The predicted elemental maps were compared to the ground truth STEM EDX profiles to assess the accuracy of our methods.

Results

Multislice simulations show that the aberration-corrected HAADF-STEM intensity of atomic columns is influenced by their composition, atomic configuration, and thickness. As these parameters are intertwined, a simple regression-based statistical analysis of the local variations of intensity is not enough to access the full underlying information. Therefore, we developed a deep learning approach with U-Net to be able to disentangle the complex relation of these parameters and predict the composition of atomic columns of the whole particle [Figure 1]. We show that for robust prediction the training dataset is of paramount importance and it needs to span across all possible configurations from the size, and shape, up to the composition and ordering of the atoms. Here, we

will present the implementation of this method for the study of chemically disordered and ordered CuAu NPs.

Conclusion

We have developed a deep-learning approach to predict the composition of bimetallic NPs at the atomic columns scale from a single HAADF-STEM image. Our method is adapted to high throughput and on-the-fly prediction which is suitable for in situ experiments. One other advantage of this method in comparison to EDX is the quantification of the composition without contaminating and damaging the NPs.

Keywords:

HAADF-STEM, Deep Learning, Bimetallic Nanoparticles

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Leveraging FIB-SEM with Integrated ToF-SIMS for Comprehensive Characterization of Lithium-Ion Battery Materials

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Poster Group 1

In recent years, there has been significant emphasis on enhancing lithium-ion batteries, crucial for electric vehicles, stationary storage technologies, and portable electronics. Their lifespan, particularly concerning capacity fade, is primarily influenced by electrode degradation and the deactivation of active materials. Another critical factor affecting battery lifespan and performance is the solid electrolyte interphase (SEI). Choosing the right analytical technique to study lithium-ion battery degradation and SEI properties is challenging due to the need for detailed structural and chemical composition data, including the distribution of light elements like lithium.
In this study, we utilized a unique combination of a Scanning Electron Microscope equipped with a Focused Ion Beam (FIB-SEM) and a compact Time-of-Flight Secondary Ion Mass Spectrometer (ToF-SIMS) [1,2] to explore the topography and chemical composition of both non-cycled and cycled lithium-ion battery electrodes. Our goal was to pinpoint degradation mechanisms, including parasitic chemical reactions [3]. By integrating SEM observations with ToF-SIMS and other analytical techniques such as Energy Dispersive X-ray Spectroscopy (EDS) [4] and Raman spectroscopy [5] on the same FIB-SEM system, we achieved comprehensive 2D and/or 3D characterization of lithium-ion battery materials. This approach offers insights into degradation processes, SEI properties, and electrode composition, enhancing our understanding of battery behavior at its core.

Keywords:

lithium-ion battery, FIB-SEM, ToF-SIMS, tomography

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Morphological characterization of the electric field aligned block copolymers containing liquid crystal moiety

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Poster Group 2

Background incl. aims
Block copolymers (BCP) arose to be an interesting class of materials for the energy storage application. The conventional system consist of at least two covalently bound homopolymer blocks which are usually thermodynamically incompatible. It results in the phase separation at nanoscale, providing a wide array of self-assembled morphologies. Nanostructured periodically spaced discrete regions of different phases are characterized by respective properties similar to the pure homopolymers.
Solid polymer electrolytes (SPE) are composed of salt dissolved in a polymeric host. Here, BCPs are an attractive choice as a matrix due to the aforementioned qualities. Phase separation enables the possibility of merging intuitively contradictive functionalities in one material: solid state electrolyte with competitive to liquid alternative ionic conductivity, and mechanical stability. It is achieved by connecting the soft block which has ability to solvate the salt and favors ion transport, with rigid block, ensuring robustness. The length scale and type of the obtained microstructure can be swiftly tuned by careful molecular design of the polymer, including molecular weight, chain architecture, relative volume fractions, number and type of the constituent blocks. However, the phase separation itself is not the only requirement for BCPs sufficient performance as battery component. Due to the membrane thickness, the final structure is mostly isotropic and consists of multiple grains with different spatial orientation. This forces to consider not only intragrain ion transport, but also intergrain to fully understand the material performance as SPE. Liquid crystalline block copolymers (LC BCP) could be the pathway to manipulate the nanochannels orientation and ensure grain continuity. The addition of the mesogenic groups to the system allows to influence diamagnetic and dielectric constant properties of the materials, giving the promise of the highly ordered structures upon subjecting the material to external electric or magnetic fields. The main objectives of this study were to investigate the native hierarchical microstructure of LC BCP and to identify the self-assembled morphologies during solvent casting without and with the applied electric field. The analyzed material is composed of soft, ion conducting block and high glass transition temperature block with LC moiety. Both pristine and LiFTSI salt doped systems were structurally analyzed, as it was expected that addition of the ion to the matrix may change the system response to the external stimuli. As the next step, we examined alignment, grain size along with orientation and phase behavior of diblock copolymers while subjected to the applied (either alternating current (AC) or direct current (DC)) electric field during film casting. We also related both aligned and non-aligned electrolytes performance in terms of ionic conductivity to derive how the material morphology translates to its application as SPE.
Methods
Morphological characterization of the BCP was performed by means of SAXS (Small angle X-ray Scattering) and cryoTEM (cryogenic Transmission Electron Microscopy). SAXS provided statistical information of the BCP microstructure and alignment, whereas TEM provided more insight regarding

the grain size and magnitude of the long-range order. Ionic conductivity was investigated by impedance spectroscopy.
Results
Phase separated BCP of conventional morphologies were obtained, where the hierarchical self assembly at two length scales was recognized (LAM-in-HEX for salt doped and LAM-in-LAM for pristine). The addition of salt to the BCP caused phase transition from lamellar morphology to hexagonal, which can easily be explained by the swelling of the conducitive phase due to solvating of the salt and hence actively increasing its volume fraction. Salt/BCP composites casted in presence of electric field resulted in increase of the ionic conductivity of the system by magnitude. Samples casted in AC field were characterized by perpendicular alignment of BCP in regards to the applied electric field and small grain size, whereas samples casted in DC field showed similar tendency in direction of reorienting the grains. However, the domain size was significantly increased.
Conclusion
The spatial grain orientation and size of BCP containing liquid crystal moiety was manipulated both via in DC and AC field, resulting in enhancement of the longer-range order and grain continuity. The increase of the ionic conductivity can be directly correlated to beneficial influence of electric field on the nano-channels alignment, which was proven by means of SAXS and TEM. This work highlights the importance of designing the nanostructured system which is susceptible to electric field directed self-assembly for BCP to find wide application in the energy storage field.

Keywords:

Solid polymer electrolytes, liquid crystals

Reference:

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TEM investigation of AlN-Cu(O) with variable amounts of copper

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Poster Group 2

Some nitrides can have a good potential for photocatalytic applications, notably for antibacterial applications. AlN combined with copper could be one of those. The action of photocatalytic materials being conditioned by the value and electrochemical positions of electronic bands, it is necessary to start by specifying precisely the nature of composing phases of the nitrides after their synthesis.

Although recent theoretical calculations [1] (using ab initio DFT –Density Funtion Theory- and thermodynamics) can predict the stable and metastable routes of bi-metal nitrides on high ranges of composition, nothing can replace the microscopic observation of materials experimentally synthesized by an out of balance technique such as reactive magnetron sputtering.

AlN-Cu(O) material was prepared under the shape of thin films by D.C. -Direct Current- reactive magnetron sputtering under synthesis conditions chosen for facilitating film nanostructuration. The progressive addition of a third element to the binary nitride AlN could either lead to solid solution AlCuN or to a material composed of AlN and Cu rich phases. Whereas, XRD –X-ray Diffraction- was systematically performed for preliminary checking, TEM-Transmission Electron Microscopy-investigation is an essential and ideal step to discriminate the actual microstructure of the films and to position the experimental synthesis route towards the limit of solubility of copper in the nitride. Electron diffraction patterns exploited through collection of rotational signals allowed the use of EVA software classically used by X-ray Diffraction experimentators to confront diffraction data to ICDD - International Center for diffraction Data- database. This work lead to the identification of phases. Chemical localization of copper could be detected by chemical mapping (STEM-EDS: 4DSTEM-ScanningTEM-), and crystallized zones could be tracked by diffraction and subsequent filtering (dynamical dark fields or numerical ones thanks to FFT –Fast Fourier Transform- of HR-High Resolution-TEM images).
The modification of the microstructure (change of nature of phases, their chemistry, crystalline structure, changes of morphology…) will be given as a function of the amount of copper used to elaborate the samples.

Keywords:

Nanostructure, morphology, AlN, copper, cermet

Reference:

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FRET and TIRF microscopy for single molecule characterisation of synergistic antimicrobial peptides in artificial bilayers

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Poster Group 1

Background incl. aims
Droplet Interface Bilayers (DIBs) function as artificial membrane models for electrical and optical measurements [1]. This project uses total internal reflection fluorescence (TIRF) microscopy and Förster Resonance Energy Transfer (FRET) in DIBs to investigate the dynamics of peptides and proteins interacting with membranes. Membrane pores have vital roles in neuronal and cardiac signalling, as well as in the mechanism of certain antibiotics.
The lytic antimicrobial peptides Magainin 2 (Mag2) and PGLa, part of the immune system of the African clawed frog (Xenopus laevis), have a synergistic antimicrobial effect when applied to Gram-negative bacteria in a 1:1 ratio. The aim of this project is to uncover the mechanism behind this synergy at the membrane level.
Methods
When an agarose surface and a droplet are submerged in a lipid/oil environment, a monolayer spontaneously self-assembles at the aqueous-oil interface. DIBs form when the lipid monolayers make contact. The protein or peptide of interest, contained within the droplet, can then form pores in this bilayer. Ions flux through these pores, and this pico-ampere current can be measured by electrophysiology. Simultaneously, TIRF microscopy is used to observe ion flux by illuminating the fluorescent calcium binding dye, Fluo-8, with totally internally reflected laser light.
Alternatively, the single-molecule dynamics of fluorescently tagged peptides on the membrane surface can be investigated. By tagging PGLa with a donor fluorophore and Mag2 with an acceptor fluorophore, single molecule FRET with TIRF microscopy can act as a so called "molecular ruler"[3]. When the donor fluorophore is being excited and is less than 10nm from the acceptor fluorophore, a non-radiative energy transfer can occur from the donor to the acceptor, causing the acceptor to fluoresce. This offers distance-dependent insights into the interaction and dynamics of Mag2-PGLa. The formation of heterodimers and higher order structures is of particular interest in these experiments.
Results
Spatiotemporal data from electrophysiology and TIRF experiments has revealed that the Mag2-PGLa pore is a highly dynamic pore that visits multiple conductance states. Single molecule FRET data of Mag2 and PGLa has been obtained for the first time, confirming the existence of Mag2-PGLa heterodimers. Data suggests that very few Mag2-PGLa heterodimers exist at any time. These experiments have allowed better understanding of the interaction of the peptides with one another and with the membrane, thus shining a light on the mechanism behind the Mag2-PGLa synergy.
Conclusion
DIBs, TIRF microscopy, and single-molecule FRET have been employed to investigate the dynamics of Mag2 and PGLa interacting with one another and the membrane. Experimental results reveal the highly dynamic nature of the Mag2-PGLa pores and confirm the existence of Mag2-PGLa heterodimers. These findings provide valuable insight into the molecular mechanism driving the Mag2-PGLa synergy. Future work will focus on achieving pseudo-simultaneous pore-imaging, electrophysiology, and FRET of Mag2 and PGLa.

Keywords:

TIRF FRET AMPs DIBs

Reference:

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Structural and Compositional Investigation of Ag-Incorporated CsPbBr3 Nanocrystal Heterostructures

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Poster Group 2

Background

The fabrication of halide perovskite-based heterostructures represents a promising avenue in the field of optoelectronics due to their unique properties arising from the heterointerface between a perovskite domain and a domain of another material [1]. When CsPbBr3 is interfaced with a compound with a different band gap, the heterointerface acts as a barrier for charge carriers, affecting their transport properties and recombination dynamics, which consequently leads to significant changes in electronic structure [1, 2]. Such tunable optical and electronic properties, together with increased structural rigidity introduced into the perovskite lattice, allow for the development of novel optoelectronics with improved performance and functionality compared to the standalone CsPbBr3-based devices. Nano-scale understanding of the structural and compositional characteristics of such heterostructures within the two domains as well as across the interface plays a pivotal role in the design and engineering of advanced optoelectronic devices. Herein, we conduct a study to characterize the structural features in CsPbBr3/AgBr heterostructures obtained via a colloidal synthesis method [3] by using advanced electron microscopy techniques at the nanoscale.
By combining HRSTEM, hyperspectral imaging, and harnessing advanced data treatment approaches, we sought to explore the structure, morphology, and subtle compositional changes at the heterointerface as well as the crystal domains of such beam-sensitive materials for optimization of optoelectronic performance.

Methods

The heterostructures were prepared for TEM analysis by dispersing the powders in hexane and then drop-casting them onto Cu grids with a carbon support film. Preliminary investigations of the nanocrystals were carried out using an image-Cs-corrected JEOL JEM-2200FS with a Schottky emitter gun operated at 200 kV, equipped with a Bruker XFlash5060 silicon-drift detector (SDD) for energy-dispersive X-ray spectroscopy.
To visualise the crystal lattice of the heterostructure domains, scanning transmission electron microscopy (STEM) images were acquired using Annular Bright Field (ABF) and High-Angle Annular Dark Field (HAADF) detectors on a probe-aberration-corrected ThermoFisher Spectra 300 S/TEM microscope with an X-FEG source, operated at 300 kV. For precise structural imaging, a drift correction frame integration technique in Velox was used to minimize image distortions. Elemental distribution maps and line scan profiles at high spatial resolution were recorded by collecting Energy-Dispersive X-ray (EDX) signals on a Dual-X system, which comprises two EDX detectors positioned on

either side of the specimen, with a total collection solid angle of ca. 1.76 sr, allowing for efficient signal acquisition from nanocrystals.
Furthermore, we combined a machine learning-based segmentation algorithm with hyperspectral EDX datasets to obtain a comprehensive statistical understanding of the chemical composition of the nanocrystals.

Results

STEM-HAADF images showed different possible morphologies for the nanocrystal heterostructures. We identified well-defined heterostructures with a sharp heterointerface in lateral projection, composed of 2 structural domains. EDX elemental maps revealed that Ag was localized in the higher contrast domains correlating with a uniform distribution of Br, which was consistent with the retention of AgBr composition in such domains. The lower contrast domains contained predominantly signals from Cs, Pb, and Br, corresponding to the CsPbBr3 according to the quantification of the compositional EDX spectra. In these heterostructures, we also noted the segregation of metallic Ag within the structural features, which we ascribed to local electron beam damage.
In addition to intact cubes of CsPbBr3 nanocrystals, CsPbBr3/metallic Ag as well as non-stoichiometric silver bromide nanostructures, compositional maps additionally showed the formation of potential heterostructures with a spherical morphology in which Ag was mainly localized at the nanocrystal edges, producing a core-shell structure. We also characterized nanocrystals preserving the superimposition of AgBr and CsPbBr3 stoichiometries without a clearly defined interface. However, differentiation of such potential heterostructures from other nanocrystals in this nanosystem is difficult due to similarity in elemental composition, resulting in insufficient contrast in HAADF images. While the analysis of individual particles by hand is often inconclusive, we apply machine learning-based routines to segment nanoparticles in hyperspectral images on large fields of view, followed by quantification of composition for each, enabling us to obtain operator-agnostic statistical insight into the composition of nanocrystals in this complex system.
Furthermore, by combining ABF and HAADF imaging, we were able to visualise atomic columns of light and heavy elements in CsPbBr3/AgBr lateral heterostructures simultaneously, providing crucial details about the crystal lattice structure and the interface.

Conclusion

Through the combined use of electron microscopy techniques, including imaging and spectroscopy, we achieved a comprehensive understanding of the structural and compositional characteristics of heterostructures formed via the incorporation of Ag-precursor during colloidal synthesis of lead halide perovskites – in particular CsPbBr3 – at nanoscale resolution. This spatially-resolved structural information, together with high-throughput analysis of compositional hyperspectral EDX datasets using machine learning-assisted methods, contributes to the efficient interpretation of unique optical properties such as tunable band gap, new charge carrier transmission modes, and high photoluminescence quantum efficiency in such materials for application in high-performing optoelectronic perovskite devices.

Keywords:

Hyperspectral Mapping, Perovskites, Heterostructure, Heterointerface

Reference:

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1025

A novel pipeline to elucidate the adaptation response to the G2/M DNA damage checkpoint

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Poster Group 1

Background
The DNA damage checkpoint (DDC) is a surveillance mechanism evolved to preserve genome integrity in response to DNA lesions. Checkpoints halt cell cycle progression to provide cells with the opportunity to repair the damage before dividing. When the DNA damage is successfully repaired, the checkpoint is satisfied and cells can resume the division in a process called checkpoint recovery. Conversely, when the DNA damage is irreparable, cells eventually die. Possibly, a few cells reenter the cell cycle with damaged DNA. This process, known as adaptation to the DDC, poses a threat to genomic stability, as daughter cells can accumulate genomic aberrations. Indeed, evidence exists that adaptation contributes to genomic instability and may drive the selection of therapy-resistant cells in oncology patients. Despite the dangerous nature and the clinical relevance of the process, adaptation is largely overlooked and has not been investigated in depth, likely because of the difficulty in experimentally tackling the question. Most commonly, adaptation is explored either by assessing the phosphorylation status of DNA damage checkpoint proteins, detectable solely by Western blot analysis on bulk population. Since adaptation is an asynchronous process (cells undergo adaptation with their own kinetics) and partial (only a fraction of the population adapts), we set to investigate adaptation in single cells by live-cell imaging measuring cell cycle parameters, with the ultimate goal of enhancing the reproducibility and reliability of adaptation studies.

Methods
We investigated adaptation to the G2/M DNA damage checkpoint in Saccharomyces cerevisiae, a highly amenable microorganism, whose genome is easily manipulated. Since in yeast checkpoint activation blocks cells in metaphase by preventing sister chromatid separation and segregation, via inhibition of cohesin cleavage and spindle elongation, respectively, these two processes can be considered the cell cycle events defining adaptation. To follow cohesin and spindle elongation, the Scc1 subunit of the cohesin complex was tagged with GFP (SCC1-yEGFP) while alpha-tubulin was tagged with the mCherry protein (mCherry-TUB1). In our experimental setup, cells are synchronized in G1 and released in presence of unrepairable DNA damage in a microfluidic chamber for live imaging where cells are immobilized and continuously supplemented with fresh media. Images are taken every 7.5 minutes for 20 hours. To analyze the resulting movies, we developed a Python script to automatically segment and measure the mitotic spindle for each cell at each timeframe, as well as to measure the SCC1 signal intensity. Anaphase onset (i.e., adaptation) is defined as the first timepoint where either reduction of the SCC1 signal or spindle elongation was observed. We applied our script to all imaged cells, gaining information about the timing of adaptation for each cell, and the overall percentage of adapting cells in the population.

Results
As a proof of concept, we analyzed wild-type (WT) cells (i.e. adaptation-proficient), as well as cells carrying mutations known to result in an adaptation-defective phenotype, including the well-characterized cdc5-ad mutant. In WT cells, we were able to discriminate adapting cells from arrested

ones in the heterogeneous population, while in cdc5-ad mutant cells, we detected only metaphase arrested cells, as expected for an adaptation defective mutant. Interestingly, certain mutants previously classified as adaptation-defective in the literature were discovered to be capable of adaptation but unable to progress through subsequent stages of the cell cycle. These data, alongside highlighting the reliability of our approach in discriminating checkpoint-arrested cells from those that escaped the checkpoint surveillance in the whole population, strongly support the idea that assessing cell cycle progression is key to understanding the molecular bases of adaptation.

Conclusion
Given the limits associated with both the experimental procedures in use and more generally with population-based experiments, we propose to investigate adaptation by live-cell imaging monitoring cell cycle parameters. Taken together, by coupling the power of live-cell imaging, which allows to investigate dynamic processes at a single-cell level, with the power of cell cycle parameters, which provide a clear picture of cellular behaviors, could provide direct insights into the molecular processes underlying adaptation and survival in cells that divide with damaged DNA, thus contributing to making the study of adaptation reproducible and reliable.

Keywords:

live imaging, DNA damage

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Differentiation of phases in phase change memory materials using 4D STEM

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Poster Group 2

Background incl. aims
With the rapid development of modern information technology, the novel approaches for storing and processing large volumes of data are being developed, from general concepts such as memory in computing, multi-level storage and neuromorphic computing to specific techniques such as new designs of circuits and storage devices. Different electronic devices are under development, such as magnetoresistive random access memory, resistive random access memory, phase change memory (PCM) and ferroelectric memory. PCMs that are based on chalcogenide materials are promising choices for data storage because phase transitions between amorphous and crystalline phases are quick and efficient. Such phase transitions result in changes between high and low resistance states, which correspond to “0” and “1” logic levels in data storage, respectively. They are also promising for realizing multi-level storage. However, the resistance of the amorphous phase in a PCM cell can drift over time. It is important to determine the underlying mechanism of resistance drift and to develop methods that can be used to differentiate between local crystalline and amorphous phases efficiently. 4-dimensional scanning transmission electron microscopy (4D STEM) enables structural identification at nm resolution. In this project, new analysis methods based on 4D STEM are applied to phase identification in phase change materials.
Methods
With the help of a fast pixelated detector (EMPAD), full 4D STEM datasets of diffraction patterns were collected as the electron beam was scanned across areas of interest. Approaches based on fluctuation electron microscopy (FEM) and radial Fourier analysis (RFA) were applied to analyse the datasets to create radial spectra from the diffraction patterns. Multivariate statistical analysis based on principal component analysis and non-negative matrix factorization was used to distinguish between different phases in the PCM materials, allowing the creation of maps showing the distribution of amorphous and crystalline phases.
Results
The approach was applied to study crystalline nanomaterials on amorphous layers to differentiate between different phases. First, test samples were studied to find optimal experimental parameters. The developed method was then applied to the PCM material Ge2Sb2Te5 (GST). Crystalline clusters in the amorphous GST layer could be distinguished using both FEM and RFA based methods. The two methods were compared with each other. RFA was found to have better robustness to noise.
Conclusions
We have developed a method that can be used to differentiate between amorphous and crystalline phases in PCM materials by combining 4D STEM with FEM and RFA. Both FEM and RFA could distinguish successfully between amorphous and crystalline phases. However, RFA was more robust to noise than FEM. The two methods are complementary and can be used alongside each other to study unknown phase change materials, ultimately during switching.

Keywords:

phase change memory materials, 4D-STEM

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Imaging-based methods to identify prognostic and predictive biomarkers for Hereditary Spastic Paraplegia

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Poster Group 1

Background
Hereditary spastic paraplegias (HSPs) are rare motor neuron diseases characterized by axonal degeneration involving the lateral corticospinal tracts. The most common is caused by haploinsufficient mutations in the SPG4 gene, which encodes spastin, a microtubule (MT) severing ATPase, that controls cytokinesis, endosomal traffic, lipid droplets (LDs) homeostasis, and axonal transport. Clinically, HSP-SPG4 age of onset and the severity of symptoms are strongly variable even among individuals belonging to the same family. Given this heterogeneity, it is important to identify prognostic biomarkers. To date, no effective disease-modifying therapies are currently available, but approaches based on drugs counteracting dysfunctional mechanisms or spastin-elevating treatments are emerging, so it will be crucial to identify biomarkers that can help to monitor the effects of spastin recovery treatments.
We have developed an automated, simple, rapid, and non-invasive cell imaging-based method to quantify the organization of the MT-cytoskeleton. By using this method, we demonstrated that the dcnc-parameter, measuring the distance between cell and nucleus centroids, is able to distinguish HSP-SPG4 from healthy donor (HD) lymphoblastoid cell lines (LCLs) and peripheral blood mononuclear cells (PBMCs).
We are now extending the dcnc-based method to a larger cohort of SPG4 patient cells to evaluate its sensitivity and specificity in relation with molecular and clinical patient features and to detect the effects of different spastin-elevating drugs. Additionally, we are also focusing on other subcellular components affected by spastin mutations, such as LDs.

Methods
The study included 13 HDs and 18 SPG4 patients with different types of mutations (12 truncating mutations and 6 missense mutations). Cell image analysis was performed using PBMCs or LCLs stained with specific antibodies (anti-β-tubulin-cy3 for MT-cytoskeleton) and dyes (Bodipy™ 493/503 for LD and Hoechst for Nucleus). Automated image acquisition was achieved by using the inverted Nikon Eclipse-Ti microscope and the JOBS module of NisElements 5.11 software to obtain a file containing more than 50 images and 500 cells related to each single sample. The acquired images were then analyzed using Cell Profiler 4.3 software, with an ad hoc designed pipeline that recognizes cellular compartments using thresholding segmentation and measures several parameters, including “dcnc” and “number of LD in each cell (nLD)”.

Results

We performed correlation analysis between the “dcnc” parameter and molecular patient features, showing that SPG4 cells from patients with missense mutations have higher “dcnc” than those with truncating mutations. Correlations with clinical features, such as the age at onset of the disease or the Spastic Paraplegia Rating Scale (SPRS) score, are ongoing.
By assessing the effect of three different spastin-elevating drugs, we observed that dcnc-based method is able to detect the reduction of MT defects induced by spastin recovery in cells from SPG4 patients carrying truncating mutations. Similar analyses on cells carrying missense mutations are ongoing.
We have implemented our pipeline to evaluate other cellular components, such as LD, so it automatically recognises the staining of the nucleus, cytoskeleton and LD, in our cells. By using this tool, we have analysed the parameter “nLD”. Correlation analysis between dcnc and “nLD” was performed, showing a positive correlation in SPG4 patients.

Conclusions and future perspectives
Our analyses revealed that the dcnc-based method is able to sense the effects of spastin elevating drugs in cells from SPG4 patients carrying truncating mutations, suggesting a predictive role. Now, we are performing correlation analyses among molecular and clinical patients features and the “dcnc” and “nLD” parameters to evaluate whether our method might have prognostic value. These results will open the possibility to identify new prognostic and predictive tools for HSP-SPG4.

Keywords:

HSP-SPG4
biomarker
neurodegenerative disease

Reference:

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Characterizing Magnetic Properties of Nanoparticle Systems: Insights from Electron Tomography and Micromagnetic Simulations

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Poster Group 1

Background incl. aims
Nanoscale 3D imaging techniques, such as electron tomography, are increasingly essential in material science and engineering. Extracting quantitative information from these techniques requires robust processing methods. This study presents several approaches for evaluating magnetic properties, such as magnetic shape anisotropy energy and demagnetization factors, as well as of magnetic nanoparticle ensembles using electron tomography data.
Methods
We extend a previously developed in-house software for processing 3D data to retrieve quantitative morphological information about nanoparticle systems [1]. Our focus is on iron oxide (magnetite) nanoparticle systems prepared via coprecipitation methods. Transmission electron microscopy (TEM) tomographic series were acquired using a JEOL 2100 TEM, and the reconstruction was performed using GENFIRE software. The raw 3D data is segmented with an in house developed software and the retrieved quantitative morphological information on nanoparticle size and shape is saved in a suitable format for use by the Object Oriented Micro Magnetic Framework (OOMMF) [2].
Results
The micromagnetic simulations are employed to obtain several magnetic features including demagnetization energy maps of the nanoparticles as well as a statistical perspective of magnetic shape anisotropy energy and demagnetization factors of the studied system. By comparing magnetic information obtained from OOMMF simulations with approximation based morphological data, we seek a better understanding of how morphological features influence the magnetic phenomena.
Conclusion
Morphological information, such as size and shape, coupled with magnetic parameters like demagnetization factors and shape anisotropy energy, holds significant importance in diverse fields such as gas sensing [3], catalysis, spintronics, and magnetic hyperthermia-based therapies [4]. By integrating electron tomography with micromagnetic simulations, this study aims to advance our understanding of the intricate relationships between morphology and magnetic behavior in nanoparticle systems.

Keywords:

Electron tomography, micromagnetic simulations

Reference:

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Correlative microscopy of creep cavitation in steels using image processing of SEM, FIB-XeF2 and EBSD

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PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Background incl. aims
Creep cavitation is an important degradation mechanism in metal alloys at high temperatures, such as in aerospace engines, gas turbines and nuclear fission and fusion reactors. During operation, metal components such as 316H stainless steel boiler headers are subjected to elevated temperatures and stresses, particularly close to weldments and geometrical features [1]. Over extended service lifetimes, these temperatures and stresses result in vacancy and dislocation movement that can gradually accumulate at microstructural features in the alloy, resulting in the formation of creep cavities that can develop into cracks and eventual failure of the component. This mechanism starts at the microstructural level, and to understand the process, it is important that the evolution of creep cavitation from nucleation to growth is characterized using advanced microscopy.
Previous work [1] has shown that in ex-service material after 65,000 hours in service in an advanced gas-cooled nuclear reactor (AGR) at 490°C–530°C, the heat-affected zone close to a weld in a 316H boiler header became extensively cavitated as a result of creep cavitation. Detailed microstructural analysis using scanning electron microscopy (SEM), electron backscatter diffraction (EBSD) and transmission electron microscopy (TEM) showed that cavities in this material were correlated with precipitation on the grain boundaries of the steel, most significantly at the interface between $M_{23}C_6$ carbides and ferrite which had formed during thermal ageing. This detailed understanding of the interaction between microstructure and creep cavitation is essential to determine component lifetimes and design new creep-resistant materials.
However, to fully understand the interactions between creep damage and microstructure, it is beneficial to characterize this interaction over larger regions of an affected material, whilst retaining the high resolution structural, crystallographic and elemental information of individual microscopy images. In this work, we present a new methodology that combines these techniques with image processing to provide spatially-identified datasets of creep cavities, precipitation and grain structure with nanometre resolution, but across millimeter length scales of a component.
Methods
Type 316H austenitic stainless steel material was removed from a boiler component, initially service-exposed for 65,000 hours at 490°C–530°C in an AGR. Material was extracted away from high stress regions of the component and was characterized to ensure it had thermally-aged microstructure but no existing creep cavitation. The material was subsequently machined into a notched creep specimen with a gauge length of 40mm, a notch radius of 6mm and a notch acuity of 5. The creep specimen was subjected to 25 cycles of tensile stress relaxation at an initial net section stress of 390 MPa and a temperature of 550°C, and then allowed to relax in pure strain control. After testing, the crept specimen was sectioned axially, ground and polished using diamond slurry and vibropolishing to an EBSD-quality mirror finish.
An area of 3.2x1.2 mm across the centre of the sectioned specimen around the notch was imaged using 162 backscattered electron (BSE) images with a 30% overlap between neighbouring images

using a Zeiss SigmaHD FEG-SEM, as well as EBSD and FIB-XeF_2 imaging across the same region. The FIB-XeF_2 imaging approach used a ThermoFisher Scios2 focused ion beam instrument, and utilized a XeF_2 gas injection system to enhance contrast between the metal matrix and precipitates, as described in [2]. The software package Dragonfly (Object Research Systems Inc, Montreal, Canada) was used for post-image processing, segmenting the BSE images to identify cavities and the FIB-XeF_2 images to identify precipitates using an Otsu threshold selection methodology [3]. EBSD data was corrected using an in-house Matlab script and all three datasets were then overlaid to enable correlation between the cavity, precipitate and grain boundary information across the entire 3.2x1.2mm region to be characterized statistically. Further detail on the methodology can be found in [4].

Results

The graphic shows an example of this correlative workflow. Figure (a) shows a stitched FIB-XeF_2 image of a 3.2 mm x 1.2 mm region of the creep specimen, with the notch at the left of the image. The stiched image comprises 32 individual FIB-XeF_2 images, which along with the 162 BSE images were image processed to identify creep cavities, cracks and precipiates by grayscale contrast and morphology. Two example frames with image processing are shown in (b) and (c), where yellow represents identifed $M_{23}C_6$ carbide precipitates (verified by transmission electron microscopy diffraction) and blue represens creep damage. (d) shows the statistical overlay of the identified precipitates in yellow with the grain boundary locations obtained by EBSD in blue, across the entire sampled region. Similar maps of damage were also obtained. The statistical analysis of these data described in full in [4] showed that damage showed very little correlation with grain-to-grain differences in Schmid factor (which many computational models of creep assume to be a key factor in damage location), but in this material precipitation, grain boundary angle and localised strain (as indicated by the kernal average misorientation in the EBSD data) showed much clearer correlation with damage, indicating that in this material the trapping sites at grain boundary carbide precipitates are the primary initation sites for creep damage.

Conclusion

Creep cavitation is a complex degradation mechanism, which starts at the atomic or nanometre scale and over extended times can build to failure of entire components. The methodology presented here shows the effectiveness of advanced microscopy combined with image processing to statistically correlate microstructural features with damage locations in crept material, enabling much better understanding over large areas of the contributing factors to this process.

Keywords:

Creep, steel, correlative microscopy

Reference:

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Insights into Lithium-rich Oxides from Synthesis and Characterization Studies

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Poster Group 1

Lithium-ion batteries (LIBs) have dominated the market as preferred energy storage system since their commercialization. However, there is growing need to improve the system in order to accommodate the advancements in different sectors, with electric vehicles being the main driving force. Electric vehicles require high energy and power density, which translates to greater distances traveled and faster charging, respectively. In this respect high capacity and voltage cathodes are a promising path forward, and Li rich oxides (LROs) are amongst the most promising cathode materials for increasing efficiency of LIBs. In addition, the most widely studied composition of LROs, $Li_{1.2}Mn_{0.54}Co_{0.13}Ni_{0.13}O_2$, reduces the amount of nickel and cobalt, making it more environmentally friendly and less expensive. However, these cathode materials suffer from severe drawbacks of voltage decay, low initial coulombic efficiencies, transition metal migration and dissolution, oxygen loss etc. In order to target and resolve these issues, it is first important to understand the pristine structure of LROs, which is still under debate in the scientific community. There are two main hypotheses for its structure i.e., nano-domains of two phases or solid solution of a single phase. The two phases correspond to a rhombohedral $LiMO_2$ phase (space group R-3m) and a monoclinic Li_2MnO_3 phase (space group C2/m). These phases share a similar oxygen lattice, which increases the difficulty in distinguishing them from one another. As such, further studies on the structural and electrochemical properties of LROs are needed to reach its wide-scale commercialization.
In this study, we synthesized a series of LRO samples with the composition, $Li_{1.2}Mn_{0.54}Co_{0.13}Ni_{0.13}O_2$, through sol-gel synthesis. We used two different chelating agents; citric and oxalic acid, and the resulting foam was calcinated at two temperatures; 850°C and 900°C. We recorded X-ray diffraction (XRD) patterns at ~15keV (0.8265 Å), over the 2θ range of 8-60 degrees, at the MCX beamline at Elettra Synchrotron, Italy. The powder samples were measured in borosilicate glass capillary tubes (0.3mm diameter) in transmission mode. We analyzed the particles using a JEM ARM200CF, probe Cs corrected scanning transmission electron microscope (STEM), at 80kV. A GIFQuantum ER dual-EELS system (GATAN-AMETEK, Pleasanton, USA), was used for electron energy loss spectroscopy (EELS). Figure 1(a) shows the XRD pattern, in which the peaks correspond to that of a rhombohedral phase, excluding the weak reflections between ~11-15 degrees. These weak peaks correspond to a monoclinic phase and are highly asymmetric. The asymmetric peak shapes are related to the presence of stacking faults in the material. Figure 1(b) shows the STEM-High-angle annular dark-field (HAADF) image of the particle and the corresponding EELS spectra at O K-edge and Mn L-edge. Three spectra for each elemental edge are shown, moving from the center to the edge of the particle, as indicated in Figure(b). The white line of oxygen K-edge is seen to vary with depth; increasing towards the center of the particle. This change can be attributed to the change in valence state of the surrounding transition metals. On the Mn L-edge spectra, more prominent on the inner-most spectra, a shoulder appears which is similar to that seen in pure Li_2MnO_3.

By combining XRD and EELS in this study, we have shown the need to complement bulk probing techniques with local probing techniques, respectively. The weak reflection for the monoclinic phase in the XRD pattern make data analysis difficult but local EELS spectra indicate the presence of different environments within a single particle. Our findings contribute to a deeper understanding of the pristine structure of LROs and highlights the need for multiscale characterization.
This work has received funding from the European Union Horizon 2020 research and innovation PhD program DESTINY under grant agreement No. 945357. Co-financing from the Slovenian Research Agency ARIS (core program funding P2-0423, projects J2-3050 and JV-4637) is acknowledged.

Keywords:

li-rich oxides, EELS, XRD, sol-gel

Reference:

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U-NET Enhanced 4D-STEM/PNBD: Advancing Microscopy Image Reconstruction

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Poster Group 2

Background
This work is focused on our recent improvements of 4D-STEM/PNBD method (Four-Dimensional Scanning Transmission Electron Microscopy/Powder NanoBeam Diffraction) [1-3], which provides a user-friendly way to use a modern SEM as a fast powder electron diffractometer. SEM must be equipped with a 2D-array detector of transmitted electrons (pixelated STEM detector). The pixelated detector yields standard STEM/BF images in the form of a standard scanning matrix. Moreover, each position within the scanning matrix is a 2D nanobeam diffraction pattern, which is captured thanks to the 2D-array of pixels within the pixelated detector. In 4D-STEM/PNBD method, the sum of the post-processed individual diffraction patterns is then used to obtain powder diffractogram.
The reduction of the 4D-STEM dataset to 2D-powder diffractogram and eventually to its 1D-radially averaged profile can be automated by means of our open-source Python packages STEMDIFF and EDIFF. The latest available versions support fast parallel processing on multiple cores, user-friendly Jupyter notebook interface and independence from any third-party software.
The current development aims to improve individual diffraction patterns so that even samples usually very challenging to study (such as specimens with high amorphous background and low diffraction power) can be processed satisfactorily. The 4D-STEM-in-SEM datasets of these difficult samples can be quite challenging to process and get the diffraction patterns that could compete with the quality of standard TEM/SAED diffractograms. Conventional image enhancement methods have failed because they cannot remove the background and noise without affecting the diffraction intensities. Therefore, deep learning methods were used to develop a model that would sufficiently suppress unwanted image deterioration. In this contribution, we introduce a U-Net Autoencoder (U-NAE) for noise and background removal in 4D-STEM-in-SEM complex datasets.
Methods
As the ideal target 4D-STEM-in-SEM data (clean, noise- and background-free diffractograms) were not available, an artificial dataset must have been simulated. The synthetic target data were derived from actual datasets of several different crystals (network input data) using multi-scale filtering, image transformations, thresholding, and manual corrections (a lengthy and inefficient procedure). After collecting the paired dataset (input/target data), each was split into 3 sub-datasets – 60% for training, 30% for validation and 10% for testing. Then finally, an architecture of a denoising neural network could have been designed. Given the limited dataset, the convolutional U-Net architecture was a suitable choice because it can find key features in the data from which it reconstructs the desired output. It consists of three parts – encoder (contracting path which captures contextual information), bottleneck (low-dimensional representation of the input), and encoder (extending path generating a clean image from the bottleneck representation).
The U-NAE encoding part consists of 3 blocks of convolutional layers that reduce the input image of size 256x256x1 to a feature map of size 32x32x64. This bottleneck-representation is then passed through the decoding part (3 blocks of convolutional layers which also work with the information

from the encoder transferred by skip connections and reconstruct the data to its original resolution). At the end, there are 3 fully-connected convolutional layers that fine-tune the output.
The model was trained using ADAM optimizer with mean squared error (MSE) as the criterial function. Additional metric to monitor for model performance assessment was mean absolute error (MAE). The validation loss after each epoch was the key score when assessing the network's learning state, as it is indicative of its generalization capabilities and how well it performs on newly introduced data. Training data was fed to the network in batches of 32 images, and the learning rate was adapting during training as it was designed to gradually decrease when approaching the global optimum.
Results
During training, the model's validation MSE dropped from 8135.73 to 25.31, and the MAE from 23.58 to 2.47. The model with the lowest validation loss was saved to avoid possible overfitting in the final training epochs. It was then tested on a test dataset that had not yet been introduced to the network and has shown to improve the quality of datasets acquired by our 4D-STEM-in-SEM technique, even for the challenging samples. Clear evidence of the functionality of U-NAE is shown in Fig. 1, where even for the most primitive method of obtaining a diffractogram (simple summation) the improvement in the quality of the output image is clearly visible, as well as the noticeable improvement of the radial distributions of the diffractograms.
Conclusion
The U-NAE has been proven effective on various challenging datasets, yet limitations arise in handling heavy noise levels, potentially leading to unsatisfactory results. Future efforts will prioritize optimizing the U-NAE architecture to address these challenges and explore alternative training strategies to enhance its performance. Incorporating domain-specific knowledge may further broaden its applicability in material science research. In addition, further work to refine the simulation of the target data could also improve model performance.

Keywords:

4D-STEM-in-SEM, U-NET, 4D-STEM/PNBD, diffraction, denoising

Reference:

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Anomalous effects of strain in selected area diffraction patterns due to boron doping in silicon

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Poster Group 2

Semiconductors have a significant impact on our lives due to their role in the fabrication of electrical devices. The sensitivity of its conductivity to temperature, illumination, magnetic field, and minute amount of impurity atoms make semiconductors one of the most important materials for electronic applications. It is important to study defects since it can significantly alter the electrical properties of a semiconductor. Microscopic lattice defects such as point defects, dislocations induce local electronic density of state changes and distort the surrounding lattice structure. The effect these interruptions of the crystal lattice have on the scattering of photons or charged particles such as electrons can be used to study the defect.

The effect of static atomic displacements associated with misfitting boron atoms in silicon (B-Si) has previously been studied using high angle annular dark-field (HAADF) imaging by Perovic et al (1). It was found that the HAADF contrast of the boron doped silicon (B-Si) layer is brighter than elemental silicon (Si), despite the decrease in atomic number. This could be due to static atomic displacements arising solely from the displacement of Si atoms from their equilibrium lattice sites adjacent to substitutional boron atoms (2). This type of contrast is called Huang scattering contrast. In addition, high concentrations of boron can also modify the phonon properties of the material (3), which affects the thermal diffuse scattering (TDS). Perovic et al. (1) had attributed the anomalous HAADF contrast to TDS, although the effects of Huang scattering were not considered.

We have used transmission electron microscopy (TEM) to analyse strain around point defects in silicon highly doped with boron, i.e., degenerate doping (B-Si). Fig 1 shows selected area diffraction patterns (SADPs) of elemental Si and B-Si from [100] and [110] zone-axis orientations obtained at room temperature. For [100] Si (Fig 1a) there is streaking arising from (largely acoustic) phonon scattering along [010] and [001] crystallographic directions around the Bragg reflections. The halo ring pattern around the unscattered beam is thought to be due to FIB specimen preparation artefacts. For [100] B-Si (Fig 1b), the streaking is reduced, which could be due to the smaller thickness of this sample. However, diffuse scattering in the form of a cross-shaped pattern between the unscattered and 040 Bragg beams is evident (dashed lines in Fig 2b). The diffuse scattering is not observed for elemental Si, suggesting modification of phonons due to boron doping. This is also consistent with our Raman results, which shows significant peak broadening upon degenerate doping. It should be noted that anomalous low angle scattering due to boron was not observed in other zone-axis orientations, such as [110] (Figs. 1c and 1d).

Radial intensity profiles from Si and B-Si are obtained from the [100] and [110] zone-axis diffraction patterns (Fig 2). The total intensity has been normalised for a direct comparison. For [100] the (t/□) specimen thickness ratio, measured using electron energy loss spectroscopy, was 0.75 for B-Si and 1.01 for Si. Therefore, at high scattering angles the intensity for Si should be higher than B-Si due to the larger atomic number (Z) and specimen thickness. However, the opposite trend is observed (Fig 2a), which is also consistent with the previous results of Perovic et al. [1]. The anomalous high angle scattering is also observed for [110], although its effects is smaller (Fig 2b; the (t/□) ratio for the two samples were similar, i.e., 0.71 for B-Si and 0.67 for Si).

The diffraction intensity at high scattering angles is due to localised scattering, and therefore we expect the intensity to be dominated by Huang scattering from the boron point defect atoms. On the other hand, the diffuse scattering in the zero order Laue zone pattern is likely to be dominated by phonons, and here we find evidence of changes in the phonon configuration due to boron doping. We acquired diffraction data of B-Si under liquid nitrogen cooling, which suppresses TDS but not Huang scattering, to investigate if the diffraction intensity at high scattering angles is solely due to Huang scattering, as boron doping in silicon induces local vibrational modes above the optical mode frequency of 16 THZ (4). Fig 3 illustrates the SADPs alongside a comparison plot detailing the radial intensities of B-Si at room temperature and -173 degrees Celsius. Due to nitrogen cooling, phonon modes above the frequency of 2.07 THz were supressed, which includes the TDS due to local phonon modes from boron doping. The radial intensity of B-Si at higher scattering angles in Fig 3b did not decrease; rather, it slightly increased upon cooling. This suggests that the intensities at high scattering angles were not predominantly contributed by TDS, as most of the phonon modes were suppressed. Hence, the intensity at higher angles is more likely to be contributed by Huang scattering, given that the vibration modes contributing to TDS were supressed.
In summary, the effect of strain due to point defects has been studied in boron doped silicon using SADPs from [100] and [110] zone axes. Anomalous diffuse scattered intensity is observed at both small and large scattering angles. It is concluded that boron gives rise large angle to Huang scattering due to the intrinsic strain in the lattice, as well as altering the phonon configuration, and hence thermal diffuse scattering.

Keywords:

High angle scattering, TEM

Reference:

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Van der Waals heterostructures of nanopatterned 2D materials for novel device geometries

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Poster Group 2

Background
As Moore's Law starts to stagnate and the continuous pursuit of smaller and faster devices persists, there is a growing need for new approaches to further push the boundaries of nanofabrication. The modification and stacking of two-dimensional (2D) materials has significant importance in this context [1]. High precision structuring tools, such as a focused electron beam, can be employed to mill a wide range of structures into 2D materials [2], e.g., to modify their electronic properties. For example, creating a band gap in graphene [3] enables its use as a semiconductor in a variety of applications. By using customized sheets of 2D materials as building blocks, it should then become possible to produce almost any three-dimensional (3D) structure with properties that have been finely tuned to suit desired requirements [4].
Methods
2D materials are structured by using scanning transmission electron microscopy (STEM). Clean samples are essential to the quality of the resulting structures. Therefore, to minimize contamination on the sample, a micro-electro-mechanical system (MEMS) chip is used for its ability to provide in situ heating. Investigation of the manufactured structures is done by transmission electron microscopy (TEM).
Results
Atomically clean graphene samples have been successfully produced using the in situ heating of the MEMS chip. Furthermore, a wide range of structures using different STEM patterning parameters have been investigated. In order to open up a band gap within the graphene, large areas (600x600 nm) with small holes with a diameter of only 2-3 nm and a periodicity of 8 nm have been patterned. According to theoretical calculations, this introduced a band gap of approximately 0.6 eV into the graphene.
Conclusion
The ability to work with contamination free 2D materials and the consequent high patterning resolution of the structures is very promising. Smaller structures will be in reach soon and thus, the possibility of larger bandgap openings within graphene becomes available. Ultimately, novel van der Waals heterostructures will be created by stacking modified layers of various 2D materials.

Keywords:

graphene, 2D materials, vdW heterostructures

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Fabrication of electron transparent membranes and nanostructures in fluidic devices by NIL and “Flow-Through”-gas-phase deposition

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Poster Group 2

Background incl. aims
Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are invaluable tools for visualizing and analyzing samples with nanometer resolution. Integration of liquid cells with these microscopy techniques has expanded their capabilities, allowing dynamic imaging and real-time analysis under controlled liquid conditions [1,2]. However, existing liquid cells still face limitations such as high production cost, restricted geometries, and reliance on slit-like chambers formed by connecting two membranes with spacers.

Methods
We present a novel fabrication method for producing micro-, and nano channels and 3D structures, self-sealed with electron transparent thin Al2O3 membranes, as sketched in Fig. 1a), 1b) and 1c). For this, we make polymeric foils (<10 µm) containing micro-, and nanofluidic structures. Fabrication of the fluidic devices involves direct UV nanoimprint lithography (NIL) using a customizable and versatile stamp design adaptable to specific geometries [3]. Then, the channels are coated selectively from the inside with Al2O3 by using our self-developed gas-phase deposition [4]. These inorganic electron-transparent structures can be suspended by removing the polymer material around them, enabling their use as liquid cells for investigating dynamic molecular behavior within confined spaces, such as in TEM.

Results
Our method allows fabrication of liquid cells with a variety of lateral dimensions, even down to nanometric channels, and complex 3D structures with graded depths and widths. The fluidic device contains two microchannels which connect the inlet and outlet holes. These microchannels are interconnected by several nanochannels. For fluidic devices fabrication, a stamp is placed and aligned onto a polycarbonate plate which has been covered with a UV curable polymer containing pre-patterned holes. The assembly is then cured with UV-light, and the substrate and stamp are separated manually. Next, a polymer coverslip is used to seal the channel system of the fluidic device. The Al2O3 coating is achieved through a specialized gas phase deposition (GPD) reactor operating in a "flow-through" mode (ftGPD), providing conformal coating of the various structures [4]. The reactor's gas and vacuum ports align with the holes in the polycarbonate plate, facilitating connection to the imprinted fluidic system. By precisely controlling precursor gas flow across the microchannel, a controlled pressure gradient is established, enabling conformal deposition of Al2O3 onto various structures, including slits, chambers, and micro- and nanochannels. This method enables to tune the Al2O3 thickness, ranging from a few nanometers to hundreds of nanometers, allowing for adjustment of mechanical stability and electron transparency as needed. This strategy effectively circumvents existing constraints associated with liquid cell geometries, particularly those limited to slit-like chambers. In subsequent fabrication steps, the micro-, nanochannels or membranes can be selectively suspended by masking and reactive ion etching (RIE). Fig 1a) shows

hollow Al2O3 microchannel which are used for flow tests. Fig. 1b) shows an example of a suspended, hollow Al2O3 nanochannel with a cross section of 500 x 500 nm and a suspended length of 20 µm. To validate the electron transparency of the Al2O3 membrane, we created a TEM grid sample coated with an Al2O3 membrane fabricated in our GPD reactor. Subsequently, polystyrene beads were deposited both above and below the Al2O3 membrane, and imaged using SEM and TEM techniques (Fig. 1 d) e) and f), respectively). SEM images (Fig. 1 (d)) captured the same area with two different detectors, where polystyrene beads beneath the membrane were detectable solely by the secondary electron secondary ion (SESI) detector. In contrast, TEM imaging (Fig. 1 (e)) of the sample in transmission mode at a separate location demonstrated the feasibility of imaging beads through the Al2O3 membrane. We also made a sandwich, by placing another Al2O3 membrane on top, and imaged the beads in TEM at 200 keV, as shown in Fig 1 f). Furthermore, we also imaged gold nanoparticles placed below the Al2O3 membranes deposited and suspended from the top in one of our microchannels. The particles were visible in the SEM at an acceleration voltage of 8kV (Fig. 1 c)). All these results confirming the electron transparency of the membranes deposited using our method. To deploy this system as liquid cells in a TEM specimen holder, detachment of the structured and coated polymer foil from the substrate and subsequent cutting to a 3 mm total diameter are necessary.

Conclusion

This fabrication method offers a versatile approach for creating liquid cells with complex geometries, overcoming limitations of existing slit-like chamber designs. The electron-transparent Al2O3 membranes enable dynamic imaging of samples within confined spaces using SEM and TEM, with potential future applications in nanoscale research and analysis. Further investigations using these liquid cells inside SEM and TEM will be presented at the conference.

Keywords:

electron-transparent membrane, liquid flow cell

Reference:

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Electron microscopy studies of Ni/GDC fuel electrode in solid oxide fuel cell

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Poster Group 1

To increase the commercialization of solid oxide fuel cells (SOFCs), it is necessary to reduce their cost and extend their lifetime [1]. By operating SOFCs at temperatures below 650 °C, it is possible to make use of low-cost materials and reduce the performance degradation of SOFCs [2]. However, in the case of yttria-stabilized zirconia (YSZ) electrolytes, the electrochemical performance dramatically decreases at lower temperatures due to the low ionic conductivity of YSZ, leading to a high ohmic resistance [2]. The ionic conductivity of gadolinia-doped ceria (GDC) is significantly higher than that of YSZ at temperatures as low as 500 °C [3]. Ni/GDC fuel electrodes therefore provide superior properties compared to Ni/YSZ [4]. The microstructural integrity of Ni-based fuel electrodes is necessary for the long-term operation of SOFCs. Therefore, it is necessary to understand the structural and chemical degradation mechanisms of SOFCs to enhance and predict the lifetime of SOFCs [5].
In this work, the effect of operation temperature and duration on the structural and chemical degradation of Ni/GDC fuel electrodes was studied using (scanning) transmission electron microscopy ((S)TEM) and focused-ion-beam/scanning-electron microscopy (FIB-SEM) to correlate the structural and chemical evolutions with the results of electrochemical impedance spectroscopy. By using FIB-SEM, it is possible to reconstruct the 3D structure of the porous electrode to acquire structural parameters such as tortuosity, particle size distribution, specific active area, and triple-phase boundary length. The TEM sample preparation and 3D reconstruction of the Ni/GDC fuel electrodes were carried out using a Helios G4 FX dual-beam instrument (Thermo Fisher Scientific). STEM imaging and energy-dispersive X-ray spectroscopy were performed using a Tecnai OSIRIS ChemiStem (Thermo Fisher Scientific). TEM and SEM results of the Ni/GDC fuel electrodes at different aging states indicate a significant change in the microstructure, e.g. Ni agglomeration at higher operating temperatures, leading to an increase in polarization resistance.

Keywords:

FIB, SEM, TEM, SOFC

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Assessing feasibility of detecting photogenerated charge carriers in photocatalysts via transmission electron microscopy: simulation study

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Poster Group 2

Background
In the face of global energy and climate crises, the necessity for sustainable solutions has never been more pressing. Photocatalysts, which use sunlight to drive chemical transformations, offer hope in this quest. Among their many applications, one of the most promising lies in the conversion of carbon dioxide (CO2) into fuels, presenting a dual opportunity for reducing atmospheric CO2 levels and generating sustainable energy.1 However, the effectiveness of photocatalysts depends on the complex interaction of light absorption, charge carrier dynamics, and catalytic activity, particularly in the context of poorly understood steps such as charge carrier accumulation.1-3

Methods
Taking advantage of the fact that the accumulation of charge carriers in photocatalyst will induce local alterations in the atomic structure affecting parameters such as nuclear charge, atomic/ionic radius, and chemical bonding.3 These alterations, potentially originating from selective reduction of cations at a specific nanocrystal (NC) active surface facet,4 can manifest as local phase shift variations in transmission electron microscope (TEM) images. The goal of this study is to identify visible light-induced phase shift variations by extracting phase images from reconstructed exit wave functions corresponding to specific NC surface facets under various conditions. Advanced image simulations will provide valuable insights into the detectability of photogenerated charge carriers in TEM experiments.

Results
In this study, ab initio Transmission Electron Microscopy (abTEM) simulations5 was employed to assess the feasibility of detecting photogenerated charge carriers at the interface of photocatalysts under light irradiation, in the TEM. By creating a model photocatalytic system comprising facet engineered NCs, where photons are absorbed on the active facet during reaction, leading to the accumulation of photogenerated charge carriers. Simulating the structural and electronic properties of these photocatalysts under illumination, we aim to elucidate whether charge carrier accumulation at the interface is detectable in the TEM. I will present both high resolution TEM (HRTEM) and integrated differential phase contrast STEM (iDPC-STEM) imaging techniques and assess whether they are sufficiently sensitive to detect the potential difference arising from the slight change in nuclear charge.

Conclusion
This simulation study provides a crucial insight on the sensitivity of TEM techniques to subtle changes in potential and chemical reactivity induced by photogenerated charge carriers, providing insights into the feasibility of using TEM for monitoring photocatalytic reactions. This serves as a crucial step towards using advanced microscopy techniques for probing the dynamics of photocatalysis at the

nanoscale, ultimately facilitating the design and optimization of efficient photocatalytic systems for renewable energy generation and addressing environmental issues.

Keywords:

Photocatalyst, charge carriers, abTEM simulations

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Unraveling the regulation pathway of photosynthetic AB-GAPDH

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Poster Group 1

Oxygenic phototrophs perform carbon fixation through the Calvin–Benson cycle. Different mechanisms adjust the cycle and the light-harvesting reactions to rapid environmental changes. Photosynthetic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a key enzyme in the cycle. Different photosynthetic GAPDHs exist in higher plants: the most abundant is formed by hetero-tetramers of A and B-subunits (AB-GAPDH), and the least abundant by A4 homotetramers. Regardless of the subunit composition, GAPDH is the major consumer of photosynthetic NADPH, and its activity is strictly regulated. While CP12 regulates A4-GAPDH, AB-GAPDH is autonomously regulated through a pair of cysteines located at the C-terminal extension (CTE) of B-subunits and by the substitution of NADP(H) with NAD(H) in the cofactor binding domain. Together, these conditions result in changes in the oligomerization state, thereby inhibiting enzyme activity. By combining small angle x-ray scattering coupled with size exclusion chromatography (SEC-SAXS) and cryo-electron microscopy (cryo-EM), we revealed the presence of several AB-GAPDH oligomers [(A2B2)n-GAPDH oligomers with n=1, 2, 4 and 5], co-existing in a dynamic system [1] (See Figure A). Moreover, the A8B8-GAPDH oligomer was present in two different conformers. Despite the sample's significant compositional and conformational heterogeneity, we solved the two A8B8-GAPDH hexadecameric conformers at around 3 Å (See Figure B). The other GAPDH oligomers were instead solved at intermediate resolution. In all GAPDH oligomers, we observed the same oligomerization/inhibition mechanism based on the mutual exchange between adjacent B-subunits of their CTEs, effectively preventing the binding of the substrate 1,3-bisphosphate-glycerate in the B subunits. The nearly atomic resolution we achieved allowed us to detail the molecular interactions among enzyme subunits at the tetramer interface, between the CTE and the active site residues, and between the CTE and the coenzyme.

Figure. A, AB-GAPDH oligomers cryo-EM density maps co-existing in the chloroplast leaf stroma. B, the cryo-EM electron-density map of the A8B8-GAPDH hexadecamer resolved at nearly atomic resolution (3 Å). The cryo-EM electron density maps are fitted by models derived from the crystal structure of oxidized A2B2 complexed with NADP+ (2).

Keywords:

photosynthesis; cryo-electron microscopy; GAPDH

Reference:

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Resolving short-range order in Carbon Nitride-based catalysts using EELS

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Poster Group 1

Background and aims
Carbon Nitrides (CNs) attract great attention as promising materials for water remediation, gas sorption, energy storage devices and photocatalysis, among others. This variety of applications stems from a set of specific properties of CNs: chemical inertness, biocompatibility, high thermal and mechanical stability, and non-toxicity [1,2]. Tuning the functionalities of carbon nitrides requires precise control over their structure and composition. Many CNs are poorly crystalline or even amorphous materials, thus requiring specific methods to describe their local structure (short- and medium-range order). In this work, we explore the possibility of obtaining quantitative information about the local structure of CN-based compounds using electron energy loss spectroscopy (EELS) in (S)TEM. Element-specific radial distribution functions (RDFs) can be derived from the extended energy loss fine structure (EXELFS) of EELS spectra [3]. Taking into account that modern analytical microscopes are much easier to access than spectroscopic beamlines at synchrotrons, this method holds great potential for fast analysis and prescreening of high amounts of samples. Here we investigate two challenging CN systems to establish the limitations of RDF analysis from EELS spectra in application to polymeric compounds: (1) the heterojunction of two photocatalysts (poly(triazine imide) (PTI) and potassium poly(heptazine imide) (K-PHI)) is used to check the ability to distinguish between two structurally closely related polymeric materials; (2) Ce-doped CNs (Ce-CNs) are used to check the limitations regarding sensing the local environment around single-atom catalysts.
Methods
All samples were first investigated using high-resolution (scanning) transmission electron microscopy (HRTEM) and electron energy loss spectroscopy (EELS) using a double aberration-corrected Jeol JEM ARM200F equipped with a cold field emission gun and a Gatan Imaging Filter (GIF) Quantum spectrometer. HRTEM images were acquired on an Oneview (4k × 4k) camera, EELS spectra were collected on a US1000 (2k × 2k) camera. EELS spectra for the PHI/PTI sample were collected using a monochromated TFS Themis Z 80-300 microscope operated at 80 kV and equipped with a GIF Continuum 1065ER spectrometer (convergence and collection angles of 22 mrad) at energy dispersion of 0.15 eV/ch. For all EELs spectra, a power law model was used for background subtraction. Multiple scattering effects were removed using the Fourier-ratio method, implemented in the Gatan Digital Micrograph Suite (GMS), version 3.4. EXELFS data were normalized and Fourier-transformed using the Athena software package. FEFF9 was used for fitting of the experimental data.
Results
The results will be described in two sections following the scientific questions defined above.
(1) Samples of PTI, K-PHI and a heterojunction between them (PTI/K-PHI) were analyzed [5]. Our study confirmed the formation of a heterojunction between the PTI and the K-PHI phase. The RDF obtained from the EXELFS part of the N K-edge of the PHI/PTI sample shows five prominent peaks. The first peak at around 1.3 Å arises due to scattering from the nearest carbon neighbors in both PTI and K-PHI. The second and third peaks correspond to the N-N (2.1 Å) and N-C (2.75 Å) distances

within the aromatic rings. The fourth peak represents the summed contribution of the shortest N-N (3.3 Å) and N-C (3.58 Å) distances between adjacent layers of K-PHI. The fitting of RDFs obtained from individual phases and the heterojunction is in progress and should allow us to quantify the amount of each phase.
2. We investigated Ce-CNs employed in photocatalytic wastewater dephosphorylation. By varying the amount of Ce(NO3)3·6H2O precursor, samples with different concentration of Ce were obtained (9.5, 17.0, 30.5 and 39.8 wt.%). HAADF-STEM images of the samples showed that Ce is present in the CN matrix in different forms: single atoms, clusters and nanoparticles. To resolve the average coordination around Ce atoms and distinguish between the aforementioned types of structures, RDFs obtained from the EXELFS part of the M4,5-edge of Ce and from EXAFS at the Ce L2,3-edge were compared. The EXELFS signal obtained from the 9.5 wt.% doped sample was found to be too low in intensity to perform reliable RDF analysis. The position (~2.5 Å) and the high intensity of the first peak on RDFs obtained from the Ce-CNs samples with 17.0, 30.5 and 39.8 wt.% suggest that Ce atoms bind to C or N and that a considerable fraction of Ce forms single atoms. On the RDFs from the CeO2 reference the most intense peak, at 3.5 Å, corresponds to the Ce-Ce distance. Different structural models for Ce incorporated into the CN matrix were constructed and the refinement of these models is currently in progress. In addition, we collected X-ray absorption spectra from these samples in order to compare RDFs obtained from bulk samples and locally from EXELFS spectra.
Conclusion
The local structure in two soft polymeric systems utilized in energy-conversion (K-PHI/PTI heterojunction) and photocatalytic water treatment (Ce-doped CNs) was studied using element-specific RDFs obtained from EELS spectra. The results suggest that a clear discrimination between the structurally closely related PHI and PTI phases is challenging; accurate data fitting (which is currently in progress) is required to make a final conclusion. The formation of Ce single atoms manifests itself by considerably higher intensity of the C/N-Ce peak on RDFs. Conclusions about the possibility to quantify the amounts of co-existing phases (CeO2, Ce-clusters and Ce-single atoms) can only be made after accurate fitting of RDFs.

Keywords:

EELS, Carbon Nitrides, short-range order.

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Ex Vivo Metabolic Imaging for Parotid Tumors: Implications for Precise Diagnosis and Customized Treatment

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Poster Group 1

Background incl. aims
Primary parotid neoplasms necessitate specialized attention due to their diverse histological and clinical features [1,2]. Accurate classification and treatment selection rely on a thorough comprehension of both the phenotypic and molecular attributes of these tumors. Molecular markers and interactions within the tumor microenvironment critically influence their behavior. Therefore, identifying new markers using routine techniques such as needle aspiration to visualize tissue metabolism in vivo is essential for refining diagnostic accuracy and treatment strategies. Our study aims to preliminarily delineate the complex metabolic profiles of parotid gland tissues, especially focusing on distinguishing between healthy subjects and patients with squamous cell carcinoma, employing both Fluorescence Ratio (FRIM) and Fluorescence Lifetime (FLIM) Imaging Microscopy for precise quantification.

Methods
In our investigation, we conduct ex vivo analysis using advanced two-photon metabolic imaging techniques to examine the morphological, molecular, and functional aspects of parotid neoplasms. Fine needle aspiration biopsy is utilized to obtain cells for analysis, bypassing the need for invasive procedures and minimizing patient discomfort. Through this approach, we analyze metabolic markers such as NADH and FADH2, employing Fluorescence Ratio Imaging Microscopy (FRIM) and Fluorescence Lifetime Imaging Microscopy (FLIM) for precise quantification [3].

Results
Through the application of Metabolic Imaging techniques to analyze NADH and FADH2 levels in ex vivo samples, our objective is to complement traditional morphological assessments with a comprehensive evaluation of tissue metabolic states. We employed both microscopy imaging techniques to analyze the behavior of these two molecules in both healthy and diseased subjects. For instance, in the case of FRIM, we introduced an innovative workflow pipeline (depicted in Figure 1), which commences with autofluorescence imaging to derive redox ratio images by analyzing the blue and red channels. A redox ratio value approaching 0 indicates a state of reduction, while nearing 1 signifies oxidation. Subsequently, mitochondrial and cytoplasmic masks are applied to these images, enabling the assessment of tissue metabolic activity.

Figure 2 displays images in the blue channel, red channel, and the RR image generated by the analysis pipeline respectively for the healthy subject (top) and the subject affected by squamous cell carcinoma (bottom). These latter images feature a scale bar indicating areas of heightened metabolic activity within the tissue.

As shown in Figure 3, controls exhibit higher RR_mit values compared to patients, suggesting greater oxidative activity in healthy tissue.
RR_mit indicates the balance between reduced and oxidized pixels in relation to mitochondria, reflecting metabolic activity and cell viability. A higher RR suggests greater metabolic activity and more viable cells, while a higher RR low may signal metabolic alterations, as observed in squamous cell carcinoma. So, the higher RR_mit in healthy subjects compared to patients highlights significant disparities in tissue metabolic health.

We delved into metabolic dynamics by identifying the quantity of mitochondria exhibiting reduced and oxidized states within both healthy and diseased tissue, leveraging suitable algorithms for precise analysis.

Conclusion
Introduction of an innovative pipeline for autofluorescence image analysis has yielded significant findings. Notably, the results reveal a distinct contrast between healthy and diseased tissues. Moving forward, these insights are poised to influence diagnostic protocols and personalized treatments, with a focus on enhancing patient well-being. Such advancements hold promise for refining our understanding of tissue pathology and optimizing therapeutic strategies tailored to individual patient needs.

Keywords:

Metabolic Imaging, parotid carcinomas,metabolic markers

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Using XeF2 FIB imaging to contrast and quantify precipitation in metal.

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Poster Group 2

Background incl. aims
Precipitation within a material is known to affect material properties, from mechanical to chemical [1]. Depending on the type, location, orientation, and distribution of precipitates, these properties can vary significantly [2]. In this study we utilize the XeF_2 gas injection system (GIS), a common feature on dualbeam microscopes, in combination with FIB imaging to drastically enhance the contrast of precipitates and therefore make image processing easier. By combining the FIB-XeF_2 technique with low magnification and high resolution, it allows the extraction of large amounts of statistical data using software such as Dragonfly [Computer Software] 2022.2.
Methods
The FIB-XeF_2 contrast mapping procedure and micrograph capture were collected using a ThermoFisher Scios 2 dual-beam FIB system, which contains a gallium ion source. A full procedure can be found in previous work [3]. We have completed this technique using three different materials, all of which had different initial conditions and reasons for evaluating. First, an experimental Fe9Cr1Mo steel exposed to high-temperature and-pressure CO_2 environments, second, a 316H steel that had undergone creep testing, and third, an Alloy 690 nickel-based alloy that has undergone a surface mechanical process.
First, the surface was initially exposed to a high current FIB beam (30kV:50nA) to remove the surface oxide layer and expose hard precipitates in the matrix. The GIS needle was inserted and XeF_2 gas was flowed across the area of interest. Once the GIS needle was retracted, the area of interest was then exposed to a much lower FIB current of 30kV:1nA. Under the influence of this FIB beam, the XeF_2 gas binds preferentially to the metallic matrix and less to the precipitates, resulting in an enhanced contrast effect. This imaging was continued until the maximum contrast between the precipitates and the metal matrix was obtained, following which a high-resolution image was taken. In this study, the final images were taken at a resolution of 6144 x 4096, integrated 4 times with a dwell time of 1 µs. This produced an image of significant quality for quantitative analysis without altering the contrast too much. The precipitates were also individually characterised with Transmission Electron Microscopy (TEM) diffraction, Energy-dispersive X-ray spectroscopy (EDS), and Transmission Kikuchi diffraction (TKD) to identify their crystal structure on elemental composition and diffraction pattern. Once a characteristic of a particular morphology was identified using TEM / EDX / TKD, precipitates of similar contrast and morphology in the FIB-XeF_2 images were labelled as this precipitate identity.
Results
FIB-XeF_2 images were processed using image recognition in Dragonfly [computer software] 2022.2. The features within each image were segmented according to greyscale contrast using an Otsu threshold selection method [4]. This allowed the separation of precipitate from bulk, voids, and subsequently segmented different precipitates into separate virtual objects. The graphic shows an example of this approach – top left shows the raw FIB-XeF_2 image of a region of Fe9Cr1Mo steel, showing a lighter metal matrix and several precipitates with different greyscale contrasts and morphologies. The subsequent subfigures show in pink, blue, and yellow the segmentation of these features into three distinct populations based on greyscale contrast and morphology that were

identified using TEM diffraction as $M_{23}C_6$, M_7C_3, and M_2C respectively. These segmented features are spatially located datasets, that can subsequently be parsed for the following statistical information: area fraction as a function of distance, distribution, size, orientation, and type. This enables quantification of microstructure over large areas with much greater confidence.
For 316H, precipitate locations were combined with grain boundary locations and compared with voids within the sample to obtain directional correlation information across the sample. Complete results for 316H steel can be found in previous work [5].
For the Alloy 690 sample, FIB-XeF_2 images provided clear microstructural information, such as precipitate pocketing along grain boundaries that was missed in other techniques.
Conclusion
Precipitation is an important factor when considering the chemical and mechanical behaviour of a material, especially for a material exposed to extreme environmental conditions. By re-utilising the XeF_2 gas module most dual beam FIB instruments have, it is possible to bring out the contrast of different precipitates against the bulk in such a way that image processing software can easily extract statistical information. This data will lead to a greater understanding of the behaviour of the material.

Keywords:

XeF_2, FIB, Contrast, Dragonfly

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Towards Automation of the Transmission Electron Microscope

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Poster Group 1

The Transmission Electron Microscope is a powerful tool investigating samples at the nano-Ångström scale. Despite its increasing popularity due to its unmatched spatial resolution, operation of the microscope is time-consuming and tedious. At times, even trivial tasks may require hours of manual operation. Automation has thus become attractive in the field, as it would lower the threshold and workload for advanced material investigations.
Scanning TEM (STEM) differential phase contrast (DPC) is a technique utilizing movement of the centre beam to determine electric and magnetic domains. For a magnetic sample with domain formation, the beam will be deflected in different directions depending on the in-plane magnetic alignment in the sample, see graphic. This slight change can be measured using e.g. a pixelated detector. Nanomagnets with dimensions 225-75 nm are shown to have interesting monodomain properties, being currently widely studied for reservoir computing [1,2].
Problems arise when using the TEM as the magnetic field is typically too big for domain-formation. For this reason, the objective lens must be turned off, reducing the spatial resolution. With the objective lens turned off (so-called Low-Mag), the magnetic field can be estimated to be of the order of tens of millitesla. This is sufficiently low for ASI-samples to show hysteretic behaviour, and in-situ studies of the dynamics can be performed by either tilting the sample, increasing the magnetic field from the objective lens or rotating the sample.
Once the TEM is aligned, the STEM-DPC technique is rather trivial, that is: acquire data, then change the magnetic field, repeat. This experiment is typically referred to as “continuous-tilt STEM-DPC” and can take hours to complete. The task however, is trivial and using human operating time is both inefficient and prone to human errors. This procedure is easy to automate, and allows other in-situ experiments such as varying the temperature over critical regions.
ASI structures will be prepared from TEM samples with 20-50 nm Si windows with top layers of permalloy and aluminium using FIB, creating nano-magnets with mono-domain behaviour. Continuous-tilt STEM-DPC will then be performed with either a MerlinEM direct electron detector or a conventional ADF detector followed by procedural post-processing. This experiment will be a proof-of-concept, and the choice of detector will depend on access to the Merlin detector.
The project aims to gain complete control of both the microscope and the detector- and scanning systems and build a python-library allowing experienced microscopists to automate their own experiments. Creating possibility for feedback-controlled automatic decision-making will also be done. Even further, programmable input-output devices for in-situ experiments will allow scripted exploration of the parameter space of all TEM samples using custom in-situ chips.
Further, given control of the scanning system, random and quasi-random sequences for data-acquisition will be implemented, reducing the necessary acquisition time for sufficient statistic. This procedure will also in particular benefit samples prone to beam damage.

Keywords:

TEM, Automation, in-situ

Reference:

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In situ SEM of slip localization and its relation to the onset of ductile fracture

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Poster Group 2

We present surface deformation measurements by in situ SEM tensile tests. The surface displacement field is obtained by tracking gold nano-droplets formed by laser dewetting. A thin film of gold of a few nm is deposited on the sample surface. The laser fluence and the thickness of the deposit can be varied to modify the diameter of the droplets and their surface density, enabling a fine-tuning of the spatial resolution of the field measurement. Diameters in the range of 10 to 100 nm, densities up to 2000/µm2 can be obtained. The advantage of tracking the center of mass of the droplets with respect to digital image correlation using a correlation window is to limit the smoothing of the displacement discontinuities. Therefore, it is well adapted to the measurement of slip band thicknesses. The field of view of a typical SEM image is of the order of 3 µm. It is enlarged up to 25 µm by automatically acquiring a series of images and stitching them together (Fig. 1). Thus enabling slip system widths as thin as 50 nm to be measured. The tracking of the center of mass of the droplets along the tensile test is done by a homemade code based on the python libraries scikit-image, scipy and run on a computer cluster. It enables a real time image treatment and the acquisition of high magnification images in the regions of interest. For example, the change of shape of the droplets within the thick slip bands or the shearing of droplets by thin slip bands have been observed in situ.
The method is tested on Eurofer 97, a tempered martensite ferritic steel for nuclear application where slip localization might be important for the initiation of ductile fracture.

Keywords:

SEM, Python scripting, tensile tests

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In-situ micro-mechanical tests under SEMs

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Poster Group 2

Background
Materials exhibit varying mechanical behaviors and properties at lower dimensions, a phenomenon often termed as the size effect. Understanding this deformation mechanism and the relationship between structure and properties in nano- to micro-sized materials requires the capability to conduct in-situ mechanical tests under an electron microscope. In this study, we performed tensile and compression tests on synthetic and natural samples using our custom-built tensile tester.

Methods
To enable in-situ mechanical testing under SEMs, we developed a setup integrating a nano-manipulator for displacement control, load cells with various spring constants, and sample clamps mounted on a frame to create a tester stage. This configuration can serve as a plug-and-play accessory for conventional SEMs or environmental SEMs and can be easily adapted for use with optical microscopes..

Results
Our mechanical stage not only facilitates tensile testing but also enables compression testing when equipped with suitable sample holders. Examples such as tensile tests on carbon and cotton fibers, along with compression tests on silk needles, will be provided.

Conclusion
Through the integration of off-the-shelf components, we have built a micro-mechanical testing apparatus capable of conducting tensile, compression, and fracture toughness tests under both SEMs and optical microscopes. With appropriate design modifications to the sample holding fixtures, this device can be converted into a 3(4)-point-bending apparatus. Such a mechanical stage offers versatility in studying the micro mechanics of samples across material and life sciences domains.

Keywords:

In-situ, micro-mechanics, tension, compression

Reference:

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Electron Diffraction on Biological Samples

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Poster Group 2

Background incl. aims
Animals have the ability to generate a diverse array of minerals at various locations within or surrounding their cells. An example of this mineralization process is evident in the development of rigid skeletal structures. Especially skeletal hard parts like bones, teeth, shells, and exoskeletons are prime examples of biomineralization [1]. Carbonate apatite crystals constitute a significant portion of various mineralized tissues in vertebrates. In the mentioned structures, like bones and mineralized tendons, these crystals typically manifest as thin, irregularly shaped plates [2].
Electron diffraction experiments provide valuable insights into lattice parameters and symmetries and sometimes help uncover hidden crystals. Directing electron beams at these samples can explain their crystal arrangements, which helps to understand the mechanical properties of the materials, mineral composition, and integrity. A comprehensive understanding of the composition and characteristics of biomineralizations can be invaluable in diagnosing pathological calcifications, which are implicated in several severe diseases. Identifying and analyzing these crystals in tissues can provide crucial insights into underlying mechanisms and progression of such conditions, aiding in early detection, accurate diagnosis, and effective treatment strategies. Electron diffraction proves instrumental in diagnosing conditions such as Alzheimer's disease, which is characterized by abnormal accumulation of metals in the brain [3].On the bone samples, electron diffraction aids in identifying hard tissue calcification, which compromises elasticity, hardens these tissues and disrupts their function.
Electron diffraction offers a significant advantage in its ability to analyze a much smaller area, typically at the nanometre scale [4], compared to X-ray diffraction, making it feasible to detect locally ordered structures (nanocrystals).
This technique is also generally suitable for examining thin layers, such as 2D crystals. Consequently, this method proves particularly valuable for studying membrane proteins that readily form 2D crystals but face challenges in forming 3D structures [5].

Methods
Low Voltage Transmission Electron Microscopy (LVEM) is a valuable technique in life science and materials research. LVEM operates at lower voltages, typically below 25 kV. This lower voltage regime offers distinct advantages, especially enhanced contrast. When assessing micrograph quality, spatial resolution and image contrast are key criteria. Low voltage improves contrast mechanisms, which is particularly beneficial for samples containing light elements, such as biological specimens. Enhanced image contrast helps to identify suspicious crystal structures in tissues. Moreover, low-voltage electron diffraction has a distinct specific advantage yielding from the dimensions of the Ewald sphere, providing more information [6].
The Low Voltage Electron Microscopes (LVEMs) by Delong Instruments integrate TEM and STEM (including dark fields in both regimes) and SEM (BSE) imaging modes. The LVEMs offer Electron

Diffraction (ED) and eventually Energy Dispersive X-ray Spectroscopy (EDS), which makes them altogether highly versatile tools for material analysis.
The samples were sectioned with 35° diamond knives at a feed of 20-30nm using ultramicrotomy method.

Results
This study performed electron diffraction on selected biological samples, namely manganese-bound bacteria, bones, shells, and urea, and explored the advantages of analyzing biological samples using low voltage electron diffraction. The experiments were carried out using low-voltage electron microscopes LVEM 25E with an accelerating voltage of 25 kV and LVEM 5 operating at approximately 5 kV.

Conclusion
In summary, electron diffraction proves invaluable for analyzing biological materials, offering insights into crystal structures and tissue properties. Particularly, it aids in studying thin layers and 2D crystals, contributing to understanding membrane proteins and the structural complexity of hard biological materials like bones and shells. Additionally, it shows potential in diagnosing diseases such as Alzheimer's. This study highlights the utility of low-voltage transmission electron microscopy in such analyses, providing enhanced contrast and analytical precision for diverse biological samples.

Keywords:

Electron Diffraction, LVEM, biological samples

Reference:

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Measurement of atom mobility of gold nanorods via coarse-sampling in quantitative 4D-STEM

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PS-05 (3), Lecture Theater 3, august 30, 2024, 10:30 - 12:30

Metal nanoparticles have attracted significant interest due to their distinctive properties which show significant potential in applications including drug delivery, chemical sensing, and biological imaging. A common method for synthesizing such nanoparticles is wet chemical synthesis; however, despite decades of development in synthetic methodologies, the precise mechanisms governing symmetry breaking and shape modification of these particles remains ambiguous and subject to debate. It is widely recognized that the introduction of different surfactants affects nanoparticle morphology.

In this work, we aim to study the influence of surfactants on the surface energies of nanoparticle facets. Our approach uses quantitative scanning transmission electron microscopy (STEM) to obtain a measure of surface mobility — as a proxy to estimate surface stability and hence energy [3]. We investigate the optimum set of parameters, including course sampling, for acquisition of a four-dimensional STEM dataset, such that the location and number of atoms in each atom column can be accurately determined while minimizing the electron dose. We quantify dose versus accuracy across these different parameter sets.

We apply these parameters to investigate the relationship between dose and atom mobility on a gold nanoparticle, with the use of double aberration-corrected microscopes (FEI Titan3 80-300 FEG TEM and a newly installed Thermo Fisher Scientific Spectra ϕ FEG TEM) (see Fig.1). We further apply this to measure surface mobilities on different gold nanoparticle morphologies.

This approach aims to offer insights into the fundamental processes underlying nanoparticle synthesis and shape evolution, paving the way for tailored nanoparticle design and optimization in various applications.

Keywords:

4D-STEM, nanoparticles, surface mobility

Reference:

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1047

Cryo-EM as a tool for observing alginate-based hydrogels

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Poster Group 2

Background
Hydrogel is an organic-based material, which finds its use in various fields ranging from well-known employment in medicine (wound treatment, scaffolds,...) to rising involvement in agriculture (superabsorbents, controlled release of fertilisers,...)[1]. While hydrogels containing chemical fertilisers provide valuable nutrients directly, the unused nutrients remain in the soil, where they accumulate, which negatively influences biodiversity, soil fertility, etc [2]. An alternative approach relies on the use of biological fertilisers (bioinuculants) in the form of plant growth-promoting bacteria (PGPRs). PGPRs positively stimulate the growth of plants using several mechanisms (phytohormone production, nitrogen fixation,...), while simultaneously reducing the growth of pathogenic microorganisms or chelating heavy metals [3]. One of the PGPRs is Azotobacter vineladii, a microorganism interesting not only for its plant growth-promoting properties but also for its production of various polymers. Namely polyhydroxyalkanoates (PHAs), biopolymers praised for their properties similar to petrochemical plastics, or alginate, polysaccharide capable of forming a hydrogel. A. vinelandii releases alginate to form a capsule around the cells, which protects them from drying out and from other hostile environmental conditions. The production of alginate is a significant advantage of using A. vinelandii as bioinoculant since there is no need to add the hydrogel-forming polymers to the bacteria for encapsulation, the polymer already in the media is crosslinked and the resulting hydrogel is then processed into the final form of bioinoculant suitable for employment in agriculture [4]. This work aimed to study the morphology of hydrogel formed using different crosslinking agents (namely CaCl2 and glucono-D-lactone), a step necessary to determine the most suitable crosslinker. Since alginate hydrogels are composed of polysaccharides and a substantial amount of water, chemical processing for EM could severely alter the hydrogel ultrastructure. Therefore, cryogenic fixation followed by freeze-fracture and cryo-SEM was proposed to be the most promising technique to study the polymeric net the most closely to the native state.
Methods
Cultures of A. vinelandii were cross-linked using various agents (2% CaCl2, 1M GDL + 0,5M CaCO3). The resulting hydrogels were cut using a scalpel to fit into 6mm carriers for high-pressure freezing and fixed using EM ICE (Leica Microsystems). No cryoprotectant was added. Frozen samples were transferred under liquid nitrogen into a cryo-vacuum preparation chamber (ACE600 Leica Microsystems), where they underwent freeze-fracturing followed by sublimation at -95°C for 7min. Samples containing hydrogel-encapsulated bacteria were then imaged in a scanning electron microscope (Magellan 400/L, FEI) equipped with a cryo-stage, at −120 °C using a 1–2 keV electron beam.
Results
Cryo-SEM imaging revealed cells of A. vinelandii containing polymeric granules, consisting of polyhydroxyalkanoate (PHA). As was previously proved, PHAs in the form of intracellular granules stay elastic even at temperatures of liquid nitrogen. They can be seen as needles sticking out of the freeze-fractured cells, as was visible also for A. vinelandii. Hydrogel encapsulating the cells showed

different structures for both crosslinking agents. While hydrogel formed using CaCl2 showed a net of individually distinguishable fibres, the gel formed by GDL showed a dense mass surrounding cells. The changes in hydrogel ultrastructure seen in cryo-SEM support the difference in the macromorphological structure of the hydrogels visible immediately after cross-linking. Some polymer net was visible also for not crosslinked samples, possibly because of the trance concentration of Ca2+ ions in the cultivation media for the cells. However, the density of the net in the images as well as the overall amount of hydrogel was considerably lower. Similar results, supporting the hypothesis, were also obtained in the preliminary STEM experiments for freeze-substituted samples.

Conclusion

Cryo-SEM together with high-pressure freezing was proven to be a capable method for studying the structure of hydrogels. It was possible to determine the changes in the hydrogel structure based on the type of crosslinker used. Since the preliminary STEM data supported the cryo-SEM findings it is proposed to use the combination of these methods for the evaluation of other types of hydrogels.

Keywords:

Hydrogel, alginate, bacteria, freeze-fracture, cryo-SEM

Reference:

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The effects of B4C network microstructure on the thermoelectrical properties of spark plasma sintered SiC

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Poster Group 1

Thermoelectric (TE) devices convert waste heat to electricity and can be used in industrial waste heat recovery utilizing TE materials which may play a vital role in developing sustainable and renewable energy-harvesting technologies. TE materials should have high electrical conductivity and Seebeck coefficient as well as low thermal conductivity for a high dimensionless figure of merit, ZT, which represents the efficiency of TE conversion. Since these properties are related to each other, e.g. a high electrical conductivity results in a high thermal conductivity value, different composite approaches or doping must be maintained for optimization of these properties. Our recent studies explored the effects of transitional metal carbide/boride network microstructures on the TE properties of SiC and B_4C which revealed simultaneous improvement of electrical and thermal conductivities with conductive network and increased phonon scattering, but deterioration of the Seebeck coefficient due to the metallic nature of the network microstructure. To minimize this problem, a B_4C network microstructure was implemented to the SiC matrix in this study, aimed to increase the electrical conductivity with minimal Seebeck coefficient loss thanks to the semiconductor nature of B_4C.
Polycrystalline bulk samples of SiC with B_4C network structures were prepared by a simple dry particle coating method followed by spark plasma sintering (SPS). 1–8 vol.% B_4C-coated SiC granules were consolidated in an SPS furnace (HP 25D, FCT GmbH, Germany) at 2000°C by applying 40 MPa pressure under a vacuum atmosphere with a 5-minute holding time. Phase and microstructure analyses were carried out on polished surfaces of the sintered bulk samples. Electrical conductivities and Seebeck coefficients of the samples were measured simultaneously by the four-point probe method between 323 and 923 K. Thermal diffusivity and heat capacity values were measured by the laser flash analysis and differential scanning calorimetry, respectively, to calculate the thermal conductivity. Density values were obtained by using the Archimedes method at room temperature.
The implementation of the conductive B_4C network microstructures given in the figure (light phase is SiC matrix, dark phase is B_4C network) improved the electrical conductivity of SiC simultaneously with the decrease in thermal conductivity due to the increased phonon scattering with increased grain boundary concentrations. Optimization of B_4C content resulted in a minimal decrease in the Seebeck coefficient resulting in a composite with increased ZT values and TE performance.
To conclude, SiC granules were successfully coated with 1–9 vol.% B_4C powders and then spark plasma sintered to obtain conductive B_4C network microstructures to improve the TE performance of SiC by simultaneously increasing electrical and decreasing thermal conductivities while limiting the decrease in the Seebeck coefficient to a minimum.
This study was financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (219M514) and The Eskisehir Technical University Scientific Research Project (23ADP195).

Keywords:

SiC, B_4C, Network-microstructure, SPS, Thermoelectric

Reference:

Ozer, S.C., Arslan, K. & Turan, S. Thermoelectric properties of carbide ceramics:a comparative analysis of thermoelectric properties of B_4C, SiC and TiC. Journal of the Australian Ceramic Society 60, 407–418 (2024). https://doi.org/10.1007/s41779-023-00979-4
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Ozer, S.C., Arslan, K. Metin, E. & Turan, S. The effect of in-situ TiB_2 segregated network structure on the thermoelectric properties of spark plasma sintered B_4C ceramics, Journal of the European Ceramic Society, 43 (16), 7508-7515 (2023). https://doi.org/10.1016/j.jeurceramsoc.2023.08.005.

1049

Revealing Nanostructural Dynamics: Exploring Inelastic Scattering in Electron Microscopy

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Poster Group 2

Background incl. aims:
Inelastic electron-matter interactions are pivotal for probing the dynamic behavior and electronic structure of materials at the nanoscale. Electron microscopy techniques like electron energy loss spectroscopy (EELS) and energy-filtered transmission electron microscopy (EFTEM) utilize inelastic scattering as a powerful tool for identifying chemical composition, mapping elemental distributions, and elucidating bonding configurations with exceptional spatial resolution [1,2].
A captivating consequence of inelastic scattering is that each electron beam, arriving at the sample after inelastic collisions, interacts with a different (e.g. excited) state. With inelastic processes like ionization, each collision contributes to the establishment of a plasma-like state within the sample. Consequently, it is pertinent to ascertain the final state of the sample after these interactions. Also, what is generally referred to as radiation damage, which may progressively change the very structure of the sample, is entirely a consequence of inelastic collisions. It becomes then extremely important to determine the state of the sample during and after electron irradiation, including, among many other effects, electron-hole recombination.
In this context, since that the relevant cross-sections are modified alongside the evolving sample, we aim to track the formation and dynamics of new molecules, molecular fragments and generated electrons resulting from inelastic scattering.
Additionally, exploring the effects of structured or shaped beams, adds another dimension to reveal the benefits and hindrances of inelastic scattering in electron microscopy. By employing beam-shaping techniques, it may become possible to modulate and control different scattering channels thereby driving the evolution of the sample.

Methods:
Our study commences with an analytical derivation of the wave function, decomposed in elastic and inelastic partial waves, originating from the electron-hydrogen atom interaction [2,3,4]. This choice has the unique advantage of providing analytical results from beginning to end. We present our findings with a set of plots describing visually the ionization of a hydrogen atom, and the subsequent formation of a secondary electron, highlighting the distinctions between elastic and inelastic scattering.

Results:
Representing the primary beam as a plane wave interacting with the hydrogen atom, we obtain scattered spherical outgoing waves modulated in amplitude and phase by the elastic and inelastic scattering factors. The latter (left side of Fig.1 a) shows a more pronounced forward-scattering angular distribution than the former, and, strictly speaking, should also exhibit a longer wavelength. This occurs because the primary beam loses momentum, transferring it to the hydrogen atom's electron. With an energy loss set at 20 eV in this example, the electron is expelled through ionization

and becomes a secondary. However, being proportional to the momentum transfer, the wavelength variation of the primary remains very small. The ejected electron is also depicted as a plane wave for simplicity, although it should be more correctly represented by a Coulomb wave function [3,4].

Conclusion:
In this preliminary study, we demonstrate how even a simple specimen as a hydrogen atom, undergoes modifications due to electron beam irradiation. The monitoring of any sample is fundamental in electron microscopy to track the alterations occurring in it and to control its behavior. This is crucial, as inelastic scattering leads to heating or damage effects, emphasizing the necessity of comprehending these interactions for proficient sample analysis and manipulation.

Figure caption:
Fig. 1 (a) Elastic and inelastic scattering processes representation. k_i and k_f are the primary initial and final momentum respectively, while q is the transfer one.
ksin is the initial momentum of the electron bounded to the hydrogen atom, while ksfin is that of the ejected electron according to momentum conservation: k_s^in+q= k_s^fin.
The angular distribution of the scattered wave function is broader for elastic collisions than for inelastic ones. This is reflected in the scattering factor f(θ) which appears narrower in the inelastic case (b).

Keywords:

Electron microscopy, Inelastic scattering

Reference:

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1050

Fast mass spectrometry imaging for immunohistochemistry

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Poster Group 1

Immunofluorescence microscopy (IFM) is the current "golden standard" in histopathology and biomedical research. IFM enables simultaneous imaging of multiple fluorescently tagged antibodies, but multiplexing beyond seven different fluorophores is impractical without photobleaching and re-staining, which it may cause sample degradation and difficulties with registering the separately-taken images. Other disadvantages are autofluorescence which can hinder detection of low-abundance proteins and signal bleeding. These disadvantages are absent in mass spectrometry-based approaches. Most mass spectrometry imaging (MSI) immunohistochemistry (IHC) is performed in “microprobe mode” which images pixel-by-pixel [1] and is limited to approximately 1,000 pixels per second, limiting the field of view. To overcome this limitation of typical MSI, fast mass microscopy (FMM) has been developed to acquire pixels, and collect many spectra, in parallel with a continuously moving stage, enabling orders of magnitude faster imaging [2]. Here we describe advancements in FMM for biomedical applications for the detection of multiple isotopically enriched metal tags that enable multiplex antibody panels, thus facilitating studies of multiple proteins in one imaging experiment.

Methods

MIBI staining with metal-conjugated antibodies (CD11b 155Gd, CD3 159Tb, Keratin 165Ho, α-SMA 164Dy) is performed similarly to traditional IFM. Flash-frozen tissue blocks were sectioned with a thickness of 12 µm using a Leica cryostat microtome. Serial sections from the same tissue block were obtained and collected on clean indium tin oxide coated glass slides. The stained human intestinal tissue and mouse tissue sections were analyzed with time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging using a PHI nanoTOF instrument II (Physical Electronics, Chanhassen, MN, USA) with tandem MS capability equipped with a liquid metal ion gun, C60 ion gun, and Ar cluster ion gun. FMM-IHC was done using an instrument based on the TRIFT II mass microscope (Physical Electronics, Inc. (PHI) Chanhassen, MN, USA) and equipped with a C60 ion beam (IOG C60-20S, Ionoptika, Chandler’s Ford, UK), with a Timepix3 ASIC-based camera (TPX3CAM, Amsterdam Scientific Instruments, Amsterdam, NL).

Results

The acquired ToF-SIMS and FMM mass spectral images of biological tissue show promise for antibody detection comparable to those observed using standard microscope-based workflows. Using IMS-IHC, it was possible to visualize elemental ions in fresh-frozen tissue sections with a high spatial resolution. The results were correlated with adjacent serial sections using optical microscopy and standard histological staining (H&E staining). A proof-of-concept study demonstrated the use of FMM as a tool for imaging by measuring the unfrosted part of a microscopy slide (23.5 x 40 mm) in under 4.5 minutes, at a pixel size of 900 nm.

Conclusion

The first steps of implementing FMM as a technique for MSI-IHC experiments instrumentation are presented. The elemental-mass-based multiplexed analysis, used to visualize protein expression on fresh frozen tissue, bypasses the limitation of both the traditional staining techniques relying on

optical absorbance or fluorescence signals and mass spectrometry imaging performed in "microprobe mode".

Keywords:

mass spectrometry imaging, mass microscopy

Reference:

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2. Körber, A., Keelor, J. D., Claes, B. S. R., et al. 2022 Anal. Chem. 94, 14652–58.

1051

Multiscale 3D organization of human auditory ossicles unveiled by synchrotron small-angle X-ray scattering tensor tomography

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background

The transmission of sound through the human middle ear is a complex process that relies on the biomechanical properties of the ossicular chain, comprising the malleus (hammer), incus (anvil), and stapes (stirrup). These ossicles are the smallest bones in the human body and are responsible for amplifying and efficiently transmitting sound vibrations from the tympanic membrane to the cochlea [1]. Their biomechanical properties are intricately linked to their structure, spanning from the molecular scale (e.g., collagen triple helix) to the millimetric scale (e.g., foramen).
In cases of conductive hearing loss resulting from an impaired ossicular chain, reconstructive surgeries are necessary to restore hearing function. In these procedures, the incus plays a crucial role as either a support for prostheses or as a material to be sculpted itself, serving as an autologous passive implant [2]. However, the optimal positioning of prostheses on the incus and the effects of the carving process on incus biomechanics remain largely unknown and would strongly benefit from an improved knowledge of the ossicles' organization across several length scales.
The ossicles display a hierarchical bone organization, beginning with collagen triple helices that further assemble into fibrils with a periodic d-spacing of approximately 67 nm. These fibrils are mineralized by hydroxyapatite (HA) forming mineral platelets that mostly align with collagen fibrils. However, the shape of these nano-mineral particles and the orientation of collagen fibrils across a significant volume of ossicle, as well as their interactions with the vascular and nutritional foramen network, remain largely unclear to date.
Recently developed Small-Angle X-ray Scattering Tensor Tomography (SAS-TT) has emerged as the preferred method for 3D nanostructural characterization of mineralized tissues like bone over extended spatial regions. SAS-TT combines principles from Small-Angle X-ray Scattering (SAXS), which provides insights into anisotropic non-crystalline structures at sub-um scales, with X-ray tomography, enabling 3D volume measurements. The complete 3D reciprocal space (scattering directions) is reconstructed for each voxel, leading to detailed information about the 3D main orientation and degree of orientation of the set of nano-structures within each voxel.
In this study, our objective is to elucidate the organization of nano- and micro-structures within the human incus, clinically the most crucial auditory ossicles in surgery, using SAS-TT. We aim to gain insights into the preferential orientations induced by sound propagation and identify potential sites of bone remodeling. Additionally, we aim to understand the effect of the sculpting process and erosive pathologies on the inner organization of the incus body.

Methods

Our study includes 5 human incudes in various conditions: 3 healthy, 1 sculpted during ossiculoplasty, and 1 pathological with cholesteatoma, obtained from anonymous donors. The samples were further cut and milled down to a size of approximately 2.5 x 1.5 mm2 each. In the end, the long crus of 2 healthy incudes, and 3 sub-volumes from the body of 1 healthy incus, 1 sculpted incus, and 1 pathological incus, were examined using SAS-TT. The experiments were conducted at the PXI beamline of the SLS (Swiss Light Source, PSI), using a focused beam of 25 x 25 µm² at 12.4 keV and a 4M EIGER detector enabling acquisitions at 300 Hz, resolving a wide energy range. Detector data were reintegrated into 32 azimuthal bins in the range of q = 0.014 – 0.36 Å−1 and symmetrically averaged to eliminate detector gaps. The remaining 16 azimuthal bins were used as input for the SAS-TT reconstructions using the mumott Python library (https://mumott.org).

Results

First results were obtained on the long crus of 2 human incudes. The samples comprised the long process followed by the lenticular process that articulates with the stapes, the last bone of the ossicular chain. We were able to extract the main orientation of the mineralized collagen fibrils and their anisotropy, enabling the identification of 3 regions along the long process that exhibit a higher degree of orientation. These 3 regions with anisotropic collagen arrangement are located along the main vascular channel in the center of the long process, and converge at the bony pedicle, the junction between the long and the lenticular processes.
Further q-resolved analysis revealed the presence of three distinct phases corresponding to various shapes and sizes of mineralized particles within the incus, specifically distinguishing the inner part of the long process from the annular shell around the lenticular process.
Subsequently, we conducted a comparison of 3 incus bodies in various conditions (healthy, pathological and sculpted). The distribution of orientations and anisotropy were extracted for each condition. While no significant differences were observed between the healthy and sculpted samples, the incus body affected by cholesteatoma exhibited a more isotropic collagen fibril organization, with a broader orientation distribution across the sample and lower local degrees of orientation. Additionally, we used the mean intensity as a measure of the mineral density to quantify the prevalence of low-density tissue in the pathological case, suggesting that bone remodeling occurred at different sites.

Conclusions

SAS-TT enables access to structural parameters at both nano- and micro-scales throughout the entire volume of samples. When applied to investigate human auditory ossicles, it revealed anisotropic pathways at the collagen fibril level, which may play a crucial role in guiding sound waves through the bone. Additionally, it identified regions with diverse nano-structuring, pinpointing sites of potential bone remodeling. Furthermore, it shed light on changes occurring in the bone in cases of erosive pathologies such as cholesteatoma. These findings could provide valuable insights for optimizing middle-ear surgery, with wide-ranging benefits for patients with conductive hearing loss.

Keywords:

X-ray Scattering, Tomography, Hearing Bones

Reference:

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Carbonic anhydrase immobilization for microscopic investigation of enzyme activity

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Poster Group 1

Background incl. aims
Carbonic anhydrase (CA) is an efficient catalyst for CO2 absorption in solvent-based carbon capture. 1 However, replenishment of CA could be required, because the solvent regeneration temperature is often higher than the enzyme's thermal tolerance, leading to CA deactivation. Alternatively, immobilizing the enzyme on solid carriers can improve its stability and longevity, allowing for catalyst recovery and reuse, and reducing overall costs. 2,3 We are developing immobilization techniques to address this challenge, which calls for high-resolution visualization techniques to design the distribution of enzymes immobilized at condensed matter interfaces and optimize their accessibility with gases and liquids. 4

Methods
We use transmission electron microscopy (TEM) and fluorescent microscopy (FM) to investigate the interaction of the immobilized enzyme with the support nanostructure and substrate, with the aim of uncovering attributes that maximize the biocatalytic interaction.

Results
Various immobilization techniques were employed to achieve the desired surface coverage of the CA enzyme on carbon nanotubes. Two different methods were used - secondary π-π interaction and EDC/NHS covalent coupling. After fluorescent labeling and immobilization, the enzymes were found to be active. High-resolution TEM and fluorescent microscopy were used to further characterize the immobilization process, which showed a high enzyme coverage and activity on the surface.

Conclusion
With the systematic development of immobilization chemistry and imaging techniques, we would unlock new insights into the dispersion and structural aspects of immobilized enzymes.

Keywords:

carbonic anhydrase,TEM, fluorescent microscopy

Reference:

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Exploring Local Order/Disorder in Relaxor Ferroelectric Materials via TEM and STEM Methods

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Poster Group 1

Background incl. aims
Perovskite relaxor ferroelectric materials are very interesting for a variety of applications due to their extraordinary piezoelectric properties. However, understanding the complicated local order/disorder phenomena within these materials is crucial for optimizing their performance. Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) techniques were employed to study the local order/disorder phenomena in (1-x)$Na_{1}/_{2}Bi_{1}/_{2}TiO_3$-x$BaTiO_3$ (NBT-BT) compositions with varying $BaTiO_3$ concentration (x = 2.5, 7.5, and 15 mol%).

Methods
The samples with mol fractions of 0.025, 0.075, and 0.15 were prepared using conventional synthesis methods for mixed oxides. High-purity powders of Bi_2O_3, Ti_2O, Na_2CO_3, $BaCO_3$, and K_2CO_3 were combined in stoichiometric ratios and then milled in a planetary mill at 250 RPM for 2 hours. After drying, the mixture was calcined three times at 920°C for 5 hours to ensure the formation and purity of the perovskite-type phase. The resulting powder was ground again and passed through a 100 µm sieve. Afterward, the powders were compacted uniaxially and isostatically at 180 Mpa pressure into discs, then sintered at 1080°C for 10 hours in a powder bed of the same composition to minimize element volatilization. Cross-sectional samples were prepared using the ThermoFischer Scios 2 DualBeam in the Scientific and Technical Resources Service of the Universitat Rovira i Virgili.
Our research was aimed at understanding the chemical composition, domain configurations, and structural evolution of these materials. The crystalline structure was analyzed by selected area diffraction (SAED); local changes in lattice parameters were monitored using HAADF-STEM and the presence of polar nanodomains (PNRs) was assessed by iDPC imaging techniques. The samples were observed in a ThermoFisher SPECTRA (S)TEM 300 at 300 kV (ALBA synchrotron), in a JEOL 2010F TEM, and in a JEOL 2100 TEM, both working at 200 kV (Scientific and Technological Centers of the Universitat de Barcelona).

Results
Our findings showed the presence of various phases within the samples. For lower x value, R3c symmetry coexisted with Pm-3m, but higher BaTiO3 ratios resulted in a transition from Pm-3m to P4bm symmetry. For the sample with x=0.15 mol, only P4bm symmetry was observed. Conversely, for x=0.025 mol, R3c and Pm-3m symmetries were detected, and for x=0.075 mol, all three symmetries were observed in distinct grains. The analysis of the lattice parameters through HAADF and iDPC images also showed the transition from a rhombohedral phase for x=0.025, to a cubic phase

for x=0.075, and to the tetragonal phase for x=0.15 mol. Polarization maps from iDPC technique will highlight the presence of PNRs. Electron Energy Loss Spectroscopy (EELS) also exhibited particular features in the spectra when increasing the $BaTiO_3$ ratio. Low-loss energy loss spectroscopy was also conducted to observe variations in the optical response and to compare them with Density Functional Theory (DFT) simulated data of $BaTiO_3$, $BiTiO_3$, and $NaTiO_3$ using the Vienna Ab initio Simulation Package (VASP).

Conclusion
This study explores the general panorama of domain configurations and structural complexities in these materials, as well as the spectroscopic properties observed, including the dielectric function and ELF, as the $BaTiO_3$ ratio is incrementally increased.

Keywords:

Relaxor-ferroelectrics, TEM, STEM, NBT-BT

Reference:

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Structural characterisation of a phage-like bacteriocin from Pseudomonas sp. by cryo-Electron Microscopy

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Poster Group 1

Background incl. aims
Antibiotic resistance is a global health crisis with the ever growing need to develop novel antibiotics and strategies to treat resistant infections. Bacteriophage therapy is often highlighted as an alternative approach due to its high specificity to kill a certain bacterial strain. However, bacteriophage propagate through a replication cycle within the target bacterium, with the potential to generate mutations with detrimental consequences. Strains of Pseudomonas sp. produce phage tail-like bacteriocins (PTLBs) which have evolved from bacteriophage. Although sharing many similarities, they differ from bacteriophage lacking a capsid and therefore the ability to replicate. These unique features highlight the potential of PTLBs as an alternative therapy to treat bacterial infections as they can be tittered to a specific dose. However, for PTLBs to be implemented as a bactericidal treatment, further information is needed regarding their structure, mechanism of action and how they recognise their target strains. We have isolated a contracting PTLB from an environmental strain of P. veronii and determined its structure by cryo-EM.

Methods
The PTLB was purified by ammonium sulphate precipitation and visualised by cryo-EM. The structure of the PTLB was determined using a combination approach of single particle and helical analysis.

Results
The structure was determined of a new clade of contracting PTLBs in both its uncontracted and contracted states. We also identified the lack of a ‘ruler protein’ for the purified PTLBs, observing varying lengths in the collected micrographs.

Conclusion
We solved the structure of a novel contracting PTLB and show that it shares structural similarities with the previously characterised contractile nanomachine from P. aeruginosa. We also observe that inconsistent lengths of PTLBs does not appear to affect the lethality of the PTLB to its target strain.

Keywords:

Bacteriocin, filament, cryo-EM

Reference:

Carim, S. et al. ISME J 15, 2289–2305 (2021)

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Structural characterization of partially relaxed hybrid radial (Pb,Sn)Te/WZ-GaAs nanowires as candidates for topological insulator nano-devices

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Poster Group 2

Background incl. aims
Topological crystalline insulators (TCIs) belong to extensively investigated research area of topological quantum matter. The interest in TCIs stems from the presence of topologically protected Dirac states on high symmetry surfaces of TCI crystals. (Pb,Sn)Te solid solution is one representative of TCIs, with topological phase occurring above some critical Sn content. Since topologically protected states in TCIs are surface properties we have studied this material in quasi one dimensional nanowire (NW) geometry providing high surface-to-volume ratio. To further enhance the surface-related properties we have chosen to investigate core-shell NW heterostructures with wurtzite (WZ) GaAs cores and (Pb,Sn)Te shells instead of uniform NWs.
Methods
Core-shell NW heterostructures have been grown by molecular beam epitaxy using two distinct MBE systems dedicated to III-V, and IV-VI semiconductors. The interface structure of WZ-GaAs/(Pb,Sn)Te NWs is investigated using wide range of characterization techniques, such as high resolution transmission electron microscopy (HR-TEM), scanning transmission electron microscopy (STEM), geometric phase analysis (GPA) and energy dispersive x-ray spectroscopy (EDX).
Results
Misfit dislocations are observed as a direct result of the lattice mismatch between the core and the shell materials. Measured distances of moiré fringes match calculated spacings of misfit dislocations observed to be higher than the calculated values, suggesting the presence of a residual strain within the structures.
Conclusion
We have shown that (Pb,Sn)Te can be successfully grown as continuous full or half-shells on the sidewalls of GaAs NWs. This provides opportunity for investigation of topological surfaces of TCIs in the tubular geometry.
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Keywords:

TEM, Nanowires, Topological Materials

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1056

Histopathologic Alterations of Cerebellum in the VPA-Induced Autism Model of Rats

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Poster Group 1

Background
Autism Spectrum Disorder (ASD), classified under neurodevelopmental disorders, is characterized by persistent deficits in social communication and interaction, along with repetitive behavioral patterns. Histomorphological changes occur in various brain regions in ASD. This study aims to investigate pathophysiological alterations in the cerebellum of rats with valproic acid (VPA)-induced autism model.
Methods
Adult female and male Sprague-Dawley rats were allowed to mate overnight. Rats with confirmed vaginal plugs the following day were considered pregnant, and embryonic day 0 (E0) was recorded. VPA (500 mg/kg) was injected intraperitoneally (i.p.) on embryonic day 12 (E12). On postnatal day 21 (P21) the genders of the offspring were determined and weaned. Rats were sacrificed at P46, and cerebellar tissues were collected. The sections were firstly stained with cresyl violet, and then random micrographs of the cerebellar cortex were captured from three serial sections at three different areas for each section. Afterward, the number of Purkinje cells was divided by the line measurement expressed as µm between the molecular and granular layers.
Results
In light microscopic examination, the number of Purkinje cells per unit length was significantly lower in the VPA-treated groups compared to the control groups in both sexes ($p<0.001$) (Female-control:0,0296±0,00263, male-control:0,0286±0,00365, Female-VPA:0,0224±0,00280, Male-VPA:0,0216±0,00254). There was no statistically significant difference between genders.
Conclusion
The number of Purkinje cells in cerebellum decreased in VPA-induced autism model. Additionally, there was no difference between genders. These results may consider that autism is also related with Purkinje cells.

Keywords:

Autism Model, cerebellum, Purkinje cell

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Accessible low-cost, long range, optical autofocus module for open-source multiwell plate and slide scanning microscopy

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Poster Group 1

Background incl. aims
We have previously presented novel optical autofocus modules that simultaneously provide extended range of operation (>100 µm) and high precision (<600 nm) using machine learning [1] in a 2-step approach or providing closed loop "single-shot" operation over up to ±37 µm with <50 nm accuracy [2]. These levels of performance are realised by focusing an infrared laser beam onto the microscope coverslip with the back reflection being imaged on a dedicated autofocus camera. We derive a metric that quantifies defocus from the size of the light distribution at the autofocus camera that can be independent of laser power and insensitive to drift in the optical alignment. The operating range and precision depend on the confocal parameter of the autofocus laser beam after being focused by the objective lens. This can be adjusted by changing the diameter of the autofocus laser beam incident at the objective lens. By contriving a different beam diameter in two orthogonal planes using either a rectangular aperture [1] in the collimated autofocus laser beam, or using different orthogonal cylindrical lenses [2] to collimate the autofocus laser beam emerging from the single mode fibre that delivers it to the autofocus module, we can maximise precision (with maximum beam diameter) and extend operating range (reducing orthogonal beam diameter), making both measurements simultaneously by resolving the autofocus camera image along orthogonal directions.
While these two approaches can provide months of stable operation, they each have their drawbacks. The machine learning approach with the rectangular apertured beam [1] requires a convolutional neural network to be trained to determine magnitude and sign of defocus from the autofocus camera image and we found it necessary to train it over ~10 days to make it independent of any system variations impacting the autofocus camera image. For the second approach [2], we slightly offset the collimation of the cylindrical lenses such that the measured defocus is different for the two planes defined by the orthogonal cylindrical lenses, and this enables the magnitude and sign of the defocus to be calculated from a single autofocus camera image following calibration of the system. However, while the system reported in [1] utilised a low-cost single-mode fibre (SMF)-coupled laser diode, we used a superluminescent diode (SLD) in the system reported in [2] since its performance was impacted by interference between the autofocus laser beam reflected from the coverslip and unwanted beam(s) reflected from other surfaces in the optical system. Using the SLD removed this interference. Unfortunately, SLDs are significantly more expensive than laser diodes, and availability can be intermittent. Accordingly, we are redesigning the optical system and analysis method to enable the closed-loop approach of [2] to be used with a simple fibre-coupled diode laser for implementation in slide scanning and automated multiwell plate microscopy.
Methods
We determined that the primary source of unwanted back reflections of the autofocus laser beam were from the microscope objective lens, and we modified the optical system such that the curvatures of the unwanted back-reflected wavefronts are different from the desired autofocus

beam reflected from the coverslip. Utilizing a modified background subtraction and signal processing algorithm we were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens that was controlled using MicroManager [3]. To independently measure the performance of the autofocus system, we configured the microscope for brightfield transillumination imaging of a USAF test chart and imaged the edge of a bar to derive a metric of defocus from the steepness of the gradient of this edge. We are also working on a fluorescent bead image-based approach utilising machine learning to determine defocus from a single bead image for real-time monitoring.

Results

We were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens. When imaging a test chart in transillumination, focus was maintained in closed loop within 200 nm over 5000 seconds – and within < 50nm over 500 seconds. The autofocus system can recover focus with single-shot operation within a range of ~60 µm and up to ~80 µm in a multi-step mode [2]. We are working to improve the autofocus precision and range and are cross-validating the measurement of defocus between the autofocus readout, the transillumination edge measurement and the machine learning approach applied to bead images. We will also explore using higher power multimode laser diodes that exhibit shorter coherence lengths [4] although these present additional laser safety considerations.

Conclusions

We have demonstrated that we can implement an optical autofocus using a low-cost diode laser that can provide closed loop “single-shot” operation and is suitable for multiwell plate imaging and slide scanning. This is important for the development of cost-effective instrumentation, including modular openFrame-based instruments for pathology and high content analysis. We will present the latest design together with methods to independently validate the correction of defocus.

Keywords:

Optical microscopy, autofocus, slide-scanning, characterisation

Reference:

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Investigation of self-assembly dynamics of magnetic nanoparticles in liquid phase by transmission electron microscopy

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Poster Group 2

Background incl. aims
Magnetic fluids, colloidal suspensions of magnetic nanoparticles (MNPs), exhibit intriguing self-assembly behaviors that are essential for numerous applications in biomedical, engineering, and artistic fields. Despite numerous simulations, experimental insights into the nanoscale self-assembly process in liquid phases remain scarce. The present study aims to address this deficit by employing advanced transmission electron microscopy (TEM) techniques to elucidate the intricate relationship between the self-assembly phenomena and the magnetic properties in magnetic fluids, in particular in those composed of iron oxide nanoparticles.
Methods
Our investigation involves a multi-scale approach using state-of-the-art TEM methods. First, in-situ observations of magnetic field-induced chaining are conducted using liquid cells, allowing real-time visualization of the self-assembly dynamics. Subsequently, advanced characterization techniques will be employed. These include off-axis electron holography to analyze the magnetic and structural properties of MNP assemblies, and precession-assisted 4D-STEM for orientation mapping. Quantitative measurements of magnetic property distributions based on first-order reversal curve (FORC) diagrams obtained by electron holography will also be performed. With a special focus on electron holography data, the development of software tools for automated data acquisition and analysis is described.
Results
Preliminary results show the successful characterization of self-assembly processes by cryo-TEM. The formation of diverse structures such as chains, rings and networks is demonstrated. Statistical analysis shows excellent agreement between experimental observations and Monte Carlo simulations. Electron holography measured on several assemblies suggests a competition between magnetic dipolar interactions and magnetic anisotropy energy, which deeply affects our understanding of the self-assembly process. In addition, we were able to establish a relationship between macroscopic magnetometry and nanoscale structural organization by combining experimental and simulated FORC diagrams. This is a promising approach for the measurement of magnetic nanostructures with different geometries and chemical compositions.
Conclusion
In summary, our study demonstrates the ability of advanced electron microscopy techniques to resolve the intricate dynamics of self-assembly processes in magnetic fluids. By studying magnetic interactions and structural organization at the nanoscale, our results provide insights that can aid in the efficient design and optimization of magnetic fluid-based technologies. In addition, the development of software tools for data acquisition and analysis offers potential improvements in the accessibility and applicability of advanced TEM methods in the study of magnetic nanomaterials.

Keywords:

Magnetic fluid, holography, FORC diagrams

Reference:

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3D reconstruction and temporal development analysis of dendritic spines

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Poster Group 2

Background
Recent advancements in live cell microscopy have significantly improved our ability to study the dynamics of dendritic spines—membranous protrusions with a role in synaptic transmission (1,2). Even though several spine analysis tools have been published, it is still difficult to satisfyingly capture and analyze both spatial and temporal aspects of the data. To address this limitation, we developed Irbis, a new comprehensive software tailored for analyzing live cell fluorescence microscopy images of dendritic spines.
Methods
The software was designed to process 3D/4D fluorescence microscopy data. This data is used to create a 3D reconstruction, that is subsequently segmented into individual spines. To create the 3D reconstruction, several algorithms from 3D modelling, medical imaging and LiDAR technologies are utilized, among others Marching cube algorithm and Poisson surface reconstruction (3).
To be able to separate individual spines from the rest of the dendrite, we developed a method for approximation of the dendritic shaft. The approximation model is combined with the 3D reconstruction of the whole dendrite and used to separate the spines. Subsequently, individual spines are matched across time and their temporal dynamics can be analyzed.
The software was developed in Matlab with incorporation of Python libraries, such as PyMeshLab for 3D mesh processing (4).
Results
Irbis is a software tool capable of 3D reconstruction of the dendrites and subsequent separation and matching of dendritic spines over time. The resulting 3D model facilitates intuitive understanding of the biological system, as well as serving as a basis for subsequent quantitative analysis.
The software is designed as a standalone application with a user-friendly graphical user interface. Its design simplifies complex data analysis tasks, making it accessible to researchers without requiring any coding skills.
Conclusion
Irbis effectively bridges the gap in current analytical capabilities by providing robust tools for detailed 3D and temporal analysis of dendritic spines. This enables a deeper understanding of spine dynamics and their role in synaptic transmission. Its ease of use makes Irbis a valuable tool in neuroscience research.

Keywords:

dendritic spines, 3D-reconstruction, data-analysis software

Reference:

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Fast large-area EDS characterization of additive manufactured steels

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Poster Group 2

Fast large-area EDS characterization of additive manufactured steels

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Background

Powder Plasma Arc Additive Manufacturing (PLAAM) is a state-of-the-art approach for producing large-scale metallic samples with targeted geometry. One of this technology's defining features is its ability to produce samples with variable chemical composition.

However, further developments in novel additive manufacturing processes, such as PLAAM, require further development in characterization concepts and methodologies. Due to varying alloy composition, unknown process-microstructure-property relations and complex time-temperature-location profiles for each specimen, which result in evaporation, dilution and diffusion, there is a need for a method for time-efficient large-area characterization to become statistically relevant.

Methods

Steel samples were manufactured using the PLAAM process. A layer of steel with the length of 1 meter was welded onto a steel substrate. The chemical composition of the sample was varied gradually from a composition of a low alloyed Carbon Steel to a highly alloyed stainless steel (316L).

Using a Flatquad detector (Bruker®), a detailed energy dispersive spectroscopy (EDS) study was performed to investigate the varying chemical composition in the PLAAM steel samples. Thereby, the SEM electron beam scanning strategy was tested, and has been verified in respect to the results when characterizing the entire sample. In detail, the scanning strategy and parameters were optimized for analyzing for the varying chemical composition along and perpendicular to the PLAAM build direction as well as at the steel substrate interface with HT 20kV, current source 20 nA and dwell time 15µs. Additionally, the effects on diffusion from the thermal history of the build platform were analyzed.

Results

Optimization of the SEM beam scanning strategy and measurement system resulted in findings that were statistically consistent with large-area characterization of the samples using long dwell times (i.e., slow image acquisition).

The use of shorter dwell times, i.e. faster large-sample-area EDS mapping, significantly shortens the experimental time while introducing less significant statistical noise.

Further insights into the effects of local composition control were gained to further optimize the PLAAM process.

Conclusion

Using the Flatquad EDS detector allowed for a higher collection angle compared to normal angled EDS. Combined with an optimized SEM beam scanning strategy, we were able to significantly reduce measurement time required for large-area measurement of PLAAM samples and increased through-put to gain statistically relevant insights into the process-microstructure-property relationship of PLAAM structures.

Keywords:

EDS, Additive Manufacturing, Metals Characterization

Reference:

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Novel nanobody-based tools for studying the synaptic vesicle life cycle

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Poster Group 1

Synaptic vesicles are important organelles in neurotransmission and their precise composition is essential for information transfer from neuron to neuron. While many aspects about their functions at the synapse are known, open questions remain regarding how these stoichiometrically highly-precise organelles are formed and maintained to mature to functional SVs that are able to release neurotransmitters at the synapse. To accurately study SV biogenesis, it is important to maintain the highly-precise stoichiometry of SV components in place and untouched. This renders experiments involving overexpression of a particular SV protein of difficult interpretation and instead requires endogenous expression.
One powerful approach to access endogenous localization, distribution and movement of proteins are intrabodies: small probes based on high-affinity nanobodies that can be expressed inside the cell. Here, I present the characterization of an intrabody against the SV Ca2+-sensor Synaptotagmin-1 (Syt1), that was named iNbSyt1. As shown in our recently published paper (https://doi.org/10.1002/smtd.202300218), this intrabody enables not only the direct live imaging of SVs (mScarlet-/mNeonGreen-iNbSyt1), but also allows to detect single action potentials thanks to a highly sensitive synaptically localized Ca2+-sensor, jGCaMP8s-iNbSyt1. Meanwhile, expression of iNbSyt1 does not affect vesicle mobility, synaptic localization, or fusion capacity, which makes this highly modifiable tool optimal to study synaptic processes in living neurons without genetic perturbation of the SV protein target. Likewise, we show that the underlying nanobody also is an ideal probe for several super-resolution imaging techniques, including STED microscopy, Structured Illumination Microscopy (SIM), DNA-PAINT and Expansion Microscopy.
Overall, the NbSyt1 is a versatile small imaging probe that offers adaptation to divers experimental requirements and will find a broad use in the microscopy field.

Keywords:

neuroscience, nanobody, synaptic vesicle, live-imaging

Reference:

Queiroz Zetune Villa Real K, Mougios N, Rehm R, Sograte-Idrissi S, Albert L, Rahimi AM, Maidorn M, Hentze J, Martínez-Carranza M, Hosseini H, Saal KA, Oleksiievets N, Prigge M, Tsukanov R, Stenmark P, Fornasiero EF, Opazo F. A Versatile Synaptotagmin-1 Nanobody Provides Perturbation-Free Live Synaptic Imaging And Low Linkage-Error in Super-Resolution Microscopy. Small Methods. 2023 Oct;7(10):e2300218. doi: 10.1002/smtd.202300218. Epub 2023 Jul 8. PMID: 37421204.

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Correlative TOF-SIMS/SEM for subcellular molecular profiling of snow microalgae

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Poster Group 1

Background incl. aims
Visualizing the metabolism within its structural context is key to understanding the biological phenomena at stake. The microalga Sanguina nivaloides was recently identified as the responsible fopr the reddish color on snowfields, notably in the Alps. This organism is yet uncultivable in laboratory conditions, rendering most classical 'bulk' metabolomics analysis largely impossible. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables the combined visualization of elements and molecules and is becoming more and more popular in the field of life sciences, especially for the investigation of tissue samples. Even though the ultimate lateral resolution of TOF-SIMS ought to be sufficient for subcellular imaging, very few studies have yet pushed the lateral resolution that far.
Methods
In this context, we have investigated how the subcellular architecture and molecular composition of Sanguina nivaloides is affected by the extreme environment it thrives in. For this purpose, we have performed TOF-SIMS imaging, combining both high lateral resolution (up to 100 nm) and high mass resolution (up to 9,000) over resin-embedded specimen using a NanoTOF II (ULVAC-PHI) instrument. We also performed over the same zone correlative SEM (10 nm) in order to identify subcellular compartments and used this image to segment the hyperspectral TOF-SIMS dataset. We are thus combining the strength of both techniques and increasing the resolution of TOF-SIMS imaging using SEM-derived information.
Results
In these algae, we have been able to locate proteins, carbohydrates, micronutrients, etc. across several subcellular features, including cell walls as thin as ~ 100 nm, nuclei, pyrenoid, starch and chloroplasts. High molecular weight molecules are being detected in the lipid droplets, however the sample preparation induced molecular damage does not allow for the identification of these species. Interestingly, the bacteria located around the cells display a higher phosphate content than the cells themselves. Our study also reveals the presence of cell wall specific compounds, while most of the micronutrients with the exception of phosphate and calcium are preferentially located in the mineralized particles located around the cells, hinting for a possible way that the alga collects micronutrients from its environment.
Conclusion
We have been able to perform molecular profiling of the microalga S. nivaloides at the organelle scale and revealed how this organism is allocating its resources to the different compartments so as to bloom in a cold and nutrient-deprived environment. We show that our approach of correlating high lateral and mass resolution TOF-SIMS with SEM is an effective manner to decipher the organelle content of microorganisms.

Keywords:

mass spectrometry imaging microalgae TOF-SIMS

Reference:

Ezzedine, Jade A., et al. "Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging." Nature Communications 14.1 (2023): 7500.

1064

Correlative TOF-SIMS/SEM for subcellular molecular profiling of snow microalgae

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Poster Group 2

Background incl. aims
Visualizing the metabolism within its structural context is key to understanding the biological phenomena at stake. The microalga Sanguina nivaloides was recently identified as the responsible fopr the reddish color on snowfields, notably in the Alps. This organism is yet uncultivable in laboratory conditions, rendering most classical 'bulk' metabolomics analysis largely impossible. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables the combined visualization of elements and molecules and is becoming more and more popular in the field of life sciences, especially for the investigation of tissue samples. Even though the ultimate lateral resolution of TOF-SIMS ought to be sufficient for subcellular imaging, very few studies have yet pushed the lateral resolution that far.
Methods
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Results
In these algae, we have been able to locate proteins, carbohydrates, micronutrients, etc. across several subcellular features, including cell walls as thin as ~ 100 nm, nuclei, pyrenoid, starch and chloroplasts. High molecular weight molecules are being detected in the lipid droplets, however the sample preparation induced molecular damage does not allow for the identification of these species. Interestingly, the bacteria located around the cells display a higher phosphate content than the cells themselves. Our study also reveals the presence of cell wall specific compounds, while most of the micronutrients with the exception of phosphate and calcium are preferentially located in the mineralized particles located around the cells, hinting for a possible way that the alga collects micronutrients from its environment.
Conclusion
We have been able to perform molecular profiling of the microalga S. nivaloides at the organelle scale and revealed how this organism is allocating its resources to the different compartments so as to bloom in a cold and nutrient-deprived environment. We show that our approach of correlating high lateral and mass resolution TOF-SIMS with SEM is an effective manner to decipher the organelle content of microorganisms.

Keywords:

high resolution mass spectrometry imaging

Reference:

Ezzedine, Jade A., et al. "Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging." Nature Communications 14.1 (2023): 7500.

1065

Innovative Microfluidic chip for Raman spectroscopy and advanced electron microscopy techniques

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Poster Group 1

Background
In microbiology research, there is a growing need for equipment that allows the study of biological samples with high resolution and preservation of their structural integrity. In this article, we present a new device, a microfluidic chip, designed for high-pressure freezing (HPF) of biological samples, freeze-fracture and their subsequent analysis using a combination of Raman spectroscopy and focused ion beam scanning electron microscopy at cryogenic temperatures (cryo-FIB-SEM). Microfluidic chips, created by microfabrication techniques, manipulate with a small amount of fluid volumes using intricately designed channels and chambers. Their precision and efficiency offer advantages over traditional methods, leading to the applications in bioanalysis, medicine, and environmental monitoring. Ongoing research focuses on enhancing chip design and integration with complementary technologies to extend their capabilities further [1]. Microfluidic chip technology has revolutionized fluid handling and analysis at a miniature scale, offering unprecedented control over fluidic processes through intricate networks of microchannels, chambers, valves, and pumps fabricated using advanced microfabrication techniques. This miniaturization enables precise manipulation of small fluid volumes, leading to applications in bioanalytical chemistry, biotechnology, medicine, and environmental monitoring.
The cryo-fixation of the microfluidic chips is very limited by a thickness of the material (sample) that must be frozen [2]. The only known microfluidics freezing system used for cryo-fixation of cells was published in 2014 using direct freezing in the optical microscope. The use of HPF presents the possibility of rapid freezing of even thicker samples (up to 600 nm) with subsequent observation by electron microscope under cryogenic conditions.
In our study, we present the application of a microfluidic chip in the research of microorganisms producing Polyhydroxyalkanoates (PHAs), which have the potential to serve as an alternative option to petrochemically produced plastics [3].

Methods
The microfluidic chip is designed primarily for frozen biological samples using a combination of Raman spectroscopy and focused beam scanning electron microscopy (cryo-FIB-SEM). This advanced chip allows the manipulation of biological samples that are either dissolved or mixed with liquid, providing the ability to perform detailed analysis and manipulation at the microscopic level. Biological samples are fixed using (HPF) and stored in liquid nitrogen. HPF is the only method used for freezing thick biological samples without the effect of ice crystalization. Fabrication of the structures that make up the microfluidic chip is done by soft lithography followed by the use of oxygen plasma to fuse the structures.

Results
The structure of the microfluidic chip consists of two thin layers of polydimethylsiloxane (PDMS). One of these layers contains channels for the flow of the sample liquid and a chamber for higher sample concentration (the chamber passes through the entire layer), while the other layer does not contain

these structures. These PDMS layers are then bonded using oxygen plasma, which provides a tight bond and allows the sample to move only within the channels. The microfluidic chip also includes a sapphire disk that allows the sample to be observed inside the chip and serves as a heat transfer medium for fast freezing processes. Ports are also attached to the inlets of the channels for easier introduction of liquid samples, which also allows for various manipulations such as mixing liquids. Once the chip is filled with the sample liquid, analysis is performed using Raman spectroscopy and the possible use of Raman tweezers to manipulate cells or particles. Subsequently, the chip is modified using a unique punch device to be inserted into the HPF. The sample is rapidly frozen using HPF and preserved in liquid nitrogen, allowing the sample to be studied in the state in which the biological material was at the time of freezing. The sample can then be inserted into a scanning cryo-electron microscope and analyzed using a focused ion beam.

Conclusion

Overall, this microfluidic chip represents an innovative tool for studying biological samples and using high-pressure freezing; high resolution is achieved by preserving their structural integrity. The combination of Raman spectroscopy and scanning cryo-electron microscopy enables detailed analysis and manipulation of biological samples, which has the potential to push the boundaries in microbiological research and biomedical science.

Keywords:

Microfluidic chip, electron microscopy, HPF

Reference:

[1] Whitesides, G. M., et al.: Nature, 442(2006), 368-373
[2] Mejia, Y. X., et al.: Lab on a chip, 14(2014), 3281-3284
[3] Anjum A., et al.: International Journal of Biological Macromolecules, 89(2016), 161-174

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Electrons phase reconstruction using Kramers-Kronig relations

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Poster Group 2

Background incl. aims
Phase imaging plays an important role in electron microscopy, particularly in mapping electric and magnetic fields at the nano and atomic scale. In this presentation we introduce a new electron phase imaging approach based on Kramers-Kronig (KK) relations, which is originally developed for optical imaging [1, 2].

Methods
According to the Kramers-Kronig relations, the real and imaginary parts of a complex function transform into each other when the function is analytical in the upper half-plane. This results in a clear link between the amplitude and phase of the exit wave. As a first attempt, we applied such KK method to reconstruct the phase of off-axis electron hologram. The experiments were performed on a Thermo Fisher Scientific Themis Z transmission electron microscope fitted with an electron biprism. An off-axis electron hologram of nano particles was then recorded using a CETA -S camera (see Fig.1). We applied the KK reconstruction on the electron holograms, the detailed reconstruction procedure is illustrated in Fig. 1. We also performed the conventional Fourier transform reconstruction for comparison.

Results :
The reconstructed amplitude and phase images based on KK relations are shown in Fig.1. Further comparsion with Fourier transform method reveals that the KK reconstruction results are consistent with Fourier transform method.

Conclusion
Here, we show that the Kramers-Kronig relation can be used to recover phase information from electron holograms. This method not only provides an alternative to conventional Fourier transform reconstruction but also helps surpass bandwidth limits when using a digital aperture in conventional Fourier transform reconstruction.

Keywords:

holography, Kramers-Kronig relations

Reference:

[1] Y. Baek and Y. Park. (2021). Nat. Photonics 15, 5.
[2] Y. Baek, K. Lee, S. Shin, and Y. Park. (2019). OPTICA 6, 45.

1067

Compartmentalization of synaptic ER studied by correlative FLIP and FIB-SEM

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LS-04 (2), Lecture Theater 4, august 29, 2024, 14:00 - 16:00

In hippocampal pyramidal cells, the endoplasmic reticulum (ER) comprises of small sheets connected with tubular network spreading to the tip of the axons and dendritic branches where it participates in the local protein synthesis required for synaptic transmission. The ER is known to be present in some of the dendritic protrusions called spines and it is highly dynamic in nature (Breit et al., 2018, Konietzny et al., 2023). In large spines, the ER forms a specialized organelle, the spine apparatus whereas in smaller spines the ER can comprises only one single ER tubule. Thousands of spines protruding from a single neuron differ morphologically and functionally from each other. To response to activation independently, each spine is supposed to have a distinct microenvironment e.g., the concentration of calcium ions differs from that in the dendrite and other spines (Breit et al., 2018). The spine morphology supports the compartmentalization, however other mechanisms are needed to obtain unidirectional diffusion and separated environment. The aim of this research was to substantiate whether the synaptic ER can be compartmentalized in the dendritic spines.

To study the compartmentalization of the synaptic ER, primary hippocampal neurons were cultivated on etched cover slips and transiently transfected with fluorescence markers for the ER and cytoplasm. The target dendrite was subjected to FLIP (fluorescence loss in photobleaching) where a part of the dendrite was bleached by pulsed light and the amount of fluorescence at the target spines after each pulse was detected. After FLIP the samples were chemically fixed and prepared for FIB-SEM (focused ion beam scanning electron microscopy). The target areas imaged with light microscope were correlated in FIB-SEM using a back scattered electron detector and the datasets were collected using 6 or 8 nm isotropic pixels. The dendrites in the FIB-SEM datasets were modelled using semi-automated segmentation tools in Microscopy Image Browser software (MIB, Belevich et al., 2016).

The FLIP studies indicated that the ER proteins may be in compartments closed off from the rest of the dendrite, preventing them from being affected by the repeated photobleaching. FIB-SEM applied to the correlated spines confirmed the results and revealed a subset of the non-continuous, compartmentalized synaptic ER. A single continuing ER tubule in a synaptic spine may have a diameter ca. 20 nm, however the volume-EM was required to enable the pinpointing the target spine in the complex neuronal network. In addition to compartmentalized synaptic ER, FIB-SEM data revealed variety of different shapes and size of dendritic spines as well as ER with two different densities. The ER with clearly different density joined and emerged throughout the whole volume of dendrites and even in some of the synaptic spines. No protein coat (possibly septin protein) could be detected around the spine neck, however, some of the spines with compartmentalized synaptic ER had a very long and narrow neck.

As a conclusion it can be said that correlative FLIP and FIB-SEM is a powerful tool to study temporally and spatially rare event in cell cultures and it allows to analyze the structures like a single tubular ER in a large neuronal network. Applying correlative FLIP and FIB-SEM verified the compartmentalization of the ER in a subset of synaptic spines. More studies must be done to further elucidate compartmentalization of the synaptic ER and its role in maintaining of synaptic strength and supporting neuronal function and plasticity.

Keywords:

CLEM, FIB-SEM, FLIP, volume-EM, ER

Reference:

Belevich I, Joensuu M, Kumar D, Vihinen H, Jokitalo E. Microscopy Image Browser: A Platform for Segmentation and Analysis of Multidimensional Datasets. PLoS Biol. 2016 Jan 4;14(1):e1002340. doi: 10.1371/journal.pbio.1002340. PMID: 26727152; PMCID: PMC4699692.

Breit M, Kessler M, Stepniewski M, Vlachos A, Queisser G. Spine-to-Dendrite Calcium Modeling Discloses Relevance for Precise Positioning of Ryanodine Receptor-Containing Spine Endoplasmic Reticulum. Sci Rep. 2018 Oct 23;8(1):15624. doi: 10.1038/s41598-018-33343-9. PMID: 30353066; PMCID: PMC6199256.

Konietzny A, Wegmann S, Mikhaylova M. The endoplasmic reticulum puts a new spin on synaptic tagging. Trends Neurosci. 2023 Jan;46(1):32-44. doi: 10.1016/j.tins.2022.10.012. Epub 2022 Nov 22. PMID: 36428191.

1068

Investigation of the Presence of Telocyte-like Cells in Human Patellar Fat Pad Tissue

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[1]Istanbul University - Cerrahpasa, Cerrahpasa Medical Faculty, Histology and Embryology Department , Istanbul , Turkey , [2]Department of Orthopedics and Traumatology, Istanbul Bakirkoy Dr. Sadi Konuk Education and Research Hospital, University of Health Sciences, Istanbul, Turkey

Poster Group 1

Stromal cells remain the focus of research in tissue biology from past to present. It is known that the stroma hosts mainly fibroblasts, pericytes, neurons, endothelial cells and immune cells. These cell types also have various distinctive features due to their different morphologies and functional properties. In addition, other different cell types have also been described in the stroma, including the interstitial cells of Cajal and telocytes. Telocytes (TC), which are characterized with small cell bodies and long telopods, have been identified in the connective tissue of many organs and are strategically positioned between target cells, near nerve endings and capillaries. Telocytes coordinate tissue homeostasis by integrating information from multiple sources and their extracellular vesicles provide bidirectional communication between them and the other stromal cells. They are able to regulate stem cell proliferation and differentiation through their secretome and play crucial roles in embryogenesis, angiogenesis, and various diseases, including cancer. Human telocytes have so far beenidentified in the heart, lung, brain, eye, thyroid, skeletal muscles, skin, gastrointestinal tract and accessory glands, urinary system, and male and female reproductive systems. But there is no information about the relationship between the telocyte cells and the Infrapatellar Fat Pad (IPFP). The approach to serious joint diseases such as osteoarthritis (OA), which is highly prevalent in our society and causes significant morbidity in the population and treatment is currently limited to microfracture treatments, autologous chondrocyte transplantations and prosthetic surgery. The incidence of symptomatic OA in the obese and elderly population and the high costs of joint replacement surgeries are increasing every year. Therefore, in addition to current treatments, the search for tissue engineering and stem cell-based research continues to meet the needs for alternative treatment methods. In recent years, tissue engineering studies have emerged as promising methods in orthopedic treatment approaches. Difficulties that may be experienced in obtaining mesenchymal stem cells (MSCs) obtained from various sources may also lead to undesirable pathological conditions such as heterogeneity in the obtained stem cell populations and teratoma formation in new cartilage cell differentiation. For this reason, researchers are now focusing on different cellular sources. Infrapatellar fat pad (IPFP), also known as Hoffa; fat pad due to its proximity to articular cartilage, in recent years has been the focus of researchers working on the relationship between cartilage regeneration and the surrounding stem cells. Studies have shown that IPFP has a good stem cell reserve and is very suitable for studies based on cartilage retrieval. On the other hand, there are also studies showing that the use of IPFP-derived stem cells in repairing joint cartilage damage may be limited due to their heterogenity. In recent years, the presence of a unique cell group called telocytes, which is in close relationships with the stem cell niche, has been described in the stroma of many organs. These cells can stimulate stem cells with the microstructures they secrete called exosomes and induce tissue regeneration by creating cellular differentiation. The

existence of infrapatellar fat pad cells with surface markers same as telocytes have been reported in only one article in the literature. TC markers including CD34, vimentin, PDGRF-α and PDGRF-β, c-kit, and α-SMA are frequently used in telocyte studies and are regarded trustworthy. Double immunofluorescence is the most accurate and precise method for identifying these cells.

Aims: This research aims to advance our understanding of the heterogenity of IPFP cells and detect the presence of TCs by employing techniques such as immunohistochemistry and immunofluorescence. Through the investigation of their morphology and identification of telocytes in the IPFP stroma, this study endeavors to provide novel insights into IPFP architecture and its implications for regenerative medicine.

Methods: In this study, IPFP tissues were obtained from 5-10 patients preparing for knee replacement surgery at Sadi Konuk Training and Research Hospital, Orthopedics and Traumatology Clinic. After obtainiing the specimen one small piece of it underwent tissue preparation procedures for light microscopy and immunohistochemical staining was performed using CD34 which is the most common marker for telocyte identification. The rest of the specimen was used for cell culture from which these cells were isolated using tissue digestion agent such as collagenase II and cell strainers of different diameters in order to separate them from other stromal cells with the cell adhesion method and subculturing the media in which the telocytes were present during 96 hours. The cells were photographed at different incubation times such as at 24h, 48, and 96h and were examined under the inverted microscope. Finally the cells resembling to telocytes with their long projections were analyzed by double immunofluorescence staining used CD34/c- kit, CD34/vimentin, CD34/PDGFR- α and β, and CD34/ α-SMA markers for telocyte identification. This comprehensive approach aimed to illuminate telocyte presence and distribution in the IPFP stroma, offering future insights into IPFP physiology and pathophysiology.

Results: In this study, the existence of telocytes within the IPFP was identified by their different shape and immunophenotypes. We identified telocytes by immunohistochemical analysis, labeling with CD34, the most extensively utilized telocyte marker. We used immunofluorescence analysis with double labeling of CD34/c-kit, CD34/vimentin, and CD34/PDGFR α and β, CD34/α-SMA in order to distinguish these cells from fibroblasts and pericytes in cell culture.
We found out that TCs were strongly positive for CD34, PDGFR α and β, and vimentin and weakly positive for α-SMA. On the other hand fibroblasts and were found to be CD34 negative, while strongly positive for vimentin and PDGFR α and β. Notably, our findings delineate telocyte cells from fibroblasts and pericytes, underscoring their unique cellular identity within the IPFP microenvironment. TCs were recognized for their long and thin prolongations and small cell bodies primarily situated around blood vessels and dispersed throughout the IPFP stroma.

Conclusion: Our study adds greatly to our understanding of IPFP telocyte biology by shedding light on their presence, distribution, and morphological properties. As a result, these cells have the potential to be a unique progenitor cell source for cartilage regeneration, and they need full attention from researchers in the tissue engineering field.

Keywords:

Infrapatellar Fat Pad, Telocytes

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WDS-supported Bayesian Peak Deconvolution for optimized Standardless EDS-Quantification

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Poster Group 1

EDS software often has the weakness that the (standardless) quantitative measurement result depends strongly on the energy calibration when peaks with evident overlap have to be deconvoluted. A typical solution is that the elements with the overlapping peaks are quantified using a WDS-analysis with much improved energy resolution and results are integrated into the EDS software via K-ratio values. Unfortunately, this WDS-analysis is then not standardless but standard based.
A combined EDS-/WDS-quantification method that is completely standardless is presented here. The novalty is that net-count ratios of the lines measured with WDS are taken into account in the Bayesian deconvolution for the standardless EDS-analysis. The by WDS measured net count ratios are used as additional boundary conditions of the probability theory based (Bayesian theorem) EDS spectrum deconvolution [1]. In this way, uncertain deconvolution of the EDS spectra is supported by additional real measured values. We use the software Benchmark-EDS and its standardless quantification model eZAF. In the latest program version Benchmark-EDS 2.01, an accuracy with relative result deviations of < ±10% was achieved (for 95% of all results) through dedicated model adjustments by empirical databases for ZAF method [2]. We will present additional EDS measurements on challenging samples on the poster, which are particularly suitable for evaluating the accuracy of standardless EDS-analysis.
The example of PbS (figure) shows how well the WDS supported deconvolution works. The spectra were measured with EDS and WDS spectrometers on a JEOL microprobe JXA-8530F. By varying the energy calibration from 5.00 eV/channel to 4.97 eV/channel, the pure EDS evaluation provides quite varying results (between 29 and 57 at% for S and 71 and 43 at% for Pb). The correct EDS quantification (50:50 at%) is achieved with 4.98 eV. With the WDS measurement support this influence of energy calibration can be eliminated. The normalised result (and at% then also) for this binary sample is determined exclusively by the deconvolution of the overlapping S- and Pb-lines. By taking into account the WDS measured line ratios, the quantification result is then stable at 50.64 at% / 49.36 at% . Uncertainties in the energy axis or other systematic errors of deconvolution of the pure EDS spectrum are then suppressed or, as in this case (binary sample, no other elements), no longer have any effect at all. It is worth noting that an EDS evaluation is still performed, but with additional WDS measurement knowledge and a probability-theory based deconvolution. The method works independently of different detection efficiencies of EDS- and WDS-detectors since peak-ratios are regarded only and the efficiency differences can be neglected since the line energies are similar. The method works even with more than 2 elements in the specimen and also if only a part of all elements is measured with WDS spectrometer, if applied only for selected EDS peak overlap problems. Additional application examples are included in the poster presentation.
In conclusion, the integration of standardless WDS-spectra acquired net-counts-ratios of lines that overlap in EDS spectra improves their peak deconvolution and makes the standardless EDS-quantification for EDS bad resolved line series independent from systematic peak deconvolution errors like the energy calibration.

Figure 1: EDS-/WDS-spectrum evaluation of PbS; a plot of deconvolution results [cps] depending on energy calibration w/wo WDS measurement support (the related quantitative results in at%)

Keywords:

Bayes-Deconvolution, standardless EDS-Quantification, standardless WDS-application

Reference:

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DOI: 10.1017/S1431927622002732
[2] F. Eggert DOI: 10.13140/RG.2.2.26778.04804

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Visualizing atomic structure of novel three-dimensional covalent organic frameworks by 3DED and high-resolution (S)TEM

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PS-07 (1), Plenary, august 29, 2024, 15:00 - 16:00

Covalent organic frameworks (COFs) are a family of porous materials constructed through organic blocks connected by covalent bonds. Its versatility of functionality, its well-defined pores, and its high surface area / high porosity have cultivated many applications in gas storage, catalysis, and separation areas.
Determining the structure of three-dimensional COFs is important for understanding their structure-function relationships and enables the rational design of materials with better performance. The most trivial way to gather the structure information is through the single-crystal X-ray diffraction (SCXRD) method. However, the small crystal sizes of the COFs hindered the common routine of ab initio structure solution. Additionally, the poor crystallinity and polymorphism of COFs also make them difficult to analyze using powder XRD data.
Electron microscopy is widely used for structure characterization in material science and gained great success on the elucidation of many complex structures. It can investigate bulk structures of small crystals by three-dimensional electron diffraction (3DED) technique and local structures by imaging. Acquiring atomic-resolution images on 3D COFs remains challenging, especially due to the electron beam damage. In recent years, the development of electron detectors and image-acquisition methods have enabled high-resolution (S)TEM with ultralow electron doses, largely overcoming this challenge. The poor crystallinity, disorders, guest molecules, and flexible characteristics of COFs still undermine the determination of the COFs' structure.
We use the 3DED technique under cryogenic conditions to get the averaging atomic structure of novel 3D COFs. The accumulated electron dose is 6~15 e-/Å2. The structure is validated with corresponding low-dose high-resolution electron microscopy (HREM) images with relatively low position offsets. We hope this investigation will cultivate the further development of structure-solving on COFs and other organic materials.

Keywords:

3DED, Low-dose HREM, 3D COFs

Reference:

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Investigation of GaAs-based nanowire heterostructures using tomography based on STEM-HAADF tilt-series[1]

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Poster Group 1

In the development of on-chip electronics, group III/V semiconductor nanowires (NWs) hold great interest, due to their small footprint allowing compatibility with lattice mismatched substrates like silicon while greatly increasing the chemical flexibility.[2] Within this work GaAs-NWs with optically active axial InGaAs heterostructures and an AlGaAs capping layer are investigated. These spatially confined optical cavity structures facilitate low loss propagation of optical modes and show a high refractive index contrast.[3] We aim to create a better understanding of the exact structure and the resulting structure property relationship of the InGaAs heterostructure based on previously measured low-temperature photoluminescence spectra.[1]. As the functionality of these heterostructures, like efficient carrier confinement, is closely linked to their 3D structure and chemical composition, tomographic reconstructions employing STEM HAADF tilt-series were carried out. To this end a tilt series with a tilt range from -75° to 75° was measured on a probe corrected FEI Titan Themis electron microscope and reconstructed using a weighted SIRT algorithm as well as finite support correction after preprocessing and alignment. The resulting tomogram is shown as an isosurface in Figure 1a. It consists of six sidewall facets perpendicular to the main [111] growth direction terminating in aflat capping layer. Assuming Lambert-Beer's law, the obtained attenuation coefficients can be qualitatively correlated to atomic numbers by using two reference areas of the tomogram, here AlGaAs (Al and Ga assumed to be 1:1) and GaAs neglecting the very low Sb concentration. As the Z-contrast shows the average atomic number of the elements present, AlGaAs was set to Z = 27.5 and GaAs set to Z = 32 respectively. This assumes the small differences in crystal parameters between AlGaAs and GaAs are neglectable. The resulting element mapping corresponds well with the elemental compositions obtained from EDX, as can be seen by comparing Figure 1b and the corresponding EDX data acquired in the same projection direction in Figure 1c. While the facets are well measured some missing wedge artifacts and a slight density gradient based on the unaccounted carbon support are still present as seen in the slices along the growth direction shown in Figure 1e. The threefold faceted capping layers as seen in Figure 1e(i) show a truncated tetrahedral growth governed by the threefold symmetry of the ZnS type crystal structure of GaAs in combination with twinning defects. In order to validate the use of the Lambert-Beer-Law for STEM HAADF measurements, pytorch multi-slice simulations were conducted for GaAs at different tilt angles using the frozen phonon approximation to account for thermal diffuse scattering, and compared to the experimental results. Furthermore, the possibility to reducing the artifacts resulting from the missing wedge focus series are investigated by multi-slice simulations and experiments.

Figure 1: STEM tomographic analysis of the GaAsSb/InGaAs/AlGaAs NW heterostructure; (a) 3D isosurface rendering of the NW tip, illustrating the facet structure consisting of six sidewall facets perpendicular to the [111] growth direction. (b) Z-contrast volume rendering of the NW, (c) corresponding EDXS map of the same NW with comparable color code, (d) two central cuts parallel to {1-10} sidewall facets (as indicated in (a)), exposing the Z-contrast through the core. (e) Cross-

sections at different positions along the NW (as indicated in (d) by labels (i)-(iv)), revealing the Z-contrast in radial direction from top (GaAs) to bottom (GaAsSb) of the NW heterostructure.

Keywords:

Nanowires, Tomography, STEM, Simulations, GaAs

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Single-camera multi-color emission anisotropy optical splitter

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Poster Group 1

Fluorescence emission anisotropy is the ratiometric measure of the polarization of fluorescence emission, which is used to provide insights into molecular organization. In the field of optical microscopy, the typical approach for multiwavelength emission anisotropy imaging involves either sequential imaging using a single camera [1] or simultaneous imaging using multiple cameras [2]. Given the limitations of the sequential imaging method, particularly in capturing sub-second dynamic processes in live-cell imaging, a viable option is the utilization of simultaneous imaging with multiple cameras. However, when accounting for multiple deformations or aberrations, the process of image registration for estimating anisotropy becomes more cumbersome. For facilitating simplified simultaneous imaging using a single camera, we engineered a novel multi wavelength, emission anisotropy image-splitter system. The measurement of point spread function (PSF) and dynamic range in emission anisotropy measurements served as critical parameters validating the potential of the constructed configuration for high-resolution cell imaging. We conducted simultaneous visualization and quantification of actin filament orientation using various actin labels, aiming to establish a benchmark for emission anisotropy measurements across three distinct excitation wavelengths: 488 nm, 561 nm, and 640 nm. Homo Förster Resonance Energy Transfer (Homo-FRET) assessments investigating the clustering behavior of Glycosylphosphatidylinositol-anchored proteins (GPI-AP) labeled with distinct fluorophores (GFP and mRuby) facilitated the realization of simultaneous multi-color anisotropy imaging through the utilization of a single camera system. We validated the capability of the system to accurately measure subtle changes in emission anisotropy during photobleaching of homo-FRET competent molecular species. This was demonstrated through a comparative study involving photobleaching of the trimeric VSVG-EGFP where VSVG-EGFP was diluted with a non-homo-FRET competent VSVG-tdTurboRFP, showcasing equivalent precision in anisotropy measurements. Simultaneous multi wavelength, emission anisotropy imaging of photoconversion of tetrameric Kaede and monomeric Dendra fluorescent proteins, transitioning from green to red fluorescence, provided a real time view of the interconversion dynamics inherent in the photoconversion process. Monte Carlo simulations were employed to provide an understanding of the intricate dynamics underlying the photoconversion process of the tetrameric fluorescent protein, demonstrating the power of this system. Simultaneous recording of Fluorescence Anisotropy Reporter (FLARE) sensors of PKA activity with cytosolic (mVenus-cpVenus-FLARE-AKAR, mCherry-mCherry-FLARE-AKAR) and membrane (LynmVenus- cpVenus-FLARE-AKAR) sensors [3], stimulated by G-protein coupled receptor activation, illustrates the optical splitter's capability to monitor the transfer of a second messenger signal from the membrane to the cytosol in real time. This substantiates the versatility of the setup, thereby unlocking a diverse array of applications within the realm of multiband emission anisotropy imaging.

Keywords:

FRET, anisotropy, polariser, optical splitter

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Influence of damage dose on the defect formation in tungsten

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Poster Group 2

1. Introduction

The development and construction of fusion reactor, such as ITER, have become very important in recent years, as has the development of new construction materials that can withstand the new requirements. Tungsten and tungsten-based alloys are considered as most promising candidate materials for these applications. Due to its favorable thermal and mechanical properties, such as high melting point, high thermal conductivity and low sputtering rate, the alloys were selected as the plasma facing materials in the ITER divertor.
Investigations of their radiation resistance, provide valuable information on the response of components to the fusion plasma. For this propose, tungsten samples were 20 MeV tungsten ion irradiated to create displacement damage and were subsequently loaded with deuterium. The aim of these investigations was to study the cause for the evolution of deuterium retention with damaging dose for samples where the displacement damage was created at a temperature of 1080°C.

2. Materials & methods

Pure W selected for this analysis was annealed at 2000°C so that the grain size increased to ~10 - 50 µm. The W-plates were W-irradiated with 20.3 MeV energy at different damage doses (0.1 dpa, 0.5 dpa and 2.3 dpa) at 1080°C. The thickness of the damaged layer formed in tungsten under these implantation conditions was ~2.5 µm. samples were loaded with deuterium at low energy and low flux (5 eV/D, $5x10^{19}$ D/m^2s) to decorate the existing defects without creating new ones. TEM lamellae preparation was carried out using the electron microscope Crossbeam Auriga, ZEISS. After preparation an electrochemical polishing process (flashing) of the lamellae was performed. The investigations were carried out using transmission electron microscopy (TEM) to determine the existence of voids and their distribution in the matrix over the cross-section, starting from the surface.

3. Results

TEM investigations of the prepared lamellae were performed using bright field imaging for the voids as well as scanning TEM dark field imaging to observe the formation of dislocation loops. The study demonstrates the homogeneous formation of voids in the depth up to 2.5 µm. The voids have a similar average size and a similar density over the whole thickness. The formation of inhomogeneities such as size peaks or number density peaks, which are typical for the implanted layers, was not observed. Figure 1 shows TEM cross-sections along the layers exposed to 0.1 dpa, 0.5 dpa and 2.3 dpa irradiation. The clearly visible fluctuations in the lamellae thickness are caused by the flash polishing of the film. The TEM images of all three tungsten samples clearly show the existence of voids of the nanometer size in the microstructure.

Figure 1: TEM image of the W irradiated to 0.1 dpa, 0.5 dpa and 2.3 dpa with finely distributed voids of different sizes.

4. Conclusions

The microstructure of tungsten irradiated to the damage dose of 0.1 dpa, 0.5 and 2.3 dpa at 1080°C were studied in detail using TEM. In all three samples, the existence of voids could be clearly demonstrated.

Keywords:

Fusion, Tungsten, FIB, TEM

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Label-free biological composition predictions in EM images

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Poster Group 2

Background incl. aims
Large-scale and volume electron microscopy (EM) allow biologists to understand structural relations in biological systems from the nanometer up to the millimeter scale and beyond. However, EM data can be difficult and time-consuming to interpret and process due to a lack of biological specificity. Fluorescence microscopy (FM) can be used together with EM in correlative light and electron microscopy (CLEM) to add biologically relevant data to the EM images. CLEM does come at the cost of increased complexity and restrictions in sample preparation and measurement workflow. Furthermore, high-throughput EM methods like multi-beam scanning electron microscopy (MB-SEM) can be hard to combine with CLEM. We aim to develop a machine-learning model that can extract biologically relevant information from EM images, which is generalisable to different methods of EM.
Methods
Convolutional neural networks (CNNs) have been shown to perform well on various image-translation tasks in biomedical contexts. We explored the use of a CNN to extract biological information from EM images by imitating real fluorescence. We used a network architecture inspired by U-net, only with a truncated up-sampling arm to account for the resolution mismatch between EM and FM data. The network, named CLEMnet, was trained on CLEM datasets obtained using an integrated array tomography workflow. The dataset consists of rat pancreas tissue samples. The islet of Langerhans was stained with Hoechst, which binds DNA and RNA and immunolabeled for insulin using Alexa 594.
In regular EM, it can take a long time to acquire large volumes of EM data. To solve this, high-throughput EM methods have been developed. One of these methods is multi-beam optical scanning transmission electron microscopy (MB-OSTEM). In MB-OSTEM, contrast is generated by the number of transmitted electrons. As CLEMnet is only trained on data from one microscope, using only backscatter electrons as a detection method, it is not directly applicable to datasets imaged under different conditions like with OSTEM. To still be able to use CLEMnet with MB-OSTEM data, we propose to transform the MB-OSTEM data such that it appears as backscatter electron (BSE) SEM using another CNN. Imaging biological samples using EM introduces beam damage into the sample. This makes generating training data for a CNN by sequential imaging in both an SEM and MB-OSTEM unviable, as the damage would appear during the second acquisition. Using generative adversarial networks (GANs), a network structure called Cycle-GAN can perform image-to-image translation tasks without the need for paired training data. We thus trained a Cycle-GAN network to be able to transform MB-OSTEM data such that it becomes similar to BSE SEM images, like the ones CLEMnet is trained on. This could thus allow for the automatic extraction of biologically relevant data from high-throughput EM techniques.
Results
Network predictions of CLEMnet show a good qualitative agreement with the recorded fluorescence signal. By superimposing the recorded and predicted signals, we can see overlap between the

recorded and predicted signals. The Hoechst signal is localised to nuclei and the endoplasmic reticulum. The network trained on the immunolabeled insulin is capable of distinguishing between the different types of granules in the sample. Quantitative network performance was evaluated using the Pearson correlation coefficient (PCC). The Hoechst-based network achieved a PCC of 0.51, and the Insulin-based network achieved a PCC of 0.765. The PCC serves as an indication of network performance. Still, inaccuracies in the recorded dataset, like inaccuracies in the EM-FM registration and bleed-through from the different fluorescent stains, can result in a spurious reduction of the PCC.
The OSTEM training data for the Cycle-GAN contains numerous staining artefacts. Despite this, we can qualitatively see that Cycle-GAN is able to translate OSTEM data into BSE SEM-like data. Furthermore, when CLEMnet trained on the Hoechst dataset is applied to this translated data, we can see that the resulting signal is localised to the nuclei and ER as expected.
Conclusion
Preliminary results demonstrate our ability to use a CNN trained on BSE SEM data to extract biologically relevant data from OSTEM images. Future work will look into extending this to MB-OSTEM data. In this future work, we will acquire a CLEM dataset using MB-OSTEM to be able to quantitatively compare the predicted signal from CLEMnet to real fluorescence data when applied to translated MB-OSTEM data. This work could help to make the interpretation and analysis of large-scale EM data more approachable, as CLEMnet is able to provide biological context to EM datasets.

Keywords:

CLEM, Machine learning, transfer learning

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Measurement of EELS standards and application on oxidation state determination of a MeOH catalyst

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Poster Group 1

Background & aims
Electron energy loss spectroscopy (EELS) is a powerful technique, foremost allowing determination of the elemental composition, but furthermore investigation of electronic properties like chemical bonding and oxidation states. This information can be collected with high spatial resolution on today´s scanning transmission electron microscopes. Most common for the analysis of EELS data is the use of cross-section models. These are often sufficient for determination of the elemental composition, but to extract information like oxidation states, a different analysis approach is necessary: Spectra of well-known reference materials (standards) improve the quantification results and allow fitting of the edge shapes to investigate and map oxidation states. These reference spectra can either be obtained by measuring well-defined materials or by using EELS reference databases [1-3]. Since their content is mostly not exhaustive to provide a set of reference spectra for each element and oxidation state for a given material, the process of obtaining commercially available materials, evaluating them for their ability to be usable as standards and using the obtained set of standards to map the oxidation states of a Cu-based MeOH catalyst [4] post-catalysis is presented.
Methods
The investigated catalyst [4] consists of Cu, ZnO and Al_2O_3. The standard materials needed to cover possible oxidation states are Cu, Cu_2O, CuO, Zn, ZnO and Al_2O_3. Of each material a commercially available sample has been obtained and investigated for its purity and oxidation state via XRD and in the TEM using EDX and EELS. Storage and sample transfer under inert conditions are considered mandatory for all samples that could oxidise further. Sample parts suitable for the measurement of standard spectra are identified, measured and spectra extracted and then imported into Gatan GMS3 as references. An EELS map of the MeOH catalyst [4] after catalytic reaction, that has been transferred under inert conditions into the microscope to preserve the oxidation states, has been acquired. This map is evaluated for materials and oxidation states based on the measured standard spectra.
Results
First it was tested via XRD if the bought materials are as described, with a focus on additional phases that can result from trace metals or different oxidation states than expected. Two materials show a deviation, Cu showed ~5% oxidation and ZnO exhibited an additional unknown phase. EDX maps of each material led to the following results: We found ~3-5% O in Cu, non-homogeneous oxidation and a slightly less O content in Cu_2O, while CuO was homogeneous as described. Some oxidation of ~5-7% was found in the Zn sample and additional O in the ZnO. The oxidation layer on the Zn appears to be amorphous, otherwise it would have been detected in the XRD. Trace elements in all cases are Al, Si and Zn for the Cu-based materials, and Al, Si and Cu for the Zn-based materials, with each amount less than 0,2 at.%. They do not explain the additional phase in the ZnO, so additional bulk EDX has been measured in SEM at low magnification, again revealing no additional elements present. Due to

the O excess likely resulting from the production process, there is a high probability that this phase determined by XRD consists of zinc hydroxides.
In all EDX maps a region on the sample can be found, where the desired material in the specific state is likely to be present. Therefore, EELS spectrum images have been acquired and evaluated as elemental mappings, allowing to identify a region of this spectrum image where the material has the desired composition and oxidation state. Of this region the standard spectra for each element and oxidation state have been obtained, integrating over several single spectra. The concurrent standards fit using these spectra on the spectrum image of the MeOH catalyst is able to differentiate between elements and oxidation state, resulting in a detailed, spatially resolved map for each (figure 1).
Conclusions
By careful combination of analysis techniques, it is possible to obtain self-measured EELS standards from commercially available standard materials. These allow to evaluate EELS data also for oxidation states with high quality, allowing deeper insight in the properties of catalysts.

Figure 1: a) HAADF image of the MeOH catalyst, from the EELS spectrum image. b) Fit result for the O K edge using self-measured standards for CuO, Cu_2O, ZnO and Al_2O_3. Mostly ZnO is present, some Al_2O_3 and very few Cu oxides. c) Fit result for the Cu $L_{2,3}$ edges using self-measured standards for Cu, CuO and Cu_2O. It shows mostly pure Cu, slightly oxidised. The region from which the line profile in e) is obtained is highlighted in this image. d) Fit result for the Zn $L_{2,3}$ edges using self-measured standards for Zn and ZnO. e) Line profile across the calculated maps, quantifying the fit result.

Keywords:

EELS, Oxidation state, Standards, catalysis

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Numerical Analysis of Temperature Calibration using Plasmon Energy Expansion Thermometry

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Poster Group 1

Background incl. aims
Transmission electron microscopy (TEM) has become an important tool for characterizing the microstructure in materials [1]. However, to gain access to the underlying structure-property relationships, real-time observations in TEM experiments are required to study the material's microstructure under operating or process conditions, e.g. at elevated temperature in in-situ heating experiments. And, with the advent of TEM nanoheaters that utilize microelectromechanical systems (MEMS) technology [2,3], atomic-scale studies at elevated temperatures became feasible.
However, the exact local temperature of a TEM sample itself remains rather uncertain, limiting the interpretation of observed structural phenomena observed in in-situ TEM heating experiments. Here, we explored how accurately plasmon energy expansion thermometry (PEET) [4] can be used to determine the local temperature. .

Methods
As our model system, a tungsten (W) sample, prepared using a ThermoFisher Hydra plasma focus ion-beam (PFIB), was placed on a DENS Wildfire nanoheater [3]. For the PEET experiment, low-loss energy electron loss spectroscopy (EELS) was performed using ThermoFisher Spectra Ultra at 300kV with an energy resolution of 1 eV and a Gatan Continuum GIF with Dual-EELS functionality.
The site-specific temperature (T) within the W lamella was estimated by measuring the T-dependent energy shift of the W bulk plasmon in the low-loss EELS. The energy dispersion of 0.01 eV was used for an increased precision for detecting the plasmon energy shift.
To accurately estimate the temperature profile within the W lamellas with uniform sample thickness, we used the finite element method (FEM) in COMSOL. Further, the GPAW package [5] was used to simulate EELS spectra, based on the dielectric function. The temperature-dependent energy of the W bulk plasmon was estimated in the GPAW environment by implementing the temperature-dependent thermal expansion of the lattice constant.

Results
The COMSOL simulation models the heat flow and the temperature distribution in our W sample with increasing nanoheater temperature, providing an estimation to simulate corresponding low-loss EELS spectra. Our simulated EELS spectra using GPAW predicted a shift of the W bulk plasmon energy ΔE of ~0.236 eV when the temperature was increased from RT to 1000℃. The value of the simulated W bulk plasmon energy is at the same order of magnitude as our experimental PEET data (ΔE ~ 0.38 eV) when increasing the temperature from RT (25.11 eV) to 1000℃ (24.73 eV) in our in-situ heating TEM experiment.

However, the deviation between our simulation and experiments indicated that more details need to be considered. One approach in the simulation is that the precision of PEET has been evaluated in our simulated EELS data from the uncertainties of the coefficient of thermal expansion and of the lattice constant at the reference temperature. Another reason could be that we have to take varying sample thickness into account in our COMSOL simulations.

Conclusion
Our study shows that PEET with W is able to measure local TEM sample temperatures in a quantitative way. The COMSOL/GPAW simulation can be used to predict the T-dependent plasmon energy and estimate the uncertainties for spectra in such experiments. This advances our approach in determining the local temperature across the sample in in situ TEM heating experiments.

Keywords:

Thermal expansion, EELS, PEET, Tungsten

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Corrosion of Alloys Suitable for Very High Temperature Systems (VHTRs) Exposed to High Temperature Helium

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Poster Group 2

Background and Aims
During operation of Very High Temperature Reactor (VHTR) systems, the temperatures will be operating at above 700°C where having a high creep strength and corrosion resistance to the high temperature atmosphere is of vital importance [1].

Helium is used within the VHTR systems as the gas is “inert” and helium, in comparison to other potential coolants, does not become radioactive when exposed to neutron radiation. Complications occur due to the presence of carbon (from the graphite in the reactor), any impurities within the helium gas and potential water ingress from the environment[2,3]. These factors, coupled with the very high temperatures of operation, can lead to complex interactions taking place between the helium coolant gas and the metal. These can include oxidation, carburization and decarburization [1,4] and in certain high temperature alloys, for example on Alloy 800 the surface corrosion layer can delaminate which will have a detrimental effect on the physical properties of mechanical components.

These interactions will affect the physical properties of the alloys as the chemistry is altered during exposure to helium at high temperatures and may negatively affect the physical properties of the alloys, for example decarburization will lead to a reduction in carbides which may affect the physical properties of the alloys.

The work here has characterized three high temperature nickel based alloys (Alloy 617, Alloy X and Alloy 800) using advanced microscopy techniques after exposure to high temperature helium (740°C) for various durations with the aim to better understand the interactions taking place within high temperature helium systems.

Methods
Various nickel based alloys have been exposed at 750°C to a helium atmosphere for various durations inside a high temperature tube furnace. These alloys (Alloy 617, Alloy 800 and Alloy X) were selected based on their physical properties and corrosion resistance. Upon removal from the furnace, samples were characterised using advanced microscopy techniques including Scanning Electron Microscopy (SEM) and Focused Ion Beam Microscopy (FIB). These techniques allowed for characterisation of the changes taking place during their exposure to high temperature helium. Elemental Dispersive X-Ray Spectroscopy (EDS) was used to characterise the elements within the corrosion layer and to highlight the elemental changes which take place at these high temperatures.

Results
Analysis of the samples shows differences in the oxidation/corrosion behaviour of the different alloys dependant on exposure duration. A comparison between three alloys exposed at 750°C to helium for

1000 hours is shown in the Figure where different morphologies can be seen across the surface of the alloys. The corrosion layer thickness and morphology varied for the different materials with precipitates and oxide nodes developing near to the surface of the exposed surface.

Conclusion
The corrosion behaviour of alloys exposed to high temperature helium is of importance to understand for use within VHTR systems. The work here has characterised three alloys and shown the differences in the oxidation characteristics for each alloy. This will help to better understand corrosion process taking place within a high temperature environment when exposed to helium.

Keywords:

Oxidation, High Temperature Corrosion, Microscopy

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Optimizing Soft X-ray Spectroscopy for Silicon Anode Lithium Mapping

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Poster Group 1

Background incl. aims
Silicon has emerged as a promising candidate material for anodes in lithium-ion batteries due to its high theoretical capacity (about 10 times that of graphite). However, the practical use of silicon faces challenges related to its large volume expansion (~300%) upon lithiation, which leads to mechanical degradation and pulverization of the electrode over repeated charge/discharge cycles. This degradation results in loss of electrical contact, reduced cyclability, and capacity fading.
To address these issues and optimize silicon-based anodes, extensive research efforts are underway. One critical area of investigation involves understanding the distribution and behavior of lithium within silicon structures during battery operation. The distribution of lithium impacts the electrochemical performance and stability of the battery.
Various techniques are employed to study silicon-based anodes, including microscopy (e.g., scanning electron microscopy, transmission electron microscopy) for structural analysis, spectroscopy (e.g., X-ray diffraction, X-ray photoelectron spectroscopy) for chemical characterization, and electrochemical methods for performance evaluation. But the lithium distribution in the anodes cannot be found by these techniques.
However, soft X-ray emission spectroscopy (SXES) is a powerful technique that can provide detailed insights into the lithium distribution within silicon anodes with high sensitivity and spatial resolution. SXES allows researchers to map the chemical states of lithium in different states of charge (SOC) and investigate phenomena such as lithium trapping, where lithium becomes immobilized within the silicon structure, contributing to capacity loss and reduced battery efficiency over time.
By studying these fundamental processes, we aim to develop strategies to mitigate silicon degradation, enhance lithium utilization, improve battery cyclability, and ultimately advance the performance and lifespan of lithium-ion batteries. Furthermore, this study aims to establish optimal measurement conditions for SXES when analysing silicon-based anodes in lithium-ion batteries. By identifying and demonstrating the most effective measurement parameters and techniques, our goal is to streamline SXES procedures for battery research, facilitating broader adoption and use by other researchers in the field.

Methods
This research focuses on investigating silicon-based anodes derived from lithium-ion batteries fabricated in our laboratory using micrometer-sized silicon particles as the anode material. The batteries are assembled in coin cell half-cell format, featuring electrodes with a diameter of 18 mm. Assembly and subsequent disassembly for post-mortem analysis are meticulously conducted within an argon atmosphere glovebox to ensure controlled environmental conditions and prevent exposure to moisture and oxygen.
After disassembly, the electrodes undergo a thorough cleaning process using dimethyl carbonate (DMC) and are then dried under vacuum to eliminate any residual contaminants. For SXES analysis, a plane surface is essential. Therefore, a small portion of the electrode is extracted and a cross-section is meticulously prepared using the IB-19520CCP cross-section polisher from Jeol under a cooled atmosphere at -120°C. The transfer from the glovebox to the ion polisher and subsequently to the

scanning electron microscope (SEM) is carried out in an inert transfer vessel to ensure that the sample remains shielded from air or moisture throughout the process.
SXES investigations are performed using a JSM-IT800 SEM equipped with the JS50XL SXES detector from Jeol. This advanced setup enables high-resolution analysis of lithium distribution and chemical states within the silicon-based anodes.

Results
Our investigation revealed significant influences of probe current, excitation voltage, and exposure time on the spectroscopy results. Identifying optimal settings for these parameters is crucial, balancing high signal intensity with minimal sample degradation during measurements. Additionally, variations in lithium (Li) distribution within the analysed particles were observed. By employing mapping techniques, we were able to spatially resolve and identify regions enriched with lithium, providing valuable insights into its distribution within the silicon-based anodes. This detailed elemental mapping enhances our understanding of lithium behavior in battery materials and underscores the importance of precise measurement conditions in SXES for comprehensive battery research.

Conclusion
In conclusion, our study underscores the significance of SXES in elucidating the complex interplay between lithium distribution and silicon-based anode performance in lithium-ion batteries. The challenges associated with silicon's volume expansion upon lithiation and subsequent mechanical degradation highlight the critical need for advanced characterization techniques to optimize battery materials.
Through our investigation, we have demonstrated the pivotal role of SXES in mapping lithium distribution with exceptional sensitivity and spatial resolution. Our findings emphasize the impact of probe current, excitation voltage, and exposure time on spectroscopy results, emphasizing the importance of optimizing measurement parameters to balance signal intensity and sample integrity. The observed variations in lithium distribution within the anode particles provide valuable insights for developing strategies to mitigate lithium trapping and enhance battery cyclability. By enhancing our understanding of lithium behavior at different states of charge, we aim to contribute to the development of more efficient and durable lithium-ion batteries.

Keywords:

SXES, Silicon-based Anodes, Lithium-Ion Battery

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Operando TEM Studies of Re@Cu2O-SnO2 catalysts during CO2 reduction reaction with optimized liquid flow configuration

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PS-05 (1), Lecture Theater 1, august 28, 2024, 10:30 - 12:30

Background

The energy transition is nowadays a topic of huge attention, as a measure to face the global energy crisis and the more and more impacting climate change. In this framework, the production of carbon-based chemicals and fuels by exploiting anthropogenic CO2 is nowadays considered a way-out to leave the traditional oil-based technology. In fact, renewable and green approaches to CO2 valorisation are aimed at minimizing the worrying impact of its emission to the environment, and to drive the transition to a new circular economy approach in chemistry and energy production. A strategic method to reduce CO2 concentration in the atmosphere is to consider it as a valuable raw material, collecting it from industrials point sources and electrochemically reducing it into value-added products. This green approach can contribute to the development of alternative energetic vectors, or organic molecules normally derived from fossil resources. Among many products that can be obtained, which depend on the catalyst characteristics, reaction conditions and electrolyte, the CO2 reduction reaction (CO2RR) to carbon monoxide (CO) or formic acid (HCOOH) are up to now the most economically viable processes and can challenge conventional production routes [1]. In order to design efficient catalysts for CO2RR with high activity, selectivity and stability, it is important to understand the fundamental mechanisms involved in the electrochemical processes. In this context, in situ / operando characterization techniques provide insight into the correlation between physical-chemical properties and the electrochemical performance. Specifically, electrochemical liquid phase transmission electron microscopy (EC-LPTEM) yields temporally and spatially resolved morphological, structural and chemical information regarding catalytic materials under electrochemical stimulation [2]. Within this framework, in this paper, EC-LPTEM experiments on molecular Re@Cu2O/SnO2 catalysts for CO2RR are presented and compared to the lab-scale experiments.

Methods

EC-LPTEM experiments are typically carried out in miniaturized liquid cell TEM holders with controlled liquid flow, where the electrochemical functionality may be provided with different technological approaches. Poseidon liquid phase TEM holder and related electrochemical commercial and modified chips have been used to perform EC-LPTEM on a FEI TECNAI F20ST microscope. Molecular Re@Cu2O/SnO2 catalysts for the CO2 electroreduction to syngas have been prepared by wet precipitation of Cu2O/SnO2 followed two functionalization steps with vinyltriethoxysilane

(VTES), to form stable surface Si-O bonds, with electropolymerizations on the silanized Cu2O/SnO2-VTES NPs with vinyl-tagged Re complex.
For the EC-LPTEM, a dispersion of the catalyst in ethanol is drop casted on a Glassy Carbon working electrode in a microchip-based electrochemical set-up. This chip, together with an optimized prototype chip, provided by Protochips, are used to compose the electrochemical cell. The catalyst was studied in 0.1M KHCO3 saturated with CO2. The electrochemical activity for the CO2RR was tested by means of CV and Chrono Potentiometry (CP) analyses.

Results
One major challenge in conventional liquid cell TEM setups for CO2RR operando experiments in aqueous electrolyte is that the evolution of gaseous products at the electrodes causes the formation of gas bubbles. Due to the miniaturized volume of the liquid cell, in few seconds the cell is completely filled with gas, in electrochemical conditions which are relevant for CO2RR. Once the cell is filled with gas, the electrolyte-electrode interface is dramatically affected, resulting in uncontrollable experimental conditions and elusive data interpretation. In this work, we use a customised liquid cell geometry with optimized liquid flow configuration, which minimizes the formation of gas bubbles in the liquid cell and concurrently removes the gaseous products more efficiently in electrochemical conditions relevant for operando CO2RR studies in aqueous electrolyte [3], [4].
With this improved experimental capability, we investigated the morphological dynamics during the life cycle of Re@Cu2O/SnO2 catalysts for the CO2 electroreduction to syngas. What emerged is that the catalyst during electrochemical activity experiences dissolution and re-crystallization phenomena, that partially change the particle size and in turn can possibly change the catalytic performance.

Conclusion
In-situ EC-LPTEM helped to shed light on the changes the material undergoes during electrocatalytic activity, and thanks to improved cell we have been able to study this catalyst at relatively wide range of potentials for prolonged periods of time, which are close to those of interest for the applications. The obtained results on the catalysts hold significance from the fundamental point of view, and, in addition, the optimised design of the LP flow TEM cell allowed to perform stability tests, which are of huge interest for the future application of these catalysts in real devices.

Ackowledgements
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This article was also funded under the National Recovery and Resilience Plan (NRRP), Mission 4 "Education and Research" - Component 2 "From research to business" - Investment 3.1 "Fund for the realization of an integrated system of research and innovation infrastructures" - Call for tender No. n. 3264 of 28/12/2021 of Italian Ministry of Research funded by the European Union – NextGenerationEU - Project code: IR0000027, Concession Decree No. 128 of 21/06/2022 adopted by the Italian Ministry of Research, CUP: B33C22000710006, Project title: iENTRANCE.
This study was carried out within the Ministerial Decree no. 1062/2021 and received funding from the FSE REACT-EU - PON Ricerca e Innovazione 2014-2020.

Keywords:

Operando EC-LPTEM, CO2RR, Cu-based catalyst

Reference:

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CAR T cell dynamics in a 3D collagen matrix: migration and interactions with cancer cells

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Poster Group 1

Background incl. aims
Chimeric antigen receptor (CAR) T cell therapy is a therapy in which T cells are engineered to target a specific antigen on a cancer cell and elicit a T cell response that ultimately leads to the death of the cancer cell. CAR T cell therapy has proven effective against blood cancers, but solid tumors are more difficult and complex targets due to their tumor microenvironment. In order to develop efficient CAR T cell therapies against solid tumors, we need systems in which we can study, challenge, and improve CAR T cells in extracellular matrix-like 3D environments. Here, we have developed a 3D model based on rat tail collagen to study CAR T cells and their interactions with cancer cells.

Methods
We used CD19 CAR T cells, i.e. T cells targeting CD19 antigen, and A375 melanoma cells engineered to express the CD19 antigen. CAR T cells and cancer cells were mixed into a rat tail collagen matrix and spinning disk microscopy was used to record 3D time-lapse videos of the cells. We developed a quantitative 3D analysis and analyzed the T cell tracks to elucidate the migration of T cells and their interactions with cancer cells. We furthermore conducted a 72-hour treatment of mature melanoma spheroids using CAR T cells to investigate the efficiency of the therapy in the 3D collagen matrix. In all experiments, we compared with untransduced T cells, i.e. normal T cells that do not express the CD19 CAR.

Results
Our analysis of CAR T cell migration in the collagen matrix showed that CAR T cells generally move slower compared to untransduced T cells. While they both have the same number of interactions with cancer cells, CAR T cells tend to interact longer.
72-hours treatment of melanoma spheroids with CAR T cells in the collagen matrix resulted in a substantial reduction in spheroid growth. Spheroids treated with untransduced T cells increased in size comparable to untreated spheroids. These results demonstrate the efficacy of CAR T cells in targeting cancer tumors in the 3D collagen matrix.

Conclusion
We have successfully employed time-lapse imaging and quantitative 3D analysis to study the migration of CAR T cells and their interactions with cancer cells. We propose that the 3D model and the quantitative analysis presented here can be used to test various CARs, antigens, solid tumor types, and matrix modifications.

Keywords:

CAR, collagen matrix, time-lapse, migration

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Analysis of the local chain orientation in conjugated polymer films

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Poster Group 2

Background incl. aims
Conjugated polymers have applications in organic, flexible electronics, with their mobility and conductivity largely dependent on parameters such as crystallinity, grain size, and chain orientation [1]. However, probing their structure at the nanoscale with electron microscopy remains challenging due to their radiation-sensitive nature. Development of fast direct electron detectors has helped open up the studies of these materials, with HRTEM and 4D-STEM data highlighting order and at the nanoscale not possible from x-ray approaches [2,3,4]. In this work we use low-dose scanning electron diffraction (SED), a variant of 4D-STEM, to investigate the nanoscale structure of conjugated polymers, with a workflow for determining chain orientation of conjugated polymers presented. We determine quantitatively chain orientation and grain size and compare to bulk experiments to observe how these quantities effect the underlying properties of the conjugated polymers.

Methods
TEM samples of a thiophene-based conjugated polymer – with potential to be used as an organic mixed ionic-electronic conductors (OMIEC) [4] – were prepared via spin-coating and a lift-off approach onto Quantifoil, amorphous carbon grids. SED data, comprised of diffraction patterns (256 x 256 pixels), acquired over a raster scan of 256 x 256 pixels was acquired on a Thermo Fisher Spectra at 200 keV, with a low convergence angle of 1 mrad and with 5pA probe current. Each diffraction pattern was acquired using a Quantum Detectors Merlin camera and analysed using the pyXem software suite. Polymer chain orientation is visualised using 'flowline maps' based upon the orientation of Bragg spots due to π - π stacking in the conjugated polymer structure.

Results
The results from one of the polymers studied, p(g0T2-g6T2), is shown in Figure 1 to illustrate the analysis now possible of conjugated polymer thin films. The bright field (BF) image results from using a 'virtual' aperture placed around the direct beam in each of the patterns acquired and plotting the summed intensity as a function of probe position. The annular dark field image is plotted similarly but using a virtual annular aperture with radius range 0.25 Å-1 – 0.3 Å-1. The mean diffraction map is proportional to the summation of all the diffracted intensity across the field of view, with a bright spot present in the bottom left due to a hot pixel on the detector during acquistion. The 'variance' map plots the variation in scattered intensity in a narrow annulus centred on the strong Bragg 'ring' (corresponding to π - π stacking) – in essence this highlights areas of strong crystallinity / order. The final map illustrates the variation in chain orientation across the field of view through the use of a colour wheel (to plot the local Bragg vector for π - π stacking) and flow lines to illustrate more graphically the local polymer structrure. In particular, here we highlight (see arrows) the appearance

of disclinations (with k = +1/2 topology), commonly seen in polymer liquid crystals. By careful analysis of the 'texture' around the disclination core, quantitative measurements of the splay and bend charcterisitcs may be determined.

Conclusions
The advent of 4D-STEM techniques, coupled with the progress in detector technology and the rise of computational power, provides an ideal platform for the study of conjugated polymers at lengthscales considerably smaller than is possible with other techniques such as x-ray diffraction. In the example we present here, chain orientation, grain size and the presence of disclinations have all been observed in the conjugated polymer p(g0T2-g6T2). The authors thank the EPSRC for funding under grant number EP/R008779/1.

Keywords:

Conjugated polymers, SED, 4D-STEM, low-dose

Reference:

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Resolving single-electron and multi-electron distribution functions with event-based electron detectors

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Poster Group 2

Background
The combination of electron probe pulses with a synchronized optical sample excitation as applied in ultrafast transmission electron microscopy (UTEM) has enabled the investigation of ultrafast dynamics on the nanoscale [1]. Besides the traditional optical-pump/electron-probe approach, sufficiently fast event-based electron detectors, based for example on the TimePix3 chip architecture [2], can detect electron events with a temporal bin width of about 1.6 ns. Due to the stochastic electron trajectories in the sensor chip the spatial and temporal resolution is often limited, in particular at higher electron energies [3,4], and requires further data processing to overcome these limitations. In addition to the nanosecond temporal resolution of event-based detectors, such a detection scheme gives access to spatio-temporal electron-electron correlations and has been recently applied to the investigation of Coulomb-correlation within a photoelectron bunch generated by femtosecond photoemission from a Schottky emitter [5]. In general, these new kinds of detectors in principal provide a measurement of multi-electron distribution functions both in electron beams and electron pulses containing additional information with respect to averaged micrographs traditionally recorded.
Methods
To gauge the potential of event-based detectors for UTEM, the temporal response of a TimePix3 detector (Cheetah T3, Amsterdam Scientific Instruments) was characterized utilizing 200-fs electron pulses (400-kHz repetition rate, 200-keV electron energy, 1.3 electron/pulse) as generated in the Regensburg UTEM. As a photoelectron source, both a laser-driven Schottky and cold-field emitter were employed. In a first experiment, we experimentally collected a dataset of electron detection events for up to 4 x 10^6 electron pulses (Fig. 1 (a)). For each event, the TimePix3 collects relative detector position, time of arrival (ToA) and time over threshold (ToT). The events are clustered depending on their ToA and position, resulting in an average of 6.5 events per cluster (Fig. 1 (b) and (c)). The data is synchronized by assigning a timestamp to each photoemission laser pulse. A fully connected deep neural network model is trained using event data from 1.6 x 10^5 time-scrambled event cluster, so that it becomes possible to predict the ToA of the primary electron.
In a second experiment, electrons are emitted by a cold-field emitter gun (CFEG) and around 5 x 10^7 electrons are detected with the TimePix3. To find Coulomb-correlated electron pairs, only clusters with a small mean ToA-difference (< 50 ns) from the dataset are selected. The electron pairs are statistically analysed and the influence of optical excitation, extraction fields and spatial distribution are examined.
Results
Using the non-clustered ToA events leads to a temporal spread of about 10 ns (Fig. 1 (d), grey curve). If a cluster algorithm is used and the mean ToA for each cluster is calculated, then the temporal spread can be reduced to 7 ns (Fig. 1 (d), red curve). The neural network reduces the predicted temporal distribution (for a part of the data not involved in the training) to 4.7 ns (FWHM) (Fig. 1 (d), green curve). In the case of the neural network the distribution is almost Gaussian while the ToA distribution in the previous cases shows a tail for larger ToA.

Applying closely spaced electron double-pulses with a temporal separation of 8 ns to TimePix3 detector (shorter than the intrinsic temporal resolution) shows a broadened flat top distribution (Fig. 1 (e), grey curve). With the implementation of the neural network, the two peaks can be distinctly separated (Fig. 1 (e), green curve), demonstrating the more general applicability of the approach.

Conclusion

In conclusion, we demonstrate, that the accurate prediction of the ToA by a neural network trained by femtosecond electron pulse data, resulting in an increased temporal resolution for the TimePix3 detector. This improved temporal resolution will also be helpful for the mapping of temporal correlations in the imaging of non-static nano-objects and non-trivial multi-particle correlations within electron pulses.

Keywords:

ultrafast-TEM, femtosecond-electron-pulses, laser-driven-cold-field-emitter, TimePix3, electron-electron-correlations

Reference:

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Structured illumination near-field electron ptychography

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Background incl. aims
Ptychography measures a correlated matrix of interference or diffraction patterns with sufficient overlap between adjoining illuminated area, and reconstructs both the object and the probe with their amplitude and phase components. For a ptychographically reconstructed image, its image pixel size is determined by the detector sampling in the Fourier space rather than the scanning step size in the real space [1]. This would allow a coarser scanning sampling and therefore a larger field of view with reasonable number of scan pixels or a lower dose with a practical probe current. In order to boost the sampling efficiency further, which would enable much larger field of view phase contrast imaging (e.g. weak contrast cellular structures) or fast time-resolved phase contrast movies (e.g. electromagnetic field dynamics), we developed a new ptychography imaging mode called near-field electron ptychography [2]. This is based on the classic ptychography principle but adopts a full-field parallel beam illumination and measures Fresnel (near-field), instead of Fraunhofer, diffraction patterns. In this case, structured illumination is of vital importance. Otherwise, each near-field diffraction image carries the same information except for laterally shifted, and therefore the ptychography reconstruction reduces to a single phase retrieval loop. In addition, a properly structured illumination will bring in a mixed range of spatial frequency to the diffraction patterns and open up the contrast transfer function over a wider range of frequencies.
Methods
A variety of diffuser designs and optical setups have been tested for the structured illumination near-field electron ptychography. A silicon nitride-based phase plate with patterned local thickness variation were firstly used as a phase diffuser. This can introduce almost arbitrary phase patterns to the illumination but the profuse inelastic scattering from the silicon nitride thin film will add an incoherent background to the measured diffraction patterns, which becomes more severe when working with lower energy electrons. An amplitude mask diffuser, consisting of a few narrow but opaque, random oriented, curved lines within a round aperture (see the enclosed figure), was found to be also very effective for this application [3] because in the near-field “ptychograms”, the Fresnel fringes, which comes from the hard edges of those lines, carry exactly the additional frequencies that are required for structured illumination. In this case, the inelastic scattering from the diffuser becomes negligible. Such a diffuser can be placed in either selected area aperture plane or condenser aperture plane. The latter is preferred because then only the area that is going to be measured will be illuminated, and also this will allow variable size of structured parallel illumination if three condenser lenses are available on the microscope. In order to realise the “shift invariance” principle of ptychography, the stage shift was originally used, which is flexible enough for large distance travel but suffers from jigging unless waiting enough time for stabilisation. In the latest development, we implemented a parallel beam 4D STEM acquisition mode that drives instant beam shift and

synchronised fast detector readout for this application, which reduces the acquisition time by at least two orders of magnitude.

Results

We demonstrated, with as few as 9 diffraction patterns, near-field ptychography can already reconstruct micron square field of view phase images [2] and this can be easily extended to hundreds micron square field of view with a small number of diffraction patterns. Applying this method in magnetic field free TEM mode, we can visualise a permalloy magnetic domain structure using near-field ptychography [3] and the reconstructed phase image is almost equal to that measured by off-axis electron holography (see the enclosed figure). When switching to the parallel beam 4D STEM setup, ultralow dose phase reconstruction becomes possible (see the enclosed figure) with only second acquisition time and minutes reconstruction period.

Conclusion

A sampling efficient phase contrast imaging method is developed based on ptychography principle but with a bit adjusted optical setup including structured illumination and near-field diffraction measurement. It holds great promise to study large cellular structures with enhanced phase contrast or measure time-resolved dynamics of electronic or magnetic devices. Further discussions on how to push further its spatial resolution will be discussed in this presentation.

Keywords:

Structured illumination, near-field ptychography, diffuser

Reference:

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Geometric phase analysis-based characterization of skyrmion lattice equilibria

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Poster Group 1

Magnetic skyrmions are, topologically protected, vortex-like magnetization patterns which can form a two-dimensional lattice. Skyrmion lattices, just as crystal lattices, can be deformed by both internal and external influences, resulting in dislocations or skyrmion grain boundaries. Understanding the rise of skyrmion lattice deformations is important to find the correlation between local and external influences on skyrmion lattice formation and stability. We apply geometric phase analysis (GPA), a widespread image analysis technique for measurement of deformation fields in high-resolution transmission electron microscopy images (TEM), to simulated Lorentz TEM images. Lorentz TEM images have been calculated by means of micromagnetic simulations based on FeGe chiral magnet which supports stabilization of skyrmion lattice. We have simulated Lorentz TEM images with and without the application of the spin-polarized current. The strain and dislocation maps were obtained by applying GPA to all simulated images. Our findings suggest that applying spin-polarized currents can lead to further stabilization of skyrmion lattice compared to ground state. We demonstrate that GPA can be successfully used for the analysis of Lorentz TEM images both at static and dynamic conditions. Strain analysis of skyrmion lattice in Lorentz TEM images can further enhance the knowledge of deformation mechanisms and skyrmion lattice dynamics.

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Keywords:

GPA, LTEM, Magnetic skyrmions

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Electron microscopy characterization of grain boundaries in Nb1-xTixFeSb based half-Heusler thermoelectric materials

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Poster Group 1

Background incl. aims
Thermoelectrics are a group of semiconductors which generate electrical power by conversion of heat into electrical energy. A thermoelectric power generator requires a hot and a cold side to use the temperature gradient for this conversion of energy.
Microstructure engineering has been used in the thermoelectric material research community to reduce the phonon mean free path and thus led to reduction of thermal conductivity which is crucial for thermoelectrics. This can be done by grain refinement, i.e. reducing the grain size for more phonon scattering. The challenge remained to keep a high electrical conductivity since increasing the grain boundary fraction usually led to decrease of electrical conductivity. To achieve the desired properties in thermoelectrics, it is important to understand the dependence of the microstructure of grain boundaries and correlate it to the thermoelectric properties. For this, detailed electron microscopy investigations covering several length scales from the µm to an atomic scale need to be conducted.

Methods
Scanning electron microscopy (SEM) analysis including electron backscatter diffraction (EBSD) and energy-dispersive X-ray spectroscopy (EDX) has been done to observe the µm down to nm scale microstructure. The grain boundary characterization down to atomic scale was achieved with (scanning) transmission electron microscopy ((S)TEM). In addition, atom probe tomography (APT) was used to get a chemical analysis on the composition of the grain boundaries with highest accuracy and spatial resolution.

Results
EBSD analysis of the grain size of the Nb1-xTixFeSb based half-Heusler thermoelectric materials synthesized at two different temperatures and two different Ti contents of 5% and 20% replacing Nb revealed a significant difference related to the grain size while all samples showed a random Mackenzie distribution for the grain misorientation (see Figure 1a). The later observation means that specific low angle grain boundary types were not dominating.
To uncover this mystery and proove our hypthoesis of resistive and conductive grain boundaries, we combined APT analysis with STEM analysis at the grain boundaries. We observed a defect grain boundary phase at random grain boundaries (Figure 1b) with chemical composition and lattice parameters differing from the one of the matrix phase of the half-Heusler material. The parameters are close to an FeSb phase in all four samples (different doping content and grain size) but with an additional segragation of Ti content for the high Ti doped samples which was shown with APT (see Figure 1c). This defect grain boundary phase proved to be the reason for increased electrical conductivity by providing conductive pathways in the high Ti doped sample.

Conclusion
Scale bridging electron microscopy was key to understand the correlation between microstructure and thermoelectric properties of NbFeSb based half-Heusler p-type thermoelectric material.[1] We have revealed a way of decoupling the electrical and thermal conductivity to tune the properties for maximizing the materials performance. This way of grain boundary alloying will provide a new road map for microstructure engineering in the future and should also be applicable to other half -Heusler materials.

Keywords:

SEM, EBSD, STEM, APT, Thermoelectrics

Reference:

R. Bueno Villoro, D. Mattlat et al., Grain Boundary Phases in NbFeSb Half-Heusler Alloys: A New Avenue to Tune Transport Properties of Thermoelectric Materials, Advanced Energy Materials, 2204321, (2023)

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Interference based optical instrument for high-throughput characterization of nanoparticles in complex biofluids

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Poster Group 1

Background incl. aims
Extracellular vesicles (EVs) are biological nanoparticles found in biofluids such as blood. EVs have been suggested to play an important physiological role, and potentially serve as markers for various diseases. However, EVs are often confounded with other bio-nanoparticles, such as lipoproteins and protein complexes. Currently no method can detect and characterize EVs in a high-throughput manner. We present an optical instrument with spatial resolution to detect and characterize EVs based on light scattering measurements. The underlying technology relies on creating a standing wave of two interfering lasers, of which one is variably phase modulated to displace the interference pattern within the intersection of the two laser beams (Fig. 1A).

Methods
The core concept is inspired by amplitude modulation radio. Specifically, this optical instrument encodes spatial information in the illumination source, manifested as an interference pattern that can variably sweep in the interrogation zone. By phase delaying one of the laser beams, we can produce a sweeping interference pattern with a known scrolling frequency (Fig. 1A-B). As nanoparticles traverse the sweeping interference pattern, the light scattered by these particles oscillate with the scrolling frequency. This enables “locking-in” on the narrow bandwidth of the oscillating signal and exclusion of the majority of broadband noise (Fig. 1C). Hereby we can increase the signal-to-noise ratio (SNR) of these already dim scattered light intensities from nanoparticles, and thus improve the lower detection limit. Furthermore, it is possible to determine size of nanoparticles and extrapolate the refractive index from the measured signal. Thus, the inherent nature of this optical instrument allows us to distinguish between different classes of bio-nanoparticles in a label-free manner (Fig. 1D). To validate the presented optical instrument, a range of nanostructures with known sizes and refractive indices were fabricated and used to probe produced light field. This was done to characterize (1) the actual wavelength of the standing wave, and (2) the extent of the interrogation zone. We are further validating that scattered light from bio-nanoparticles can be expressed with Lorenz-Mie theory to develop an accurate model for extrapolation of particle refractive index.

Results
Currently, we have constructed a prototype of the optical instrument. This serves to prove the core concepts and demonstrate the feasibility of this instrument to (1) improve the SNR for dim light scatter signals, (2) distinguish between nanoparticles based on size and refractive index. To assess these features, we are fabricating a range of nanostructures with differing shape, size, and material properties, which we can use to characterize the performance of the optical instrument.

Conclusion
The complex bio-nanoparticle landscape is yet to be explored, and no methods have been demonstrated to have adequate sensitivity for high-throughput characterization and distinction between different classes of bio-nanoparticles. In this poster abstract we present an optical instrument that addresses these short-comings by utilizing a scrolling interference pattern to characterize bio-nanoparticles capable of measuring size and modelling refractive index. The project is still in an early stage, and the technology is yet to be fully demonstrated.

Keywords:

Extracellular vesicles, Light scattering, Label-free

Reference:

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Observing the in-situ formation of the lead-free piezoceramic potassium sodium niobate (KNN) with SAED

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Poster Group 1

Background incl. aims
The use of PZT has continued despite the restriction of hazardous substances (RoHS) directives being passed by the European union (EU) to limit the use of hazardous materials – such as lead (PZT) – in electronic equipment. This is influenced by a lack of available substitutes that can be implemented and scaled in a reasonable time to eliminate the use of PZT (RoHS Annex III). The lead-free alkali-niobate, potassium sodium niobate (KxNa1-xNbO3, KNN), is a potential replacement for PZT, since it can exhibit equivalent, if not better, piezoelectric properties without compromise at temperatures >120 °C. The challenges in the synthesis of KNN, however, have hindered its use as an alternative. Notable challenges include difficulty in achieving the 50% K+ and Na+ occupancy required to make stochiometric KNN (i.e., K0.5Na0.5NbO3) and a lack of control in the formation of phase boundaries. Therefore, developing a reproducible method for synthesising stoichiometric KNN, bodes well for the implementing KNN as a commercial piezoceramic.
Methods
A simple and robust method for synthesis of the lead-free piezoceramic material KNN has been developed via an aqueous route. Stochiometric KNN (K0.5Na0.5NbO3) was prepared by combining alkali-nitrate solutions (NaNO3 and KNO3) with the nano-sized, water-soluble, niobium precursor hexaniobate ([HxNb6O19]8-x, Nb6), followed by sintering at elevated temperatures. Ex-situ studies on the formation of solid-state niobium oxides from Nb6 indicate formation of amorphous niobium oxides ca 400–500 °C. However, observing the dynamics accompanying this crystallization process are limited with conventional solid-state methods. We therefore probed the transformation of Nb6 in the presence of alkali-cations, to yield crystalline KNN from an amorphous oxide precursor. This was possible via in-situ heating with simultaneous selected area electron diffraction (SAED). Whereby, the amorphous Nb6-KNN precursor was mounted on a micro-electromechanical systems (MEMs) chip (Norcada Inc.), which is heated via a tungsten filament coil. In-situ heating experiments proceeded with heating from 400 to 1000 °C, at ramping rate of ca 0.25 °C s-1 in vacuum.
Results
KNN can be synthesised with heating NaNO3 and KNO3, with the water-soluble hexaniobate (Nb6) precursor. The results from SAED indicate the amorphous Nb6-KNN precursor becomes crystalline above ca 550 °C, without degradation after further heating up to 1000 °C. The SAED patterns at 800, 900 and 1000 °C, were acquired along the (101) axis, and agreed with the simulated diffraction pattern of stoichiometric KNN reported previously. The SAED patterns <800 °C exhibited sporadic spots which were incongruent with the simulated diffraction pattern. However, below ca 550 °C there were no obvious spots in SAED patterns, which is consistent with the presence of an amorphous oxide. These findings merit the use of a water-soluble niobium precursor, such as Nb6, as an alternative to accessing KNN in the solid-state.
Conclusion

In-situ heating with SAED is a suitable technique for monitoring the real-time transformation of amorphous precursor oxides, to yield crystalline lead-free piezoceramics. Formation of the lead-free piezoceramic KNN is achievable using the water-soluble, niobium precursor Nb6, which is advantageous compared to conventional solid-state methods. This makes it an attractive alternative approach for developing lead-free piezoceramic materials.

Keywords:

Lead-free, hexaniobate, KNN, in-situ, TEM

Reference:

Reference:
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Quarantotto: a 48-segment STEM detector for enhanced STEM performance and new applications

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Poster Group 2

Background incl. aims

In scanning transmission electron microscopy (STEM) the image formation process requires the acquisition of convergent beam electron diffraction (CBED) patterns, at every position of the probe on the sample. Traditionally, disc or ring detectors capture only part of CBED signal within their area, enabling annular bright field (ABF) or annular dark field (ADF) STEM imaging. The center-of-mass (COM) of the CBED pattern provides an essential information on the sample, as the COM vector represents the projected electric field of the sample, the gradient (differential) of the projected electrostatic potential [1, 2]. In thin samples, the projected electrostatic potential is encoded in the phase of the electron wave passing through the specimen, and for this reason these COM-based techniques are referred to as differential-phase-contrast (DPC) techniques.

DPC-STEM techniques have emerged as a powerful tool, offering unprecedented insights into electric and magnetic properties of materials at nano and atomic scale. Integrated DPC-STEM technique (iDPC-STEM) [2] allows efficient imaging of light elements (including hydrogen) next to heavy ones, beam sensitive materials including Zeolites, Metal-Organic-Frameworks (MOFs) at sub-Å scale, as well as cryo-biological nanoparticles at near-atomic resolution [3].

DPC-STEM and iDPC-STEM are implemented in the simplest way by exploiting a four-quadrant detector [1], as this is sufficient for obtaining a good representation of the COM vector. At the other extreme, cameras with larger number of pixels can be used to record the full CBED patterns. This approach is known as 4D-STEM and enables additional reconstruction techniques such as ptychography. However, due to readout limitations, the acquisition speed of cameras (10 to 100µs dwell-time) is much lower than of single/multi-segment detectors (100ns dwell-time). While the precision of 4D-STEM methods benefits from larger number of pixels, many practical applications require dwell-times of 1µs, or smaller, making the use of cameras not an ideal choice.

In this work, we propose an intermediate solution based on a 48-segment detector, called Quarantotto. With this approach, the acquisition speed can be as fast as with a single segment, while, at the same time, the larger number of segments improve the COM measurement. This leads to contrast transfer improvement for DPC-STEM and enables implementation of new applications, such as aberration measurements [4] and segmented ptychography.

Methods

Although four segments provide very accurate results for most applications, there are cases (e.g. thicker samples and heavy atoms) where the effects of the 4-fold symmetry of the detector is

reflected in the corresponding contrast-transfer-function (CTF). The CTF improves by increasing the number of detector segments as the COM vector can be precisely computed and an ideal version of iDPC-STEM, called iCOM-STEM, can be obtained. When a smaller number of larger segments is used, the positions of their geometrical-gravity-centers are used (the DPC mask in Fig. 1a-d bottom) to map their integral signals and compute COM of the CBED.

Quantification of the atomic electric field based on COM was used to demonstrate how the number of segments in segmented-STEM detector affects the accuracy of the measurement compared to cameras [5].
Quarantotto, the newly proposed solid-state detector, Fig. 1a-d top row, consists of 48 segments arranged in 3 rings of 8, 16 and 24 segments of equal area. All 48 segments can be simultaneously read out at a speed of 100ns dwell-time. The improvement in the CTF for the DPC-STEM and iDPC-STEM techniques with the 48-segment detector, is demonstrated against the three sub-configurations of the detector. The first two sub-configurations are two orientations of a regular 4-quadrant detector (Fig 1a and b), the third one is an 8-segment detector consisting of 8 pie-pieces (Fig. c).

In addition to DPC-STEM and iDPC-STEM, the 48-segment STEM detector enables efficient implementation of new techniques, such as segmented-ptychography, where the reconstructed CBED patterns are matched with the measurement segment by segment, and fast probe aberration measurements, where the shifts between the 48 segment images based on the aberration function are matched with the measured segment image shifts. In the past, these techniques have not been fully explored because of the lack of a detector featuring both enough segments and high speed, but now possible with the 48 segments detector.

Results

Quarantotto was used to demonstrate a more rotationally symmetric CTF when compared to four quadrant detectors. Figure 1 shows a direct comparison between iDPC-STEM images of gold islands on amorphous carbon, acquired at 300keV and convergent semi angle of the beam of 30mrad, obtained with the two possible 4-quadrant configurations (Fig. 1a-b), 8-segment azimuthal segment configuration (Fig. 1c) and the full 48-segment detector (Fig. 1d), respectively. For each image, the bottom row shows the FFT where the bottom-left insets show the DPC mask (x-direction), while the bottom-right insets show the theoretical CTF [1] using given detector configuration.

The FFTs of the iDPC-STEM images reflect the corresponding symmetry of the CTFs. The possible undesired effects of the noncentral symmetrical CTFs of 4-quadrant configurations are largely mitigated with 8-quadrant configuration and eliminated using the 48-segment configuration. The CTF of the 48-segment configuration is practically indistinguishable from the ideal rotationally symmetric situation of a camera.

The detector has been successfully utilized to measure and tune the aberrations of the microscope. This was achieved by measuring the shifts between the 48 segment images and fitting them to the expected shifts based on the aberration function. The image in Figure 1 was obtained after correcting for these measured aberrations.

Quarantotto has also been benchmarked against EMPAD to validate the precision of the electric field measurement across a GaN-GaAlN nanowire structure.

Conclusion

The 48-segment detector was used to demonstrate an improvement in (i)DPC-STEM performance when compared to the 4 and 8-quadrant detectors. It has been successfully used to measure the electric fields across a GaN-GaAlN nanowire structure and to tune the optical aberrations of the microscope. Overall, the Quarantotto detector represents a significant advancement, offering improved imaging at ultimate speed, larger field of view measurements, and the implementation of new techniques.

Keywords:

Multi-segment-detector, iDPC-STEM, iCOM-STEM, Ptychography, Aberration-Correction

Reference:

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Correlative Microscopy Analysis of Jack Hills Zircons by Photonic Atom Probe

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PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

Background incl. aims
The application of Atom Probe Tomography (APT) to geological materials has recently disclosed new possibilities into the nanoscale characterization of these systems, yielding important results in the interpretation of the processes that characterize their growth and the subsequent modifications [1, 2]. APT may indeed be exploited because of its 3D nanoscale resolution within a multiscale approach including a set of microscopical and microanalytical techniques. Optical emissions in some minerals have been related to the presence of impurities and defects within a matrix, and can be used in correlative nanoscale analyses targeting both chemical – possibly structural – and optical properties. In this framework, cathodoluminescence (CL) has already been applied as a spatially and spectrally resolved technique [3].
Zircon is a robust geochronometer and one of the most widely used minerals across the geosciences to quantify the nature, timing, and duration of geological processes. Zircons exhibit a strong CL signal and a significant optical emission with spatially varying luminescence properties, also due to the presence of luminescent quartz inclusion within the zircon matrix. In this work we aim to provide more in-depth nanoscale information on such luminescent systems using correlated analysis obtained by coupling APT with in-situ optical spectroscopy, using the recently developed Photonic Atom Probe (PAP) [4].
Methods
As a model system, zircon ($ZrSiO_4$) grains from Jack Hills were embedded in resin, then carbon coated to enable SEM imaging. CL and EDX were carried out to reveal chemical and optical properties, and help determine suitable regions of interests. The samples were then prepared into sharp needles using the standard site-specific FIB lift-out method followed by annular milling to enable subsequent APT analysis. The APT and photoluminescence (PL) analyses were performed simultaneously in the PAP, where a deep-UV laser was used to trigger both the field-evaporation of the surface atoms and the optical emission.
Results
Following CL and EDX analysis, samples from several zircon grains were analyzed and compared using the PAP. Data collected from different phases highlight varying PL signals that were correlated to the measured chemistry, thus demonstrating the capability to discriminate microscale features. Our exploratory results indicate the possibility to obtain different types of correlation at different scales: (a) the correlation of CL or EDX hyperspectral maps with the APT analysis of tips whose location is controlled on the crystal surface (b) the correlation of optical PL spectra and 3D APT reconstructions within the in-situ approach enabled by the PAP (c) the multi-scale correlation of CL and PL spectra providing a useful insight on the optical properties of the samples.
Conclusion

The optical properties of zircons at the micron and nanoscale were exploited to carry out multi-scale correlative chemical and structural analysis. Varying PL spectra obtained in-situ from different phases were correlated to CL and APT data, demonstrating the photonic atom probe as a new correlative nanoscale analysis characterization technique for the geosciences.

Keywords:

Zircons, APT, Photonic Atom Probe

Reference:

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Fabrication and high-resolution transmission electron microscopy characterization of nanopores in silicon nitride and 2D materials

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Poster Group 2

Background
Solid-state nanopores are an emerging technology for single molecule sensing of biomolecules including DNA, RNA, and proteins [1]. They provide a more resilient alternative to tradition biological nanopores, which are sensitive to temperature, pH, and other environmental factors. Specifically for solid-state nanopores, 2D materials provide a good foundation that is easy to modify, robust, and reusable [2,3]. 2D materials have unique properties that only appear when bulk materials are reduced to the nanoscale. Specific attributes can be generated in the 2D material and alter the dynamics of molecules travelling through the nanopores. Materials of interest include graphene, hexagonal boron nitride (h-BN), and transition metal dichalcogenides (TMDCs).
Methods
Different techniques for manufacturing solid state nanopores are investigated. These include transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM), and helium ion microscopy (HIM). From these microscopy methods, nanopores are created in SiNx membranes as well as in 2D materials.
Results
Pores in the range of tens of nanometres are milled into SiNx using the HIM. Single nanopores as well as arrays of nanopores were created to investigate various milling parameters (Figure 1. A, B). DNA translocations were observed by ionic current measurements, validating the presence and function of the nanopore. For 2D materials, a scaffold of SiNx must be used to create freestanding 2D materials. Graphene and h-BN are transferred onto HIM milled or gallium focused ion beam (FIB) milled holes in SiNx membranes. The resulting membranes are imaged with high resolution TEM (Figure 1.C).
Conclusion
Electron microscopy and ion beam milling are promising techniques for the production of solid-state nanopores. Various pore geometries will continue to be explored and methods will be developed to create novel nanopores in 2D materials. The combination of 2D materials results in unique properties in nanopore structures which would otherwise be difficult to achieve without the use of electron microscopy and nanostructuring.

Keywords:

Nanopores, 2D materials

Reference:

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Microstructure of light-emitting phosphor of (Sr, Ca)AlSiN3:Eu2+

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Poster Group 2

If the peak half-maximum width of the emission spectrum is wide, the emission of light at wavelengths far from the target emission peak wavelength may increase, and the emission color indicating the desired chromaticity coordinates may not be obtained. In addition, in the case of red phosphors, the emission of light increases in wavelength regions with low specific visual sensitivity, such as 700 nm or more, which contributes to a decrease in the conversion efficiency of the light-emitting device. For this reason, phosphors with a narrower peak half-maximum width are required.

(Sr, Ca) AlSiN3:Eu2+ (SCASN) is a red phosphor [1] with a narrower peak half-width in the emission spectrum than CaAlSiN3:Eu2+ (CASN). These phosphors are highly reliable because they replace the Al site of AlN with Sr, Ca, and Si. In this study, we present the first microscopic results obtained for this type of materials.

SCASN and CASN have the same crystal structure, space group: Cmc21, which was investigated by the convergent beam electron diffraction (CBED) technique and the integrated differential phase contrast STEM (iDPC-STEM) technique. In the case of SCASN, the structure of the spatial group: Pcbn was also confirmed in the selected area of diffraction pattern, which was found to be due to the local atomic arrangement of the stacking fault. It was found using scanning moiré fringe imaging [2] that the higher the amount of Sr added in SCASN, the more dislocations and stacking fault co-exist, and that the disturbed stress distribution settles through the stacking defects in the region with high dislocation density. In addition, in SCASN manufactured by the high-pressure method, the distance between the stacking fault was far apart, and the stress distribution was stable.

These experiments have allowed the achieving of understanding of the close relationship between the ratios of Sr and Ca and the stress distribution and the extraction of significant features like the dislocation in the crystal, the stacking fault that it expanded, and the first neighbourhood distances between defects.

Keywords:

(Sr,Ca)AlSiN3, phosphor, iDPC-STEM

Reference:

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Molecular dynamics simulation of the Brownian motion of biomolecules in liquid phase electron microscopy

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Poster Group 2

Background incl. aims
Liquid phase electron microscopy (LP-EM) revolutionizes our ability to observe dynamic processes within liquids at nanoscale resolution. It allows for the direct observation of biological, chemical and physical phenomena in their native liquid environments which was not possible with conventional or cryogenic electron microscopy (Cryo-EM). While previous successes of the technique have predominantly been demonstrated with rigid materials such as gold nanoparticles [1], it is anticipated that the application of the technique on soft materials such as biological macromolecules may yield novel insights into the functioning mechanisms from the observed dynamics [2].
In this work, we aim to investigates the behaviour of biomolecular samples in a liquid environment and consider the effect of the Brownian motion.
Methods
We employed molecular dynamics simulations to study the behaviour of the biomolecules in liquid at the nanosecond scale. The structure of the horse liver alcohol dehydrogenase (ADH) enzyme was used for this study [3], as shown in figure (a). We simulated multiple trajectories of the ADH enzyme using the OpenMM library [4], and the ff14SB force field along with the TIP3P water model was used for all simulations [5].
Results
As expected, the diffusivity of the protein is found to be increasing with temperature from the simulations. The protein exhibits significant deviations due to Brownian motion, which can be characterised by a random walk model, in figure (b), we have shown a snippet of the trajectory of the centre of mass of the ADH at 300 K to confirm that the global motion of the protein is indeed random. When the thermal energy of the water molecules is low but still above the freezing point, the diffusive motion of the protein tends to favour the direction with the smallest solvent accessible surface area, this was due to the irregular shape of the ADH protein. Upon further increase of temperature to 300 K, this anisotropy diminishes, as the protein diffuses more isotopically potentially due to more frequent interactions between the solvent and itself.
The protein exhibits thermal vibrations across all temperatures, which collectively influence the overall deviation of its structure. These vibrations are temperature-dependent and exhibit a relatively stable magnitude throughout the period of observation. Additionally, higher temperatures can induce further localised deviations within the protein structure, particularly in regions with higher flexibility, such as the sequence termini, where deviations can be notably pronounced compared to the rest of the protein.
Conclusion
Overall, this study enhances our understanding of the effect of Brownian motion inherent to biomolecules in liquid and may inform the development of more effective experimental designs for LP-EM. Future studies could extend this analysis to samples of different configurations, as well as longer simulation times for more robust estimations over larger timescales. The authors would like to acknowledge funding from the BBSRC International Institutional Partnership Fund.

Keywords:

Liquid-phase, electron microscopy, molecular dynamics

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Multifunctional hybrid nanocomposite films: linking inorganic nanoparticles using α-synuclein as molecular linker

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Poster Group 2

Background incl. aims
Organic-inorganic hybrid nanocomposites can combine mechanical properties and particular functionalities exploiting organic macromolecules and inorganic nanoparticles. Inorganic nanoparticles can induce peculiar properties that can be exploited in several technological fields. For instance, luminescent nanomaterials are useful in nanomedicine as contrast agents, while proteins can act as stealth agents.
Methods
The interaction of inorganic nanoparticles with proteins is a matter of particular interest when studying their behaviour in a biological environment, especially for the development of new hybrid materials. Recently, investigation on hybrid monolayers of gold nanoparticles and α-synuclein, an intrinsically disordered protein associated with some neurodegenerative diseases such as Parkinson's disease, have appeared in the literature. Due to particular variations of the chemical environment, α-synuclein undergoes structural changes from a disordered state to a β-sheet conformation that leads to the formation of fibrils, useful for the formation of hybrid films. This behavior makes α-synuclein suitable for the development of nano-biocomposite nanomaterials such as nanofilms and filaments.
Results
Hybrid films composed of inorganic nanoparticles and α-synuclein have been developed. The hybrid films have been prepared, using a wet chemistry procedure, using various types of inorganic nanoparticles, as lanthanide doped alkaline-earth fluorides, to obtain nanocomposites with strong emission in the optical range. In particular, the structure of the inorganic nanoparticles and the α-synuclein monolayer is under study by means of STEM and HREM; the nanoparticle upconversion emission, a particular process involving emission of light (e.g. in the UV-visible range) upon excitation with electromagnetic radiation of lower energy (e.g. in the Near InfraRed, NIR, range) of the nanocomposites is described. The peculiar emission of the lanthanide ions in the optical range can be very useful for applications in nanomedicine, working in the so-called biological windows, exploiting the transparency of biological tissues in certain NIR ranges. Moreover, combination of different kinds of nanoparticles (e.g. gold and luminescent fluoride based nanoparticles) have been considered to develop multifunctional films with luminescence and thermometric capability for possible applications as biosensors.

Keywords:

nano-biocomposite nanomaterials, inorganic nanoparticles, upconversion.

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Quantitative EMCD analysis of Fe thin films on MgOx substrates

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Quantitative EMCD analysis of Fe thin films on MgOx substrates
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Background

The structure and electronic structure of interfaces determine their magnetic properties in many modern materials. Yet, X-ray and neutron-based analysis techniques, albeit their fantastic contributions to the field, cannot give the real space image of magnetization. And among the TEM techniques, the close-to-atomic scale analysis of magnetic interfaces remains a challenge. All three magnetic analysis techniques in the TEM, electron holography, Lorentz microscopy, and electron energy loss magnetic circular dichroism (EMCD) have now established the first results demonstrating that with TEM techniques, we can obtain magnetic information at this length scale [1-4]. In the EMCD technique, magnetic information originates from the interference of electrons inelastically scattered into different beams. This interference giving rise to the EMCD signal is appearing at well-defined and conjugated q-vectors called C+ and C- positions. It bears an intensity that can be interpreted as resulting from the orbital and spin magnetic moments of the involved magnetic atoms. The technique can address both ferro- and antiferromagnetic materials.
Here, we present EMCD measurement on thin Fe films on MgOx substrate and show how accurate alignment and experimental parameters like convergence and collection angles modify the EMCD signal and interpret this change in magnetic signal to obtain magnetization and orbital/spin magnetic moment ratio in the Fe and at the Fe/MgO interface.

Methods

Fe films were grown on MgOx (001) substrate by sputter deposition. The cross-sectional and plan view samples were analysed using the EMCD technique in STEM mode. Spectra of the L3 and L2 edge of Fe corresponding to the C+ and C- positions were acquired at 2 beam condition using a Titan/Themis probe corrected TEM at an acceleration voltage of 200 - 300 kV with Gatan energy filter and a CEFID energy filter equipped with a direct electron camera ELA. The q-selection of the EELS spectra was carried out by using a custom-made script to shift the diffraction pattern and by placing a slit aperture into the EELS entrance aperture holder, see schematic Figure below. Each acquisition contains between 2000-10000 spectra which enables the treatment of individual spectra or sum spectra as well as the use of statistical methods in data analysis.

Results

Refinements of both, EELS analysis conditions as well as the sample quality enables us to improve the EMCD signal strength from the original paper of around 4 % to 17 % as shown in the figure below,

which was taken at small convergence angle and using the conventional circular EELS entrance aperture. Though, aiming to approach atomic resolution in magnetic measurements, high convergence angles in the EMCD analysis are needed, which will be typically larger than 5 mrad. The other limit of such EMCD analysis is beam damage. We analysed the EMCD signal as a function of convergence angle and optimized acquisition conditions in view of limiting beam damage. With those conditions, we analyse EMCD signals at the Fe/MgOx interface with down to 2Å resolution [4,5].

Conclusion
In summary, we have optimized and analysed signals in EMCD measurements on Fe/MgOx . An excellent EMCD difference signal as well as improvement in the signal-to-noise ratio is evident due to the crystalline quality. We have obtained interfacial profiles of EMCD signals with down to 2Å resolution.

Keywords:

EMCD, HRSTEM, Magnetic measurement.

Reference:

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Laboratory Soft X-ray Microscopy for Biomedical Applications

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Poster Group 2

Background incl. aims: Soft X-ray microscopy is a powerful tool for three-dimensional investigation of biological material [1]. The water window energy range between the absorption edges of carbon (284 eV) and oxygen (543 eV) provides a strong natural contrast for aqueous samples and offers the possibility for both a high resolution of a few tens of nanometer and a high penetration depth of up to 10 µm. Within the Collaborative Research Center 1340 (CRC) “Matrix in Vision” funded by the German Research Foundation, we use soft X-ray microscopy to investigate the role of the extracellular matrix (ECM) in diseased tissue, as the components and properties of the ECM play a major role in the regulation of cell and tissue function.
Methods: Alteration of the ECM plays a pivotal role in the progression of inflammatory diseases such as atherosclerosis. Understanding the underlying processes contributing to ECM alterations can aid in early detection during the initial stages of development. The ECM of cryofixed model cells (THP-1 cell line derived from an acute monocytic leukemia patient) has been studied with a laboratory soft X-ray microscope (L-TXM) located at the Berlin Laboratory for innovative X-ray Technologies (BLiX) at TU Berlin. A correlative workflow was developed by integrating a visible light microscope into the L-TXM setup [2], allowing a fast transition between the two modalities, and facilitating sample localization to accelerate 3D cell imaging. Additionally, various upgrades have been performed on the L-TXM setup to enhance stability and increase sample throughput in order to streamline the workflow and address the specific demands of the samples investigated within the CRC.
Results: The upgraded L-TXM at TU Berlin offers enhanced stability and increased sample throughput for biomedical research within the CRC, resulting in consistent imaging quality over extended acquisition times. Laboratory-based soft X-ray microscopy was able to resolve the THP-1’s glycocalyx – an intricate fragile extracellular structure. Nevertheless, different sample preparations resulted in different thicknesses and lengths of the glycocalyx’s interlocking meshy structure.
Conclusion: Laboratory soft X-ray microscopy provides unique flexibility and access to high resolution 3D imaging. Recent upgrades of the laboratory setup now enable a more streamlined workflow. The resulting higher sample throughput will help to establish suitable preparation techniques for various cell types for soft X-ray ECM research within the CRC. Combining results from X-ray based analytics with clinical diagnostics adapted for ECM research will help to better understand the role that the extracellular matrix plays in (inflammatory) diseases.

Keywords:

Soft X-ray Microscopy, Extracellular Matrix

Reference:

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1096

Nanowire field emitters fabricated using focused electron, gallium and helium ion beam methods

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Poster Group 2

Background incl. aims
Devices whose operation is based on the field emission phenomenon are widely used and continuously developed. Among the numerous categories, vacuum microelectronics stands out. In previous decades, the fabrication of field emitters based on the Spindt method was developed[1]. This is a multi-step method based on etching and deposition of selective areas on a volumetric substrate. The extremely attractive topic of integrating field emitters with microelectromechanical systems (MEMS), acting as nano- and picometrology sensors, has now emerged[2]. However, the fabrication of multicomponent micro- and nanodevices in a single process line is a challenge. Therefore, combining different manufacturing technologies should be considered. A method that enables the creation of planar and three-dimensional nanodevices is a combination of focused-electron-beam-induced deposition (FEBID) and focused-ion-beam-induced deposition (FIBID)[3]. This is a one-step, so-called direct-writing method. The material of the deposited nanodevice is defined by the precursor used. Its shape is determined by the scanning motion of a focused beam with nanometre and sub-nanometre cross sections. The equipment that enables the fabrication of ultra-sharp field emitter tips is the scanning electron microscope (SEM) with a focused gallium ion beam (Ga-FIB) and the helium ion microscope (HIM). A commonly used material is tungsten[4] due to its good thermal and electrical conductivity, low work function and the ability to sustain high current densities. However, platinum is also of interest due to its high chemical inertness[5]. In this work, nanowire platinum and tungsten field emitters fabricated by the FEBID and FIBID (Ga and H) process will be shown for the first time. The field emitters will be integrated into microcantilevers manufactured by photolithography, acting as nanosensors of their deflection. The field emission phenomenon will be studied and the operation of the nanosensors will be evaluated.
Methods
A Helios Nanolab 600i SEM with FIB equipped with MeCpPtMe3 precursor and a Zeiss Orion Nanofab HIM containing W(CO)6 precursor were used for microcantilever modification and deposition of nanowire field emitters. During the FEBID and FIBID process, precursor molecules are injected into the vacuum chamber of the microscope. The primary focused electron/ion beam induces secondary electrons from the sample surface, which have the required energy to decompose the precursor molecules. Hence, in the vicinity of the scan area the material grows, leading to the fabrication of the field emitter nanowire. In order to prepare the microcantilever for field emitter deposition and eliminate the risk of short-circuiting the electrodes due to the existence of the halo effect, the microstructures were modified by milling with a focused gallium/neon beam. Leakage currents between the electrodes were measured before depositing the field emitters on them. The field emission phenomenon was characterised in the vacuum chamber of the microscope, using a self-designed electronic setup, a source measurement unit Keithley 2614 and software written in the

LabVIEW environment. Analysis of the nanowire material composition was conducted with an energy-dispersive X-ray detector attached to a transmission electron microscope.

Results

Calibration of the growth of platinum and tungsten nanowires was performed using SEM-FIB and HIM, respectively, for specific values of beam current, accelerating voltage, dwell time, beam spacing, and precursor pressure. Field emission enhancement factor calculations were made to optimise the emitter shape. Microcantilever modifications were performed using a focused gallium and neon ion beam (Figure 1a). Spatial holes were milled between adjacent electrode paths dedicated to the integration of field emitters on the microcantilever. The aim was to separate them permanently, minimising the risk of short-circuiting due to the halo effect associated with the FEBID and FIBID process. Leakage currents between adjacent electrode paths were measured in the range of +/-100 V. Measurement values oscillated in the pA regime. Planar nanowires, as field emitters, were then deposited with a distance of about 100 nm between them (Figure 1b,c). In situ field emission measurements were conducted for the fabricated structures. Field emission from 50 V was recorded for the platinum field emitters with a distance of 150 nm between the electrodes. For tungsten field emitters and a cathode-to-anode distance of 120 nm, the threshold voltage of field emission was equal to 70 V. The stability of the emitter operation over time was verified, together with an analysis of the emitter material composition before and after operation.

Conclusion

This work presents a fabrication technology for nanowire field emitters by the FEBID and FIBID process, using two different precursors - MeCpPtMe3 and W(CO)6. The technique of integrating FEBID/FIBID nanodevices with microcantilevers produced in photolithography steps was developed. The characterisation of the field emission phenomenon was undertaken as a basis for investigating the usability of a microcantilever deflection sensor.

Acknowledgements

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Keywords:

Field emitters, SEM, HIM, MEMS

Reference:

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Investigation of Lateral and Vertical Heterostructures of MoS2/WS2

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Poster Group 2

Background
Two-dimensional (2D) materials have the potential to transform semiconductor technology. Their rich compositional and stacking diversity, especially when deposited as heterostructures, allows tailoring of material properties to enable a wide range of device applications [1]. A prominent class of these 2D materials are the transition metal dichalcogenides (TMDs). With improved synthesis and fabrication capabilities, heterostructure concepts have been developed that show promising material properties for ultrathin optoelectronic devices. While these heterostructures can be fabricated by mechanical pattern transfer for small areas with high precision, these approaches are difficult to scale up. Therefore, alternative approaches such as metal organic chemical vapor deposition (MOCVD) have been developed to fabricate 2D materials and their heterostructures. Depending on the process parameters, several growth modes can dominate and thus a variety of structure combinations can be created. Since optical and electronic properties typically depend on the orientation, high-resolution measurements of the number of layers and feature sizes of each material can help to understand macroscopic optical measurements and guide the optimization of growth processes towards desired layer structures.
Methods
The MOCVD grown WS2/MoS2 heterostructures were transferred using a PMMA transfer process with successive cleaning and baking steps. Scanning transmission electron microscopy (STEM) measurements were performed using a JEOL JEM-2200FS. By tuning the convergence angle to less than 2 mrad, we obtained scanning diffraction data leading to nanoscale resolved alignment information. In addition to these diffraction measurements, we use energy dispersive X-ray spectroscopy (EDX) to resolve the chemical composition of the few-layer heterostructures. The structure is further resolved by conventional quantitative annular darkfield STEM image simulations and 4D-STEM measurements.
Results
We find evidence for both vertical and lateral heterostructures of MoS2/WS2 and a homogeneous but complex 2D film with few layers and pronounced island growth. The number of layers can be deduced by quantitative EDX mapping supported by image contrast simulations (Figure 1a). We find a nearly coalesced WS2 monolayer with additional multilayer island growth. The MoS2 layer grown on top can be shown to form either from facets of the multilayer WS2 (Figure 1b) or from nucleation without facets on top of the tungsten TMD. Figure 1c shows an example of a presumably vertically stacked MoS2/WS2 heterointerface. It can be seen that the contrast is dominated by the tungsten columns of the underlying film, which highlights the need for quantitative image simulations.
Conclusion

We can show the microscopic orientations of the TMD heterostructures in the case of MoS2 and WS2 grown by MOCVD. The combination of analytical techniques such as EDX and quantitative image simulations helps to unambiguously identify the complex layer structure of this few layer material system. We are also exploring the possibility of identifying relevant material domains by large area scanning nanobeam diffraction using 4D STEM imaging.

Keywords:

2D Materials, STEM, Heterostructures, TMDs,

Reference:

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In-Situ microscopy study on self-healing process of vitrimers

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Poster Group 1

Vitrimers are reprocessable and recyclable polymers with a wide range of adjustable properties. One keypoint of the reprocessability is the thermoreversible associative dynamic exchange mechanism which makes a vitrimer behave like viscoelastic fluid at elevated temperatures and like a thermoset at low temperatures. This change in behavior also has the potential to design polymers with "self-healing" characteristics where scratches or fractures are removed by applying heat. In this work we track this self-healing mechanism of a scratched vitrimer surface with in-situ microscopy and surface reconstruction using a segmented backscatter electron detector. The vitrimer used in this study is a tailor-made vinylogous urethane vitrimer with self-assembling binary and ternary block and random copolymers.[1]
The surface height of the sample is obtained with a shape-from-shading algorithm that uses the angular dependency of backscattered electrons and the orientation of the surface inclination to the detector. This technique does not need sample tilting and allows for live in situ topographic view of sample surfaces.[2]
The topographic view of the scratch show the displaced material as a 2 µm high ridge on both sides right next to the 5 µm deep scratch. After heating the sample to 115 °C for 2 minutes the ridges have flattened to a large extent due to the transition of the polymer to a fluidic state.
This initial result show the great potential of topographic imaging in in-situ experiments as conventional SEM imaging only result in 2D-images without or only obfuscated height information.

Keywords:

shape-from-shading, in-situ microscopy, vitrimers

Reference:

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Deep learning-based single cell volume segmentation for soft X-ray microscopy data

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Poster Group 2

Background and Aims:
Soft X-ray microscopy within the water window spectral range offers non-destructive imaging capabilities with nanometer resolution and a penetration depth of up to 10 µm. This enables the investigation of biological samples in a near native state in three dimensions. The interpretation of X-ray microscopy images of biological cells can be challenging due to the similar absorption characteristics of carbon-based structures within these samples. To address this challenge, we have developed a contrast enhancement protocol, to improve the interpretability of the acquired images. Subsequently, an efficient segmentation technique based on deep learning is deployed for the extraction of biologically relevant information from the large amount of data generated by fast tomogram acquisition.
Methods:
We employed a laboratory-based soft X-ray microscope operating within the water window (500 eV photon energy). To enhance the contrast in acquired images, we developed a contrast enhancement protocol incorporating an adaptation of the Paganin filter as well as an unsharp masking filter. Additionally, we deployed a neural network model utilizing a U-Net architecture for the segmentation of THP-1 cell tomograms. This approach leverages the natural higher contrast of lipid membranes to facilitate segmentation.
Results:
The implementation of the contrast enhancement protocol resulted in improved contrast-to-noise ratios, thereby enhancing the interpretability of acquired images. Moreover, the neural network-based segmentation technique efficiently extracted biologically relevant information from tomograms, as confirmed by organelle volume calculations that align with tabular data. This segmentation can enable quantitative insights at the subcellular level, including the location and distances between different organelles, as well as their concentrations.
Conclusions:
Our study offers a comprehensive approach to soft X-ray microscopy analysis, addressing challenges in image interpretation and data segmentation. The developed contrast enhancement protocol and segmentation techniques facilitate efficient and accurate investigation of biological samples at the sub-cellular level. For the generalization of the segmentation process, increasing both the volume and quality of the data, as well as refining sample preparation, is necessary. Nevertheless, our advances contribute to analyse soft X-ray microscopy data more efficiently.

Keywords:

Soft X-ray microscopy; Tomography; Segmentation;

Reference:

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Insights into the formation of polycrystalline seed layers for the solution growth of semiconductor nanorods

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Poster Group 2

Background incl. aims

ZnO, and Ga2O3 nanostructures are the subject of interest in many research groups. In particular, ZnO and Ga2O3 nanorods (NRs) have considerable potential for applications in gas sensors, piezoelectric nanogenerators, photovoltaic devices, and pressure sensors. Most applications require position-controlled ordered arrays of physically identical nanorods (NRs). Position-controlled nanorod arrays can be obtained by selective-area epitaxy (SAE) on a suitable seed layer (SL) [1].
The size, shape, and preferential orientation along the c-axis, as well as the in-plane orientation of the crystallites within the SLs, are crucial for successful nucleation, good alignment, and crystallinity of the NRs. Crystallographic, morphological, also optical, and electrical properties of the crystallites and nanocrystals can be influenced and enhanced by heat treatment [2, 3].
The impact of the SL on the orientation of the NRs is even more significant and visible during the SAE, where the NRs do not nucleate and grow in close proximity, and the geometrical selection is suppressed. Every deviation from the preferential orientation of crystallites along the c-axis causes a visible misalignment of the grown NRs.
Another property of wurtzite ZnO crystals, which not only influences the optical and electronic properties but also affects the nucleation and growth of ZnO and NRs, is the polarity of the crystals. Spontaneous polarization in the ZnO and GaN wurtzite structures is present because of the missing center of symmetry; therefore, the [0001] direction and [000-1] direction are not equivalent.
We provide novel insights into the formation and investigation of the properties and quality of SLs used for the growth of ZnO and Ga2O3. We utilize SAE-grown arrays of single NRs to scrutinize the misalignment of crystallites within SLs prepared under different conditions without the influence of geometrical selection. By enhancing the crystal quality, controlling the Ga2O3 phase, and understanding of the ZnO polarity, we are moving the ZnO and Ga2O3 nanostructures closer to the applications.

Methods

ZnO and Ga2O3 arrays were prepared by chemical bath deposition (CBD) on sol-gel-deposited SLs patterned by EBL. The SEM provided the first morphological characterization of the nanostructures. With the assistance of machine learning during SEM image processing, dimensional changes were evaluated in nanostructures prepared under different growth conditions.
The crystallographic structures of ZnO and Ga2O3 were further investigated using TEM and HRTEM. Plane and line defects, which significantly influence the electronic and optical properties of the nanostructures, were examined using HRTEM, DF, and WBDF.
The epitaxial relationship between the NRs and SLs was investigated in more detail by automated crystal orientation mapping in TEM (ACOM/TEM-ASTAR), where a high spatial resolution map of the

crystallite lattice orientation was obtained [2]. The interfaces between the NWs and SLs were investigated not only crystallographically but also chemically using EDX in the TEM.
Piezo-force microscopy (PFM) was used to examine the polarity of the crystallites within the ZnO SL. PFM is an AFM mode that can map the piezoelectric and ferroelectric properties of a material. The polarity of ZnO polar wurtzite NRs was investigated using convergent beam electron diffraction (CBED) and annular bright field (ABF) measurements.
In situ TEM heating experiments were performed on polycrystalline ZnO SL (Fig. 1) and Ga_2O_3 NRs to provide insight into the phenomena that occur at elevated temperatures.

Results
The SAE-grown arrays of NRs showed the expected behaviour. When the NRs showed significant misalignment on the non-patterned SL, the NRs in the arrays exhibited the same behaviour. In addition, the NRs showing good alignment on non-patterned SL sometimes grew well aligned in the arrays. However, a discrepancy was observed on a significant number of ZnO SLs, where the alignment of NRs from patterned and non-patterned SLs was not in agreement. This discrepancy was caused by geometrical selection during nonpatterned growth on SLs with a low texture.
Generally, the alignment of crystallites within ZnO SLs improves with elevated temperatures at the expense of reproducibility. The results of the ZnO SL sintering experiments in different atmospheres and on various substrates indicated the simultaneous occurrence of oxygen and zinc diffusion and preferential alignment. Depending on the conditions, this phenomenon occurs at different temperatures. Surprisingly, preferential alignment was also observed in the in-situ TEM heating experiment, where the substrate was not present. Furthermore, PFM investigation of the crystallite polarity evolution during the heat treatment of the ZnO SL suggests the unification of ZnO crystalline polarity at Zn-polar at elevated temperatures.
To move towards applications and achieve monocrystalline SAE-grown ZnO NRs on ZnO SL, the correlation between the size of an opening in the patterning mask and the size of crystallites in the SL was crucial. If the size of the opening was equal to or smaller than the average size of the crystallites in the SL, the probability of nucleation and growth of monocrystalline NR from a single crystallite increased significantly. The requirements for SL were still a good crystalline preferential orientation along the c-axis, maximum crystallite size, and minimized dispersion of crystallite sizes within the SL. Investigation of the crystal polarity dependence between the ZnO SL/ZnO NR indicated a hereditary relationship between the ZnO SL/ZnO NR polarities.
Although Ga_2O_3 has several polymorphs, the monoclinic β-Ga_2O_3 phase was mostly observed in the Ga_2O_3 nanostructures. SAE of β-Ga_2O_3 NRs on SnO SL showed no preferential alignment and uncovered a significant role of the geometrical selection. Furthermore, no epitaxial relationship was observed between the SnO SL and the β-Ga_2O_3 NRs during the in situ TEM heating experiment.

Conclusion
Using microscopy techniques and SAE, we delved into the behaviour of ZnO SLs during heat treatment. We break the common belief that vertical orientation of NRs on polycrystalline SLs can be directly linked to the quality and preferential orientation of the SL. With the help of SAE, we showed that this conclusion can be made only on arrays where the distances between the NRs are high and geometrical selection affecting the growth is not involved. We further demonstrated that the development of the preferential orientation of the crystallites in the SL during heat treatment is not related to the presence of the substrate.

Keywords:

Nanorods, semiconductors, ZnO, polar crystals

Reference:

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1101

SoftGrids: towards disease modelling in CryoEM

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Poster Group 1

Background incl. aim
In vitro cells derived from pluripotent stem cells (iPSCs) show some changes in their phenotype compared to their adult counterparts in vivo. Studies have shown that the application of external stimuli to provide a more natural environment has a major impact on many characteristics of cultured cells. Of particular interest is mechanical stimulation with a material whose stiffness is adapted to the tissue in which the cell type naturally occurs. Typical electron microscopy substrates such as EM grids do not offer this possibility. These usually consist of a combination of very hard grid bars and a very soft film and therefore do not offer the optimal conditions for some cell types. The aim of this work is to offer a workflow for cryo electron tomography (CryoET) that closes this gap by coating grids with a material whose mechanical properties can be easily modified.

Methods
In order to show the influence of the stiffness of EM grids coated with substrates of different stiffness, mesenchymal stem cells (MSCs) are seeded onto the grids and then examined for the quantitative expression of specific markers proteins using fluorescence microscopy. The markers selected were those that are usually expressed to a high degree in cells with strongly differentiated natural stiffness domains. They are categorized into neurogenic, myogenic and osteogenic markers. In addition, control cultures are created from progenitor cells that express the specific markers to a particularly high degree. The stiffness of the respective substrates is measured using atomic force microscopy.
For the integration into the CryoET workflow, iPSC-derived neurons and iPSC-derived cardiomyocytes are seeded on the grid and plunged after 7-10 days. With the help of a FIB, thin lamellae of about 200 nm are prepared. In a transmission electron microscope, tilt series are generated by gradually tilting the stage, from which tomograms are then generated.

Results
The measured intensities from the marker expression in MSCs show a maximum for a certain stiffness and a decreasing signal for cultures on grids whose stiffness leads further away from the ideal value.
The generated tomograms show vitrified content. The lamellae can be produced in a comparable time compared to non-coated standard grids. By using stripe grids for the topological stimulation of cardiomyocytes, the cells can be polarized efficiently. This configuration allows a very high slat density per grid.

Conclusion
We have shown that the cultivation of cells on our grids has an effect on the phenotype of certain cell types compared to standard EM grids and that this effect depends on the stiffness of the material. This behavior has been sufficiently proven for non-grid substrates in many studies. With the provided workflow for CryoET, the possibility has been created to perform model-based research

with iPSC-derived cells in structural biology. This opens another door, particularly for desease research on neuonal and cardiac model systems.

Keywords:

Cryo electron tomography, Mechanical stimulation

Reference:

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1102

Room temperature viscoplastic response of amorphous olivine films revealed by ex/in-situ TEM nanomechanical testing

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Poster Group 2

Olivine is a silicate which controls the rheology of the Earth's mantle down to ca. 410 km depth. Recently, we have discovered a new deformation mechanism of olivine where grain boundary sliding involves amorphization of grain boundaries under high stresses and further plastic flow along this amorphous layer [1]. We are convinced that this mechanism has a fairly general application to hard materials under conditions of high stress. This idea has been previously advocated by Idrissi et al. [2]. In the present case of olivine, the implications are mainly in the lithosphere in a ductile brittle context and at the boundary between the lithosphere and the asthenosphere. This led us to characterize the mechanical properties of amorphous olivine, but this material is mostly available in the form of thin films. It is therefore necessary to employ nanomechanical techniques: nanoindentation, in-situ TEM deformation and Lab-on-Chip (Figure 1). These tests have in particular the capacity to extend the solicitations to very low deformation rates relevant in geodynamics. Advanced TEM characterizations have allowed to identify the underlying microscopic mechanisms either in-situ or in ex-situ deformed samples. These data will allow in the future to feed mesoscopic mechanical models of olivine-rich rocks of the upper mantle. We also observe a significant influence of the electron beam on the viscoplastic behaviour of amorphous olivine. Special attention was paid to elucidate the origin of such feature.

Keywords:

TEM, Olivine, Nano-mechanical testing, Lab-on-chip

Reference:

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1103

Low-dose 4D-STEM cryo-tomography of biological samples

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Poster Group 2

Background incl. aims
4D-STEM provides a copious amount of information that must be reduced for interpretion. The challenge is compounded in the context of low dose exposure in beam-sensitive specimens. We describe several data processing approaches for tomographic reconstruction that result in improved 3D mapping of biological samples.

Methods
4D STEM collects a 2D diffraction pattern at each pixel in real space. Summation of electron counts over masked areas define virtual detectors in the form of annular rings and azimuthal sectors. A tilt series of projection images provide data for tomography, from which 3D reconstructions are generated for each virtual detector individually. Principal component analysis (PCA) of annuli yields a separation of amplitude and phase contrast. Alternatively, center of mass (COM) and differential phase contrast (DPC), generated from azimuthal segments, reveal phase gradients. By manipulation of parallax corrections, which appear as image shifts, the phase contrast extracted by integrated virtual differential phase contrast (iDPC) can be separated into parts that originate from distinct contributions of the contrast transfer function, aiding in interpretation. Finally, by collection of a large scattering range, information accessible in the virtual rings in the dark field is also interpretable as a differential cross section, which decomposes to elastic and inelastic scattering contributions depending on the scattering angle.

Results
3D reconstructions are applicable on either the virtual rings or sectors. Applying parallax manipulation over the virtual DPC is particularly useful and shares common aspects with ptychography techniques. The differential cross section data fits predictions in elastic scattering and plasmon generation in several amorphous non-metallic materials.

Conclusion
4D-STEM in tilt series can be applied routinely to generate 3D imaging that demonstrates improvement over simple dark-field or incoherent bright-field STEM. In parallel, the direct measurement of differential scattering cross section provides indications on atomic composition.

Keywords:

4D-STEM tomography; ptychography; DPC; cryo-TEM

Reference:

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1104

Ultra-low-cost, high-dynamic-range, additively manufactured CMOS spectrometers with UV, visible, and NIR sensing functionality

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Poster Group 1

Background incl. aims
Ultraviolet (UV)-Visible and infrared (IR) spectroscopy are widely used to characterize material and chemical samples. Inexpensive and high-performance spectrometers would broaden the impact of these tools. Educational spectrometers often lack accuracy, resolution, and repeatability, whereas industrial spectrometers are often expensive and hard to customize. We used consumer microprocessor development boards, image processing, and additive manufacturing (AM) to create an ultra-low-cost spectrometer platform, capable of modularly converting between UV, visible, and near-IR (NIR) measurements. The system can also track reaction progress via tandem temperature monitoring, with the possibility of further General Purpose Input/Output (GPIO) channels. This device offers high precision and flexible customization within a simple and inexpensive package.
Methods
This work comprises 1) hardware, 2) electronics, 3) firmware and software. The hardware architecture uses design for AM (DfAM) principles to minimize the part count, while achieving high performance, and low cost. We explored Fused Deposition Modeling (FDM) and stereolithography (SLA) manufacturing strategies. The electronics are based around an ESP32S3 development board from Seeed Studio, which has a high pseudostatic random-access memory (PSRAM) capacity that enables long-term data-gathering and GPIO expansion. The board is paired with complementary metal oxide semiconductor (CMOS) OV2640 and OV5640 camera modules from Seeed Studio, with the former offering low costs and the latter's backside-illuminated (BSI) construction enabling UV sensing. Temperature sensing is via a thermistor. The firmware is written in C/C++ using ESP32 camera libraries. The control software and user interface are written in Python using standard serial communication, image processing, time series analysis, and graphical user interface libraries. Features include automatic wavelength calibration using curve registration algorithms and an option for manual exposure control. Debvec's algorithm constructs a camera response curve from the bracketed exposures to produce a high-dynamic-range (HDR) reading.
Results
Using these inexpensive CMOS arrays, rapid data collection with high-precision sensing is achieved. Camera module and development board costs are less than USD 15 combined, and each scan takes on the order of 10 milliseconds (excluding HDR processing, on the order of seconds). For a 500-nanometer detection range, the 1600-pixel CMOS array width corresponds to approximately three pixels of data per nanometer. Practical resolution is limited by the choice of diffraction grating. NIR functionality relies on the intrinsic NIR sensitivity of CMOS arrays. UV functionality requires the removal of the Bayer filter from a BSI sensor, increasing fabrication cost and complexity. Software calibrations correct for color casts (i.e., intrinsic tints from the sensor) and uneven black levels. Raw RGB sensor data is not accessible due to poor library and hardware support, but raw grayscale data yields low noise (70dB peak signal-to-noise ratio for non-HDR data). JPEG compression is available given resource limitations. Tandem temperature monitoring successfully tracks reaction progress. SLA manufacturing requires fewer parts, while FDM achieves similar performance with simpler post-

processing. Further quantification of usable resolution, noise performance, dynamic range, and wavelength limits is ongoing.

Conclusions

Desktop AM systems, low-cost development boards, and library support for CMOS sensors enable the design of ultra-low-cost and compact spectrometry systems that can be tailored to specific experimental needs. With appropriate image processing and hardware, these systems achieve the repeatability and precision of commercial solutions that are more expensive and less modular. The approaches presented here enable low-cost, high-performance, agile spectrometry solutions that experimentalists can tailor to their specific needs, including in distributed IoT and other non-laboratory systems.

Keywords:

Spectroscopy, Additive manufacturing, CMOS

1105

Correlative microscopy for the discovery of novel nano-grains in spintronic materials.

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Poster Group 1

Background incl. aims
Spintronics is an expanding field in nanoscience, attracting attention in the last decade due to its potential to revolutionize CMOS based logic and data devices by utilizing the spin of electrons. Spin manipulation via spin current generation, propagation, and spin injection and detection, are key steps for the realization of fast, non-volatile, and low power computational devices. The successful achievement of these goals is challenging as it requires atomic level control of interfaces and thin film structures in multi-layered heterostructures. The 100% spin polarization in ideal L21 structured Heusler alloys is very sensitive to both extended defects (i.e. dislocations) due to strain effects, as well as anti-site defects, and which results in variety of lower symmetry ordered Heusler phase, like B2, DO3, and A2. Hence methods that can detect differences between phases in nano-volumes is critical for optimizing performance of spintronic devices based on Heusler alloys.

Methods
We have investigated the nano-grain structure of Co2FeSi films using correlative microscopy, fusing simultaneously acquired Energy Dispersive X-ray (EDX) spectroscopy and 4D-Scanning TEM datasets, on a Thermo Fisher Talos F200X operated at 200kV. The convergence angle achieved was 1.5 mrad, using a 10 µ C2 aperture. A Thermo Fisher Super-X SDD detector, with 0.9 srad collection angle, was used to acquire EDX. CBED patterns were recorded using a Quantum Detectors Merlin Quad detector with 512x512 pixels. For the scans used in this study a dwell time of 2–25 ms was used to scan regions with a step-size of 1–2 nm. Unsupervised machine learning algorithms have then been employed to identify nano-grains in the Heusler Co2FeSi thin films. Fuzzy C-means clustering, using a Gustafsson-Kessel approach allowing for elliptical clustering, was applied to a robustly scaled and dimensionally reduced combination of CBED and EDX data. The dimensional reduction was achieved using Principal Component Analysis. Density Functional Theory (DTF) studies were performed using the Vienna Ab-inito Simulation Package (VASP) using the SCAN functional with PAW pseudopotentials. A k-point mesh of 8 x 8 x 8 and planewave cut-off of 700eV were used for geometry optimisations with a finer density used for spectral calculations.

Results
Clustering of individual signals from the 4D-STEM and EDX of Co2FeSi thin films was unable to identify any higher ordering within the Heusler structure. However when the signals were combined the finer details of the film texture became apparent. Specifically, the correlative acquisition has allowed the unsupervised clustering algorithm to separate regions inseparable via noncorrelative techniques. The analysis of the clustered nano-grains, of differing structural and chemical composition, revealed a novel phase of CoFeSi that has been confirmed via further HAADF imaging. The structure has been optimised using DFT calculations and shown to be more stable than other

known CoFeSi phases, emphasising the structure's relevance. Further computational calculations have predicted the structure to be a semi-metal rather than a half-metal and as such it's inclusion in a Co2FeSi is potentially a, previously hidden, defect compromising spin polarisation.

Conclusion
By combining Energy Dispersive X-ray (EDX) spectroscopy and 4D-Scanning TEM datasets, we have a unique structure and chemical composition that is invisible when the data is treated independently. Subsequent analysis of the identified nano-size secondary phase shows that it contains ordering peaks that overlap with the full-Heusler L21-ordering, making it difficult to detect via standard characterization techniques (e.g. X-Ray Diffraction). Furthermore, we show that this new phase has unique ordering nor reported for Heusler phases in current literature. Additional magnetic measurements and density functional calculations have shown that the secondary phase is not fully spin polarized, has lower magnetic moment per unit cell, which can be detrimental when films with such inhomogeneities are incorporated in a device structure.

Keywords:

4D-STEM Unsupervised Learning DFT

1106

Hyperspectral Imaging with 'Spectromics': Label-free Chemical Mapping in the NIR-SWIR for Tissue Diagnostics

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Poster Group 1

Background, Motivation and Aims

The incidence of Osteoarthritis (OA) is increasing across the world with an expected 1 billion individuals suffering from it in the year 2050 [Lancet Rheumatol. 2023 PMID: 37675071]. OA is the most common degenerative joint disease, which presents as degradation of articular cartilage that results in pain and limited mobility. There is an unmet need for patient friendly paradigms for clinical assessment that do not require ionizing radiation (CT), exogenous contrast enhancing dyes (MRI), biopsy, and/or invasive instrumentation approaches (arthroscopy or endoscopy). Hence, techniques that use non-destructive, near- and shortwave infrared (NIR, SWIR) light may be ideal providing for providing non-invasive, label-free and deep tissue interrogation. NIR and SWIR spectral regions (700 nm to 2500 nm) are well suited for deep tissue penetration given the reduction in scattering with an increase in wavelength and decrease in absorption by tissue components. This results in 'biological transparency windows' in the NIR and SWIR that can be used for deep tissue penetration of light. With this motivation we utilized the chemical 'fingerprinting' techniques of NIR Raman scattering and NIR-SWIR absorption spectroscopy to develop a new approach to tissue diagnostics. We aimed to develop the multimodal 'spectromics' approach, wherein we carry out low-level abstraction data fusion of Raman scattering and absorption spectroscopy data to provide an enhanced, interpretable 'fingerprint' for diagnosis of OA in human cartilage.

Methods

Articular cartilage samples, obtained with full ethical approval and patient consent (REC reference 18/NW/0231), were excised from human femoral heads. Cartilage tissue slices were taken parallel to the femoral head surface, as deep as the subchondral bone. Cartilage slices were fixed in 4% paraformaldehyde (PFA) for 72 h and stored, refrigerated, in phosphate buffered saline before spectral analysis.
Raman spectroscopy measurements were carried out on a Renishaw InVia microscope system with samples excited using a 785 nm laser focused through a Leica 50x (0.75 NA) short working distance (~200 µm) objective. NIR-SWIR spectroscopy of the cartilage samples was carried out on a homemade benchtop system. Incident excitation light was provided by a broadband halogen lamp (HL-2000-FHSA-LL, Ocean Insight) emitting as a blackbody across the NIR-SWIR range, and signal collected via an OceanOptics NIR Quest 2.5+ spectrometer. Hyperspectral mapping of each sample was achieved by moving the sample stage and for each position, a mean average spectrum of 3 acquisitions with exposure time of 5 s for Raman and 10 ms for NIR-SWIR absorption spectroscopy were recorded. A modal average of 3 samples were investigated for each patient.
Raman spectra were preprocessed (baseline corrected, denoised and normalised). NIR-SWIR absorbance spectra were first treated with a 1st-derivative transformation and subsequently

smoothed, baseline corrected and normalised. A rubber-band baseline correction was applied to both spectra before concatenating them. The spectromics 'fingerprint' was created by concatenating the mean average pre-processed Raman spectra to the mean average pre-processed NIR-SWIR data from a given patient. Multivariate data analysis and classification was carried out using principal component and linear discriminant analysis as well as supervised machine learning using support vector machine.

Results
Under multivariate statistical analysis and supervised machine learning, tissue was classified to high precision: 100% segregation of tissue classes, and a classification accuracy of 95% (control) and 80% (OA), using the combined spectroscopic data was obtained. There was a marked improvement (5 to 6-fold for multivariate analysis) in classification performance using the spectromics fingerprint compared to results obtained solely using Raman or NIR-SWIR data. Furthermore, discriminatory spectral features in the enhanced fingerprint elucidated clinically relevant tissue components (OA biomarkers). Clinically relevant spectral features from the tissue allowed interpretable feedback from the enhanced fingerprint.
In order to take tissue heterogeneity into account we further created a new setup and performed automated 3D goniometric hyperspectral mapping of OA cartilage on whole human femoral heads post hip arthroplasty for spatially correlated spectromics of OA and age-matched controls. Spatially resolved spectral analysis highlighted morphologically relevant regions and chemical signatures with potential to aid early diagnosis, using spectromics-based OA biomarkers.

Conclusions
This proof-of-concept study examined Raman scattering and NIR-SWIR absorption spectroscopy for chemometric assessment of articular cartilage tissue in an elegant low-level abstraction data fusion approach termed spectromics. The improved classification potential of the enhanced spectromic fingerprint was shown. Our study demonstrated the potential of the spectromics approach for OA diagnosis with significant therapeutic diagnostic implications for an aging demographic. Our results lay the foundation for label-free chemometric diagnosis of OA and potentially other diseases that can be implemented in the clinic in a minimally-invasive or completely non-invasive ways.

Keywords:

Raman, infrared spectroscopy, hyperspectral, diagnostics

1107

Simultaneous nanoscale mapping of strain and electric field in semiconductor heterostructures using 4D-STEM

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

Background incl. aims
High-performance semiconductor devices are the basis of modern electronic equipment and have a very important impact on the processing, transmission and storage of information and energy fields. In many of those devices, the built-in electric field, depending on the distribution of dopants, plays a vital role in their performance and operating characteristics. On the other hand, introducing strain in the semiconductor structures, either during epitaxial growth or by external stresses, has been often used to optimize the functional properties of the semiconductor materials. Therefore, how to measure the electric field and strain at the nanoscale accurately and ideally simultaneously is crucial to the development and improvement of miniaturized semiconductor devices. Transmission electron microscopy (TEM) would be able to offer such a spatial resolution, but it is usually required to adopt different imaging modes to measure the strain and electric field. This would make it particularly difficult when measuring them during in situ straining experiments as the measured strain and electric field may not correspond to the same state over serial measurements.
The emergence of four-dimensional scanning transmission electron microscopy (4D-STEM) has enabled an alternative solution to measuring electromagnetic field and strain at the nanoscale. 4D-STEM, employing a pixelated electron detector for recording 2D images of convergent beam electron diffractions over a 2D grid of beam positions, offers a wide range of applications. Its resolutions in high-precision strain measurement and electromagnetic field determination can be up to the sub-nanometer scale. The technique allows nanoscale field mapping by measuring the center-of-mass (CoM) shift of the bright-field (BF) disk which is linearly proportional to the field, and the integration of CoM (iCoM) leads to the projected phase shift comparable to other techniques such as electron holography. For strain mapping, the variation of the reciprocal distance between a pair of diffraction disks is typically quantified to measure the strain.
Methods
Precession-assisted 4D-STEM dataset including both dark field disks and bright field disks were acquired. Precession was used to overcome the effect of dynamic diffraction. The long range electric field will cause a physical shift of the whole disk rather than the intensity redistribution inside the disks. In view of this, an edge detection method was used to determine the position of the diffraction disks, in contrast to the traditional CoM method. In theory, the shift of the bright disk is only caused by the electric field (EdgeBF), while the shift of the dark disks contain the effects from both electromagnetic fields (EdgeD¬¬¬¬_field) and the strain (EdgeD_strain). Since the EdgeD_strain in a pair of diffraction disks are equal in magnitude and opposite in direction, we can add the vectors of a pair of diffraction disks together and divide by two to obtain the pure contribution from the electrical field.

Results
Applying this method with semiconductor, the electric field and strain result are obtained simultaneously in a same 4dstem data. In principle, one pair of dark field spots are enough if they are the zone axis we want and uniform and bright enough, but if we can get 3 pairs of diffraction spots

edge results respectively and average them up is also credible. In second figure, there is a demo experiment result with this method, the EdgeBF, from bright disk, is compared with the average EdgeD¬¬¬¬_field, especially the profile and the difference in (d), the dark field is same direction of (b) and (c) in vertical, the (e),(f) and (g) is the strain along x and y direction.

Conclusion

In this contribution, we describe the procedure for decouple the influences attributed to strain and electromagnetic fields from the observed edge detection of a pair of diffraction patterns within a same 4D STEM dataset, as shown in the Figure. This procedure is applied with semiconductor samples to ensure the perfect correspondence between electric field and strain data acquired under identical conditions. After comparing with electric field from bright disk, the EdgeD_strain result is credible to some extent.

Keywords:

4DSTEM, Electric field, Strain, Semiconductors

Reference:

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1108

Novel In-situ TKD Nano-tensile Testing: Insights into Nanoscale Crystal Plasticity and Grain Boundary Mechanics

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Poster Group 2

Background

Advancements in nanoscale characterization techniques are crucial for understanding the complex deformation mechanisms of metal alloys, coatings and nanolaminates. Traditional post-mortem Transmission Kikuchi Diffraction (TKD) and Transmission Electron Microscopy (TEM) analyses, although powerful, often fail to capture the complete sequence and nature of deformation mechanisms as they occur [1]. This limitation is due to their inability to observe the initiation, progression, and interaction of deformation features such as dislocations or twins, leading to potential misinterpretations involved in material deformation.
In-situ TEM and in-situ EBSD nano- and micromechanical testing are both well-established techniques that offer detailed characterization of evolving crystal plasticity [2-3]. However, in-situ TEM is technically challenging and is limited in its scale. In contrast, In-situ EBSD is more feasible and allows larger field of views, yet its spatial resolution is typically limited to ~50 nm, which can miss finer structural details critical in nanoscale materials. In-situ TKD, on the other hand, would offer a significantly enhanced spatial resolution down to ~5 nm [4], while still performed inside a relatively accessible SEM.
Given these challenges and opportunities, this study introduces in-situ TKD integrated into nano-tensile testing as a novel methodology designed to provide comprehensive insights into the evolution of twinning, dislocation plasticity, and grain boundary mechanics under applied loading. This method will bridge the gap in understanding the intricate behaviors of complex nanolaminate metal/ceramic structures, while also providing an ideal platform to study phase transformation and twinning in advanced alloys.

Methods

A specialized experimental methodology was devised, combining (i) focused ion beam (FIB) processing for specimen preparation, (ii) a push-to-pull device for in-situ tension application, and (iii) an SEM configured for in-situ TKD with an Alemnis indenter. As proof of concept, we investigate deformations of advanced Cu1-x–Alx (X=0-12 at.%) multi-layers with interlayer interfaces of 2-5 nm amorphous Al2O3 for improved microstructure control and strength. These were synthetized in a novel deposition chamber from Swiss Cluster AG (https://swisscluster.com/), combining physical vapor and atomic layer depositions (PVD and ALD) without breaking the vacuum.
Specimens were prepared through site-specific FIB lift-out, subsequent fixation to the push-to-pull device using Pt deposition [5], a dedicated procedure for thinning to ~150 nm, and creation of a precise gauge section of several µm2. We designed and employed a custom configuration of an Alemnis nanoindenter frame inside a Tescan SEM, configured to fit with an Oxford Instruments Symmetry 2 EBSD detector for in-situ TKD and simultaneous STEM detection. Specimens are loaded with the push-to-pull device under continuous in-situ STEM observation, with intermittent loading pauses for detailed TKD mapping.

Results
In-situ STEM and TKD nano-tensile testing provides detailed and rich insights into the microstructural behavior of nanolaminate CuAl/Al2O3 films. We observe the development of twinning, dislocation plasticity and interactions at grain boundaries from the undeformed state all the way to the formation of a localized shear band. Thereby, we demonstrate the capability of this methodology to analyze complex mechanical interactions at the nanoscale on a specimen of several micrometers in size.

Conclusions
This study establishes in-situ TKD nano-tensile testing as a valuable approach for analyzing nano- and micromechanical deformation mechanisms of complex metallic specimens. The integration of TKD with nano-tensile testing offers a new perspective for exploring deformation mechanisms, providing insights essential for the design and improvement of nanolaminated metallic materials, as well as advanced alloys that show phase transformation and twinning. The findings emphasize the utility of this method in advancing materials science and engineering.

Keywords:

TKD, STEM, twinning, micromechanics, nanolaminates

Reference:

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Physical and chemical parameters determining the formation of gold sp-metal (Al, Ga, In, Pb) nanoalloys

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Poster Group 2

Background incl. aims.

Alloying is a key step towards the fabrication of advanced nanomaterials with multiple and unique properties demanded by the most innovative nanotechnology solutions. In particular, the interest in Au nanoalloys is expected to increase with the capabilities in tailoring and modelling new compounds, such as bimetallic NPs containing the sp-metals, which have appealing plasmonic and electronic properties for a wide range of applications in optics, catalysis, nanomedicine, sensing and quantum devices. However, for these systems little is known about the thermodynamic and synthetic factors leading to successful alloying at the nanoscale.

Methods.
Laser ablation in Liquid (LAL) is currently one of the reference techniques for the green and straightforward preparation of colloids of nanoalloys with conventional (e.g. Au-Ag, Au-Cu) or unconventional composition (e.g. immiscible elements such as Au-Fe, Ag-Fe, Ag-Co, Au-Co); LAL has, in addition, several advantages such as being a green, low-cost and self-standing process.
In this work, Au-M nanoalloys, with M = Al, Ga, In, or Pb, have been synthesized by LAL using anhydrous (acetone) and not-anhydrous (ethanol) liquid environments to delve deeper in the key parameters leading to the successful alloying in the typical operating conditions of LAL.
Electron Microscopy Techniques, SAED, NBD and HREM, have been applied to the study of the synthesized systems together with a multiparametric analysis that was performed considering the mixing enthalpy from DFT calculations and other alloying descriptors like the Hume-Rothery parameters.

Results.
The series of prepared four alloy nanosystems (Au-Al, Au-Ga, Au-In, and Au-Pb) by laser ablation in liquid (LAL) has the purpose to move along the 13th group for studying the effect on the products composition, structure and morphology. In case of Al, Ga, In, and Pb, these are either miscible with Au at some proportions or form intermetallic phases, but they also form stable oxides with a strongly negative standard formation enthalpy. Therefore, oxidation and passivation at the surface of NPs, as well as segregation due to oxidation of the sp metal during the synthesis, may occur. To confirm that the sp metals are alloyed with gold and identify the alloy phase among the various possibilities (substitutional SS, intermetallic compound, or phase-segregated), the XRD patterns of the ten dried colloids were collected and the phases were identified through the Rietveld refinement. In all samples, at least one substitutional alloy or intermetallic phase was detected, confirming the presence of the nanoalloys. The composition of each Au sp metal alloy was different in anhydrous acetone and in ethanol. It is worth noting that the NPs obtained by LAL have a relatively broad size distribution; hence, the composition obtained from the XRD analysis is the average composition of the sample, and it does not give information about the possibility that the chemical composition

changes with the size of the NPs. Therefore, alloying was also assessed at the nanoscale using SAED, NBD and HRTEM (see Figure) on the samples obtained in anhydrous acetone, which are those with the highest fraction of Au–sp metal phases. HRTEM analysis evidenced the polycrystalline structure of the NPs, which is also systematically observed in single-element metal particles obtained by LAL. Consequently, the SAED patterns on groups of NPs are rich in reflections coming from different crystalline domains, even inside the same particle. Nonetheless, it was possible to identify the reflections of the various Au–sp metal alloys previously identified using XRD analysis, where there is no ambiguity about the discrimination from the pure Au FCC reflections. This includes the AlAu4 phase with space group P213, the Au7Ga2 phase with space group P-62m, and reflections compatible with both the Au3In phase with space group Pmmn and the Au9In4 phase with space group P-43m. In the case of the Au–Pb sample, the very low abundance of the alloy compared to that of pure Au and hydrocerussite does not allow for the unambiguous identification of the Au2Pb phase in the SAED and NBD patterns, but it was possible to find a match with HRTEM of a single Au–Pb NP, exhibiting the interplanar distance and the Hanning masked FFT pattern of the Au2Pb phase with space group Fd-3m. Overall, the NBD and HRTEM results, along with the Hanning masked FFT patterns on selected nanometric regions, further confirmed, at the single NP level, all results obtained by SAED on groups of NPs and by XRD on the powder samples.

Conclusion.
The physical and chemical parameters determining the formation of gold–sp metal nanoalloys were studied as a function of the sp metal period, moving from Al to Ga, In and Pb, and under real synthetic conditions involved in the nanosecond laser ablation of bimetallic targets in two different liquid environments (anhydrous acetone and ethanol). The successful formation of Au nanoalloys with sp metals has been demonstrated in most cases and the LAL is confirmed to be a versatile approach for the production of innovative nanoalloys, provided that appropriate synthesis parameters are adopted. Especially relevant is the oxidizing capability of the solvent which must be balanced with the tendency of the metals to undergo oxidation, to avoid phase segregation and byproduct formation. The physical and chemical parameters leading to the formation of alloyed versus phase segregated NPs were analyzed systematically. Good agreement with the typical rules for alloying is found only in the absence of remarkable chemical interactions with the metals; otherwise, the tendency to undergo oxidation prevails. These findings lead to an immediate and deeper understanding of the phenomena that affect the composition of nanoalloys under real physical synthetic conditions. This will provide a crucial support for guiding the realization of next-generation multifunctional metallic nanostructures with remarkable applicative potential in optics, catalysis, nanomedicine, sensing and quantum devices.

Keywords:

nano-alloying, Laser Ablation, NBD, SAED

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1110

Sample holder design for TEM in-situ straining experiments on 2D materials

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Poster Group 2

Background incl. aims
Two-dimensional (2D) materials consisting of single- or few-layer atoms such as graphene, transition metal dichalcogenides (TMDs, e.g. MoS2, MoSe2, WS2, WSe2) and hexagonal boron nitride (h-BN) show unique physical, chemical and mechanical properties owing to their planar atomic and band structures making them promising candidates for future device applications. For the fabrication of stable and reliable nanodevices such as for example energy storage and memory storage devices, optical switches, nanofilm displays and superconducting devices, it is crucial to understand the deformation behavior of the 2D materials used as building block of the respective device. In situ TEM tensile testing can give insights into the mechanisms of nanomechanical deformation, cleavage and sliding of the strained 2D material at the atomic level. [1]
For uniaxial in situ TEM tensile testing of freestanding 2D materials, either commercially available push-to-pull devices or custom-designed straining holders are needed. The custom fabrication of straining holders allow for the flexible adaptation of the utilized design to the needs of the transferred 2D material and/or the employed TEM techniques.
2D flakes mechanically cleaved from the bulk material are typically very small in size, but the exposed sample surface is very clean. To preserve this clean sample surface, a transfer process not involving stabilizing polymer layers (for example polymethyl methacrylate (PMMA) or polyvinyl alcohol (PVA)) should be used. For the Polydimethylsiloxane (PDMS) mediated dry stamping transfer of mechanically cleaved 2D materials a sample holder with a small central “viewing window” is required because the transfer small flakes is facilitated if they span over the whole central window. Furthermore the material surrounding the central window must be sturdy enough to withstand the slight pressure applied during the stamping transfer.
For the transfer of PMMA stabilized CVD grown 2D materials it can beneficial to have a longer viewing window (length about 20 µm) allowing the determination of the layer number of the 2D material by tilting the flake in Darkfield-TEM [2].
In this work, we report the design and fabrication of Cu support, which was used as substrate for the transfer of both mechanically exfoliated MoS2 and CVD grown bilayer graphene in situ TEM straining combined with 4D scanning transmission electron microscopy (4D STEM) were carried out to study the mechanical properties of 2D materials.

Methods
As substrate material for in-situ TEM tensile tests on 2D materials using a single-tilt Gatan straining holder 50 µm thick copper foil (Karl Schlenk AG) was chosen. As the transfer of 2D materials on the substrate requires a very smooth surface, the Cu-foil was polished to a surface roughness of 0,25 µm using diamond paste. After polishing, custom-designed dog bone shaped sample holders were sculpted from the Cu-foil, tailored to fit the Gatan straining holder 654, using a microPREP PRO laser

ablation system (3D micromac). The sample holder design, measuring 12,0 mm in length, 2,4 mm in width and 1,0 mm width in the center, was generated using AutoCAD software (Autodesk). A small window with a length of 5 µm or 20 µm (depending on the transferred material) was cut into the middle of the dog bone-shaped Cu-holder for the transfer of desired 2D flake. Two longer lateral slits (length 300 µm) were added to the design close to the central window to prevent exceeding the force limit of the Gatan straining holder 654 during tensile testing. To eliminate residual debris from the rim of the laser ablated central windows and to adjust the shape of the windows, the central area of the Cu support underwent precise ion beam refinement using a FEI Helios NanoLab 660 instrument (scanning electron microscope-focus ion beam, SEM-FIB). [3]
If the Cu-holder was used for straining molybdenum disulfide (MoS_2) the polished copper surface was coated with a thin gold film (thickness 30 nm) to facilitate PDMS mediated dry transfer. The MoS_2 flake to be transferred was first exfoliated to a PDMS stamp attached to a glass slide using wafer tape. The glass slide was then mounted into a micromanipulator which was used to align the flake under a stereomicroscope with the central windows of the sample holder and then to bring it in contact with its surface. To prevent slipping and deformation of the Cu-holder during the transfer process, the holder was attached on both ends with 2 strips of thermal release tape (RA-95LS(N), Rephalpha, release temperature 100-105 °C) to a glass slide prior to transfer. Once the MoS_2 flake was in contact with the rim of one of the central small windows of the Cu-holder, the PDMS stamp was gently peeled off and the glass slide was placed on a hot plate at 100°C for several minutes to detach the thermal release tape.
CVD grown bilayer graphene (ACS materials, Trivial Transfer Graphene) was directly transferred to the customized Cu support by a “fishing” method. The PMMA coated CVD graphene was first immersed in deionized (DI) water, collected on a piece of filter paper and sectioned into tiny rectangles. One of these rectangular PMMA/ graphene films was then detached from the filter paper by once again immersing the filter paper in DI water and was fished out covering one of the central windows of the custom-designed Cu-holders. After drying for one hour, the PMMA was removed using acetone. [3]

Results & Conclusion
We designed straining holders tailor made for both the PDMS mediated transfer of mechanically exfoliated MoS_2 as well as the polymer-assisted transfer of CVD grown bilayer graphene. After successful transfer, in situ straining experiments combined with 4D STEM were performed on both materials giving insights into the mechanisms of nanomechanical deformation at atomic level (for more details on this straining experiment on bilayer graphene see Zhou et al.- A twist to superlubric sliding in bilayer graphene uncovered by in situ TEM) .

Keywords:

in-situ straining, MoS_2, graphene

Reference:

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Synthetic Bispecific RBD Antibody Effectively Neutralizes SRAS-CoV-2

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Poster Group 2

Background incl. aims
Coronavirus Disease 2019 (COVID-19) pandemic is severely impacting the world, and tremendous efforts have been made to deal with it. Despite many advances in vaccines and therapeutics, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants remains an intractable challenge. We employed non-antibody protein binder called repebody and designed a dimeric form of repebody A6 which can effectively neutralizes viral infection.
Methods
Using a library of non-antibody protein binder called repebody, and rational maturation, we have produced an A6 repebody that binds RBD (KD of 2.2nM) and blocks its interaction with ACE2. By using both cryo-EM and X-ray crystallography, we successfully determined A6 repebody mode of action. We utilized ITC, SPR to measure the binding kinetics and FRNT to test A6 neutralizing ability. In-vivo neutralization test was conducted on the mouse model expressing human-ACE2.
Results
We present a bivalent Receptor Binding Domain (RBD)-specific synthetic antibody, specific for the RBD of wild-type (lineage A), developed from a non-antibody protein scaffold composed of LRR (Leucine-rich repeat) modules through phage display. We further reinforced the unique feature of the synthetic antibody by constructing a tandem dimeric form. The resulting bivalent form showed a broader neutralizing activity against the variants. The in vivo neutralizing efficacy of the bivalent synthetic antibody was confirmed using a human ACE2-expressing mouse model that significantly alleviated viral titer and lung infection.
Conclusion
In the present study, we have demonstrated that a bivalent repebody, specific for the SARS-CoV-2 RBD, effectively neutralizes the SARS-CoV-2 virus and its variants. Despite their small size, target-specific synthetic antibodies developed from the repebody scaffold through phage display offer larger interface areas. The present approach can be used to develop a synthetic antibody showing a broader neutralizing activity against a multitude of SARS-CoV-2 variants.

Keywords:

SARS-CoV-2, Repebody, Cryo-EM, Synthetic antibody

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1112

Structure and stability of core-shell AuTiOx nanoparticles for CO oxidation

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Poster Group 2

Background
A catalyst's stability and longevity under operating conditions is key for their long-term application in industrial processes. This calls for an understanding of deactivation mechanisms to advance catalyst design strategies that can counteract such mechanisms.
Here we focus on gold (Au) nanoparticles (NPs) as a catalyst for the CO oxidation reaction (1). Specifically, we demonstrate that NPs formed by titanium (Ti) and Au alloying enables the development of an anchoring shell over the Au NPs, that suppresses sintering during operation. This new design strategy could be generally applicable and offers a higher degree of flexibility in preparing sinter-resistant catalyst nanoparticles.
Here we report the synthesis, structural characterization and stability testing of colloidal core-shell AuTiOx nanoparticles, displaying improved stability under thermal CO oxidation conditions on silicon nitride and TiOx supports (2).
Method
An Au0.5Ti0.5 alloy target was used to synthesize mass-selected nanoparticles of 160k amu under high vacuum conditions onto material supports that were used for the present characterization. Ion Scattering Spectroscopy (ISS), X-ray Photoelectron Spectroscopy (XPS) and High-Resolution Transmission Electron Microscopy (HRTEM) were used to elucidate the structure and chemical composition of the synthesized NPs. The catalytic characteristics of the AuTiOx NPs were then studied by performing comparative studies of the NPs and reference Au NPs under CO oxidation conditions. The NPs deposited on silicon nitride were studied by HRTEM under reactive conditions, by introducing a few mbar CO and O2 at various sample temperatures in a FEI Titan ETEM operated at 300 kV. Precautions were taken to acquire images of areas that were previously unexposed to the electron beam to differentiate beam-induced and environment-induced phenomena. The CO oxidation activity of the NPs deposited on TiOx were studied by running flow reactor experiments with the samples and reactive gasses at various temperatures and measuring gas conversion via a quadrupole mass spectrometer.
Results
ISS of the as-synthesized NPs, produced from the Au0.5Ti0.5 alloy, showed that only a small portion of the outermost layer contained Au atoms. HRTEM imaging of the nanoparticles after air exposure demonstrated that the NPs were composed of a 2.1±2 nm metallic Au core phase surrounded by a lighter ~0.8 nm shell. By XPS measurement the shell layer was identified as primarily consisting of TiOx. Imaging of the NPs in the reactive environment showed that a filament growth mechanism was present in the interface between the Au NPs and silicon nitride support at temperatures ≥200 °C, mobilizing the Au NPs. The AuTiOx NPs maintained their core-shell shape and size up to at least a temperature of 400 °C and did not demonstrate any growth mechanisms. This finding suggests that the TiOx shell acts as a protective layer for the Au NPs in this instance. Comparative activity measurements of the AuTiOx and Au NPs deposited on a TiOx support showed an improved stability

of the NP activity for the AuTiOx NPs in comparison to the Au NPs. Furthermore, the AuTiOx NPs showed a characteristic in which their activity increased over time after the sample temperature was increased. In certain cases, the activity would increase by >2 fold over the span of hours while maintaining a certain temperature. This phenomenon was observed to be reversible when lowering the sample temperature. Nonetheless, the average NP site turnover frequency (TOF) was lower for the AuTiOx NPs than the Au NPs, likely due to a blocking of active sites on the core-shell structure.
Conclusion
Characterization of the AuTiOx alloy nanoparticles revealed an Au core and TiOx dominant shell containing small traces of Au atoms. Supported on silicon nitride, the Au NPs were observed to facilitate a filament growth under CO oxidation conditions while the phenomenon was not observed for the AuTiOx NPS, suggesting that the TiOx shell can act as a protective layer. Activity measurements of the NPs supported on TiOx, showed a time dependent deactivation was observable for the Au NPs and not for the AuTiOx NPs. The average TOF of the core-shell NPs was observed to be lower than the Au NPs, which could be explained by a blocking of NP active sites by the shell layer. Treatment methods to enhance the shell layer porosity could likely improve the activity of the core-shell nanoparticles while maintaining their stability characteristics. Development of such colloidal alloy NPs could lead to significant advances in producing highly stable catalysts for industrial use.

Keywords:

Stability, alloy nanoparticles, electron microscopy

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3. The Center for Visualizing Catalytic Processes is sponsored by the Danish National Research Foundation (DNRF146).

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MEMS Monochromator

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Poster Group 1

Background and Aims
Present ultra-high-resolution SEM and S(T)EM commonly use high brightness electron source such as Schottky source (SFE) or cold field emitter (CFE) with a relatively high reduced brightness of about 1x108 and 2x108 [A/Sr.m.V] respectively. A typical energy spread of these electron sources are 1eV and 0.4eV respectively. For most applications requiring narrower energy spread, these are too large. Two examples of these applications could be: 1]- Extremely low voltage imaging with electrons known as LVSEM with a typical landing energy (LE) of only a few tens of electron-volts where chromatic aberration limits the resolution, 2]- Energy resolved applications such as high-resolution electron energy-loss spectroscopy (HREELS) where an extremely lower energy spread of only a few meV is required. A lower energy spread could only be obtained when a monochromator is used.
In the current monochromators, the increased demand on performance has increased the complexity of the monochromator designs, decreasing their user friendliness: to reach a few meV energy resolution, multiple power supplies have to be tuned to reach an optimal performance. The complexity in terms of large number of power supplies with extremely accurate temporal stability can partly be attributed to the form factor of the design. For instance, the main feature of a monochromator is a large deflection angle, with an associated three dimensionally shaped macroscopic geometry with inherently large aberrations, while also being very sensitive to small mechanical misalignments . These geometric and parasitic aberrations require compensation using extra multipole correctors. Moreover, adding extra multipoles in the design, extends the geometry of the monochromator, and consequently requires a reduced probe current for suppressing the energy broadening due to the Boersch effect. The increasing complexity demands a new design capable of achieving similar output in terms of energy resolution and current while reducing the number of required voltage supplies and their combined stability.
Methods
We present a new fully electrostatic monochromator based on Micro-Electro-Mechanical-Systems (MEMS). This manufacturing approach allows fabrication of monochromator parts with an unprecedentedly accuracy. While conventional monochromator deflectors can be approximated using 2D methods with multipole approximation, this approach is inadequate when the aperture sizes are large compared to the curvature of an electron beam's path. A 3D approach is required in the case of free form MEMS-based electron optics. To do so, a BEM method, is used for design and optimization of a new, fully electrostatic design. The optimization is partially automated by tuning the voltages in the design based on fitted optical aberrations.
Results
The new monochromator consists of 8 power supplies (including ground), and has a 1-to-1 ratio between voltage supply and beam energy drift. The new 5 mm long monochromator images / disperses the input plane to a selection slit plane with a unit magnification and a dispersion of 6 µm/eV at a beam energy of 500 eV. At the slit plane, an energy resolution better than 20 meV with a probe current of 5nA can be selected. The dispersion is a result of a (large) deflection angle using Fring Field deflectors. These fringe fields are created by extremely low aspect ratio MEMS multipoles.

Accurate fabrication and lower aspect ratio arrangement of main deflection fields eliminates the need for additional multipoles to compensate for geometric and parasitic mechanical misalignments.
Conclusions
In conclusion, monochromators represent a field of technology where the integration of MEMS techniques has shown novel promise in reducing complexity while maintaining crucial parameters. In addition to energy filtering, the newly found configuration could be used for other related applications such as energy analysis. The next step in the project will thus be to manufacture the new design for integration and testing in an electron microscope.
Figure:
Cross-section of monochromator layout, with an exaggerated initial energy distribution (+/- 5 V). The electrodes in the design are a series of stacked electrodes, with differing shapes of apertures created in each layer. The colours of the elements depict the surface charge densities resulting from the application of optimal voltages in the geometry. The dispersed beam enters in the left of the image, and is deflected and imaged towards the output plane on the right. The field free energy selection slit has been left out to favour simulation and make show the dispersed spot in the image plane.

Keywords:

Monochromator Spectrometer MEMS LVSEM stability

Reference:

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Structural characterization of single wall carbon nanotubes via AI assisted transmission electron microscopy

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PS-01 (3), Lecture Theater 3, august 30, 2024, 14:00 - 16:00

Correlating single wall carbon nanotubes (SWCNT) structural properties with growth conditions during catalytic chemical vapor deposition (C-CVD) is the key to understand the fundamental mechanism involved during this process. However, characterization of individual SWCNTs has always been a challenge, especially under real growth conditions. Recently, homodyne polarization microscopy was able to perform in-situ observations of SWCNT growth inside a CVD reactor [1]. While this technique provides high-throughput growth kinetic measurements, it is still unable to determine the atomic configuration, i.e. chirality, of each individual tube. On the other hand, transmission electron microscopy (TEM) is the technique of choice to directly image SWCNT at the atomic scale and therefore unambiguously determine their chirality [2]. Combining both characterization on the same tubes would reveal significant information about the growth mechanism of CNT. However, TEM sample preparation often relies on random CNT dispersion onto a TEM grid preventing any individual tube identification inside the microscope. In addition, manually determine the chirality for each tube from high-resolution (HR) TEM images is a long process limiting the analysis to a small number of tubes. Here, we aim to develop a new sample preparation protocol to transfer SWCNTs from a substrate to a TEM grid while preserving their location and allowing high quality atomic scale imaging. Furthermore, our goal is also to speed up chirality determination using artificial intelligence (AI) based image analysis.

First, SWCNTs were grown on a quartz substrate inside a custom CVD reactor allowing in-situ optical microscopy imaging. After growth, Au markers were deposited by optical lithography and scanning electron microscopy of the sample was performed to locate tube positions relative to the markers. Before transferring SWCNTs onto a TEM grid, the latter was covered with a graphene monolayer using the PMMA-mediated graphene-transfer method [3]. This layer acts as a mechanical support, preventing tube breaking above grid holes, and facilitating charge evacuation during TEM imaging. Then both SWCNTs and Au markers were transferred onto the graphene coated TEM grid using a similar wet transfer method. Atomic scale images of SWCNT on graphene were then obtained using an aberration corrected TEM equipped with a field emission gun and a monochromator. Finally, fast chirality determination was performed using an open source deep learning code based on convolutional neural networks [4]. This AI code is intended to analyze only SWCNT images; hence, the graphene layer signal must be removed. We developed a new filtering method called Geometric Amplitude and Phase Interpolation (GAPI) based on Fourier transform (FT) filtering that allows us to subtract the graphene signal even when it is superimposed over the SWCNT signal in both real and reciprocal space.

SWCNTs were successfully transferred with the Au markers on the custom TEM grid coated with graphene. Nanotubes, previously observed by in-situ techniques were imaged at high resolution inside the TEM. Both AI and manual methods were used to determine the chirality of the tubes of interest. The strong agreement between the chiral indices measured by both methods highlighted the efficiency of the AI algorithm. In comparison to other FT-based methods, the GAPI filtering method significantly improved AI chirality determination. Our protocol offers the possibility to track down defects and eventual chirality changes induced during growth. However, residual polymer contamination from the transfer processes prevented the analysis of all tubes across their entire length. Nevertheless, we were able to follow single chirality tubes across 10s of µm, providing a direct correlation between their structure and previously measured growth kinetic.

For the first time, the same individual SWCNTs were characterized by both in-situ optical and ex-situ HRTEM observations. The combination of a new image filtering method with automated atomic structure determination using AI, enabled a fast and unambiguous determination of their chirality providing a direct correlation between growth kinetics and tube structures. While the TEM characterization is still limited by polymer contamination, tubes chirality could still be measured in different areas, tens of µm apart.

Keywords:

Carbon nanotubes, HRTEM, artificial intelligence.

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Investigating nanoparticle interactions with the human blood-brain barrier in vitro

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Poster Group 1

Background
Transporting currently used and potentially anticipated active substances to tissues through drug carrier systems, particularly nanocarrier systems or nanoparticles (NPs), is feasible. The unique properties of NPs facilitate increasing the concentration of active substances in the bloodstream, extending their half-life, and optimizing dosing frequency to enhance active substance solubility and stability. Moreover, NPs may enhance treatment efficiency in systemic applications by accelerating the passage of active substances through biological barriers such as the blood-brain barrier (BBB), thus potentially improving therapeutic outcomes. There is a growing body of research on NPs crossing the BBB, although most studies are conducted on experimental animals, particularly rodents. Therefore, understanding the interaction of nanoparticles with the human BBB is crucial for advancing biomedical applications.

Methods
The Brust-Schriffrin method was utilized to synthesize gold nanoparticles (AuNPs) for cell imaging purposes. These AuNPs, acting as markers, were subsequently encapsulated within human serum albumin (HSA) and bovine serum albumin (BSA) nanoparticle structures using the desolvation method. Another nanoparticle formulation employing AuNPs as markers is the nano lipid carrier (NLC), synthesized via hot homogenization. Furthermore, Zr-based metal-organic framework (MOF) structures were utilized as markers in cell imaging, with Zr nanoparticles encapsulated within Polylactide-co-glycolide (PLGA) nanoparticles. A concentration dose of 62.5 µg/ml of these NPs was administered to primary human microvascular endothelial cells (1oh BMECs) and human brain vascular pericytes (HBVPs) for 3 hours. Subsequently, the cells were subjected to light microscopic and transmission electron microscopy (TEM) analysis.

Results
NP formulations of HSA, BSA, and PLGA were observed within cells by light microscopic analysis using the silver enhancement method. In contrast, no NP uptake was observed for NLC formulations in both 1oh BMECs and HBVPs after a 3-hour incubation period. Furthermore, TEM analysis of 1oh BMEC revealed no significant ultrastructural alterations following NP applications. Moreover, NLCs were not detected under TEM, whereas the other NP formulations were observed within the cells, consistent with the findings from light microscopic analysis. Interestingly, HSA was found within some autophagic vacuoles, randomly associated with myelin figures, and within numerous vesicles, particularly at the peripheral region of the cytoplasm.

Conclusion

There are various types of NP formulations showing promise as potential solutions to enhance drug transport to the BBB. However, their interactions within the cells of the human BBB are poorly understood. Therefore, it is crucial to comprehend the mechanisms and behavior of NPs within the human BBB.

Keywords:

Nanoparticle, BBB, Endothelial cells, Pericytes

1116

Effects of swimming training on orexin receptor 2 distribution in brain damage of rats

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Poster Group 1

Background incl. Aims
The World Health Organization defines obesity as hyperplasia and hypertrophy of adipose tissue that poses a health risk. Obesity is increasing in Western countries and is one of the major epidemic problems. In particular, the high-fat diet (HFD) is one of the most important factors in the spread of obesity in these countries. It is also known that HFD causes cognitive dysfunction and impaired memory, and exercise can improve cognitive function in animals. It is known that neuroinflammation, which is associated with progressive neuronal loss, is exacerbated by cognitive decline and obesity. Orexin neurons are projected throughout the central nervous system, including the hippocampus. Orexin neurons are also involved in operant activities, and cognitive functions. In addition, the loss of orexin also impairs memory. Experimental studies have shown that mice lacking orexin neurons exhibit increased cognitive deficits, and the brain also becomes more susceptible to neuronal insults and increased levels of neuroinflammation. HFD induces impairment of long-term memory, and HFD exposure and orexin loss in mice lead to increased inflammatory markers. In this study, we focused on the possible neuronal degenerative effects of HFD on brain tissue and the protective effect of swimming training. The aim of this study is to investigate HFD-induced brain damage and the putative healing effect of exercise on orexin expression on cortical neuroinflammation and cognitive decline.
Methods
Male Sprague-Dawley rats were fed either standard chow (Control group, 6% fat) or a HFD (HFD group, 45% fat) for 18 weeks. Half of the animals fed with each diet were trained by swimming exercise (1 h/day, 5 days/week) for the last 6 weeks of the experimental period (Ex and HFD-Ex groups). At the end of the study, an object recognition test was applied to evaluate cognitive function, and brains were collected for routine light and transmission electron microscopy (TEM) and biochemical analysis. Histopathological evaluation was performed in hematoxylin-eosin (H&E)-stained paraffin sections and Orexin-2 (OX-2) and Glial fibrillary acidic protein (GFAP) immunohistochemistry were performed. Malondialdehyde (MDA) and glutathione (GSH) levels were measured in the brain homogenates. The histological and biochemical data were evaluated statistically and a p-value <0.05 was accepted as significant.
Results
Deteriorated object recognition test was observed in the HFD group compared to the rats in the control group, which was ameliorated with exercise. Morphological evaluation revealed increased neuronal degeneration compared to that of the control groups in the HFD group ($p<0.05$). The HFD+Ex group showed decreased neuronal degeneration relative to the HFD group ($p<0.05$). Altered GFAP immunohistochemistry and OX-2 immunofluorescence observed in the HFD group were similar to controls in the HFD+Ex groups. Increased MDA and decreased GSH levels of the HFD group compared to control and Ex groups ($p<0.001$) were observed. However, decreased MDA levels ($p<0.001$) and increased GSH levels ($p<0.01$) in the HFD-Ex group relative to the HFD group.
Conclusions

Our study revealed HFD-induced neuronal damage and increased oxidative stress in the brain tissue. Swimming exercise protects the high fat diet-induced brain damage including the alteration of GFAP and OX-2 immunoreactivity in the cortex of rats by modulating oxidant/antioxidant balance. It is also thought that the regulation and neuronal development of the rodent cerebral cortex is influenced by HFD and that Ox-2 expression may contribute to this cortical development.

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Keywords:

HFD, Exercise, Brain, Orexin-2, GFAP

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1117

Towards Single-Pattern Absolute High angular Resolution EBSD without using Simulated Patterns as Reference

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IM-06 (3), Lecture Theater 1, august 30, 2024, 14:00 - 16:00

Background
Over the years, High angular Resolution EBSD (HR-EBSD) has evolved into a reliable tool for measuring misorientations and relative stresses (elastic strains) in crystalline materials with high sensitivity [1]. However, in the absence of stress-free intragranular reference points, absolute stress measurements are not yet routinely performed, while they could potentially provide new insights in the micromechanics of a range of (poly)crystalline materials.
The extension from relative to absolute cross-correlation based HR-EBSD, by using a simulated EBSD pattern as a reference, is under continuous development in the literature [2]. However, there are limitations by factors such as the inaccurate simulation of EBSD patterns and the uncertainty of the experimental EBSD geometry [3]. Consequently, an absolute HR-EBSD approach without using simulated reference patterns, or any experimental stress-free reference pattern, would be of huge interest to the community.
In the past years, several developments were proposed to extend HR-EBSD from a local cross-correlation based approach to a global full-field correlation [4]. Besides improved accuracy and robustness, such consistent correlation frameworks have shown the potential for non-simulation based absolute HR-EBSD, by exploiting crystal symmetry, simultaneous correlation between multiple (intergranular) patterns and co-correlating the pattern centre [5]. However, experimental validation is non-trivial on polycrystalline materials and excess-deficiency features (band asymmetry) in the patterns were expected to cause problems.

Methods
Therefore, we have continued developments on this framework to reduce the complexity towards an absolute non-simulation-based HR-EBSD correlation of only a single pattern. Specifically, we correlate one area within a pattern to another area of the same pattern by using crystal symmetry operators, as visualized for a single symmetry operator in the figure attached. Using all available symmetry operators, we correlate multiple areas within the same pattern in parallel, using absolute in-plane stress components, absolute orientation, and the pattern centre parameters as degrees of freedom.

Results
In this presentation, we will first show how the framework works and how it performs on idealized simulated patterns. Further testing on single experimental stress-free patterns, acquired with a direct electron EBSD camera, will show how excess-deficiency features can significantly degrade the experimental accuracy, as verified on dedicated dynamically simulated patterns. Therefore, the

method is extended with an integrated excess-deficiency correction approach which shows how non-simulation based absolute HR-EBSD is finally within reach.

Conclusion
In summary, we advance absolute HR-EBSD while avoiding the use of simulated patterns as a reference. Through single-pattern correlation in an advanced DIC framework, we show the potential for measuring absolute elastic strains at high resolution.

Keywords:

HR-EBSD, EBSD, absolute stress, DIC

Reference:

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1118

Microscopy to discern cells behaviour on different nano/microstructured calcium phosphates ceramics

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Poster Group 1

Background incl. aims
The formation bone tissue and the mineralization are positively influenced by the nanostructure or microstructure of calcium phosphate ceramics and by pore architecture of implants (1). Moreover, the release of Ca2+ was shown to induce cell growth, and PO43- released into medium induced osteogenic genes expression (2). The nano- or microstructure may significantly influence cell adhesion, proliferation and differentiation. The aim of this work was to study the effect of hydroxyapatite (HA) and beta tricalcium phosphate (β-TCP) of different structure on osteosarcoma cells adhesion, proliferation and osteogenic differentiation using microscopy techniques.
Methods
Porous ceramics from hydroxyapatite (HA), nanostructured HA (N-HA), calcium-depleted HA (CDHA), β-tricalcium phosphate (β-TCP), and nanostructured β-TCP (N-β-TCP) were prepared by 3D printing and sintering, and were characterized with SEM, XRD and tested in vitro with osteosarcoma SaOS-2 cells, seeded at a density of 368 x 103 cells/cm2 cultured in McCoy's 5A, 15% foetal bovine serum, 1 % of antibiotics (Penicilin/Streptomycin), and 40 µg/mL ascorbic acid. Cell proliferation was evaluated by dsDNA assay, and the metabolic activity was tested using MTS assay on days 7, 14, and 21. Focal adhesions were visualized using mouse monoclonal antibody Anti-Talin (T3287, Sigma Aldrich, 1:200), secondary Anti-mouse IgG Fab2-AlexaFluor488 (1:400, Molecular probes) and beta-actin was stained with Phalloidon-ATTO633 (1:1000, Sigma Aldrich) on day 1. Moreover, gene expressions of alkaline phosphatase (ALP) activity, Runx2, osteocalcin, collagen type I were tested. RNA was isolated from discs using RNeasy Mini Kit (74104, Qiagen, Hilden, Germany). The isolated mRNA was reverse transcribed to cDNA using qScript cDNA Synthesis Kit (QuantaBio, USA), following the manufacturer's protocol. Polymerase chain reaction was performed on Light Cycler 480 (Roche, Basel, Switzerlands), primers and probe were obtained from ThermoFisher Scientific. Data were evaluated using the 2−ΔCp method, for each gene relative to the housekeeping gene GAPDH.
Collagen type I, a midterm marker, was visualized using monoclonal antibody anti collagen I M-38c (1:50, DSHB, Iowa City, USA) and Anti-mouse IgG Fab2-AlexaFluor488 (1:400, Molecular probes) and propidium iodide (cell nuclei) on day 7 and 14. Osteocalcin, a late marker of osteogenic differentiation, was stained with Rabbit Anti-Osteocalcin (1:20, T4744, BMA Biomedicals) and Alexa Fluor 633 goat anti-Rabbit IgG (H+L) (1:200, Invitrogen) and Hoechst 34580 (2 µg/mL, H21486, Invitrogen) on day 21. All proteins were visualized using confocal microscopy and quantified using ImageJ. Statistical analysis was performed using GraphPad Prism 8.1.2. software, using One-way analysis of variance (ANOVA), according to normality test. The significance was set at $p < 0.05$.
Results
CDHA, N-HA and N-β-TCP had a nanostructure made of plate-like crystals. In contrast, HA and β-TCP had a microstructure of polyhedral grains. All nanostructured samples showed dot-like talin shape

with low density of focal adhesions. Oppositely, both microstructured HA and β-TCP ceramics showed well visible and fibrous-like talin structures.
β-TCP showed significantly higher proliferation of Saos-2 cells compared to all groups on day 7. CDHA showed lower proliferation than HA, and β-TCP on day 7, and 14, respectively.
Gene expression of collagen I, Runx2 were highest in HA and N-HA samples on day 1, and 7, Runx2 was lowest in β-TCP on day 14. ALP expression of HA and N-HA was higher than on CDHA on day 7 and higher than N-β-TCP on day 1. Osteocalcin showed significantly increased expression on β-TCP compared to HA, N-HA and N- β-TCP on day 14. CDHA showed higher osteocalcin expression on HA on day 7. Interestingly, both collagen I and osteocalcin synthesis were positive in high amounts in all scaffolds without significant differences.
Conclusions
Nanostructured calcium phosphate ceramics significantly influenced the localization of focal adhesions. HA scaffolds supports early and midterm marker expression, while β-TCP supported osteocalcin gene expression. Collagen I and osteocalcin synthesis were highly produced in all scaffolds but were not influenced by different ceramics structures or chemical composition.
Project was supported by MEYS of the Czech Republic, the program IMPROVE V (CZ.02.01.01/00/22_010/0002552), from European Regional Development Fund – Project Excellence in Regenerative Medicine" CZ.02.01.01/00/22_008/0004562, and MSCA RISE project iP-OSTEO (824007).

Keywords:

microscopy, nanostructures, osteoconductivity, adhesion, differentiation

Reference:

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Towards direct imaging of defects in carbon nanotubes with 4DSTEM

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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

4-dimensional scanning transmission electron microscopy (4DSTEM) based imaging techniques, in particular multislice electron ptychography, demonstrated unprecedented lateral spatial resolution while also providing depth-resolved imaging of the sample [1]. It is therefore, one of the most promising technique for 3D imaging of TEM samples at the atomic scale. Carbon nanotubes (CNT) are an ideal test subject for multislice ptychographic reconstructions using electron beams. While CNT atomic scale imaging can be achieved by high-resolution HR(S)TEM imaging, resolving their complex atomic structure is still a challenge, mainly because of the overlapping signal coming from both side of the tubes [2]. It becomes even more difficult when one wants to solve the structure of atomic defects inside CNTs. Here, we investigate the efficiency of 4DSTEM experiments combined with multiple iterative phase retrieval methods to resolved topological defects in CNTs. In particular, our goal is to resolve separately both sides of nanotubes using multislice ptychographic reconstructions of a single projection.

We used defective carbon nanotube structures, obtained by molecular dynamic calculations in a previous work [2], to simulate 4DSTEM datasets. Diffraction pattern simulations were computed using the open source abTEM package [3]. These simulated datasets serve two purposes: optimizing experimental conditions for high resolution imaging of nanotubes and evaluating the efficiency of phase retrieval algorithms. We performed phase images reconstruction using the open source py4DSTEM package [4], which features several iterative phase retrieval algorithms. We focused our work on the following methods: differential phase contrast, parallax, single and multislice ptychography [5]. 4DSTEM experiments were conducted on a double corrected TEM operating at 80kV and equipped with a Schottky field emission gun (X-FEG) and a direct electron detector. Experimental parameters were set as close to the optimum previously determined by simulations and phase images were reconstructed using the previously presented methods

In order to preserve tubes from contamination and irradiation damage, 4DSTEM experiments must be carried out in less than ideal conditions; i.e. low beam current (<20pA), short dwell time per pixel (<2ms), large real space step size (>1.5Å) and defocused STEM probe (tens of nm). In these conditions, the efficiency of the iterative ptychographic reconstructions strongly depends on the first guess given for the incident probe wave function. To optimize the determination of the initial probe parameters, i.e. defocus and residual aberrations, from experimental datasets, we computed differential phase contrast and parallax reconstructions [5], prior to the ptychographic reconstructions. We also acquired a vacuum probe reference in a separated dataset to constrain the incident wave function intensity. This method allowed the reconstructions to converge and strongly improved the resolution of CNTs images compared to high-resolution STEM images. However, phase images did not achieve the ultimate spatial resolution. Using simulated 4DSTEM datasets, we found

out that both lateral and depth (in the case of multislice ptychography) resolutions were strongly degraded when dealing with partial temporal coherence, i.e. energy spread, of electron beams. The energy resolution of the microscope used in this study is limited to a FWHM of 1.5 eV. We showed that with a greater energy resolution, which is available on microscopes equipped with a cold FEG gun for example, it is possible to improve lateral resolution and even perform CNT depth sectioning using multislice electron ptychography to capture a direct image of topological defects.

4DSTEM based high-resolution imaging techniques, are very promising to obtain direct imaging of topological defects in carbon nanotubes. By conducting two parallel studies, one based on simulation and one based on real 4DSTEM experiments, we were able to improve the resolution of CNT STEM images by performing iterative phase retrieval reconstructions. We demonstrated that 3D imaging of carbon nanotubes is even possible using multislice electron ptychography when the electron beam's energy spread is reduced. We argue that this limitation can be overcome by state-of-the-art-microscopes and improved reconstruction algorithms.

Keywords:

4DSTEM, electron ptychography, carbon nanotubes

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1120

Multimodal imaging accelerates the analysis of composition in bone implant sites

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Poster Group 1

Multimodal imaging approaches are essential for the characterisation of biomaterials and tissue-biomaterials interactions. We focused on multi-modal imaging techniques to acquire combined structural, molecular, and chemical information.
Confocal Raman imaging uses the inelastic scattering of photons to provide molecular bonding information and chemical identification. Hyperspectral Raman mapping gives the discrete distribution of chemical compounds within a material. Backscattered Electron and X-ray (BEX) imaging is the combined acquisition of backscattered electron (BSE) and x-ray data in a scanning electron microscope (SEM). BEX provides fast and automated mapping across a large area, collecting ultrastructural and composition data simultaneously.
We used a multimodal approach that combines Raman, SEM and BEX for investigating the interface regions between hydroxyapatite implants and bone tissue, connecting materials and tissue analysis. Resin embedded bone samples were polished to expose the implant regions. For SEM imaging we used a Zeiss 460 (Carl Zeiss Microscopy GmbH, Germany) operated at 10kV, 800pA - 1nA probe current, at 8.5mm WD, in variable pressure mode (30 Pa). BEX imaging was collected in the SEM with Unity (BEX detector, Oxford Instruments, UK) combined with an Ultim 100 (Oxford Instruments, UK). Implant areas were collected automatically, using cartography mode.
Regions of interest (ROIs) were identified with low magnification (500x magnification), large field of view (2 x 1.5mm ROIs, 30min acquisition time) rapid mapping using BEX (Fig 1a, b). High resolution images (2500x magnification) show that the implants are heterogenous, with distinct higher contras foci, rich in phosphate (Fig 1d, e). The same ROIs were subsequently imaged with Raman mapping using a 785 nm laser at 50 mW focused using a 100x objective with a spatial resolution of less than 500 nm.
We observed differences in electron density between bone and implant, as well as variability within the implant itself (Fig 1a, d). The combination of BEX and Raman analysis of the ROIs shows that electron density variation is explained by differences in concentration of calcium and phosphate (Fig 1g) and molecular composition, apatite vs hydrogenphosphate (Fig1 f, h).
The fast mapping of large samples using BEX was vital for an efficient selection of multiple ROIs of implant-bone interface. In addition, the speed of data acquisition using BEX permits the imaging of beam sensitive samples with compatibility for Raman imaging (no coating and lower electron dose related damage). The compositional information from BEX and Raman provides valuable context for the interpretation of bone and implant ultrastructure.

Keywords:

Multimodal imaging, Raman, BEX imaging

1121

Investigation of composition and origin of the intermediate layers at the Ga2O3/AlN and Ga2O3/Al2O3 interfaces

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Poster Group 2

Background incl. aims
Intermediate layers have been observed at the interfaces between Ga_2O_3 and Al_2O_3 [1] as well as Ga_2O_3 and AlN [2]. These layers exhibit the crystal structure of the substrate but with altered composition. For the first heterostructures, stabilization of the intermediated layer due to strain was proposed under the assumption of pure α-Ga_2O_3. Recently, it was shown that the composition is about 25% Ga [3] only. To better understand their physical origin, the composition of samples grown under different conditions were investigated using TEM and complemented by DFT computations.
Methods
For composition determination different methods were employed: energy dispersive x-ray spectroscopy (EDX) evaluated using ThermoFisher's Velox software, composition determination from quantitative HAADF-STEM [4] and strain state analysis from measurement of atomic distances [5]. Measurements were carried out in a probe-corrected ThermoFisher Spectra 300 and an imaging-corrected FEI Titan 80-300 on specimens prepared using a FEI Nova 200 FIB by the lift-out technique. DFT studies were performed using VASP [6]. Specimens were grown by molecular beam epitaxy (MBE), MOCATAXY [2] and suboxide MBE [2].
Results
Fig. 1 a) and b) show a HRSTEM image and a Ga concentration profile measured on a Ga_2O_3/Al_2O_3 interface formed by MBE in conditions, where actually no growth can be observed. The afore mentioned α-$(AlGa)_2O_3$ intermediate layer with a Ga concentration of about 25% can be seen as the layer with higher intensity. The composition of this sample formed at 300 °C for 3 minutes was rather similar to samples obtained at 700 °C for 180 minutes and at 700 °C for 3 minutes. This result shows that the composition significantly differs from the one (100% Ga) proposed in Schewski et al. [1]. To elucidate whether the layer stabilizes due to strain for the low concentration, DFT computations were carried out. For that, strained and unstrained α- and β-Ga_2O_3 unit cells were generated and Al atoms according to different concentrations were substituted. All possible atomic configurations in 1x1x1 cells were considered. Their mean values and respective standard deviations are displayed in Fig. 2 a) as function of Ga concentration. In general, the strained β-phase has significantly higher energy compared to all other cases due to the very large strain. In the limits (100% Ga and 0% Ga) unstrained β-Ga_2O_3 and α-Al_2O_3 are found to be energetically lowest as expected. This means that critical concentrations exist at which the energies of the 3 cases cross: Strained α-$(AlGa)_2O_3$ was found lower in energy up to Ga concentrations of 33% compared to β-$(AlGa)_2O_3$. β-$(AlGa)_2O_3$ becomes lower in energy than unstrained α-$(AlGa)_2O_3$ above about 62 % Ga. This shows that the occurrence of the layer can be explained just by its low concentration. In order to elucidate its origin further, Al_2O_3 surface slabs were generated in which two Al atoms were substituted by Ga atoms, respectively. Fig. 2 b) shows the energy difference between a cell, in which both Ga atoms were in the 2 topmost layers compared to cells where one Ga atom is in the topmost layer and the second

atom is placed deeper in the slab as a function of distance of the second atom to the top atom. Two cells could be identified for which the second atom being deeper in the crystal was energetically lower than both atoms close to the surface. These special positions were found within the topmost 3 atomic double layers and thus, it could be possible that Ga atoms chemisorbing can diffuse into the topmost 3 double layers of the substrate resulting in an effective concentration of 33% Ga. Fig. 1 c) and d) show HRSTEM images of MOCATAXY (with In as catalyst) and suboxide MBE grown Ga_2O_3/AlN samples. A bright layer with wurtzite type crystal structure can be observed similar to the Ga_2O_3/Al_2O_3 interfaces. In e) respective relative lattice distance profiles are shown for both samples. Simulated lattice distances as a function of Ga concentration are depicted in f). For the suboxide MBE the comparison results in a nearly pure GaN composition of the layer, whereas for the MOCATAXY sample the measured lattice distance is larger than all simulated ones indicating that In atoms have been incorporated into the layer. Note, that we neglected O incorporation in these considerations. Further studies in this direction are under way.

Conclusion

Our measurements and computations for the Ga_2O_3/Al_2O_3 interface show that the intermediate layer's composition is significantly smaller than assumed in previous publications and its occurrence can be explained by the low Ga concentration. For the Ga_2O_3/AlN interface a nearly pure GaN layer was found, possibly containing In in the case of In-mediated MOCATAXY.

Keywords

Composition determination, DFT, Ga_2O_3

Keywords:

Composition determination, DFT, Ga_2O_3

Reference:

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1122

Cryo-electron microscopy for the study of sensitive energy materials

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Poster Group 1

Background incl. aims

As current batteries are nearing their theoretical limits, future generation high-energy-density battery chemistries demand a fundamental understanding of their operation and failure pathways at the atomic level. However, most energy materials and most notably lithium (Li) are sensitive and unstable under the electron beam in conventional TEM. This problem has been addressed in recent years by the application of Cryo-EM methods, borrowed from structural biology.
A major obstacle hindering the implementation of Li-metal batteries is the limited understanding of the Li nucleation and growth mechanisms. Furthermore, the solid-electrolyte interphase (SEI) is essential for the reversibility of the lithium Li-metal electrode, but the lack of in-depth understanding of its structure and unclear formation/evolution mechanisms have significantly hampered its rational design. We applied Cryo-EM and Cryo-ET to unravel the morphology and inner structure of Li deposits and to investigate the morphological evolution of Li deposition and the interface confined SEI.

Methods

We have used a JEM2100 Cryo-TEM equipped with a bottom mounted 4k TVIPS F416 camera and a Gatan 914 cryo-transfer holder. We have used a cryo-transfer procedure where the TEM grids containing the Li deposits are transferred to the TEM column without exposure to the ambient environment. Cryo-EM micrographs and tilt series have been acquired using SerialEM. Tilt series have been processed with IMOD and segmentation has been done in Amira.

Results

In one work, Cryo-EM revealed two distinct types of Li deposits, depending on the current density: Li-balls, which were found to be primarily amorphous and Li-whiskers, which were found to be highly crystalline. Additionally, their solid electrolyte interface (SEI) layers showed a difference in structure and composition, correlated to the underlying deposition mechanism. In another work, we used Cryo-EM to reveal the morphological evolution of the SEI layer during Li plating, showing a thick (~100 nm) and wrinkled SEI layers forming in the initial stage, which progressively stretched and thinned to up to 7nm after 24 min of Li deposition. Cryo-EM and Cryo-ET also revealed in detail the morphologies of the Li deposits.

Conclusion

Cryo-EM and Cryo-ET, which are methods well established in the field of structural biology, could be well adapted to the study of sensitive energy materials.

Keywords:

Cryo-TEM, Cryo-ET, SEI, Energy Materials

Reference:

[1] K. Dong et al., ACS Energy Lett. 6 (2021), pp. 1719-1728
[2] Y. Jie et al., Cell Rep. Phys. Sci. 3 (2022), 101057

1123

Automated Identification of Slip System and Twinning Activity Fields from Digital Image Correlation Data

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Poster Group 2

Background
Plastic deformation in metals predominantly occurs through crystallographic slip, which requires identification to advance the understanding of mechanical behaviour and micromechanical deformation mechanisms. Current identification methods include (i) the use of the Schmid Factor (SF), (ii) matching of observed experimental slip traces to theoretical slip traces as determined by, e.g., EBSD and, (iii) calculation and matching of the 'Relative Displacement Ratio' (RDR) along a pre-determined slip trace, derived from SEM-DIC data. However, these methods require the presence of clear and straight slip traces in the strain field. To identify plasticity which involves, e.g., cross-slip, curved slip, and/or diffuse slip, a method is required that performs a one-step identification, locally, on the SEM-DIC displacement/strain field, i.e., without requiring an initial identification of slip trace lines in the strain map.

Methods
This paper proposes a novel slip system identification framework, termed SSLIP (for Slip Systems based Local Identification of Plasticity), in which the measured displacement gradient fields (from Digital Image Correlation) are locally matched to the kinematics of one or multiple combined theoretical slip systems, based on the measured crystal orientations. To identify the amount of slip that conforms to the measured kinematics, an optimization problem is solved for every datapoint individually, resulting in a slip activity field for every considered slip system.

Results
The identification framework is demonstrated and validated on an HCP virtual experiment, for discrete and diffuse slip, incorporating 24 slip systems. Experimental case studies on FCC and BCC metals show how full-field identification of discrete slip, diffuse slip and cross-slip becomes feasible, even when considering 48 slip systems for BCC. Additionally, recent developments now also allow identification of slip and twinning systems on challenging HCP Zn coatings.

Conclusion
In summary, we will show how the SSLIP method can be very useful for the materials science community, especially since we provide the codes in open-source format on Github [1,2].

Keywords:

crystallographic slip, crystal plasticity, DIC

Reference:

[1] Vermeij, T., Peerlings, R. H. J., Geers, M. G. D., & Hoefnagels, J. P. M. (2023). Automated identification of slip system activity fields from digital image correlation data. Acta Materialia, 243, 118502. https://doi.org/10.1016/j.actamat.2022.118502
[2] Open-source code on Github: www.github.com/TijmenVermeij/SSLIP

1124

SEM Automated Quantitative Mineralogy Method-development with Mineralogic: 200-nm-resolution Quantitative Assessment and Mineral-specific element mapping

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Poster Group 2

The study of fine-grained materials at the sub-micrometer scale with energy-dispersive X-ray spectroscopy (EDX) analysis using a scanning electron microscope (SEM) is often challenging. The primary electron beam's interaction-volume typically exceeds the grain size to be analyzed, resulting in mixed signals from individual grains. Consequently, EDX is rarely applied to such fine-grained materials. However, by employing a low primary beam acceleration voltage, a large aperture, and a dedicated mineral classification within the Zeiss Mineralogic software platform, mixed signals can be deconvoluted down to a spatial resolution of 200 nm [1]. This enables EDX and automated quantitative mineralogy (AQM) investigations of sub-micrometer-sized grains. Here, we would like to demonstrate reliable quantitative mineralogy on three case studies with geological samples with fine-grained material: 1) Fine-crushed fault gouge exhibiting a heterogeneous mineralogy, 2) fine needles in glass particles of a meteorite impact crater, and 3) rims in glauconite clasts affected by burial diagenesis.

Background

Automated Quantitative Mineralogy (AQM) has been applied since the early 1980s, applying technologies like QEMSCAN and the more recently TIMA-X. These techniques involve controlling the electron beam to scan the sample surface at a user-defined spatial resolution, capturing unquantified energy-dispersive spectroscopy (EDX) spectra. These unprocessed spectra are then matched to a reference library of known EDX spectra to identify minerals. This approach is very fast, however it provides only a rough assessment of mineralogy when elemental concentrations exceed ca. 10 wt%.

Method

The introduction of ZEISS' Mineralogic software advance AQM capabilities by integrating matrix corrections, and peak deconvolution in EDX spectra for each analysis point rather than matching spectra. Minerals are classified based on stoichiometry values. Importantly, the software retains the chemistry information for each pixel, allowing reinterpretation of mineralogy after analysis. Samples were polished and coated with carbon and examined using a Zeiss SIGMA 300VP SEM equipped with two Bruker XFlash 6|30 EDX detectors (129-eV energy resolution) and the Zeiss Mineralogic AQM mineralogy software platform. A specific region of interest was imaged to create a high-resolution back-scattered electron contrast mosaic [2]. Additionally, a 200-nm step-size AQM analysis was performed using Mineralogic, resulting in a detailed mineral map.

X-rays are generated from an interaction volume, which size depends on the primary energy of the beam and the mean atomic number of the sample. The interaction volumes for geological samples (silicates) typically ranges from 2 to 5 µm when using a 20-kV primary electron beam. For minerals with finer grain sizes however, accurate chemical analysis and mineral classification based solely on EDX spectra is therefore challenging as the resulting spectrum will consist of a compositional "mixing" of multiple grains. To address this, the interaction volume was reduced by setting the

acceleration volume of the primary beam to 10 kV, while applying a 60-µm aperture providing a 1.8-nA beam current, to minimizing the interaction volume and therefore the amount of signal mixing, while keeping a high-enough signal to resolve all relevant elements in silicates. Despite this reduction, Monte Carlo simulations indicated that minerals with the lowest average atomic number (Z) still yielded an interaction volume diameter of approximately 1 µm, while high-Z phases reached down to 400 nm. The 200-nm EDX pixel size allowed for oversampling and captured a significant volume of mixed pixel signatures. But the integration of fully quantitative energy-dispersive X-ray spectroscopy (EDX) classifications within the Zeiss Mineralogic software allows for precise analysis at the pixel level [2]. By quantifying the weight percent contribution of elements on a per-pixel basis, the software discriminates minerals effectively, even when dealing a certain amount of contamination resulting from mixed signals. Even for minerals with a low atomic number (Z), where the interaction volume is relatively high, Monte Carlo simulations demonstrate that a significant portion of chemical information originates from the localized area where the electron beam strikes the sample. By leveraging this insight, the software accurately translates mixed signals into mineral classifications, ensuring correct identification and analysis of these particles [1].

To perform mineral-specific element mapping, the technique above is further adapted in the ZEISS Mineralogic software platform to be able to visualise the element distribution in selected minerals, while masking out the other minerals simultaneously [3].

Results
In the presentation will be shown that polymineralic fault gouge down to 200 nm in diameter can be differentiated, classified from the data for that pixel, and its grain size distribution can be assessed [1]. Micrometer and submicrometer scale features in grains collected from the Hiawatha impact crater reveal the history of the area during and after the impact. Acicular orthopyroxene microlites, with a 1-2 µm width, were crystallized from melts above the solidus under different degrees of undercooling. Emulsion microstructures with siliceous melt droplets in an iron-rich melt phase show internal zonation at µm-scale, interpreted as incomplete mixing between two shock melts with different compositions forming equant, micrometer-sized microlites. Felsic melt grains contains abundant, closely packed microspherulites of 1-3 µm mordenite, giving evidence for a hydration event [4]. Rimmed glauconitic clasts reveal different trends during burial of glauconitic sandstone: the composition of the glauconite grains changes with depth, they become on average more K-rich and Fe-poor, with exception those being chlorite-replaced. This is an alteration effect, depending on temperature, distance to embedding mudstones and/or fluid composition, and the illitization of glauconitic clasts, at increasing depth. Nearly all glauconitic clasts have rims richer in Al and poorer in Fe and K, related to late diagenetic alteration, either resulting from the reaction to smectite or due to interaction with brine or hydrocarbons [3,5].

Conclusions
The ability to quantify the EDX spectrum at each analyzed point into its primary chemical components, and the use of in-built matrix corrections and peak deconvolutions, provide a robust analytical framework to provide high-quality quantitative chemical and mineralogical data that open up for a range of new applications in AQM.

Keywords:

Automated-Quantitative-Mineralogy
high-resolution EDS

Mineral-specific element-mapping

Reference:

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1125

Depositing biological segmentation datasets FAIRly

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Poster Group 2

Background incl. aims
Segmentation of biological images identifies regions of an image which correspond to specific features of interest, which can be analysed quantitatively to answer biological questions. This task has long been a barrier to conducting large-scale biological imaging studies as it is time- and labour-intensive. Modern artificial intelligence segmentation tools can automate this process, but require high quality segmentation data for training, which is challenging to acquire. Biological segmentation data has been produced for many years, but this data is not often re-used to develop new tools as it is hard to find, access, and use. Recent disparate efforts [1-4] have been made to facilitate deposition and re-use of these valuable datasets, but more work is needed to increase re-usability. In this work, we review the current state of publicly available annotation and segmentation datasets and make specific recommendations to increase re-usability following FAIR (findable, accessible, interoperable, re-usable) principles [5] for the future.
Methods
A collection of publicly available segmentation and annotation datasets for 3-D volumetric electron microscopy and associated metadata was assembled from searches in the EMDB, EMPIAR, and Open Organelle databases, and a literature search with Pubmed from 2012-2023. Characteristics about these datasets were collected and trends over time were investigated, e.g., the purpose of the segmentations, data formats, biological feature type and size scale, imaging modality, and others.
Results
Whilst there were many examples of publicly available segmentation data that could be easily reused, these datasets were few and far between. We identified barriers to reusing published segmentation data according to FAIR principles. Many publications we reviewed were not findable or accessible as they were deposited at defunct URLs, were only available on request, or required researchers to search separately through citations or online to download the data. We found that data was deposited across at least 12 different formats, and 9 different online repositories, limiting the interoperability of these datasets as significant effort would be required to parse these formats into a single unified database for training segmentation tools. We found that there were considerable differences in the definitions of certain terms such as “segmentation”, “reconstruction”, and “ground truth”. A consensus is required across the breadth of the bioimaging community to ensure that these datasets can be re-used appropriately without misinterpretation.
Enhanced metadata capture and search capabilities would help developers find suitable datasets for their use case and determine how much (if any) manual curation would be required to bring segmentation data to the required standard for their study. This metadata could include the number and size of the feature being segmented relative to the image, the quality of the segmentation or the intended use of the dataset (qualitative visualisation, quantitative morphology, etc).
Conclusion
Re-use of biological segmentation data is important for enhancing development of segmentation software tools, particularly those using artificial intelligence techniques requiring large amounts of training data. Enhanced metadata capture, quality evaluation, community consensus in ontology and

standardisation in data formats is required for encouraging re-use of these precious segmentation datasets.

Keywords:

Segmentation; annotation; volume electron microscopy

Reference:

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Mapping Li in thin-films and heterostructures by EELS and ptychography: case of Li7La3Zr2O12 and LiCoO3

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PS-04 (4), Plenary, august 27, 2024, 14:00 - 16:00

Background incl. aims
Improving upon the current generation of lithium-ion batteries requires a fundamental understanding of electrochemical and structural/chemical changes due to work-cycles of the electrodes/electrolyte heterostructure. Multiple mechanisms exist for cell degradation, whether this be through structural fatigue in the bulk of the electrodes or via formation of solid electrolyte interfaces (SEIs). With resolutions achievable below 1 Å in aberration corrected transmission electron microscopy (TEM), structural analysis of grains, grain boundaries, as well as interfaces between electrode (LiCoO2, LCO) and electrolyte (Li7La3Zr2O12, LLZO) as well as formed current induced SEIs is possible on atomic scale. Determining the structural characteristics of the boundaries and interfaces will allow us to build atomistic models for calculating the electronic properties for lithium diffusion. The observation of defects and interfacial composition down to atomic resolutions is fundamental to understanding the specific characteristics changing the efficiency of lithium-ion movement through the device. By combining ptychography and high resolution EELS we demonstrate that Li can be uniquely traced through the grown films, and more importantly at the grain boundaries that delimitate higher with lower regions of Li.

Methods
The thin films were grown by pulsed laser ablation (PLD) on several substrates including Al2O3, GGG and SrTiO3. STEM-EELS was done using Nion UltraSTEM100MC – Hermes Scanning Transmission Electron Microscope (STEM), operated at 60kV. The instrument is equipped with a cold field emission source, with energy spread of around 6meV.
The electron energy loss spectroscopy (EELS) spectrum images (SI) were acquired with a probe with an energy spread of 150 meV with a collection half-angle of 22 mrad. The HAADF-STEM imaging and 4D STEM imaging was done by aberration corrected JEOL Grand ARM at 300keV.

Results
Single layer films of LCO, annealed at various temperatures, have been imaged in HAADF-STEM with regions of rhombohedral, spinel and cubic phases identified. As temperature increases during the annealing process the films show a reduction in rhombohedral phase grains and an increase in number of cubic grains. This fits with the assumption that the spinel and cubic phases are the stabilisation of an increased reduction in lithium content, respectively. Grain boundaries between the three phases of the deposited films have been studied via EELS in order to map the Li content and potential channels between the nanograins. Further to this electron ptychography was performed showing the Li position in the grains and across boundaries, corroborating the spectroscopic data.

Heterostructures of LCO and LLZO grown on GGG substrates have also been studied via STEM, EDS and EELS datasets to understand the details of both electrode and electrolyte as well as features appearing at the interface. Interfacial decomposition appears most dependant on temperature, with the scale of intermixing greatly reduced when subjecting heterostructures to applied bias. LCO will grow crystalline on LLZO (LCO/LLZO) allowing a crystalline electrode/electrolyte interface to form, whereas the electrolyte remains amorphous when deposited on top of crystalline LCO (LLZO/LCO). However, LLZO/LCO structures promote the formation of $LaCoO_3$ interfacial layers and crystallites of cobalt-containing structures growing through into the LLZO layer. These contribute to a complex system formed during thermal decomposition and intermixing occurring at the solid-state interface.

Conclusion

The position of Li atoms, within three phases of differing Li content, in LCO have been imaged. This insight can help engineer LCO films with optimal Li transport properties, and provide insiights of the effect of annealing in crustalisation process of these thin films and heterostructures. LCO and LLZO interfaces have been grown and studied via STEM-HAADF, and STEM-EELS. The orientation of the substrate only allows for crystalline LCO/LLZO interfaces whilst LLZO/ LCO heterostructures have an intermixing $LaCoO_3$ layer and large crystallites that pierce into the LLZO film.

The combined approach of imaging and spectroscopy have been shown to be rather beneficial in determining the structure and Li content in the thin films and heterostructures of Li based oxides used as electrolyte and electrodes.

Keywords:

STEM-EELS, Li batteries, Ptychography

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Spectrally resolved brightfield widefield microscopy by an ordinary digital camera

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Poster Group 1

Background
The signal at the digital sensor of the microscope camera contains much more information than is typically extracted by commonplace visualization software. Combined with spectra-based calibration (1) and quasi-spectral analysis (2), we obtained an information set with a visible range spectrum for each camera point. Instead of an image, we have a physical dataset completely independent of the measuring system by which the image was obtained. Multiple types of visualization and physical analysis may be applied.
Methods
We use the simplest possible set-up -- a telecentric objective, a reflected light source, and a black background -- with no optical path modifications. In this way, we reduce the number of aberrations to the minimum. The optical signal is detected by a full frame colour digital camera sensor. Interpolation in the high-dimensional spectral space compensates for geometrical limitations caused by the construction of the camera sensor.
Samples are unmodified as possible: cells and tissues are unstained in their native states; materials are only commonly surface treated.
Physico-chemically different regions in the sample are determined as regions of similar visible light spectra.
Results
We show results of quasi-spectral analysis on living leukocytes (Fig. 1 and 2), unlabeled necrotic lung tissue (Fig. 3), and a polished metal surface (Fig. 4). In each case, we observe regions of ~100 nm in diameter with homogeneous spectra.
Conclusion
There is much more information in the primary dataset of the digital microscope camera sensor than is typically utilized. By combining the knowledge of spectra of the incoming light, transparency spectra of the camera RGB sensor filters, and geometric considerations, we approach the physical and information limit of light microscopy. The results are comparable to standard results obtained by stained samples and contain more chemically specific information than scanning electron microscopy imaging. The experiment requires minimal sample preparation. Most practical tasks may be heavily simplified.
No artificial intelligence is required up to this point, only physical measurement, geometry, and advanced statistics.
The large sensor enables the obtaining of excellent statistics on observed sample properties.
Using maximum physical and information analysis on the primary dataset is essential before applying artificial intelligence. Otherwise, we follow the rule "garbage in, garbage out".

Panel 1. Overview of the image of unstained human leukocytes. The APS-H camera sensor captures the image of 3.08x1.92 mm2 with the pixel size of 230×230 nm2. The image typically contains over 2000 cells.

Panel 2. Detail of the image of unstained human leukocytes. The details of organelles allow us to identify the types of cells, even much more details may be found than in the stained image.

Panel 3. Histological section of necrotic lung tissue after massive fat embolism. Tissue was generally unlabeled, fat aggregates are oil red stained (pink objects) and cells and nuclei appear as bright objects inside the tissue.

Panel 4. Weld of two types of steel of different composition and internal structure.

Keywords:

Visible light spectrum, geometry, statistics

Reference:

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Nanoparticle Self Diffraction in the TEM: A proposal

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Poster Group 2

Background and aims
Entanglement in the TEM has been proposed to study the superposition of mesoscopic quantum states and their decoherence via the reduced density matrix of the probe electron [1]. Here, we take this idea further and discuss the possibility to prepare a massive nanoparticle in a quantum superposition of distinct momentum states and observe its interference. Contrary to previous proposals for nanoparticle diffraction at optical standing-wave gratings [2], the spatial and temporal coherence requirements are substantially lower. TEM-assisted diffraction might be therefore a viable alternative route towards new mass records in matter-wave interferometry.
Methods
We propose to diffract a single electron on a (crystalline) nanoparticle of about 50 nm radius and lattice plane distance d. By virtue of momentum conservation, each Bragg reflection gn=2 π n/d imparts a momentum of nhg/2 π onto the center of mass (CM) of the nanoparticle (red dot in Fig. 1A). Detecting the electron in the image plane (Fig. 1B) then prepares the nanoparticle in a quantum superposition of wave packets with different momenta – a Schrödinger kitten state with a relative phase conditioned on the detected electron position. As the particle propagates freely in time (Fig. 1C), the wave packets disperse and interference fringes form. A position measurement, e.g., in an optical dipole trap (Fig. 1D), would reveal these fringes, demonstrating the quantum wave nature of the nanoparticle's CM. This can be viewed as resulting from the diffraction of the nanoparticle on its own crystal lattice.
Results
We predict fringe patterns caused by quantum interference, different from both a classical mixture of individually spreading states that would not show fringes, and from a semiclassical model of Moiré fringes, which presumes that the Wigner function of the nanoparticle be non-negative at all times. For didactical reasons, we demonstrate the principle in Fig. 2 showing the density matrix of a hypothetical particle's CM with an initial position spread of the pure state of d/20. ξ/d is the main diagonal, η/d shows the degree of coherence. The red curve is the density profile. A realistic scenario with a crystalline nanoparticle in the mass regime of 10^6 to 10^8 atomic mass units will be benchmarked against the semiclassical model.
Conclusion
In a dedicated TEM the observation of macroscopic quantum interference should be feasible.

Keywords:

Entanglement, matter-wave interference, cat-states, coherence

Reference:

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1129

Tungsten nanoparticles generated in an atmospheric pressure plasma jet

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Poster Group 2

Background
Atmospheric pressure plasma sources have proven to be a cost and space efficient technology for the preparation of various nanostructures. However, synthesis of high melting point metal nanoparticles remains an ongoing challenge. In this work, tungsten nanoparticles are synthesized by the HelixJet atmospheric pressure plasma source using a tungsten wire as a source material and an oxygen free argon plasma. This offers the opportunity to fabricate a wide range of nanoparticles in terms of particle size, morphology and chemical composition in a single preparation step.
Methods
Tungsten nanoparticles were synthesized in an atmospheric pressure plasma jet (APPJ) with helix electrodes (HelixJet) and a metal wire inserted into the plasma. The metal wire was heated by the interaction with the plasma. The vaporized material is carried in the gas stream and condensates to form nanoparticle nuclei. The synthesized nanoparticles were measured with a commercial scanning mobility particle sizer (SMPS) spectrometer. The morphology and the crystal structure of the resulting particles were analyzed by bright field transmission electron microscopy (TEM) and selected area electron diffraction (SAED). Energy dispersive X-ray spectroscopy (EDX) combined with scanning TEM was used to gain local information on the chemical composition of the particles.
Results
TEM along with SAED and EDX showed monocrystalline tungsten nanoparticles with an average size of 14 nm, consisting of beta-tungsten with a thin oxygen-rich layer on the surface. The composition of such particles was studied with EDX profiling and analyzed using a so called sub-shell approach revealing that the core of the particle is composed of pure tungsten while the approximately 1.5nm surface layer has a tungsten to oxygen ratio of around 1:9. When increasing the plasma power, the average size of the nanoparticles also increased. The weakest plasma power used led to nanoparticles of average size of 12 nm. As the working gas flow rate increased, the time spent by the nanoparticles in the plasma decreased and the resulting size decreased. For the highest flow rates, when the residence time dropped to 0.4 s, the resulting particles had a thick oxide layer on the surface, while for the lowest flow rates with residence times above 1.8 s, the resulting particles were composed mainly of alpha-tungsten. Nanoparticles agglomerate into clusters, which are then measured by SMPS. The resulting size spectrum was fitted and individual peaks were sequentially assigned to agglomerates with increasing number of particles. It turns out that the SMPS measures the mobility of agglomerates as if they were larger particles than their actual size given by the sum of individual volumes or masses of particles in the agglomerate, making them to appear significantly larger.
Conclusion
Synthesis of monocrystalline tungsten nanoparticles with an average size as low as 12 nm and with a fairly uniform distribution has been shown. The HelixJet APPJ gives the possibility to prepare nanoparticles of high melting point materials and due to its versatility allows to prepare particles with complex morphology (alloys, oxides, core shells).

Keywords:

Tungsten nanoparticles, Atmospheric plasma

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Development of multiciliated cells in the respiratory and esophageal epithelium of the chicken embryo

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Poster Group 1

Background and Aims
Cilia are highly conserved cell organells which emerge from the cell surface. They are frequently involved in signaling functions as well as in the coordinated motion of extracellular fluids and particles along epithelial surfaces. While monocilia are present in many cell types, the generation of multiple elongated cilia in multiciliated cells is restricted to specialized epithelia, e.g. the respiratory epithelium covering the trachea and the nasal conchae.
Until now there is still limited knowledge on the development of multiciliated cells, specifically regarding the details of their cell surface morphology during the ciliogenesis process. Using scanning electron microscopy (SEM) we have investigated the appearance and spatial extension of multiciliary cells in the trachea, in the concha nasalis media and adjacent regions of the embryonic chicken addressing developmental days E12 to E18. In addition, the esophageal epithelium bearing a more restricted lining of multiciliated cells was analyzed from E10 onwards.

Methods
Embryonic tissues were collected from fertilized chicken eggs (var. white leghorn) at the indicated days of incubation (E) and, after fixation, were processed for critical point drying for SEM using pieces with longitudinal cuts from trachea or oesophagus and coronal slices for evaluation of the nasal conchae. Additional processing for histology included resin embedding for transmission electron microscopy (TEM), or paraffin embedding for immunohistochemistry. Here, antibodies to acetylated tubulin have been used as a ciliary marker.

Results
At E12 to E14 short monocilia are detected with SEM on the surface of epithelial cells of the trachea and concha nasalis media. Following E14 the formation of much more elongated monocilia is observed and at E14,5 first multiciliated cells are found next to many cells with elongated monocila. At E15 an increased number of multiciliated cells is present showing multiple growing cilia with different length profiles whereas fewer cells with extended monocilia remain next to the clustered multiciliated cells until E16. At E17 monociliated cells are largely absent and most of the multiciliated cells have grown a large number of individual cilia to their final length of 5-6 µm. Here and in the following stages, the epithelial surface of the trachea and concha nasalis media is almost completely covered by cilia, whereas epithelial grooves that have emerged during development and have gradually increased in depth show a strongly reduced number of multiciliary cells, e.g. at E18 in the conchae nasalis media.
Interestingly, a distinct earlier onset of this ciliogenesis process was noted in the nasal septum adjacent to the former conchae, where cells with elongated monocilia and multiciliated cells are detected starting at E13. Accordingly the full coverage of the epithelium with cilia does occur earlier in the nasal septum i.e. at E16. Again, a much earlier onset of ciliogenesis is observed in the developing epithelium of the esophagus where at places we have detected nascent multiciliary cells

at E10 onwards which are located next to cells with short monocilia and some cells with extended monocilia. While much less multiciliated cells are formed during development in the esophageal epithelium compared to the respiratory epithelia examined above these findings suggest a similar sequence of ciliogenesis in multiciliated cells of the esophagus epithelium that involves transitory monocilia and a specific temporal development.

Conclusions
Our findings indicate that the appearance of cells with extended monocilia is a morphological marker for cells that are likely to further develop as multiciliated cells in an epithelium and will emerge with multiple cilia in the following. The observed developmental sequence of multicilated cells is completed in a relatively short period of time comprising approximately 3 days after onset in the chicken embryo.

Keywords:

multiciliated cells, chicken, electron microscopy

1131

In-situ synthesis of thin kesterite films using high voltage TEM

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Poster Group 1

A promising route for the synthesis of kesterite solar cell absorber layers consists in the annealing of thin layers in vapours resulting in alloys of kesterites, a route that has recently also be used for the synthesis of van der Waals materials. This straightforward annealing procedure includes several structural transformations of the absorber thin film layer such as phase transformations, diffusion processes and grain growth. The disentanglement of these different processes is difficult and TEM analysis is essential to understand the structural changes during the annealing of those materials. The best approach to follow the structural changes during the synthesis process of such kesterite materials is to carry out the related annealing steps in situ in the TEM. Since the layers have a certain thickness and the annealing must be carried out in a Se atmosphere, the high voltage TEM (HVTEM) is an appropriate method for this type of in situ annealing which we carry out in this work.

Methods
For the TEM in-situ synthesis of kesterite structures of Cu2ZnSnSe4, we used the JEOL-1000K RS HVTEM at Nagoya University. The starting materials are Cu2ZnSnS4 and AgCu2ZnSnS4, which are annealed in a Se atmosphere to obtain a Cu2ZnSnSSe phase with as high as possible Se content. The temperature is increased in steps of 50-100 °C up to 500 °C while the Se-enriched carrier gas streams along the sample surface. At each temperature ramp, the sample is analysed by electron diffraction, TEM and STEM imaging as well as by EELS spectroscopy. In addition, the TEM is equipped with a highly sensitive mass spectrometer, which enables us to analyse the gases coming from the sample during annealing.

Results
We started the synthesis either from the metallic stacks or from Cu2ZnSnS4 which are exposed to Se vapours. Though the analysis of the metallic stacks has promising results, the thickness increase of about a factor of 2 during the selenisation process made the sample so thick that it was difficult to analyse nanometric changes in the sample happening during the synthesis process. This situation changed by using thin Cu2ZnSnS4 and AgCu2ZnSnS4 films as a starting material since these films preserve their thickness during the synthesis.
During the in-situ annealing of AgCu2ZnSnS4, we observed the nucleation of the kesterite Cu2ZnSn(S)Se4 phase, which is precedented by the formation of Ag- and Se-containing nanoprecipitates. Those partially disappear during higher annealing temperatures, when the AgCu2ZnSnSe4 grains grow. By in-situ EELS analysis and post-annealing EDS measurements of the thin layers we could follow the chemical modifications of the crystallites forming a core-shell structure.
When the Cu2ZnSnS4 is synthesised, the replacement of S by Se causes an expansion of the lattice parameter. We applied a Hough transform to extract the lattice plane spacing from the diffraction patterns and conclude that in Cu2ZnSnS(4-x)Sex, Se is inserted at a concentration of 50±10 %. One goal of this annealing process is to increase the grain size to reduce the impact of grain boundaries

on carrier transport. Whereas the starting kesterite has a grain size in the 10 nm range, after the annealing process, a grain size of 50 nm is reached.

Conclusion
Using in situ HVTEM synthesis of Cu2ZnSn S(1-x)Sex kesterite structures, we could successfully demonstrate the grain growth and replacement of S by Se in the kesterite crystal. This replacement reaction happens at elevated temperatures of 400-500 °C.

Keywords:

high voltage TEM, insitu, kesterite

1132

Morphology evolution and phase transition of $Co(OH)_2$ and Co_3O_4 investigated with STEM-tomography and in-situ XRD

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Poster Group 2

Background incl. aims
Transition metal hydroxides ($TM(OH)_2$) have garnered significant attention in research due to their diverse applications in the energy and environmental sectors. These hydroxides exhibit promising capabilities in catalysis, supercapacitors, and battery materials [1-3]. For instance, cobalt hydroxides ($Co(OH)_2$) can decompose to form cobalt oxides (e.g., Co_3O_4), which are materials known for their applications as catalysts in the Fisher-Tropsch process, water splitting, and N2O decomposition [1,4].

Catalytic processes typically occur at elevated temperatures. Understanding the morphological changes and phase transitions of Co_3O_4 upon heating is crucial for controlling its properties in various applications, including those listed above. Furthermore, studies suggest that besides particle size and morphology, the distribution of exposed facets in Co_3O_4 plays a role in the catalyst's activity during N_2O decomposition [4].

In this work, a visualization of the morphological evolution of Co_3O_4 crystals synthesized by calcination of $Co(OH)_2$ at different temperatures is carried out through ex-situ (S)TEM imaging of samples. Furthermore, the facet distribution of a Co_3O_4 particle, revealing the concentration of exposed surfaces, is determined and visualized in 3D by combining STEM-HAADF electron tomography (ET) and high-resolution TEM.

Methods
In an in-situ XRD experiment, the precursor $Co(OH)_2$ underwent calcination to 1200°C in steps of 25°C in synthetic air (20%O_2), mapping the crystal-size evolution of Co_3O_4 and its phase transitions. For visualization of the sample morphologies by electron microscopy, several Co_3O_4 samples were prepared separately by calcining $Co(OH)_2$ at varying temperatures. The choice of temperatures was based on the in-situ XRD experiment.

Transmission electron microscopy was carried out using a probe-corrected Spectra 200 (S)TEM (Thermo Fischer Scientific), operated at 200 kV in scanning mode (STEM), or in parallel beam mode (TEM) for high-resolution imaging. Electron tomography tilt series were acquired in scanning mode using the high-angle annular dark field detector (STEM-HAADF) and a low convergence angle of 5.6 mrad for enhanced depth-of-field [5]. The tilt-series, recorded from +70 to -70 degrees in 2-degree intervals, were reconstructed into a tomogram with voxel size $(0.43\ nm)^3$ using fiducial marker tracking (10nm) and weighted back projection (WBP) in Inspect3D v.4.5. The crystallographic orientation of the reconstruction was determined from additional high-resolution TEM images.

Finally, the facet distribution of the 3D reconstructed Co_3O_4 particle was evaluated from a 3D printed model of the reconstructed volume, by identifying the facets.

Results

The decomposition of $Co(OH)_2$ in synthetic air to form Co_3O_4 initiated at 150°C. As the calcination process continued, the mean crystal size of Co_3O_4, as measured by XRD, gradually increased from about 10 nm to 500 nm, indicating sintering. The maximum sintering rate was observed around 850°C, right before an abrupt full phase transition into CoO, which occurred between 875°C and 900°C. Upon cooling, the CoO phase fully transformed back to a Co_3O_4 structure with an average crystallite size (XRD) of ~150 nm. (S)TEM images confirm the presence of both large and small Co_3O_4 nanocrystals in the residual sample.

STEM imaging reveals that the cobalt spinel samples, calcined at 350°C, 450°C, and 550°C, are present in a pseudo-morph structure, resembling the overall shape of the hydroxide precursor, across all three samples. This morphology can be characterized as monocrystalline mesoporous nanograin sheets. While the sheet morphology is consistent across all three samples, an increase in the size of both the grains and pores is observed with increasing calcination temperature.

STEM images of a sample calcined at a high sintering rate (850°C), revealed large particles which appear to be partly faceted. One particle was reconstructed in 3D, revealing clear facets with the (100) facet being predominantly present, followed by (111) and (110). 24% of the surface could not be identified due to rounding, suggesting that the facet formation was not fully completed or equilibrated. This observation was supported by a 50-hour XRD experiment which showed that the mean crystal size of Co_3O_4 initially converges after several hours at 850°C, revealing that the 1-hour heated sample was captured in a dynamic sintering process.

Conclusion

By utilizing electron microscopy – specifically tomography – we were able to observe the morphological changes of Co_3O_4 synthesized by calcination of $Co(OH)_2$ at various temperatures. The Co_3O_4 samples exhibited a sheet-like monocrystalline mesoporous nanograin structure at 350°C and underwent drastic morphological changes as the material sintered to form large particles at higher temperatures. The shape and facet distribution of a Co_3O_4 crystal (calcined at 850°C for 1 hour) were determined using electron tomography combined with high-resolution TEM imaging, revealing the (100) facet as predominantly present, followed by (111) and (110). This approach provides valuable insights into the complex transformation processes (e.g., sintering) occurring in catalytic processes.

Keywords:

Co_3O_4, morphology, sintering, facet distribution

Reference:

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1133

FLIPs: Novel genetically encoded biosensors for polarization microscopy

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LS-02 (2), Lecture Theater 4, august 26, 2024, 14:00 - 15:00

Background
Genetically encoded fluorescent biosensors convert specific biomolecular events into optically detectable signals. Typically, such probes utilize a suitable fluorescence quenching process in order to modulate the absorption or emission spectrum the fluorophore, or the fluorophore's lifetime. However, an unrelated detection principle, directionality of optical properties of fluorescent molecules, should also allow development of genetically encoded biosensors. Remarkably, despite numerous potential advantages of such biosensors (ratiometric readout, resistance to bleaching artifacts, information about protein structure, possibility of multiplexing, orthogonality to other approaches), optical directionality has remained largely unexploited as a detection principle. Our goal was to make optical directionality of fluorescent molecules widely applicable to imaging of biomolecular processes of cell signaling.

Methods
Using techniques of molecular biology, we set out to develop a series of genetically encoded biosensors suitable for observations of molecular processes of cell signaling by polarization-resolved fluorescence microscopy.

Results
We have now identified a novel design of genetically encoded fluorescent biosensors, which we term FLIPs(1,2,3) (Fluorescence anisotropy and LInear dichroism Probes). FLIPs offer an extremely simple design, high sensitivity, multiplexing capability, ratiometric output and resilience to bleaching artifacts. Importantly, FLIPs allow their targets to remain non-modified. The probes are applicable to imaging cellular activity of G protein coupled receptors, G proteins, arrestins, receptor tyrosine kinases and other signaling proteins, using simple microscopy instrumentation. The sensitivity of the probes allows even imaging of endogenously expressed targets.

Conclusion
The simplicity, modularity and versatility of the FLIP design allows rapid creation of biosensors of desirable properties. By exploiting a simple, universal, yet long overlooked detection principle, FLIPs open a wide new area of development both in genetically encoded fluorescent biosensors and in optical instrumentation.

Keywords:

Polarization microscopy, biosensors, GPCR signaling

Reference:

1) Miclea P., Nagy Markova V., Bondar A., Sakhi A., Lazar J.; Novel Genetically Encoded Biosensors for Functional Imaging of Cell Signaling by Polarization Microscopy; https://www.biorxiv.org/content/10.1101/2024.02.23.581811v2
2) Lazar J., Bondar A., EPO patent application 21209717.4
3) 'FLIP' and 'FLIPs' are are trademarks used by Innovative Bioimaging, s.r.o.

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Unsupervised and supervised machine learning for feature classification in atomic resolution images

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IM-10 (1), Lecture Theater 3, august 29, 2024, 10:30 - 12:30

Background
High resolution scanning and transmission electron microscopy (S/TEM) enable the observation and exploration even of complex materials and interfaces down to the atomic level. It is often desired to uncover the atomic scale building blocks of materials, which is pivotal in understanding their physical nature and to tailor material properties. Identifying local features of the nanostructure of a material and deciphering latent attributes of them is of vital importance to discover new material phenomena. However, the increasing rate at which atomic resolution data is generated in modern electron microscopes makes human-based analysis tedious and renders it nearly impossible in the near future. This requires the development of automatic data analysis approaches to automatically extract meaningful physical information from local image features.

Methods
We present unsupervised and supervised machine learning approaches to classify phases and interfaces in atomic resolution microscopy images. An unsupervised image segmentation approach based on local symmetry descriptors to detect crystallographic features without prior knowledge of the underlying crystal structure is introduced [1]. The segmentation algorithm relies on self-similarity measures based on local symmetry operators that map the image into a symmetry score vector space. The dimensionality of the local descriptor is reduced by principal component analysis and the pattern labels are assigned by K-means clustering. We then show a supervised image classification framework that automatically labels crystal symmetries and orientations as well as interface regions in atomic resolution STEM images [2]. The underlying convolutional neural network is trained on simulated images of pristine crystal structures, while using the fast Fourier transform of local window regions as the descriptor. Typical noise sources, lattice distortions and rotations are taken into account by augmenting the training data.

Results
We show that the unsupervised segmentation approach can identify the different crystalline regions across a grain boundary in atomic resolution STEM images. By including rotational symmetry, it is even possible to segment the interface itself in an automatic fashion. We then apply the segmentation to atomic scale compositional faults in tetrahedrally complex phases and demonstrate its robustness on noisy data. In a final example, we show that the approach is even capable to segment image regions in an in situ atomic resolution video sequence. The supervised image classification is demonstrated on atomic resolution STEM images of grain boundaries in fcc, bcc and

hcp systems of pure metals. By adopting a Bayesian neural network, the uncertainty estimates of the prediction are considered, which provides information even on structures not contained in the training data. To test the model, we first apply it to synthetic polycrystalline images and demonstrate that amorphous regions can be indirectly identified by high uncertainty estimates. We then show the applicability to experimental STEM images of grain boundaries, where the mutual information is used to identify the interface regions automatically. The higher-dimensional neural network representations are explored via unsupervised learning and we find that it does not only provide information on the different crystal symmetries, but also the interface types.

Conclusions
We developed unsupervised and supervised machine learning approaches to automatically segment and classify image regions in atomic resolution images. It is demonstrated that the supervised segmentation is robust against noise in images and can be applied to image video sequences for in situ experimentation. The supervised learning provides quantitative classification of atomic resolution images of crystalline phases and is even capable to identify structural features not contained in the training data.

Keywords:

Atomicßresolution, machine learning, segmentation, classification

Reference:

[1] Wang, Ning, et al. “Segmentation of Static and Dynamic Atomic-Resolution Microscopy Data Sets with Unsupervised Machine Learning Using Local Symmetry Descriptors”, Micr. Microanal. 27 (2021) 1454-1464

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1135

Synthesis and Characterization of Ultrathin Unconventional Mixed 2H-HCP/FCC Phase Au Nanowire

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Poster Group 2

Background incl. aims
Crystal structure engineering enables the cultivation of noble metal nanostructures featuring unique crystal phases, showcasing innovative optical, catalytic, and electronic characteristics. Variations in atomic stacking sequences within these nanostructures are termed polymorphs, often showcasing distinct properties and functionalities despite being of the same material. The crystal structure of nanocrystals is frequently influenced by synthesis conditions such as reaction temperature, surfactants, precursor properties and gas environment. Polytypism, evidenced by the coexistence of several phases within a single nanostructure, defines this phenomenon. Compared to the typical stacking arrangement of "ABC" along the [111] fcc direction in fcc gold, the 4H/2H gold structure exhibits a stacking sequence of "ABCB/AB" along the [001] 4H/2H direction. 1,2
Ultrathin gold nanowires (NWs) with a diameter of approximately 2 nanometers hold significant interest for both nanoscience research and nanotechnology applications. Due to their high surface area and quantum-confined dimensions, these nanowires exhibit fascinating properties such as quantum conductance and ballistic conduction.3 The synthesis of high-quality, single-crystalline ultrathin gold NWs is essential for accurate physical property measurements and further application exploration. Recent reports have surfaced, highlighting successful advancements in the synthesis of these ultrathin gold NWs. However, reports on the synthesis and characterization of ultrathin 2H/4H Au nanowire is still very limited and a detailed structural and optical study of this nanostructure is nedded to be examined closely.1,2,4,5

Methods
In a typical synthesis process, 4.08 mg gold chloride hydrate (HauCl4, H2O) were dissolved into a mixture of 220 µL of oleylamine, 3.54 mL hexane and 250 µL of 1,2-dichloropropane, in a 20 mL glass vial. The solution was shaken for complete dissolution of HauCl4. Next, 80 mL de-ionized water is added into a 500 mL beaker, which was heated to 58°C. The glass vial is kept on the beaker for 16h and after that the solution is centrifuged at 5000 rpm for 2 minutes to filter the residue. The residue was further washed using hexane 3 times. For TEM characterization the Au nanowire solution in hexane was drop-casted on a C coated Cu grid. The HRTEM, selected area diffraction (SAD) were done in an image corrected FEI TECNAI F-30 operated at 300 kV. HRSTEM, energy dispersive X-ray spectroscopy (EDS) were carried out in a probe-corrected FEI TITAN TEM equipped with high-brightness gun and an Oxford Instruments Ultim X-MaxN 100TLE detector for EDS measurements.

Results
We have observed uniform formation of ultrathin Au nanowire. The diameter of the nanowire is between 1.9 nm – 2.3 nm and the length varies between 50nm to 300 nm. The EDS meaurement on them clearly shows the naowires are indeed Au. HRTEM measurement shows formation of 2H-HCP phase mostly on the top of the nanowire and a mixed 2H/HCP and FCC phase in the middle portion as

observed form the IFFT image also. A detailed study on the structural evolution of this nanostructure upon electron beam irradiation will be prersented in this work.

Conclusion

We have done successful synthesis of ultrathin Au nanowire consisting of mixed 2H-HCP/FCC phase. Interestingly the nanowire contains bead like structure as observed from the HRSTEM and HRTEM images and they are observed to be very much sensitive to the electron beam.

Keywords:

Au nanostructure, 4H/2H-HCP Au, Nanowire

Reference:

Reference:

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1136

Utilization of TEM in archaeology to gain in-depth information on historic artifacts

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Poster Group 2

Background incl. aims

Although inherently dealing with the past, archaeology has profited immensely from the use of modern advanced analysis techniques like electron microscopy [1,2]. Especially TEM can provide inaccessible insights, for example when only miniscule amounts of material are available or faint traces are studied [3,4]. Furthermore, the combination of in situ techniques (like heat treatment) with replicas can lead to vital insights into the exact methods and paramters used for the fabrication of historic artifacts.

In Sweden, two copper axes were found buried in the soil. Both axes were from the late neolithic and comparable to the axe found alongside “Ötzi”. First measurements with X-ray fluorescence spectroscopy (XRF) indicated a silver-rich surface layer next to the obvious strong corrosion. These axes are historic artefacts and therefore a non-destructive sample preparation is necessary. A FIB cut TEM analysis of the surface layers could be carried out adhering to the strict rules of artifact preservation.

The very first settlers in Europe used cow dong as a reinforcement in pottery around 7000 B.C. Mechanical testing measured a higher mechanical strength at certain firing conditions for the reinforced pottery. Apart from the astonishing finding that composite (nano) materials were utilized at this time the exact nature of the strengthening mechanism is not known: is it mechanical (carbon fibres from the grass) or chemical (cow dung ash reacts with clay) in nature? Detailed analysis using TEM can help to better understand the mechanism as well as identify the fabrication procedure of ceramic artifacts.

Methods
The axes were first investigated via XRF and XRD to validate the field measurements. Additionally, the axes were loaded into a FIB-SEM and lamellae were taken out and analyzed via SEM-EDX. The FIB lamellae were subsequently analyzed utilizing a FEI Tecnai F30 G2 and a Jeol JEM-200F NEOARM.
In order to better understand the formation mechanism of silver enrichment and the corrosion layer a replica artifact was produced and artificially aged using suspension in humic acid for multiple weeks.

For the analysis of the reinforced pottery visible grass fibres were extracted with tweezers, ground in a mortar while suspended in butanol and deposited on the TEM substrate via drop coating . The same process was used for the archaeological sample, the unfired replica and several fired replicas. In addition a small amount of material was transferred onto an in situ heating chip (DENS solutions

Wildfire) and heat treated up to 1100 °C in order to demonstrate the feasibility of in situ TEM observation of ceramic sintering and give insight into the mechanisms of the reinforced pottery during firing.

Results

XRD Rietveld analysis of the axe material showed a chemical composition in good agreement with XRF analysis . Since both techniques utilise X-rays the penetration depth is similar resulting in the same relative surface sensitivity. Analysis of the FIB-cut reveals the difference between the unoxidized bulk and the oxide layer, where in the CuO layer, precipitations of Ag are identified (see graphic). Additionally, the brittleness of the surface layer can be linked to the high amount of fractures visible in the CuO layer. The bulk material is uniform in contrast and has an Ag content of 0.8 at%.

In situ heating of a non-reinforced ceramic showed that for temperatures below 1000 °C the material showed a decrease in crystallinity as evidenced be loss of contrast in BF TEM and weaker diffraction rings in SAED. Above 1000 °C the material, which initially consisted of numerous crystallites, fused into larger crystals. Sophistaced backround subtraction in rotational averages of the SAED patterns allowed their evaluation even for faint diffraction patterns.

TEM analysis of the plant fiber reinforced ceramics did not reveal the presence of carbon nanofibers in the pristine as well as heated (in situ and ex situ) state. Carbon rich areas in the specimens could be found, yet not unambiguously linked to originate from the plant fibers. During heating no distinct differences to the non-reinforced ceramic were observed.

Conclusion

The investigation of the axes showed that the Ag-rich surface is a result of the corrosion process of the falutype copper, where the oxidization of the Cu reduces the AgO back to pure Ag, which then forms the grains visible in the EDS map. Consequently, the silver spots were not introduced deliberately, and thus, an initially ritualistic origin of the axes is disproven.

The feasibility of in situ TEM observation of ceramic sintering could be successfully shown. Due to the absence of carbon nanofibers for all sintering conditions mechanical reinforcement of the ceramic on the nanoscale can likely be ruled out. Due to the difficulty of unanbigously identifying specimen areas that originated from plant fibers the identification of the strengthening mechanism is the subject of ongoing investigation.

Keywords:

archeology, pottery, traceology, insitu, heating

Reference:

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INSIHGT - Scalable, Accessible, Homogeneous Deep Multiplexed Immunolabelling Platform for 3D Spatial Biology

Dr Hei Ming Lai[1,2], Mr Jacky Tin Shing Hung[2,3], Mr Juno Chun Ngo Yau[2], Mr King Ngai Chow[2], Mr Thomas Chun Yip Wong[2]
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LS-02 (2), Lecture Theater 4, august 26, 2024, 14:00 - 15:00

Biological systems are complex, encompassing intertwined spatial, molecular and functional features. However, methodological constraints always limit the completeness of information that can be extracted. Here, we report the development of INSIHGT, a minimally perturbative and cost-efficient three-dimensional (3D) spatial biology method utilizing solvent perturbative chemistry. This allows quantitative, highly multiplexed and multi-modal readout of tissue biomolecules in up to centimeter-scale biological systems. Diverse antigens, mRNA transcripts, neurotransmitters, and post-translational and epigenetic modifications, are well-preserved and visualized, allowing multi-round molecular probing for high-dimensional spatial biology and compatibility with downstream traditional histology. With INSIHGT, we mapped previously undescribed podocyte-to-parietal epithelial cell microfilaments and demonstrated their geodesic clustering in mouse glomeruli, catalogued sparsely located neurofilament-intensive inclusion bodies in the human cerebellum, and identified NPY-proximal cell types defined by spatial morpho-proteomics in mouse hypothalamus. We anticipate INSIHGT can form the foundations for 3D spatial multi-omics technology development and holistic systems biology studies.

Keywords:

3D spatial biology, systems biology

Reference:

Lai et al. (2022). Antibody stabilization for thermally accelerated deep immunostaining. Nature Methods. 19, 1137–1146.
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Nestin expression in the myocardium of normotensive and spontaneously hypertensive rats during aging

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Poster Group 1

Background incl. aims
Nestin, an interesting intermediate filament protein, is expressed in several tissues in the stem cells or within a certain period of development and reoccurs in the newly formed cells in adulthood. It is a proven marker of skeletal muscle regeneration and angiogenesis. Nestin is shortly expressed during the development of the heart and in some cell types in the adult myocardium, particularly in pathological conditions such as myocardial infarction or fibrosis. In this work we described expression and distribution pattern of nestin and its copolymerizing tissue-specific proteins desmin and vimentin in the intact and hypertrophic, adult and aged rat myocardium with the aim to contribute to understanding the reappearance of nestin in the diseased heart.
Methods
Nestin was detected by enzyme indirect two-step immunohistochemical method in the left ventricle myocardium of normotensive Wistar Kyoto (WKY) rats and in the hypertrophic left ventricle myocardium of spontaneously hypertensive (SHR) rats, both at the age of 1 and 1.5 year. Nestin expression in all samples of the myocardium was quantified by image analysis and statistically evaluated. For double immunofluorescent detections a novel method was introduced to enhance intensity of nestin signal based on application of Dako EnVision+ System-HRP Labelled Polymer.
Results
In the intact myocardium of normotensive rats nestin was expressed only in the endothelial cells of some blood vessels in 1.5-year-old animals, whereas in the 1-year-old WKY rats no nestin immunoreactivity was noticed. In the hypertrophic myocardium of SHR rats of both ages nestin was rarely detected in desmin+ vimentin- cardiomyocytes and in some vimentin+ interstitial cells that were usually accumulated forming the clusters and differing in intensity of desmin immunoreactivity. In addition, nestin was sparsely expressed in myocardial capillaries and in the endothelium of larger blood vessels in one-year-old SHR rats.
Quantitative image analysis of nestin expression in the myocardial samples confirmed significant increase in 1.5-year-old WKY rats and in SHR rats of both ages compared to the intact 1-year-old WKY rats.
Conclusion
This work firstly describes re-occurrence of intermediate filament nestin in some cardiomyocytes and in certain interstitial cells in the hypertrophic myocardium of adult and aged spontaneously hypertensive rats. Nestin re-appearance in the endothelial cells of the blood vessels documents important role of angiogenesis during aging in the intact and early hypertrophic myocardium. Nestin re-expression during cytoskeletal remodelling in the different cell types accompanies complex changes in the chronically pressure over-loaded heart and is related not only to myocardial hypertrophy and aging, but also likely to cardiac regeneration.

Keywords:

nestin, hypertension, myocardial hypertrophy, rat

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Studying nano-catalysts degradation with an identical location STEM approach

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Poster Group 2

The local structure and morphology of a material is usually modified when submitted to an electro-catalytic process depending on the cycling and potential used. One way to learn more about the effects happening on the material is by using identical location, that is, examining a specific region of interest of the material before and after the electrochemical process takes place. In this work, we show different examples using identical location analytical scanning transmission electron microscopy (IL-STEM) to track specific changes of the material by observing before and after states. In this way, it is possible to study degradation mechanisms directly in the region of interest. Since some of the changes are occurring at the atomic level, understanding them can shed some light on the structural-properties relationship, including the metal support interaction. Most of the analyses shown in this work correspond to Pt-based nanoparticles typically employed in fuel cells and electrolysers that have been submitted to a particular electrochemical cycling protocol. The structural and morphological information gathered from the initial and after states suggests the occurrence of dissolution from specific atomic columns and atomic sites as well as redeposition for different columns and sites. These observations can lead to the conclusion that certain facets may be affected more than others, or in other cases, as it will be shown, to reveal how the support interacts with the catalysts. In this regard, the analysis of regions that suffered modifications can give us certain clues on how degradation started or evolved. Hence, the incorporation of advanced electron microscopy analytical techniques coupled with electro-chemistry experiments can provide crucial insights for a better catalyst design, including stability and durability.

Aknowledgements:
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Keywords:

Nanoparticles, Catalysis, Identical location STEM,

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Visualisation of lepidopteran silk gland morphology using X-ray micro-computed tomography scanning technique

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Poster Group 1

Background incl. aims
Lepidopteran caterpillars are one of the most significant insect silk producers. Depending on diverse life strategies of the two sister families of Bombycoidea, Saturniidae and Sphingidae, the caterpillars have evolved various ways of using silk. Most of the sphingiid larvae use silk exclusively in their first instars to produce rescue silk fibres that prevent the tiny caterpillars from falling off the host plant. In contrast, their closest relatives, the Saturniidae, save all of their silk for the end of larval development to construct complex silk cocoons that serve as a protective outer shell for the individual during its pupal stage. In general, silk is secreted by specialised ectodermal cells that form a silk gland. The silk glands of individual lepidopteran families differ significantly in their overall morphology. This research aims to analyse the in situ localisation of silk glands of several representatives of the Saturniidae and Sphingidae using micro-computed tomography (microCT). The data obtained allow the generation of detailed 3D projections of a caterpillar body, including its internal organs. Here we compare the advantages of the Surface module for segmentation and surface rendering in three different versions of the Imaris software (Imaris 9.3, Imaris 10.0 and Imaris 10.1).
Methods
Last instar caterpillars of the Saturniidae and Sphingidae representatives were submerged in Bouin-Hollande's fixative and contrasted in Lugol's solution. X-ray microCT SkyScan, model 1272 (Bruker microCT, Belgium) was used to visualise iodine-contrasted specimens. High-resolution 3D output tomography data were reconstructed in SkyScan’s volumetric NRecon software version 2.2.0.6 (Bruker microCT, Belgium). 3D models were created in Imaris software versions 9.3, 10.0 and 10.1 (Bitplane AG, Oxford Instruments, UK) using the Surface module: Surpass - Contour Surface, and Machine learning segmentation based on Artificial Intelligence.
Results
Non-invasive microCT is the ideal technology to project internal structures and organs in situ without destroying the specimens. Using Imaris analysis software, we found that Imaris 9.3 is excellent at creating semi-transparent surfaces with adjustable transparency, while the latest Imaris 10.1 does not allow this. On the other hand, Imaris 10.1 uses Machine learning segmentation based on Artificial Intelligence, which makes surface creation much faster and easier.
Conclusions
The procedure for preparing caterpillars for microCT has been optimised. Detection of silk glands in members of the Saturniidae and Sphingidae families based on microCT scanning reveals their exact location in the larval body. This approach allows detailed comparison of the silk gland morphology in relation to the location of other internal organs. Imaris 9.3 and Imaris 10.1 are a suitable combination for complex 3D renderings of biological objects.

Keywords:

microCT, silk glands, Imaris

Reference:

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TEM structural analysis of photocatalytically active mesoporous single crystalline LaTiO2N particles

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Poster Group 2

Background incl. aims
Single crystalline mesoporous oxynitride particles have been studied as prospective catalysts in solar hydrogen-generating devices [1]. Both, photocatalytic and photoelectrochemical applications benefit from the high active surface area of catalytic particles in combination with good charge-transport properties [2]. A suitable combination of desired properties is achieved in the case of large mesoporous LaTiO2N single crystalline particles (up to 1 µm). The absence of grain boundaries reduces charge carrier recombination while at the same time their porosity results in increased surface area [3][4]. LaTiO2N mesoporous particles are obtained by transforming La2Ti2O7 particles in a thermal ammonolysis process. In this contribution, we focus on a structural description of pore formation and pore arrangement concerning the LaTiO2N orthorhombic lattice.

Methods
The photocatalytically active mesoporous single-crystalline LaTiO2N particles were synthesised via a thermal ammonolysis process $La2Ti2O7 + 2\ NH3 \rightleftharpoons 2\ LaTiO2N + 3\ H2O$, T > 900 °C.
A dual beam microscope Thermo Fisher Scientific Helios 5 FX was used for SEM imaging (immersion mode, in-lens detector, 3 kV, 100 pA) and orientation-specific preparation of electron transparent samples. Chunks of mesoporous particles were lifted out and transferred onto a compustage holder. The thinning was performed at ion accelerating voltage ranging from 30 to 2 kV and beam current ranging from 2 nA to 25 pA. During the thinning process, the sample was aligned using α and β tilts of the compustage holder in combination with a pixilated 4D STEM detector.
TEM/STEM analyses were performed using a JEOL F200 cold FEG microscope operated at an accelerating voltage of 200 kV. For the STEM-EELS analyses, CEOS CEFID energy filter equipped with a Tvips XF 416 CMOS camera was used. Tomograms were obtained by performing tilt series with a step width of 2° from −54° to 78° in STEM HAADF mode. For tomography, a 200 mesh copper grid coated with a continuous carbon thin film (Plano GmbH) was used. The 3D reconstruction was carried out with ImageJ and the 3D representation with the Amira software (Thermo Fisher Scientific).

Results
The topotactic thermal ammonolysis process conserves the overall size of the particles while at the same time creating a network of both closed and opened pores. Closed volume porosity was estimated to 12% based on a STEM tomography reconstruction. The orientation and arrangement of the pores were compared to the orientation of the crystal. TEM images in combination with SAD patterns show long axes of the pores (ranging from 20-60 nm) oriented along the [001] and [010] directions of the orthorhombic LaTiO2N Imma structure (ICSD-168551). The EELS data was recorded over closed pores and the surrounding matrix. The nitrogen K-eddge (402 eV) shows a higher content of nitrogen in the closed pores. This suggests the presence of entrapped nitrogen gas in the mesoporous structure.

Conclusions

Micrometer-sized mesoporous LaTiO2N particles were prepared by an ammonolysis process from La2Ti2O7 particles. The structural arrangement of the pores was analysed using TEM and STEM tomography techniques. The pores have their long axes oriented along the [001] and [010] lattice directions. The closed pores have a volume fraction of 12% and contain encapsulated nitrogen gas.

Keywords:

TEM, 2N, Mesoporous, Photocatalysis

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Correlating microscopy methods: the case of precipitation in lean, bioabsorbable Mg alloys

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Poster Group 2

Background incl. aims
Many of the properties of metallic systems are driven by nanoscale precipitation. We present our workflow in the correlative microstructural study of "ZX" lean Mg alloys, which contain zinc and calcium, as a test case. Such alloys are promising candidates for bioabsorbable implants due to their excellent biocompatibility and degradation rate matching tissue regeneration. Because the nanoscale intermetallic precipitates within the alloys drive the mechanical properties and degradation behavior, it is critical to understand their type and role to tailor the alloys for specific applications.
Methods
We are deploying a correlative approach, utilizing mainly atom probe tomography (APT) and transmission electron microscopy (TEM), to cover a wide range of spatial length scales and obtain quantitative information. Despite its power, the correlative approach presents technical hurdles: sample preparation requires meticulous methods, including focused ion beam. Ideally, the same region of interest should be analyzed across all instruments, which can be challenging considering the vastly different size scales involved. Minimizing damage or corrosion during transfer between instruments, which usually occurs in air, is also crucial. Finally, to fully leverage the harvested data, one must apply data-treatment techniques, including AI and simulations to aid data interpretation.
Results
Starting from the solid solution, we were able to elucidate the precipitation sequence via APT at the smallest scales, and, as precipitates matured and grew in size, with STEM imaging and EDS chemical mapping supported by simulations. We revealed that the debated equilibrium phase of the ternary precipitates is based on the Ca2Mg5Zn5 crystal [1]. However, its composition derived from EDS and APT presented a broader range, which we also noted in the correlative nanoscale analysis of Fe–Cr alloys [2, 3]. This showed us that the correlative evaluation of APT- and EDS-derived results is beneficial when assessing a composition. We conclude with an outlook on further correlative approaches, including X-ray nanotomography.
Conclusion
This study showed us that correlating APT and TEM is beneficial for the study of precipitation of metallic systems, starting from the solid solution, and when assessing the composition of nanoscale precipitate. We conclude our presentation with an outlook on further correlative approaches, including X-ray nanotomography, which allows to further bridge the gap between spatial scales.

Keywords:

Mg-alloys, Fe–Cr, correlative-microscopy, APT, TEM

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Imaging Chiral Spin Textures with Electron Interferometry and Polarimetry

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PS-08 (2), Lecture Theater 2, august 27, 2024, 14:00 - 16:00

Background incl. aims
Magnetism and spintronics play increasingly significant roles in modern technologies such as data storage and logic devices, sensors, quantum computing, and transportation and electricity generation. The three-dimensional nanoscale spin texture within magnetic materials plays a large role in the behavior of these technologies, and also provides a rich area of fundamental study. Electron, x-ray, and scanning probe microscopies can be used to image 2-D projections of spin textures, yet each technique has strengths and limitations. Lorentz TEM, for example, projects images of the average magnetic field inside a specimen while exposing it to an external field or temperature, but it is insensitive to the field component along the beam path. We aim to combine these techniques with new ones to obtain the 3-D structure of magnetic spin textures.

Methods
Here we discuss the use of specialized electron microscopies, STEM holography and scanning electron microscopy with polarization analysis (SEMPA), to understand the 3D structure of nanomagnetic spin textures such as skyrmions. STEM holography [1,2] is an interferometric 4D-STEM technique where electrons in the beam are coherently divided into a superposition of two or more separate probes and then scanned over the specimen. If one probe passes through vacuum while the other transmits through the specimen, the resulting phase shift can be measured by recording the interference pattern formed in the far-field at the detector. That is, the bright field discs of the two probes overlap at the detector, forming interference fringes (Fig. 1). Unlike defocus- and deflection-based magnetic imaging techniques such as Lorentz TEM or DPC which are sensitive to phase gradients within the specimen, STEM holography is directly sensitive to the phase relative to the reference beam. With this, STEM holography can be used to directly measure the component of the magnetic vector potential parallel to the beam.
SEM with polarization analysis (SEMPA) [3] is a surface-sensitive technique that can image all three vector components of the surface magnetization. Special detectors can measure the average spin polarization of secondary electrons. Each detector returns information about two components of the magnetization vector; one returns in-plane (Mx and My) and the other returns one in-plane (Mx) and the out-of-plane component (Mz). Therefore, two consecutive scans provides complete, quantitative imaging of the in-plane and out-of-plane magnetic nanostructure as well as normal SE contrast.

Results
STEM holography and Lorentz TEM were used to provide images of the skyrmion magnetic field projected through the thickness of the specimen, and SEMPA was provided images of the magnetization at the surface. We find that whereas crystalline specimens can host magnetic skyrmions with whorl-like magnetic fields that extend uniformly through the thickness of the material, here we observe a more intricate magnetic structure. TEM indicates that in the bulk of the material, the magnetic flux wraps around the core like a vortex, yet SEMPA indicates that near the surface the magnetic flux points radially [4], such that the 3D structure is akin to that of a vortex ring.

Conclusion

We used SEMPA, STEM holography, and Lorentz TEM on the same Fe/Gd multilayer materials to understand the 3D structure of magnetic skyrmions, observing knotted vortex ring-like structures. This illustrates that multimodal magnetic electron microscopy can be used to provide 3D information about magnetic skyrmions that cannot be observed with a single technique alone.

Keywords:

Skyrmions, magnetic microscopy, electron holography

Reference:

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IMG Electron Microscopy Core Facility

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Poster Group 1

Background and objectives
Imaging techniques are essential for modern biomedical research. State-of-the-art microscopy technologies are becoming increasingly sophisticated and require not only expensive equipment, but also highly qualified personnel to operate the instruments, collect high-quality data and analyse the results. This is why imaging core facilities are becoming increasingly important, helping scientists to use advanced imaging techniques in a time and cost efficient manner. The Electron Microscopy Core Facility at the Institute of Molecular Genetics in Prague provides open access to a wide range of imaging, analysis and sample preparation methods in biological electron microscopy.
Methods and results
Our team's expertise is supported by state-of-the-art equipment for sample preparation and ultrastructural imaging. A high-end transmission electron microscope (TEM) operates at up to 200kV and offers high resolution TEM, imaging in STEM mode, 3D analysis by TEM or STEM tomography, cryo-electron microscopy and STEM-EDS elemental analysis. A standard 120kV TEM with the user-friendly Limitless Panorama application is used for routine observation. Recently we have incorporated a cryoFIB-SEM microscope and set up cryo workflows for cryoTEM tomography or diffraction analysis on FIB-milled frozen-hydrated lamella. For precise targeting of the region of interest within the sample, light fluorescent imaging in a specialized cryoCLEM microscope can be used.
Standard and advanced techniques are also used in sample preparation. Starting from routine chemical fixation and resin embedding or negative staining of weakly stained specimens, we can progress to better preservation of natural specimen appearance by cryofixation using plunge freezing or high pressure freezing, followed by freeze substitution, cryosectioning or freeze fracture replica labelling. Detection of specific moleculles within the cells or tissues can be achieved using pre- or post-embedding immunolabeling techniques using gold nanoparticles of different sizes.
The facility can accommodate a wide range of biological samples for processing and imaging, including human and animal cell cultures, plant and animal tissues, worms, microorganisms, lipid micelles, isolated DNA, and purified proteins. Furthermore, the facility offers tailored development and optimization of sample preparation, based on a longstanding expertise and fruitful collaborations with companies manufacturing equipment for electron microscopy.
Conclusions
The Electron Microscopy Core Facility is part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node. It offers open access to its technologies and expertise, and is prepared to welcome users from all fields.

Keywords:

Electron microscopy, open-access, Czech-BioImaging, Euro-BioImaging

Reference:

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Interplay between microstructural properties with ionic/electronic conductivity in Na3PS4-based composite cathodes for Na-SSBs

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Poster Group 1

The potential of having high energy and power density in all-solid-state batteries (ASSBs) makes them attractive candidates for energy storage systems of the future [1]. They also have several advantages over traditional liquid electrolyte-based systems, including safety and a wide operating temperature range [2]. Furthermore, the higher abundance of sodium relative to lithium makes solid state sodium ion batteries a more cost-effective alternative [3]. One challenge is the limited ionic transport capabilities of current solid electrolytes (SE), but also the development of a suitable cathode, which is as crucial as having a promising SE for practical applications [4]. An optimized composite cathode (CC) should provide a percolating network of ions and electrons with short transport pathways and a large active interphase area between SE and cathode active material (CAM) [4,5]. Therefore, the composite cathode must be carefully built by optimizing the phase fractions between CAM, SE, and CA (conductive additive), particle characteristics, and phase distribution to ensure percolation and adequate ionic and electronic transport.
In this work, our motivation is to gain a deeper understanding of the relationship between the microstructure and the ionic/electronic conductivity of the composite cathode. Within this aim, as a CAM, NaCrO2 was mixed with the conductive matrix (CM), which consists of Na3PS4 as a SE and vapor growth carbon fiber (VGCF) as a CA. The effective ionic and electronic conductivity of the composite cathodes were measured using electrochemical impedance spectroscopy (EIS) with two different configurations (ion blocking and electron blocking). The cross-sectional microstructures of CCs were investigated via scanning electron microscopy (SEM) and FIB-tomography. Our findings revealed that reducing the Na3PS4 (SE) and VGCF (CA) content simultaneously results in a balanced ionic and electronic conductivity (70 wt. % CAM contained CC) at 10 MPa stack pressure. Additionally, both ionic and electronic conductivity values increase with increasing stack pressure. 2D microstructural information from SEM images showed slightly reduced density but a better CAM distribution by reducing CAM content. To obtain 3D information from the microstructures XRM & FIB tomography analyses will be performed and presented in this study.

Keywords:

Sodium solid-state batteries, Microstructural design

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ESEM technique for monitoring the geo-polymerization mechanism of the wet ash-cement mixture

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Poster Group 2

Background
Portland cement is one of the most important material of any country infrastructure development. Due to the massive global production and consumption of Portland cement, the cement industry is responsible for almost 10% of the world's carbon dioxide emissions associated with anthropogenic activities [1] and consumes about 3% of the world's energy [2]. Recent studies have demonstrated that fly-ash (FA) can be used as a mineral admixture in the cement industry to reduce the consumption of the most common calcareous raw materials [3].
Herein, we report on the benefits of the ESEM (Environmental Scanning Electron Microscopy) technique to expand the in situ knowledge of the geo-polymerization mechanism of the FA-cement mixture in the wet state. Moreover, X-ray diffraction (XRD) results about the phase composition and crystallinity of the initial ash, as well as in-situ evaluation for hydration of cement pastes, are reported.

Methods
Ash and slag from the Turceni thermal power plant deposit were used as supplementary cementitious materials (SCMs) to replace ordinary Portland cement.
The microstructure of the cement pastes and the in-situ reaction products formed during the first minutes to hours were studied using the Versa 3D Scanning Electron Microscope, which allows the adjustment of pressure, temperature, and humidity. Imaging was performed at a temperature of 2 0C and relative humidity of 90%, with a beam voltage of 30 kV, at a working distance of 9 mm. Morphology changes during hydration of cement pastes were in-situ ESEM monitored under a relative humidity of 90% and at a temperature of 2 0C. According to the pressure-humidity-temperature diagram of the liquid-vapor phase diagram for water, the expected pressure in the working chamber was estimated to be ~640 Pa. It is worth mentioning that the is situ ESEM dynamic experiments were performed on uncoated fresh wet cement mixture pastes. Each sample was monitored for a period of 3 to 6 h (depending on the specificity of the observed morphological changes) and the images were acquired every 10 min at magnifications of ×5,000 and ×10,000. Scanning electron microscopy (SEM) analysis of the ash powder and cement pastes after several days of curing was performed using an FEI Inspect F50 microscope coupled with an energy dispersive spectrometer (EDS) (ThermoFisher, Eindhoven, The Netherlands).
The phase composition and crystallinity of the initial ash, as well as in-situ evaluation for hydration of cement pastes, were studied by X-ray diffraction (XRD) using PANalytical Empyrean equipment. The equipment was used in transmission geometry and was equipped with a hybrid monochromator and

a 1/20 divergent slit on the incident beam side and a programmable anti-scattering slit mounted on the PIXCel3D detector on the diffracted beam side. The sample was investigated in the range of 4–480 2θ angle. Measurements were performed every 15 min for 24 h. Reduction of X-ray diffraction data and full pattern fitting by the Rietveld method were performed using HighScorePlus 3.0.e software (PANalytical, Almelo, The Netherlands).

Results

The morphologies for the reference sample C0 (100% cement) and the sample C45 (55% cement and 45% ash) are shown in Figure 1. The legends show the time of acquisition, with the first image taken immediately after the “dead” time limit, called t0. In the micrographs in Figure 1a) of hydrated cement paste (sample C0) it can be seen the evolution of the first hydration products cement, with the formation of acicular structures of ettringite and CSH/CASH (calcium/aluminium silicate hydrates) [4, 5]. Smaller particles are more reactive compared to larger ones. The hydration products tended to crystallize into cracks and pore spaces. A significant amount of cement hydration reactions appeared to have occurred during the “dead” period. The soft and fragile bride’s veil morphology of CSH appeared to have emerged from all surfaces. As revealed by Figure 1b), the addition of high ash content (sample C45) induced a delay in the hydration reactions, with the formation of prismatic sheet structures over time. The hydration products appeared even more crystalline. The usual amorphous CSH veil structure appeared at later stages (420 min) of hydration. As the paste hydrates over time, the hydrogel resulting from the partial hydration of mineralogical compounds such as dicalcium silicate, tricalcium silicate, and tricalcium aluminate, covered the spaces between the particles of the specimen. At this highest loading content of ash into cement pastes (sample C45), the formed crystals appeared to have better-defined edges. Ca(OH)2 platelet structures could be captured after the “dead” time. The gel-like hydrosilicates covered the ash particles very well, and the needle structures responsible for the subsequent mechanical strengths of the binder material can be visualized in the advanced moments of hydration (t0 + 20’, t0 + 420’).

The XRD patterns obtained for the C0 and C45 sample, at the initial time point and after different time intervals from 15 min to 24 h, are depicted in Figure 2. According to the recorded spectrum, the intensity of the peaks decreases with time, as a consequence of a decreasing in the degree of crystallinity (anhydrous phase content). As seen in Figure 2, the cement substitution with FA in a proportion of 45% did not drastically alter the phase evolution during hydration.

Conclusions

The herein reported in-situ ESEM and in-situ XRD has revealed new insides about the geo-polymerization mechanism of the FA-cement mixture in the wet state. The addition of ash in different proportions affects the hydration kinetics in the sense that some hydration reactions of the mineralogical binders are delayed with increasing ash content whereas Ca-initiated ones are accelerated.

Sustainability through the reuse of accessible economic and social resources is a way to achieve environmental balance while ensuring long-term development. The obtained results have confirmed the ash from Turceni thermal power plant features as SCM material to replace ordinary Portland cement.

Keywords:

ESEM, IN-Situ Hydration

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Molecular insights into rapid cell death involving endoplasmic reticulum and nuclear remodeling p

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Poster Group 1

Conformationally fluid peptide-lipid complexes have been identified as potent tumoricidal agents in cellular models and clinical trials (1, 2). Here, we identify the mechanistic details of how the peptide-lipid complex, alpha1-oleate, achieves this outcome by rapidly engaging with the largest membranous organelle in the cell, the endoplasmic reticulum (ER). We identify a previously unknown mechanism of tumor cell death, involving ER and nuclear remodeling and the creation of a joint ER and nuclear compartment for the scavenging of cellular contents. Induced by the membrane-active alpha1-oleate complex. This response also affected molecular mechanisms of cancer and cell adhesion gene networks. Cell death was not affected by inhibitors of apoptosis, necrosis or autophagy but was sensitive to ion channel inhibitors. In addition, the novel cellular changes triggered by alpha1-oleate, included massive detachment of dying cells loaded with alpha1-oleate was also observed.

The results identify a new, ER-driven mechanism for capturing constituents of dying cells for final processing and shedding from treated tumors, to avoid tissue toxicity.

Keywords:

Endoplasmic reticulum, nuclear remodeling, cancer

Reference:

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Effects of Myricetin on Mesenchymal Stem Cells Exposed to Oxidative Stress

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Poster Group 1

Background: The mesenchymal stem cells (MSCs) have been widely used both in cell therapies. Myricetin is a flavonoid which suppresses oxidative stress and inflammation, reduces bioactivation of carcinogens, and affects cell signaling.
Aims: The aim was to evaluate the effects of myricetin in the hydrogen peroxide (H2O2) oxidative stress model in MSCs by immunocytochemical staining and electron microscopy.
Methods: The groups were determined by control, H2O2, myricetin and myricetin + H2O2 groups. The MSCs were treated with concentrations of 500 µM H2O2 and 5 µM myricetin for 24 hours. The MSCs ultrastructure and caspase-3, TIMP17 and TNF-α expressions were analyzed using transmission electron microscope and immunocytochemistry.
Results: The effects of myricetin against H2O2 oxidative stress damage in the MSCs, an increase in cell count, decrease in caspase-3 and TNF-α immunocytochemistry staining, increase in Tim17 immunocytochemistry staining and decrease in degenerative cell morphology in electron microscopic were observed.
Conclusion: Flavonoid myricetin may be a promising target for an alternative treatment option for increased cell viability, anti-apoptotic and anti-inflammatory effects in the MSCs.

Keywords:

Mesenchymal stem cell, myricetin

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Impact of In Situ and Ex Situ Annealing on Al-Cu Heterogeneous Nanostructures: A Comparative Study

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Poster Group 2

Background incl. aims
Water-based aluminum batteries are a promising alternative to commonly used lithium batteries due to properties such as low production costs, safety in handling, and high theoretical capacity [1]. However, the formation of an oxide layer prevents achieving the desired electrochemical properties. By annealing Al-Cu multilayer heterogeneous nanostructures, the formation of a dendritic Al2Cu structure enveloped by pure aluminum can be achieved by a eutectic transformation [2]. Techniques such as FIB (focused ion beam) [3] for sample preparation and in-situ TEM analyses enable detailed monitoring of phase transformations and structural dynamics of materials at high temperatures. Research in this area is key to the development of new materials with potential applications in energy, especially in the context of improving performance and efficiency of water-based aluminum batteries.
The main objective of this work is to analyze the structural and phase changes of multilayer nanostructures at elevated temperatures through in-situ annealing in TEM. Another goal is to compare the processes occurring in lamellae annealed in-situ with lamellae created from ex-situ annealed material. The results contribute to a deeper understanding of properties of Al-Cu multilayer heterogeneous nanostructures and their potential for applications. Moreover, they validate the experimental in situ methods employed in studying the microstructural processes.
Methods
Al-Cu multilayer heterogeneous nanostructures, consisting of alternating thin layers of Al and Cu nanoparticles, were prepared using magnetron sputtering. The transmission electron microscopy (TEM) and scanning TEM (STEM) with high-angle annular dark field (HAADF) imaging on a Jeol 2200FS was used for the characterization of samples. This was complemented by energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM) analyses and automated orientation and phase mapping (ASTAR). Cross-sectional lamellae were prepared using a focused ion beam (FIB) on a Zeiss Auriga SEM. In situ annealing was conducted within the TEM using a GATAN heating holder.
Results
Films of the Al-Cu heterogeneous nanostructures, with thicknesses of 200 nm and 1 µm, were produced. The thin films were annealed in situ to investigate structural changes. Cross-sectional lamellae made from the 1 µm thick films revealed a gradient structure, showing an increasing amount of Al towards the bottom of the specimen. The impact of film thickness on the resultant structures was examined through comparative analysis of in situ annealed lamellae of two different thicknesses and lamellae prepared from ex situ annealed film. Surface modifications, grain size, and the distribution of Al2Cu within Al were mapped using SEM, EDS, and HAADF detectors together with ASTAR imaging techniques.
Conclusion
This study demonstrates the capabilities of in-situ annealing for exploring the structural and phase transformations in Al-Cu multilayer heterogeneous nanostructures. Through characterization techniques, including TEM, STEM, EDS, and ASTAR, a gradient structure with varying aluminum concentration was observed, particularly in multilayer films. The comparisons between in-situ and

ex-situ annealed lamellae, highlight the thermal behavior and potential of these nanostructures for advanced material applications, such as in next-generation battery technologies.

Keywords:

Al-Cu multilayer nanostructures, In-situ TEM

Reference:

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The effects of resveratrol on liver damage and ferroptosis in fructose-streptozotocin induced diabetic model

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Poster Group 1

Background
The liver is the main detoxification organ in the body and regulates normal glucose homeostasis. There is a relationship between diabetes and liver disease. Pathological changes such as hepatic steatosis, accumulation of fatty acids, and fibrosis have been demonstrated in the livers of diabetic patients. Due to the connection between Type 2 Diabetes Mellitus (T2DM) and progressive liver disease, further research is needed to understand the pathogenesis of diabetic liver disease (1). Studies on liver damage in diabetes have focused on inflammatory and insulin signaling pathways, as well as oxidative stress. Recent studies have found that ferroptosis plays a significant role in acute or chronic liver damage (2).
Ferroptosis is a form of non-apoptotic cell death characterized by excessive lipid peroxidation, iron dependence, and is associated with various pathological conditions in the liver. Increased interest has been shown in the role of ferroptosis in liver diseases because excessive iron overload and oxidative stress are major triggers for liver damage and disease progression in many liver diseases. Therefore, targeting ferroptosis could provide a promising new therapeutic strategy for the treatment of liver disease in affected patients (2,3).
A fructose-rich diet impairs aerobic capacity and leads to diabetes and fatty liver disease. It triggers various metabolic disorders, including hypertriglyceridemia, hyperglycemia, insulin resistance, and glucose intolerance. Fructose reduces antioxidant activity and harms the livers of animals (4).
Resveratrol (RSV) is a compound belonging to the stilbenes group, found in grape skins and leaves. It exhibits properties of phytoalexins, which are produced by plants in response to fungal or bacterial infections, and it prevents cellular damage caused by free radicals. In addition to its antioxidant and anti-inflammatory effects, resveratrol has protective effects against cancer, aging, obesity, diabetes, cardiovascular, and nervous system diseases. Resveratrol has various beneficial effects, such as normalizing the activities of antioxidant enzymes like catalase, superoxide dismutase, and glutathione S-transferase, as well as lowering blood sugar levels. Low doses of resveratrol can reduce blood sugar levels and improve insulin sensitivity in diabetic patients (4,5).
The aim of this study is to demonstrate the role of ferroptosis in the formation of liver damage in a diabetic model created with a high fructose diet and streptozotocin (STZ) and to investigate the potential effects of resveratrol treatment on this process.
Methods
In the study, 8-week-old Sprague-Dawley rats were divided into four groups: 1) Diabetic group (D), fed with a 10% fructose solution for two weeks, injected with STZ (40mg/kg) at the end of the second week, and then fed with a 10% fructose solution for three more weeks. 2) Diabetic + resveratrol group (D+RSV), treated with 1mg/kg/day resveratrol for four weeks. 3) Non-diabetic rats treated with resveratrol (C+RSV) (1mg/kg/day) for four weeks. 4) Control group (C). At the end of the experiment (9th week), all rats were sacrificed, and liver tissues were collected. Throughout the experiment, calorie intake, body weight, and blood sugar levels were measured. The liver tissue sections were

immunostained with GPX-4, COX-2, and H2AX antibodies. All values were analyzed using statistical methods.

Results

In the fasting blood glucose measurements of the rats that were administered STZ (40 mg/kg, i.p.) after 10% fructose for 2 weeks ($p < 0.001$), a highly significant difference was detected between the diabetic groups and the control groups. At the end of the experiment, there was a significant difference between the diabetic group and the control groups ($p < 0.001$). Additionally, a significant difference in blood glucose levels was detected between the control and D+RSV groups ($p < 0.05$). The calorie intake in the second and fifth weeks was significantly different in the D and D+RSV groups compared to the control groups ($p < 0.001$). However, at the end of the study, there were no differences between all groups.

H&E, Van Gieson, and Prussian blue staining of liver tissue sections revealed vacuolization of hepatocytes, an increase in collagen fibers in the portal areas and around the central vein, which is an indicator of fibrosis, and iron accumulation in the tissue in the diabetic group. In the D+RSV group, it was found that the damage caused by diabetes was recovered and iron accumulation in liver tissue was inhibited. Decreased GPX-4 protein expression and increased COX-2 expression were detected by immunohistochemistry staining in the diabetic group. Additionally, GPX-4 immune positive cell numbers were significantly higher in the D+RSV group. H2AX is used by many researchers as a tool to measure induced DNA damage. In the D group, an increase in the number of cells marked with phosphorylated H2AX antibody was observed, while there was a decrease in the D+RSV groups.

Conclusion

We suggest that the high-fructose diet and low-dose STZ administration increased blood glucose levels, caused liver fibrosis, and led to iron accumulation. Additionally, resveratrol treatment decreased blood glucose levels in diabetic rats, positively affected oxidative stress by increasing GPX-4 expression, the main purifier of lipid peroxides in cells, and prevented iron accumulation in hepatocytes, thereby protecting liver tissue from ferroptosis.

Keywords:

Ferroptosis, Streptozotocin, Fructose, Resveratrol, Liver

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Multiscale characterization of Al-4Fe alloy grown by additive manufactoring

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Poster Group 2

The advent of Additive Manufacturing (AM) processes in recent years has given us access to parts with complex geometries that are difficult to machine using conventional manufacturing methods. However, Additive Manufacturing leads to microstructures and metallurgical states that differ from those obtained by conventional processes. In this context, it has become necessary to develop new alloy grades dedicated to Additive Manufacturing in order to take full advantage of the specific features of these processes. In the aeronautics sector, there is a strong interest in the study of lightweight titanium- and aluminum-based alloy grades [1,2].

In this work, we are interested in a model Al-Fe alloy, which is not possible to produce using conventional processes due to the precipitation of the embrittling Al13Fe4 phase. The use of the L-PBF (Laser Powder Bed Fusion) additive manufacturing process avoids this pitfall, thanks to a very rapid cooling rate that favors precipitation of the metastable Al6Fe phase. It should also be noted that in the Al-Fe system, due to the very low solubility of Fe in cfc aluminum, it is not possible to carry out post-elaboration homogenization annealing. The aim is therefore to optimize the processing parameters in order to obtain an alloy with the desired mechanical properties at the end of the process. This type of alloy is particularly interesting in the context of repairing parts by AM, since annealing the part is often either not possible or not desirable in order to preserve the mechanical properties of the other parts of the part.
The L-PBF process is an additive manufacturing process involving the successive fusion of layers of powdered material using a laser. This process is characterized by very short interactions between the raw material (powder) and the laser, resulting in very rapid cooling rates (of the order of 106 K/s). The result is a complex microstructure organized on several size scales, which we have characterized in the Al4wt%Fe alloy and which is presented in the figure : At the hundred-micron scale, we observe the melt baths generated by laser melting; Inside the baths, we observe a structure of often columnar grains; Inside the grains, we observe a cellular structure, whose walls are enriched in Fe; Finally, inside the cells, we sometimes observe precipitates, as well as dislocations formed during solidification due to the presence of strong thermal gradients.
We are particularly interested in the hardening mechanisms operating in this microstructure. In addition to a Hall-Pech effect and hardening caused by iron-rich precipitates and cells and by dislocations, hardening due to the presence of iron solute is likely to be significant. Indeed, it is well known that the very rapid cooling rate during the L-PBF process leads to solute trapping in the matrix [3]. Furthermore, it has been shown that the presence of a low concentration of Fe in an aluminum matrix has a significant contribution to yield strength, far greater than that of usual solutes such as copper [4]. For our alloys, it is therefore necessary to measure the Fe composition inside the cells in order to quantify its impact on alloy hardening.

Another point of clarification is the composition of the cell edges, which are also enriched in Fe: this is expected to deviate from the composition of the metastable Al6Fe phase reported in less rapid solidification processes.
A final point that is not stabilized for our samples, as for similar alloys in the literature [5], is the presence of oxygen. Oxygen is present due to the inevitable oxidation of the powders, and may also originate from the gas present in the preparation chamber. However, its presence and location in Al-Fe alloys produced by L-PBF needs to be investigated. Indeed, the lattice parameters of L-PBF Al-Fe alloys, measured by X-ray diffraction, deviate from the values expected for these alloys, and the explanation put forward in the literature is the presence of oxygen, which would deform the aluminum lattice.
To achieve these goals, detailed characterisation by transmission and scanning electron microscopy (Figure), X-ray diffraction and atomic probe tomography has been carried out on samples produced using different parameters. The impact of manufacturing parameters on microstructure is highlighted, particularly the fact that the size of the solidification cells largely controls the mechanical behaviour. The results also reveals the correlation between the Fe distribution in the microstructure and the material's local hardness. Using transmission electron microscopy enables analysis of the interactions between nanometric precipitates and dislocations resulting from the thermal process, providing a better understanding of the hardening mechanisms. Atomic probe tomography allows to estimate the remaining Fe in the Al matrix in the as-built alloy and local enrichment in iron that lead to precipitation or segregation in the solidification cells.
The results allow the contribution of the different microstructural elements to hardening to be assessed using a phenomenological model. This model, developed based on mechanical tests conducted on various metallurgical states, can be used to propose chemical optimization paths. The purpose is to define an alloy which, in the as-built state, will benefit from a mechanical behaviour combining work and precipitation hardening.

Keywords:

Al alloys, additive manufacturing, solidification

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Introducing a FAIR RDM infrastructure for electron microscopy and other materials science data

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IM-10 (3), Lecture Theater 5, august 30, 2024, 14:00 - 16:00

Digitization and an increase in complexity and price of electron microscopy hardware and characterization techniques, as well as the maturation of machine learning tools to extract patterns from large amounts of very diverse (annotated) data, promise to accelerate materials development by synergistically combining research data from many sources. While some labs have started uploading their (raw) research data to data repositories, this is only a first but insufficient step to realize the above-mentioned potential, as such repositories are typically either specific to a very particular technique or agnostic to much of the domain-specific content of the uploaded data [1,2]. In both cases the research data cannot be easily compared and integrated with experimental data from other sources or numerical predictions, and certainly not without significant human effort,. Therefore, working towards an interoperable knowledge representation for experiments and computer simulations [3-6] is the main motivation for implementing FAIR research data management. This highlights the need for tools for information extraction and semantic mapping. Fundamental to these tools' effectiveness is the creation of thorough and transparent documentation. This needs to be made more complete, shared openly, and should benefit from activities where representatives of the communities agree on defining and using standardized knowledge representations.
We will report on recent progress by the FAIRmat NFDI consortium [7] in extending NOMAD, the world's largest data base for ab-initio computational materials data, to also host experimental research data on the synthesis and characterization of materials in a machine-accessible manner, i.e. annotated with well-defined and interoperable metadata that establish links between related (experimental and computational) quantities [8-10]. We will report on our work on developing a comprehensive data schema for electron microscopy and related techniques, and the corresponding software tools for data converting, visualizing, and online-processing. We have integrated these tools as customizations into NOMAD Oasis to offer a locally-installable version of the NOMAD research data management system to complement its note keeping, file format parsing, cloud-based domain-specific data analyses, and information retrieval capabilities.

Keywords:

FAIR data managament, database

Reference:

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[8] M. Scheidgen et al., (2023), https://doi.org/10.21105/joss.05388
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[10] https://gitlab.mpcdf.mpg.de/nomad-lab/nomad-FAIR

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A silicon micro-heater chip for in-situ transmission electron microscopy

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Poster Group 1

For years, the monolithic integration of III-V compound semiconductors on silicon substrates has thrived, capitalizing on the superior photoelectric properties of III-V materials and the abundant availability of silicon. Among various integration methods, direct epitaxial growth is coveted for its optimal cost-to-performance. However, the heteroepitaxy process inevitably introduces defects due to lattice mismatch, crystal polarity, and different thermal properties. In the case of GaAs, which are extensively applied in solar cells, dislocations are prone to form due to the 4% misfit. Additionally, antiphase boundaries are anticipated concerning the polarized GaAs thin films grown on non-polarized Si surfaces.
While research has sought to understand growth mechanisms, reliance on ex-situ techniques limits real-time insights. Incorporating a silicon-based MEMS(micro electro-mechanical system) chip, we achieved epitaxial growth of GaAs crystal islands on silicon substrates within a source-equipped transmission electron microscope. The custom-made chip, compensated with FEM simulation and Raman spectroscopy, are further calibrated and optimized for different application purposes.
Through our direct visualization of the dynamic processes, encompassing the formation of crystal islands and facets, coalescence of islands, transitions from layer growth to island formation, and vice versa, have been thoroughly examined.

Keywords:

MEMS-chip, Heteroepitaxy, in-situ TEM

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Electron Microscopy of a Gas-Atomized NiSiV Powder

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Poster Group 2

Background incl. aims
Gas-atomization is a powder production technique involving rapid solidification of molten metal by high-pressure gas jets. Molten metal is poured through a nozzle into a chamber filled with inert gas where it is rapidly cooled and fragmented into fine, spherical particles by gas jets. The resulting particles are generally spherical with a high degree of uniformity in composition. There is a wide range of applications, from additive manufacturing and as feedstock to thermal spray coatings. In this study, a nickel silicide powder alloyed with vanadium has been investigated, with composition as stated in [1]. Nickel silicides are well-known for their resistance to high temperatures, corrosion, and oxidation, particularly in demanding environments such as in offshore applications. The powders are known to has high brittleness, but the incorporation of transition metals such as vanadium has been shown to improve the ductility. By using electron microscopy, both the surface and the internal structure can be investigated to gain a deeper understanding of the properties of the powder.
Methods
First, the particles surface and polished cross-sections were studied by scanning electron microscopy (SEM). A thin cross-section of a particle was prepared using a focused ion beam (FIB) and then investigated using transmission electron microscopy (TEM) imaging and energy-dispersive X-ray spectrometry (EDS) mapping. Scanning precession electron diffraction (SPED) maps were acquired to obtain high-resolution information on crystal phases and orientation relationships. The data was processed and investigated using the open-source Python libraries Hyperspy [2] and pyxem [3].
Results
SEM imaging reveals spherical particles with diameters from a few hundred microns down to a couple hundred nanometres. The particles are mainly uniform in shape but show signs of irregularities such as helmets and satellites. The surfaces are not smooth, but show signs of parallel stripes, revealing a lamellar dual-phase structure. The same stripes are visible in SEM images of the cross-sections. When looking at TEM bright field images, lamellae with a thickness of around 100 nm can be seen. EDS maps confirm that the lamellae are two distinct phases, alternating between vanadium-rich and silicon-rich, while the nickel is evenly distributed. Vanadium is very soluble in nickel at low quantities, and SPED was used to identify the vanadium-rich areas as the nickel fcc phase, in which vanadium has a high solubility. Equally, the diffraction patterns from the silicon-rich areas suggest a nickel silicide phase. While the crystal structure of nickel is very uniform along the stripes, the nickel silicide phase exhibits signs of misorientations and planar defects.
Conclusion
Gas-atomized nickel silicide powders alloyed with vanadium were studied using electron microscopy. SEM images reveal defects such as helmets and satellites, and structures both on the surface and on the cross-section. TEM methods such as EDS and SPED were used to study this further, and we were able to identify stripes with thicknesses of around 100 nm of alternating nickel solution with vanadium, and nickel silicide. Nickel is shown to have a slightly increased lattice parameter with increasing amount of vanadium [4], which might make it a better lattice match with the nickel silicide

phas and could possibly explain the improved ductility of the material when alloyed with vanadium. Further work includes obtaining a deeper understanding of the variations in the nickel silicide phase along the stripes and what effect this has on the properties, as well as the orientation relationships between the two phases.

Keywords:

TEM, SEM, SPED, Atomization, NiSiV

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1155

Structural investigation of the 40S hnRNP particles

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Poster Group 2

Heterogeneous nuclear ribonucleoproteins (hnRNPs) constitute a prominent family of RNA-binding proteins abundant within the nucleus. They play pivotal roles in various aspects of nucleic acid metabolism, encompassing mRNA stabilization, alternative splicing, transcriptional and translational regulation, RNA export, and degradation [1]. Early investigations in the 1970s revealed that upon lysing nuclei without RNase inhibitors, a significant portion of pre-mRNA formed a distinct protein-RNA complex, sedimenting at 40S [2]. Notably, the core constituents of this complex were identified as hnRNP C1/C2, hnRNP A1/B2, and hnRNP A2/B1. This observation brought up the intriguing proposition that the 40S hnRNP particle might serve as an analogue to the DNA nucleosome [3]. Our objective is to describe the biogenesis of the 40S particle, provide a structural description of the 40S particle using cryo-electron microscopy (cryo-EM) and visualize it in its native context. We have generated TRex-293 cell lines expressing FLAG-tagged hnRNPC1/C2 proteins, isolated the 40S particles and analyzed them by negative staining EM. Furthermore, to investigate the ribonucleosome within intact cells, we have immunostained the key ribonucleosome components and generated thin lamellae using cryo-focused ion beam scanning electron microscopy (cryo-FIB/SEM). Using cryo-electron tomography we have acquired data from FIB-milled lamellae.

Keywords:

40S particles, immunostaining, cryo-FIB, cryo-ET

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Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution

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Poster Group 1

The field of cryogenic electron microscopy (cryo-EM) has seen remarkable advancements, enabling researchers to visualize biomolecular structures with ångström-level resolution. While conventional transmission electron microscopy (CTEM) combined with single-particle analysis (SPA) has played a crucial role in these developments, it faces challenges with very small proteins and large biomolecules. Specifically, small proteins often exhibit insufficient contrast, and large biomolecules can produce opaque images due to multiple scattering.
Electron ptychography, a technique derived from 4D scanning transmission electron microscopy (4D-STEM), presents promising solutions to these issues. It enhances contrast through phase retrieval, aiding in imaging small proteins, and manages multiple scattering effects, which is vital for examining thicker specimens. Electron ptychography has already proven to be a powerful method for room temperature material science samples, achieving unprecedented resolutions[1]. However, the application of STEM-related techniques to biological specimens is relatively rare[2,3], and ptychographic imaging at low-dose conditions (less than 50 e/Å²) remains largely unexplored.
In our study, we applied this technique to frozen hydrated single protein samples and achieved sub-nanometer resolution with a relatively small number of particles, yielding micrographs of enhanced quality (signal-to-noise ratio, SNR). The scattering potential of single protein particles was iteratively reconstructed using the open-source software, py4DSTEM[4]. We present structures of apoferritin at 5.8 Å using 11,552 particles, a phi92 sheath structure at 8.4 Å using 1,600 particles, and a tobacco mosaic virus at 6.4 Å using 2,120 particles.
The application of ptychography to biological specimens, particularly when integrated within the SPA framework for smaller frozen hydrated protein particles or combined with tomography tilt series for thicker samples (e.g., bacteria, tissue), holds great promise.

Keywords:

4D-STEM, ptychography, cryo-EM, low-dose

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Xenon PFIB-milling and cryo-electron tomography of bacterial anti-phage effectors

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Poster Group 2

Introduction
Bacterial abortive infection responses to bacteriophage infection play critical roles in bacteria population survival by limiting viral propagation, through activation of programmed cell death or metabolic arrest of the infected bacteria. Cyclic oligonucleotides produced by the bacteria in response to phage infection are responsible for activating certain abortive infection effector proteins. One such example is TIR-SAVED, which has been shown to degrade NAD+ when activated by cyclic tri-adenylate. Previous analysis by cryo-electron microscopy (cryo-EM) demonstrated that TIR-SAVED oligomerises into extended helical filaments in vitro when activated by cyclic oligonucleotide binding [1].
Here, we develop a high-throughput approach based on the Waffle method [2] to imaging filaments in situ in high numbers of high-pressure frozen bacteria using xenon plasma ion sources for lamella preparation. We use cryo-electron tomography (cryo-ET) and subtomogram averaging (STA) to characterise the structure and higher-order organisation of these filaments in a cellular context, as well as the morphological changes to the bacterial cells upon activation of the TIR-SAVED system. This research will provide insights into ancestral mechanisms of antiviral defence, and further our understanding of the innate immune systems of prokaryotes.

Materials and Methods
E. coli C43 (DE3) transformed with plasmids as for the plasmid immunity assay specified by Hogrel et al. [1] were incubated overnight at 37 °C in LB before the addition of D-lactose and L-arabinose to induce activation of the TIR-SAVED system.
Bacteria were vitrified by high pressure freezing and lamellae milled using xenon and argon plasma with an Arctis dual-beam FIB/SEM microscope following an adapted version of the Waffle Method [2].
Dose-symmetric tilt series were collected with a Titan Krios equipped with a Falcon 4i camera and Selectris energy filter. Warp was used for gain correction, CTF estimation, motion correction and tilt series stack generation. AreTomo was used for tomogram reconstruction. Particle picking for subtomogram averaging used a combination of IMOD, EMAN2, STOPGAP and custom scripts, and RELION was used for subtomogram averaging.

Results
Full induction of the TIR-SAVED system resulted in cell death of the bacteria. When partially induced to capture an intermediate state before total cell death, electron-dense regions were observed at the poles of cells. These regions of uncharacterised composition displayed increased sensitivity to radiation damage caused by electron beam exposure during tilt-series acquisition. Bacteria cells

containing a TIR-SAVED mutation (E84Q) that inhibits its NAD+-degrading activity did not typically contain these inclusions.
Filaments were observed in reconstructed tomograms of bacteria containing TIR-SAVED-E84Q in both single filaments and as tight bundles of filaments. Subtomogram averaging of the bundles has revealed the in-situ structure of a TIR-SAVED-E84Q helical filament at a resolution of 12 Å. This analysis shows large variations in helical parameters such as pitch along the length of each filament, indicating significant flexibility in the oligomeric structure. Mapping these averaged structures back into the cellular volumes reveals that the bundled filaments are arranged into hexameric assemblies.

Conclusion
Here, we present the first in-situ investigation of bacterial abortive infection systems by cryo-electron tomography. Our study gives insights into the structure and higher-order organisation of TIR-SAVED in cells, in addition to the morphological effects of the activation of TIR-SAVED on the whole bacteria.

Keywords:

Cryo-ET, FIB-milling, STA, bacteriophage infection

Reference:

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Time ecology method

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Poster Group 1

"[One] cannot solve a problem with the same level of thinking that created it" said Professor Albert Einstein. A new modeling [1] developed as 3 layers 4:3 dimensions frames may contribute to understanding how macromolecular complexes ultrastructure can influence cell physiology, and the reverse. As those 3 frames, 4D:OLI:LOI, connect to the concept of time and the dynamic perception of the flows in time ecology [2].
Are labeled [3] microorganisms oscillatory responses healthy or would those entrain inflammatory loop cascades? Is it fundamental, to gain an understanding of live cells, those forming living systems, to account for time and sound?
Background incl. aims.
The LOI (Location-Ownership-Internalization) dynamic conceptual positioning, enveloped in OLI (in existence since 1977), a static envelop, and the 4 structuring environmental dimensions, the chemotaxis motives of the environmentally active actor, as the: market, resources, efficiency (in existence since 1974) and strategic asset: associated with the notion of the time internalization, are various theoretical tools to conceptualize about time, timing, and transitions inside living systems. Internalization is a synonym for assimilation, for instance to quantify the assimilation of radioactive labeled or stable-isotope-labeled substrate (in single cell) to study the cell ecophysiology [3]. Combining LOI:OLI:4D frames together form a wider system view, as if several foreign languages would be simultaneously used to characterize one unique situation. This approach could be more specifically used to distinguish between macroscopic and microscopic entropy, to develop inferential statistics (see Results).
One purpose would be to conceptualize the formation of sound matter, based on a theoretical understanding of past literatures [4] to close a gap between cognitive psychology and the perception of sound as music.
Methods.
Imaging of microorganisms can be performed using the expansion microscopy to visualize intra- and extra-cellular components as well as the cellular ultrastructural context (the environment). Based on the tridimensional thinking described and the understanding of HOH molecules configurations dynamics [1] an understanding of time ownership could be formed from a multidimensional spectral sensing of 3 dimensions: hearing (sound goes faster than neural transmission speed. [2]), color vision and olfaction (most odorants do not contain nitrogen). The research could also focus on linear thinking, or one single ribbon, with the focus on the role of neuropeptides (one CKK, -4 or -8S), and NO; neuropeptides as signaling molecules, e.g. bombesin like peptides, can modify nerve impulse. CCK may have a universally conserved role in cognition depending on how significant lipids are in individuals' diet. I could defend the relevance of odd-chain fatty acids for the stability of thinking (gluconeogenesis) about complex stressful situations.
Time to be owned must be internalized. An envelope is thus necessary as a rigid frame to enable the dynamics to become dense enough to be observed.
The logic for dynamics to emerge is: first a location (minimal viable cavitation) will be required for resources to be gathered, which may become internalized or future resources would first be owned, before to become further internalized.

Results.
Accounting for time in research would imply to reveal time soft matter, beyond time measurement, dynamics. Or unpacking signals from noise using gentle ultraweak-photon emission intensities as non-invasive [3] spectroscopic tool for diagnosis of internal states.
Going back to the Viennese physicist Ludvig Boltzmann's aim to discover one objective interpretation of the law of increase of entropy in terms of microscopic mechanical properties of the system, the LOI [1] model of time internalization could be applied to understanding thermodynamics: hypotheses could be formulated regarding variables causing structural or transactional flows failure. The flow is driven by the hopping from electrons: a quantum-mechanical tunneling. Hopping is a long-range transfer across redox chains (oxidized radicals) using multi-step tunnelling. The process is required in several natural enzymes, it involves vibrational mode from hydrogen tunneling, producing volatile organic compounds.
Conclusions.
"Thermodynamic quantities should be assigned, not to single systems, but to ensembles of systems having a given probability to occupy this or that point in […] space." [5]. The LOI [1] dynamics based on the dipole moment of HOH mirror important biological models. Those would be forming, or contributing to form, the 'awareness' or 'consciousness' factor of human cognition, or a 'Zeitgeist'. The "chance factors" forms the major influence from the surrounding. It is important to account for the constant novelty provided by one surrounding. These can be observed through taking a standard view (proposed here). A system view is a simultaneously joint exploration of dynamics and the intersections: the fluids inside their enveloping structures. A system view can be systematically developed around 3 dimensions, as the dynamic framework: LOI, which is also embedded inside a static 3 dimensional frame: OLI.

Keywords:

Time ecology. Time internalization. Ecophysiology.

Reference:

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1159

Real-Time Studies of Resistive Switching Mechanisms

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Poster Group 2

Background incl. aims
Demands for increased information density in nanoscale devices used for applications in artificial intelligence and the internet of things (IoT) are resulting in the need for improved data storage and processing technologies. As current memory technologies are approaching their fundamental limits, so the development of architectures for novel memory devices, such as in-memory computing and neuro-inspired computing devices, is becoming more important. Memristive or resistive random-access memor (ReRAM) devices promise to provide non-volatile, fast, low-power and cost-effective technologies, and are leading candidates to replace flash memory in a broad range of applications, including high-density memory, low-power IoT devices, neuromorphic computing, sensors and security applications [1-5]. Phase change memory (PCM) devices based on chalcogenides and valence change memory (VCM) devices based on oxides are promising candidate materials for non-volatile ReRAM devices because they offer possibilities to encode information reversibly and rapidly.
The switching mechanisms of such devices are typically based on local changes in resistivity between a high resistance state (HRS) and a low resistance state (LRS). Local high spatial resolution imaging and spectroscopy, in combination with real-time electrical measurements, are required to understand fundamental aspects of resistive switching in both PCM and VCM devices, and in turn to achieve improvements in scalability, reliability and speed. Transmission electron microscopy (TEM) is a powerful technique that can be used to provide information about local variations in crystal structure, composition, phase, and electronic structure of materials. Therefore, studies of the details of where, when and how resistive switching processes take place in different material systems using TEM are of great interest to unravel resistive switching mechanisms.

Methods
In order to realize real-time electrical biasing in the TEM, either home-made chips with electrical contacts were fabricated using micro-electromechanical systems (MEMS) technologies, or a Nanofactory probing specimen holder was used to provide a moveable electrical contact to a TEM specimen. Reproducible switching of different materials systems was recorded using different TEM techniques, such as off-axis electron holography, four-dimensional scanning TEM (4D-STEM), energy-dispersive X-ray spectroscopy, and electron energy-loss spectroscopy (EELS). Efficient and reliable methods to extract meaningful features from complex and large datasets were developed using machine learning technologies, for example for differentiating between crystalline and amorphous phases, and between different valence states in oxides.

Results
For PCMs, scanning nanobeam electron diffraction in 4D-STEM was used to study $Ge_2Sb_2Te_5$ (GST) thin films to investigate local variations in their crystalline structure. Data processing methods were developed to differentiate between crystalline and amorphous phases, in order to identify local

distributions of crystalline phases in amorphous GST thin films. For VCMs, resistive switching mechanisms in TiO_2-based ReRAM devices were investigated in real time using correlative transmission X-ray microscopy in the synchrotron and EELS in the TEM. The formation of conductive filaments in oxides was confirmed by extracting features from noisy spectra using non-negative matrix factorization.

Conclusions
Advanced TEM techniques combined with real-time electrical biasing and machine learning allow resistive switching mechanisms to be studied in different material systems. Local changes in chemical composition, microstructure and electronic properties in PCM and VCM devices during resistive switching can be investigated successfully. These approaches are expected to lay the foundation for the development of advanced next-generation memory devices.

Keywords:

TEM, resistive switching, real-time electrical-biasing

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Visualization of the in situ molecular architecture of tau pathology in the murine brain

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Poster Group 2

Background incl. aims
The accumulation of pathological tau protein aggregates is a hallmark of numerous neurodegenerative diseases, including Alzheimer's disease. The accumulation of misfolded tau in neurons is toxic, it disrupts cellular physiology, leading to neuronal death and the propagation of tau misfolding throughout the brain. Effects of tau pathology include disrupted axonal transport, mitochondrial and lysosomal dysfunction, and synapse degeneration. Despite advancements in understanding tau pathology, the relationships between initial tau misfolding, fibril formation, pathology propagation across connected neurons, and subsequent cytotoxicity on the level of individual neurons remain unclear. We aim to visualize the pathological changes in molecular architecture directly in the vitrified brain tissue of the murine model for tauopathy.

Methods
To visualize the native ultrastructure we use vitrified fresh brain without staining or fixation. We combine cryo-plasma-focused ion beam milling (FIB) and bio-contrast scanning electron microscopy (SEM) imaging with cryo-electron tomography (cryo-ET) on lamella. The cryo-plasma-FIB/SEM setup of the Helios Hydra V microscope allows imaging of non-stained vitrified hydrated biological samples with high biological contrast in nanometer resolution permitting volume imaging covering a much wider area than typical lamella used in cryo-ET.

Results
In this poster, we present our in situ visualization workflow and showcase preliminary bio-contrast cryo-plasma-FIB/SEM images and tomographs of murine brain tissue affected by tauopathy.

Conclusions
We showed that the novel bio-contrast cryo-plasma-FIB/SEM imaging workflow can be used for ultrastructural characterization of pathological tissues without chemical fixation and that the combination with lamella lift-out and in situ cryo-ET provides an excellent tool for uncovering the details of cellular mechanisms of neurodegeneration.

Acknowledgment
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Keywords:

in-situ cryo-ET, cryo-plasma-FIB/SEM, bio-contrast, tauopathy

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Novel scan coil design for high spatiotemporal-resolution imaging in the scanning transmission electron microscope

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Poster Group 1

The Scanning Transmission Electron Microscope (STEM) is a highly versatile tool that is used to study a wide range of materials, from semiconductors to biological cells. It operates by scanning a beam across a specimen and then recording the beam that has been transmitted through the specimen at these points. STEM is a powerful technique for probing specimens down to the atomic scale, but the slow imaging speeds can result in excessive beam exposure, and therefore damage, and the inability to capture dynamic in-situ events. Increasing the scanning speed is therefore an attractive proposition for better control of the dose-rate on the sample as well as improved temporal resolution

Currently, the main limitations to the scanning time in a STEM are imposed by the response time of the scanning coils, determined largely by their inductance. For this reason conventional imaging requires a flyback wait time between scan lines to reduce inductive hysteresis. Flyback time may be eliminated using novel scan paths, though at fast scan speeds the inductive effects are still problematic. Similarly, techniques such as compressed sensing can increase frame-rates but retains issues regarding the hysteresis of the scanning coils. Previous work by Ishikawa et al. shows scan coils with an inductance ~240 times less than the conventional scan coils, though with a limited scan area.

In this work we present a scan coil system to greatly improve the response time of the scanning system. Alongside the existing, main scan coils, we add additional deflectors with lower inductance to work simultaneously with the main scan coils. By using the second coils to counteract the effects of hysteresis in the main scanning coils, imaging at lower dwell and flyback times without image distortion or compromising scan area. The additional coils themselves are not perfect and further sets of coils could be used to compensate remaining hysteresis. Equally, the use of electrostatics due to the rapid response time and lack of hysteresis could be deployed in tandem. With our design, we hope to make fast scanning in STEM practicable and enable high-resolution imaging to improve dose-control and in-situ measurements.

Keywords:

Coils, Hysteresis, Induction, Flyback

Reference:

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In-situ cryo-biasing heating TEM sample holder with full-range temperature control from -170°C up to >1000°C

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Poster Group 1

Background

Traditionally in-situ TEM biasing, heating and cooling have been split capabilities, requiring different TEM holders. In-situ cryogenic cooling and biasing of specimens during scanning/transmission electron microscopy (S/TEM) has previously enabled the in-situ characterization of various quantum interfaces and chemical phase interactions in battery materials and other strongly correlated systems [1]. Quantum material responses must be studied under cryogenic conditions because many of the relevant properties in these materials only manifest below certain temperatures, while battery material interfaces are sensitive to electron beam damage when not cooled.

Combined in-situ sample heating with biasing has only become available in recent years with the use of the chip-based experimental platform, which has enabled capture of high-temperature microstructural and microchemical evolution alongside measurement of high-temperature electrical response in materials systems. Now, with the inclusion of on-chip biasing and thin film heater chip designs, we have developed a novel in-situ cryo-biasing heating TEM holder which enables simultaneous electrical stimulus and imaging of a sample across the full temperature range from cryogenic temperatures to over 1000 °C, while matching room temperature (RT) microscope spec for resolution and drift. The basic functionalities of heating, cooling, biasing, and imaging are demonstrated alongside discussion of different applications.

Methods

The TEM sample holder has an attached LN2 dewar that cools the sample down from RT to the lowest operating temperature (< -170°C) and can be controlled via software with closed-loop feedback at any temperature up to RT. Below RT where on-chip temperature measurements become increasingly inaccurate, precise temperature control is maintained using a conventional resistance heater and miniature thermocouple at the sample in the TEM holder tip. Nine electrical contacts to the user's sample or device allow on-chip biasing. Biasing experiments can now also be extended above RT to >1000°C using thin film sample heating with reliable on-chip temperature sensing. Since the heated area is small, the response time to temperature changes is fast and sample drift is minimized across the entire temperature range, resulting in image stability that matches the RT performance. Here, we present an example of a battery process after cooling a single nanowire system from RT down to liquid nitrogen temperature ~ -170°C [2].

Results

In Fig 1a, electrical biasing was performed on a nanowire sample bridging the electrodes on a biasing chip using this holder at RT down to near-liquid nitrogen temperature to protect damage in these beam-sensitive materials [2]. With constant current applied at cold temperatures, the voltage drops across the nanowire as a reaction proceeds with the plating of a dendrite layer on the surface, as shown in Fig 1d. In Fig 1b, atomic lattices are shown as a demonstration of resolution. Temperature control of the cooled and heated ranges is also shown in Fig 1c and 1e, respectively. The figures exhibit the core functionalities of the tool: stable and reliable sample biasing, heating, and cooling under atomic resolution TEM.

Conclusions

The In-situ cryo-biasing heating TEM sample holder presented here enables, for the first time, simultaneous electrical stimulus and high-resolution imaging of a sample across the full temperature range, from cryogenic up to high temperatures. This instrument will accelerate the development of the next generation of electronic, quantum, and energy storage materials devices. Combination of the two temperature regimes expands the range of temperatures available for dynamic temperature experiments, wherein multiple processing, imaging, or synthesis steps must be performed at various temperatures. High-resolution in-situ observation of operating (solid-state) battery interfaces in their full operational range of -40°C to 80°C is traditionally difficult to achieve due to air sensitivity, electron beam damage at RT, and lack of temperature control in TEM biasing platforms [3-5]. With this new tool, batteries may now be electrochemically cycled in situ at their entire operating range and then lowered to cryogenic temperature for imaging to combat electron beam effects, without the need for sample transfer between tools. With increasing demand for batteries that function at high temperatures, quantum material responses, synthesis, and processing, and material phase information from cryogenic to high temperature, the cryo-biasing TEM holder will provide the expanded versatility required of temperature-controlled in-situ electrical biasing systems.

Keywords:

cryogenic, heating, transmission electron microscopy

Reference:

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1163

Spatiotemporal observation of ultrafast magnetization dynamics with 4D Lorentz Transmission Electron Microscope

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Poster Group 2

Background incl. aims
Since the discovery of the sub-picosecond-timescale ultrafast demagnetization induced by femtosecond laser pulse by Beaurepaire et al. in 1996, utilizing ultrafast light to manipulate the magnetization has opened up a new field compared with the conventional ways to control the spins by magnetic fields, charge, or spin currents. Known as 'femtomagnetism', ultrafast optical control of magnetization at femtosecond or sub-picosecond timescales is the currently fastest route known to demagnetize or switch a magnetic material, which conforms to the ever-increasing demand for energy-efficient high-speed magnetic storage devices or random-access memories. To promote further understanding of light-spin interaction and potential technological applications, however, exploiting experimental tools to characterize and understand the light-spin dynamics at the sub-picosecond and nanometer scale plays a decisive role.
Methods
We combine Lorentz Transmission Electron Microscope with transient optical gratings to control laser-induced gradients of the in-plane magnetization. The light absorption distribution is characterized by Photo-induced Near-Field Electron Microscopy (PINEM) technique.
Results
We have spatiotemporally analyzed patterned ultrafast demagnetization dynamics in Ni80Fe20 near the sample discontinuity excited by a transient optical grating. Our findings demonstrate that the propagating surface plasmon polariton (SPP) modulates the ultrafast demagnetization process in space, time, and amplitude. Furthermore, we demonstrate the sub-picosecond all-optical switching dynamics in GdFeCo at a local scale by utilizing the Lorentz contrast from the Bloch domain wall induced by local switching in constructive interference areas. Last but not least, we observe the laser-induced coherent precession in GdFeCo with both FMR mode and exchange mode.
Conclusion
Our findings demonstrate 4D Lorentz Transmission Electron Microscope as an effective tool for investigating laser-induced magnetization dynamics with sub-picosecond and nanometer spatiotemporal resolution.

Keywords:

Ultrafast demagnetization, all optical switching,

Reference:

1 Cao, G., Jiang, S., Akerman, J. & Weissenrieder, J. Femtosecond laser driven precessing magnetic gratings. Nanoscale 13, 3746-3756 (2021).
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3 Fan, Y., Cao, G., Jiang, S., Akerman, J. & Weissenrieder, Spatiotemporal observation of all optical switching in GdFeCo with 4D Lorentz Transmission Electron Microscope, to be submitted.

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Quantum Measurements using Interferometric STEM-EELS

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IM-03 (3), Plenary, august 29, 2024, 10:30 - 12:30

Background incl. aims
Nanoscale amplitude beamsplitters for electrons enable flexible electron interferometry in STEM instruments. Nanofabricated materials phase gratings provide a way to coherently diffract electron wavefunctions into different paths, and can easily be placed in probe-forming apertures of unmodified TEM instruments. These tools provide a way to manipulate both the phase, amplitude, and momentum of electrons [1]. This lends itself to the implementation of new interferometric methods in electron microscopy [2-4], with the ultimate goal of performing quantum measurements in the TEM.

Methods
We used a pair of nanoscale phase gratings as diffractive amplitude beamsplitters to provide a Mach-Zehnder electron interferometer inside an unmodified TEM [2]. One phase grating beamsplitter coherently divides the electrons into separated probes before the specimen, and the second beamsplitter recombines the paths after the specimen, creating a set of discrete interfering outputs. This enables several new imaging and measurement modalities. For example, phase contrast imaging can be provided if one of the probes transmits through a specimen region while the other passes through vacuum, acquiring a relative phase shift that can be recorded by monitoring a discrete electron beam output.
The setup also enables quantum-inspired measurements, such as interfering electron paths that have lost energy to the specimen. If the interferometer is tuned to provide destructive interference at the detector, blocking one of the probe paths with an object eliminates the destructive interference, allowing a non-zero probability current to be incident on the detector. This is a quantum interrogation method sometimes called “interaction-free” measurement, because a detection event in a dark detector indicates the presence of an absorbing sample; the electrons that did not scatter from the sample indicate its presence.

Results
We demonstrated using the 2-grating electron interferometer for STEM phase imaging of nanostructures [2], even using only inelastically scattered electrons [3]. We used this to determine that fast electrons passing on either side of a metallic nanoparticle that excite a plasmon acquire a relative π phase difference, corresponding to the symmetry of an optical dipole excitation. We also used the setup to demonstrate a quantum “interaction-free” measurement of the presence of an opaque object [4]. In this case, single electron events recorded at the detector indicate the presence of an object without scattering from or transmitting through it.

Conclusion
We used nanoscale phase gratings to implement electron interferometry in STEM without modifying the instrument other than an aperture change. Potential advantages of the techniques this enables relative to other methods will be discussed.

Keywords:

Electron interferometry, electron holography

Reference:

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[2] Johnson, C. W., Turner, A. E. & McMorran, B. J. Scanning two-grating free electron Mach-Zehnder interferometer. Phys. Rev. Research 3, 043009 (2021).
[3] Johnson, C. W., Turner, A. E., García de Abajo, F. J. & McMorran, B. J. Inelastic Mach-Zehnder Interferometry with Free Electrons. Phys. Rev. Lett. 128, 147401 (2022).
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[5] The author gratefully acknowledges collaboration with Pieter Kruit (Delf University) and Javier García de Abajo (ICFO). This material is based upon work supported by the National Science Foundation under Grant No. 2012191.

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Exploring polar ordering in lead-free K0.5Na0.5NbO3 ferroelectrics using in situ biasing and 4D STEM techniques

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Poster Group 1

The effectiveness of ferroelectric materials is fundamentally dependent on their ability to switch polarization, a process driven by complex dynamics that include the movement of ferroelectric domain walls (DWs), domain growth, and the nucleation of new domains. Traditionally, the behaviour of ferroelectric domains and DWs has been examined using indirect methods, such as nonlinear ferroelectric / piezoelectric measurements or in situ x-ray diffraction techniques. While these methods have significantly enhanced our understanding of the relationship between domain structures and functional properties, they only offer an averaged, collective response that may mask the details of individual events. In contrast, in situ transmission electron microscopy (TEM) provides a direct observation method for ferroelectric switching and domain dynamics. This approach reveals phenomena that are often concealed in macroscopic studies, offering a clearer, more detailed view of the microscale processes that govern the macroscopic properties of ferroelectric materials.
One key aspect of ferroelectric research involves determining the direction and magnitude of polar ordering, typically achieved through precise measurements of atomic displacements from their equilibrium positions in centrosymmetric structures. The adoption of advanced atomic-scale Scanning Transmission Electron Microscopy (STEM) technologies, especially those equipped with 4D STEM pixelated detectors, combined with the insights from STEM image simulations and first-principles calculations, has not only enabled the direct determination of polar directions but has also expanded the research to include analysing various defect types such as oxygen vacancies, strain fields, and charge density distributions around defects. Moreover, the use of in-situ biasing/heating holders for structural examinations under applied external stimuli has enriched our understanding of the dynamic nature of ferroelectrics.
This presentation will focus on a series of structural studies exploring the dynamic properties of potassium sodium niobate. The STEM analyses were conducted using Jeol ARM 200CF and Thermo Fisher Scientific Spectra 300 Cs-corrected microscopes, both equipped with advanced Merlin and EMPAD pixelated detectors, respectively. Our studies of potassium sodium niobate materials focus on directly observing domain growth, coalescence, and interactions among different types of domain walls within the microscope's environment under applied voltage. Through these investigations, we aim to elucidate how the type, quantity, and dynamics of structural defects influence local material properties, offering opportunities for tailored manipulation and optimization.
In the study, we explore the voltage-driven dynamics of mobile, needle-like domains and DWs within a $(K,Na)NbO_3$ single crystal (KNNsc) utilizing in situ Transmission Electron Microscopy (TEM) in a miniaturized capacitor setup [1]. Our findings suggest that the immobile DWs act as random bound pinning centres, capable of pinning larger regions while the edges of the sample facilitate the nucleation of new domains. The process of domain growth and coalescence is not consistently continuous; specific voltages can disrupt it, leading to fine domain splitting and the creation of nanoscale domains. Discontinuities in the functional response also occur when two orthogonal,

needle-like domains intersect, resulting in soft-pinning events. These insights deepen our understanding of ferroelectric domain behaviour and may extend to other perovskite-based ferroelectric materials that exhibit similar domain morphologies or coexistence of dynamic and stationary DWs.
Slovenian research and innovation agency (ARIS) is acknowledge for financial support through projects J2-3041, J7-4637, P2-0105 and P2-0421.

Keywords:

KNN, ferroelectrics, 4DSTEM

Reference:

[1]. O. Condurache et al., Applied physics letters, 2023, 20, 202902-1-202902-7

1166

The use of EELS for the characterization of diamond interfaces: the ohmic contact

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Poster Group 2

The use of EELS for the characterization of diamond interfaces: the ohmic contact
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Background
Electron energy-loss spectrometry (EELS) is a technique that provides information about the energy distribution of electrons that have come through the specimen. Moreover, EELS in scanning transmission electron microscopy (STEM-EELS) has information about atomic bonding configurations, local electronic information, oxidation, and spin states through the core-loss spectrum at the interface, indicating configurational changes to a metal oxide's crystal field with a resolution of nanometers. In this regard, STEM-EELS can be used to measure the energy bandgap (Eg) of a material. The energy bandgap refers to the difference between the highest energy-filled electron state (valence band) and the lowest energy-empty electron state (conduction band), which can determine the electrical properties of the material. It represents the minimum energy required to move an electron from the valence band to the conduction band, allowing it to conduct electricity. Among many materials existing, diamond is an example of a wide-bandgap one, since it is about 5.5 eV. This large bandgap makes it an interesting material for applications in electronics, such as high-power and high-frequency devices, as well as for optoelectronic applications, including ultraviolet (UV) light-emitting diodes (LEDs) and detectors. However, measuring the Eg by STEM-EELS is not a simple process because of the appearance of different effects on the energy loss spectrum that can lead to an ambiguous measurement. For that, low acceleration voltages of 60 keV are employed to avoid the Cherenkov effect. This event is produced by relativistic electrons and different plasmon peaks, which are collective excitations of charge carriers of the diamond. Another artifact to consider in an EELS spectrum is the zero-loss peak (ZPL). The ZPL depends on the local thickness of the specimen and needs to have a robust subtraction to interpret the low-loss region and to determine the local band gap.
For that, the aim of this research is the determination of bandgap for WC and Au-based ohmic contacts on diamond using the STEM-EELS technique.
Methods
For this study, two samples were employed:

- Sample #1: Chemical vapor deposition (CVD)-growth boron-doped ([B] ~ 10^{19} atoms·cm-3) diamond layer was contacted to a W layer (40 nm thick) deposited by electron beam-induced deposition (EBID). The sample was subject to a thermal treatment with the temperature ranging from 450 to 900 K for 30 min each. This process is expected to induce tungsten carbide (WC) formation at the interface.
- Sample #2: CVD-growth boron-doped ([B] ~ 10^{20} atoms·cm-3) diamond layer was contacted to an Au layer (50 nm thick) deposited by sputtering. In this case, the thermal treatment was not

employed because the ohmic behavior was obtained with Au as-deposited, and there is not a carbide formation with Au and diamond.
Regarding the electron microscopy analysis, a FEI-TITAN 3 THEMIS electron microscope at 300 kV was used for chemical and structural analysis. The structural analysis was performed using the High-Angle Annular Dark-Field STEM (HAADF-STEM) image, High-Resolution STEM (HRSTEM), and Energy-Dispersive X-ray Spectroscopy (EDS) techniques. Electron Energy Loss Spectroscopy (EELS) data of the metal-diamond interface were acquired at 60 keV, using a monochromator.
Results
The EELS spectrum recorded on the < 80 nm lamella of WC and Au samples is generally composed of a variety of phenomena such as the zero loss peak (ZLP) (0-2 eV), Cherenkov effect (4-6 eV), or plasmonic peaks, which can mask the assessment of the electronic transitions and bandgap determination. At 60 keV, the bandgap nature of WC and Au is deduced to be indirect by fitting the energy loss spectrum to a (Eloss-Egap)3/2 function. The measured bandgap of WC and Au is 3.21 and 3.10 eV, respectively. These values are higher than the bandgap of tungsten oxide and gold nanoparticles, which are attributed to the bonding with carbon, in the case of tungsten-diamond, and the good adhesion between gold-diamond. Moreover, the ZLP contribution was deconvoluted using a logarithmic tail fit to the range 2 eV to 5 eV of the spectrum, leaving a plateau in the 5 to 8 eV region, as no contribution was expected at these energies. No Cherenkov-effect-related contribution was observed in the spectrum due to the low beam energy used (60 keV).
Conclusion
It was reported a method to measure the bandgap energy at the nanometer scale using the STEM-EELS technique. For that, W and Au contacts on the diamond were annealed at 800 K to obtain an ohmic behavior and thermal stabilization. An indirect bandgap of 3.10 eV is deduced for WC which is in good correspondence with literature. The bandgap of the Au-C contact is reported to be constant among the layers on a nanometric level with a value of 3.21 eV.

Keywords:

diamond, EELS, interface, nanostructure

Reference:

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Large-angle Lorentz 4D-STEM for Simultaneous Magnetic and Atomic Structure Mapping

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Poster Group 1

Achieving a correlative measurement of both magnetic and atomic structures at the nanoscale is imperative to understand the fundamental magnetism of matters and for fostering the development of new magnetic nanomaterials. Conventional microscopy methods fall short in providing the two information simultaneously. Here, we develop a new approach, large-angle Lorentz 4-dimensional scanning transmission electron microscopy (LA-Ltz-4D-STEM), to simultaneously map the magnetic field and atomic structure at the nanoscale (figure 1).
To achieve field-free conditions for studying magnetic materials, both the upper and lower pole pieces of the objective lens must be turned off. This limits the probe diameter and thus the spatial resolution of the scanned image. At the same time, as the diffraction lens is located far away from the sample (more than 10 cm for uncorrected systems), the liner tube of TEM acts as a physical aperture and blocks diffracted beams at high angles, thus hindering the recording of atomic-level structural information (e.g., the recording angle is limited to < 2.2 mrad, corresponding to observable lattice spacings larger than ~ 0.9 nm in a TEM). Only the direct beam and surroundings can be used for the imaging of magnetic domains. In order to overcome this issue and to get access to highly scattered electrons containing lattice information < 1 Å, we employ the Lorentz lens, designed for regular field-free TEM, in the field-free STEM mode. The Lorentz lens allows the lens to capture highly scattered electrons before they are obstructed by the liner tube, all while ensuring that the magnetic field acting on the sample remains unaffected. As a result, the highest accessible diffraction angle in the new setup is increased to about 25 mrad without introducing significant distortions. Thereby, the attainable maximum structural information was enhanced from approximately about 9 Å (in the conventional field-free mode) to < 0.8 Å (in the new mode). Arrays of diffraction patterns containing both unscattered and highly scattered beams can be captured using 4D-STEM with the new lens setup, which we refer to as LA-Ltz-4D-STEM.
The LA-Ltz-4D-STEM data offer rich structural and magnetic information through reciprocal space. For instance, in the case of crystalline materials, the diffraction spots convey details of the crystal symmetry, lattice parameters, and orientation (refer to Figure 1b, top). For amorphous matter characterized by a set of diffuse rings due to the lack of long-range order (Figure 1b, bottom), the diffraction rings provide information on the short/medium-range atomic arrangement e.g., the inter-atomic distance and atomic coordination. The in-plane component of the magnetic fields inside of the sample deflects the electron beam through the Lorentz force. Therefore, the center position of the diffraction pattern at each probe position reflects the direction and strength of the local in-plane magnetic field that the electron probe has passed through.
This method enables precise measurement of the characteristic atomic and magnetic structures across an extensive field of view, [1] a critical aspect for investigating real-world ferromagnetic materials. The pixel-by-pixel correlation of the different information offers comprehensive visualization and statistical evaluation of the nanoscale magnetic phenomena. We applied the new method to directly visualize the magnetoelastic coupling as well as the competition between magnetoelastic and magnetostatic energy in an amorphous ferromagnet. [2] This approach opens

new avenues for in-depth studying the structure-property correlation of nanoscale magnetic materials.

Keywords:

LA-Ltz-4D-STEM, magnetic imaging, magnetic structure

Reference:

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[2] Silveyra et al., Science 362, 418 (2018)

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Mapping the Space Charge Region in BaTiO3 and SrTiO3 using 4-Dimensional Scanning Transmission Electron Microscopy

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PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

Ceramic capacitors are passive electronic components designed to store and release electrical energy through an electric field [1]. Polycrystalline barium titanate (BaTiO3) and strontium titanate (SrTiO3) have been widely employed in ceramic capacitors due to their reliable dielectric properties, compact size, and cost-effectiveness [2]. However, this material experiences degradation, such as increased leakage current, as a result of diminishing resistance under elevated temperatures and voltage stress. According to the ionic de-mixing model, the primary cause of this degradation is the migration of oxygen vacancies, which move toward grain boundaries and eventually reach the cathode [3].
The migration of oxygen vacancies can be effectively altered by doping with elements such as Fe and Mn. This doping modifies the Fermi level and the space charge region (SCR) at grain boundaries. The SCR is the result of an accumulation or depletion of oxygen vacancies and cations around grain boundaries, limiting their migration and preventing degradation. However, this general description lacks validation of the details such as the influence of grain boundary character on the SCR due to limited microscopic observations of grain boundaries and SCR in ceramic capacitors, making it challenging to further develop the theory.

In this study, we employ 4-dimensional scanning transmission electron microscopy (4D-STEM) [4] in combination with electron precession to map electric fields at grain boundaries in Fe-doped BaTiO3 and SrTiO3. Unlike conventional differential phase contrast (DPC) analysis, electric field mapping by 4D-STEM reduces diffraction and channeling artifacts thus enabling accurate measurement of electric field and charge density at the grain boundary. Combining it with an in-situ biasing TEM setup, we can visualize SCRs under different biasing conditions at various temperatures. Through our presentation, we aim to showcase our methodological developments and observations of SCRs in BaTiO3 and SrTiO3. These findings promise to significantly advance the fundamental scientific understanding of ceramic capacitor performance and illuminate the degradation mechanisms under high voltage stress.

Keywords:

Space Charge Layer, 4D-STEM

Reference:

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[4] Kang, Sangjun, et al. "Direct observation of quadrupolar strain fields forming a shear band in metallic glasses." Advanced Materials 35.25 (2023): 2212086.

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Direct observation of quadrupolar strain fields forming a shear band in metallic glasses

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Poster Group 2

The promising application of metallic glasses is limited by their catastrophic failure at low strain due to shear banding [1]. For decades, scanning/transmission electron microscopy (S/TEM) techniques have been employed to analyze shear bands in metallic glasses and understand their formation [2]. However, due to a lack of direct information in reciprocal space, conventional S/TEM cannot characterize structural variation, e.g. local atomic strain, of amorphous materials, which are key to describe the deformation of glasses. With this work [3-4], we solved the longstanding difficulty of experimentally imaging atomic packing structure and local strain of amorphous materials using 4-dimensional scanning transmission electron microscopy (4D-STEM). Figure 1a schematically shows the 4D-STEM setup. A quasi-parallel electron probe is focused to ~5 nm diameter on an electron transparent sample. 4D-STEM records 2D images of local diffraction patterns over a 2D grid of each probe position by stepwise scanning of the probe over the area of interest. As shown in Figure 1b, the local strain is quantified by the elliptical deviation present in each diffraction pattern. We obtain the principal strains from the long and short axes of the ellipse indicated by qmax and qmin. The strain tensor is obtained by algebraic transformation of the principal strains to the loading coordinates. Moreover, a PDF analysis is performed as a structural descriptor based on the 4D-STEM dataset to analyze the local atomic structure of metallic glasses. Thereby, the 4D-STEM approach provides a correlative visualization of the nanoscale strain field and the atomic structure information. We used a Fe85.2Si0.5B9.5P4Cu0.8 (at.%) metallic glass ribbon as an example in this study, which receives attention owing to its soft ferromagnetism. The metallic glass was deformed by scratch testing at ambient conditions using a diamond tip. We observe residual strain fields concentrated at inclusions (Figure 1c). It provides for the first time an experimental visualization of the Eshelby-like inclusions surrounded by quadrupolar strain fields aligned on a shear band in deformed metallic glasses. The results provide direct experimental evidence for a concrete scenario for the initiation of a shear band: the dilatated Eshelby inclusions are the result of local plastic atomic displacements in the glassy matrix, which concentrate a stress field with quadrupolar symmetry. The quadrupolar stress field perturbs the surrounding material in a vortex-like manner and percolates neighboring inclusions. This eventually leads to the formation of a shear band. This provides a new understanding of the formation of shear bands in metallic glass. Our new method is also expected to initiate broad research possibilities for solving questions in amorphous matters.

Keywords:

Metallic glass, 4D-STEM, Strain field

Reference:

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CARS microscopy for studies in skin physiology and pharmacology

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LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

The application of Coherent anti-Stokes Raman Scattering (CARS) microscopy in biomedical research represents an extension in our ability to study complex biological systems with unique resolution and specificity. This presentation highlights the use of CARS microscopy to explore various aspects of skin physiology and pharmacology, demonstrating the technique's versatility and its potential to extend our understanding of biological tissues and treatment efficacy.
Firstly, utilizing CARS microscopy enabled the direct observation of time-dependent, spatially resolved diffusion of water (D2O) within human skin tissue, unveiling significant variations in diffusion coefficients across different strata of the Stratum Corneum (SC). This heterogeneity challenges the prevailing notion of the SC as a monolithic barrier, instead positing it as a complex, layered defense mechanism. This study show CARS microscopy's ability to quantitatively measure diffusion coefficients at varying tissue depths and locations.
Further, the examination of dissolvable microneedles for transdermal drug delivery underlines CARS microscopy's unique ability to visualize penetration of the microneedles and follow their morphology within the skin. By providing detailed images of microneedle degradation and drug dispersion, CARS microscopy offers valuable insights into the mechanisms of drug release and absorption, supporting the development of more effective transdermal therapeutics.
Additionally, employing CARS microscopy in conjunction with a perspiring skin simulator to assess sunscreen substantivity offers a novel approach to studying the interaction between topical formulations and physiological processes like sweating. This application highlights the technique's potential to dynamically follow the redistribution of specific active ingredients in the sunscreen during perspiration. Thus, evaluate product performance under realistic conditions, informing the design of more resilient and effective sunscreens.
Overall, these studies exemplify the power of CARS microscopy to provide detailed, molecule-specific insights into complex molecular interactions in tissue.

Keywords:

CARS, Drug delivery, Micro needles

Reference:

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Exploring Melanoma Dynamics: Insights from a 3D Cell Culture Model with Vemurafenib Treatment

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Poster Group 1

Background and aim
Malignant melanoma represents a difficult challenge in oncology due to its high mortality rates and frequent development of treatment resistance mechanisms. Phenotype switching, a recognized phenomenon in melanoma cells wherein they transition from a highly proliferative/low invasive state to a low proliferative/highly invasive state, is implicated in the development of treatment resistance. To enhance our comprehension of this dynamic process, elucidating the underlying mechanisms is imperative to identify optimal therapeutic targets for novel treatments.
Despite the necessity for effective treatments, the use of animal models in drug development is hindered by time constraints, high costs, and ethical considerations, underscoring the urgency for improved in vitro models. Utilizing 3D human tissue models presents a promising avenue to address these challenges, offering cost-effective and expedited experimentation while closely mimicking human physiology. Therefore, improving 3D in vitro human tissue models is pivotal in drug development and biomedical research in general.
For these models to be suitable for preclinical studies, it is important to thoroughly characterize and recognize their restrictions and applicability. Our study aims to establish a simple yet robust 3D melanoma model and employ multiple assays to investigate the dynamics of melanoma spheroids, facilitating their utility as a platform for drug screening, notably utilizing the MAPK inhibitor, vemurafenib, a key agent in melanoma therapy.

Methods
A viability assay and migration assay were performed on spheroids in suspension and spheroids in a collagen matrix, respectively, after 96-hour treatment with the MAPK inhibitor, vemurafenib. The viability assay was performed using a sensitive colorimetric assay, CCK-8. For the migration assay, images were obtained every 24 hours for a total of 96 hours, and the spheroid core and migrating cells were identified.
Volume measurements were performed in GFP-expressing human melanoma spheroids, after performing live-cell imaging of full thickness human melanoma skin models treated with vemurafenib for 72 hours, using confocal microscopy.

Results
Treatment with vemurafenib led to a significant reduction in spheroid viability and attenuated migration compared to untreated controls. Live cell imaging revealed a marked decrease in spheroid volume following 72-hour vemurafenib treatment

Conclusion
Our findings demonstrate the efficacy of vemurafenib treatment in 3D melanoma spheroids across multiple assays, highlighting the utility of this model in assessing diverse melanoma therapies.

Keywords:

Melanoma, cancer skin model,

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Development of an automated pipeline for segmenting and analyzing organelle contacts in Volume Electron Microscopy.

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Poster Group 1

Background incl. aims
Volume electron microscopy (vEM) is recognized as a powerful imaging tool capable of providing detailed insights into the 3D structure of cells, tissues, and model organisms at the nanometer scale. However, the manual segmentation process required for analyzing vEM datasets is time-consuming and limits the application and throughput of this technique. In response, this study aims to address these limitations by implementing deep learning approaches for automated segmentation based on a Panoptic-DeepLab (PDL) architecture. Specifically, the researchers aim to develop an automated segmentation workflow using Empanada-Napari plugins for reconstructing airway cells and efferent ductules of the male reproductive system in 3D. Furthermore, the study seeks to demonstrate the adaptability of these tools for generating tissue and organelle-specific segmentation models trained on relatively few 2D images, with the goal of improving efficiency and accuracy.
Methods
The researchers employed a deep learning-based approach utilizing the PDL architecture for automated segmentation of vEM datasets. Empanada-Napari plugins were developed to facilitate the 3D reconstruction of airway cells and efferent ductules, originally designed for segmenting mitochondria. The segmentation models were trained on a limited number of 2D images, highlighting a strategy for achieving robust results with minimal training data. Additionally, custom Python scripts were developed to extract cells of interest from the automatically segmented volumes, enabling single-cell quantitative analysis. Adapted image analysis methods were utilized to quantify spatial relationships between distinct organelles within the cell volume, including identifying points of contact on both semantic and instance segmented objects.
Results
The study demonstrates the effectiveness of the automated segmentation workflow in accurately segmenting airway cells and efferent ductules from vEM datasets. The segmentation models, trained on a relatively small number of 2D images, yield exceptional results when compared to human-validated data. The developed tools enable precise quantitative analyses of cellular metrics such as volume, surface area, and number of organelles, enhancing understanding of cellular processes and functions. Furthermore, the adapted image analysis methods successfully quantify spatial relationships between organelles, providing insights into organelle topology within cells.
Conclusion
The automated segmentation workflow and accompanying tools presented in this study represent a significant advancement in the analysis of vEM data. By alleviating the laborious process of manual segmentation, these tools enhance the efficiency and throughput of vEM imaging, thereby enabling comprehensive studies of cellular structures and functions. The adaptability of the segmentation models to different tissues and organelles underscores their versatility and potential for widespread application in biological research. Overall, the study provides a robust framework for accurate and

effective quantitative analysis of vEM data, offering unprecedented insights into mammalian cellular biology.

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The Impact of Dietary Haemoglobin on Nymphal Stages of I.ricinus: The gut volume EM reconstruction

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Poster Group 1

The tick is a ubiquitous parasite whose life is highly dependent on the haemoglobin of its host. Tick ability to synthesize its own haem group has been lost in the course of its evolution. The haem group is an inevitable part of the tick's reproduction, as the haem group is deposited on the eggs. Previous studies have shown that the adult females can survive even without the haem group, but the requirement of the nymphs is still unknown.
We observed the morphology of the tick nymph gut through SBF-SEM (Serial Block Face Scanning Electron Microscopy), followed by a 3D reconstruction of its inner structures, including the granules, which were identified by a parallel immunolabelling study of host albumin and haemoglobin. The total volume of 0.003 mm3 was reconstructed using MIB/deepMIB[1, 2] and then visualised using AMIRA (Thermo Fisher Scientific).
The results of our work presented here show the distribution of haemoglobin and albumin in the midgut cells and, in addition, the distribution of lipid droplets, which occupy the majority of the cells at this feeding time.
All these components are important for the metamorphosis of the nymph and for the further life of the adult males and females.
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In-situ liquid-cell dynamic TEM observations of liquid crystal nanocomposite phase transitions

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Poster Group 1

Background incl. aims
Liquid crystals (LCs) possess distinctive, anisotropic optical properties, and because of their fluidity combined with a partially ordered structure, they are easily influenced by external stimuli, making them extensively utilised in technology. The primary techniques used for their examination include polarised light microscopy (PLM), differential scanning calorimetry (DSC), small angle X-ray scattering (SAXS), and nonlinear optical imaging methods. Transmission electron microscopy (TEM) is known for its exceptional resolution and analytical capabilities. Although liquid crystals have been recognised for more than a century, only one study has reported TEM imaging of liquid crystals in their native state in the air [1]. This is partly due to the high vacuum environment of microscopes. To observe dynamic processes in a liquid environment, samples must be isolated from the vacuum, as imaging under high pressure leads to electron scattering and vacuum pump issues, hindering conventional TEM methods [2]. Another challenge, particularly for hydrated samples such as liquid crystals, is the impact of the electron beam, which requires precise control over the electron dose for reliable results.
Methods
In our study, we investigated the liquid crystal 4’-Octyl-4-biphenylcarbonitrile (8CB) and its nanocomposite that contains gold nanoparticles using polarizing microscopy and in-situ liquid-cell TEM.
Results
Our findings demonstrated the possibility of studying not only the arrangement of nanomaterials within the matrix but also the phase transitions kinetics of liquid crystal using the liquid-cell TEM approach. Polarising microscopy revealed changes in the optical properties of the nanocomposites upon the addition of Au nanomaterials, along with a uniform distribution of defects in the smectic A mesophase. We will discuss these results along with sample preparation for liquid-cell electron microscopy, the interaction between the electron beam and the sample, and dynamic observations of phase transitions.
Conclusion
Observations of LCs phase transitions and nanoparticle dynamics within the LCs matrix using TEM are possible with specified electron dose restrictions. Electron beam damage at low doses was negligible, and the phase transition was fully reversible. The dynamic behaviour of nanoparticle aggregates inLCs is linked to the electron dose value.
Keywords
Liquid-cell, TEM, in-situ, liquid crystals, nanocomposites

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Revealing the molecular architecture of the cell using Ultrastructure Expansion Microscopy (U-ExM)

Prof. Paul Guichard

LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

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Precise targeting for volume electron microscopy, a multimodal approach

Mr. Yannick Schwab

IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

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Adding Dimensions to Intravital Imaging to Better Eavesdrop on Biology

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LS-02 (2), Lecture Theater 4, august 26, 2024, 14:00 - 15:00

Imaging of living specimens can animate the wealth of high-throughput molecular data to better understand complex events ranging from embryonic development to disease processes. We are advancing this approach despite the unavoidable tradeoffs - between spatial & temporal resolution, field of view, limited photon budget - by constructing faster and more efficient light sheet and laser-scanning microscopes that maintain subcellular resolution.
Our two-photon light-sheet microscope combines the deep penetration of two-photon microscopy and the speed of light sheet microscopy to generate images with more than 10x improved imaging speed & sensitivity. Better engineering of the detection objective's point-spread-function improves this another 3-fold. Two-photon excitation light is far less scattered, permitting subcellular resolution to be maintained better than conventional light sheet microscopes, resulting in 4D (3D over time) cell and molecular imaging with sufficient speed and resolution to unambiguous trace cell lineages, movements and signals in intact systems.
To increase the 5th Dimension (# of simultaneous labels), we are refining new multispectral image analysis tools that exceed the performance of our previous work on Linear Unmixing by orders of magnitude in speed, error propagation and accuracy. Novel denoising strategies using machine learning permit imaging at far lower light levels, yielding rapid and unambiguous analyses without perturbing even fragile multiplex-labeled specimens.
Parallel refinements in label-free approaches extend imaging to patient-derived tissues and even human subjects. The low concentrations of these intrinsic labels required us to refine fluorescence lifetime imaging (FLIM), and combine it with multispectral and advanced denoising tools, to perform intravital imaging in such challenging settings.
Combined, these imaging and analysis tools offer the multi-dimensional imaging required to follow key events in intact systems as they take place, and allow us to use noise and variance as experimental tools rather than experimental limitations.

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Dynamics of molecules at the nanoscale: from RESOLFT to STARSS

Ilaria Testa

LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Molecular interactions and complex formation are the base of cellular processes and therefore human activities. However, it is very hard with state-of-the-art techniques to precisely, efficiently and specifically follow their formation directly in physiological relevant system such as living cells. The goal of this project is to develop a novel apparatus for measuring the formation of large molecular complexes with unprecedented level of precision and in living cells, providing new capabilities in the functional aspects of imaging.
Measuring rotational diffusion properties of biological macromolecules is an extremely useful tool in cell biology because of its high sensibility to molecular weight changes, giving direct insight into several biological processes such as molecular binding, enzymatic activity, and protein complex formation. Here, we present a novel methodology based on the time-resolved fluorescence anisotropy experiment (TR-FA), and named Super Time-resolved Fluorescence Anisotropy with Switchable States (STARSS), which aims to measure complex formation in living cells by providing rotational accuracy far beyond the current fluorescence lifetime temporal limit. STARSS translates powerful and fundamental ideas from the field of super-resolution microscopy, which deals with increasing the precision of the determination of the spatial coordinates of fluorescent probes, into rotational accuracy. We used STARSS to measure the maturation state of viral-like particles as well as chromatin packing and the oligomerization of protein in cells.

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Mechanics of blastocyst morphogenesis

Jean-Léon Maître

LS-02 (1), Lecture Theater 4, august 26, 2024, 10:30 - 12:30

During pre-implantation development, the mammalian embryo forms the blastocyst. The architecture of the blastocyst is essential to the specification of the first mammalian lineages and to the implantation of the embryo. Consisting of an epithelium enveloping a fluid-filled lumen and the inner cell mass, the blastocyst is sculpted by a succession of morphogenetic events. These deformations result from the changes in the forces and mechanical properties of the tissue composing the embryo. Combining microscopy (spinning disk, light sheet and lattice light sheet confocal microscopy), image analysis, biophysical tools (such as optical tweezers) and genetics, we study the mechanical and cellular changes leading to the formation of the blastocyst.

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Integrated structural cell biology of pathogen-host interactions

Prof Kay Grünewald

LS-08, Lecture Theater 4, august 28, 2024, 10:30 - 11:30

1182

Scanning patterns evaluations towards FIB-SEM/SIMS low-dose high-speed acquisition

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Poster Group 1

Two fundamental artifacts that affect resolution in Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) and Secondary Ion Mass Spectrometry (SIMS) are beam damage and shot noise. Beam damage is a well-known factor that causes structural modifications and impacts the image quality of sensitive samples. On the other hand, shot noise limits the signal-to-noise ratio (SNR) of the image, increasing distortion. These limitations affect the mentioned techniques and reduce their capabilities to analyse beam sensitive materials properly [1].
For image acquisition, the scanning process involves focusing the ion (or electron) beam to scan the sample row by row. At the end of each line, the beam is returned to the start of the next line, a movement known as “flyback”. This conventional raster is the most used due to its simple application and ease of reconstruction. However, for fast scan, it suffers from deformations due to flyback, thus requiring a delay time to avoid distortion. Also, an extra dose is injected into the sample during the flyback, which may further alter sensitive samples [2].
Reducing the dose rate can be achieved by accelerating the scan. This can be done by decreasing the dwell time, but this reduction will lead to a low SNR. Other solution is to implement a continuous scanning method to eliminate the need for flyback. A moderate number of studies have focused on the development of alternative scanning patterns to reduce beam damage or increase image acquisition speed in FIB-SEM/SIMS. Patterns like bi-directional, spiral, Hilbert, and Z-order are pointed out for this application. These different patterns move the ion or electron beam continuously along the raster, aiming to improve acquisition time and reduce beam damage [1].
The bidirectional (serpentine) scan pattern, also simple to implement, improves scanning by avoiding flyback but encounters distinct distortions for leftwards and rightwards trajectories, necessitating post-processing for image improvement. The spiral scan pattern also avoids flyback delay and benefits from smooth movements that reduces distortions, yet it produces non-uniform image quality and requires post-processing. The Hilbert pattern is a space-filling curve that changes the scanning direction by no more than two steps, resulting in small changes in both axes during the scan. This method avoids flyback delay, follows an isotropic path, and reduces acquisition time but experiences distortions when using small dwell times. Similarly, the Z-order scan pattern provides an isotropic path and reduces acquisition time, with the drawback of potential distortions due to short flyback times in small dwell times [2, 3].
Lastly, the sparse scan pattern reduces acquisition time by scanning only a portion of the total pixels and minimizes distortions caused by the finite response time while maintaining an isotropic path. However, it is sensitive to drift and necessitates a reconstruction procedure. Its implementation requires a higher level of complexity [4].
Each pattern offers unique benefits and challenges, influencing their suitability for different microscopy applications.
As an aid to implementing the scanning methods previously discussed, the application of deep learning algorithms contributes to the smart low-dose high-speed acquisition and reconstruction. However, their implementation involves increasing the complexity of the system and requires more computational resources [4].

Our objective is the implementation of new scanning methods that allow for low-dose high-speed acquisition in a high-vacuum FIB-SEM platform (Scios) from Thermo Fisher Scientific equipped with a SEM column, a Ga liquid metal ion source (LMIS)-based FIB column (Ga-FIB), and a magnetic sector SIMS system [5].
To achieve this goal, we have successfully implemented and tested the different patterns mentioned before to control the raster of ion and electron beams to form secondary electrons images in the Scios instrument.
To control the deflection system and collect data from the Everhart Thornley Detector (ETD), we employed a custom-built acquisition system that uses a USB-6351 acquisition card from National Instruments. The system is divided into two parts: i) scan control and ii) data acquisition. The scan control was managed using the two analog outputs of the acquisition card, with each signal controlling one coordinate position of the beam (X, Y). The data acquisition involved receiving secondary electron signals from the ETD via the digital input. A LabVIEW program was developed to manage the system.
To test the functionalities of the system, we used a non-sensitive solid sample (FeTi). The system can set the image size in pixels, dwell time, and scan pattern. Other parameters, such as accelerating voltage, field of view (FOV), or beam current, must be defined using the microscope's internal system.
The novel raster schemes being explored for low-dose high-speed ion or electron beam imaging are expected to accelerate materials research by enabling high-resolution imaging of transient processes and/or radiation sensitive samples.

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Keywords:

FIB/SEM, High-speed low-dose acquisition, scan-patterns

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Quantification of Potential Drops Across Semiconductor Heterointerfaces Using 4D-STEM: prospects and pitfalls

Prof. Dr. Kerstin Volz

PS-03 (1), Lecture Theater 2, august 29, 2024, 14:00 - 16:00

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Geological Materials and Bio-mineral systems

Dr David McNamara

PS-06, Lecture Theater 1, august 29, 2024, 10:30 - 12:30

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Probing the structure and dynamics of active molecular materials with cryo and liquid EM

Professor Joe Patterson

PS-07 (2), Plenary, august 30, 2024, 10:30 - 12:30

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Pushing the limits of Coupled Extreme Environments during In-situ TEM

Professor Khalid Hattar

PS-12, Lecture Theater 2, august 29, 2024, 10:30 - 12:30

Background
In-situ TEM and STEM have pushed our understanding of the nanoscale and atomistic mechanisms active in materials exposed to thermal, mechanical, electrical, magnetic forces. Understanding at this length scale has been instrumental in the validation and refinement of many models developed to understand and predict materials degradation. In many ways, the ability of in-situ electron microscopy to explore a range of coupled extreme environments has become a path to an integrated materials science characterization toolbox. [1]

Methods
In the subfield of TEM with in-situ irradiation, it has been commonplace to combine in-situ ion irradiation with heating or cooling, mechanical loading, or both simultaneously. These advancements have been greatly advanced in the last decade or two with advancements in miniaturization that permit the inclusion of multiple probes into the same often small pole-piece gap, automation to permit the user to run multiple aspects of the experiment without assistance, and advanced manufacturing that permit costly development of parts for a specific microscope. One such example of a facility was the In-situ Ion Irradiation TEM (I3TEM) that was developed at Sandia National Laboratories to explore such coupled extreme environments. [2] This facility was unique in that it coupled two lasers into the I3TEM to permit both dynamic TEM (DTEM) imaging with 6 ns resolution and laser-based heating above 2000 °C for some samples. [3] Such examples raise the question of what the physical limit for is coupled in-situ TEM experiments and what are the limits set by historical presidents or engineering costs.

Results
Within the last year, the Tennessee Ion Beam Materials Laboratory (TIBML) has taken on the challenge of pushing the limit in coupled in-situ experiments in various extreme environments. This facility will be based on a JEOL 2100+ STEM with PyJEM compatibility. This microscope was chosen due to a combination of robust nature and ease of use. The high tilt pole-piece and LaB6 options were chosen to permit greatest access to the sample and excellent imaging of dislocation loop structures in two-beam and related imaging conditions, respectively. The JEOL 2100+ has already been modified by JEOL/IDES for the inclusion of a C0 lens for increased brightness, a laser port aimed at the electron gun for potential DTEM experiments in the future, a laser port for sample heating, and a port for ion optics coming in perpendicular to the electron optics. In addition, the microscope has been outfitted with a Gas Injection System (GIS) from Waviks, the ability to do Automated Crystallographic Orientation Mapping (ACOM) from Nanomegas, and real-time defect analysis via edge computing from Theia Scientific. The process for the development of this instrument, the current status of the microscope, and the planned vision for coupling it with 20 W 1064 nm laser, 20 W 532 nm laser, 20 kV ion source, and 3 MV tandem accelerator will be presented.

Conclusion

Similar to advancements in electron microscopy resolution resulting from advancements in aberration correction, advancements in miniaturization, automation, and advanced manufacturing have greatly increased the potential for various coupled in-situ TEM experiments. This work will discuss the physical limits of coupled in-situ experiments and compare them to the current engineering limits. It will also highlight the current status of the Tennessee in-situ ion irradiation STEM that is being developed to explore these extreme coupled environments.

Figure: The Tennessee In-situ Ion Irradiation Scanning Transmission Electron Microscope with several of the already implemented and planned upgrades labeled.

Keywords:

In-situ TEM; extreme environments; electron optics; ion optics

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1190

Exploring the dynamics of semiconductors with an ultrafast transmission electron microscope

Sophie Meuret

PS-11, Lecture Theater 2, august 28, 2024, 14:00 - 16:00

1191

Breaking Resolution Limits with Ptychography using Topological Materials

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IM-03 (1), Plenary, august 28, 2024, 10:30 - 12:30

Electrons play a pivotal role in stabilizing matter, but they are also tools that can reveal the underlying physics of complex systems from high energy physics to condensed matter. One advantage of electrons is that they can also be used imaging probes, where properties of matter such as ferroelectricity, magnetism or topology can be observed atom-by-atom. While previous works have used vortex beam to uncover changes in orbital angular momentum [1,2], we use a generation of new direct electron detectors [3] and develop a mathematical algorithmic approach to uncover the torque transfer of an electron beam as it imparts upon a topological ferroelectric vortex [4]. Furthermore, using these new detector systems, we can also push imaging resolution on a conventional, non-aberration corrected STEM to sub-angstrom 0.5 resolution with electron ptychography [5,6].

Firstly, I will discuss a new type of electron probe which can image orbital angular momentum and torque transfer of topological structures in ferroelectrics. Using these new electron probes, we show that the presence of an electric toroidal moment transfers a measurable torque and orbital angular momentum to a localized electron beam in the ballistic limit. In Figure 1, we showed that the torque transfer could be measured where the point of reference is at the center of the vortex [4]. Depending on the rotation of the polarization vortices in our structure, we obtain a signal for the change in orbital angular momentum in either a counter-clockwise or clockwise direction (Figure 1).

Secondly, I will show how electron ptychography, an iterative computation imaging technique, can improve resolution beyond the numerical aperture of the electromagnetic lenses to the sub-angstrom limit in a conventional electron microscope for a two-dimensional moiré system (Figure 2, right image). Using this technique, we essentially use a 'computation lens' approach to imaging, opening opportunities to explore new physics in emergent materials beyond physical lenses and providing high-resolution imaging access to institutions that lack the funds to house such expensive equipment [6].

Finally, the interplay between technological and computational progress will be discussed in my talk, including the challenges that still arise for electron microscopy. Moreover, our research efforts described in these works were pursued using non-aberration-corrected electron microscopes. We believe that combining new detector technologies with computation, we can make electron microscopy more accessible while still developing new efforts for investigating new physics in emergent topological materials.

Keywords:

ptychography, 4D-STEM, orbital angular momentum, topological materials, 2D moiré

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Studying the crystallization of inorganic materials using correlated transmission electron microscopy by Brydson et al

Mr. Rik Drummond-Brydson

PS-09, Lecture Theater 2, august 26, 2024, 14:00 - 16:00

1196

Exploiting dynamical diffraction theory in the crystal structure determination from 3D electron diffraction data

Dr. Lukas Palatinus

IM-06 (2), Lecture Theater 1, august 30, 2024, 10:30 - 12:30

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Quantifying nanoscale diffusion phenomena using in situ TEM

Dr. Peter Schweizer

IM-07, Lecture Theater 2, august 26, 2024, 10:30 - 12:30

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Data analysis workflows to measure material properties and structure using 4D-STEM

Colin Ophus[1], Stephanie Ribet[1], Georgios Varnavides[1,2], Steven Zeltmann[3], Karen Bustillo[1], Alexander Rakowski[1], Benjamin Savitzky[1], Min Chen[2], Yang Yang[2], Andrew Minor[1,2], Mary Scott[1,2]
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IM-06 (1), Lecture Theater 1, august 29, 2024, 14:00 - 16:00

Background including aims
Scanning transmission electron microscopy (STEM) has become an essential tool for materials science research, where it has been applied to atomic-scale imaging, diffraction, spectroscopy, and 3D tomography of many materials. High speed direct electron detectors have now become ubiquitous, allowing researchers to record full 2D diffraction patterns for each electron probe position in a 2D grid, producing very large 4D-STEM datasets [1]. Extracting material properties and structures from 4D-STEM datasets requires efficient and robust software methods. In this talk, I will highlight various 4D-STEM analysis workflows using our open source py4DSTEM toolkit [2].

Methods
The py4DSTEM toolkit is an open-source python library hosted on GitHub, along with many tutorials and example datasets. It contains various analysis modules including those aimed at calibration, classification, diffraction pattern simulation and matching, Bragg diffraction analysis, amorphous diffraction analysis, phase contrast imaging, and others. To analyze nanobeam diffraction patterns generated by crystalline samples, we use template matching for disk detection, correlation for orientation mapping, and lattice fitting to measure strain [2,3]. Phase contrast imaging modes are implemented using iterative gradient descent with regularization [4].

Results
We have performed high resolution 4D-STEM strain mapping on metallurgical samples and energy materials, both ex situ and in situ. We have also demonstrated improved precision and accuracy for nanobeam strain measurements by using patterned apertures [5]. We have also used 4D-STEM to characterize the morphology of highly beam-sensitive soft matter samples, including block copolymers with both crystalline and amorphous constituent phases. We have also used dictionary lookup methods to determine the crystalline phases of complex samples, and machine learning methods for blind estimation of crystal systems, space groups and lattice constants. Finally, we have also used STEM phase contrast imaging methods to measure the atomic structure of materials in 2D and 3D. I will show how each of these results were generated using the py4DSTEM code. I will also demonstrate how modern machine learning methods can be used to analyze the most challenging 4D-STEM experiments: those where multiple scattering and dynamical effects from thick samples produce highly nonlinear diffraction contrast.

Conclusion
While 4D-STEM is a powerful tool to characterize the material structure and properties, modern high speed electron detectors produce a truly prodigious amount of data. These experiments therefore require highly robust and efficient software analysis tools to analyze the results. We have

implemented many algorithmic methods in the open source py4DSTEM code, and we welcome additional contributions from the microscopy community.

Keywords
Scanning Transmission Electron Microscopy, 4D-STEM, Electron Diffraction

Graphic

(top) Geometry of a 4D-STEM experiment where many diffraction patterns are recorded from a sample. (bottom) Examples of analysis methods implemented in py4DSTEM. Adapted from [2].

References

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Transdifferentiation of human dental pulp-derived mesenchymal stem cells into neurospheres and transplantation into aganglionic hindgut

Msc Adam Soos[1], MSc. Emoke Szocs[1], Bendeguz Sramko[2], Anna Abbas[2], Dr. Anna Foldes[3], PhD Karolina Pircs[2], Prof. PhD. Nandor Nagy[1]
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Poster Group 1

Background incl. aims
The enteric nervous system (ENS) regulates the function of the gastrointestinal tract. Numerous congenital neurointestinal disorders underlie its defective function, mostly affecting the migration and differentiation of neural stem cells of neural crest origin. Treatments for these diseases are limited; surgery treats the symptoms only, and no regenerative cell therapy is available yet. Direct cell reprogramming (transdifferentiation) is a novel technique where patient-derived somatic cells are reprogrammed into neuronal lineage without going through an intermediate proliferative pluripotent stem cell stage, providing a promising strategy for stem cell therapy to treat neurointestinal diseases. Human dental pulp-derived mesenchymal stem cells (DPSCs), similar to the ENS, are neural crest-derived cells found in adult teeth. Our primary aim is to use transdifferentiated DPSCs for intestinal transplantation to restore the enteric nervous tissue in the congenital aganglionic colon.
Methods
We initiated our experimental work by generating neural cell aggregates (neurospheres) from DPSCs transdifferentiated to neural phenotype using viral transfection. Immunocytochemical methods were used to characterize the neurospheres, and their nervous tissue forming capacity was studied by transplanting the neurospheres into aganglionic segments of 5-day-old chicken embryonic hindgut.
Results
Serial sections of neurospheres generated from transdifferentiated human DPSC demonstrate ubiquitous TUJ1+ neuronal expression and scattered SOX10+ precursors. Cells from neurospheres transplanted to the aganglionic chicken hindgut and transplanted onto the chorioallantoic membrane of host E9 chick embryos or cultured in 3D collagen gel for 72 hours migrate out and differentiate to neurons.
Conclusions
Transdifferentiated postnatal DPCS behave like embryonic neural crest-derived cells. The avian hindgut environment is permissive to engraftment by human DPSCs and supports migration and neuro-glia differentiation of these cells following transplantation in vivo. Our findings suggest that neurospheres generated from DPSCs hold significant potential as cell-based therapy for future treatment of neurointestinal disease, but only in early stages of neural differentiation.
Keywords
Dental pulp stem cells, transdifferentiation, neurosphere, transplantation, enteric nervous system
Funding: Supported by the ÚNKP-23-3-II, OTKA-K-138664, Semmelweis 250+ Scholarship for PhD Excellence

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Understanding how microalgae cells remediate heavy metals using Atomic Force Microscopy (AFM)

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IM-09, Lecture Theater 5, august 29, 2024, 14:00 - 16:00

Background incl. aims
Microalgae have recently emerged as a promising biomass for the biosorption of heavy metals from aqueous environments, by offering an efficient and cost-effective alternative compared to conventional remediation techniques. However, to date, the mechanisms by which microalgae can remediate these pollutants is still not entirely clear. In this work, we investigate the role of extracellular polymeric substances (EPS) of a green microalgae species, Parachlorella kessleri, isolated from the polluted Reconquista River in Argentina, in the biosorption of zinc.
Methods
For that, we use an original approach based on atomic force microscopy (AFM) experiments. This technique, developed in 1986, relies on the control of a force acting between a sharp tip and a surface, while scanning a sample. In force spectroscopy mode, AFM can record force-distance curves, where the force experienced by the probe is plotted as a function of the probe-sample separation distance (typically from 0.5 to 6 µm). These curves can then be analyzed to extract several information on the sample morphology, on its nanomechanical properties, and on the physico-chemical nature and strength of interactions that can take place between the tip and the sample. In addition, while the tip used can be bare, it can also be functionalized with any type of molecules or particles, making it possible to access specific interactions between these functionalized tips and cell surfaces.
Results
A first step in the study was to optimize a culture medium favoring the production of EPS by cells. It was showed that cells cultivated in the presence of nitrates could produce EPS while the cells had much less EPS when cultivated with ammonium as a nitrogen source. This was confirmed by nanomechanical experiments performed with AFM that showed that cells cultivated with nitrates were covered by a thick layer of a soft material (200 Pa approximately), corresponding to the EPS layer. Then, our hypothesis is that EPS play a key role on the biosorption of metals. To test this hypothesis, AFM force spectroscopy experiments were performed using zinc-functionalized AFM tips, this way allowing to probe directly the interactions between zinc and cell surfaces at the molecular level. The results obtained showed that zinc could interact with the EPS present at the surface of cells cultivated with nitrates with a force of up to 1 nN. Since no interactions were recorded when cells cultivated with ammonium were probed, this confirms that the EPS of P. kessleri can interact directly with and absorb zinc, thereby remediating it from the medium.
Conclusion
Overall, these experiments provide a new understanding of biosorption mechanisms using an original biophysical approach, and show the potential of P. kessleri for heavy metal biosorption.

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Revealing foliar nanoparticle uptake dynamics: integrating nano-CT, confocal microscopy and LA-ICP-MS insights

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Poster Group 1

Revealing foliar nanoparticle uptake dynamics: integrating nano-CT, confocal microscopy and LA-ICP-MS insights
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Background incl. aims
The agriculture sector calls for urgent action on novel strategies that can enhance fertilizers' efficiency in order to increase crop production while reducing its environmental footprint. Conventional soil fertilization methods often suffer from low efficiency due to nutrient immobilization, leaching, and volatilization. Foliar fertilization presents key advantages as it bypasses soil limitations but encounters obstacles such as leaf scorching and limited nutrient translocation1. Recent progress in bionanotechnology offers potential solutions by utilizing nanoparticles (NPs) with tailored properties to improve nutrient delivery to plant tissues. Comparably bigger than their ionic counterparts, yet small enough to penetrate all the relevant plant barriers e.g. cuticle, stomata, cell wall and vasculature, NPs can deliver plant mineral nutrients right where needed, overcoming the limitations of ions mobility and enhancing nutrient use efficiency1. However, unravelling the intricate fate and behavior of NPs within plants hampers the potential implementation of a nano-approach into foliar fertilization. Employing advanced bioimaging techniques can provide valuable mechanistic insights regarding plant interaction with and assimilation of NPs, which can positively contribute to our understanding and strengthen the case for nanotechnology adoption in plant production.
The objective of our research is to develop an NP-based system that allows for efficient delivery of Mn, one of the essential plant mineral nutrients, via foliar fertilization. To this end, formulation and NP physicochemical properties are optimized to enable effective penetration of the leaf surface, securing the delivery of adequate amounts of bioavailable Mn, whilst reducing the risk of scorching. At the same time, we want to characterize NP uptake pathways, distribution and assimilation of NPs in Mn-deficient barley plants. In this poster, we present a comprehensive investigation employing confocal imaging, nano-computed tomography (nano-CT) and laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to elucidate the dynamics of foliar-applied NPs.
Methods
25 nm poly(acrylic acid)-coated Mn oxide (PAA-MnO) NPs were produced via one-pot polyol method2. NP physicochemical properties such as size, shape, surface charge, dissolution and Mn oxidation state, were characterized using different techniques, including dynamic light scattering, TEM, ICP-MS and XPS. Fluorophore-labelled (DiI-PAA-MnO) and Ce-spiked (Ce-PAA-MnO) NPs were produced for confocal and LA-ICP-MS applications, respectively.
Mn-deficient 21-day-old barley plants were used for the NP application experiments. To study the NP leaf uptake, fluorescent DiI-PAA-MnO NPs were imaged 2 to 5 hours after the leaf application using a confocal microscope (Leica Stellaris 8). A small portion of the dosed area was excised with a scalpel, placed on a microscope glass slide, and submerged into perfluorodecalin 95% (Sigma-Aldrich) before imaging.

The nano-CT experiments were performed at the European Synchrotron Radiation Facility id16b beamline in Grenoble (FR). For these, pristine PAA-MnO NPs were dosed on barley leaves. 5 hours after treatment, a small leaf cut was placed inside a 200 µL pipette plastic tip and mounted on top of a rotating sample stage. The beamline was then set up in a projection geometry with a 29 keV pink beam focused to 50 x 50 nm^2. Each sample was scanned at four sample-to-detector distances with 900 angles over 360 degrees of rotation. The phase was retrieved using an iterative contrast transfer function (CTF) approach. Finally, the data was reconstructed and post-processed in Dragonfly, which was also used for visualizations.
For the LA-ICP-MS experiment, Ce-PAA-MnO were used. 5 hours after the application, a small portion of the leaf exposed area was cut, embedded in OCT mounting media and frozen in dry ice-cooled liquid hexane. The OCT molds were sliced at -30°C using a cryotome (Leica CM3050S) and freeze-dried overnight. Thin 14 µm cross-sections were ablated using a nanosecond LA unit (Iridia 193 nm excimer laser ablation system, Teledyne CETAC technologies).
Results
Our experiments document the early stages of NP uptake in plant leaves. Confocal analysis showed that NP uptake in barley leaves occur as early as two hours after the application and that stomata appear to be the main gateway for NP entry into the leaf. To validate the confocal imaging findings, nano-CT was used to study the uptake pathway of pristine PAA-MnO NPs. Nano-CT results corroborates the data obtained via confocal imaging, as NPs were detected inside the mesophyll below the stomata area. Despite nano-CT high resolution, NP clusters were visibile only when the leaf was pre-treated with a CaCl2 solution allowing for NPs aggregation inside the mesophyll. Finally, the correlation of nanoparticle distribution patterns observed via confocal imaging and nano-CT was determined by LA-ICP-MS, which confirmed the stomatal pathway as the main gateway for NP entry into the mesophyll, and highlighted the potential presence of NPs inside the leaf vascular tissue.
Conclusion
Our research highlights the strengths of three different, yet complementary, bioimaging techniques. Easy procedures for sample preparation and cellular resolution make CLSM a powerful technique to visualize NP uptake and distribution within plant cells and tissues. However, the imaging depth of confocal microscopy is limited to the superficial layer of the sample. Scattering, photobleaching and noise are few factors that restrict its use to study processes occurring a few hundred microns below the leaf surface. To overcome some of these issues, we integrated nano-CT imaging to obtain high-resolution, real three-dimensional images of pristine NP clusters inside the leaf. Complementing this, laser-ablation ICP-MS was used for elemental mapping, allowing us to study NP concentration gradients and NP spatial distribution at the whole leaf level. By combining these complementary techniques, we gained a comprehensive understanding of the processes occurring in the early hours after the leaf application of NPs.
This poster underscores the pivotal role of cutting-edge bioimaging techniques for unraveling the complex dynamics of foliar-applied NPs within plant systems, paving the way for the rational design and implementation of nanoparticle-based agricultural innovations.

Keywords
Manganese, nanoparticle, uptake, bioimaging

Reference:
1. Husted et al., 2023 – DOI: 10.1016/j.tplants.2022.08.017
2. Marasini et al., 2021 – DOI: 10.3390/app11062596

1206

Streamlining Graphene Liquid Cell Preparation: VitroTEM's Naiad

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Poster Group 1

Graphene Liquid Cells (GLCs) offer unprecedented capabilities for imaging samples in their native hydrated state, providing insights at atomic resolution [1]. However, manual assembly of GLCs presents significant challenges, limiting their widespread adoption. This poster presents VitroTEM's Naiad system, a revolutionary approach to GLC preparation. By employing a layered structure consisting of monolayer graphene sheets on standard TEM grids, the Naiad system rapidly constructs GLCs, encapsulating liquid samples for imaging [2]. Our poster showcases images of ferritin particles in their native environment, demonstrating the system's efficacy in biological materials research. Additionally, we present atomic resolution images of Au nanoparticles, highlighting its utility in nanomaterial science. The Naiad system simplifies GLC assembly, enabling researchers to focus on sample imaging rather than grappling with graphene preparation. This poster emphasizes the Naiad system's potential to accelerate discoveries in diverse fields reliant on high-resolution imaging of liquid-phase samples.

Fig. 1: Ferritin particles encapsulated in GLC pockets.
Fig. 2: High resolution TEM image of Au nanoparticles encapsulated in a very thin GLC pocket.

References:
[1] Park, Jungjae, et al. ACS nano 15.1 (2021): 288-308.
[2] van Deursen, Pauline MG, et al. Advanced Functional Materials 30.11 (2020): 1904468.

Keywords:

Graphene Liquid Cells, CryoEM, Insitu

Reference:

[1] Park, Jungjae, et al. ACS nano 15.1 (2021): 288-308.
[2] van Deursen, Pauline MG, et al. Advanced Functional Materials 30.11 (2020): 1904468.

1208

Visualizing electronic correlations in quantum materials at millikelvin temperature

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PS-10, Lecture Theater 5, august 26, 2024, 15:00 - 16:00

In this talk I will discuss the discovery of two-dimensional heavy fermions (2DHF) made of 5f electrons with an effective mass 17 times the free electron mass. These 2DHF present quantized states at terraces. The energy separation between quantized levels is of a fraction of a meV and the level width is set by the interaction with correlated bulk states [1]. Interestingly, we find a new connection between bulk and surface features. I will also describe recent new insight in the Josephson effect of ultra small Josephson junctions, which lead to an improved Josephson microscopy, called feedback Josephson microscopy.

[1] Quantum-well states at the surface of a heavy-fermion superconductor, Edwin Herrera, Isabel Guillamón, Víctor Barrena, William J. Herrera, Jose Augusto Galvis, Alfredo Levy Yeyati, Ján Rusz, Peter M. Oppeneer, Georg Knebel, Jean Pascal Brison, Jacques Flouquet, Dai Aoki & Hermann Suderow, Nature 616, pp 465-469 (2023).

1215

HR STEM study of anion/cation exchange in colloidal lead halide perovskite heterostructures

Iurii Ivanov[1], Mr Nikolaos Livakas[2], Ms Juliette Zito[2], Dr. Ivan Infante[3], Prof. Liberato Manna[2], Dr. Giorgio Divitini[1]
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Poster Group 2

Introduction Lead halide perovskite nanocrystals (LHP NCs) with composition $CsPbX_3$ (X = Cl, Br, I) have been widely studied due to their high photoluminescence quantum yield (PLQY) and narrow emission bandwidth, covering the whole visible spectral range. These properties, together with a remarkable tolerance to surface defects, make LHP NCs quantum dots (QDs) promising candidates for various light-emitting applications. Emission tunability in such materials is typically achieved by halide alloying, produced either directly during synthesis or as a result of post-synthetic halide (anion) exchange reactions. Cl-Br and Br-I exchanges are now well understood and we have recently provided a clearer view of the $CsPbCl_3$ → $CsPbI_3$ halide exchange [1]. Another way to tune outstanding optical properties of such QDs is to bond them with other semiconductors – metal chalcogenides (MC), which also adds the possibility of employing post-synthetic cation exchange. Here we present a detailed structural and compositional study of the interfaces between LHPs and MCs and their role during post-synthetic anion/cation exchange. Methods High resolution scanning transmission electron microscopy (STEM) studies were performed in an aberration-corrected ThermoFisher Spectra 300 operated at 300 kV. Samples were prepared by drop-casting diluted solutions of NCs onto ultra-thin carbon film grids. Atomic resolution images were acquired in both HAADF and iDPC modes. Compositional maps were obtained with a Dual-X EDX detector using Velox and rapid rastered scanning of the beam. Results Figure 1 shows HR STEM HAADF images of two colloidal epitaxial LHP-MC heterostructures used as a basis for post-synthetic anion/cation exchange: $CsPbCl_3$–$Pb_4S_3Cl_2$ and $CsPbCl_3$-PbS. The Cl-I halide exchange in $CsPbCl_3$–$Pb_4S_3Cl_2$ makes it possible to stabilise two perovskite domains ($CsPbCl_3$ and $CsPbI_3$) within one colloidal NC, despite the huge difference in lattice parameters (9%). HAADF, iDPC and GPA analysis revealed a complex 3D strain distribution and key role for the LHP-MC interface during halide exchange. The newly synthesised $CsPbCl_3$-PbS epitaxial heterostructure is shown in Figure 1b,c. Based on it we could prepare, by post synthetic anion exchange, heterostructures in which the perovskite domain is either $CsPbCl_3$, $CsPbBr_3$ or alloyed $CsPb(Brx/Cl1\text{-}x)_3$. HRSTEM confirmed that anion exchange only affects the perovskite region and does not affect the PbS domain. Next, through post-synthetic cation exchange, we created heterostructures in which the MC is either PbS or Cu_2S. HR STEM and STEM-EDX data were used to construct 3D atomic models of all heteroepitaxial NCs before and after post synthetic anion/cation exchange reactions. Conclusion Using STEM, we constructed atomic models of a series of recently discovered LHP-MC heteroepitaxial colloidal QDs and linked them to the optoelectronic properties, in particular emission tunability by post synthetic anion/cation exchange reactions.

Keywords:

Quantum dots, perovskites, HRSTEM

Reference:

[1] Livakas, N. et al. J. Am. Chem. Soc. 2023, 145, 37, 20442–20450

1217

Multi-scale characterisation of laser-induced defects in the production of heterojunction photovoltaic cells

Dr Anacleto Proietti[1], Dr Luca Buccini[1], Dr Pierfrancesco Atanasio[1], Dr. Giancarlo La Penna[1], Dr. Chiara Mancini[1], Dr. Corrado Di Conzo[1,3], Dr. Francesco Mura[1,2], Dr Marco Galiazzo[4], Dr Nicola Frasson[4], Prof. Daniele Passeri[1,2], Prof. Marco Rossi[1,2]
[1]Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Rome, Italy, [2]Research Center for Nanotechnology applied to Engineering of Sapienza University of Rome (CNIS), Rome, Italy, [3]Department of Applied Science and Technology (DISAT), Polytechnic of Turin, Turin, Italy, [4]Applied Materials Italia, , Italy

Poster Group 1

Background incl. aims:
Silicon-based HeteroJuncTion solar cells (HJT or SHJ) are a family of photovoltaic cells based on the heterojunction formed between two materials with different bandgaps. They are hybrid devices combining the technology of classical crystalline silicon-based cells and thin-film cells.
HJT are now a well-established reality as they guarantee high efficiency and mass production [1], however they suffer of slight losses of the cell integrity when they are cut and assembled with a shingled structure. In this study, a mass-produced HJT cell surface was scribed for half of its thickness employing an ns-IR laser. Subsequently, the cell is separated in sub-cells by mechanically cleaving. This process is both stressful for the cell structure due to the high level of thermalization and is also locally removing the passivation layer of the newly cut edges. These mechanisms induce a drop in performance at the edges of the cell, hindering the advantages of shingled technology [2].
In this study, defects induced by the cut were characterized using different multi-scale techniques to identify the technological solution that would allow the cell structure to be protected as much as possible.
Methods:
The sample object of the study is a HJT cell with a bulk structure based on n-type silicon and a multi-layer passivation surface composed of 3 thin layers of intrinsic amorphous silicon doped with hydrogen (15-20 nm), n-doped amorphous silicon doped with hydrogen (15-20 nm) and indium thin oxide (ITO) (70-80 nm) provided by Applied Materials. The cut was performed with an ns-IR laser (Rofin Powerline F50) on an entire wafer (156,75 mm side) on several lines, while a second pristine wafer was used as a comparison.
The techniques used were X-Ray Diffraction (Bruker D8 ADVANCE), Raman and Photo-Luminescence spectroscopy (Renishaw InViaTM), Scanning Electron Microscopy (Zeiss Auriga) equipped with EdX Spectroscope (Bruker Quantax) and with Focused Ion Beam (Physics d'Orsay Cobra) and Atomic Force Microscopy (Oxford Instruments Cypher VRS).
Results:
The analyses, focused on the detection and identification of defects in the pristine and post-cut wafer, were initially concentrated on the physical-chemical characterization of the basic structure. The XRD analyses show that after laser cut, the ITO main peak disappears or consistently decreases its intensity, thus indicating a loss of crystallinity or even a detachment from the surface. Moreover, the silicon beneath ITO layers seems affected by the high energy laser treatment and in particular the strongest peak of Si (400) shows an increase in the strained component. In fact the pristine HJT sample shows, by means of XRD, a double peak of Si (400) with two relative maximum values at 30.3 deg and 30.4 deg (with a Mo tube). The relative amount of strained silicon (i.e. the intensity of the

peak at lower angle and therefore higher interplanar distance) appears increased, such behaviour might indicate an annealing of the sample due to the high energy treatment.
Through Raman and PhotoLuminescence (PL) analyses, it was then possible to assess the presence of crystalline defects within the silicon of both the pristine and post-cut wafers, with a clear splitting of the silicon peak in the case of Raman spectroscopy and a decrease in the Band To Band transition of the silicon in the case of PhotoLuminescence spectroscopy.
The morphology and chemical composition of these defects were then studied using scanning electron microscopy and atomic force microscopy. The surface structure is composed of square-based pyramids necessary to maximize the active surface of the cell. The pristine wafer presents mechanical defects derived from the production phase which cause the removal of the surface layer of ITO and thus an initial random decrease in the cell's performance. Once the laser cut has been performed, however, a complete destruction of the structure can be seen in the proximity of the cut. Moving away from the cut up to about 1 mm, a gradual improvement of the integrity of the structure can be seen until its complete recovery. Afterwards, in addition to the vast damage to the structures around the cut area due to the very high temperatures, the presence of silicon-based particles of various sizes scattered throughout the sample was observed causing a masking effect which decreases the cell's efficiency. Finally, viscoelastic and EDX maps were made on both the intact and cut zones unveiling that the ITO is not completely ablated; on the contrary, it tends to follow the reorganization of the silicon-based substrate, as also demonstrated through FIB-SEM. The viscoelastic maps revealed that the hardness of the sample strongly depends on the size of the ITO layer and, in the areas away from the cut, there is also a clear directional trend in hardness and thus in ITO thickness. This variation in thickness, although not critical, causes a heterogeneity that makes it easier to ablate the ITO in areas close to the cut.
Conclusion:
A complete characterization and identification of defects within HJT cells (both pristine and post-cut) was conducted. Several defectivities, of structural, mechanical and thermal nature were identified and characterized. The defects on the pristine wafer show that there is still room to optimize the synthesis of these materials in order to improve their final performances. Finally, through the study of the cut-induced defects, it was possible to demonstrate the causes of the decrease in the performances of this type of cell after the cutting and shingling process, something which has been already widely reported in literature.

Keywords:

Photovoltaic, Multiscale Characterisation, Defect Analysis

Reference:

[1] X. Ru, & al., "Silicon heterojunction solar cells achieving 26.6% efficiency on commercial-size p-type silicon wafer", Joule, Vol. 8 (4), pp. 1092-1104 (2024).
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3D imaging, visualisation, and analysis services at Lund University Bioimaging Centre

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[1]Lund University Bioimaging Centre, Lund, Sweden

Poster Group 1

Background and methods
Recent advances in optical clearing methods, which render tissues transparent by homogenization of the reflective index throughout the sample, allow for deep imaging into organs and even entire animals [1]. Optical clearing can unveil spatial relationships within tissues, providing a new dimension of understanding across various fields of biology and medicine. Light-sheet fluorescence microscopy (LSFM) offers an unparalleled combination of spatio-temporal resolution, ideal for imaging large biological samples [2].

Results and conclusions
Lund University Bioimaging Centre (LBIC) has made strategic investments in LSFM and optical clearing since 2019, currently possessing two LSFM systems and employing multiple clearing techniques, including the recent acquisition of a high-throughput commercial clearing system [3]. Here, we introduce LBIC's lineup of service offerings related to 3D-imaging and subsequent customised visualisation and analysis workflows.

Keywords:

Optical clearing
Light-sheet fluorescence microscopy

Reference:

[1] Richardson D.S. et al. Tissue Clearing. Nature Rev. Methods Primers 1(1):84 (2021).
[2] Dodt, H.-U. et al. Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. Nat. Methods 4, 331–336 (2007).
[3] Park, Y.-G. et al. Protection of tissue physicochemical properties using polyfunctional crosslinkers. Nat. Biotechnology 37, 73–83 (2019).

1219

An efficient method for quantifying the degree of neurodegeneration in an insect brain

Jakub Opelka[1,2], Lucie Pauchova[1], Andrea Bednarova[1], Radka Zavodska[1,3], Michal Sery[1,3], Ivo Sauman[1,2], Hana Sehadova[1,2]
[1]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [2]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [3]Faculty of Education, University of South Bohemia, Ceske Budejovice, Czech Republic

Poster Group 1

Background
For decades, the fruit fly Drosophila melanogaster has served as a model organism for studying the molecular and genetic basis of many human diseases, including neurodegeneration. In this study the comparative analysis was performed using the Drosophila model that mimics the human pathological condition associated with spinocerebellar ataxia type 1 (SCA1), which is characterized by progressive problems in movement (3). In Drosophila, such anatomical evidence can be obtained by viewing serial paraffin sections of the mutant brain under a light or fluorescence microscope. This method was originally developed by Heisenberg and Böhl (2). The extent of brain damage was determined subjectively based on the frequency of brain neuropathology on the 6-point scale from "none" to the highest level of neurodegeneration (1). Our study aimed to address this limitation by developing a novel method for accurate quantifying of brain damage in Drosophila models of SCA1, using Fiji (ImageJ) and Imaris 3D modeling software on Z-stacks of whole adult Drosophila brain.
Methods
Flies were anesthetized under CO2 and prefixed in 4% paraformaldehyde (PFA) in phosphate buffer (PB). The prefixed flies were then washed in PB and the brains were dissected in PB. The dissected brains were fixed in 4% PFA. After fixation, brains were rinsed in PB-Tween and were blocked with 5% normal goat serum in PB-Tween for 2 hours as blocking solution. After thorough rinsing with PB-Tween (four times for 15 min at room temperature), the preparations were transferred to the goat anti-mouse IgG secondary antibody conjugated with Alexa Fluor 448 diluted 1:200 in the blocking solution and incubated overnight at 4 ºC. Preparations were rinsed with PB-Tween and mounted on microscopic slides (Figure 1). The confocal laser scanning microscope (FV 3000 Olympus) was used to acquire images of the Drosophila brain using a high sensitivity detector with a resolution of 1024x1024 pixels and a pixel acquisition time of 8 µs. To match the size of the Drosophila brain, the UPLANSApo 20XO objective was used. Serial Z-stack images were acquired with the optimal thickness. The data in oir format obtained with the confocal microscope were uploaded to the Fiji software and subjected to analysis by Threshold and Analyse particles modules. The obtained data were summed and multiplied by the layer thickness to obtain the exact volume of neurodegeneration in µm3.Three-dimensional models of areas of neurodegeneration were reconstructed in Imaris software (Bitplane) using the Surpass module - > Contour Surface. We correlated the obtained values to the volume of the whole brain thus determined the percentage loss of the brain volume caused by the neurodegeneration.
Results
In this study, two analytical approaches are presented to determine the degree of brain tissue damage in the Drosophila adult brain, and they are compared in terms of the time required and the accuracy of the measurements. The first method of raw data analysis was primarily based on the use of the publicly available software Fiji. The second method of brain damage extent analysis uses the

commercial image software Imaris. In both types of analyses (Fiji and Imaris), statistical analyses using Student t-test confirmed a significant difference in the degree of neurodegeneration between mutant and control brains, p-value was $P < 0.001$. To compare results obtained by these two analytical methods, the extent of neurodegeneration in mutants measured by Fiji and Imaris software was subjected to statistical analysis using Student t-test and linear regression. The results showed no significant differences.

Conclusion

In Fiji software, the Analyze Particles function enables automatic selection of neurodegenerative holes. However, subjective adjustment of the Threshold level may introduce measurement error. Conversely, Imaris software requires manual selection of each hole across all optical sections of the Z-stack using the Contour Surface module, which is time-consuming. Thus, the choice between the two methods depends on the degree of neurodegeneration. For low levels, Imaris is recommended for its 3D visualization capabilities, albeit at the expense of time and effort. Conversely, for high levels of neurodegeneration, Fiji is more efficient.

Keywords:

neurodegeneration, insect, Fiji, Imaris, confocal-microscopy

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1220

Vacuum ultra violet emission spectra observed by newly designed grating with improved diffraction efficiency

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Poster Group 1

Introduction

In the research and development of new functional materials, obtaining information on their chemical bonding states is one of the most important subjects for understanding the physical properties of the materials. In the soft X-ray and vacuum ultra-violet (VUV) energy regions, various characteristic X-ray emissions owing to electronic transitions from the valence bands (bonding electron states) to the inner-shell electron levels are observed. In other words, the analysis of these emission spectra will lead to an understanding of the chemical bonding states and the physical properties of the materials. For the purpose described above, Terauchi et al. fabricated a soft X-ray emission spectrometer (SXES) accommodated to a transmission electron microscope (TEM) and demonstrated a high energy resolution of 0.22 eV for Al L-emission spectrum [1]. Based on their work, JEOL Ltd. commercialized a SXES, which can be mounted on a general-purpose scanning electron microscope (SEM) / electron probe micro analyzer (EPMA) using newly developed varied-line-spacing laminar-type gratings in 2013. This model, named SS-94000SXES, accepted the energy range of 50–210 eV. SS-94000SXES has been applied successfully to analyze battery materials and contributed to a trendy discussion of Li bonding with Si anode materials [2]. On the other hand, in Li oxides, the Li spectrum is predicted to shift significantly to the lower energy side compared with that of Li metal. However, it has not been accessed with SS-94000SXES due to its limitation of acceptable energy range.
To overcome this difficulty, a new diffraction grating which covers an energy range of 35–100 eV and a larger reflection efficiency with a coating was developed [3]. This grating system allows the observation of the whole band-spectrum of Li in the compounds with high sensitivity [4]. In addition, this energy range accepts to measure whole Mg L-emission spectrum. In this paper, some preliminary results obtained with the new grating system are presented.

Experiments

The modified SXES with the new grating was installed to an EPMA (JEOL JXA-8230 and JXA-iSP100). Samples used for the present experiments were selected for emphasizing the fascinating detection of lower-energy emission, The energy axes of these spectra shown in this paper are calibrated by C K-emission (277.0 eV) [5] and its higher order diffracted lines of HOPG.

Results

Figure 1 shows Al L-emission spectra of aluminum metal obtained by using the new grating (hereafter called as NEW) and SS-94000SXES system (hereafter called as CONVENTIONAL). Labels of L_3-M, L_2-M and L_1-$L_{2,3}$ are the assignments of related electronic transitions between shells. This shows that the intensity of NEW is more than two times larger than that of CONVENTIONAL. Besides, an intensity enhancement of approximately 5-times is obtained for Mg L-emission [4]. Inset (a) is the

enlargement of Fermi-edge region of Al L-emission, where intensities of the two spectra are normalized by L_3-M peak for comparison. It is seen that the energy resolutions of NEW and CONVENTIONAL are the same. The L_2-M intensity, which is separated from L_3-M by 0.4 eV, is clearly observed. Inset (b) is the enlargement of Al L_1-$L_{2,3}$ emission region, which is out of the energy range of SS-94000SXES. Furthermore, the spectrum of NEW shows the second-order Al $L_{2,3}$-M spectrum because of the extended performance of NEW down to 35 eV. This means that NEW covers the energy region of shifted Li K-emission intensities of compounds.
Figure 2 shows $M_{2,3}$-$M_{4,5}$ emission spectra of Manganese and Cobalt. These transition metals were selected to show the applicability to battery cathode materials. Spectral intensities of Mn and Co are normalized by peak intensities at 46.2 eV and 58.1 eV, respectively. The vertical dotted line is the lower limit of the detection energy of CONVENTIONAL. Then, the Mn $M_{2,3}$-$M_{4,5}$ emission spectrum shown here can only be measured by using NEW. Furthermore, this Mn $M_{2,3}$-$M_{4,5}$ emission spectrum shows asymmetric intensity profile with an additional structure in the high energy side (49.0 eV) indicated by a red vertical line, which reflecting the chemical bonding state of the material.

Conclusion
VUV region emission from 35 eV could be observed by using the NEW grating system. Compared to CONVENTIONAL, the intensity of Al L-emission has improved more than 2 times (more than 5 times for Mg L-emission [4]). Furthermore, Mn $M_{2,3}$-$M_{4,5}$ emission spectrum, which could not be observed with CONVENTIONAL, reflecting the chemical bonding state was observed by NEW.

Keywords:

SXES, VUV, detection efficiency

Reference:

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Visualization of surface plasmon propagation and emissions by cathodoluminescence

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Poster Group 1

- Background -
Plasmonic materials are capable of manipulating light-matter interactions at nanoscale dimensions through the control of material morphology. Metallic plasmonic waveguides with extended geometrical nanostructures, such as planar surfaces, periodical gratings, and nanowires, can support eigenmodes of surface plasmon polaritons (SPPs), which are defined as surface electromagnetic waves propagating along the interface between a metal and a dielectric medium. The intrinsically two-dimensional nature of SPPs provides flexibility in engineering integrated nanophotonic circuits, facilitating the transfer of optical information across given distances due to their confined and enhanced fields near the interface [1]. However, despite the importance of SPP modes in applications such as optical communications and photonics, detailed studies on their propagation and emissions upon excitation are relatively underexplored. In this work, we present an extensive study on the behavior of SPPs on one-dimensional plasmonic crystals (1D-PlCs), delving into their propagation properties as well as the simultaneous correlation of their emission positions relative to the beam irradiation spots.
- Methods -
A 1D-PlCs with a periodicity of 600 nm and terrace dimensions of 420 nm width and 100 nm height, patterned on an otherwise flat 200 nm-thick silver surface, served as the samples. Mode excitation and data acquisition were both performed in cathodoluminescence-based scanning transmission electron microscopy (STEM-CL) at an accelerating voltage of 80 kV. This modified STEM-CL system is capable of resolving the emission angles θ and energies E of the detected CL radiations, providing full post-access on the fields of the excited modes [2]. The vertically-polarized emissions from different sample setups were analyzed, namely the “flat at front” and “flat at rear” setups with half-grating-half-flat structure oriented symmetrically along the x-direction (see Figure 1).
- Results -
Distinctive dispersion curves with predominantly opposing slopes were observed. The “flat at front” setup exhibits negative-slope dispersion lines attributing to backward-propagating SPPs (in the negative x-direction), while the “flat at rear” setup with a geometrically symmetric flip shows positive-slope dispersion lines referring to forward-propagating SPPs (in the positive x-direction), as shown in Figure 1(a,b). These differences are due to the presence of periodic structures on only one side of the samples, causing the SPPs propagating towards the planar surface to experience no SPP-scattering structure. Moreover, the effect of the interference of the outcoupled SPPs as photons with transition radiation (TR) generated at the beam impact position was also clearly observed as additional dispersion lines appearing in-between, as marked by numbers in Figure 1(a,b) [3].
Photon maps analysis further elucidates the characteristics of the excited modes and spatially visualizes the interference phenomena. Two modes with distinguished hotspot distributions were observed at θ = 20°±5°: the symmetric mode (S-mode) at around E = 2.50 eV (Figure 1(d,f)) and the antisymmetric mode (A-mode) at E = 1.47 eV (Figure 1(c,e)). The former S-mode has opposite sign charges located alternatively at the terrace and groove centers, while the latter A-mode distributes

the charges along the terrace edges. Additionally, periodic-like fringes with varying spacings were observed on the planar surface, depicting a spatial image of the interference of SPP-induced radiation with TR. For the A-mode, for instance, given the measured fringe spacings of 645 nm and 1190 nm respectively for the “flat at front” and “flat at rear” setups, the average SPP wavelength was estimated to be around 838 nm [4]. Such a wavelength, close to but less than its free-space wavelength of the A-mode at 843 nm, indicates the bound nature of SPPs on a flat surface.
A study on the optical emission spots was also performed to evaluate the positions of the photon emissions from the propagating SPPs upon scattering by grating structures. By synchronizing emission imaging with beam scanning on the sample, signals of the emitted CL light were captured and digitally mapped as emission spots, with each pixel in the emission space storing information about spatial emission positions in the sample space [5]. Depending on the sample setups (i.e., the “flat at front” or “flat at rear” setups) and beam excitation positions (i.e., at the terrace, groove, or flat area), the obtained emission spots varied in number (i.e., one, two, or three spots). By extracting the signals of these emission spots, known as emission-spot-decomposed CL maps, it was uncovered that photons emerge not only at where the beam is irradiated but also at more delocalized locations, providing strong evidence of the scattering of propagating SPPs. Moreover, shifts of the emission spots with beam irradiations across the samples show a nonlinear trend, depicting a modulation of the emission spots by the presence of the surface charge distributions.
- Conclusions -
In conclusion, our studies uncover how SPPs interact with 1D-PlCs through their propagation behaviors, as revealed by dispersion curves with opposing slopes, CL photon maps with differing fringe widths, and optical emission spots with emission-spot-decomposed CL maps showing emission positions. This work, with in-depth details on the SPP propagation, could provide valuable insights for designing nanophotonic devices.

Keywords:

Surface plasmons, transition radiation, cathodoluminescence

Reference:

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Nanocrystals with dilated interplanar distances in the carburized surface case of Inconel-718 gas-processed at 570°C

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Poster Group 1

It is utterly important to find appropriate methods of surface processing with the aim of enhancement of the properties of engineering interest of the stainless alloys (SS) and even of superalloys, as are hardness, wear resistance and even corrosion resistance. At Swagelok Co. Ltd. (Solon, OH, USA) it was invented the surface carburization processing at low temperature (LT) in gas-atmosphere (LTC) which matches the engineering requests for many SS alloys and for Inconel-718 (IN-718) Ni-Fe based superalloy [1]. The effect of LT gas-carburization consists in the formation at the alloy's free surface of a so-called carburized case (tens of µm-s thick) which is suggested to consist in crystallographically slightly modified alloy grains having properties that are matching the engineering requests. The theory currently accepted for explanation of the modified crystalline structure is based on a supposed supersaturation with carbon (CSS). It is claimed that carbon diffuses in the interstitial locations of the initial alloy grains (of face centered cubic structure (FCC)), leaving unchanged the initial grain structure and crystallography of the alloy, except for a dilatation of cubic lattice parameter due to the colossal-stuffing with carbon [2]. This theory is currently considered as valid for explanation of the enhancement of all the surface properties of the carburized IN-718 at carburizing temperatures of 843K, 803K and 783K [3]. We report that the low-temperature carbon supersaturation theory (LTCSS) is not valid in the case of IN-718 carburization at 843 K even when a surface carburized case is formed and revealed through chemical etching. We herewith report that the initial micrometer sized grain structure is replaced by a nanostructure located at least in the uppermost layer 1-2 µm thick, generated by the gas-carburization of the surface at LT.
The uppermost layer 1-2 µm thick of the free carburized surface at 843K of IN-718 was investigated by HRTEM by using a JEM-ARM200F aberration corrected (HR)(S)TEM microscope. In view of reaching the highest achievable precision in our measurement of the interplanar distances by using the HRTEM images of the observed nanocrystals, the Fourier transform of the Bragg reflections - mainly of the alloy matrix (111) reflections which have the highest structure factors in transmission electron diffraction (TED) - were analyzed. The usual disk samples of 3 mm diameter were prepared for TEM by following the usual steps: (a) mechanical cutting of a ≈1.5 mm thick sheet along a plane parallel to the free carburized surface of a bulk piece of carburized alloy, (b) mechanical polishing until a ≈80÷100 µm thickness is achieved, (c) ultrasonic punching of Ø=3mm disks, (d) dimpling of the disks until a remaining minimum thickness of ≈20µm is reached at the carburized face and (e) final dual beams milling with Ar+ ions in a Gatan PIPS device. The operations (b), (d) and (e) were done only at the uncarburized face of the disk samples.
A Cu contamination of the free carburized surface was our permanent mark for checking that the observed nanostructure belongs to the uppermost layer of the carburized case, whose entire thickness was reported as ≈20µm [3]. We have investigated the same IN-718 material sample as the one reported in [3], where no mention is made concerning the existence of a surface nanostructure due to the LT gas carburization. We collected a large number of directly measured d(hkl) values from HRTEM images of atomic resolution, regarding mainly the d(111) interplanar distances. We measured d(hkl)-s also for other observed nanocrystalline orientations. Interplanar distances of carbides were also measured, but they are not the object of our interest here. The distribution of the directly

measured d(hkl) values revealed that most of the observed matrix (austenite) nanocrystals have enlarged crystallographic interplanar distances in comparison to the uncarburized crystalline FCC structure of the IN-718 austenite matrix. The values measured by us are higher far beyond the errors that could affect our measurements. A much lower number of IN-718 austenite nanocrystals with non-modified lattice constant was observed. We took as a reference value the IN-718 lattice constant measured via neutron diffraction in a fully aged IN-718 superalloy [4].
Fig-1(a) is a negative inverted photo (used for better visibility of the nanostructure details) and shows the direct HRTEM image of an area located at the rim of a hole resulted by ion milling. It reveals the presence of nanocrystals embedded in an amorphous phase located in the uppermost layer of the carburized case. Fig-1(b) - not allowed by the template of the abstract text - was a reverse Fourier filtered image generated by mask selection of only the (111) reflections in the Fourie transform (FT) of the image shown in Fig-1(a). In many areas similar to the depicted one (Fig-1(a)) we measured several surprising values of the interplanar distance d(111), as for example 2.07, 2.08 , 2.11, 2.18 Å which correspond respectively to the FCC lattice cell parameter of the matrix nanocrystals of 3.58, 3.60, 3.65, 3.77 Å. These are as a rule largely higher than the most precisely measured lattice constant - done by means of neutron diffraction - of 3.5954÷3.6005 Å [4] - in a fully annealed IN-718 alloy.
The image shown in Figure (1a) (the FT of Fig-1a was not allowed by the template of the abstract text) is only an example taken from a wealth of HRTEM recorded images showing similar nanostructures. We got similar d(hkl) values also by measurements done at the recorded transmission electron diffractograms. This large collection of directly measured d(hkl)-s are clearly evidencing that the FCC crystalline cells of the nanosized fragments of the carburized IN-718 matrix are most probably generated via a massive diffusion of carbon in the alloy grains, as claimed by the LTCSS theoretical model, which instead of simply dilating the crystalline cell of the initial alloy grains, is inducing the fragmentation of them. The ultimate evidence concerning the occurrence, the mechanism and the crystallography of this fragmentation process - occuring deeper in the alloy - is obtainable only by means of neutron diffraction or by synchrotron XRD. Furthermore, it should be revisited the original evidence supplied via classical XRD which supports the claimed basic idea of the LTCSS model.

Keywords:

low-temperature gas-carburization, Inconel-718, lattice dilatation, LTCSS model

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Ghostbuster – a phase retrieval diffraction tomography algorithm for cryo-EM particle refinement

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Poster Group 1

Background incl. aims
Continual advances in methods development for single particle imaging in cryogenic electron microscopy (cryo-EM) have paved the way towards ever-increasing resolution for three-dimensional (3D) particle reconstruction. For thicker particles, one must further correct for the effects of multiple scattering within the particle. This is commonly associated with the correction of the Ewald sphere curvature, and existing algorithms have recently successfully pushed the resolution limit beyond what was possible. However, the physics of cryo-EM image formation is better understood as in-line holography. This implies that the phases of the complex-valued field arriving at the detector are lost upon the measurement of particle images. Therefore, the Fourier coefficients inferred from these phase-less images, which are subsequently used in Ewald sphere curvature correction, cannot fully remove the effects of multiple scattering. This results in ghost-like artifacts in the reconstructed particle. Our developed algorithm, Ghostbuster [1], aims to minimize these artifacts by accounting for multiple scattering in cryo-EM particle refinement through phase retrieval.

Methods
Ghostbuster refines the 3D particle through batch stochastic gradient descent by minimizing the error between the estimated images from a multislice-based forward model and actual cryo-EM measurements. Our algorithm implicitly recovers the lost phases of the measured particles, enabling the inversion of the multislice method through wave backpropagation. Particle refinement is further accelerated through the inclusion of a sparsity constraint as well as symmetry priors.

Results
Here, we compare Ghostbuster's reconstructed particle against state-of-the-art software such as CryoSPARC and RELION. Using a simulated dataset of Methanococcoides burtonii Rubisco (PDB: 5MAC), Ghostbuster demonstrates improved resolution beyond that of CryoSPARC's Ewald sphere curvature correction algorithm based on Fourier shell correlation (Fig. 1). Preliminary tests on experimental datasets deposited on EMPIAR show that Ghostbuster performs close to, if not, similar, to both CryoSPARC and RELION (Fig. 1).

Conclusion
In the absence of confounding noise sources in experiments, we show that the effects of multiple scattering can be minimized using Ghostbuster, even for particles previously assumed to be thin. Further work is to be done to investigate the efficacy of Ghostbuster under experimental conditions.

Keywords:

Phase retrieval, diffraction tomography, multislice

Reference:

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Heterogeneous dissolution of Au nanoparticles under constant electrochemical potential as observed via in-situ liquid-cell TEM

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Poster Group 1

Background incl. aims
Better synthesis control of nanostructured catalysts and their durability in electrochemical systems can be achieved by an in-depth understanding of the electrochemical deposition and dissolution processes. Electrodeposition and electrodissolution studies of nanomaterials are conventionally investigated over large specimen areas. The thermodynamics and kinetics of both NP nucleation and dissolution are affected by the specific surface interactions between NPs and substrate, which depend on the heterogeneous substrate's chemical, structural, and morphological properties. Although some high-sensitivity electrochemical techniques allow recording electrochemical signals of the growth or dissolution of single NPs, a direct visualization of these processes at high-spatial resolution can provide invaluable information on the underlying mechanisms. Recently developed liquid-cell transmission electron microscopy (LC-TEM) holders allow in-situ study of materials reactions on a nanoscale. Such specialized holders contain liquid cells that can withhold the liquid in a confined environment, allowing the imaging and spectroscopy of samples in the reaction media. Combining the capabilities of LC-TEM holders with micro-size electrodes printed on a chip enables us to study dynamic phenomena during electrochemical reactions. In this work, we have employed the LC-TEM to directly visualize the dynamic dissolution of electrodeposited gold nanoparticles (NPs) on an electrode and tap into the durability and stability of Au electrocatalysts. Combining the in-situ electrochemical LC-TEM, ex-situ nano-scale, and macro-scale measurements provided a unique perspective on the interaction between the metallic particles and the substrate during the electrochemical process [1,2].
Methods
The in-situ EC-LTEM experiments were performed using a JEOL JEM 2100 TEM at a 200 kV accelerating voltage in the bright-field mode. Experimental images and videos were recorded at the standard illumination conditions for real-time imaging with controlled electron beam damage effects. The electrochemical measurements in the TEM were carried out using a Protochips Poseidon 500 liquid holder, which allows observation of dynamic electrochemical processes in the liquid. The electrodeposition process of Au NPs was conducted from 1 mM HAuCl4 + 0.1 M NaCl solution in ultrapure deionized water. The electrochemical dissolution of these Au nanoparticles was observed under a constant applied potential during a potentiostatic regime at 1.5 V vs Pt pseudo reference.
Results
The in-situ electrochemical deposition of Au NPs from the HAuCl4 electrolyte resulted in fairly homogeneous particles of around 20 nm in size. The real-time observation of the dynamic electrochemical dissolution of these particles revealed that these Au NPs were dissolved heterogeneously and consecutively (one after another) during anodic polarization in a time span of

more than 30 seconds. Moreover, it was shown that Au NPs form a core-shell structure, with the shells being more resistant to the dissolution process.

Conclusions

The unprecedented combination of in-situ electrochemical LC-TEM and the macro-scale experiments revealed important information from the electrochemical deposition and dissolution of supported metal NPs. This approach opens up new opportunities for the rational design of functional nanostructured materials for catalytic applications and for evaluating their durability under electrochemical polarization from the perspective of their resistance to electrochemical dissolution.

Keywords:

In-situ liquid-cell TEM, electrochemical dissolution

Reference:

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Tracking the Ultrastructure of Life with Serial Block-face Scanning Electron Microscopy

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Poster Group 1

Background incl. aims
Life happens in 3D. To unlock the secrets of life we must consider and understand different aspects. Images acquired with an electron microscope reveal insights into the ultrastructure with highest resolution. Scanning electron microscopy (SEM) is a versatile method to obtain high-resolution information on the nanometer scale. While traditionally used for topography measurements, modern SEMs in biomedical research are utilized increasingly to obtain large volume data of biological samples. Single images are snapshots of a complex three-dimensional architecture reduced to a 2D image. The acquisition of volume data is necessary to understand this complex architecture. New developments in hardware and software as well as in electron optics enable an ever-larger range of applications in 3D. Different techniques like serial section tomography (Array Tomography), Serial block-face SEM (SBF-SEM) and focus ion beam SEM (FIB-SEM) allow us to understand these volumes. On this poster, we present the latest developments in Serial block-face imaging realized as ZEISS Volutome. ZEISS Volutome enables the acquisition of large volumes at nanometer resolution in an automated, unattended way. Regardless of which biological sample is to be analyzed - whether individual cells surrounded by pure, non-conductive resin or large, densely packed tissues such as brain - ZEISS Volutome is developed to image any type of resin-embedded biological sample.

Methods
Serial block-face SEM (SBF-SEM) was first developed and communicated by Denk et al. in 2004 [1]. In SBF-SEM, an ultramicrotome inside the SEM chamber can cut down to 25 nm thick sections from a resin-embedded sample block. The exposed sample surface is imaged with an electron beam, then new sections are cut away with a diamond knife, and the newly exposed block-face surface is imaged. This cutting and imaging process is repeated until the structure of interest is completely imaged. The acquired EM images are processed and digitally aligned into a 3D data set. Cell compartments can be easily identified and segmented from this z-stack. The segmented 3D data set can be visualized, investigated, and statistically analyzed.

Results
Serial block-face Imaging is the appropriate solution to image and follow neurons with long and thin protrusions, such as dendrites and axons. It is well suited to trace neurons within the large volumes necessary to capture the often-unpredictable paths of these cells. ZEISS Volutome enables acquisition of large mosaic images over all three dimensions at high resolution. This is supported by the stability of the ultramicrotome stage solution. Once the cutting and imaging parameters are set-up, the experiment runs automatically and autonomously. Sections as thin as 25 nm with pixel sizes as small as 3 nm can be cut to follow the dendrites and axons precisely over long distances (Figure 1A).
Going beyond image acquisition, ZEISS arivis, the software for image processing, segmentation, and visualization, facilitates the analysis of your ultrastructural 3D data set to elucidate the path of the individual neurons and the network of cell organelles (example shown in Figure 1B). High resolution

image acquisition is crucial for the identification of different cellular components; this is possible with ZEISS Volutome (Figure 1C).

Conclusion
In recent years a range of technical hurdles have been overcome and have extended the applicability of SBF-SEM. Most notably, a technique called Focal Charge Compensation has been developed to enable imaging of charge-prone biological samples [2] as well as the new high-sensitivity Volume BSE detector for high quality imaging at low kV. Unattended sectioning and imaging make this technique a convenient way to acquire large, ultrastructural 3D data sets.

Figure Caption
A, Large volume acquisition is needed to understand the neuronal network and the structure of the brain as well as to capture the unpredictable paths of these cells. B, Data set was processed and visualized with ZEISS arivis, in blue: cell nuclei, in red: mitochondria. C, Images were acquired with 3 nm pixel size, revealing different organelles. Data was acquired with ZEISS Volutome on a GeminiSEM 460, pixel size: 6nm (A+B) or 3nm (C), cutting thickness: 25 nm, EHT: 1.2 - 1.4 kV, Ip: 90 pA, dwell time: 0.8 - 1.6 µs.
Sample Courtesy of Christel Genoud, Université de Lausanne, EMF, 1015 Lausanne, Switzerland

Keywords:

volumeEM, SBF-SEM, Cell biology, Neuroscience

Reference:

Reference:
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Self-assembled nanoparticles in a thin film of water

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Poster Group 1

Background including Aims
Metal nanoparticles that are functionalised may be used as markers in Cryo-EM studies on soft materials (Ahmad 2022). An observation has been made where these particles have self-assembled into remarkably regular 2-dimensional lattices in a thin water layer, and where the interparticle separation is large. The aim is to investigate this phenomenon, the role of the water thickness, and to link it to similar observations

Methods
Au nanoparticles functionalised with NH2 groups were obtained from a commercial supplier and mixed with small unilamellar vesicles in a series of aqueous samples. TEM grids were prepared in the Leica GP1 grid plunger and transferred to the TEM in a Cryotransfer holder (Fischione Model 2550). Observations were made on a JEOL 2200FS TEM adapted for cryogenic studies and operated at 200 kV. While the main study was aimed at the interaction of the functionalised NPs with the vesicles, this presentation concerns the observation of the interaction between the functionalised NPs themselves.

Results
Functionalised NPs were found to have self-assembled into a 2D hexagonal lattice. The interaction occured only within thin layers of water. Note that the observations were made when the sample was frozen and the thin layer transformed to vitreous ice. The distance from one particle centre to another is in the 50 nm range, while the gold nanoparticle diameter is around 14 nm. Hence the gap between NP surfaces is around 36 nm, indicating a chain length of the functionalising ligand of around 18 nm.

Conclusions
Low density ordered arrays of particles forming spontaneously from various solutions are an interesting phenomenon. Previous work (Kanie 2012) found assemblies with 7 nm gaps between particles, which the authors commented was rather larger than typical gaps of 3 nm or even less. This observation of a 36 nm interparticle gap, which can still result in a particle array having a highly regular hexagonal packing, is intriguing. Further work will investigate the role of the thickness of the water/ice layer.

Figure 1 Micrograph showing self-assembled Au NPs in several patches of thin vitreous ice (200kV, TEM magnification 10kx). The distance between these NPs is around 50 nm. NPs can also be seen in thicker ice in random arrangements, and attached to the strands of lacey carbon film. Some small unilamellar vesicles appear (with low contrast in this image) in the thicker ice with sizes 70 - 100 nm; 6 are outlined in white.

Keywords:

Self-assembly Cryo-EM nanoparticles

Reference:

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Structural organization of the native Neisseria meningitids PilQ environment through an MS-EM approach

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Poster Group 1

Background incl. aims
Neisseria meningitidis are Gram-negative bacteria responsible for life threatening sepsis and meningitis and are human-exclusive pathogens. Disease is driven by polymeric fibers at the surface of bacteria called type-IV pili (T4P), assembled by a multi-protein molecular machine known as type-IV pilus machinery (T4PM). Only the outer membrane and peptidoglycan segment of the T4PM, the PilQ secretin, allows the T4P to be secreted to the extracellular medium and thus it is of great interest for therapeutical approaches and bacterial model design. Because PilQ is reported to be widely different among bacteria, the structure and assembly of this secretin in Neisseria is not known. We therefore aim to solve not only the high-resolution model of PilQ but also describe the proteins involved in its assembly. We finally use this information to develop a piliation-trapped bacteria for cryo-ET.

Methods
For electron microscopy purposes, PilQ was natively purified from N. meningitidis (Strain 8013) ΔsiaD under Biosecurity II conditions. Proteins were loaded into custom carbon-coated Au-CFlat grids and images were collected in a Titan Krios microscope equipped with a Falcon 4i camera (Thermo Fischer Scientific), then processed using diverse processing software, most notably Relion 4 and CryoSPARC 4. Modelling and refinement of PilQ and its associated proteins was performed with Coot and Phenix using information from MS, Alphafold2, deep-etch EM and cryo-EM. Peptidoglycan-binding and protein-protein interaction assays were carried out to support the working model.
Mass spectrometry was performed on cross-linked samples of N. meningitidis pellets harvested from plate cultures. Membranes were crosslinked using UV-aided click-chemistry with NNP9 and digested. Eluted peptides from Photocleavable alkyne agarose beads were analyzed by nanoLC-MS/MS using a Vanquish Neo UHPLC system coupled to an Orbitrap Eclipse Tribrid mass spectrometer fitted with an EASY-Spray Source (Thermo Scientific). Peptides were separated on an EASY-Spray PepMap Neo.

Results
Our cryo-EM map of native NM PilQ at 2.14 Å resolution allowed to fully build an atomic model of all domains of the protein, including its highly-dynamic gate but excluding the flexible AMIN1 and AMIN2 peptidoglycan region. It is a 14-fold symmetry dynamic molecule as opposed to the previously reported C12. This structure allowed to design NM strains with locked conformations of the T4PM for subtomogram averaging purposes.
Both in vitro and crosslink data, combined with knowledge from literature allowed to build the AMIN regions, which tend form dimers, thus pointing to a heptamer-of-dimers structure. Similarly, MS data on NM membranes pointed to three proteins that interacted with PilQ in-situ: TsaP, RmpM and

BamA. This data, along with PGN-binding and BLI assays, allowed to build a working model of the assembly. Crosslinks of interactor-deficient mutant strains also enabled to describe the functionality of the interacting proteins in the assembly of PilQ. TsaP dimerizes PilQ protomers and anchors them to the peptidoglycan, while BamA interacts with RmpM which in turn localizes the PilQ protomers and keeps the secretin on top of the peptidoglycan.

Conclusion
Neisseria meningitidis PilQ is a 14-mer secretin, assembled as a heptamer-of-dimers, driven by TsaP and the propensity of the AMIN region to dimerize. Transitory interactions with BamA and RmpM help localize protomers for oligomerization and position the secretin on top of the peptidoglycan. The structure of PilQ shows a highly-dynamic gate that can be blocked with mutations, thus enabling T4PM-driven piliation steps to be studied in-situ by cryo-electron tomography. Further understanding piliation may enable novel approaches to inhibiting the process and thus, disease.

Keywords:

Secretin, gram-negative, peptidoglycan, cryo-EM, cross-linking

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1233

Parent grain reconstruction of martensitic microstructures: a comparison of different methods

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Poster Group 1

Background
Metallographic techniques have traditionally been used for primary austenite grain analysis. In this study, we compare conventional metallography with selective etching to reveal primary austenite grains within the microstructure and two different parent grain reconstruction software packages, which reconstruct the primary austenitic grains based on orientation relationships between (child) martensitic phase and (parent) austenite phase.
Methods
The samples were prepared following standard metallographic procedures. The samples for optical microscopy were etched with different etchants to reveal the primary austenite grain boundaries. For EBSD analysis the samples were finished with 5 min OP-S final polishing.
For light otical microscopy, we used Microphot FXA, Nikon with 3CCD-videocamera Hitachi HV-C20A and analySIS software. For scanning electron microscopy, two different microscope set-ups were considered: Zeiss CrossBeam 550, with EDAX Hikari Super EBSD detector and OIM 8 software for data processing and ThermoFisher Apreo 2, with Oxford Instruments Symmetry 2 EBSD system and Crystal software for data processing was used.
Results
The etching process highlights grain boundaries, facilitating the identification and measurement of primary austenite grains, and providing valuable information about their size, distribution, and orientation. Complementing the metallographic approach, EBSD can also be used to determine primary austenite grains through crystallographic orientation mapping. EBSD data is processed to reconstruct parent grains, offering high spatial resolution and insights into the crystallographic characteristics of the primary austenite phase.
Conclusion
The presented work shows and compares different ways to reconstruct primary austenite grains from martensitic microstructure.
Light microscopy is by all means a much faster technique, while scanning electron microscopy and electron backscatter diffraction require advanced equipment, additional sample preparation and additional post processing of the acquired data; but can give more impartial results that are not as dependent on the skill of metallographer.

Keywords:

steel, martensite, austenite, microstructure

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Interactions between Titanium Dioxide Particles and Wood Cell Wall Ultrastructurea

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Poster Group 1

TiO_2 is known for its photoactivity and chemically inert behavior. It is widely used in many applications as a pigment or as a UV light absorber. We were interested in what happen when the particle of TiO_2 is in contact with the wood matter and irradiated by UV light. For the experiments, two wood species were chosen: beech (Fagus sylvatica) and pine (Pinus sylvestris). Molecular and physical modifications in coated and uncoated wood exposed to UV radiation were investigated with Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) and transmission electron microscopy (TEM). UV-VIS spectroscopy was used to describe the absorption of UV light by the TiO_2 planar particles chosen for the experiment. It was demonstrated that TiO_2 coating protects wood against photodegradation to a limited extent. TEM micrographs showed fissures in the wood matter around clusters of TiO_2 particles in beech wood.

Keywords:

TiO_2, TEM, UV-VIS, FTIR-ATR

Reference:

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1235

Global BioImaging: Imaging Networks Accelerate Collaboration, Exchange and Innovation in Imaging Science

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Poster Group 1

Global BioImaging mobilizes imaging scientists and facilities from 60 countries and 13 networks, accelerating resource exchange and championing the global impact of imaging. Through inclusive programs, Global BioImaging transcends barriers to foster innovation and community building under the banner of "Imaging 4 All," spanning bioimaging, biomedical, and material sciences.

Global BioImaging offers comprehensive training, job shadowing, and advocacy to empower imaging scientists and influence policy. Collaborative papers and working groups advance topics from career pathways to infrastructure development. Key discussions focus on fair career paths, funder communication, network building, training courses, showcasing core values, and data management.

Imaging networks connect researchers with advanced instruments, enhancing data quality, fostering collaboration, and improving resource efficiency. Shared facilities reduce costs and expand access to cutting-edge technologies.

The synergy between open access and imaging networks drives scientific progress. Open access disseminates high-quality data widely, while imaging networks contribute to openly accessible research, enhancing reproducibility, fostering global collaboration, and driving technological advancements.

Global BioImaging and partner imaging networks are reshaping the scientific community by democratizing knowledge and providing unparalleled tools for exploration and analysis. As anticipation grows for the annual meeting in Japan (#GBI_EoE2024), Global BioImaging continues to accelerate exchange and foster collaboration in imaging science.

Keywords:

#GlobalBioImaging #ImagingScience
#OpenAccess #Training #Innovation

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(3) https://doi.org/10.5281/zenodo.10591588

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Visualization of the Endosomal Fate of mRNA-Lipid Nanoparticles Reveals the Reason for Low Escape Rate

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Poster Group 1

Visualization of the Endosomal Fate of mRNA-Lipid Nanoparticles Reveals the Reason for Low Escape Rate

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Background incl. aims
During Covid-19 pandemic, lipid nanoparticles (LNPs) have proven an indispensable drug for fighting viral infections[1]. They also bear potential as drugs against cancer and in many other fields[2]. They are formed of ionizable cationic lipids, helper lipids, PEGylated lipids and mRNA. They assemble at low pH during manufacturing, taking advantage of electrostatic interaction of cationic lipids with negative charges of the phosphate backbone of mRNA. The LNPs are taken up by cells into endosomes. Transfer of mRNA from the endosome to the cytosol is a prerequisite for the transcription of mRNA into drug effective proteins. Ionizable cationic lipids are thought to hold a crucial functional role because at low pH in late endosomes they should interact with and destabilize the endosomal membrane[3].
Despite vaccines being efficient, it is known that endosomal escape of mRNA is a bottle neck for this technology, being in the low percent range[4]. Visualization of endosomal processing of LNPs is crucial to understand the inefficiency of release.

Methods
Using correlative light and electron microscopy (CLEM) we were able to visualize the LNPs within endosomes for 2 different LNP formulations: lab scale LNPs with mRNA encoding for Firefly Luciferase and the COVID-19 vaccine Comirnaty® produced by Pfizer/Biontech. During administration to dendritic cells, the LNPs were exposed to an RNA stain to confer fluorescence for the correlation. Cells were incubated with LNPs for 6, 16-18 and 24h. Subsequently, cells were high pressure frozen (HPF), OsO_4 and Uranyl-acetate stained, embedded in EPON and sectioned. Confocal images and EM images (SEM and TEM) were registered.

Results
After 6h incubation with lab scale LNPs, we see fluorescently stained particles of ~100nm size in endosomes on the verge of aggregation. After 16-18h the proportion of LNPs in endosomes has increased, coalescence took place resulting in dense layered structures. These structures clearly differ from endogenous multilamellar bodies but resemble TEM images of LNPs[5]. After 24h, endosomes are packed with large aggregates. Disentanglement of the dense stratified layers in some parts of the aggregates is observed. Despite filling > 90% of the endosomes, the aggregates rarely touch the endosomal membrane. However, at few places local ruptures of the endosomal membrane are visible.

Conclusion
We were able to visualize processing of LNPs in endosomes after uptake. We see massive accumulation of LNPs in endosomes over a time period of 24 hours, resulting in coalescence of LNPs. This process has rather been considered to limit the lifetime for storage of vaccine formulations. However, it also has crucial impact on endosomal escape. Release of mRNA is thought to occur via local breakup of the endosomal membrane after contact with cationic lipids followed by release of single LNPs to the cytosol[3]. Our images reveal a different scenario: PEGylation of LNPs minimizes contact with the endosomal membrane in early endosomes. Yet, coalescence of LNPs takes place due to massive accumulation of LNPs in endosomes leading to concentrations orders of magnitude above those in the formulations. Coalescence also increases the density of PEG-groups on the surface, thus further reducing contact between LNP agglomerates and the endosomal membrane. Maturation of the endosomes causes a drop of pH so that ionizable cationic lipids get increasingly positively charged. This leads to electrostatic repulsion of the lipids resulting in the observed disentanglement of the lipid layers in the agglomerates. On the surface of the agglomerates there is local breakup of the lipid layers. Helped by their positive charge, loose strands locally interact with and perforate the endosomal membrane. However, the agglomerates are too bulky to pass through the membrane with their mRNA load and diffusion of single mRNA strands is hampered by their electrostatic attraction to the cationic lipids. Only a complete breakdown of the endosomes would reincrease the pH to levels allowing for efficient mRNA diffusion to the cytosol. Our findings indicate that the very concept of LNP assembly finally limits the mRNA release. Careful adjustment of the apparent pKa value of the ionizable cationic lipids can optimize the release rate.

Keywords:

CLEM, Lipid Nanoparticles, Endosomal Escape

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3D imaging by Array Tomography using benchtop SEM

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Poster Group 1

Background incl. aims
3D imaging is helpful for revealing the internal structure of biological tissues, microbes, and plants. This technique, especially for soft materials in micromorphology, has traditionally been performed using the tomography method with a transmission electron microscope (TEM). In recent years, scanning electron microscopes (SEM) have been used for 3D imaging by array tomography [1, 2], focused ion beam SEM (FIB-SEM), and serial block-face SEM (SBF-SEM). These SEM tomography methods are expected to be useful for many biological specimens because SEM can observe a wider region than TEM. Especially, the array tomography method is very useful due to the following features: fewer limitations on specimen size, the ability to re-observe sections, and the capability to perform correlative light electron microscopy (CLEM).
The array tomography method requires long acquisition times because it involves taking large number of photos over a wide area and through many sections. This method is generally performed using a high-resolution SEM, which is expensive and has a large footprint. Since benchtop SEMs are cheaper and have a smaller footprint, using multiple benchtop SEMs can be expected to increase throughput for large microstructures within a limited budget. Additionally, efficiency can be improved by first using 3D images obtained from benchtop SEMs to narrow down regions of interest. These selected regions can then be analyzed in detail with high-resolution SEMs.
The array tomography method using benchtop SEMs, which are manually operated to acquire images one by one, has been reported [3]. Modern benchtop SEMs, which have a motorized stage, can automatically perform array tomography similarly to high-resolution SEMs. In this report, we compare the 3D image quality between benchtop SEMs and high-resolution SEMs and discuss the possibility of the array tomography method using benchtop SEMs. Furthermore, we introduce the stage position linkage between both SEMs for the purpose of the efficient detailed analysis.

Methods
Paramecium was used as the model sample. Paramecium was cultured in water with gold, silver, and silica nanoparticles for 1 hour. The specimen was fixed with glutaraldehyde and OsO4 and then stained with uranyl acetate. The specimen was embedded in resin and sliced to a thickness of 200 nm. Three hundred sixty-six serial sections were placed on a carbon substrate (Ultra Flat Carbon: UFC, JEOL Ltd.). The serial sections were observed using a benchtop SEM (JCM-7000, JEOL Ltd.) and a high-resolution SEM (JSM-IT800<SHL>, JEOL Ltd.).

Results
3D reconstruction from the images of the serial sections was successful even with the benchtop SEM. The 3D reconstructed image obtained by the benchtop SEM is shown in Fig.1. In the cross-sectional image from the 3D reconstructed image, the paramecium (approximately 150 µm) and the phagosomes (approximately 10 µm) inside the paramecium were clearly recognized. However, in the benchtop SEM 3D image, the aggregation (approximately 1 µm) of nanoparticles in the phagosomes could not be clearly identified. This is because the spatial resolution of the benchtop SEM is poorer

than that of the high-resolution SEM. Despite this, the benchtop SEM was able to perform array tomography and identify the macrostructure of the paramecium.

Conclusion
We succeeded in automatically capturing the images of the serial sections and demonstrated the effectiveness of array tomography on the benchtop SEM. The stage position linkage system between the benchtop SEM and the high-resolution SEM makes it possible to re-analyze the region of interest in detail and effectively.

Keywords:

Array Tomography, Benchtop SEM

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Electrochemical sensors for detection of benzotriazole in water

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Poster Group 1

Background incl. aims
Benzotriazole (BTA) and its derivatives are extensively utilized in various industries for their properties as corrosion inhibitors, UV radiation filters, and plastic stabilizers. BTA is, therefore, found in food packaging, dishwashing detergents, textiles, lubricants, antifreeze, aircraft de-icing fluids, and other commercial and industrial products. [1,2]
Due to its solubility in water and resistance to biodegradation, BTA can persist in the environment. The inclusion of BTA as an additive in dishwashing detergents and tablets leads to its direct discharge into wastewater treatment plants, where it is only partially removed during the treatment process. As a result, BTA is prevalent in natural waters at nanomolar concentrations. While these concentrations may not pose an immediate threat to human health, the persistent nature and potential bioavailability of BTA can lead to long-term environmental consequences, which have yet to be sufficiently investigated. [1,2]
Currently, the primary methods for analyzing BTA in environmental samples involve solid-phase extraction followed by gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry. However, there is a need for cost-effective, fast, reliable, in-situ detection of BTA for real-time monitoring of environmental samples. Given that BTA and some of its derivatives can be electrochemically reduced at low potentials, electrochemical detection on screen-printed electrodes (SPE) emerges as a promising approach. SPEs offer a more cost-effective, portable alternative to traditional electrochemical setups, which use conventional electrodes such as glassy carbon and mercury drop electrodes. In this work, a sensor based on carbon nanotube-Nafion-modified SPE is proposed for the detection of BTA. The modification of SPE was fully characterized via electrochemical and electron microscopy techniques.
Methods
1 mg/mL dispersions of carboxyl-functionalized single-walled or multi-walled carbon nanotubes (SWCNT, MWCNT) were prepared in a 1:1 (v/v) mixture of DMF and water containing 1 wt.% Nafion. The sensor was fabricated by drop-casting 4 µL of the CNT suspension on the working electrode of the SPE (DRP-150, Dropsens, Metrohm) and allowing it to dry at room temperature overnight.
BTA solutions were prepared in Britton-Robinson buffer (pH 2.4) and analyzed using cyclic voltammetry (CV) and square wave voltammetry (SWV). The electrochemical measurements were carried out by the PalmSens4 potentiostat.
The surface morphologies of the commercial SPE-C and modified SPE-SWCNT and SPE-MWCNT electrodes were investigated by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The samples for SEM were embedded in metallic sample holders using conductive, carbon adhesive tape and observed at various magnifications in a field-emission-gun scanning electron microscope (FEG-SEM Verios G4 HP, Thermo Fisher Scientific, USA). Preliminary examinations of the samples showed that they were sufficiently electrically conductive for the SEM analysis. The experimental conditions for imaging were set to an accelerating voltage of 4 kV, a beam current between 20 pA and 50 pA, and a working distance of 3 mm. The electron micrographs were recorded with two detectors: (i) a through-the-lens detector (TLD) for secondary electrons (SE) and

(ii) a mirror detector (MD) for backscattered electrons (BSE) positioned within the objective lens. To observe the SWCNT and MWCNT structure and size, transmission electron microscopy (TEM) with energy-dispersive X-ray spectroscopy (EDS) was used (JEOL JEM-2100, Jeol Ltd., Tokyo, Japan). The samples for TEM observations were prepared by dispersing the nanoparticles after ultrasonic deagglomeration in water onto lacey carbon-coated Cu grids.

Results

The sensors' receptor element was based on modified commercial screen-printed electrodes (SPE). The drop-casted material's coverage of the working electrode (SWCNT or MWCNT) was assessed using electron microscopy to observe the morphology, composition, and conductivity of the material. As seen in Fig. 1a, the SWCNTs are anchored to the working electrode by a Nafion membrane, resulting in satisfactory surface coverage and conductivity. The MWCNT showed similar results with a grass-like structure and Nafion presence.

At the next step, the electrochemical behavior of BTA on the SPE-C/SWCNT was evaluated via CV. BTA exhibited a single reduction peak around –1,4 V with no observable oxidation peak during the reverse scan. The SPE-C/SWCNT exhibited significant adsorption capacity for BTA. As a result, SWV analysis was performed after a 60 s preconcentration period at –1 V. With this approach, a limit of quantification (LOQ) was determined, and the limit of detection (LOD) was calculated from the calibration curve (Fig 1b). The LOQ was 10 2 µM and LOD of 2 µM. This LOD is comparable to that obtained for BTA detection using commercially available SPE with a SWCNT working electrode [2], indicating that homemade modified SPEs offer a versatile alternative to commercial counterparts. Furthermore, by adjusting the composition, it may be possible to achieve an even lower LOD, comparable to those achieved using modified glassy carbon electrodes [3], making the proposed sensor suitable for real sample monitoring.

Figure 1. a) SEM image of the working electrode surface modified with SWCNT and Nafion and b) Calibration curve of the reduction peak current recorded on SPE-C/SWCNT.

Conclusion

Benzotriazole and its derivatives are ubiquitous in industries due to their diverse applications, yet their persistence in the environment poses ecological concerns. This study proposes a carbon nanotube-Nafion-modified screen-printed electrode for electrochemical BTA detection, addressing the need for cost-effective and rapid monitoring methods. Electrochemical analysis revealed a single reduction peak, leading to an LOD of 2 µM with square wave voltammetry. This approach offers a comparable alternative to commercial electrodes, underscoring its potential for environmental monitoring.

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Keywords:

Benzotriazole, electrochemical sensor, SWCNTs, microscopy

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Microstructural characterization of neutron-irradiated ITER specified tungsten grades

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Poster Group 1

Tungsten, as a candidate material for the plasma-facing component of the ITER reactor, will endure severe operational conditions. Recent studies have reported radiation-induced hardening and DBTT profiles for various tungsten grades, which needs to be supported by microstructural analysis.
In this study, neutron-irradiated, various tungsten grades are investigated using transmission electron microscopy (TEM) in order to comprehend the microstructural stability of tungsten under fusion-relevant conditions. Irradiation experiments were conducted at BR2 reactor at temperatures 600 C and 1000 C with 1 dpa dosage, which is the expected lifetime in ITER. Characterization of the three main radiation-induced defects (dislocation loops, nanovoids and transmutation precipitates) includes defect density, size, morphology, decoration and spatial distributions for each tungsten grade. Chemical analysis provides insights into the decoration of ODS precipitates and radiation-induced defects. The observed trend of increased radiation-induced hardening from irradiation temperatures of 600 C to 1000 C is attributed to changes in defect densities and chemical composition.
This research enhances our understanding of tungsten's microstructural response to neutron irradiation, offering valuable insights for the development and performance assessment of plasma-facing materials in fusion reactors.

Keywords:

ITER, tungsten, neutron-irradiation, TEM

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Volume Electron Microscopy: A Powerful Tool for Studying Embryonic Pineal Organ Development in Birds

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LS-07 (2), Lecture Theater 5, august 28, 2024, 14:00 - 16:00

The structure of the pineal organ in birds exhibits significant species variability, with three main types identified: saccular, tubule-follicular, and solid, along with several intermediate forms. The parenchyma of the most commonly observed tubule-follicular type includes the pineal canal, a primary evagination of the neuroectoderm of the diencephalon roof, and numerous follicles. Three types of pinealocytes (receptor pinealocytes, rudimentary-receptor pinealocytes, and secretory pinealocytes) and two types of supporting cells (ependymal-like and astrocyte-like cells) have been identified in the avian pineal organ at the electron microscopy level. However, two-dimensional studies have been insufficient to answer many questions about the spatial organization of pineal parenchyma.
This study utilized volume electron microscopy to investigate the structure of the embryonic pineal organs of the domestic turkey and the domestic goose, chosen for their substantial differences in pineal parenchyma organization. The embryonic turkey pineal organ features follicles with a large lumen surrounded by a thin layer of parenchymal cells, resembling pseudostratified epithelium. In contrast, the pineal parenchyma of goose embryos consists of two distinct parts: follicular areas composed of elongated cells surrounding the follicular lumen and parafollicular areas, which are peripheral to the follicular region and consist of several layers of cells.
The pineal organs of 18-day-old turkey and goose embryos were fixed and contrasted according to a modified protocol by Deerinck et al. (1). Serial block face imaging (SBFI) was performed using 3View 2XP (Gatan, USA) and OnPoint detector (Gatan, USA) operating in EF-SEM Gemini 450 (Carl Zeiss, Germany) with the focal charge compensation system. Stacks of 300-1000 images (15,000 x 15,000 pixels, pixel size 5 nm, section thickness 50 nm) were manually segmented using MIB software (2), and the models were visualized using Amira 3D (Thermo Fisher Scientific, USA).
In the embryonic pineal organ of the domestic turkey, two types of parenchymal cells were identified and classified as embryonic rudimentary receptor pinealocytes and ependymal-like supporting cells. These cells were present in both the pineal follicles and the pineal canal, with structural differences depending on their location. SBFI revealed that rudimentary receptor pinealocytes typically do not contact the basement membrane limiting the follicles and the canal. The basal parts of rudimentary receptor pinealocytes in the follicles had the shape of an inverted cup, with their outer surfaces in contact with ependymal-like supporting cells. In the pineal canal, pinealocytes formed long basal processes that created flattened fragments lying in invaginations of ependymal-like supporting cells. Up to five flat parts of different rudimentary receptor pinealocytes were found in one invagination. Ependymal-like supporting cells had a broad basal portion resting on the basement membrane. Both rudimentary receptor pinealocytes and ependymal-like supporting cells had regular internal organization. In pinealocytes, the organelles were distributed as follows: 1) nucleus in the basal part of cell, 2) accumulation of endoplasmic reticulum cisterns on the upper pole of the nucleus, 3) Golgi apparatus, and 4) mitochondria. The apical protrusion of pinealocytes had a 9+0 cilium and contained endoplasmic reticulum and mitochondria. A key feature of rudimentary receptor pinealocytes was the accumulation of mitochondria in the upper part of the cell, with SBFI showing these mitochondria as elongated and parallel to the cell's long axis. Some mitochondria formed long strands from the cell

apex to the perinuclear region. In supporting cells, mitochondria were present throughout the cytoplasm, including the basal process. Secretory pinealocytes and astrocyte-like supporting cells were not found in the turkey pineal organ.
Special attention was given to mitotic cells since precursor cells of pinealocytes and supporting cells have never been described in the avian pineal organ. Cell divisions were more numerous in the pineal canal than in the follicles. Mitotic cells always contacted the canal lumen by short apical processes and had long basal projections ending in numerous fine, parallel-running processes that did not attach to the basement membrane. Daughter cells maintained contact with the lumen and remained connected by a thin cytoplasmic bridge near the canal lumen for an unknown period. These cells lacked a polarized distribution of organelles, leading us to hypothesize that differentiation results in the characteristic distribution of organelles seen in pinealocytes or supporting cells.
Rudimentary receptor pinealocytes in the pineal canal varied in the proportion of cell body length to basal process length. Cells with short bodies and long basal processes exhibited numerous deep invaginations of the nuclear envelope. Occasionally, large parts of the cytoplasm of these pinealocytes, connected to the cell body by a narrow band, were located in the lumen, suggesting that the upper parts of these cells are removed into the lumen. The same process was observed in the pineal follicles. Since undifferentiated precursor cells were not found, we hypothesize that old pinealocytes lose the upper part of their cytoplasm and re-enter the cell cycle.
In the goose pineal organ, follicular areas were mainly composed of cells resembling rudimentary receptor pinealocytes, which created long processes penetrating into the parafollicular areas. Parafollicular areas were primarily composed of embryonic secretory pinealocytes. Supporting cells were exclusively found close to the basement membrane and were largely infrequent due to the parenchyma's poor division by connective tissue, resulting in the majority of cells not contacting the basement membrane. The cells of the parafollicular region formed specific contact sites bordered by tight junctions, created by both cell bodies and cell processes. Secretory pinealocytes showed significant variability in shape and the presence of very long processes. Their cytoplasm often contained three zones with the Golgi apparatus, endoplasmic reticulum, and mitochondria, though not arranged in series.
Funded by the Minister of Science under the Regional Initiative of Excellence Program.

Keywords:

3D-ultrastructure, VEM, pineal organ

Reference:

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1248

TEM strain measurement methods on SiGe/Si films for accuracy tests

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Poster Group 1

Background incl. aims
Transmission electron microscopy (TEM) is still considered a key analysis technique when high spatial resolution is required, even considering the generally lengthy, difficult and sometimes troublesome sample preparation. Such important advantage applies to imaging, structural and diffraction analysis and analytical investigations. The analysis and quantification of strain fields in crystalline materials also takes advantage of the TEM spatial resolution and over the last decades several TEM based techniques were developed [1].
In the framework of the CHALLENGES project financed by the EC (Grant agreement 861857 [2]), several methods have been used to validate the results of Tip Enhanced Raman Spectroscopy (TERS) in the characterisation of semiconductors (Si based) wafers and structures. Strain was one of the physical quantities measured in either thin film or patterned structures and TEM methods were used as standard reference. A series of three Si1-xGex heterostructutres, deposited as thin films onto a Si substrate, at different Ge concentration and thickness (see Table I), were used as 'standard strain' samples in order to compare all the techniques adopted (that includes optical based as well as diffraction based ones, such as TEM and X-ray Diffraction, XRD) onto the same samples. The Ge nominal concentration and SiGe film thickness were #1: 20%/45 nm, #2: 30%/29 nm and #3: 40%/22nm, respectively.
We therefore took this opportunity to test different standard TEM strain analysis methods, namely Convergent Beam Electron Diffraction (CBED), Geometric Phase Analysis (GPA) and standard Selected Area Diffraction (SAD), in such samples using a conventional TEM, with the aim of evaluating the accuracy of the measurements with particular reference to the strain relaxation related to the lamellae thickness.

Methods
Thin cross-sectioned lamellae for TEM analysis have been prepared from the wafes using a Zeiss XBeam 340 FIB, with Ga ions accelerated at 30 KeV, then at 5 and 2 KeV for a final polishing to reduce the amorphous and crystal damaged layers thickness at the thinned surfaces. In each lamella a series of 3 thin regions at different thicknesses were prepared. The local crystal thickness in each region was evaluated by means of two-beams (004) CBED and resulted of about 100 nm, 200 nm and more than 450 nm, from the thinner to the thicker ones in each lamella.
The TEM measurements were performed in a FEI Tecnai F20T equipped with a Schottky emitter and operated at 200 kV accelerating voltage.
The samples film strains have also been measured with high resolution XRD using a Rigaku SmartLab X-ray diffractometer.

Results
CBED strain analysis was performed in STEM mode by obtaining diffraction pattern along several lines proceeding from the substrate to the surface after having oriented the sample along the <230> zone axis [3]. The CBED measurements were performed only in the thicker (>450 nm) regions of the

sample. It was possible to obtain good and measurable patterns only up to regions close to the Si/SiGe interface. In the centre of the film the High Order Laue Zones (HOLZ) lines split or blurred out, thus preventing any measurement.
Since strain values were of the order of 10-2 and the film thicknesses were at least 20 nm it was possible to use also conventional SAD that showed comparable results with those obtained with the more sophisticated and instrumental requiring Nano Beam Diffraction (NBD). The SAD measurements showed a split of the (400) reflection family, corresponding to the Si and the SiGe regions, confirming that the crystal deformation was uniaxial in all samples, i.e. along the growth direction, as expected in a tetragonally distorted crystal. The mismatch values obtained vary considerably in the same sample at different thicknesses of the lamella: in Table 1 the mismatch values obtained in the thicker regions are reported.
Finally, good quality HREM images were recorded only in the thinner area of the samples, in both film and substrate, and from these we obtained the corresponding mismatch values from GPA analysis. From this analysis too the distortion showed an uniaxial behaviour but also smaller mismatch values if compared with those obtained by SAD in thicker regions.
All the results obtained are summarised in Table I, together with the measured Ge concentration (as obtained by energy dispersive x-Ray spectroscopy) and High Resolution XRD (HRXRD) measurements. From the results shown in the table it is quite evident that phase analysis gives lower values for the measured mismatches, and it reasonable to assume that a larger strain relaxation occurs here since the sample should be thinned to a lower thickness value to collect HREM images. Moreover, samples #1 and sample #2 show a similar residual strain, while sample #3 keeps a much higher value. This could be due an increased rigidity in sample #3 related to the much smaller film thickness. The small discrepancy between SAD and HRXRD results suggests that small strain relaxation could still occur even in thicker areas.

Conclusion
The application of well established TEM techniques for crystal strain evaluation in simple SiGe/Si heterostructures showed some limitations. Despite the High strain sensitivity, CBED confirmed to be ineffective in samples regions were an high strain gradient or a strong strain relaxation occur. Both GPA and conventional diffraction were able to measure the uniaxial deformation, but best results were obtained in thick lamellae (>450 nm), thus confirming that the relation between strain vs. local sample thickness plays an important role in measurement accuracy.

Keywords:

Transmission Electron Microscopy, Crystal Strain

Reference:

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1249

FlexAble Labeling of Primary Antibodies with Fluorescent Dyes and Biotin for Multiplex Experiments

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Poster Group 1

Background incl. aims:
Multiplex immunostaining has become an increasingly important approach in current biomedical research. However, the availability of directly labeled primary antibodies often poses a bottleneck for multiplex methods. Here we aimed to develop an antibody-labeling process that enables fast and easy conjugation of primary antibodies to fluorophores at a microgram to milligram scale, e.g. for the amount needed for one immunostaining experiment.
Methods:
For the development of this labeling procedure, we employed a broad range of methods including in silico protein design, molecular cloning, protein expression, protein purification, site-directed conjugation, BLI, DLS, cell culture, IF, IHC, flow cytometry and X-ray crystallography. Successful application of the developed labeling technique takes around 10 minutes and does not require any special equipment or proficiency in chemical conjugation or antibody purification.
Results:
We designed peptide-based multivalent linkers that bind to primary IgGs from rabbit, mouse, rat or human species with an apparent dissociation rate koff (1/s) below 0.0001 and covalently conjugated them to different fluorophores (CL+405/488/555/647/750). We validated the specificity of the binding between the linkers and their target IgGs and confirmed the proposed molecular arrangement with X-ray crystallography. Since these linkers are fluorescent, they offer a simple and convenient tool to functionalize primary IgGs from the aforementioned species with the desired fluorophores. We called this labeling approach FlexAble labeling because it embodies the simple steps of mixing and briefly incubating linkers and primaries. This procedure can be performed virtually in any laboratory for any amount of primaries right before applying the antibodies in the desired assay. Moreover, primary antibodies can be in any buffer, since glycerol or other additives do not interfere with FlexAble labeling.
Here, we show that FlexAble-labeled antibodies are suitable for immunofluorescence stainings, especially when multiplexing of primaries from the same species is required. Also, we present examples of FlexAble applied to flow cytometry, WB, tissue IF, live time-lapse imaging, cyclic IF, and other more advanced experiments employing immunofluorescence detection of the target molecules.
Conclusion:
We have established FlexAble, a novel antibody labeling process that enables rapid conjugation of primary antibodies to fluorophores and facilitates multiplex immunofluorescence experiments. We are expanding this method further to expedite labeling of primary antibodies with reporter proteins (HRP, PE, APC), biotin, DNA oligonucleotides, rare-earth metals etc. to support multiplex flow cytometry, nucleotide barcoding (e.g. 10x Genomics) and IMC (e.g. CyTOF).

Keywords:

Multiplex, immunofluorescence, antibody conjugation, FlexAble

Reference:

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Comparative Analysis of Sample Preparation and Imaging Methods for Metallographic Examination of Archaeological Silver

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Poster Group 1

Background
There are two principal considerations for metallographic examination of historical metal artefacts: first, the selected portion of the material must be representative of the object; second, the sample preparation procedure must ensure minimal destruction to the archaeological object. Sample preparation is a critical step in the metallographic analysis of archaeological metals and is a prerequisite for accurately determining the microstructure and elemental composition using SEM, SEM-EDS, EPMA, and EBSD techniques. The quality of the sample surface is crucial for accurate metallographic analysis: surface damage, surface contamination, and limited material availability are key factors that limit the reproducibility and accuracy of structural and analytical results. The sample state (corrosion), area of interest (surface vs. bulk), analytical technique, and intended purpose (information required) play major roles in selecting the optimum sample preparation.
Methods
The 17th century silver coins and artefacts from the Batavia shipwreck of the Dutch East India Company's (VOC) flagship from Western Australian Museum collection have been analysed using STEM and SEM detectors in FEI Verios 460 SEM and FEI Helios FIB-SEM. The samples for SEM examination have been prepared by ultramicrotomy (Leica EMFC7 Ultramicrotome Microsystem equipped with Diatome diamond knife), focused ion beam milling (the FEI Helios Nanolab G3 CX DualBeam FIB), and laser micromachining tool (3D Micromac microPREP).
Results
Various sample preparation methods and their outcomes have been compared to reveal their advantages and limitations for metallographic applications in the archaeological silver artefacts. The outcomes are demonstrated below in Figure(a) 100nm slice prepared from a silver alloy using ultramicrotomy, imaged at 30kV using the TLD detector; Figure(b) sectioned and polished silver alloy sample with the laser micromachining tool, imaged at 20kV using CBS detector; Figure(c) cross-section prepared by focused ion beam milling, imaged at 5kV, 1.4nA, using the ICD detector.
Conclusion
All methods are excellent sample preparation options for archaeological metal samples, such as silver objects and coins when the best surface quality and minimal destruction is required. Additionally, the most suitable protocols for sample preparation, imaging, and analytical studies have been found specifically suitable for historical silver alloys.

Keywords:

metal alloys, heritage metals, SEM

Reference:

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D.A.Scott, R.Schwab in " Metallography in Archaeology and Art" Springer 2019

1251

Phase Transformation of GeO2 Glass to Nanocrystals via High-Temperature Annealing

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Poster Group 1

Background
Germanium dioxide (GeO2) is an advanced semiconductor material characterized by an ultrawide band gap ranging from approximately 4.6 to 6.2 eV [1], depending on its concrete crystal phase and doping level. This wide gap renders GeO2 well-suited for high-power and high-frequency electronic devices. Establishing precise correlations between thermal processes, structure evolutions, and electrical properties is essential for understanding fundamental mechanisms and achieving high-performance semiconductor materials.
This study utilizes SEM, XRD, and TEM to investigate the phase transformations of GeO2 from amorphous to crystalline phases triggered by a controlled annealing process.
Methods
An approximately 1 µm thick amorphous GeO2 layer was deposited on c-plane sapphire (Al2O3 {0001}) wafers at 300 °C by plasma-assisted molecular beam epitaxy (PAMBE). These layers were subsequently subjected to annealing in an oxygen environment at temperatures of 1000°C, 1100°C, and 1200°C for a duration of six hours. The resulting samples were directly analyzed by SEM and XRD. And the cross-section samples were prepared by FIB for TEM investigations.
Results
The figure depicts bright field TEM images, high-resolution TEM images, and corresponding fast Fourier transition (FFT) patterns of GeO_2 layers subjected to thermal treatment at temperatures of 1000°C, 1100°C, and 1200°C, respectively. After annealing at 1000°C for 6 hours (figures a-c), mainly circular, with occasional hexagonal crystalline grains of quartz phase GeO2 (α-GeO2) emerged in the amorphous matrix, typically between 10-20 nm in size. With further heating to 1100°C, these nanograins, as seen in figures d-f, evolved within the amorphous matrix to larger crystals between 10 and 60 nm. Further annealing under 1200°C (figures g-i) completely transformed the amorphous phase into crystalline. Meanwhile, a phase transition from the hexagonal quartz to the tetragonal rutile was observed. This transition coincides with non-homogeneous grain growth in GeO2, i.e. one grain grew to over 20 times the mean grain size depicted in figures (g, h). Such grain growth highlights the dynamic nature of the phase transformation process within the GeO2 layers when subjected to elevated temperatures. Further results, such as the XRD patterns and ESD analysis, will be presented on-site.
Conclusion
This comprehensive investigation provides insights into the structural evolution and phase transformations of GeO_2 under varying thermal conditions. It offers valuable information for further understanding of the properties and behaviors in different temperature regimes.

Keywords:

GeO2, phase transformation, grain growth

Reference:

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1253

Computational analysis of the anisotropy of SEM images to quantify nanowire verticality and surface roughness

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Poster Group 1

Background incl. aims
Surface texturing on micro and nanoscale plays a fundamental role in many applications of nanotechnology due to its effects on almost all surface properties such as wetting, wear, reflectance, bioactivity to name just a few. A chief feature of surface texture is its isotropy/anisotropy. Anisotropy (i.e. directionality of surface texture) is encountered in a wide gamut of cases since it can be caused by a plethora of processes on different scales and can have strong impact on applications at a variety of scales. Based on its importance, the characterization of surface texture anisotropy has been incorporated in the ISO 25178-2 and two parameters have been proposed to characterize the degree and directionality of anisotropy using the autocorrelation function and Fourier transform respectively. The ISO characterization assumes full measurement of 3D topography by 3D profilometer or AFM. However, since height information is not critical in anisotropy identification, SEM images can also be used and provide a straightforward way to surface anisotropy characterization.

Moreover, surfaces with nanowires have been used in various fields of applications from electronics and photonics to bio-devices and energy harvesting systems. One of the critical properties of nanowire patterns is their alignment and verticality since it tunes the contact area with the attached body and hence their performance in their applications.

The aim of this work is firstly to implement a methodology for the quantification of SEM image anisotropy based on 2D Fourier spectrum analysis to conclude about surface anisotropy and secondly to characterize indirectly the verticality of nanowires (NWs) developed on substrates.

Methods
Here, we propose a fast and easily implemented method to characterize the degree of surface anisotropy1, which can also be applied to surfaces with nanowires to assess their verticality. First, we apply a threshold to the Fourier spectrum values to identify the low-frequency region in the 2D Fourier space with amplitudes higher than this threshold. Then, we calculate the anisotropy ratio A by dividing the smallest radius of this region by the largest one and then substract it from 1 (see Fig. 1). An A value close to 0 indicates an isotropic surface, whereas a value close to 1 indicates an anisotropic surface.
A top-down SEM image of a fully vertical nanowire (NW) with a cylindrical shape is isotropic, as it displays the circular shape of its cross-section. When the NW is tilted from verticality, the SEM image shows a portion of its axis, indicating local anisotropy. Therefore, the deviation from full verticality can be quantified by an index characterizing the anisotropy of NW spots in top-down SEM images.

Results
Our methodology was applied to SEM images of inclined polymer surfaces after plasma etching with oxygen gas. We observed that at the bottom part of these surfaces, near the electrode plate of plasma reactor, anisotropic morphologies were emerged, forming horizontal ripples at large

inclination angles2 (see Fig. 2). As we are moving upwards, there is a noticeable transition from anisotropy to isotropy with ripples qradually replacing by nanowire bundles. Using Fourier-based anisotropy indices, we can quantify this transition and establish a link to plasma etching conditions and inclination angles. Figure 2 illustrates the change in low-frequency Fourier contour lines (blue for bottom areas vs. red for top areas), from which we calculated the anisotropy index A increase from 0.13 at the top to 0.82 at the bottom.
To study the change in the verticality of the nanowires, Figure 3 presents the anisotropy index used to assess the verticality of nanowires on the polymer surface before and after water condensation. The anisotropy index, A, decreased from 0.51 to 0.26, indicating degradation of nanowire verticality by nearly 50%.

Conclusion
In conclusion, we developed a fast and easily implemented method to characterize surface anisotropy, which is also applicable to assessing the verticality of surfaces with nanowires. This method successfully quantified the transition from anisotropic to isotropic morphologies on inclined polymer surfaces after plasma etching with oxygen gas, linking these changes to etching conditions and inclination angles. The anisotropy index proved to be an effective tool in determining nanowire verticality, demonstrating significant sensitivity and accuracy. These findings pave the way for optimizing plasma etching processes and enhancing the design and fabrication of nanowire-based materials and devices.

Keywords:

anisotropy, Fourier spectrum, Nanowire verticality

Reference:

1. Quantitative characterization of nanowire verticality using SEM images (to be submitted)
2. Plasma-induced maskless formation of periodic nanoripples on polymeric substrates (to be submitted)

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Cathodoluminescent and Characteristic X-ray-emissive Rare-Earth-doped Core/Shell Protein Labels for Spectromicroscopic Analysis of Cell Surface Receptors

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Poster Group 1

By unlocking previously unattainable scales, microscopy proved pivotal for our today's understanding of the fundamental processes in living systems and enabled future-shaping discoveries. However, understanding the interactions of biomolecules at the nanoscale and putting them in a cellular context remains a major challenge as light diffraction events limit the achievable resolutions. Electron microscopy (EM), unlike light-based techniques, gives access to the cellular ultrastructure yet results in grey-scale images and averts unambiguous (co-)localization of biological targets without electron-dense labels or correlative approaches.
This work presents multimodal nanoparticle-based protein labels for correlative cathodoluminescence electron microscopy (CCLEM) and energy-dispersive X-ray spectromicroscopy (EDX-SM). By incorporating rare-earth dopants in a low phonon host lattice and shielding them from their environment using an inert shell, the characteristic cathodoluminescence (CL) and characteristic X-ray emissivity of sub-20 nm nanoparticles were utilized as unique spectral fingerprints for precise label identification and localization. The core/shell nanoparticles were decorated with either folic (terbium-doped) or caffeic acid (europium-doped), bridging the single-particle response to a biological entity. Their potential for (protein-)labelling was examined using HeLa cells expressing different surface receptors that bind to folic or caffeic acid, respectively.
Single-particle cathodoluminescence along with a distinctive energy-dispersive X-ray signal was successfully presented for both populations, with the latter outcompeting CL as response for ColorEM. EDX-SM persuaded with swift imaging times well below 2 mins per µm² while offering high resolution with a pixel size of 2.78 nm, enabling the observation of biological relevant areas.
Taken together, these results pave the way for multi-color labelling based on electron spectromicroscopy and show its unleashed potential for the study of structure-function relationships.

Keywords:

Nanoparticle, Ultrastructure, Protein Targeting, Multi-Color

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Observation of spontaneous fluctuations in product selectivity of the acetylene hydrogenation reaction in operando TEM

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Poster Group 1

Background incl. aims
The selective hydrogenation of acetylene to ethylene is an important step in the purification of feed streams for the production of polyethylene.¹ A key finding from Teschner et al. describes the beneficial influence of subsurface C species on the selectivity of the reaction.² In that study, spontaneous fluctuations of the product selectivity were also observed under adiabatic conditions. The aim of our ongoing study is the detection of dynamic motifs and structural changes, which govern the occurrence of the fluctuations in the product selectivity. Specifically, we focus on the detection and elucidation of the role of transient and non-transient C species, as mediator for the catalytic performance.
Methods
In the framework of the CATLAB project, the development of thin film catalysts are conducted using existing preparation tools previously employed for thin film photovoltaics. Using a sputter deposition technique results in open networks of aggregated nanocrystals of the catalyst metals of interest, termed laterally condensed catalysts (LCCs). In this fashion, nominally 3nm thick Pd or Pd₆₀Au₄₀ alloy LCCs were deposited on DENS Solutions climate chips (SiNx windows). Using a home built gas-feeding station coupled to a quadrupole mass spectrometer, the reaction and product composition can be followed in real-time.³ Simultaneously, the structural parameters of the LCC are observed by TEM, electron diffraction (ED), and pair distribution function analysis of ED (ePDF).⁴
Results
To the best of our knowledge, the (semi)-hydrogenation of acetylene has not been observed in an operando TEM study prior to our experiments. A surprising result of our first experiment conducted at 150 °C was the observation of the aforementioned fluctuations in selectiviy and activity. and, second, that the product composition did not change, when the chip temperature was reduced to 20 °C (Figure 1). The observation of the fluctuations was confirmed by a second experiment conducted entirely at RT, while the absence of any product formation or fluctuations was confirmed in a blind experiment conducted with the empty operando cell. The high rate of conversion at RT exemplifies the exceptionally high activity of the LCC. However, this also indicates that catalyst material present in the non-heated and non-observable areas of the operando cell may have a significant contribution to the signal detected by the MS. Further, the results of supporting operando TEM experiments are discussed. These show the high potential of the ePDF method for operando measurements on nanocrystalline and amorphous catalysts in showing the formation/decomposition of the beta-PdH phase, and the measurement of crystalline domain growth, sintering and dewetting of the LCC.
Conclusion
In conclusion, the LCC seems to present itself as a highly promising platform for operando TEM, as its preparation is highly reproducible, the particle distribution very homogeneous, the metal layer very thin, and due to the high activity in the acetylene hydrogenation reaction, the product detection is

facile. So far, we could not detect structural changes that correlate with the fluctuations in the MS data. However, this raises the question about how local the origins of these effects are. Our future experiments aim to limit the sputter-deposited LCC to the heatable and observable region of the operando chip by using a specifically designed sputter mask.
Caption to Figure 1. Mass spectroscopy of the acetylene hydrogenation reaction showing spontaneous fluctuations observed in an operando TEM experiment. A Plot of the heater chip temperature and measured heater power (top), together with partial pressures of the relevant educts and products detected by QMS (bottom). B Calculated rel. selectivity (top) and activity (bottom) for the ethylene formation.

Keywords:

Operando, catalysis, ePDF, acetylene hydrogenation

Reference:

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A systematic study on PtRu alloy composition for catalytic applications

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Poster Group 1

Background incl. aims
In order to reduce CO2 emissions in the areas of energy supply and consumption the use of fuel cells (FC) is inevitable. The choice of the catalyst material for the electrochemical reaction to convert H2 in electricity plays a major role. For this reaction the system of PtRu catalyst nanoparticles is well studied and already used in commercial application. However, for the PtRu phase diagram at ambient temperatures empirical data is not available. Thus, only Thermo-Calc generated phase diagrams down to room temperature exist.
Until now just few compositions of the PtRu nanoparticles are investigated in terms of structure and their performance since it is challenging and time consuming to synthetize nanoparticles with different specific compositions. In this work we perform a systematic investigation on the different chemical compositions of PtRu alloys and the influence on their micro- and nanostructure using a thin film.

Methods
The thin film was deposited on an oxidized silicon wafer via combinatorial magnetron sputtering. With positioning the Pt target and the Ru target in an 180° angle to each other a geometrical concentration gradient is generated. The surface of the thin film was analyzed in a Scanning Electron Microscope (SEM) followed by Energy dispersive X-ray Spectroscopy (EDXS) to determine chemical composition and distribution of the elements. Using a focused ion beam (FIB) lamellae were lifted out on specific targeted areas. Theses were analyzed with a Transmission Electron Microscope (TEM) to obtain the local structure and composition. The software Thermo-Calc was used to generate a phase diagram of the PtRu alloy down to room temperature.

Results
The deposited thin film had a smooth surface with no macroscopic defects. The atomic concentration range goes from Pt:Ru 58:42 at % - 17:83 at % along the sample. The thickness of the thin film was determined to be 115 nm ± 3 nm based on TEM micrographs. Additionally, the TEM micrographs taken from cross sections of the thin film showed columnar grains with 13 nm ± 4 nm width and the height of the thin film. With lamellae from areas of targeted positions EDXS was performed in the TEM to verify the validity of the calculated phase diagram. EDXS maps revealed that a two-phase material exists in regions where the calculation suggests a single phase material.

Conclusion
Combinatorial magnetron sputtering can be used to create thin films with a concentration gradient. A systematic study on the influence of the chemical composition on the structure was performed. This is necessary for a follow-up investigation of the electrochemical performance and its dependence on the composition and atomic structure. Correlation of structure and electrochemical experiments is key for future catalyst design.

Keywords:

PtRu, thin film, composition, catalyst

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Correlative light electron microscopy for improved investigation of subcellular GLUT4 distribution in human skeletal muscle

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Poster Group 1

Background incl. aims
Insulin stimulated glucose uptake into skeletal muscle tissue is facilitated by translocation of the Glucose Transporter Type 4 (GLUT4) from intracellular storage sites to the cell surface, a well-established site of impairment in obesity linked insulin resistance. Despite impaired GLUT4 translocation, canonical signal transmission via the Insulin receptor substrate-1 is frequently observed to be normal, and human fractionation studies indicate that intracellular GLUT4 is missorted in individuals with insulin resistance. Exercise acutely promotes skeletal muscle glucose uptake by insulin-independent GLUT4 translocation and improves skeletal muscle insulin sensitivity independent of present insulin resistance in the hours following exercise - a phenomenon coexisting with the redistribution of GLUT4 to well-known insulin responsive subcellular compartments in metabolically healthy individuals. Whether the subcellular localization of GLUT4 is distorted in obesity linked insulin resistance and is corrected by a single insulin-sensitizing bout of exercise has not yet been investigated by high resolution microscopy-based approaches.
The aims of this study were 1) to establish and apply a quantitative correlative light electron microscopy (CLEM) workflow on human skeletal muscle biopsies, and 2) to investigate the subcellular distribution of GLUT4 in skeletal muscle single fibers of metabolically healthy vs. insulin resistant individuals prior to, immediately after and 3 hours into recovery from a single exercise bout.
Methods
Skeletal muscle biopsies were obtained from m. Vastus Lateralis of normal weight (BMI: 22.8 ± 1.7) and obese (BMI: 36.2 ± 3.1) young men (age 25-35y) (n=5) before, immediately after, and 3 hours into recovery from an acute bout of bicycling exercise (65% VO2max, 30 min). Immediately after excision, biopsies were chemically fixed for light and electron microscopy. Muscle fiber bundles were teased apart to single fibers, labelled with a GLUT4 antibody and subsequently FluoroNanogold, counterstained with Hoechst, embedded in agar on a glass slide, sealed with a coverslip and imaged using a Leica SP8 confocal microscope equipped with a 40x oil objective. For each muscle fiber, a full fiber surface tile scan was obtained using transmitted light followed by a single-tile 10µm z-stack for the wavelengths of interest. The specific tile was chosen based on visual landmarks (bends, curves, non-fiber substances etc.) for optimal recognition of the area in the subsequent electron microscopy imaging. Following gentle removal of the coverslip, in-agar fibers were further processed directly on the glass slide for electron microscopy. The procedure included silver enhancement of the FluoroNanogold particles, chemical post-fixation, lipid and nucleic acid staining and graded resin infiltration and polymerization. The specific areas of interest from confocal imaging were identified under a stereomicroscope and targeted for in-resin ultrathin (70 nm) sectioning. The sections were mounted on grids and imaged on a Tecnai T20 transmission electron microscope. Tile imaging was performed blinded along the edges of the fiber prior to low-magnification, full-section overview imaging. Overview images containing bleached areas were used in combination with confocal and

stereomicroscope images to overlay the corresponding light and electron microscopy images and confirmed by the shape, size and distance between nuclei.
Results
Insulin resistance was confirmed in participants with obesity both in the fasting state by the homeostasis model assessment 1 of insulin resistance (HOMA1-IR) (obese: 3.7 ± 2.1 vs. lean: 0.7 ± 0.1, p=0.02) as well as in response to an oral glucose tolerance test by the Matsuda Index (obese: 3.4 ± 1.5 vs lean: 15.7 ± 3.6, p=0.0001). The CLEM workflow was successfully implemented, and data are currently being analysed for the accumulation of GLUT4 in various subcellular compartments prior to, immediately after, and 3 hours into recovery from a single insulin-sensitizing bout of exercise.
Conclusion
In this study, we successfully established a novel quantitative CLEM workflow to investigate the subcellular distribution of GLUT4 in human skeletal muscle fibers with high spatial precision, achieving resolution down to the nanometer scale while ensuring ultrastructural integrity. Our methodology enabled detailed information on the localization of GLUT4 in single muscle fibers from both metabolically healthy and insulin-resistant individuals, and ongoing analyses aim to elucidate whether insulin resistance is associated with distorted subcellular distribution of GLUT4 and whether this is corrected by a single bout of exercise. This research advances our understanding of GLUT4 dynamics and provides a foundation for potential therapeutic strategies targeting insulin resistance through exercise. Moreover, this advanced imaging approach can be utilized to investigate various other subcellular processes in muscle tissue, expanding its applicability in muscle biology research.

Keywords:

muscle, GLUT4, insulin, exercise, CLEM

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Synthesis, structural and electrochemical characterization of nanostructured Ir/TiO_2 for the oxygen evolution reaction

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Poster Group 1

Background incl. aims
To reduce the problem of global warming, alternative eco-friendly energy sources such as solar cells or fuel cells should be used. For fuel cells, hydrogen is an attractive energy carrier.1) To produce hydrogen in a sustainable way, we need to improve the efficiency of hydrogen generation in electrolysers. For this, efficient and stable electrocatalysts are required. One promising class are Ir-based catalyst which are known to speed up the oxygen evolution reaction (OER) but its stability needs to be improved. 2) In our work, we want to improve the stability of Ir and reduce the amount of Ir by using TiO_2 support.
Methods
Ir/TiO_2 nanostructures were synthesized on fluorine doped tin oxide coated glass slide (FTO) using a facile hydrothermal approach. After synthesis, films were prepared for scanning electron microscopy (SEM) by using copper tape attached to an aluminum SEM holder. ZEISS Gemini SEM 500 and ZEISS Merlin FE-SEM (field emission gun) were applied for examining the morphology of samples. For the (scanning) transmission electron microscopy ((S)TEM) analysis, the nanostructures were collected on a TEM holey carbon-copper (C/Cu) grid. A JEOL 2100 and an image Cs-corrected Titan Themis 80-300 from Thermo Fisher were used for the examination.
Results
Electrochemical characterization of the films showed that higher contents of Ir lead to an improved oxygen evolution reaction activity. SEM and (S)TEM analysis revealed that Ir/TiO_2 films consist of an assembly of facetted nanowires on the FTO substrate with the facets in according to the Wulff shape. The width of the individual nanowires was evaluated and it was observed that it increased from 95 nm to 114 nm with increasing amount of Ir used during the synthesis. Electron diffraction in TEM shows that Ir/TiO_2 maintains a rutile structure independent of the Ir content. Finally, energy dispersive X-ray spectroscopy (EDS) demonstrates that small Ir clusters with a size of less than 1 nm form which are homogenously distributed. However, until now it is not yet clear whether Ir is placed on top of the TiO_2 or embedded in the TiO_2 lattice.
Conclusion
To reduce the amount of Ir catalyst for the OER, TiO_2 is used as support. By changing the amount of Ir during the synthesis, the width of the nanowires increased in proportion to the amount of Ir. It is found that Ir/TiO_2 has a rutile structure but further studies are needed to determine whether Ir is incorporated into the lattice or adsorbed on the surface.

Keywords:

Electrocatalyst,nanowires,TiO2,Ir,OER,SEM,TEM

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Insights into the mechanisms of silver phase formation vial electrochemical liquid-cell TEM

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Poster Group 1

Background incl. aims
Understanding the mechanisms involved in nanoparticle formation, especially at the initial stages, is a significant challenge in materials science. The knowledge of early stages provides essential information regarding design strategies during nanomaterial synthesis1; silver is one such material of interest. This metal is significant for catalysis2 and sensing3 applications. Different silver nanoparticle synthesis strategies were investigated previously through chemical and electrochemical routes. However, there is a lack of comprehensive dynamic knowledge regarding the mechanisms of its reactions in solution. The control of essential parameters such as nanoparticle size, size distribution, and morphology will benefit from proper description and direct observation of nanoscale reactions. In-situ Liquid-Cell Transmission Electron Microscopy (LCTEM) is an experimental microscopy technique that allows observation of these nanoscale processes close to the native environment, directly in the liquid. Electrochemical reactions, in particular, can be studied and imaged using a miniaturized three-electrode electrochemical cell setup MEMS chip. Therefore, in this work, we are employing a correlative methodology for in-situ electrochemical LCTEM experiments to study the electrodeposition of silver nanoparticles.
Methods
The in-situ EC-LCTEM experiments were performed using a JEOL JEM 2100 TEM at a 200 kV accelerating voltage in parallel beam mode. The electrochemical measurements in the TEM were carried out using a Protochips Poseidon 500 liquid holder, which allows observation of dynamic electrochemical processes in the liquid. Cyclic voltammetry (CV) technique was used to obtain the characteristic curve for the electrolyte/electrode deposition system, potential window between -300 mV to 200 mV vs. Pt. A chronoamperometry (CA) experiment at -300 mV vs. Pt was carried out to study the growth of silver nanostructures in liquid. The electrolyte solution containing 2 mM AgNO3 + 50 mM KNO3 in ultrapure deionized water was used for the electrodeposition experiments. The electron beam dose was estimated to be below 1000 e-/A2·s.
Results
The in-situ LCTEM CV measurements pinpointed the peak potentials for nucleation of Silver onto the clean electrode measured at -150 mV vs Pt, and the potentials for reduction and oxidation of Ag, which were measured to be around -60 mV vs Pt and 70 mV vs. Pt. Due to this overpotential value, a potential of -300 mV vs. Pt was selected. The growth of small nuclei was recorded immediately after biasing started. The size of this first nuclei was around 6 nm. The growth rate of these particles was observed to be much higher at the beginning of the experiment due to the higher availability of electrolytes. As the electrolyte was not as available to the particle's growth, diffusion-limited growth of needle-like nanostructures extended from these nuclei shortly after.

Conclusion
The LCTEM technique is a powerful experimental method for probing dynamic chemical processes at the nanoscale. Electrochemical deposition, in particular, exhibits mechanisms that are still unclear and might follow non-classical phase transformation routes. This study allowed us to explain the mechanisms of silver nanostructure's electrochemical nucleation and growth.

Keywords:

In-situ TEM; Liquid-Cell TEM; Electrodeposition

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Analysis of transcription dynamics via single cell imaging of chemically inducible system in S. Cerevisiae

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Poster Group 1

Background incl. aims
Transcription is stochastic switching between an active and an inactive state, known as bursting. Advances in microscopy and imaging methods have enabled visualization and quantification of transcription cycles in single living cells, enhancing our understanding of transcription regulation. It is well-studied how the native inducer, for example, GAL1, regulates the transcription activation. Recently, many synthetic transcription factors (TFs) have been used in synthetic biology, for example, the GEV system and zinc finger proteins, which can rapidly regulate gene expression by simply supplying chemical inducers. In most research projects, it has been shown that by using different inducer concentrations, different translation levels could be observed. However, it is still unknown how the chemical inducer concentration affects transcriptional bursting.
In addition to the inducer dependent transcriptional bursting, it is also unclear whether the GC content of the gene of interest will directly influence the initiation rate, elongation rate, or pausing during the transcription. It has been shown that in different organisms, the elongation rates differ. By using our synthetic GOI oin combination with MS2/PP7 system, we could visualize and analyze the promotor state and the elongation rate under identical conditions by using the same concentration of inducer and inserting at the same genomic integration site. This helps to directly understand the dynamic changes caused by varying the GC content of the GOI.
In the future, we intend to use moment-based variational inference for approximate Bayesian estimation of transcription rate parameters from time-lapse observations of the MS2/PP7 system in identical living cells. Utilizing the totally asymmetric exclusion process with extended particle size (l-TASEP) and a switchable promoter state, we model the transcription dynamics in detail. The model accurately describes parameter regimes of high polymerase density, as expected for high GC content. Given the computational intractability of the full model, we investigate principled approximations.

Methods
To test how chemical inducers affect the transcription dynamics, a chemical inducible LexA-ER-AD system was used, combined with the MS2-PP7 system. Before adding the chemical inducer beta-estradiol, LexA-ER-AD is constitutively expressed in the cytoplasm. Beta-estradiol can bind to the LexA-ER-AD estrogen receptor domain, and form a complex causing LexA-ER-AD relocalization, receptor binding and activate transcription through the VP16 domain. By using the LexA-ER-AD system, we can easily control the transcription at any time without toxic effects. Once the transcription starts, the MS2 and PP7 stem loops up- and downstream of the GOI will begin to be transcribed and form hairpin structures. The PP7 and MS2 coat proteins, fused with fluorescence reporters will bind to the specific loop structures and show as a bright spot.
To quantify the number of fluorescence proteins in one spot, an absolute calibration tool using self-assembling nanocages was applied, which has never been tested in S. Cerevisiae. Each monomer of the nanocage was tagged with one or two fluorophores, leading to 60- or 120mers, and was tethered to the outer membrane of the endoplasmic reticulum (ER). To avoid aggregation, an aTC-inducible system was used to control the expression level.

To image all the dynamics at a single cell level, we used a microfluidics chip, containing a custom designed cell trap. This allows us to image the same cell over a long time period, supplying fresh media and inducing the cell at any time point.

Results
To visualize the transcription dynamics in living cells, the MS2-PP7 system is crucial, especially the repeat numbers. To ensure that the transcription site can be visualized under a widefield microscope, we used yeast-optimized GFP fused with the PP7 coat protein and mScarlet fused with the MS2 coat protein. We tested different numbers of stem-loops up and downstream of the GOI and found that we could visualize the transcription sites with 24 repeats of PP7 stem loops and 16 repeats of MS2 stem-loops.
Additionally, the absolute calibration using nanocages was successfully established in S. cerevisiae, where the 120mer nanocage has exactly twice the fluorescence intensity compared to the 60mer.

Conclusion
- Chemical inducible LexA-ER-AD system can quickly respond to beta-estradiol
- Stem loops upstream and downstream of the GOI enable analysis of elongation rate
- MS2-PP7 system allows to assess rate parameters of transcription for different inducer concentrations and thus facilitates the analysis of transcriptional bursting
- Absolute calibration applying self-assembling nanocages allows to separate detection noise from system fluctuations

Keywords:

Transcription dynamics; I-TASEP；microfluidics chips；time lapse

Reference:

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Combining 3D-ED and Z-contrast imaging: crystallographic structure solution of Ca2MnO3Cl

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Poster Group 1

Background incl. aims
Mixed-anion compounds are much less explored than monoanionic materials such as oxides. However, the possibility of forming heteroleptic coordination polyhedra can result in significantly different emergent properties and the different anion properties such as charge, ionic radii, electronegativity, and polarizability add new degrees of freedom to control and tune the electronic and atomic structure of materials. We have recently succeeded in preparing Ca_2MnO_3Cl (powder) which adopts a novel structure type. However, obtaining the structure of this new compound was tricky because of the powder's extreme sensitivity to humidity, and because of the presence of secondary phases, which made indexing the powder X-ray diffraction pattern particularly hard.
Methods
Particularly well-suited to the study of nanomaterials, Transmission Electron Microscopes (TEMs) have recently become indispensable in nanoscale crystallography, thanks to major technological advances such as the invention of spherical aberration correctors, the development of fast, sensitive pixel detectors, cryogenic methods, beam precession, etc... which have led to the advent of 3D Electron Diffraction (3D-ED) methods for solving nanocrystal structures. The microscopist now has reliable diffracted intensities at his disposal, enabling him to calculate a structural model ab initio, then refine it on the basis of dynamic theory, thanks to the development and constant updates of dedicated crystallography programs [1-3], or use X-ray or neutron diffraction. He can also compare the resulting structure with atomic resolution images and maps, recorded in parallel in a state-of-the-art TEM. We performed 3D-ED, Z-contrast imaging and Energy Dispersive X-ray Spectroscopy (EDX) to determine the crystallographic structure of the new mixed anion oxyhalide Ca_2MnO_3Cl. Then the structural model was refined using powder X-ray and neutron diffraction [4].
Results
Ca_2MnO_3Cl crystallizes in a novel structure type stabilized by the small ionic radius of Ca and the strong Jahn-Teller effect of $Mn^{3}+$. The resulting structure (9.752 Å × 6.4946 Å × 6.5763 Å, space group Cmcm) contains 1-dimensional chains of MnO4 square planes, with an angle ~120 ° between neighboring planes. At low temperatures, this material adopts magnetic arrangements with ferromagnetic chains coupled antiferromagnetically. On applying a magnetic field, it experiences a spin flop transition leading to a magnetic unit cell four times larger than the nuclear one.
Conclusion
We will describe the process leading to the structure of the new oxychloride Ca_2MnO_3Cl, emphasizing the importance of the TEM part to solve ab initio its crystal structure, which opened the doors to the comprehension of its magnetic state.
Acknowledgments
The TEM facility JEOL NEOARM at CNRS Institut Néel was co-financed by the European Union under the European Regional Development Fund (ERDF, contract no. RA0023813).

Keywords:

3D-ED, Z-contrast, mixed-anion compound, crystallography

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Sub-4-Å cryo-EM structure of apoferritin from a basic 120 keV TEM with thermionic electron gun

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Poster Group 1

Background incl. aims: Cryogenic electron microscopy (cryo-EM) has developed into one of the most powerful techniques in structural biology since direct electron detectors (DEDs) became available some 10 years ago. High resolution cryo-EM structures are usually solved with high-end microscopes (e.g. the Thermo Fisher Titan Krios) or well-equipped medium range microscopes (e.g. Thermo Fisher Galcios). These microscopes operate at 200 kV or 300 kV and are equipped with field emission guns (FEGs), DEDs and often with energy filters. Here we analyzed the performance of a current entry-level transmission electron microscope (TEM) with an up-to-date scintillator camera.
Methods: We used a Thermo Fisher Talos L120C with a Gatan 698 Elsa cryo-transfer holder to solve the structure of mouse heavy chain apoferritin (mFTH1) by single particle cryo-EM. This microscope is equipped with a thermionic LaB6 electron source and a Thermo Fisher CETA-F scintillator camera. It does not have an energy filter and is usually used for conventional EM, negative staining EM and only for very basic screening of cryogenic samples.
Results: Here we present a 3.8 Å structure (FSC = 0.143) of apoferritin solved by single particle cryo-EM using a Thermo Fisher Talos L120C. At this resolution the protein backbone is clearly visible and most amino acid side chains produce recognizable densities. To our knowledge this is by far the highest resolution for a single particle cryo-EM structure solved with an entry-level 120 kV TEM. We claim that the decent detector quantum efficiency (DQE) and fast readout speed of the CETA-F camera contributed to achieving high resolution.
Conclusions: A resolution below 4 Å for single particle reconstructions was first achieved about 15 years ago and this resolution range was reserved for high-end and medium range microscopes until now. Our results show that a modern compact and affordable electron microscope can reach near-atomic resolutions in single particle cryo-EM and can be used for much more than analyzing particle distribution and ice quality.

Keywords:

SPA, cryo-EM, structural biology

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Nano-characterization of Sodium-ion cathodes fabricated using economically and environmentally friendly organic routes

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Poster Group 1

Developing new materials for battery energy storage is a significant challenge and a key factor in technology. Among the limitations, new materials for more efficient cathodes have been required, but until now there is no perfect candidate. Sodium ion batteries have emerged as a promising solution, although they still present some disadvantages. In addition to new cathode materials, fabrication routes also need to be improved, and economically and environmentally friendly synthesis routes are desirable. When new candidates were sought, in this study, a promising transition metal oxide cathode for Sodium-Ion Batteries (SIBs) was characterized. The synthesis was performed following a variant of an organic synthesis approach developed within the research group [1]. This method uses metal acetates, and Vaseline (hydrophobic component) and oleic acid (surfactant). To further test this route, different combinations of hydrophilic and surfactants have now been employed to validate the use of this route for the synthesis of the cathodes. Notably, the use of non-polluting, economically and safe reagents and the generation of non-contaminant reaction by-products render this approach environmentally friendly. Moreover, it has shown speeds and efficiency higher than those of conventional dry-milling or sol-gel methods, with significant potential for scalability. Preliminary X-ray diffraction and transmission electron microscopy results confirmed the structure of the materials, similar to those obtained by other fabrications routes.

Methods
To achieve the emulsions studied in this work, metal acetates were mixed for 4 minutes in a shear mixer with the other organic compounds to be tested. Products such as coconut oil or mineral oil were used as the hydrophobic component. Whereas glycerol or, sodium dodecyl sulphate (SDS), were tested as surfactants. The achieved emulsion was heat treated for 10 hours at 900 ° C. The crystallinity of the obtained Na compounds was studied by X-ray diffraction (XRD) and the microstructure, morphology and size of the particles as well as the composition by (Scanning)Transmission Electron Microscopy ((S)TEM).

Results
After sintering, XRD was performed to investigate the structure of the synthesized particles, revealing the formation of the desirable products in all cases, similar to those obtained using other techniques, i.e., the stable P2 structure. Scanning electron microscopy revealed hexagonal-shaped particles, with sizes consistent with those in the literature [2]. EDS mapping in STEM mode was used to corroborate composition and the homogeneous distribution of elements which the particles.

Conclusions

In summary, the use of economical and common products to fabricate cathodes following this organic route is a suitable and environmentally friendly technique as well as a scalable route, while drastically decreasing mixing times down to a few minutes. Furthermore, the use of organic products guarantees that the side products are non-contaminant.

Keywords:

Sodium-batteries; cathodes; (S)TEM; nanoparticles

Reference:

Reference:
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1264

Epitaxial LiNi0.33Mn0.33Co0.33O2 thin films as a model cathode material system for Li-ion batteries

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Poster Group 1

LiNi1-x-yMnxCoyO2 (NMC) belongs to a class of layered oxide materials renowned for their solid energy storage performance, making them ideal for use as cathodes in lithium-ion batteries. NMC's structure consists of a two-dimensional layered arrangement where lithium and transition metal ions layers alternate, affecting how lithium ions move from the bulk to the surface within the crystal structure. These materials have already reached the stage of commercial performance with Tesla currently utilizing the NMC622 ($LiNi_{0.6}Mn_{0.2}Co_{0.2}O_2$) formula. However, the performance of NMC cathodes tends to degrade with battery cycling, which is linked to structural deterioration of NMC, although the precise degradation mechanisms remain poorly understood. To explore the performance and structural integrity of NMC cathodes and to understand the directional transport properties of Li ions, we created a thin film model cathode system using NMC111 ($LiNi_{0.33}Mn_{0.33}Co_{0.33}O_2$).

NMC111 thin films were grown on $SrRuO_3$/0.5% wt. Nb-doped $SrTiO_3$ (SRO/Nb) substrates with varying surface terminations by means of the pulsed laser deposition (PLD) technique. X-ray diffraction was used to confirm their epitaxial nature, showing distinct out-of-plane orientations (104), (118), and (003) corresponding to the (001), (110), and (111) substrate orientations. Detailed microstructural analysis through conventional and high-resolution transmission electron microscopy (TEM) showed twinned domains in films on (001) and (110) SRO/Nb substrates due to angled layer growth, with no twinning observed on the (111) SRO/Nb substrate. Electrochemical behavior of the produced thin films with different orientations was tested via galvanostatic cycling at different cycling rates. It revealed that crystal orientation markedly influences NMC's electrochemical performance, especially at higher charge and discharge rates, highlighting the anisotropic nature of lithium ion movement within NMC.

Acknowledgements: This work has received funding from the Slovenian Research Agency ARIS through core program funding P2-0423 and projects J2-3050 and JV-4637.

Keywords:

Thin films, Li-ion batteries, NMC

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1265

Investigating VSV-GP replication using TEM & STEM tomography of high pressure frozen, freeze substituted samples

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Poster Group 1

Background: With a growing demand of viral products for gene therapy and as oncolytic agents, understanding the production process of viruses in the biopharmaceutical environment becomes increasingly important. For this, besides controlling classical bioprocess parameters like infectious titer, there is a need for additional methods to characterize the Drug product. Up to now, electron microscopy (EM) remains the only way of directly visualizing cellular processes during virus replication. Here, we aimed at the development of a protocol using high pressure freezing and subsequent freeze substitution for studying manufacturing of VSV-GP, a recombinant vesicular stomatitis virus (VSV) pseudotyped with the glycoprotein of lymphocytic choriomeningitis virus (LCMV-GP).
Methods: Transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) tomography were used to characterize VSV-GP replication kinetics. Samples were prepared by high pressure freezing and subsequent freeze substitution. The ultrastructure was compared with the number of viral genomic copies, quantified by qPCR.
Results: High pressure freezing and freeze substitution of chemically fixed VSV-GP infected cells yielded a good ultrastructural preservation. At 8 hours post infection, inclusion bodies and bullet shaped virions budding from the plasma membrane could be observed. TEM and STEM tomograms indicate a colocalization of inclusion bodies and rough endoplasmic reticulum.
Conclusion: A hybrid fixation by chemical inactivation and subsequent high pressure freezing proved feasible for VSV-GP infected cells. Three-dimensional reconstruction of STEM tomograms yields information on VSV-GP replication that is otherwise not accessible by TEM imaging and that improves the scientific understanding of viral genome replication and virion morphogenesis.

Keywords:

High pressure freezing, freeze substitution

Reference:

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1266

ExpertPI: A Comprehensive Tool for Automated 4D-STEM Multimodal Analysis and Method Development

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Poster Group 1

Background including aims
The advancement of analytical techniques, particularly in scanning transmission electron microscopy (STEM), necessitates sophisticated tools that can balance ease of use for routine applications and flexibility for method development. The TESCAN TENSOR is a new analytical scanning transmission electron microscope (STEM) that is optimized for electron diffraction experiments, both by unique hardware and software. Specifically, the Expert PI package is an innovative solution designed for development and implementation of new methods and approaches in (multimodal) analytical STEM, 4D-STEM, and 3D-ED techniques.

Methods
ExpertPI is a Python-based library integrated into the TESCAN TENSOR platform, enabling full access to the microscope's functionalities. Unlike the traditional user interfaces that simplify the microscope control and operations at the cost of limited advanced capabilities, the ExpertPI enables direct and unconstrained utilization of all components and modules to their full potential. The core functions of ExpertPI include: i) full instrument control including EM optics, stage, detectors, etc., ii) customizable User Interface with powerful access to all functions via Python console, iii) possibility of integration with third-party libraries, iv) automated data acquisition and processing, v) advanced analytical techniques (such as DPC), vi) customized method development environment, and vii) AI-assisted workflows.

Results
Consequently, such comprehensive control over all electron-optical parameters, beam scanning and acquisition settings, sample stage movements, and synchronized readout of all detectors facilitates unparalleled experimental control, thereby empowering researchers to innovate and refine their techniques, thereby driving forward the capabilities of STEM analysis in material sciences and semiconductor research.

Conclusions
The TESCAN TENSOR therefore represents a highly versatile platform for both routine and advanced 4D-STEM applications. By providing full control over the microscope functionalities and integration with third-party analytical tools, ExpertPI bridges the gap between ease-of-use of routine measurements and the need for advanced method development. This dual capability ensures that the TESCAN TENSOR remains at the forefront of STEM technology, meeting the evolving demands of both application-focused users and method developers.

Keywords:

Analytical STEM, 4D-STEM, precession, development

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Fatigue fracture behaviour of high strength steels under gaseous hydrogen

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Poster Group 1

Structural steels suffer from hydrogen embrittlement (HE) at various stages of its value chain. Versatility of steel microstructures necessitates detailed investigation about how HE occurs at various microstructural hierarchies and interfaces. In this study, we examine the role of various boundaries in two key steel microstructures, i.e., direct quench martensitic steel (DQ1600) - consisting >99% martensite, and quench-partitioned martensitic steel (QP1900) - consisting up to 10% retained austenite and 90% martensite.
We used in-house built bellow-devised fatigue testing set up, to examine fatigue behaviour in pressurized hydrogen atmosphere. Fatigue tests of these microstructures were carried out using 100 bar gaseous H2 (pre-charged + in-situ charging) using 0.03 Hz frequency, under ambient condition using 86 Hz frequency, using stress ratio of -1 under variety of stress amplitudes. Tests were carried out until fracture and fracture surfaces of the selected specimens were investigated using scanning electron microscope. Selected fracture surfaces were cut across the cross-section along the primary crack propagation direction. After standard metallographic polishing, we examined the cross-sections using electron backscatter diffraction (EBSD).
We observed up to 3 orders of deterioration in fatigue life under the influence of 100 bar H2 environment compared to ambient atmosphere in both the steel microstructure. Effect of these microstructures on the fracture events, during HE, is widely different. In ambient conditions as well in pressurized N2, both steels demonstrate similar fracture behaviour, depicted by occurrence of fatigue striations. In hydrogen, on the other hand, DQ1600 steel shows prevalent plasticity mediated intergrannular (IG) fracture, whereas, QP1900 steel exhibits dominant quasi-cleavage (QS) transgrannular (TG) fracture event. Image analyses from the cross-section of the selected samples indicate that depth of secondary cracks (SCs) at 1 mm from the initiation point is twice in case of DQ1600 steel compared to QP1900 one. EBSD-assisted analyses in determining the contribution of various boundaries towards SCs propagation suggests that compared to ambient condition, in case of HE, SCs activated along different lath interfaces in both steels. Using Kurdjumov-Sachs relationship, we showed that in case of DQ1600 steel subjected to HE, block boundaries dominated the fracture events; whereas, in case of QP1900 steel, the sub-block boundaries and lath interface fracture were more prevalent.
In conclusion, local plasticity mediated decohesion, also known as hydrogen induced local plasticity (HELP) mediate hydrogen enhanced decohesion embrittlement (HEDE) is observed to be more dominant in DQ1600 steel compared to QP1900 steel, in which decohesion is more prevailing at microstructural hierarchs.

Keywords:

hydrogen embrittlement, EBSD, fatigue, interface

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1269

Understanding of electronic structure by combination of soft X-ray spectrometer with electron energy loss spectrometer

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Poster Group 1

The development of soft X-ray emission spectrometer (SXES) significantly enhances the capabilities of scanning electron microscopy (SEM) in elucidating the electronic structure of material [1]. In SEM, the primary electron beam excites inner electrons of the sample, resulting in the emission of X-rays due to energy transitions as electrons move from higher to lower orbitals to stabilize the sample. For detecting soft X-rays, a mirror and diffraction grating are employed to focus and guide the X-rays to a CCD camera within the SEM, enabling subsequent signal analysis [2]. Soft X-rays, originating from electron transitions from the valence band to the core level, provide valuable information about the bonding energy states, such as the 2s, 2p electrons from carbon. This makes SXES a promising tool for analyzing energy materials. In 2024, the installation of a new JEOL IT-800 SEM in Tumint-Energy Research GmbH, equipped with SXES, will support our efforts to characterization electronic structure of various materials. With an energy resolution 0.3 eV and the non-destructive nature of the technique, SXES holds potential for analyzing a wide range of energy materials.
Electron energy loss spectroscopy (EELS) is well-established for detecting the density of states (DOS) from the conduction band. The combination of SXES with EELS provides electron microscopists with a comprehensive approach to analyzing the total electronic structure of materials. Our goal is to integrate these two methods to gain a deeper understanding of the electronic structure of different materials.
We utilized two samples for the preliminary assessment of SXES determination. Fig.1(a) shows the SEM image of diamond crystals on a silicon wafer. Fig.1(b) presents the soft X-ray results: the black curve responds to the diamond sample, while the red curve represents soft X-ray obtained from carbon tape. A notable small peak in the circled area of Fig.1(b) distinguishes the diamond sample from the carbon tape successfully.

Keywords:

soft X-ray, electronic structure, EELS

Reference:

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1270

Structural studies of the cross-link mutant, ABC transporter BmrA by cryo electron microscopy

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Poster Group 1

ATP-binding cassette (ABC) transporters are the membrane proteins that use the energy from ATP hydrolysis to undergo some conformational changes and transport a variety of substrates across biological membranes. The flexible nature of these transporters enables them to adopt various conformation, hence accommodate different substrates. Certain ABC transporters provide multidrug and antimicrobial resistance in cancer cells and pathogenic microorganisms.
These transporters mainly function by alternating between inward-facing and outward facing conformations. ATP binding creates an outward-facing conformation that facilitates drug release, while ATP hydrolysis resets the transporter to its inward-facing conformation. The opening and closing conformation of the nucleotide-binding domains (NBDs) directly cause movements in the transmembrane helices, leading to changes in the substrate-binding pocket. Various degrees of the opening and closing of the nucleotide binding domain has been reported.
Although the amount of the structural information has been increased over the past years, there are still some missing information.
To study the effect of the separation of the NBDs on catalytic cycle, we introduced a Cysteine near the C terminal of the NBD. The structural impact of the mutation on conformational changes of the transporter was investigated by cryo electron microscopy. The mutant did not affect the function of the BmrA and there was no structural difference between mutant and WT BmrA. Two structures were highly similar, showing intermediate opening between their NBDs while their C-terminal extremities remain in close proximity. This data suggests that the NBDs of BmrA function with a tweezers-like motion.

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Atomic Structure and Electron Magnetic Circular Dichroism of Individual Rock-Salt Structure Antiphase-Boundaries in Spinel Ferrites

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Poster Group 1

Background incl. aims
Functional oxides are ubiquitous and exhibit a wide range of electric, magnetic, optical, and structural properties. Controlling the defects and interfaces in thin-film devices is the new challenge in multifunctional oxides. Antiphase boundaries are the interfaces between two crystallographically identical regions with shifted phases. Based on previous studies, the correlation between the existence of antiphase boundary and decreased saturation magnetization in oxides has been discussed [1]. Due to the resolution limitation of magnetism measurement, it is difficult to investigate the structure-property relationship at high spatial resolution.
Methods
EMCD, first experimentally verified by Schattchneider et al. [2], is a magnetism measurement method in a transmission electron microscope and is confirmed with the ability to reach spatial resolution better than 2 nm using a convergent beam [3]. This significant breakthrough enables us to study the magnetic properties of interfaces and boundaries on the nanometer scale. In the meanwhile, our group has developed site-specific EMCD [4] and atomic plane resolved EMCD [5] in complex oxides such as spinel and double perovskite.
Results
Intensity analysis of antiphase boundary (APB) in HAADF (high angle annular dark-field imaging) STEM (scanning transmission electron microscopy) images suggests that only half of the octahedral interstices are occupied at the rock salt structure interlayer of a new type APB in $NiFe_2O_4$ with a relative translation of (1/4)a[011]. High-spatial-resolution EMCD has been used to demonstrate experimentally reductions of ~46.8%±8.2% and ~38.8%±14.5% in the EMCD strengths of Fe and Ni in APB, respectively, compared to perfectly ordered $NiFe_2O_4$ [6]. DFT and dynamical diffraction calculations suggest that the reduced EMCD strengths result from the fact that Fe ions at the APB interlayer are antiferromagnetically coupled with each other. In contrast, Ni ions show a significant decrease in magnetic moment as a result of the formation of low-spin state Ni^{4+} (d^6) ions.
Conclusion
Our combined approach of using element-specific EMCD under high-spatial-resolution and first-principles calculations to resolve and identify the atomic structure and magnetic coupling of an individual APB in spinel ferrite is applicable to studies of a broad spectrum of other defects in magnetic materials.[7]

Keywords:

Antiphase-boundary, Electron-magnetic-circular-dichroism, Spinel ferrites

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Unveiling the Mechanisms of Structural Reconstruction and Magnetic Evolution in NiFe2O4 During Phase Transition

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Poster Group 1

The phase transition from spinel to rock salt structure has significant implications for information storage and energy conversion applications. Previous studies have employed various techniques, such as X-ray diffraction (XRD), neutron scattering, and X-ray absorption spectroscopy (XAS), to investigate the mechanism and pathway of this transition.[1] However, due to technical constraints, a comprehensive understanding of the transformation of local structures during this transition remains elusive, particularly regarding how local lattice and chemical environments influence the process. Moreover, the role of local structural order and chemical environment in this transition process has not been fully elucidated.[2] To bridge this knowledge gap, our study utilizes advanced transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS) techniques to monitor atomic migration and electronic structure alterations in real-time during the phase transition. By employing TEM and EELS, we aim to provide new insights into the mechanism and pathway of the spinel to rock salt phase transition, shedding light on the influence of local lattice and chemical environments on the process. These enable us to gain a deeper understanding of the local structural and chemical changes occurring during the transition, which have not been fully explored in previous studies.

Methods

This study employs a combination of advanced scanning transmission electron microscopy (STEM) techniques to investigate the phase transition in NiFe2O4's spinel structure under electron beam irradiation. Specifically, we utilize high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and annular bright-field scanning transmission electron microscopy (ABF-STEM) to dynamically observe the migration of Fe atoms within the spinel structure. In addition to the structural analysis, we employ a nanobeam electron magnetic circular dichroism (nanobeam-EMCD) method to observe the variation in magnetic information during the phase transition. This technique enables us to probe the local magnetic properties at the nanoscale and correlate them with the structural changes observed through HAADF-STEM and ABF-STEM.

Results

Through the integration of STEM image simulations and quantitative image analysis, we have unraveled the reconstruction dynamics of octahedrally-coordinated Fe/Ni atoms during the phase transition in NiFe2O4. Our findings provide valuable insights into the lattice dynamics and Fe atom rearrangement pathways across varying coordination environments. By tracking the movement of these atoms in real-time, we have gained a deeper understanding of the atomic-level mechanisms driving the phase transition.Simultaneously, we have employed real-time electron energy loss spectroscopy (EELS) to monitor the changes in the chemical valence states of Fe, Ni, and O throughout the various stages of the phase transition. This approach has allowed us to correlate the structural changes observed through STEM with the electronic structure evolution, providing a comprehensive picture of the phase transition process.Furthermore, we have investigated the

magnetism evolution during the phase transition using EMCD.[3] By correlating the structural information obtained from STEM with the magnetic information derived from EMCD, we have gained insights into the interplay between the atomic structure and magnetic properties of NiFe2O4 during the phase transition.[4]

Keywords:

Dynamic atomic study, NiFe2O4, EMCD

Reference:

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Understanding and modifying cell wall permeability to facilitate cellular nanoparticle uptake

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Poster Group 1

Background incl. aims
The green transition of agriculture requires an increased crop productivity accompanied by a low environmental impact. However, poor nutrient use efficiency (NUE) and the environmental footprint of conventional fertilizers hamper this process.
The advances within nano-biotechnology have paved the way for the development of next-generation nanofertilizers. Foliar application of nanofertilizers allows farmers to bypass the soil matrix, where a large fraction of nutrients becomes unavailable to the plant due to chemical fixation, microbial immobilization, leaching to water bodies or volatilized to the atmosphere. Moreover, these nanofertilizers can be tailored with a range of smart properties, which enable them to deliver nutrients more efficiently to the plant.
In order to increase the efficiency of foliar nanofertilization, fundamental understanding of how these nanoparticles (NPs) interact with plant tissue is essential. After foliar application, NPs can cross the leaf surface through stomata to reach the apoplast. Here, NPs must remain intact to deliver their nutrient cargo inside mesophyll cells or travel through the phloem towards developing plant tissues. Further cellular internalization of NPs requires passage through the cell wall (CW) and plasma membrane. The CW has a size exclusion limit of 10-20 nm and, thus, it represents a major barrier for NP cellular uptake.
Zwitterions have been shown to loosen the tight cell wall networks of plant tissue in a biocompatible manner. Zwitterions destabilize hydrogen bonds in the cellulose matrix of CW's and thereby enhancing its permeability.
We hypothesize that CW porosity is the major barrier for NP uptake at the single cell level. Therefore, our aim is to utilize zwitterions to enhance CW porosity and facilitate internalization of polyacrylic acid coated manganese oxide (PAA-MnO) NPs.
In this innovative approach, the impact of zwitterions on NPs uptake is investigated in single-cell cultures with a combination of Confocal Laser Scanning Microscopy (CLSM), Transmission Electron Microscopy (TEM) and Laser Ablation Inductively Coupled Plasma Mass Spectometry (LA-ICP-MS).
Methods
For the single-cell experiments, Tobacco BY-2 cells (Nicotiana Tabacum cv. Bright Yellow 2) were used.
PAA-MnO NPs were synthesized according to an established protocol and labeled with Dil dye. PAA-MnO NPs exhibited a hydrodynamic dyameter of ~ 20 nm and a zeta potential of -20 mv.
BY-2 cells were either incubated with only PAA-MnO NPs or pre-incubated (30 minutes) with PAA-MnO NPs and then treated with zwitterions. Cells were imaged with CLSM after several time points of interest. The same experimental setup is repeated to image the interaction between BY-2 cells and Ce spiked PAA-MnO NPs, using TEM and LA-ICP-MS.
Results
BY-2 cells treated only with PAA-MnO NPs for 3 hours exhibited a pronounced fluorescent signal around the cell edges. Additionally, a faint fluorescent signal was also visible within the cells, with a halo-like pattern surrounding the cell nucleus. The intensity of the signal inside the cell increased

when cells were imaged after 4 hours of incubation with PAA-MnO NPs. Interestingly, BY-2 cells incubated with a combination of PAA-MnO NPs and zwitterions for 3 hours were characterized by a strong fluorescent signal inside the cell, localized in the cytosol, around the cell nucleus and tracing actin filaments. A mild fluorescent signal was also observed around the cell perimeters. The intensity of the fluorescent signal inside the cell increased when the incubation time was prolonged to 4 hours.

Conclusions

Our findings suggest that the CW is a significant barrier to NPs internalization as it limits the uptake of 20 nm spherical NPs, while zwitterions facilitate NPs uptake in BY-2 cells. The mechanism of plasma membrane penetration is still unclear and warrants for further investigation. Furthermore, CLSM, TEM and LA-ICP-MS are powerful techniques to study the processes of NP internalization at the single cell level.

Keywords:

Nanofertilizers, single-cells, zwitterions, cell-wall, bioimaging.

Reference:

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Low energy electron microscopy and spectroscopy of 2D materials

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Poster Group 1

Carbon, as the element of many different forms, is in the center of attention of many physicists for quite some time. In the last decade, carbon science and technology have enlarged its scope thanks to the discovery of 1D and 2D carbon forms (nanotubes and graphene), and the newest 2D materials – MXenes. Numerous technological applications are rising and will still arise in the foreseeable future, from these newly discovered carbon forms. Indeed, the development of these new materials and nanostructures requires the emergence of new surface-sensitive techniques for their characterization. As regards the surface sensitivity, the ultra-low-energy electron microscopy/spectroscopy can become a very powerful tool for the true examination of these atom-thin materials, capable of confirming physical phenomena predicted to occur on their surfaces. The advantage of modern commercial scanning electron microscopes is the possibility to enable imaging and analysis by low-energy electrons even at very high magnification.

Nevertheless, the real surface studies of 1D and 2D nanomaterials in microscopes equipped with selected spectroscopic techniques are not common so far, which is due to significant problems associated with the sample contamination under the electron beam. Since the specimen contamination increases with increasing of the electron dose and decreasing landing energy, specimen cleanness is a critical factor in obtaining meaningful data by low-voltage SEM/STEM. A range of various surface cleaning methods can be applied to selected samples. Typical cleaning methods, such as solvent rinsing, heating, bombarding with ions, and plasma etching have their limitations. Even a small amount of hydrocarbon contamination can severely impact the results obtained with low-energy electrons. During the scanning of surfaces by electrons, the image usually darkens because a carbonaceous layer gradually deposits on the top from adsorbed hydrocarbon precursors. This effect is called electron stimulated deposition. The surface diffusion of hydrocarbon molecules around the irradiated area serves as a source of additional precursors responsible for an even darker frame of the contaminated field of view. On the other hand, the effect of electron stimulated desorption occurs at the same time, especially at low energies. So, the fundamental question arises, whether the deposition of desorption will dominate, which depends on parameters settings in SEMs. We are using the slow electrons to electron-induced in-situ cleaning, which is gentle, experimentally convenient, and very effective for a wide range of specimens.

Detailed knowledge of mechanisms of electron scattering and its practical consequences for very low energies are of prime importance for not only measurement techniques but also development of new
materials for next-generation electronic devices. Determining inelastic mean free path (IMFP) of electrons in bulk materials is an ongoing topic in spectroscopy. Information on IMFP for very low electron energies, $E \leq 100$ eV, is not satisfactory and it is often missing in case of 2D materials, which are promising in the semiconductor industry. Low thickness of 2D crystalline materials motivated us to develop the unique UHV device analyzing samples via transmitted electrons in a standard

microscopic regime (energy range 0 – 5 keV), and also via time-of-flight (ToF) method (focusing on energy range E ≤ 300 eV). [1]

Graphene is one of the most well-known 2D materials, it is lightweight and strong, and its other unique
properties such as excellent electrical and thermal conductivity and transparency make it an ideal candidate for study at very low energies in the transmission mode of our device. We performed experiments with multilayered graphene to obtain electron energy loss spectra (EELS). Both plasmon peaks (π and π+σ) are present in the measured spectra for 2 layer and 3-5 layers graphene samples (Figure 1). The position of the plasmon peaks increases with both the number of layers of 2D samples and the momentum transfer value [2, 3]. The experimental EELS data are used to derive IMFP values. The measured data are supported by simulated momentum-resolved EELS spectra using many-body perturbation theory (Yambo code), on top of density-functional theory (DFT), Quantum Espresso [4].

We are focusing on the precise characterization of advanced 1D and 2D carbon-based nanomaterials (mainly graphene) and comparing the obtained data with theoretical DFT simulations. Moreover, we also examine possible surface damage by low-energy electron irradiation, which is a very important parameter in the electron analysis. We are using the low- and ultra-low-energy electrons for in-situ cleaning and even for already observed possible structure recovery. The influence of electron irradiation on sample quality (recovery/damage) is studied by Raman spectroscopy in detail.

Keywords:

LVS(T)EM, ToF spectroscopy, graphene, DFT

Reference:

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Embryonic and postembryonic development of arthropods: imaging from the cellular to the whole organism level

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Poster Group 1

Background
Knowledge of arthropod development is important for understanding morphogenesis, comparative embryology and the application of these concepts in various fields of bioscience. The processes of morphogenesis and differentiation during embryonic and postembryonic development of an organism are complex and interdependent at all organisational levels, which is crucial for the establishment of the functions of individual organs. The analyses of samples of isolated organs and of intact animals complement each other, particularly in terms of understanding the 3D structure at different levels, and require the combined use of complementary imaging techniques: micro-computed tomography (micro-CT), histology, fluorescence labelling and various electron microscopy methods, including array tomography (AT) and transmission electron microscopy (TEM) tomography. In addition, the study of developmental processes demands the analysis of temporally sequential developmental stages, which range in duration from a few hours to a few days. The basis for an integrative study of developmental processes is the establishment of a staging system for the species investigated. Accordingly, the first aim of our studies is to comprehensively characterise the morphology of the developmental stages in the investigated crustacean species. Next, in the context of comparative studies of arthropod development we aim to study morphogenesis of the digestive system and the integument in detail, focusing on the differentiation of epithelia, that is crucial for shaping of the organ and establishment of vital functions.
Methods
The morphological characterization of developmental stages in the crustacean species (Porcellio scaber and Asellus aquaticus) was performed by imaging of living organisms (embryonic and postembryonic stages) by light microscopy and fluorescence labelling of cell nuclei in fixed whole-mount embryos. To study morphogenesis of selected organs and differentiation of epithelia, the samples of P. scaber mid- and late-stage embryos, postembryonic stages (marsupial and postmarsupial mancae) and adults were analysed using micro-CT, histology, fluorescence labelling of selected structures and electron microscopy. For micro-CT imaging whole animals were chemically fixed and dried, and after imaging the segmentation of the digestive system was performed. Histological and ultrastructural analysis were performed on semithin and ultrathin sections, respectively, prepared from chemically fixed, dehydrated and resin-embedded samples of whole organisms or isolated organs.
Results and conclusions
Morphological analysis of sequential developmental stages is currently being used to characterise embryonic development of a freshwater crustacean A. aquaticus, a model organism in various fields, notably ecotoxicology and evolutionary ecology. The morphological characteristics of intact embryos and mancae (postembryonic stages) were analysed by light microscopy each day of the development. Fluorescence labelling of nuclei is particularly suitable for the analysis of early embryonic development, when the arrangement of cells follows a well-defined pattern (Fig. 1A). According to the main morphological modifications, the development of A. aquaticus was divided into 10 embryonic stages, the stage of marsupial manca and the stage of postmarsupial manca (Fig. 1B, C).

During the formation of the integument, gut and digestive glands the differentiation of epithelial tissues was described in P. scaber, including changes in cell shape and ultrastructure. Using micro-CT, we obtained 3D morphology data of the digestive system in intact organisms of distinct developmental stages (Fig. 2A). Data on the changes in epithelial cell shapes in different organs during development were obtained by analysing semithin sections (Fig. 2B). The elongation of the invaginated foregut and hindgut until they fuse into a single digestive tube and the gradual incorporation of the yolk into the developing digestive glands in embryos, as well as the differentiation of the gut regions in postembryonic stages, were clearly visible. At the ultrastructural level, the processes of cell junction differentiation, apical and basal plasma membrane remodelling, and apical extracellular matrix (cuticle) formation were characterised by transmission electron microscopy (Fig. 2C), supplemented with AT for the 3D context of the cell junctions. The combination of the described imaging analyses provides a broad spectrum of information on the structure of individual cells, tissues and whole organs at the same developmental stage in the context of the whole organism. This allows us to relate modifications in detailed cell ultrastructure and tissue differentiation to morphogenetic processes at the organ and organism level during development.

Keywords:

arthropod development, micro-CT, histology, TEM

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Visualisation of tetrahedral Li in the alkali layers of Li-rich layered oxides

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Li-rich metal oxide cathode materials for Li-ion batteries, such as Li1.2Ni0.13Mn0.54Co0.13O2 can make use of the redox reaction of oxygen anions and transition metal (TM) cations, delivering superior capacities of over 250 mAh/g, much higher than the conventional metal oxides. However, fading in the capacity and voltage and the sluggish kinetics are significant problems for the Li-rich oxides1,2. Understanding the Li+ ion diffusion pathway is crucial for understanding the sluggish kinetics in anionic O2- redox. Li diffusion within the alkali layers undergoes a well-known, low-barrier octahedral-tetrahedral-octahedral (o-t-o) pathway3, however, it is less clear how Li diffuses in and out of the TM layers, particularly given the complex structural rearrangements which take place during O2- oxidation.

Here, we perform electron ptychography and simultaneous annular dark field imaging to directly visualise the Li in Li1.2Ni0.13Mn0.54Co0.13O2. At the end of the TM-oxidation region and before the high voltage O oxidation plateau, we observe Li occupying alkali-layer tetrahedral sites on opposite sides of the TM layers, forming Li-Li dumbbell configurations. The presence of Li at these tetrahedral sites is demonstrated by mathematical processing of the quantitative ptychographic phase contrast, see Figure 1. Density functional theory (DFT) calculations support the observation showing that tetrahedral Li-Li dumbbell configurations are lower in energy than corresponding tetrahedral TM configurations. We also observe the in- and out-of-plane TM migration as well as a partial phase transition from O3 to O1 stacking. In the O1 stacking phase, tetrahedral Li is absent, consistent with our DFT calculations indicating the O1 phase is not thermodynamically stable to accommodate tetrahedral Li. Upon further Li deintercalation to 4.8V, most tetrahedral Li is removed and we only observe a small amount of residual tetrahedral Li. After discharging to 2 V, we do not observe the reformation of tetrahedral Li but observe permanently migrated TMs occupying the alkali-layer sites. These migrated TMs may suppress the Li re-population of some vacant TM-layer octahedral sites by disfavouring Li occupation of the face-sharing tetrahedral sites. To maintain the maximum number of accessible tetrahedral Li sites and minimise the blocking of tetrahedral Li diffusion pathways, strategies to mitigate the irreversible TM migration and the O1 phase change should be employed.

Figure 1 Quantification of ptychographic phase contrast of Li1.2Ni0.13Mn0.54Co0.13O2 at a charging state of 4.45 V. (a) Ptychographic phase image marked with the alkali-layer tetrahedral sites showing

contrast. The scale bar is 0.5 nm. (b) Squared phase image by squaring the ptychographic phase contrast in (a). The squared phase values around each atom are integrated using Voronoi cells to obtain the integrated squared phase (ISP). The ISP values at the alkali-layer tetrahedral site (AKtetra) and TM-layer octahedral site (TMoct) define an ISP ratio between these values. Two DFT models are used to simulate the ptychographic image and deliver the ISP ratios: (c) tetrahedral Li (Tetra_Li) model with Li ions at the tetrahedral sites. (d) tetrahedral Ni (Tetra_Ni) model with Ni ions at the tetrahedral sites. In the schematic representations of the models, grey is Ni, purple Mn, blue Co, red O and green Li. The ISP ratios calculated from the collected image in (a) and simulated images of the two DFT models within a range of thicknesses are compared in (e) and (f).

Keywords:

Li-rich metal oxide; electron ptychography

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Evaluating the Efficacy of Laboratory-Based X-ray Diffraction Tomography for Crystallographic Analysis in Pure Iron

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Poster Group 1

Background
Iron (Fe) alloys are fundamental to various industrial applications due to their diverse mechanical properties and structural versatility. Understanding these alloys' grain structure and crystallographic characteristics is crucial for optimizing their performance. Here, a newly developed laboratory-based Diffraction Contrast Tomography (LabDCT) was used for non-destructive three-dimensional grain mapping and crystallographic analysis of pure iron. LabDCT, within a commercial laboratory X-ray microscope (Zeiss Xradia 630 Versa), uses a synchrotron-style tomography detection system and, combined with conventional absorption tomography, enables analysis of defects, deformation, grain growth, and phase transformation. This study aims to evaluate the efficacy of XRD-CT in characterizing pure iron, comparing the obtained grain and crystallographic data. The aim is to use the data from pure iron as a reference point for the future characterization of more complicated Fe alloys. To validate the results obtained from LabDCT, this study also employs 3D Electron Backscatter Diffraction (EBSD) tomography on the same volume. This comparative analysis seeks to highlight the potential advantages and limitations of LabDCT, advancing its application in material science and industrial contexts.
Experimental Method.
A cylindrical piece of pure iron with a diameter and height of 450 µm and 1800 µm Lab-based Diffraction Contrast Tomography (DCT) scan and X-ray absorption tomography were performed using the Zeiss Xradia 630 Versa Micro X-ray Computed Tomography (Micro-XCT) system. A helical phyllotaxis Lab DCT (HP-DCT) scan was used to cover a larger field of view. The DCT Scan was performed using an accelerated voltage of 100 kV, a power of 14 W, A letterbox aperture of (375 µm × 375 µm), DCT 4X objective detector with 2.5 mm x 2.5 mm detector beam stop to block the transmitted X-rays. The specimen was rotated 360°, over which 181 projections with an exposure time of 60 s per projection were collected. To Satisfy the Bragg condition, the source and detector were positioned within an equal distance from the sample. A helical scan was used to cover a larger field of view. For serial sectioning tomography, a dual-beam TFS Helios G5 Xe PFIB system was employed. The sectioning and EBSD scans were carried out under the following conditions and controlled by the FEI Auto-slice-and-view software. The slice thickness was set to 2 µm, and EBSD was collected every other slice for a final z-resolution of 4 µm. A current of 0.2 µA was used for slicing the block, and a 5° rocking mill was used to prevent curtaining effects. 2D EBSD maps were collected at 20 keV with a current of 13 nA using an Oxford Symmetry detector and the Aztec control software. Each EBSD map covered the entire sample face with indexing at 4 µm step size to provide approximately cubic voxels for the 3D reconstructions. In total, 75 individual slices were collected on a sequence of X-Y planes.
3D Reconstruction

To reconstruct the DCT data and grain mapping, version 3.2.5 of the data reconstruction software, GrainMapper3D by XnovoTech® was utilized. Importing the raw data, thresholding, alignment, and clean-ups, were all carried out using DREAM.3D software to reconstruct the EBSD-FIB Tomography data. Following the reconstruction, 3D datasets were visualized using Dragonfly Pro and ParaView software.

Results

Fig 1a shows the DCT-reconstructed 3D volume of the pure iron and the three 2D cross sections of the grains in the XY, XZ, and YZ planes. Fig 1 b illustrates a 3D-EBSD reconstruction of the volume corresponding to the top 300 µm of the sample. To match the EBSD and DCT results, a slice-by-slice observation was carried out to reveal the internal microstructure of the material. The 3D variation of the grain morphology and crystallography was precisely tracked through the entire volume of the material using both 3D-EBSD and DCT datasets. After identifying the corresponding 2D slice in the lab-based DCT dataset, comparisons were made between lab-based DCT and EBSD techniques in terms of grain size, shape, and texture. A grain-by-grain comparison of the DCT and EBSD data is shown in Fig 1C. Although the LabDCT effectively determined the center of the mass position and large grains' shape, the EBSD data showed more accuracy in terms of the grain boundaries and grain shapes and detection of smaller grains. Furthermore, the DCT method was able to image larger volumes in a shorter time.

Conclusions

The quality of the 3D grain maps of pure iron obtained with LabDCT and 3D FIB-EBSD was compared in terms of grain size and shape. The centre of the mass position of grains in DCT was found very close to 3D EBSD. The grain size and number of grains in DCT were underestimated compared to the grain size obtained in the EBSD. The grains in the EBSD image are more complex than the DCT grains due to the higher resolution of the EBSD method. Overall, a promising agreement was observed between the DCT and EBSD data.

Keywords:

LabDCT, XCT, 3D FIB-EBSD, Grain-mapping

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Correlative-Cryo Microscopy to Characterise Bacteria-Nanopillar Interactions: Achievements and Challenges

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Poster Group 1

Background

Nature-inspired nanopillar surfaces have been shown to kill surface-attached bacteria by physical means, providing a novel route to potentially control bacterial colonisation on implant surfaces and thus combat implant-related infections. It is proposed that structural damage to the bacterial cell wall kills bacteria as they attach to the nanopillars[1,2]. However, more than a decade since this concept was introduced, the precise mechanism by which bacteria are killed remains an open question[1-4]. Using fluorescent staining, damage to the cell wall is predicted to happen at a single time point after the interaction. However, light microscopy cannot visualise damage to the cell wall, while traditional electron microscopy (EM) techniques associated with sample processing cannot characterise hydrated samples and may introduce artefacts during processing. This work aims to enable the native interaction between bacteria and nanopillars to be visualised at nanometre resolution and so better understand the bactericidal mechanism by establishing a cryo-correlative workflow to characterise the bacterial cell wall interacting with the surface nanotopography while maintaining a hydrated environment[5]. Here, we discuss our assessment of different vitrification techniques and different cryo-microscopy techniques to characterise bacteria within a hydrated state.

Methods

The project used silicon (Si) and titanium (Ti) as test substrata and Escherichia coli as a model bacterium to develop the workflow. Freshly grown bacteria, diluted into PBS, were incubated for 30 minutes on the test substrata at the room temperature. Samples were then vitrified using different techniques: 1) liquid nitrogen slush freezing; 2) plunge freezing (PF); or 3) high pressure freezing (HPF). Both fluorescently-labelled and non-labelled bacteria were vitrified by PF and imaged by cryo-SEM to identify surface-attached bacteria. Single bacterial cells in HPF samples were identified for further analysis after screening under cryo-fluorescence microscopy. Single bacterial cells were lifted using cryo-FIB/SEM and placed onto TEM grids for cryoET. After polishing the lamella, the presence of bacterial cells was confirmed using cryo-fluorescence microscopy.

Results

Slush frozen samples could allow the visualisation of surface-attached bacteria with minimal sample preparation. Both Si and Ti samples were successfully vitrified for cryoSEM. However, the technique could not be used for further investigations as the workflow was not set up for sample transfer between different microscopes. PF and HPF resulted in vitrification of the water layer on the substrate. Bacterial cells were not visible under cryoSEM when vitrified by HPF or PF as they were buried under the surface of the liquid phase. Therefore, bacteria could only be identified when they were fluorescently labelled. Some of the PF samples showed ice crystals as blotting was not optimal. Vitrification of samples by HPF was more reproducible without ice crystal formation. However, finding and isolating individual bacterial cells for further analysis was a challenge with both techniques. Therefore, cryo-fluorescence is an essential step in this workflow. For HPF samples, estimation of depth was a challenge as the test substrate surface could not be accurately measured on the micrometer scale under the cryofluorescence microscope. When a bacterium was selected in the XY plane, it was not necessarily attached to the substrate. This could only be verified after lift-out of lamella, followed by cryo-fluorescence imaging.

Conclusions

Nitrogen slush freezing is a rapid way of vitrifying the sample and suitable for cryo-SEM to study the morphology of bacterial cells interacting with nanopillar topography. Both HPF and PF are promising techniques to vitrify samples with bacteria attached to the substrate. However, identification of individual bacteria attached to the substrate is a challenge.

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Keywords:

Mechano-bactericidal action, Nanopillars, VolumeEM, Cryo-microscopy

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3D chromatin architecture by volume EM

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Poster Group 1

Background
Detailed knowledge of the dynamic nuclear architecture is indispensable for understanding important nuclear functions such as DNA replication and repair, RNA synthesis, maturation, and transport. These processes occur in non-membrane chromatin-containing domains. Generally accepted opinion is that chromosomes reside in a certain area of the nucleus known as the chromosomal territories (CT). On the other hand, there is still disagreement over the interchromatin compartment, which is the DNA-free or DNA-poor nuclear region surrounding each individual CT. Our volume electron microscopy (EM) study set out to examine the distribution of DNA and chromatin inside the nuclear region, taking into account its extensive organization and functional role.

Methods
Light microscopy and fluorescent labels can provide an estimate of chromatin compaction and organization. For an understanding of the structural and functional organization of the cell nucleus in higher resolution, analysis of chromosomal domains at the ultrastructural level is essential.
To differentiate chromatin from other, especially, ribonucleoprotein containing structures, in EM, the cells were treated by pre-embedding staining where chromatin is observed as a highly contrasted structure. NAMA-Ur is method based on the extraction of RNA and phosphate groups from phosphoproteins by a weak alkali hydrolysis (NA) which does not affect DNA, followed by blockage of the amino and carboxyl groups by methylation and acetylation (MA). Finally, samples were stained by uranyl (Ur), which can bind to DNA, dehydrated and embedded in epoxy resin. Structural 3D acquisitions were performed using focused ion beam scanning electron microscopy (FIB-SEM). The obtained data was analysed by using Amira, NIS-Elements, and Imaris softwares. Nuclear subcompartments considered for analysis were as follows: perinuclear chromatin, perinucleolar chromatin, intranuclear chromatin, chromocenters, and interchromatin area.
Another analyses, which are currently in process, are comprised of function visualization of chromatin. To reveal the chromatin domains where gene transcription or replication take place, cells were labelled by in vivo incorporation of the nucleic acid precursors marked with halogens such as fluorine or bromine and/or by stable isotope 15N or 13C. Incorporated nucleotides were then observed either by combination of FIB-SEM with time-of-flight secondary ion mass spectrometry (SIMS) or by TEM with nanoscale SIMS (NanoSIMS). Moreover, serial sectioning of labeled cell nuclei is in progress for SEM followed by NanoSIMS as well as for array tomography.

Results
We successfully obtained a 3D datasets of NAMA-Ur-labeled nuclei by FIB-SEM acquisition with sufficient resolution and contrast for further quantification of perinuclear chromatin, perinucleolar chromatin, intranuclear chromatin, chromocenters, and interchromatin volumes.

Conclusion
The results demonstrate that the volume EM approaches performed on mammalian cell nuclei with specifically contrasted chromatin and eventually combining with spectrometry methods offer an extraordinary opportunity for study of nuclear architecture in situ and show great promise for novel achievements.

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Keywords:

Chromatin, Mammalian cells, Volume EM

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Cryo-EM structure of wild-type Orsay virus: preliminary insights into the assembly mechanism

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Poster Group 1

Background
The Orsay virus (OrV) is a non-enveloped, icosahedral virus with a bipartite, positive-sense, single-stranded RNA genome (RNA1 and RNA2). It naturally infects the nematode Caenorhabditis elegans. Previous crystallographic studies have resolved the structure of an OrV-like particle (360 Å in diameter) at a resolution of 3.2 Å [1]. One hundred-eighty copies of the capsid protein (CP) assemble the icosahedral proteinaceous shell in a T = 3 lattice. Additionally, other studies have demonstrated the presence of a pentameric fiber, which may facilitate viral attachment and infection of the host cell [2-3]. Here we aim to elucidate the structure of the wild-type virus and to provide an understanding of its assembly mechanism and role played by the genome in this process.
Methods
Wild-type viruses obtained from the infection of C. elegans were purified, vitrified, and subsequently analyzed by cryo-EM using high-resolution microscopes. RELION, Chimerax, Phenix, and Coot software were employed for map analysis and localized reconstruction technique to study the structure of the fiber and the CP-genome interactions.
Results and Conclusion
We have determined the structure of the wild-type OrV virion by cryogenic electron microscopy to 2.5 Å resolution. The reconstructed 3D electron density, in addition to showing an ordered icosahedral capsid, unravels a previously unreported defined density network beneath the viral capsid that may be related to the interaction between the CP and the bi-partite RNA genome. Our efforts are currently focused on improving the resolution and implementing symmetry relaxation techniques, as well as biochemical techniques that will allow us to structurally elucidate possible sites of interactions between the bipartite genome and the CP and its evolutionary relationship with other RNA viruses. Additionally, we are studying the structure of a pentameric virion-associated fiber and its potential role in host cells infection.

Keywords:

Virus, RNA, Cryo-EM, Capsid Protein

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Nanoscale X-ray imaging to Study Bacteria-Nanopillar Interactions

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Poster Group 1

Background
Exploitation of nanotopography found in nature with bactericidal properties was reported a decade ago as an alternative method to combat the emerging threat of bacterial antibiotic resistance.[1],[2] However, a consensus has yet to be reached on the mechanism(s) by which bacteria are killed upon contact with pillar-like nanostructures. Without knowing the mechanism by which the cell wall is damaged, it is unlikely that this concept can be further implemented for real-world applications. Using fluorescent staining, damage to the cell wall is envisioned to happen at a single time point after the interaction. However light microscopy cannot visualise the damage to the cell wall, and serial cross-sectioning and imaging in FIB-SEM can create a pseudo-3D image of bacteria, and is a destructive approach. Our FIB-SEM data shows that bacteria cell wall is in contact with nanopillars at random positions and many nanopillars under the bacterium is not touching the cell wall.[2]-[4]This project aims to investigate the use of lab based X-ray nanotomography to study the 3D volume of bacteria attached on to nanopillar surfaces non-destructively at nanometer resolution.

Methods
The project uses nanostructured Silicon (Si) as test substrate and E.coli as model organism to develop the workflow. Freshly grown bacteria sample diluted into PBS media, incubated for 30 minutes on test substrata and chemically fixed, dehydrated by ethanol series and dried by critical point drying to be compatible for electron microscopy. Then subsamples of 15 µm in size with bacteria centred was isolated and placed on a sharp needle tip using a plasma FIB, as in preparation for nanoCT. These samples are then imaged in Zeiss Xradia 810 nanoCT, with low X-ray energy, with a voxel size of 150 nm.

Results
Micro manipulation of the samples on to a needle top was successfully achieved by plasma FIB. Creating of these sub-samples of 10 µm in size is essential to enable nanoCT characterisation. Ultra-high resolution was not successful, but high-resolution imaging nanoCT data clearly shows the bacterium attached on the top of nanopillars of 50-70 nm in size. As 3D image of bacteria and it's immediate surrounding is visualised by a non-distrructive method, now TEM lamellas can be prepared from the positions where bacterial cell wall is interacting with nanopillars.

Conclusion
Use of plasma FIB/SEM to create subsamples compatible for nanoCT was successful. The analysis of the samples in nanoCT was successful as bacteria and nanopillars could be located using 100 nm voxel resolution. This allows to characterise the overall 3D shape of bacterium when they interact with nanopillars non-destructively. approach to find individual bacteria cells. This approach allows to pinpoint positions where bacterial cell-wall interact with nanopillar surface so that TEM lamellas can be prepared for high-resolution TEM tomography to study the cell-wall damages in detail.

Keywords
nanoCT, FIB/SEM, Mechano bactericidal action, nanopillars, bactericidal surfaces, electron tomography

Acknowledgements
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Keywords:

nanoCT, FIB/SEM, nanopillars, bactericidal surfaces,

Reference:

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Correlative X-ray and Electron Microscopy Workflow for Investigating Grey Matter Lesions in Multiple Sclerosis

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Poster Group 1

Background: Multiple sclerosis (MS) is a complex neurological disease characterized by a heterogeneous distribution of lesions within the brain, making their investigation challenging. Currently, the treatment of MS primarily focusses on white matter pathology. However, it is increasingly recognized that injuries in the cortex, i.e. grey matter lesions, play a critical role in progressive disease outcome. Unfortunately, these grey matter lesions are poorly understood. In order to gain a better understanding of these lesions and their underlying pathological mechanisms, we pursue an ultrastructural study. We have established a correlative microscopy workflow that combines X-ray microscopy (XRM) and electron microscopy (EM). This workflow enables us to track and analyse the diseased regions in human brain samples throughout the different stages of tissue preparation up to their ultrastructural investigation.

Methods: we apply a correlative multiscale approach based on XRM, scanning electron microscopy, and transmission electron microscopy to analyse MS lesions in human brain samples.

Results: XRM is used to identify the diseased regions during the tissue preparation process for ultrastructural imaging, starting from fixed brain tissue to the final EM samples. Through XRM, we are able to identify lesions in the brain samples. Using a coordinate system, specific regions of interest within resin-embedded EM samples are then targeted for detailed ultrastructural examination by electron microscopy. This targeted approach enables us to directly visualize and analyse the ultrastructural changes of grey matter lesions.

Conclusion: The combination of X-ray microscopy (XRM) and electron microscopy (EM) offers a systematic approach for analysing the spatial distribution and the ultrastructural changes of heterogeneously localized diseased areas.

Keywords:

correlative microscopy, XRM, EM, neuropathology

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AI-driven microscopy for analysis of xenotransplantation models

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Poster Group 1

Background and Aims
Accurate visualization and quantification of immunohistochemical data is critically important in biological applications, particularly when requiring nuance within disease models and paradigms. Manual and single threshold methods are prone to bias, inaccuracy and are wholly inefficient over a large scale. The increased demand for accurate analysis of high throughput data acquisition becomes even more critical when modelling disease as results can influence the development of treatments and new therapeutics. Therefore, the aims of this work were to develop a highly accurate pipeline for image analysis of xenografted human cells to determine how pathology may develop in Parkinson's disease (PD) mouse models, utilizing AI-driven machine learning to accomplish these aims.
Methods
Immunofluorescent stained mouse tissue grafted with human derived cells were imaged using a widefield slide-scanner solution to train a deep neural network (DNN). Individual slides were loaded and scanned automatically using a 20x overview and target cells manually labelled to create initial training datasets. Of these datasets, 80% were used to train the DNN and 20% used for internal validation. Utilising instance segmentation with the ResNet50/FasteRCNN DNN we generated, this first trained iteration was applied over new datasets for automated tissue and cell detection with manual approval/quality control checks. Additional training and optimisation of the DNN was applied until a level of accuracy and acceptability was reached with low (<5%) errors detected.
Results
All grafts within scanned tissue were able to be accurately detected and cells of interest quantified. Initial training had accuracy at approximately 84% and required alterations to the scanning parameters to ensure high quality input data was used to train and improve the DNN accuracy. The developed DNN was additionally able to provide several critical readouts important for the analysis of pathophysiology as it relates to xenotransplantation models in PD. Area and size of grafts, migration of cells from the injection site, phenotyping and quantification of cells within the graft were able to be automatically detected and accurately analysed with the developed DNN.
Conclusion
Development of this AI driven microscopy solution for analysis of grafted cells within the brain has allowed for greater efficiency, accuracy and decreased bias within this animal model. By developing and training the above DNN, we were able to efficiently and accurately provide robust commentary on large dataset using a mouse model of human disease, improving our confidence in the results. This method could be applied widely to other models and similarly improve the accuracy and efficiency of data analysis as it applied to biological questions and potential downstream therapeutic development.

Keywords:

DNN AI xenotransplantation immunofluorescence widefield

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SEM and mCT investigations on GDEs with Copper-Nafion coating for carbon dioxide reduction to ethylene

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Poster Group 1

Background incl. aims
Replacing conventional high energy consuming chemical syntheses by a carbon-neutral route is in high demand for the future chemical industries. Especially the electrochemical synthesis of ethylene through the reduction of carbon dioxide instead of cracking intermediates of mineral oil at high temperatures allows sustainable value chains with a carbon circular economy [1]. Copper turned out to be the only pure metal that reduces carbon dioxide to products requiring more than two electrons transfers with substantial Faradaic efficiencies like hydrocarbons, aldehydes, and alcohols [2]. In order to prevent agglomeration of nanoparticles and improve adhesion to the substrate material, ionomers like Nafion (perfluorosulfonic acid, PFSA) are added to the ink. The combination of hydrophobic PTFE chains and terminal hydrophilic sulfonic acid groups promotes a homogeneous dispersion of the copper nanoparticles resulting in an overall high chemical activity and serves as a robust binder between the copper nanoparticles and the gas diffusion electrode [3]. However, any variations in the catalyst layers like thickness, porosity, inner network connectivity/tortuosity and conductivity has a significant impact on activity, selectivity and stability of the electrolysis. Therefore, knowledge of the 3-dimensional structure of the catalyst layer is essential for understanding the influence of chosen ink compositions and the resulting morphology of the catalyst layer on the performance of the electrode.
This work investigates the influence of different copper-to-Nafion ratios on the 3-dimensional morphology of the catalyst layer placed on a gas diffusion electrode. SEM panorama images and mCT measurements were used to characterize the homogeneity of the spray coating as well as the network connectivity and tortuosity of copper, Nafion and pores in the catalyst layer.

Methods
Gas diffusion electrodes (GDE) were spray coated with copper-Nafion inks with a loading of 0.5 mg/cm and a Cu:Nafion-ratio of 10 : 1 and 10 : 6, respectively.
SEM data were acquired on a ZEISS Gemini 300, equipped with the EDS detector UltimMax 65 by Oxford Instruments and the software SmartStitch, Version V01.02.13 by ZEISS for generating large panorama images.
Micro-computed tomography measurements (mCT) were acquired with a ZEISS Xradia Versa 620. X-ray voltage was 40 kV, X-ray current was 75.1 mA and the exposure time was 30 s. The field of view was 763.36 x 763.36 µm and the pixel size was 0.7649 µm. The number of total projections was 1201 with a full 360° tomography. The data evaluation was performed with the Dragonfly software, version 2022.2.0.1409 for Windows.

Results
According to the panorama images, the copper-Nafion ratio has a great influence on the morphology of the catalyst layer. Though the loading over the overall GDE is quite homogeneous for both ratios

(not shown in this abstract), a higher Nafion content results in huge cracks of the catalyst layer. A higher Nafion content also seems to cause more agglomeration of the copper particles (see Figure 2) compared to the sample with minor Nafion. Additionally, the pore network is finer dispersed for smaller Nafion contents as the Nafion tends to form a covering layer on the GDE (except for the cracks). A quantification of the copper, Nafion and pores based on the grey scale of the images results in 26% copper, 65% Nafion and 9% pores for a copper-Nafion ratio of 10:1 and in 21% copper, 71% Nafion and 8% pores for a copper-Nafion ratio of 10:6.
In Figure 3 are 3-dimensional mCT images of the samples, which look quite different to the BSE images concerning the homogenity of the catalyst layers. The sample with less Nafion content seems to have an inhomgeneous thickness of the catalyst coating.

Conclusion
The Nafion content significantly changes the morphology of the catalyst layer. Higher Nafion contents result in cracks of the catalyst layer, agglomeration of copper particles and a minor dispersed pores network.

Keywords:

mCT, CO2 reduction to ethylene

Reference:

[1] doi: 10.1126/science.aav3506
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[3] doi: 10.1021/acscatal.2c05235

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Low-Bleaching Cryo-Light Microscopy with Immersion Objectives Improves Super-Resolution STED Imaging

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Poster Group 1

Background incl. aims
Precise correlation in correlative light and electron microscopy (CLEM) is crucial for detailed and accurate studies of cellular structures and functions. Super-resolution techniques, such as stimulated emission depletion (STED) microscopy, hold great promise for high-precision localization in CLEM. However, the intense laser illumination required often leads to rapid photobleaching of fluorescent dyes. This rapid bleaching not only limits the duration of imaging, but also reduces the potential for capturing weak signals, ultimately lowering the effectiveness of super-resolution microscopy in CLEM applications.
We investigate the use of cryoimmersion-light microscopy (cryo-iLM) to face this challenge as cryogenic temperatures can significantly reduce photo bleaching [1].

Methods
To evaluate the photobleaching of fluorescently labeled cells, we used a high numerical aperture (NA) cryo-immersion objective (1.3 NA, 63x). At room temperature, living cells were imaged using water immersion. For cryogenic temperatures, plunge-frozen cells were imaged using the refractive index-matched immersion medium HFE 7200, as described by Faoro et al. [2].
Photobleaching was quantified by measuring the average intensity of wide-field images over time under continuous illumination. To assess STED compatibility, we compared the resolution enhancement to confocal acquisition and evaluated STED-bleaching over a series of images.

Results
Our experiments showed a significantly lower bleaching rate at cryogenic temperatures compared to live cell imaging. This was consistently observed for a variety of fluorescent markers, including organic dyes and fluorescent proteins.
In addition, the improved stability at low temperatures allowed for more robust super-resolution STED imaging. Notably, some dyes that were not compatible with STED at room temperature became suitable for STED at cryogenic conditions. By minimizing bleaching, we achieved clearer and more precise localization of fluorescent signals.

Conclusion
Combining both, low temperatures with the high-NA cryoimmersion objective, significantly reduces photobleaching and enables the visualization of weak signals with greater clarity such as fluorescent proteins. By mitigating the effects of bleaching, cryo-light microscopy improves the feasibility of super-resolution STED imaging.

Acknowledgement

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Keywords:

Cryo-immersion-microscopy, Cryo-fluorescence-microscopy, Correlative-microscopy, Super-resolution-microscopy, Photobleaching

Reference:

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Nanosized Ti-Ni composite

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Poster Group 1

Background incl. aims
Self-propagating High-temperature Synthesis (SHS) is a relatively novel and straightforward method for producing advanced ceramics, composites, and intermetallic compounds [1]. The foundation of SHS lies in the ability of highly exothermic reactions to sustain themselves through a reaction (combustion) wave. SHS reactions have also been observed in Ni-Ti multilayer films [2], possessing great potential as an external heat source in various applications, including joining, ignitors, and intermetallic synthesis on the nanoscale. A novel approach proposes to use nanoparticles to achieve similar self-propagating reactions. This concept is particularly promising due to the ease of application of nanoparticles on various surfaces. Core-shell nanoparticles, especially those with a titanium (Ti) core and a nickel (Ni) shell, are of significant interest. This configuration ensures a stable Ni to Ti ratio while the Ni shell protects the Ti core from oxidation. This study presents basic configurations of Ti and Ni nanoparticles and their composite produced by gas aggregation source (GAS) under different conditions.
Methods
DC magnetron sputtering with two planar magnetrons was used to prepare Ti-Ni core-shell nanoparticles and a mixture of Ni and Ti nanoparticles. The sputtering system consisted of a primary gas aggregation cluster source (GAS) for creating Ti nanoparticles (NPs) and a secondary chamber for coating them with a Ni film, producing Ni nanoparticles, or depositing Ni nanoparticles on Ti ones. Ar gas with 99.996% purity was used as the working gas with a pressure of 35 Pa in the Ni and 11 Pa in the Ti chamber. A current of 400 mA on Ni magnetron and a current varying between 100 and 900 mA on Ti magnetron were used. Further details about the experimental setup are available in the work by Hanus et al. [3].
The nanoparticles were deposited onto a glass substrate. The samples for TEM analysis were prepared by depositing the nanoparticles, suspended in methanol, onto a grid with a SiN or lacey-carbon support film. A Jeol 2200FS transmission electron microscope operated at 200 kV was employed in STEM mode. Chemical mapping was done using energy dispersive spectroscopy (EDS).
Results
Pure Ti nanoparticles were prepared in the aggregation chamber (current of 400 mA). Their size is about 40 nm. Their surface is covered by a 3-5 nm thick TiO2 amorphous film, which probably forms during the exposition of nanoparticles to air. Pure nickel nanoparticles form in the secondary chamber (current of 400 mA). Their size ranges between 8-10 nm. A very thin oxide layer also forms on their surface after longer exposure to atmospheric oxide. However, its thickness is significantly smaller (less than 1 nm).
The composite nanoparticles were formed with a current of 100 mA in the GAS chamber for the Ti particle formation and 400 mA on the Ni coating magnetron. They are composed of a 30-40 nm Ti core encapsulated in a 5-10 nm thick Ni shell.
Conclusion

Titanium and nickel nanoparticles for SHS were successfully synthesized using a DC magnetron sputtering technique, which produced particles with a core-shell structure characterized by a metallic core and an oxidized shell. A thick oxide layer forms immediately on Ti nanoparticles, preventing their reactive properties. The oxide layer on Ni nanoparticles forms after longer exposure to atmospheric air, and its thickness is significantly lower. Composite nanoparticles consisting of Ti-core and Ni-shell could be prepared in the GAS with a simple engineering of the Ni/Ti ratio and used for the SHS synthesis.

Keywords:

Nanoparticles, Ti-Ni core-shell nanocomposite

Reference:

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3D Characterization of Pore Structures in Shaped Heterogeneous Catalysts Using FIB-SEM Tomography

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Poster Group 1

Background incl. aims
Heterogeneous catalysts are crucial for industrial chemical processes, with their efficiency relying heavily on physical and chemical properties across multiple scales. Scaling up the application of these catalysts necessitates a comprehensive understanding of their structures for multiscale design, particularly for industrial applications where powdered catalysts are typically pressed into shaped forms, like extrudates. These extrudates form porous medium where mass transport limitations predominantly govern the reactions kinetics, making the characterization of pore size and distribution critical.
Methods
Direct measurement of pore structures in shaped catalysts is challenging due to the local inhomogeneities and the large size of extrudates (several millimeters) compared to the nanoscale pores. Traditional transmission electron microscopy is inadequate due to its destructive sample preparation and thin sample requirement. To address these challenges, we employed a combination of focused ion beam and scanning electron microscope (FIB-SEM) tomography to achieve high-resolution imaging of the pore structures within catalyst extrudates. This approach enabled us to capture detailed images of pores ranging in size from 50 to 100 nanometers. Using Avizo software, we reconstructed the 3-dimensional microstructure of the catalysts, providing a comprehensive view of pore volumes and their connectivity.
Results
The application of FIB-SEM tomography, coupled with advanced 3D reconstruction, enabled accurate characterization of pore structures within the catalyst extrudates. Our results revealed intricate details of the pore distribution and connectivity, essential for understanding mass transport properties. The 3 dimensional images facilitated precise estimation of pore volumes, enhancing our ability to model and predict catalyst performance under various operational conditions.
Conclusion
This study highlights the importance of advanced imaging techniques in catalyst characterization. By accurately mapping the 3D pore structure, we gain a deeper understanding of the relationship between pore geometry and catalyst performance. These insights are crucial for optimizing catalyst design to ensure efficient mass transport and improved catalytic efficiency at larger scales.

Keywords:

Heterogeneous Catalysts, FIB-SEM, 3D Reconstruction

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Cryo-Volume Electron Microscopy characterization of chlorophyll deficient microalgae

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Poster Group 1

Microalgae are a novel, valuable food source with multiple benefits including high nutritional value, and potentially with a more sustainable production compared to conventional food protein sources such as soybean, corn and meat. Nevertheless, consumer studies reveal that when applied in food the inclusion levels must be kept low, mainly due to the intense green colour of the algae and taste. By using UV-radiation combined with a visual screening process, we have obtained chlorophyll deficient mutants of the two microalgae species: Chlorella vulgaris and Nannochloropsis oceanica.

Characterization and comparison of the algal mutants and wild types with respect to growth kinetics and biomass composition is ongoing. However, light microscopy indicates that the morphology of the pale mutants is very different from the wild types. Therefore, Focused Ion-Beam Scanning Electron Microscopy (FIBSEM) has been applied to describe these morphological differences and to characterize the algal mutants at the ultrastructural level. The FIBSEM is well suited for site-specific sectioning where the FIB milling exposes a surface that can be visualized either with FIB or SEM to examine the microstructure within each layer and the interfacial structure between layers. By collecting ultrastructural data from a large sample volume and in turn interrogated in silico; novel understandings of the interconnectivity between organelle through an entire cell can be deduced. To date, this has only been successfully applied to chemically processed tissue, with their associated artefacts e.g., significant shrinkage, loss of intracellular soluble content and disruption to membrane structure. Non-chemical fixation using low temperature allows cells and tissues to be studied close to their native biological state. This is a major advantage for the characterization of poorly understood ultrastructural changes such as in the novel microalgae mutants generated in our study. To elucidate and compare the cell morphology of chlorophyll deficient mutants and wild type strains of the single celled microalgae Chlorella vulgaris and Nannochloropsis oceanica we have applied cryo electron tomography by controlled milling of vitrified cells using focussed ion beam milling FIBSEM microscopy at cryogenic temperatures (cryoFIBSEM).

By using a novel cryo vEM (cvEM) approach to generate larger 3D volumes than possible with cryoET through high-resolution sectioning in a cryoFIBSEM, we have been able to observe the microalgae strains close to their native biological state. By successfully overcoming the beam sensitivity of the vitrified samples and avoiding heavy metal contrast, as well as addressing significant challenges in generating sufficient signal-to-noise ratio, we have been able to define the ultrastructural details of the algae. This new approach has revealed significant differences in the ultrastructure between the mutants and the wild types including deformation of the thylakoids and reduced volume of the chloroplast.

Keywords:

Microalgae, volume electron microscopy, FIBSEM-cryo

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Optimized Bright Field STEM Imaging for Detecting Molecules absorbed within Zeolite Pores

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Poster Group 1

Background incl. aims
Zeolites, characterized by their large and regular pores, are utilized to filter target molecules from mixtures, purify effluent by removing pollutants and in catalysis. Atomic resolution imaging of small molecules in pores will contribute to elucidate host-guest interactions between small molecules and porous structures and understand diverse molecular absorption/desorption behaviors in pores. Scanning transmission electron microscopy (STEM) is a powerful technique to capture interpretable atom-scale images, facilitating localized structural analyses. Recognizing the inherent sensitivity of zeolites and molecules to electron beam exposure, low electron dose imaging techniques, including the differential phase contrast (DPC) employing segmented detectors and 4D-STEM using pixelated detectors, have been developed. Pixelated detectors employed by 4D-STEM offer superior electron collection efficiency with fine resolution and high signal-to-noise ratio (SNR) at probe positions[1]. However, pixelated detectors entail higher costs and longer dwell time compared to segmented counterparts. The integrated DPC (iDPC) technique has shown its power in imaging light elements with low-dose conditions. Recently, a newly developed optimum bright-field (OBF) STEM technique demonstrates enhanced SNR relative to iDPC[23] OBF images are reconstructed by combining the images captured by segmented detectors. Each segmented image is weighted by a frequency filter for the contribution to the Fourier component of the reconstructed OBF image, where the frequency filters are built using phase contrast transfer functions (PCTFs) for the segmented detector[2]. Here, the OBF-STEM technique with a custom-made python package is utilized for the efficient detection of molecules within the pores of ZSM-5 zeolite using a four-segmented detector.
Methods
The theoretical framework of the OBF STEM image can be derived from the single side band (SSB) ptychography[4], where the specimen is weakly electron scattering. The exit wave function M(k ,Q) is given by the convolution of the entrance probe wavefunction ψ(k)·ψ^* (k-Q) on the specimen with the pupil function A(k) A^* (k +Q) , where Q is the spatial frequency of the probe at spaced position r on the specimen in Fourier space. Therefore, the PCTF can be calculated by T^* (k)T(k -Q)-T(k) T^* (k +Q) , where T(k) is the aperture transfer function defined as the multiplication of the aperture function with the probe wave function in reciprocal space. In a segmented detector, the STEM image intensity of the i-th segment I_i (r) is described mathematically as ∫I(k ,r) D_i (k)𝑑k, where D_i (k) is the i-th segmented detector response function in reciprocal space and I(k, r) is the inverse Fourier transform of the diffraction pattern (|M(k,Q)|^2). The OBF STEM image is reconstructed by combining the segmented images weighted by frequency filters in reciprocal space, or combining the convolution of each segmented image with a corresponding point spread function (PSF) in real space[2]. For maximizing the SNR of the reconstructed OBF image, the PCTFs in the segmented detector are optimized by incorporating real-valued functions, such as high/low bandpass filters [2]. In this work, a custom-made python package is developed to calculate the PCTFs and the corresponding real-valued functions for OBF STEM image reconstruction. The specimen of ZSM-5 zeolite absorbed pollutants was imaged with the convergence semi-angle of 21 mrad on an aberration-corrected FEI Titan Cubed Themis Z microscope operated at 300 kV. A four-segmented detector was employed to

capture images using a beam current around 4.7 pA and a dwell time of 2 µs per pixel within a collection angle range of 8-42 mrad, with a camera length of 185 mm.

Results

The ZSM-5 with pollutants in pores was imaged along the [0 1 0] direction under a dose rate of 856 e-/Å². iDPC image (Figure a) and OBF image (Figure b) were reconstructed using the same dataset. Compared with the iDPC image, OBF image demonstrates clearer structural details due to its higher contrast and SNR, evident in line profiles and the cropped image (Figure d) from the same area in iDPC and OBF images. The superior contrast and finer resolution of OBF image enable more distinct observation of pollutants (marked by arrows in the line profiles) within pores than that of the iDPC image.

Conclusion

OBF-STEM images were reconstructed using the custom-made python package. This package implements algorithms to compute the PCTFs and the corresponding real-valued filters for image reconstruction. The reconstructed OBF image demonstrates enhanced contrast and SNR, giving clearer discernment of structural features. Importantly, features within the pores of ZSM-5 were distinctly distinguished in the OBF images compared to those reconstructed iDPC images. These findings imply that the OBF technique, employing a high-speed segmented detector, represents a promising approach for low-dose imaging of molecules encapsulated within the pores of zeolites.

Keywords:

STEM, segmented detectors, low-dose imaging

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CELS-3D – Cutting edge light source for exciting fluorescence in microtome-based 3D microscopy

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Poster Group 1

It was discovered that the light entering a triangle ultramicrotome glass knife from the bottom exits the knife through its cutting edge, forming an oblique light sheet-like illumination that is suitable for side-illumination of the sample block and 3D imaging. Several challenges were successfully managed, and a working prototype of a novel serial sectioning block-face imaging microscope was constructed. The CELS-3D (Cutting Edge Light Source, Three-Dimensional) is a microscope mounted on a commercial ultramicrotome. The CELS-3D has been characterised and applied for three-dimensional imaging of human liver spheroids with a diameter of approximately 500 micrometres. The structure of nuclei and tight junctions has been successfully reconstructed over the full spheroid volume. The formation of bile sinusoids in the central region of the spheroid was identified, which is crucial for modelling hepatitis viral infection in vitro. In comparison, the confocal microscope was unable to image spheroids to a depth exceeding approximately 50 micrometres and failed to detect the sinusoids.
The CELS-3D can be utilised for the three-dimensional reconstruction of fluorescent biological and/or artificial materials, irrespective of whether they are transparent or not. This encompasses a range of applications, including operation biopsies, experimental organoids/spheroids, artificial cartilage, and bone, among others. The CELS-3D can be effortlessly mounted on the top of any commercially available ultramicrotome, and its operation is straightforward and intuitive. It is anticipated that this technology will find applications in the expanding market for 3D microscopy, which is seeking a compromise between sample size and resolution.

Keywords:

3D microscopy, Serial sectioning, CLEM

1302

Correlative microscopy of graphene with SEM, Raman spectroscopy and AFM

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Poster Group 1

Graphene has been speculated to be a suitable material for producing robust and sensitive Hall sensors [1] and biosensors [2] due to its ultra-high carrier mobility. A key challenge however is producing electronic grade, high mobility graphene at scale. Paragraf has realised this by being the first company in the world to produce graphene using our proprietary and commercially scalable growth method, directly onto target substrates without the need of a transfer process, using standard semiconductor manufacturing tools. [3] We cover all aspects of production, from the growth of graphene to processing into final devices, including Hall sensors and graphene-FETs. We also develop in-depth fundamental structural understanding of our graphene to be able to relate to device performance and electrical properties.

When investigating graphene structural features in detail, we employ a number of microscopy techniques to gain a deeper understanding of the features observed. However, as most microscopy techniques are on the micron scale, it is challenging to locate the same feature reliably using multiple techniques. To address this, we employed a relatively simple and low-cost method from [4] of glueing down a TEM grid to our samples to locate the same 30 µm x 30 µm area across the techniques used. We then performed correlative microscopy on graphene on sapphire substrate to understand how different features appear with different techniques, including scanning electron microscopy (SEM), micron-scale mapping with Raman spectroscopy, and atomic force microscopy (AFM). We were able to identify how wrinkles in the graphene appear in the three techniques, and better understand areas that appear as darker contrast in SEM in-lens (SE1) images.

Keywords:

graphene, correlative, microscopy

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1305

The synthetic chaperonin Poly-CCT5 as a nanoparticle carrier

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Poster Group 1

Chaperones assist in the de novo protein folding and prevent protein aggregation. One of the most important chaperone families are the chaperonins (Hsp60s), which are organized as two oligomeric back-two-back rings generating a cavity in each ring where the substrate is placed for its folding. The most complex and important of all chaperonins is the eukaryotic CCT (Chaperonin Containing TCP-1) whose structure and the folding mechanism are key for nanotechnological applications.

The main aim of this project is to build a stable synthetic cylindrical structure capable of encapsulating chemical reagents or small proteins. It has been shown that CCT5 is able to self-oligomerize. When compared to the eukaryotic CCT, poly-CCT5 is easier to purify, can be genetically modified in all subunits and allows a more manageable image processing. These capabilities could enable poly-CCT5 to act as a nanocontainer delivering molecules to specific targets.

Our group used negative staining EM to assess the encapsulation of various nanoparticles inside synthetic poly-CCT5. VENOFER, an iron-sucrose coating NP, produced the best results overall and was chosen for Cryoelectron microscopy (CryoEM) analysis. We generated a 3.3 Å 3D reconstruction of the NP-bound poly- CCT5, with the NP presumably held by CCT5 apical domains. As part of this project, we are now focusing our efforts on the design and the structural characterization of three poly-CCT5 mutants, which rearrange the charge distribution on the cavity, to improve nanoparticle internalization and to prevent undesired interactions.

Keywords:

Poly-CCT5, nanocarrier, nanoparticles, CryoEM

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1306

The apoptosome assembly in situ

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Poster Group 1

Background incl. aims
The apoptosome is a large protein complex crucial in initiating programmed cell death (apoptosis). It forms upon initiating apoptosis and recruits procaspase-9 to activate the caspase cascade, eventually leading to the dismantling of the cell. Although experimental structures of the apoptosome are available, the occurrence of the complex has yet to be shown in situ. In vitro, it is a heptameric assembly of the apoptotic protease-activating factor 1 (Apaf1) and bound cytochrome c [1,2]. Using Cryo-Correlative Light Electron Microscopy (Cryo-CLEM), our lab has found that dense meshworks in the cytosol of apoptotic cells correlate with foci of Apaf1-GFP. Our cell biological data indicate that these foci are functional apoptosome equivalents [3]. Interestingly, these foci disassemble when cells survive apoptotic signals, indicating a potential regulatory function. Based on these findings, we aim to uncover the underlying structure of these dense Apaf1 meshworks within cells.

Methods
To further investigate the Apaf1 dense meshwork, we increased our dataset of electron cryo-tomograms of cryo-FIB milled cells. Additionally, we used a modified sample preparation protocol to unroof cells on cryo-EM grids to obtain tomograms of the dense meshwork in a close-to-native environment without needing prior FIB-milling [4]. We are now implementing a computational pipeline to analyse the amorphous meshwork using Rasterized Subtomogram Extraction (RSE), in which subtomograms from a region of interest are sampled in regular intervals, combined with the latest Relion 5 tomography pipeline for subtomogram classification and averaging.

Results
At present, we acquired a dataset of 12 tomograms of cryo-FIB milled cells and 52 tomograms of unroofed cells showing the Apaf1 dense meshwork. In these tomograms, we can outline the dense meshwork corresponding to the Apaf1-GFP signal of Apaf1 foci. Our cell biological experiments show that the assembly is based on defined molecular interactions, indicating a higher-order structure of the meshwork. However, the dense meshwork has a continuous, cloud-like appearance, in which it is challenging to discern the repeating subunits for subtomogram averaging. We, therefore, implement a systematic approach to extract subvolumes, which we will extensively classify and align in many iterations. Furthermore, our cell biology data indicates that caspase-9 plays a role in the assembly of Apaf1 foci. We therefore consider that caspase-9 might be a structural component of the foci.

Conclusion
By Cryo-CLEM we demonstrated that Apaf1 is associated with high-density amorphous meshworks in cryo-electron tomograms of apoptotic cells. These meshworks are notably different from the currently documented, discrete heptameric apoptosome wheel and thus likely represent an alternative in situ apoptosome assembly structure. Moving forward, we aim to reveal the structure of the meshwork at high resolution. We then want to identify the potential meshwork subunits, construct structural models and understand the biological function of the assembly. In particular, we

want to understand the molecular basis of the transientness of Apaf1 foci and whether it serves to regulate apoptosome function.

Keywords:

Apoptosome, Cryo-ET, Cryo-CLEM, Subtomogram Averaging

Reference:

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1307

Morphology-Driven Photothermal Efficiency in Nanostructured Semiconductors

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Poster Group 1

Nowadays, nanostructured semiconductor design represents outstanding model material systems for examining how the precise morphology manipulation at the nanoscale allows to fine-tuning their electronic structures to boost a plasmonic effect. Recent advances in designing metal-oxide semiconductor-based photocatalysts with photo-chemical/-thermal dual effects to photo-oxidize water contaminants and water-splitting reactions to produce hydrogen have garnered significant interest[1].

So far, in semiconductors, this dual functionality has often been achieved by doping or creating heterojunctions with external photothermal agents[2] such as carbon-based materials, conjugated polymers, and noble metal-based nanoparticles[3]. In this work, we have demonstrated that the precise morphological control of SnO_2 nanostructures promotes electronic structure changes in the material that impact its band structure and induce structural defects, such as oxygen vacancies, that enhance the density of free charge carriers enabling a plasmonic behavior tuning[4]. Under irradiation, this effect confers autarchic photo-thermal capability to the nanomaterial by generating hot carriers that produce local heating, which is then transferred to the surroundings. This increased temperature improves the mobility of photogenerated charge carriers of the semiconductor and molecular collisions in the medium, consequently boosting its photocatalytic efficiency[3].

This synergistic effect of photo-chemical and -thermal processes in a single system represents a novel approach to obtain high-performance photocatalysts. Herein, we focus on SnO_2 nanostructures, precisely engineered with varying morphologies such as nanoparticles, nanorods, nanosheets, and nanoflowers, synthesized via hydrothermal methods using structure-directing agents at different temperatures that allow for control of the shape. The microstructure, size, and morphology of these SnO_2 nanostructures were analyzed by a Thermo Fisher Scientific Tecnai F20 field-emission (scanning) transmission electron microscope (FEG-S/TEM) operated at 200 kV and a Hitachi High-Technologies SU-8000 and SU-8230 field emission scanning electron microscope (FE-SEM) operated at low voltage. Demonstrating the precise control of synthesis parameters enabled the achievement of desired morphologies with different structural dimensionalities from zero-dimensional nanoparticles to three-dimensional nanoflowers.

Optical and electronic properties were investigated using diffuse reflectance (DRS) and photoluminescence (PL) spectroscopies. The optical band gap energies (Ebg) were determined using Tauc's plots, and the obtained Ebg values exhibited a nanomaterial shape-dependent response. PL analysis was to assess the nature of structural defects and how they can affect their electronic band structure by tailoring the morphology. The strong emission peaks observed in the PL spectra were linked to surface and structural defects such as tin interstitials, dangling bonds, and oxygen vacancies

(OV), which can introduce localized energy levels within the bandgap, affecting their electronic band structure[4].

Tailoring morphology together with the obtained confinement effect at the nanoscale allowed not only the fine-tuning of the electronic structure of synthesized SnO_2 nanostructures but also control over localized surface plasmon resonance (LSPR), critical for generating high-energy hot carriers and enhancing photothermal conversion[2]. Under blue laser irradiation, the temperature changes in an aqueous medium were evaluated for all nanostructures. Notably, flower-like SnO_2 displayed an increased temperature by up to 14.5°C, attributed to the enhanced local electric field at tips, edges, and corners present in this kind of shape. In parallel, the temperature gradient enhanced the charge carrier transfer, boosting higher reactive oxygen species (ROS) generation, such as hydroxyl radical, which in turn accelerates the photo-oxidation rate[5]. Moreover, under UVA-Vis irradiation, the nanomaterials exhibited remarkable light harvesting and light-to-heat conversion capabilities that significantly enhanced the photocatalytic performance for water pollutant removal and green hydrogen production through a synergistic photo-chemical/thermal effect.

On the other hand, the morphology variation studied through FESEM and HRTEM provided insights into the crystal growth mechanism based on the structural dimensionality of these nanomaterials, ranging from zero- to three-dimension. Furthermore, a potential mechanism as photo-thermo-catalyzers was also proposed. This work and the corresponding findings pave the way for the precise design of nanostructured materials with tailored morphologies for enhanced photocatalytic and photothermal applications, particularly in environmental remediation and sustainable energy production.

Acknowledgements
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Keywords:

Nanomaterials, morphology, semiconductors, photothermal, photocatalysis

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In situ cryo electron tomography enables localization-dependent structural studies: Finding symmetric nuclear pore complexes

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Poster Group 1

Nuclear pore complexes (NPCs) bridge across the nuclear envelope and mediate nucleocytoplasmic exchange. Numerous copies of over 30 distinct protein species assemble to form each nuclear pore complex. The NPC's core scaffold is symmetric across the nuclear envelope. Distinct peripheral nucleoporin complexes are attached to the cytoplasmic and nuclear face, where they ensure transport directionality and fulfill compartment-specific roles, such as genome organization. It remains enigmatic how such compositional asymmetry is established on a scaffold architecture that exposes the same interaction surfaces to either side. Here, we combine in situ cryo-electron tomography (cryoET), subtomogram averaging, and template matching with live cell imaging to address this question in both budding yeast and isolated cells from Drosophila melanogaster ovaries. We propose that the cell uses the surrounding cellular milieu as a cue for the correct allocation of components specific to the two faces of the NPC. This would predict the existence of symmetric NPCs when exposed to the same environment on both sides. To test this prediction, we study pores outside the nuclear envelope. Such ectopic NPCs naturally occur, but are either rare, transient, or prevalent in cell types that are not amenable to standard cryoET sample preparation. We use genetically engineered systems to enrich pores in either intranuclear or cytoplasmic double membranes. We show that in contrast to pores at the nuclear envelope, such NPCs are symmetric across the membrane. We furthermore demonstrate that the peripheral nucleoporin configuration depends on the nucleotide state of the small GTPase Ran. Our data indicate that the nuclear transport system is self-regulatory, namely the same molecular mechanism controls both transport and transport channel composition.
Our findings exemplify that not only structural determinants but also the local cellular environment shape the composition and architecture of macromolecular assemblies, highlighting the importance of in situ structural biology.

Keywords:

nucleus, NPC, cryoET, FIB-SEM, correlative

1313

Enhanced Photocatalytic Degradation of Methyl Orange Dye Using Two-Dimensional Ti3C2Tx MXene with TiO2

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Poster Group 1

Background incl. aims

Water pollution is a significant global issue, with various domestic and industrial activities causing a substantial decrease in the availability of clean water [1]. The emission of dyes used in the textile, cosmetics, and food industries is a major contributor to water pollution, accounting for 17 to 20% of global contamination. The production of these dyes requires approximately 150 180 liters of water per kilogram, and approximately 15% of the dyes end up in water-based textile waste [2][3].
Various methods have been explored to address this issue, for the treatment and removal of dyes, with photocatalysis emerging as a particularly effective approach. Photocatalysis enables the high degradation of contaminants under ambient temperature and pressure conditions, using sunlight or ultraviolet light as the irradiation source [4].
MXenes, a novel family of two-dimensional nanomaterials composed of transition metal carbides bonded to organic functional groups, have shown great promise in this regard. Their lamellar structure provides a high surface area, electrical conductivity, and thermal and structural stability, making MXenes ideal for the fabrication and use of nanoparticles in photocatalysis [5]. The aim of this study was to synthesize the Ti_3C_3Tx MXene and, to improve its photocalytic properties by the in-situ deposition of TiO_2 nanoparticles, which was tested by the degradation of methyl orange dye.

Methods

The Ti_3C_3Tx MXene was synthesized by the chemical etching of Al from the precursor Ti_3C_3Tx MAX phase. For this, 0,5 g of MAX phase were added to a premixed solution of 0,5 g LiF and 5 mL 9 M of HCl, and stirred for 24 hours at 50°C. The resulting mixture was washed and centrifuged using deionized water at 12.000 rpm until the pH reached was 6; being later vacuum filtrated, sonicated and dried for 48 hours at 90°C. The further delamination step was performed by sonication in water during 1 h. The MXenes before delamination are multilayer samples (ML) while delaminated MXenes are few layer samples (FL).
The in situ TiO_2 nanoparticles were formed on the surface of the MXene surface by a solvothermal oxidation process dispersing the particles in isopropanol ((CH3)2CHOH) and heating up the solution at 190°C for 24 hours. This procedure was performed in ML and FL samples to compare the effect of the number of layers on the photocatalytic response. The samples obtained from the oxidation process were named ML-TiO_2 d FL-TiO_2. The prepared materials, unoxidized and oxidized samples, were characterized by XRD, Raman spectroscopy, SEM and TEM, while the photocatalytic properties were studied by degradation of methyl orange dye under UV light exposure.

Results

Raman spectroscopy and XRD confirmed the delamination process and the formation of the Ti_3C_2Tx MXene, and the further formation of TiO_2 in the anatase phase. SEM and TEM analysis revealed the formation of 2D lamellar particles and the removal of Al from the initial MAX phase to form the MXene material. Likewise, both microscopy techniques showed the formation of TiO_2 nanoparticles on the surface of the MXene layers from the solvothermal oxidation process. The dye degradation tests showed that the unoxidized ML and FL samples exhibited better dye removal by adsorption under dark conditions, while the oxidized samples exhibited greater photocatalytic degradation under UV light exposure. The anatase nanoparticles formed on the ML-TiO_2 and FL-TiO_2 samples significantly enhanced the photocatalytic activity of the Ti_3C_2Tx MXene, with a better performance for the FL-TiO_2 sample. The tests with various dye concentrations and photocatalyst charge achieved a maximum degradation efficiency of 99.4% for the FL-TiO_2 sample, highlighting the high effectiveness of Ti_3C_2Tx sheets with TiO_2 for removing contaminant dyes.

Conclusion

This study successfully synthesized 2D Ti_3C_2Tx MXenes using chemical etching and exfoliation techniques. The subsequent solvothermal oxidation process facilitated the formation of anatase TiO_2 on the MXene particles. The photocatalytic tests demonstrated that the oxidation process of the Ti_3C_2Tx MXene enhanced the photocatalytic properties over the adsorption process of the unoxidized MXene, and that the TiO_2-coated samples exhibited superior photocatalytic degradation of Methyl Orange under UV light. The optimal degradation performance, achieving a maximum efficiency of 99.4% for the delaminated FL-TiO_2 sample, which highligts the potential of the Ti_3C_2Tx MXenes with TiO_2 for effective removal of contaminant dyes, making them a promising solution for water pollution treatment.

Keywords:

Dye-degradation Photocatalysis MXenes Anatase

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1314

Evaluation

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Background incl. aims

Keywords:

MOF, GO, removal, adsorption, photocatalysis

Reference:

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1315

DELiVR: An end-to-end deep-learning pipeline for cleared-brain cell annotation

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Poster Group 1

Background:
Automated detection of specific cells in three-dimensional datasets such as whole-brain light-sheet image stacks is challenging. Deep learning is a promising method for light-sheet image analysis, but only is as good as the training data for this specific dataset.

Methods:
We realized that virtual reality (VR) is an ideal approach to generate custom deep-learning training data. We found that VR-generated training data leads to better model performance, but more importantly is also generated much faster. We built DELiVR, a broadly applicable processing pipeline to segment arbitrarily labelled cells in light-sheet image stacks of cleared mouse brains.
We have open-sourced the entire pipeline, docker containers, FIJI plugin, and training data at https://discotechnologies.org/DELiVR/ .

Results:
Here, we present DELiVR, a virtual reality-trained deep-learning pipeline for detecting c-Fos+ cells as markers for neuronal activity in cleared mouse brains. Virtual reality annotation substantially accelerated training data generation, enabling DELiVR to outperform state-of-the-art cell-segmenting approaches. Our pipeline is available in a user-friendly Docker container that runs with a standalone Fiji plugin. DELiVR features a comprehensive toolkit for data visualization and can be customized to other cell types of interest, as we did here for microglia somata, using Fiji for dataset-specific training. We applied DELiVR to investigate cancer-related brain activity, unveiling an activation pattern that distinguishes weight-stable cancer from cancers associated with weight loss.

Conclusion:
Overall, DELiVR is a robust deep-learning tool that does not require advanced coding skills to analyze whole-brain imaging data in health and disease.

Keywords:

light-sheet, deep-learning, VR, virtual reality

Reference:

Kaltenecker, D., Al-Maskari, R., Negwer, M. et al. Virtual reality-empowered deep-learning analysis of brain cells. Nat Methods 21, 1306–1315 (2024). https://doi.org/10.1038/s41592-024-02245-2

1316

Fabrication and charaterization of plasmonic nanopillars on a thin membrane for TEM investigation

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Poster Group 1

Plasmonic resonances are characterized by a highly localized and enhanced electromagnetic field around structures the size of the excitation wave, also known as “hot-spots". This phenomenon has been exploited in recent years in fields such as sensing, imaging or catalysis. The hot-spots enhance the signal in Raman spectroscopy [1], increase sensitivity for label-free protein detection [2], or generate localized heat for catalysis [3]. In these studies, plasmonic effects are usually quantified through these secondary effects or simulations rather than the direct measurement of the electromagnetic field in the hot-spots. In this work, we present the fabrication of a plasmonic sample consisting of a nanopillar array on a thin membrane suitable for a TEM study, which will allow probing the surface plasmons (SPs) with an electron beam. Excitation of the SPs will be possible with the mounting of a white LED in the chip, and thus the electron beam will be deflected and measurable in the vicinity of the hot-spots.

For the sample fabrication, we employed a combination of resolution enhancement techniques on a DUV stepper tool namely off-axis illumination, cross- triple exposure and the addition of assist feature lines on the design [4]. We achieved a hexagonal array of nanopillars with 150 nm diameters and 350 nm pitch. Deep Reactive Ion Etching (DRIE) was employed for the silicon oxide pillars’ etch and UV lithography was used to pattern and align the openings for the membranes with an accuracy of 2 um. A KOH bath etched the back of the silicon wafer to achieve 20 nm thick membranes. Finally, a 19 nm of aluminum coating ensured the plasmonic effects. Optical characterization on a micro-spectrophotometer of the fabricated chips confirmed plasmonic resonances on the visible range, Figure 1 b). The reflectance shows a resonance dip at 500 nm wavelength that differs significantly to the reflectance in the unpatterned area of the same membrane. This proofs that the addition of nanostructures increases the absorption of light in the form of localized surface plasmons.

Additionally, the sample was mounted on a TEM grid by means of a lift-out and welding process. Using a xenon ion beam, the nanopillar membrane was released from the substrate and mounted on a TEM grid and to a TEM electrical chip welding it with tungsten, Figure 1 a). A preliminary simulation of the fabricated nanopillar presented plasmonics resonances for visible wavelengths. An example is shown in Figure 2 a) where the EM-field distribution is highly localized in the top of the pillar quadruplicating the intensity of the excitation beam of 430 nm wavelength. The spectral shape of the simulated reflectance in Figure 2 b) is a good match to the reflectance measured in Figure 1 b). The measured dip at 500 nm shifts to the simulated dip at 650 nm, still within the visible range. Thus, the model describes the physics of the structure well.

Keywords:

Plasmonic
Nanopillar
Membrane
TEM
Resonance

Reference:

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1317

TEM-based study of the effect of precursor in thermal polymerization synthesis of graphitic carbon nitride

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Poster Group 1

In the last years, graphitic carbon nitride (g-C_3N_4) emerged as an attractive alternative to carbon materials, especially for photocatalysis. Several synthesis strategies were shown to lead to a plethora of partially polymerized allotropes, while the fully polymerized form of g-C_3N_4 is hardly obtained. The 3D structure of g-C_3N_4 obtained by thermal polymerization of N-rich precursors is usually well described as consisting of tri-s-triazine building blocks arranged in layers bound by Van der Waals forces, more or less hydrogenated [1,2]. Among possible precursors, melamine (M) and urea (U) have been tested in thermal polymerization methods [3]. The mixture of these two precursors can lead to a tunable combination of high reaction yield and high specific surface area. This contribution provides a study at nanometer scale of the material obtained using M and U precursors and mixtures of them, which is key for understanding the variations in catalytic functionalities.
The material investigated has been synthesized via thermal polymerization in air at 550°C starting from powder precursors, using pure melamine (sample M100), pure urea (sample U100) and mixtures of them (U/M (mass)= 80/20 and 50/50, resulting in samples U80 and U50, respectively) [4]. The obtained powders, after grinding in a mortar, have been suspended in ethanol, sonicated and drop-cast onto holey-carbon-coated TEM grids. BF-TEM and SAED analyses (using a SA aperture of 5.8 µm) were carried out by a Tecnai F20. For HRTEM analyses, an image-Cs-corrected JEM-2200FS with a direct electron detection camera (Gatan K2 Summit) was used, which allowed to obtain large field-of-view images at minimized electron-dose-rate thus beam-induced damage, expected for this material [5]. HRTEM images were obtained at an electron dose rate of about 30 e-/(Å²·s) and a total dose of about 330 e-/Å².
Similar to what reported earlier, morphological analysis shows that, while M100 material consists of thicker and bulkier flakes, U100 is characterized by crinkled, thinner and porous flakes (BF-TEM in panels a, e). SAED analysis shows polycrystalline flakes in both samples, but a higher crystallinity characterizes M100, which exhibits a higher number of sharp diffraction rings compared to U100 (panels b, f). For both samples, SAED patterns match with an orthorhombic phase, hydrogenated ($C_{24}N_{30}(NH_2)_6$, ICSD 194747) reported for g-C_3N_4 obtained by thermal polycondensation of melamine [1]. The bulkier flakes in M100 are formed by nanometer-size crystalline domains, which give rise to sharp diffraction rings corresponding to the (hk0) planes of the structure, generated by the in-plane stacking arrangement of tri-s-triazine units. The dominant diffraction feature in SAED patterns for U100 is instead the (002), corresponding to the distance between stacked graphitic planes, while (hk0)-related features are much less visible: this stands for a much weaker in-plane order and higher defectivity in the material. FFT analyses of HRTEM images, obtained from smaller areas, show a hexagonal-symmetry pattern for M100, with rings that however show an in-plane-rotation of the crystallites forming the flake (panels c, d). HRTEM analysis of U100 confirms the totally random orientation of crystallites and the presence of (002) planes due to rolled-up flakes (panels g, h). Increasing electron doses show faster amorphization and final degradation in U100, due to the knock-on of hydrogen atoms, probably present at a higher content due to incomplete polymerization

of the urea precursor [5]. From the comparison between SAED and HRTEM results, the comparably well-defined (hk0) peaks for M100 signify long-range order within the extended layers. Intermediate compositions lead to intermediate characteristics in the nanometer-scale structure, as expected. In summary, overall the material obtained by thermal polymerization of U/M powders is characterized by a lack of long-range order. However, significant differences occur between the outcomes of different U/M precursor ratios, which can be explained in terms of nanostructure organization of crystalline domains. This tunable structure allows to control the material properties with the aim of optimizing their employ in application.

Keywords:

graphitic carbon nitride, thermal polymerization

Reference:

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1318

A pipeline for classification and segmentation of 3D quasi-ordered patterns rmormo

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Poster Group 1

Background incl. aims
Biological materials are of spectacular beauty and source of inspiration for principles of material design and synthesis. They are composed of a remarkable small number of simple building blocks which are often organized, at different length scales, in patches of periodic or quasi periodic units. Prominent examples are the cases of trabeculae in bone or skeletal elements of echinoderms, color-bearing photonics crystals structures in arthropods and pore systems in cuticular materials or bone. The structural features (3D texture) and orientation of the periodic/quasiperiodic units largely dictate the properties of the materials to which they belong. Yet, segmenting and classifying these regions in large 3D datasets --a prerequisite for a complete understanding of the material's properties-- is incredibly challenging. In fact, while it is relatively simple to obtain a binarized volume of the component these structures are made of (e.g. trabeculae, pores or channels etc...), it is not trivial to segment different regions with similar 3D organization and orientation or, even more so, recognize and classify those regions that exhibit the same texture but that are differently oriented with respect to each other.
Methods
To address this, we propose a pipeline that, with minimal supervision and without user bias, extracts, classifies and learns, in reciprocal space, 3D textural features from tomographic datasets. The learned features can then be used to segment out, in the real space, regions which differ by their structural regularity and spatial periodicity. Also, the proposed pipeline allows to easily identify regions of the dataset that are equivalent by rotation and quantitatively compare textural properties of different datasets.
Results
Using this pipeline, we were able to segment large tomographic datasets (in the order of 10^{10} voxels) of several different biological materials with minimal memory and storage imprint and user supervision. As an example, the graphic shows how the pipeline allows an unbiased and automatic segmentation of textural features in a sea urchin test plate (µCT dataset $4 \cdot 10^9$ voxels, top), lacuno-canalicular network in bone ($1.8 \cdot 10^9$ voxels, laser scanning confocal microscopy, bottom left) and a photonic crystal in weevils scales (FIB/SEM dataset $1.5 \cdot 10^9$ voxels, bottom right).
A segmentation of about 10^9 voxels dataset can be run in a few hours on a desktop PC with a small GPU (8GB of RAM) and 32 GB RAM.
Conclusion
The pipeline presented in this work allows the segmentation of large volumetric datasets that exhibit quasi-periodic features and is particularly useful in those cases where the aim of the imaging is to establish relationships between the structure and the function of the investigated.

Keywords:

Tomography, machine-learning, segmentation, biological materials

1320

In-Situ Microstructure-Mechanical Property Mapping of Multi-Component Materials Using PI 89 Auto SEM PicoIndenter

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Poster Group 1

Background
Next-generation structural materials are designed to achieve superior performance by combining different elements and phases, each contributing distinct properties such as strength, toughness, or corrosion resistance. However, the often complex distribution of these multiple phases leads to challenges in characterizing the variations in mechanical properties across different regions of the material. Understanding these spatial variations is crucial for further refinement and optimization of the chemistry and microstructure of these complex alloys. Precise knowledge of how each phase and its distribution affect the overall mechanical behavior allows researchers and engineers to fine-tune the material for specific applications, ensuring optimal performance and reliability.

Methods
One major challenge for the characterization of these materials is the alignment of separately measured mechanical property and microstructure maps. Bruker's PI 89 Auto introduces advanced automation to the Hysitron PI 89 SEM PicoIndenter to address this challenge. This tool automates the positioning of the Rotation and Tilt Stage (R/T stage) for indentation after SEM imaging and EBSD/EDS mapping. The integration of TriboScan Auto software allows high-throughput testing with precision and control, seamlessly transitioniong between nanoindentation, SEM imaging, and EBSD/EDS analysis. From an EDS/EBSD map, the PI89 auto enables the co-localized acquisition of quantitative in-situ mechanical data at user-defined regions of interest. This significantly enhances the efficiency and accuracy of correlative structure-property analysis, facilitating advancements in material science and engineering. In this study, two heterogeneous alloys, a two-component steel and an accumulative roll bonded copper-niobium (ARB Cu/Nb) nanolaminate, are selected. The steel sample demonstrates significant variations in properties resulting from differences in chemistry and microstructure introduced during the synthesis process. This steel is fabricated by laser cladding 410 stainless steel onto a 4140 steel substrate, a technique that produces distinct microstructural zones identifiable through chemistry maps from Energy Dispersive Spectroscopy (EDS) and grain orientation maps from Electron Backscatter Diffraction (EBSD). These zones include: (1) the base 4140 steel substrate; (2) the heat-affected zone; and (3) the high chromium (Cr) 410 steel cladding.

Results
Figure 1 illustrates the three microstructural zones and their mechanical properties, which are assessed through a lateral nanoindentation hardness profile. Notably, the heat-affected zone is considerably harder than both the base substrate and the cladding. This increased hardness is attributed to the microstructural alterations induced by the laser cladding process, which include grain refinement and potential phase transformations due to the severe local heating. Elevated hardness in heat affected zones is commonly observed in many structural metals, but the facilitated correlation of chemistry and structural data makes it quick to identify these regions and measured their local properties with high spatial resolution.

The accumulative roll bonded copper-niobium (ARB Cu/Nb) alloy exhibits differences in mechanical properties across its component phases. This alloy is synthesized through a process involving repeated stacking, roll-bonding, and cutting of pure copper and niobium sheets. This method results in a lamellar microstructure characterized by alternating layers of copper and niobium. An array of Berkovich nanoindentations placed across several layers of the ARB Cu/Nb material allows for precise mapping of the mechanical properties of individual phases, as illustrated in Figure 2. By aligning a chemical map from Energy Dispersive Spectroscopy (EDS) with a nanoindentation hardness map, the mechanical heterogeneity of the respective component phases is correlated. This analysis reveals that the niobium phase is stiffer than the copper phase as expected. The increased hardness of the copper relative to the niobium phase is not necessarily intuitive, and can be attributed to the distinct grain sizes within each phase. Specifically, the copper phase exhibits a smaller grain size compared to the niobium phase. This disparity arises from the greater propensity for dynamic recrystallization in copper during the accumulative roll bonding process. Dynamic recrystallization, which involves the formation of new, strain-free grains during deformation, is more pronounced in copper due to its lower melting point and higher diffusivity compared to niobium.

Conclusions

This work highlights the utility of the PI 89 Auto in correlating local mechanical behavior with differences in grain structure and chemistry. By providing seamless integration of nanoindentation with SEM imaging and EBSD/EDS analysis, the PI 89 Auto enables high-resolution, high-throughput characterization of complex materials like ARB Cu/Nb and laser-clad steel. This capability is crucial for advancing our understanding of the relationships between microstructure and mechanical properties, thereby facilitating the development and optimization of advanced structural materials.

Keywords:

SEM PicoIndenter, Mechanical Property Mapping

1322

Electron Energy-Loss Spectroscopy of Liquids

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Poster Group 1

Background incl. aims
Liquid phase transmission electron microscopy (LPTEM) makes it possible to study reactions and dynamics in liquids at atomic resolution, with application in biology, battery studies, catalysis etc. This makes it a powerful tool for understanding the underlying mechanisms of liquid phase processes and interactions with other liquids or solids.
Electron energy loss spectroscopy (EELS) enables elemental analysis of materials through energy measurements in the transmission electron microscope (TEM). From EELS measurements it is possible to extract not only the elemental composition, but also obtain chemical bond knowledge of the electronic structures from the energy-loss near-edge structure (ELNES).
Combining LPTEM with EELS makes it possible to examine the elemental composition of liquids as well as their electronic structure and thereby chemical bonding structure through the ELNES. Additionally, it will enable the analysis of the beam induced changes in sample chemistry in the liquids and can be a valuable tool to understand radiolysis of liquids.
The aim of this study is firstly to measure, analyze and understand the molecular structure of a range of different liquids through their EELS spectra with associated ELNES structures, and assess if there are prominent radiolytic effects of the beam on liquid.
Methods
Nanochannel LPTEM chips of silicon nitride, supplied by InsightChips, were used to encapsulate the water. The nanochannels allow flow and thereby exchange of liquids while inserted in the TEM and makes it possible to have thin layers of liquids, thereby enhancing the resolution of the EELS spectrum compared to other silicon nitride based LPTEM systems. Graphene based LPTEM methods do not allow flow and hence cannot be used to establish a baseline measurement where there is a need for continuous fresh supply of the liquid to avoid radiolytic effects.
To obtain a sufficiently high resolution to resolve the ELNES structure of the water a Spectra Ultra S/TEM with a monochromator was used in for the measurements in both scanning transmission electron microscopy (STEM) and TEM mode. When the beam was focused on one of the channels it was possible to generate bubbles through radiolysis, which was then analyzed using the EELS system on the microscope. This allows us a better understanding of the liquid – electron beam interaction. The bubbles are temporary and dissolve in minutes if not continuously irradiated.
Results
From the oxygen edge on the core-loss spectrum it is possible to extract information about the structure of the water. Additionally, it is possible to determine the molecular composition of the radiolytic gases generated from the interaction between the liquid and the electron beam. Finally, it has been possible to evaluate the structure of other liquids such as ethanol and hexane.
Conclusion
From the experimental measurements of liquids, we present an approach for determination of the structures of different liquids. Additionally, the aim was to obtain knowledge of the radiolytic processes that occur in liquids when exposed to a high dose electron beam.

Keywords:

LPTEM, EELS, liquid analysis.

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Structural biology analyzes of photoreceptor outer segment: a closer look at PDE6

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Poster Group 1

Background incl. aims
Sight is perhaps the most spectacular human sense. The front of the eye allows light to pass through and channels it to the photoreceptor layer on the retina. There it triggers biochemical reactions that ultimately are converted to electrical signals that are sent and processed in the brain to generate images. Many components are required to create a vision, and it took millions of years of evolution to arrive at such a phenomenal organ. The conversion of photons to electrical pulse relies on the phototransduction cascade that involves many proteins at the outer segments of the cells and on its stacking of discs. There is a long-standing observation that in addition to the phototransduction activation, light stimulus also evokes morphological changes in the photoreceptors, still, the molecular determinates of this phenomenon have remained evasive. Recent developments in the Optical Coherence Tomography (OCT) field (including Optoretinography (ORG)) allow direct imaging of photoreceptors, and offer a new biomarker with the measurement of morphological changes detected upon light stimulus. At the same time, there is now evidence that a phosphodiesterase enzyme, namely Phosphodiesterase 6 (PDE6), that is at the core of the phototransduction signal pathway and is attached to the disc in the photoreceptor's outer segments, may bridge consecutive discs. Its structure has been determined in slightly different conformations that render the different protein lengths, thus opening the possibility that PDE6 is the molecular driver of the morphological changes observed. Our goal is to test if PDE6 is the molecular driver behind the observed morphological changes and a ORG biomarker.
Methods
To test our hypothesis, we isolated rod PDE6 and full outer segments (ROS) and employed Cryo-Electron Tomography (cryoET) and Cryo-Soft X-ray Tomography (cryoSXT) to test our theory in the cellular environment. The resolutions archived are modest compared to the Cryo-Electron Microscopy (cryoEM) structures already determined for PDE6, but still adequate to measure ROS disc spacing under different conditions and add to the “big picture” of the observed morphological changes. Importantly, the measurements are done with PDE6 in its natural environment and in the presence of relevant membranes. Additionally, we are also imaging fixed ROS, isolated under different conditions with Transmission Electron Microscopy (TEM).
Results
We have established the conditions to isolate and deposit ROS in cryo-grids, freeze the samples, prepare lamellas and collect tomograms. The tomograms are then processed so that information on the ROS disc spacing, under different conditions, is collected and analyzed. Such knowledge may have implications for therapeutics diagnostics and functional imaging of photoreceptor physiology.
Initial TEM images were obtained, and larger sample sets are being prepared.
Conclusion
We are at the initial sets of this project, but we have overcome already some technical difficulties, and expect now to collect larger data sets to test our hypothesis. At the same time, we are looking

for other possible microscopic techniques that may add information to the observed morphological phenomenon.

Keywords:

Photoreceptor outer-segment, cryoET, cryoSXT, TEM

1324

Advancements in Quantifying Neuroinflammation: Leveraging AI-Based Microscopy for Enhanced In Vivo Analysis in pharmaceutical studies

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Poster Group 1

Background
In the field of pharmaceutical research, accurately quantifying imaging data is essential. This precision is crucial for assessing neuroinflammation, a key factor in numerous neurological disorders. However, powered in vivo studies, especially for pharmacokinetics (PK) and pharmacodynamics (PD) evaluations, require utilizing large cohorts of animals. Additionally, recent technological advancements have significantly increased the capacity for multiplexing, allowing for more comprehensive data collection. This increase in data complexity poses new challenges for analysis.

Methods and Results
In our current studies investigating neuroinflammation, to manage and analyze the extensive data generated, we employ AI-based microscopy analysis. This innovative approach enables automated evaluation of drug efficiency in vivo, providing a robust and scalable solution for high-throughput studies. Our findings demonstrate the potential of AI-driven methodologies to streamline the analysis process, thereby enhancing the accuracy and efficiency of neuroinflammation assessments.

Conclusions
By integrating these advanced technologies, we pave the way for more in-depth research and faster, more precise therapeutic developments. The implications of our work extend beyond neuroinflammation, offering a framework for the application of AI in various bioimaging research areas. This approach not only improves the reliability of data interpretation but also significantly reduces the time and resources required for comprehensive analysis, ultimately accelerating the drug development pipeline and improving outcomes for patients.

Keywords:

neuroinflammation, histology, AI-based

1325

Revealing the Microstructure of Binary Solvent Hydrogels: a Novel Cryo-SEM Approach

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Poster Group 1

Hydrogels' biocompatibility and resemblance to biological tissues result in diverse applications in tissue engineering, drug delivery, agriculture, and bioelectronics. Binary solvent gels, such as those in aqueous dimethyl sulfoxide (DMSO) are more stable and interact more favorably with hydrophobic compounds. The internal structure of these binary solvent hydrogels remains poorly understood due to the lack of sample preparation techniques specific to the materials systems. In this study, a benzimidazolone (BZI) derivative supramolecular hydrogel is synthesized in both single and binary solvent systems. The single solvent hydrogel was prepared in water while the binary solvent hydrogel was obtained from 75 wt.% water and 25 wt.% DMSO solvent. We provide a novel preparation method for characterizing binary solvent hydrogels using cryogenic scanning electron microscopy (cryo-SEM). The preparation conditions for the binary solvent hydrogel were obtained by introducing a soaking treatment in deionized water (DIW) and tuning the appropriate sublimation temperature and duration while ensuring specimen integrity. We demonstrate that our new cryo-SEM characterization method can successfully remove residual solvent to expose the structural details of binary solvent hydrogels which was little known previously. The method will be harnessed in the future to comprehend the microstructural evolution of the binary solvent BZI hydrogel when it is loaded/unloaded with hydrophobic drugs. This newly developed method helps to understand the other binary solvent hydrogel structures, and the structure with organic solvents in their system using cryo-SEM characterization.

Keywords:

Cryo-SEM, hydrogels, sample preparation

Reference:

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1326

Charting the molecular landscape across layers of vitrified mammalian hippocampus using electron cryotomographyaa

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Poster Group 1

Background incl. aims
Our understanding of neurological function and disease relies on knowledge of both the cellular architecture of brain tissue and the molecular organisation within neurons and glia. Electron cryotomography (cryo-ET) is a powerful technique for investigating the structure and distribution of biological macromolecules in their native, cellular environment. Traditional sample preparation for cryo-ET of tissues involving chemical fixation and microtomy leads to artefacts that can obscure high-resolution detail, impeding biological interpretation on the molecular scale. Alternatively, tissues can be studied in their native, hydrated state by preparing vitreous samples with high-pressure freezing (HPF), combined with nanofabrication approaches such as focused ion beam (FIB) milling.
The advent of plasma sourced focused ion beam (PFIB) instruments offers enhanced milling speeds afforded by the greater beam coherence at high currents that plasma sources provide [1]. This has the potential to greatly improve the throughput of sample preparation for thick tissue samples. Similarly, recent advancements in cryogenic lift-out of high-pressure frozen samples on grids have shown the ability to excise large sections of material from HPF samples, and has enabled the analysis of more complex biological models such as C.Elegans embryos and organoids by cryo-ET [2,3]. Applied to mammalian tissues, the approach promises to unlock the molecular characterisation of brain organisation. Here we outline a robust technical workflow for fabricating brain tissue lamellae using PFIB. We leverage serial lift-outs to access new sampling geometries from HPF samples spanning the mouse hippocampus CA1 stratum radiatum.
Methods
The brain of a 184 day old female C57BL6 mouse was divided into hemispheres and sectioned to 100 µm using a vibratome on ice where 2 mm biopsies of hippocampus were taken for incubation in cryoprotectant. Hoescht 33342 nuclear stain was added prior to vitrification in copper-coated gold carriers using the EM ICE HPF (Leica). The sample was loaded into the Helios Hydra G5 PFIB (TFS), where fluorescence mapping was performed using an integrated light microscope (Delmic Meteor) within the microscope chamber. A 350 µm region of CA1sr was identified and adjacent trenches were milled with 200 nA Xe plasma. Undercuts were performed at 4 nA to relieve the sample from the bulk of the material. A cryogenically cooled tungsten manipulator (TFS) adapted with a copper block attachment was then used to lift out the sample, and serial sections were deposited on a 400 x 100 mesh copper support grid (Agar Scientific). Automated lamellae thinning was performed using Xe for Rough (4nA), Medium (1nA) and Fine (0.1nA) milling followed by manual polishing with argon (60-20pA). Tilt series were acquired using a Titan Krios G4 with an energy filtered Falcon 4i detector (TFS). Tilt series were preprocessed in Warp and reconstructed with WBP in AreTomo before visualisation in IMOD.
Results
A novel milling approach was developed to isolate sections spanning mouse hippocampal layers for serial lift-out from HPF carriers. Firstly, the location of the pyramidal cell layer was identified using a

correlated light and electron microscopy (CLEM) approach. The distribution of fluorescently labelled nuclei allowed for the cell body layer of the CA1 to be identified and guided the lift-out of targets spanning from the CA1 stratum oriens to the CA1 stratum lacunosum. The use of the xenon plasma to shape the sample allowed deep trenches adjacent and above the region to be milled in less than 30 minutes. This utilised beam currents ranging from 60-200 nA, with resulting lamellae showing no visible damage artefacts. The orientation of the milling enabled undercuts to be made using a 90º relative stage rotation so that the tissue could be excised. Subsequently, we could deposit up to 42 serial sections that sample across a 350-400 µm region containing multiple layers of the CA1. Sections were thinned to TEM transparency with >90% success, where 246 tilt series were collected in the CA1 stratum radiatum (CA1sr).
From these data, the cellular organisation and content across the mammalian CA1sr could be characterised. These include multiple different cell types, such as glia with patches of ribosomes in cell bodies (n=12/251) and neuronal projections (n=79/246). Cellular features observed include microtubules (n=221/246), actin (n=75/246) and endoplasmic reticulum (ER) (n=38/246). Manipulation of the geometry of the lift out by isolating a section of tissue within the plane of the sample provided access to a previously unobserved view of the CA1 apical dendrite cytoskeletal network in 14% (n=35/246) of tomograms. This perspective revealed networks of cytoskeletal elements, ER, and mitochondria. Furthermore, 9% of tomograms in this dataset contained a confirmed synapse (n=22/246), with a further 11% (n=27/246) showing putative synapses. The quality of the tomograms reconstructed from this tissue sample is sufficient to identify canonical synaptic features including cleft proteins, post-synaptic density, active zone organisation and synaptic vesicle endocytosis.
Conclusion
This work presents a routine approach for generating lamellae for cryo-ET of multiple layers of mammalian hippocampus from a single lift out using plasma FIB. The developed pipeline incorporates a cryo-CLEM aspect to guide the lift-out of targets spanning 350-400 µm across the CA1 region, generating over 40 deposited sections of high-pressure frozen brain biopsy in a single 24-hour microscope session. Semi-automation of lamellae thinning using a hybrid Xe/Ar plasma approach produces sufficiently thin lamellae to characterise cellular organisation and content across the mammalian CA1sr. By lifting out a section of tissue in the plane of the sample, previously inaccessible views of the CA1pyr apical dendrite cytoskeletal network could be imaged in vitrified samples. The spatial relationship between sequential lamellae, which sample every 5-10 µm of the CA1sr, demonstrates the feasibility of acquiring cellular cryo-ET data with enhanced biological context using our approach. The workflow we present here has the potential to be adapted for different cryogenically preserved model systems and tissues, opening up possibilities for observing biomolecules within their most native environment. Our approach paves the way for more comprehensive, contextually rich structural studies of brain tissue at the molecular level.

Keywords:

Plasma-FIB, Cryo-ET, Lift-Out, Structural Neurobiology

Reference:

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Label-free evaluation of apoptotic cell death in pancreatic cancer cells using stimulated Raman scattering microscopy

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Poster Group 1

Anti-cancer drug treatment has been a standard approach for managing aggressive human pancreatic cancer. Effective, label-free monitoring of cell death is crucial for assessing drug efficacy and elucidating the mechanism of apoptosis. In this study, we utilized stimulated Raman scattering (SRS) microscopy to evaluate individual pancreatic cancer cells treated with a newly synthesized hydroxyl group-introduced chalcone derivative and the commercially available gemcitabine. SRS microscopy, by detecting inelastic vibration scattering of biomolecules, provided high-resolution imaging data revealing morphological and compositional alterations during apoptotic cell death. SRS imaging identified distinctive apoptotic features, including cellular shrinkage and membrane blebbing, without the need for staining. Notably, treatment with hydroxyl group-introduced chalcone derivative induced more pronounced structural and biomolecular changes, such as increased lipid droplets and decreased protein levels, compared to gemcitabine during apoptotic cell death.

Keywords:

Pancreatic cancer, Apoptosis, SRS microscopy

Reference:

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Evaluation of pH-sensitive drug delivery systems for lung cancer therapy via multiplex fluorescence microscopy

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Poster Group 1

Non-small cell lung cancer (NSCLC) is characterized by the presence of tumor-associated macrophages that can polarize into a tumor-promoting phenotype, along with elevated levels of folate receptor beta (FRβ). Additionally, calpain-2 (CAPN2) is widely expressed in NSCLC and contributes to tumor progression. To enhance anticancer treatment efficacy and minimize side effects, developing innovative drug delivery systems with precise targeting and controlled release is essential. Here, we employed multiplex fluorescence microscopy to demonstrate that pH-sensitive, FRβ-targeted liposome-based nanoparticle drug delivery systems facilitate precise targeting and acid-responsive release in NSCLC. Our findings reveal that folate-mediated targeting of FRβ in tumor-promoting macrophages and NSCLC cells significantly suppresses tumor growth, while the stimulus-responsive release mechanism reduces drug-related toxicity. Furthermore, multiplex fluorescence imaging reveals that the combined use of docetaxel (an anticancer agent) and doxycycline (an anti-CAPN2 agent) with FRβ-targeted pH-sensitive liposomes exhibits a synergistic effect in suppressing tumor progression.

Keywords:

Multiplex fluorescence microscopy, lung cancer

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Observation of phase transformation of Sn by transmission electron microscopy

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Poster Group 1

Background
Tin (Sn) experiences phase transformation from metallic β phase to semiconductor α phase at 13 °C, and to liquid phase at 232 °C [1]. During the phase transformation from β phase to α phase, the metallic luster is lost [2], and the structure becomes prone to collapse, which is known as tin pests. In recent years, lead-free solder has attracted attention where Sn is often used as a substitute for lead (Pb) in the electronic products. Since the electronic elements become miniaturized down to nanoscales, understanding of the behavior of Sn at nanoscales is important. In this study, we investigated the phase transformations of Sn nanoparticles at low temperatures using transmission electron microscopy (TEM) and also carried out the optical measurement at high temperatures.
Methods
On a TEM grid with a SiN membrane, Sn of 99.9% was deposited with a target thickness of 14 nm for a flat configuration. The Sn-deposited sample was annealed at a temperature of 300 °C for 3 hours to form particles. The prepared sample was measured in TEM after being cooled down to -177 °C using liquid nitrogen. At -177 °C, the sample was held for one hour to allow for a complete phase transformation, and the TEM images and diffraction patterns were acquired. The temperature was then raised to room temperature (20 °C) by the heater and the TEM image and diffraction pattern were acquired.
For the optical measurement, we first deposited Sn nanoparticles of different sizes on borosilicate glass at film thicknesses of 1.5 nm, 3 nm, and 6 nm. The samples for the optical measurement were annealed under the same conditions as before to convert the films into nanoparticles. The particle sizes for each sample were measured to be 21.8 nm, 30.6 nm, and 83.0 nm, respectively. The samples were then coated with a 13.3 nm thick layer of AlN to prevent oxidation. The transmission spectra of the Sn nanoparticles of different sizes were measured while changing the temperature up to the phase transition point.
Results
The obtained TEM images at -177 °C and room temperature are shown in Fig.1(a), and (b), respectively. Although volume change is expected by the phase transformation to the α phase, no clear change in the shape of the nanoparticles was observed in the bright field TEM images. The diffraction patterns at -177 °C and room temperature are shown in Fig.2(a) and (b). The sharp Debye rings are clearly observed in Fig. 2(a) at -177 °C, while the Debye rings at room temperature are broad and vague as shown in Fig. 2(b). This indicates the crystallinity change (from large crystalline to smaller one) during the phase transformation although no clear shape change in the nanoparticles was observed in the bright field TEM image. The low-temperature crystal structure was identified to be the diamond structure, while the crystal structure at the room temperature was unable to be determined.
For the optical measurement at high temperature, the transmission spectrum for the nanoparticle with means diameter of 30.6 nm is shown in Fig. 3. It can be observed that the transmission intensity and the wavelength of the resonance peak change drastically at the phase transition from β phase to liquid. Moreover, the resonance wavelength shifts to the shorter wavelength at the liquid phase

together with the decrease in the transmission intensity, and the peak width. The transmission spectra of the samples with other sizes showed similar results.

Conclusions

We have successfully observed the phase transformation of Sn nanoparticles from the β phase to the α phase from the diffraction patterns obtained using TEM. The diffraction pattern at low temperature showed only α-phase rings, indicating a complete transformation of Sn nanoparticles into the α-phase. In the optical measurement at high temperature, we successfully observed the solid-to-liquid phase transition of the Sn nanoparticles in the transmission spectra.

Keywords:

Tin(Sn), phase transformation, TEM

Reference:

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1330

Pulse counting in the SEM

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Poster Group 1

Single electron pulse counting has revolutionised low-dose TEM and STEM imaging by providing zero background noise, quantitative information, and fast detector response times. This allows the uses of lower doses with faster image acquisition times whilst maintaining image fidelity and signal-to-noise ratio. Whilst this technology is common in the context of (S)TEM, the benefits of electron pulse detection are not obvious in the context of SEM and similar techniques such as Helium ion microscopy. Beam-related modification of samples can still occur (for example in battery anode materials), particularly for insulating samples where a low dose-rate may allow charging effects to dissipate. Scanning at high speeds can achieve this but can run into problems with slow detector response times, particularly scintillator-based technologies such as Everhart–Thornley detectors. Pulse counting can mitigate this, though the effect on image contrast is not as simple as the case of (S)TEM. For example, in secondary electron imaging, each signal pulse on the detector may represent multiple electrons, with the number (and therefore intensity) determined by the secondary electron yield from the sample. In this work, we present and discuss preliminary data using the turboTEM Pulse counter combined with SEM detectors to show the benefits and potential use cases for SEM imaging, from mitigating noise for low signals, increasing detector speed, and exploring the possibilities of new imaging modes.

Keywords:

SEM, Electron Counting

Reference:

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1331

Screening of surface Fermi level pinning governs contrast of modulation-doped n-type GaN by electron holography

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Poster Group 1

Background incl. aims
Quantifying electrostatic potentials in semiconductors at high spatial resolution is essential for understanding and optimizing the performance of semiconductor devices. Various electron beam-based microscopy techniques are employed to achieve this goal. Among these techniques, electron holography in a transmission electron microscope (TEM) has proven particularly effective for demonstrating the interface potential in p-n junctions and heterostructures. This technique provides highest energy precision at nm scale resolution, while minimizing electron beam induced damage to the sample. However, previous studies have revealed intriguing results. In particularly, the electron optical phase contrast observed across p-n junctions is always smaller than anticipated, whereas the phase contrast across a n-n+ doping step is larger than expected. This work aims to elucidate the physical origin of the phase contrast measured by electron holography of doping structures, seeking to reconcile these discrepancies and enhance our understanding of the underlying mechanisms.
Methods
We use an n-n+ doping step in GaN with Si concentrations of 8×10^{17} and 3.5×10^{18} cm^{-3} as our As model system. lamellas were prepared using focused ion beam (FIB) milling. The FIB-prepared lamellas are investigated by off-axis electron holography in a TEM (FEI Titan G2 60–300 HOLO, FEI instrument) operated at 300 kV. The lamella is kept at edge-on orientation and tilted away from the [10-10] zone axis to suppress the dynamical diffraction.

Results
An example of a phase map derived from a hologram acquired across GaN doping step is displayed in Fig. 1a. The investigated lamella exhibits a crystalline thickness of 257 ± 6 nm and a damaged layer on the surface due to FIB processing. [1] The corresponding phase change profile is plotted in Fig. 1b, showing that the difference in the phase contrast across the doping step is 0.40 ± 0.02 rad. Lamellas with crystalline thicknesses ranging from 188 to 380 nm were measured, indicating an almost thickness-independent phase contrast across the doping junction. For quantitative interpretation of the measured phase change profiles, self-consistent electrostatic potential calculations are carried out, taking the presence of a surface Fermi-level pinning of the TEM lamellas into consideration. By fitting experimental data with self-consistent electrostatic potential calculations, we revealed a surface Fermi-level pinning of 0.7 eV above the valence band in III-nitride lamella. From the calculation, we find the predominant contribution to the phase contrast is shown to arise from the doping-dependent screening length of the FIB-induced surface Fermi-level pinning occurring in the defect-rich crystalline inner shell (below the outer amorphous shell). This near surface depletion region remains unchanged for lamellas with different thicknesses, resulting in an almost constant electron optical phase contrast vs. thickness. The contribution of the built-in potential is almost negligible since its value is too small for modulation doping and only relevant for large built-in

potentials at e.g. p-n junctions. Thus, the weak built-in potential of GaN doping steps adds only a small thickness dependence to the phase contrast. [2]

Conclusion

The electron optical phase contrast probed by electron holography at n-n+ GaN doping steps is found to exhibit a giant enhancement, in sharp contrast to the always smaller than expected phase contrast reported for p-n junctions. We unravel the physical origin of the giant enhancement by combining off-axis electron holography data with self-consistent electrostatic potential calculations. The predominant contribution to the phase contrast is shown to arise from the doping dependent screening length of the surface Fermi-level pinning, induced by FIB-implanted carbon point defects below the outer amorphous shell. The contribution of the built-in potential is negligible for modulation doping and only relevant for large built-in potentials at e.g. p-n junctions. This work provides a quantitative approach to so-called dead layers at TEM lamellas.

Graphic

Fig. 1. (a) Phase map across an n-n+ GaN doping step. The n-GaN layer exhibits a darker contrast. (b) Phase change profile extracted from the phase map averaged over a width of 500 nm. The n-n+ interface is positioned at 0 nm. The phase contrast across the doping step Δφ junction is 0.4 rad.

Keywords:

GaN, electron holography, surface pinning

Reference:

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Localization of SHP-1 in Natural Killer Cells Across Education

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Poster Group 1

Natural killer cells learn to differentiate self from non-self cells through a process called education, wherein inhibitory receptors on natural killer cells interact with self-MHC class I molecules on healthy cells, leading to the acquisition of functional competence. Inhibitory receptors transmit signals via the recruitment of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which downregulates activating signals, suggesting a role of SHP-1 in NK cell education.
Our recent super-resolution imaging studies have demonstrated a link between SHP-1 expression, localization, and morphology in activating synapses. Educated natural killer cells exhibited reduced SHP-1 expression and localized less to activating synapses compared to uneducated natural killer cells, indicating that SHP-1 may regulate the NK cell education process.
Interestingly, we also observed that the educating receptor Ly49A associates more extensively with SHP-1 in resting natural killer cells of uneducated cells compared to educated cells, suggesting a role for Ly49A in regulating SHP-1 involvement with activation synapses.
To further elucidate the role of SHP-1 in natural killer cell education we aim to develop novel techniques of investigating the natural killer cell synapses in real time. We will utilize high-resolution live cell imaging and super resolution techniques on primary cells and in vitro systems. In these studies, we will investigate SHP-1 correlations, localization, and dynamics, using continuously developed and refined analysis scripts to optimize and automate the data analysis process.
These investigations aim to provide a deeper understanding of SHP-1 regulation in natural killer cells and may lead to new therapeutic strategies for natural killer cell-related diseases.

Keywords:

Localization, STED, Confocal, Immunology

Reference:

Schmied L, Luu TT, Søndergaard JN, et al. SHP-1 localization to the activating immune synapse promotes NK cell tolerance in MHC class I deficiency. Sci Signal. 2023;16(780):eabq0752. doi:10.1126/scisignal.abq0752

1334

In-situ Micromechanical Testing and Optical Microscopy of Native Human Ligamentum Flavum

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Poster Group 1

Background:
Ligamentum flavum (LF) significantly contributes the development of lumbar spinal stenosis, which is a serious disease in the aging population. The aim of this study is to characterize the mechanical properties of native human ligamentum flavum and correlate them with histopathological changes.
Methods:
Mechanical property gradients across the cranial, medial, and caudal regions of LF were mapped and compared with corresponding histological sections. Coincidence analysis of mechanical property maps with pathological alterations in LF microstructure was conducted to assess the influence of these changes on micromechanical properties.
Results:
Results demonstrated significant heterogeneity in LF mechanical properties, with local variations correlating with specific histopathological features. Pathological changes, such as chondroid metaplasia and loss of elastic fibers, were found to impact the distribution of micromechanical properties.
Conclusion:
These findings highlight the importance of considering LF heterogeneity in its mechanical characterization and provide insights into the complex behavior of LF under pathological conditions.
Figure 1. Lumbar spine stenosis Sample - Cranial Region: A. Distribution of mechanical properties (Reduced Young's Modulus) across the ligamentum flavum (LF) section. B. Selected rectangle areas with different microstructure shown in detail in C, D, E and F. C. Chondroid metaplasia. D. Moderate to severe degeneration of elastic fibers replaced by collagen. E. Normal structure of LF with predominant elastin fibers. F. Area of LF showing mild to moderate degeneration of elastic fibers. G. Graph displaying average values of elastic moduli (E) from selected areas. The colors of the bar graphs correspond to the colored areas surrounding rectangles (B) from which the average E values were calculated. H. Table presenting average values of elastic moduli (E) from selected areas.
Figure 1. Lumbar spine stenosis Sample - Cranial Region: A. Distribution of mechanical properties (Reduced Young's Modulus) across the ligamentum flavum (LF) section. B. Selected rectangle areas with different microstructure shown in detail in C, D, E and F. C. Chondroid metaplasia. D. Moderate to severe degeneration of elastic fibers replaced by collagen. E. Normal structure of LF with predominant elastin fibers. F. Area of LF showing mild to moderate degeneration of elastic fibers. G. Graph displaying average values of elastic moduli (E) from selected areas. The colors of the bar graphs correspond to the colored areas surrounding rectangles (B) from which the average E values were calculated. H. Table presenting average values of elastic moduli (E) from selected areas.

Keywords:

Ligamentum flavum, nanoindentation, microstructure, histopathology

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Annular EDS in Transmission and TKD Combined for Chemical and Crystallographic Nano-Analysis in SEM

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Poster Group 1

Background and aims
The behavior of artificial or naturally occurring functional nanostructures can only be understood and / or tuned to our needs, if their chemical composition and geometric arrangement are fully understood. Correlative microscopy allows fast and specific explanation of macroscopic behavior based on nanoscale properties. We demonstrate the combination of large collection angle energy-dispersive X-ray spectroscopy (EDS) and transmission Kikuchi diffraction (TKD) [1] in SEM for studying the element composition and crystallographic properties of various materials prepared as electron transparent specimens. We show, that using the combination of these techniques in SEM can either deliver sufficient quantitative data on the nanoscale without the need for high-end STEM analysis or help to carefully prepare for and minimize the latter.

Methods
For the experiments we used an annular EDS detector [2] placed horizontally between the specimen and the SEM pole piece, thus providing a high solid angle of around 1 sr for X-ray collection. This geometry allows high count rates in case of low X-ray yield from thin lamellae, light element containing samples and in case of fast scanning needed for beam sensitive large specimens. The annular arrangement of the silicon drift detector quadrants parallel to the specimen surface around a small aperture for passing the electron beam furthermore enables a high take-off angle, minimizing absorption and enabling the spectroscopic analysis of rough topography or bent specimens. This geometry was combined with an electron detector directly underneath the specimen for on-axis Kikuchi diffraction pattern acquisition. Thus, element distribution and specimen thickness were delivered by quantitative EDS applying the Cliff-Lorimer and the EDS Zeta-factor methods [3], and crystallographic grain phase, size and orientation were accessible via TKD. For standard-based EDS, the specimen thickness was measured directly using grain size and specimen tilt.

Results
Some of the results are shown. The top part of the figure demonstrates the combination of crystallographic and chemical layer analyses of a Cu(In,Ga)Se_2 (CIGS) solar cell structure detail prepared as electron transparent lamella. Four crystallographic phases with large and small crystallites and different crystallite distributions could be identified over a wide specimen region using TKD. The respective IPF-Z colorings for each phase are shown combined to illustrate this. Knowing the crystal structure of the different functional layers and with that, their density, helps refining the accuracy of quantitative EDS to a few atomic percent. The CdS buffer layer and Ga-grading profile was quantified based on the Cliff-Lorimer method using a broadened line scan and the composition of a well-known CIGS region of the specimen as standard.
The lower part of the figure shows a similar combination of analyses for a copper connect. The specimen thickness was determined by tilt to be between 60nm and 80nm. The larger Cu plug

crystallites are in the same size range as the specimen thickness. This allows to conclude, that the overlap of Cu and Si in the spectroscopic signal is caused by the connect being slightly tapered, and that sample thickness does not cause further overlaps in that region. The quality of the Cu connect is important for the device performance. Crystallites of a few nm in diameter were identified in the Cu connect and Cu film as well, which is of interest, since crystallite size and grain boundary distribution can influence device behavior under extreme conditions.

Conclusion
Our data show, that the element composition and crystallography of device layers and interconnects can be studied quantitatively in SEM with the spatial resolution of a few nanometers and few atomic percent accuracy. The combination of annular EDS and TKD in SEM is a powerful tool and complements high-end STEM analysis. It should be applied not only to semiconductor structures. Further examples from materials and certainly life science would be valuable indeed to explore its potential.

Keywords:

SEM, STEM, chemical composition, diffraction

Reference:

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High throughput automated SEM imaging and feature identification using AI/ML models

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Poster Group 1

Background
Accelerated materials development requires integrating automation with Artificial intelligence (AI)/Machine learning (ML) based workflows along selection of material composition, production, characterization, and testing [1]. Each stage possesses unique possibility of incorporating automation and AI/ML models, supporting efficient experimental activities and ultimately computational modelling, and reducing time and cost. Materials Acceleration Platforms (MAPs) [2] address these possibilities. MAPs concept involves developing the methods for predicting optimal alloy compositions, the automation of manufacturing processes, and the implementation of advanced materials characterization techniques. Among these, the field of high-throughput characterization remains the least developed.

This work focuses on developing protocols for the automated imaging and defect detection in High-entropy alloys (HEA), through automated SEM imaging and AI/ML based models. The developed protocols are then extended to automated fatigue fracture surface analysis of 316L stainless steels and the detection of features such as facets, dimples, and striations.

Methods
Automated imaging was performed with a Zeiss Ultra plus SEM at an accelerating voltage of 15 kV. This developed facility allows imaging of random fields of HEA samples at the desired magnification using in-house developed code and SmartSEM macro functions. The collected images were segmented to identify defects and background information using a DeepLab-v3+ encoder-decoder architecture for semantic segmentation. The encoder extracts information from the input image through convolutional layers, while the decoder refines the segmentation results, thus capturing fine details and improving the localization of object edges. A dataset of 160 images (1024 x 768 pixels each) with annotated masks was utilized with 70%, 15%, and 15%, respectively, as training, validation, and test sets.

The developed SEM automation and AI/ML protocols in HEAs were subsequently applied to the fatigue fracture surfaces of fatigue of 316L stainless steels samples. Rather than inputting random fields in the case HEAs, user-defined feature selection from overview images and comprehensive fracture surface mapping of 316L stainless steels were utilized. Figure 1 illustrates the method employed for automated fracture surface imaging. Additionally, the ML algorithm developed for void detection are being refined to identify fatigue fracture surface features.

Results
With this guided automatic image acquisition, over 1000 images were captured at a rate of ~60 images per hour using SEM from multiple samples within 24 hours without human intervention. The AI/ML model predictions on the collecte data set demonstrated highly accurate segmentation of

defects in images, with only minor deviations in a few instances, achieving scores of 0.994 for IoU (intersection over union) and 0.003 for dice loss, respectively. The predicted masks, generated within seconds, provide detailed information about the size, shape, and location of defects in the SEM images, thereby aiding in the analysis and decision-making process for alloy optimization and characterization.

Conclusion and outlook
Integrating automation and AI/ML models facilitates rapid material development by streamlining the traditionally tedious workflows required for optimizing material properties. We developed techniques for high-entropy alloys (HEAs) and extended them to fracture surface analysis through automated SEM image acquisition and feature identification using AI/ML models. This approach minimizes human intervention, accelerates analysis, and significantly reduces the costs and time associated with these activities.

Keywords:

SEM, Alloys, Fracture surface, Automation

Reference:

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Effect of thin 2D support layers on the catalytic properties of catalysts

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Poster Group 1

Background incl. aims
Electrochemical catalysts composed of metals used as active materials and supports for dispersion of active materials have advantages on both activity and stability. Carbon nanomaterials are widely used as conductive supporting materials. The coverage of support materials, even by a very thin layer of graphene, has been regarded as harmful for electrochemical reactions due to the physical blockage of active sites. However, recent research reported that the catalytic performance could be optimized depending on whether the active metal surface is covered with a monolayer of graphene, which alters the adsorption and desorption energies of reactants1 Considering diverse structures from the composites of metal nanoparticles and 2D support materials, controlling their structures and investigating structure-property relationships are important for developing novel catalysts. In this work, we used Pt nanoparticles as an active catalyst, and 2D MnO2 as support to prepare Pt-exposed and Pt-covered models for structure-relationship study.
Methods
Pt nanoparticles were synthesized using polyvinylpyrrolidone as a surfactant and ethylene glycol as a reductant. 2D MnO2 nanosheets were synthesized through oxidation of Mn2+ by H2O2 in the presence of tetramethylammonium hydroxide. To obtain Pt/MnO2 composites, the electrostatic self-assembly strategy was conducted. By changing the self-assembly process, two composite models were prepared. One is Pt nanoparticles covered with 2D MnO2 nanosheets (Pt-covered), and the other is Pt nanoparticles placed on the surface of 2D MnO2 nanosheets (Pt-exposed). The local structures and relative ratio between Pt and MnO2 were analyzed using transmission electron microscopy (TEM), X-ray diffraction (XRD), and inductively coupled plasma optical emission spectroscopy (ICP-OES), respectively.
Results
Two models had a similar Pt to MnO2 ratio. The Pt-covered model showed more dispersed Pt nanoparticles in the composites with less thickness of the 2D MnO2 layer assembly. The Pt-covered model revealed a lower electron transfer number for the oxygen reduction reaction compared to the Pt-exposed model, highlighting the possibility of controlling the catalytic pathway by changing the local structure of the composites.
Conclusion
In this study, we synthesized two model structures of Pt nanoparticles and 2D MnO2. Through ORR measurement, we observed that the catalytic activity varied depending on the structural differences. This indicates that the selectivity of catalytic reactions can be adjusted by controlling the catalyst structure. The detailed structure-relationship of the two models will be discussed.

Keywords:

Catalyst,support,2Dnanosheets,Oxygen reduction reaction

Reference:

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1340

Catalytically active MoS2 support for hydrogen generation from seawater

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Poster Group 1

1. Background incl. aims
Electrocatalytic water splitting is an environmentally sustainable method to produce clean hydrogen but generally demands expensive high-purity water. Thus, direct seawater electrolysis can be attractive, yet it has serious challenges, such as inorganic precipitation and chlorine corrosion, which reduce electrocatalytic activity. Seawater acidification using a bipolar membrane (BPM) has been proposed as a promising approach to mitigate these problems.[1] Meanwhile, Pt is the most active electrocatalyst for the hydrogen evolution reaction (HER), but its high cost limits its use. Therefore, two-dimensional MoS2 has emerged as a potential non-precious metal catalyst. Hybrid electrocatalysts, specifically Pt nanoparticles anchored on MoS2 nanosheets, have also been studied to achieve the required HER activity. However, it has not yet been studied under seawater conditions. Here, we investigate MoS2 nanosheets as a catalytic support in Pt/MoS2/carbon cloth (Pt/MoS2/CC) cathode, focusing on the structure and HER performance under a BPM-based seawater electrolysis system, compared with Pt/CC and MoS2/CC.
2. Methods
2.1. Synthesis of Pt/MoS2/CC
Pt/MoS2/CC was synthesized via the hydrothermal method followed by the sintering under N2 gas.
2.2. Physical and chemical characterization
Field-emission scanning electron microscopy (FE-SEM) was used to examine the morphology of the samples. Field-emission transmission electron microscopy (FE-TEM) was conducted to analyze the morphology and crystal structure of the samples. Energy-dispersive X-ray spectroscopy (EDS) was performed to check the chemical composition of the samples in SEM and TEM. X-ray diffraction (XRD) analysis was applied to determine the crystal structure of the samples. X-ray photoelectron spectroscopy (XPS) was employed to investigate the bond between Pt nanoparticles and MoS2 nanosheets with Pt and Mo oxidation states.
2.3. Electrochemical characterization
Electrochemical measurements were performed using a potentiostat. Linear sweep voltammetry (LSV) was conducted on a three-electrode system in acidic conditions. Chronopotentiometry (CP) was conducted on the BPM-based seawater electrolyzer in simulated seawater.
3. Results
SEM images of Pt/MoS2/CC showed that carbon cloth was entirely covered with dense MoS2 nanosheets, and Pt nanoparticles were attached to MoS2 nanosheets. In addition, Pt nanoparticles were not uniform. XRD patterns confirmed that the crystal structure of MoS2 was maintained after the sintering at 400/450 °C. During HER under a BPM-based seawater electrolysis system, Pt/MoS2/CC cathode showed lower overpotential and higher stability than Pt/CC.
4. Conclusion
Based on the result, MoS2 nanosheets are anticipated to enhance the anchoring strength of Pt nanoparticles, provide additional active sites, and compel the formation of inorganic precipitates, thereby contributing to stable catalytic activity. More details about the structural interaction

between MoS2 nanosheets and Pt nanoparticles, as well as the electrode characteristics of MoS2 in seawater, will be discussed.

Keywords:

Seawater electrolysis, HER, Pt, MoS2

Reference:

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Application of dual-beam microscope with TOF-SIMS in characterization of ceramic coatings deposited on filtration membranes

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Poster Group 1

Filtration technologies are of the highest interest to human civilisation since they decrease air pollution and can expand access to drinking water. In the case of application in the food industry, bacteria and microorganisms' growth are the additional factors that influence the lifetime of membranes. Appropriate materials can be deposited on the membrane surface to limit these phenomena. However, such coatings shouldn't affect the filtration parameters of the membranes. During the work, different types of nanocrystalline ceramics coatings (CuO, AgO, ZnO and Cu+ZnO, Ag+ZnO, Ag+AgO, AgO+CuO) were deposited using two magnetron sputtering techniques: DC MS (Direct Current) and HPIMS (High Power Impulse). The influence of deposition parameters on coating thickness, surface morphology, microstructure and chemical composition was analysed. The distribution of the deposited material on the membrane surface, the interface between the coating and substrate, and antibacterial properties were analysed. The investigation evidenced that the obtained coatings were characterised by homogeneous, nanocrystalline structures, but they had different microstructures and morphology. For example, CuO coatings exhibit columnar or needle-like structures, while nano-grains in AgO coatings have more equalized shapes. Moreover, in multicomponent coatings (AgO+CuO), changes in the cathode (copper or silver) power result in differences in coating microstructure. The use of two sources leads additionally to the composite structure (matrix and fine particles) of the coating. The performed investigation also revealed that the application of the HPIMS technique allows the deposit of coatings of more homogeneous and less porous microstructure with smaller grain size compared to the DC MS technique. Coatings exhibits good antibacterial properties.

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Keywords:

Filtration membranes, composite coatings, dualbeam

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Effects of Vitamin D Administration On Testicular Tissue in Metabolic Syndrome Rats

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Poster Group 1

Background: Metabolic Syndrome (MS) includes a series of metabolic disorders such as abdominal obesity, insulin resistance, impaired fasting glucose, hypertension, and dyslipidemia, with insulin resistance being a dominant factor in these conditions (1. MS is associated with obesity resulting from a high-fat and high-sugar diet combined with a sedentary lifestyle and is increasing as a global public health issue. Infertility affects 15% of couples worldwide, with male factors playing a role in 50% of cases (2). Paternal obesity leads to reduced sperm count, increased sperm DNA damage, and long-term epigenetic changes (3). Vitamin D3 plays a role in the regulation of reproductive processes in addition to maintaining calcium and phosphorus homeostasis. Vitamin D deficiency can impair testicular development and spermatogenesis by inhibiting testicular germ cell proliferation (4). We aimed to explore the impact of metabolic syndrome on male infertility and analyze the influence of vitamin D supplementation on testicular damage, cell proliferation, and apoptosis induced by a high-fat and high-fructose diet.
Methods: To create a metabolic syndrome model, we utilized a specialized diet comprising 17% fat and 17% fructose, along with another diet consisting of 20% fructose water over a period of 15 weeks. The study involved twenty-four male Sprague-Dawley rats. Based on dietary variations and vitamin D administration, we established four groups: Healthy Control (SC), Metabolic Syndrome (MS), MS+Vitamin D (MSD), and Healthy Control+Vitamin D (HCV) groups. The Vitamin D-treated groups received oral vitamin D supplementation at a rate of 170 IU/week for 12 weeks. At the end of the 15-week period, the animals were euthanized. Testicular tissue samples were fixed in 10% buffered neutral formalin and embedded in paraffin for further analysis. Throughout the experiment, daily food intake, water consumption, weight changes, as well as fasting blood glucose levels were monitored. Immunohistochemistry techniques involving primary antibodies against aromatase, StAR, 8-OHdG, Vitamin D receptor (VDR), PCNA, and activated caspase-3 proteins were performed. Serum testosterone levels were measured using ELISA method, and TUNEL method was used to determine apoptotic cells.
Results: Statistical analysis revealed that animals in the MS group exhibited significantly higher daily caloric intake, were heavier, and had elevated fasting blood glucose levels compared to other groups. VitaminD administration led to decreased blood glucose levels inthe MSD group compared tothe MS group possibly due to its metabolic regulatory effect. Morphological examination indicated irregular seminiferous tubules with reduced diameters in the MS group compared to other groups, resulting from germ cell loss and a thickening of the basement membrane. Moreover, the number of spermatid cells within these tubules as well as the number of spermatozoa within the lumen were

significantly diminished when contrasted with other groups. Testicular morphology showed improvement in the MSD group compared to the MS group. Expressions of aromatase, 8-OHdG, activated caspase-3, and TUNEL positivity were markedly increased with in the MS group; conversely StAR, VDR, and PCNA expressions along with serum testosterone levels was substantially decreased. Conclusion: It is suggested that high fat and fructose-induced metabolic syndrome contributes to male infertility by disrupting testicular structure however, vitamin D administration can ameliorate this damage.

Keywords:

Metabolic syndrome, VitaminD, Testis, Rat

Reference:

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Global BioImage Analysts' society: CZI funded initiative and future association

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Poster Group 1

GloBIAS is a non-profit association to be officially constituted by 2025. GloBIAS aims to develop, discuss and establish the role of BioImage Analysts worldwide by expanding the NEUBIAS activities globally in the same spirit and disciplines, to provide the community with a sustainable base (educationally and financially) through a society and welcome a larger number of scientists involved in bioimage analysis.

Keywords:

Bioimage Analysis, Society, Association, Training

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3D Spatial Transcriptomics: A MERFISH Platform for High-Resolution Molecular Imaging

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Poster Group 1

Spatial transcriptomics (SP) is a rapidly growing technology in the field of genomics that gives insights into the cellular organization and their molecular interactions within complex tissues. High-resolution imaging-based SP techniques allow us to visualize and quantify numerous RNA species within individual cells even in subcellular compartments.
We have developed an in-house automatic imaging-based platform that utilizes computational algorithms to localize individual transcripts in 3D. This platform is built on the MERFISH (Multiplexed Error-Robust Fluorescence In Situ Hybridization) technology that combines the principles of fluorescence in situ hybridization (FISH) with multiplexed barcoding strategies. Our platform has been built to encompass a wide range of samples, including cell cultures and tissues. In line, we have implemented tissue clearing in our protocol to reduce background autofluorescence in various tissues - improving our signal-to-noise and spot detection.
Current tools for processing the imaging data from a MERFISH experiment are deficient when processing these large 3D data sets. Thus, we have developed a barcode decoding tool utilizing iterative clustering, which has been incorporated into a larger pipeline consisting of open-source packages. This pipeline is written in Python and is highly focused on utilizing the GPU, parallelization, and machine learning. To validate the pipeline, a simulator of 3D MERFISH data has been developed, which can be tuned to different levels of noise and bit-errors.
By combination of error-robust encoding and super-resolution microscopy, MERFISH overcomes the limitations of traditional FISH techniques, providing enhanced accuracy and sensitivity in detecting and quantifying RNA molecules. Furthermore, the versatility and adaptability of MERFISH make it a valuable tool for unraveling the intricate molecular landscape of cells and tissues, with implications in numerous fields ranging from developmental biology and neuroscience to cancer research and beyond.

Keywords:

MERFISH
Spatial transcriptomics
Super-resolution microscopy

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Coarsening mechanisms of Ni under operating conditions of Ni/YSZ solid oxide cell using Scanning 3DXRD

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Poster Group 2

Ni coarsening during operating conditions in Ni/YSZ solid oxide cells is the dominant degradation mechanism of these devices1. In this study, we showcase Scanning 3DXRD (S3DXRD) and high-energy X-ray fluorescence tomography (XRF) to map the 3D volume of a solid oxide under ex-situ heat treatment with an unprecedented spatial resolution of 100 nm. Scanning 3DXRD is highly advantageous compared to conventional tomography as it adds crystallographic information to the spatial maps. This helped us to pinpoint the areas where we observed Ni particle aggregation coarsening which is detrimental to these devices. We observe correlations between Ni grain growth quantified using crystallographic information and Ni coarsening observed through the XRF maps. These results advance our understanding of Ni coarsening mechanisms in a SOC device and can help us solve the challenge of making them more stable under operating conditions.

Keywords:

Scanning 3DXRD, Grain boundary characterization

Reference:

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