Invited Speaker

1177 Precise targeting for volume electron microscopy, a multimodal approach
Mr. Yannick Schwab

Oral Presentation

761 Multiplexed 3D imaging of single-cell organization and tissue morphology in the multicellular intestinal organoid
Nicole Repina[1], Enrico Tagliavini[1], Marius Mählen[1], Joel Lüthi[3], Manan Lalit[2], Florian Jug[2], Virginie Uhlmann[3], Prisca Liberali[1]
[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [2]Human Technopole, Milan, Italy, [3]BioVision Center, University of Zurich, Zurich, Switzerland
203 Unsupervised Deep Learning approach for image registration in Correlative Microscopy for the localization of Nanoparticles
Daksh Daksh[1], Dr. Anke Kaltbeitzel[1], Mr. Gunnar Glaßer[1], Dr. Ingo Lieberwirth[1], Prof. Dr. Katharina Landfester[1]
[1]Max Planck Institute for Polymer Research, Mainz, Germany
347 Multimodal and correlative imaging approaches to study early stages of SARS-CoV 2 infection
Dr Johannes Groen[1,2], Mr Simon Blachier[2], Dr Max Baker[3], Dr Giovanna Barba-Spaeth[3], Dr Jost Enninga[2], Dr Anne Brelot[2], Dr Anna Sartori-Rupp[1]
[1]NanoImaging Core Facility, Institut Pasteur, Paris, France, [2]Dynamics in Host Pathogen Interaction, Institut Pasteur, Paris, France, [3]FlavImmunity, Institut Pasteur, Paris, France
609 Correlative transmission electron microscopy and photoluminescence microscopy revealing enhanced fluorescence in nitrogen vacancy containing nanodiamond
Prof Shery Chang[1], Dr Haotian Wen[2], Dr Christian Dwyer[3], Dr Huan-Cheng Chang[4]
[1]Electron Microscope Unit, Mark Wainwright Analytical Centre, University of New South Wales, Sydney, Australia, [2]School of Materials Science and Engineering, University of New South Wales , Sydney, Australia, [3]Electron Imaging and Spectroscopy Tools, Sans Souci, Australia, [4]Academia Sinica, Taipei, Taiwan
658 Correlative characterization by X-ray tomography, SEM/FIB and TEM using reference markers: bridging imaging with micro-structuring
Dr Alexey Boubnov[1], Charlotte Neidiger[1], Rafaela Debastiani[1], Torsten Scherer[1], Matthias Mail[1], Di Wang[1], Christian Kübel[1]
[1]Institute of Nanotechnology, Karlsruhe Institute of Technology, Karlsruhe, Germany
900 Image Registration Based Navigation of Region of Interests in Volume Correlative Light and Electron Microscopy
Kohki Konishi[1,2], Guilherme Neves[2], Matthew Russell[2], Leanne Allison[2], Masafumi Mimura[3], Juan Burrone[2], Roland Fleck[2]
[1]Nikon U.K., Branch of Nikon Europe B.V., Surbiton, United Kingdom, [2]King's College London, London, United Kingdom, [3]Nikon Corporation, Tokyo, Japan
63 A Better Insight Into Battery Materials Using A Correlative Approach
Dr Dan Haspel[1], Dr Joshua Lea[1], Dr Jonathan Moffat[1], Dr Pedro Machado[1], Dr James Sagar[1], Dr Louise Hughes[1]
[1]Oxford Instruments, High Wycombe, United Kingdom
311 Developing a multimodal imaging pipeline for molecular biochemical studies with a 3D approach
Dr Caroline Bissardon[1], Victor Armijo[1], Dr. Sylvain Bohic[2,3], Dr. Peter Cloetens[3], Dr. Matthew W. Bowler[1], Gergely Papp[1]

[1]European Molecular Biology Laboratory, Grenoble, France, [2]Inserm, UA7, Rayonnement Synchrotron pour la Recherche Biomédicale (STROBE), Grenoble, France, [3]European Synchrotron Radiation Facility, Grenoble, France

649 Methodological development of nanoscopic defects characterization in nuclear materials: contribution of TEM-APT correlative microscopy

Alexandre Rakotomizao[1], Solene Rouland[1], Bertrand Radiguet[1], Cristelle Pareige[1]
[1]Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Groupe de Physique des Matériaux UMR 6634, F-76000, ROUEN, FRANCE

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

Dominik Pinkas[1], Samuel Záchej[2], Martina Zanova[2], Prof. Pavel Hozak[3], Vlada Filimonenko[1,3]
[1]Electron Microscopy Core Facility, Institute of Molecular Genetics ASCR, Prague, Czech republic, [2]TESCAN GROUP, Brno, Czech republic, [3]Department of Biology of the Cell Nucleus, Institute of Molecular Genetics ASCR, Prague, Czech republic

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

Msc. Jan Čermák[1], PhD. Jiří Materna[2], MSc. Ondřej Ambrož[1], BSc. Patrik Jozefovič[1], PhD. Šárka Mikmeková[1]
[1]Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic, [2]Machine Learning College, Brno, Czech Republic

955 Localisation of nanoparticles in whole cells using correlative cryo soft x-ray tomography and fluorescent microscopy

Mr Kenneth Fahy[1], Sergey Kapishnikov[1], Paul Sheridan[1], William Fyans[1], Fergal O'Reilly[1,2], Tony McEnroe[1]
[1]SiriusXT, Dublin , Ireland , [2]UCD Dublin, Dublin 4, Ireland

Poster Presentation

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

Dr Jorge Diniz[1], Miss Jennifer Ferguson[1], Mr Pete Johnson[1], Mr Tim Smith[1]
[1]Renishaw plc, New Mills, Wotton-under-Edge, UK

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

Marit Smeets[1], Deniz Daviran[1], Wessel Teunisse[1]
[1]Delmic B.V., Delft, The Netherlands

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

Senior Researcher Alba Garzon Manjon[1], Mr Miquel Vega Paredes[2], Miss Raquel Aymerich Armengol[2], Mr Daniel Arenas Esteban[3], Miss Sara Martí Sánchez[1], Miss Sara Bals[3], Miss Christina Scheu[2]
[1]Institut Català de Nanociència i Nanotecnologia, Bellaterra, Barcelona, [2]Max Planck Institut für Eisenforschung GmbH, Düsseldorf, Germany, [3]University of Antwerpen - Electron microscopy for materials science, Amtwerpen, Belgium

323 4D STEM and EELS Acquired Simultaneously with a Fast Pixelated Direct Detector with Center Hole

Dr. Martin Huth[1], Valentin Kroner[1], Yassine El Imari[1], Dr. Stefan Aschauer[1], Dr. Ryusuke Sagawa[3], Dr. Hiroki Hashiguchi[3], Dr. Akiho Nakamura[3], Prof. Lothar Strüder[2], Dr. Heike Soltau[1]
[1]PNDetector GmbH, Munich, Germany, [2]PNSensor GmbH, Munich, Germany, [3]JEOL Ltd., Tokyo, Japan

366 CL-EBSD-TUNA Correlative Multi-microscopy Study of Grain Boundaries in Pseudo-symmetric Cu(In,Ga)S2 Solar Cell Absorber

Yucheng Hu[1], Dr Gunnar Kusch[1], Miss Damilola Adeleye[2], Prof Susanne Siebentritt[2], Prof Rachel Oliver[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Luxembourg, Esch-sur-Alzette, Luxembourg

472 Illuminating the Microscopic Realm: Application of Resin R221 for CLEM in microbes and plant tissues

Andrea Elizabeth Babu Dominic Cheradil[1], Dr Julia Buchner[1], Dr Matthias Ostermeier[2], Isabella Gantner[1], Jennifer Grünert[1], Prof. Dr. Andreas Klingl[1]
[1]Plant Development & Electron Microscopy, Planegg-Martinsried, Munich, Germany, [2]Department of Molecular Plant Science, Planegg-Martinsried, Munich, Germany

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

Mgr. Jan Dobeš[1], Jaromír Bačovský[1], Radka Martínková[1], Jan Vysloužil[2], Zuzana Bytešníková[3]
[1]Delong Instruments, Brno, Czech Republic, [2]Pragolab, Brno, Czech Republic, [3]Department of Chemistry and Biochemistry, Mendel University, Brno, Czech Republic

781 The development of multimodal imaging with functional silica nanoparticle

Dr. Tomohiro Haruta[1], Prof. Michihiro Nakamura[2]
[1]JEOL Ltd., Akishima, Japan, [2]Graduate school of medicine,Yamaguchi university, Ube, Japan

861 Correlative microscopy and spectroscopy of nanophotonic materials

Michael Foltýn[1], Petr Liška[1], Peter Kepič[1], Rastislav Motúz[2], Jaroslav Jiruše[2], Andrea Konečná[1], Vlastimil Křápek[1], Tomáš Šikola[1], Michal Horák[1]
[1]Brno University of Technology, Brno, Czech Republic, [2]TESCAN Group, R&D Department, Brno, Czech Republic

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

Mr William Bowles[1,2,3], Dr Abner Velazco-Torrejon[1], Dr Siva Ramadurai[1], Dr Marcus Gallagher-Jones[1], Prof Angus Kirkland[1,4], Dr Alistair Siebert[2], Prof James Naismith[5], Dr Maud Dumoux[1]
[1]Structural Biology, Rosalind Franklin Institute, Didcot, United Kingdom, [2]Electron Biology Imaging Centre, Diamond Light Source, Didcot, United Kingdom, [3]Doctoral Training Centre, University of Oxford, Oxford, United Kingdom, [4]Department of Materials, University of Oxford, Oxford, United Kingdom, [5]Division of Structural Biology, Wellcome Trust Centre of Human Genomics, Oxford, United Kingdom

974 Strategies for Multimodal Image Data Transformation to a Common Format for Cloud Integration and Visualization

Phd Rafael Camacho[1], PhD Jens Berndtsson[1], PhD Massimo Micaroni[1], PhD Karl Zhang[1], PhD Julia Fernandez-Rodriguez[1]
[1]Center for Cellular Imaging, Core Facilities, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, Gothenburg, Sweden

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

Dr Sangjun Kang[1], Prof. Xiaoke Mu[1,3], Dr Di Wang[1], Prof Christian Kübel[1]
[1]Tu Darmstadt, Darmstadt, Germany, [2]Karlsruhe Institute of Technology , Karlsruhe , Germany, [3]Lanzhou University, Lanzhou , China

Late Poster Presentation

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]

[1]Technical University of Darmstadt, Department of Electrical Engineering and Information Technology, Darmstadt, Germany, [2]Technical University of Darmstadt, Department of Biology, Darmstadt, Germany

1302 Correlative microscopy of graphene with SEM, Raman spectroscopy and AFM

Dr Lok Yi Lee[1], Juliet Biard[1]

[1]Paragraf Ltd, Somersham, United Kingdom

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

Dr. Purvesh Soni[1], Dr. Anna Mogilatenko[2,3], Dr. Meiken Falke[1]

[1]Bruker Nano GmbH, Berlin, Germany, [2]Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik , Berlin, Germany, [3]Institute of Physics, Humboldt-University of Berlin, Berlin, Germany

1177

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

761

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

Nicole Repina[1], Enrico Tagliavini[1], Marius Mählen[1], Joel Lüthi[3], Manan Lalit[2], Florian Jug[2], Virginie Uhlmann[3], Prisca Liberali[1]
[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [2]Human Technopole, Milan, Italy, [3]BioVision Center, University of Zurich, Zurich, Switzerland

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

203

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

Daksh Daksh[1], Dr. Anke Kaltbeitzel[1], Mr. Gunnar Glaßer[1], Dr. Ingo Lieberwirth[1], Prof. Dr. Katharina Landfester[1]
[1]Max Planck Institute for Polymer Research, Mainz, Germany

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.

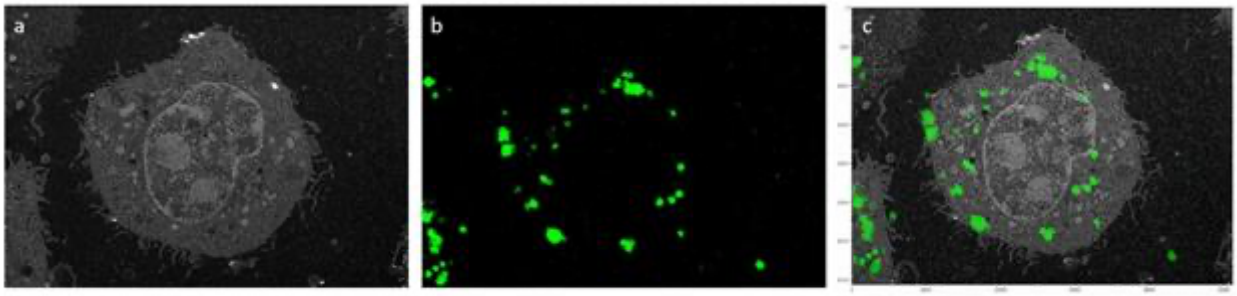


Fig. 1. Correlative Light-Electron Microscopy (CLEM) micrograph (a) represents electron microscopy image, (b) represents fluorescence microscopy image and (c) represents CLEM micrograph featuring nanoparticles (NPs).

Keywords:

Correlative microscopy, image registration, DNN

Reference:

1. G Balakrishnan et al., IEEE transactions on medical imaging 38(8) (2019), p. 1788
2. O Ronneberger, P Fischer and T Brox. in “International Conference on Medical image computing and computer-assisted intervention”, (Springer, Cham)
3. A Nan et al., Medical Imaging with Deep Learning. PMLR (2020)

347

Multimodal and correlative imaging approaches to study early stages of SARS-CoV 2 infection

Dr Johannes Groen[1,2], Mr Simon Blachier[2], Dr Max Baker[3], Dr Giovanna Barba-Spaeth[3], Dr Jost Enninga[2], Dr Anne Brelot[2], Dr Anna Sartori-Rupp[1]
[1]NanoImaging Core Facility, Institut Pasteur, Paris, France, [2]Dynamics in Host Pathogen Interaction, Institut Pasteur, Paris, France, [3]FlavImmunity, Institut Pasteur, Paris, France

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:

Bykov, Y. S., Cortese, M., Briggs, J. A. G., & Bartenschlager, R. (2016). Correlative light and electron microscopy methods for the study of virus–cell interactions. In FEBS Letters.

Collinson, L. M., Domart, M.-C., Carzaniga, R., Razi, M., Guttmann, P., Schneider, G., Pereiro, E., Tooze, S. A., & Duke, E. (2017). Soft X-Ray Tomography: Filling the Gap Between Light and Electrons for Imaging Hydrated Biological Cells. Microscopy and Microanalysis.

Jackson, C. B., Farzan, M., Chen, B., & Choe, H. (2022). Mechanisms of SARS-CoV-2 entry into cells. Nature reviews Molecular cell biology, 23(1), 3-20.

Klein, S., Wachsmuth-Melm, M., Winter, S. L., Kolovou, A., & Chlanda, P. (2021). Cryo-correlative light and electron microscopy workflow for cryo-focused ion beam milled adherent cells. In Methods in Cell Biology (Vol. 162).

609

Correlative transmission electron microscopy and photoluminescence microscopy revealing enhanced fluorescence in nitrogen vacancy containing nanodiamond

Prof Shery Chang[1], Dr Haotian Wen[2], Dr Christian Dwyer[3], Dr Huan-Cheng Chang[4]
[1]Electron Microscope Unit, Mark Wainwright Analytical Centre, University of New South Wales, Sydney, Australia, [2]School of Materials Science and Engineering, University of New South Wales , Sydney, Australia, [3]Electron Imaging and Spectroscopy Tools, Sans Souci, Australia, [4]Academia Sinica, Taipei, Taiwan

IM-13 (1), Lecture Theater 5, august 27, 2024, 10:30 - 12:30

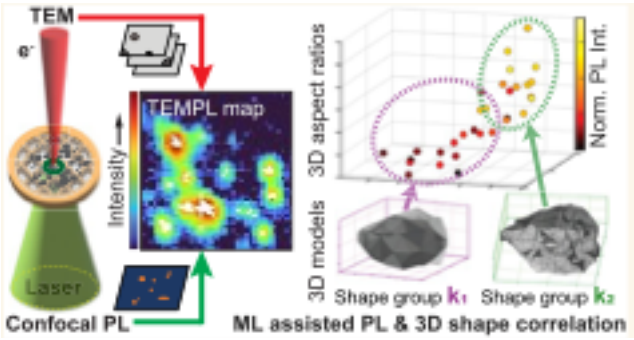
Fluorescent nanodiamonds (FNDs) are diamond nanoparticles containing color centers that emit visible light at room temperature. Among the color centers in FNDs, the nitrogen-vacancy centers (NV) have drawn the most attention due to its exceptionally stable optical properties and great prospect in sensing 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:

[1] Xiao et al, Nanoscale Research Letters (2021) 16, pp.167
[2] J Storteboom et al, Optics Express (2015) 23, 9, pp.11327-11333
[3] H Wen et al, ACS Nano (2022) 17, pp.16491

658

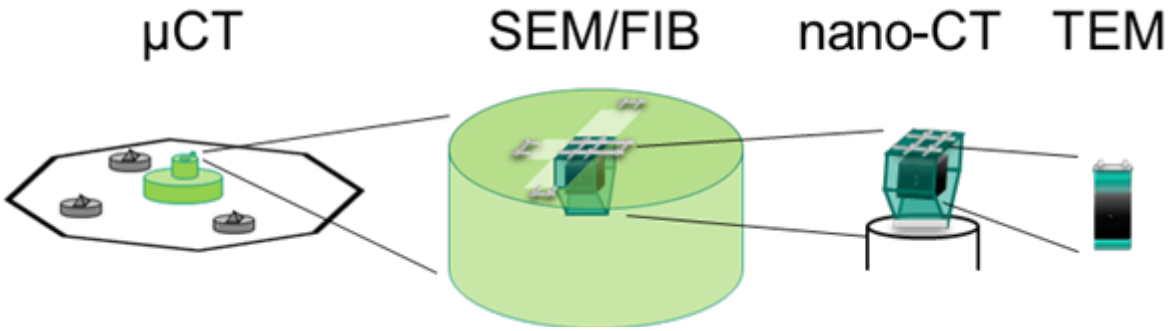
Correlative characterization by X-ray tomography, SEM/FIB and TEM using reference markers: bridging imaging with micro-structuring

Dr Alexey Boubnov[1], Charlotte Neidiger[1], Rafaela Debastiani[1], Torsten Scherer[1], Matthias Mail[1], Di Wang[1], Christian Kübel[1]
[1]Institute of Nanotechnology, Karlsruhe Institute of Technology, Karlsruhe, Germany

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

Reference:

[1] T. L. Burnett, S. A. McDonald, A. Gholinia, R. Geurts, M. Janus, T. Slater, S. J. Haigh, C. Ornek, F. Almuaili, D. L. Engelberg, G. E. Thompson, P. J. Withers Burnett, Scientific Reports 4, 4711 (2014)
[2] D. Nugmanov, A. Mazilkin, H. Hahn, Y. Ivanisenko, Metals 9(10), 1081 (2019)
[3] K. V. Raun, L. F. Lundegaard, J. Chevallier, P. Beato, C. Clausen Appel, K. Nielsen, M. Thorhauge, A. D. Jensen, M. Høj, Cat. Sci. Tech. 8, 4626 (2018)

900

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

Kohki Konishi[1,2], Guilherme Neves[2], Matthew Russell[2], Leanne Allison[2], Masafumi Mimura[3], Juan Burrone[2], Roland Fleck[2]
[1]Nikon U.K., Branch of Nikon Europe B.V., Surbiton, United Kingdom, [2]King's College London, London, United Kingdom, [3]Nikon Corporation, Tokyo, Japan

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:

[1] J. A. Bogovic et al., "Robust registration of calcium images by learned contrast synthesis," 2016 IEEE 13th International Symposium on Biomedical Imaging (ISBI), Prague, Czech Republic, 2016, pp. 1123-1126
[2] P. Paul-Gilloteaux et al., “eC-CLEM: flexible multidimensional registration software for correlative microscopies.” Nature Methods, 2017, 14, pp. 102–103
[3] B. M. Toledo Acosta et al., "Intensity-based matching and registration for 3D correlative microscopy with large discrepancies," 2018 IEEE 15th International Symposium on Biomedical Imaging (ISBI 2018), Washington, DC, USA, 2018
[4] D. Krentzel et al., “CLEM-Reg: An automated point cloud based registration algorithm for correlative light and volume electron microscopy”, bioRxiv 2023.05.11.54044

63

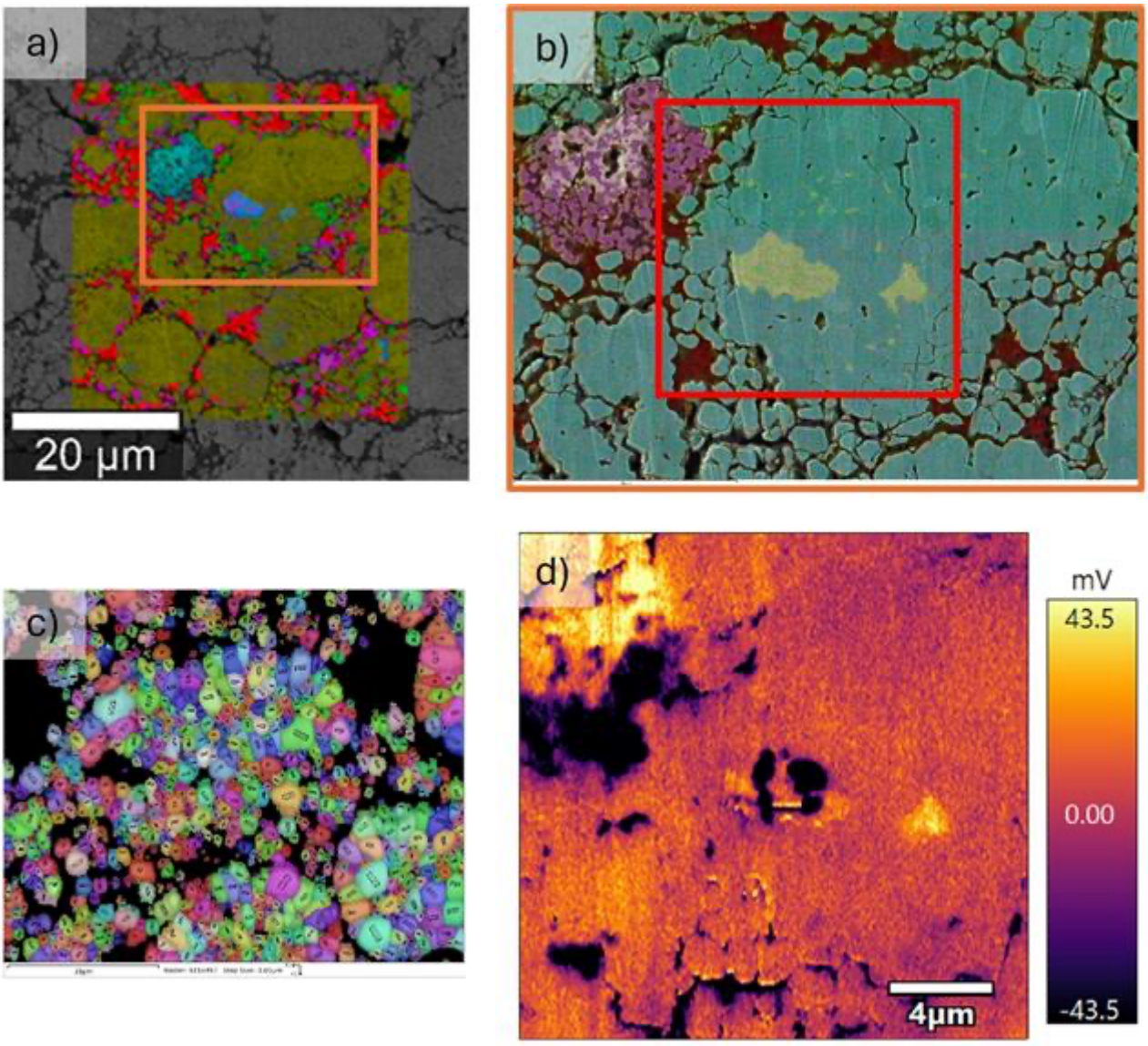
A Better Insight Into Battery Materials Using A Correlative Approach

Dr Dan Haspel[1], Dr Joshua Lea[1], Dr Jonathan Moffat[1], Dr Pedro Machado[1], Dr James Sagar[1], Dr Louise Hughes[1]
[1]Oxford Instruments, High Wycombe, United Kingdom

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

311

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

Dr Caroline Bissardon[1], Victor Armijo[1], Dr. Sylvain Bohic[2,3], Dr. Peter Cloetens[3], Dr. Matthew W. Bowler[1], Gergely Papp[1]
[1]European Molecular Biology Laboratory, Grenoble, France, [2]Inserm, UA7, Rayonnement Synchrotron pour la Recherche Biomédicale (STROBE), Grenoble, France, [3]European Synchrotron Radiation Facility, Grenoble, France

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.

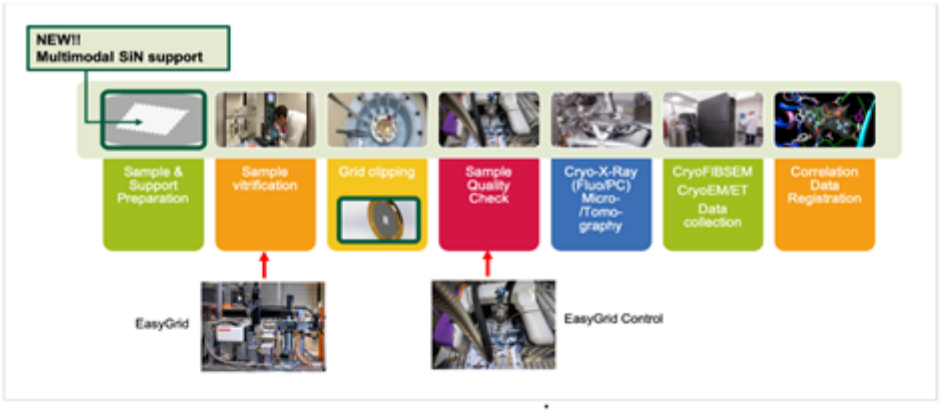


Figure 1: Experimental sample preparation and analysis setup: a new multimodal and multiscale imaging pipeline.

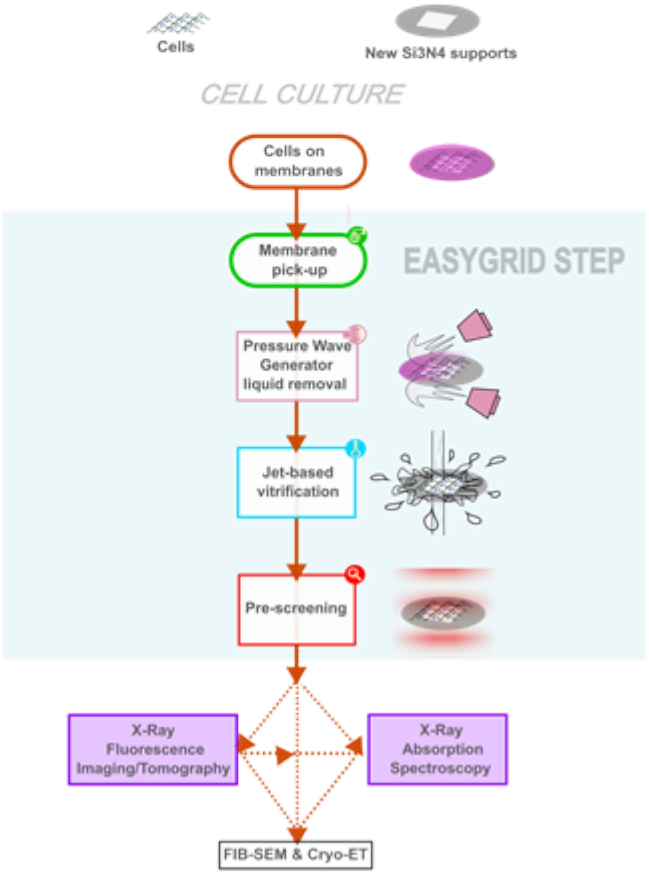


Figure 2: Cell Sample preparation from cell culture to data collection in diverse imaging modalities.

Keywords:

Electron Microscopy/Tomography, X-rayFluorescenceNanoscopie, phase contrast,EasyGrid

Reference:

[1]Gemin et al. EasyGrid. BioRxiv, in review in Nature Methods (2023)

649

Methodological development of nanoscopic defects characterization in nuclear materials: contribution of TEM-APT correlative microscopy

Alexandre Rakotomizao[1], Solene Rouland[1], Bertrand Radiguet[1], Cristelle Pareige[1]
[1]Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Groupe de Physique des Matériaux UMR 6634, F-76000, ROUEN, FRANCE

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.

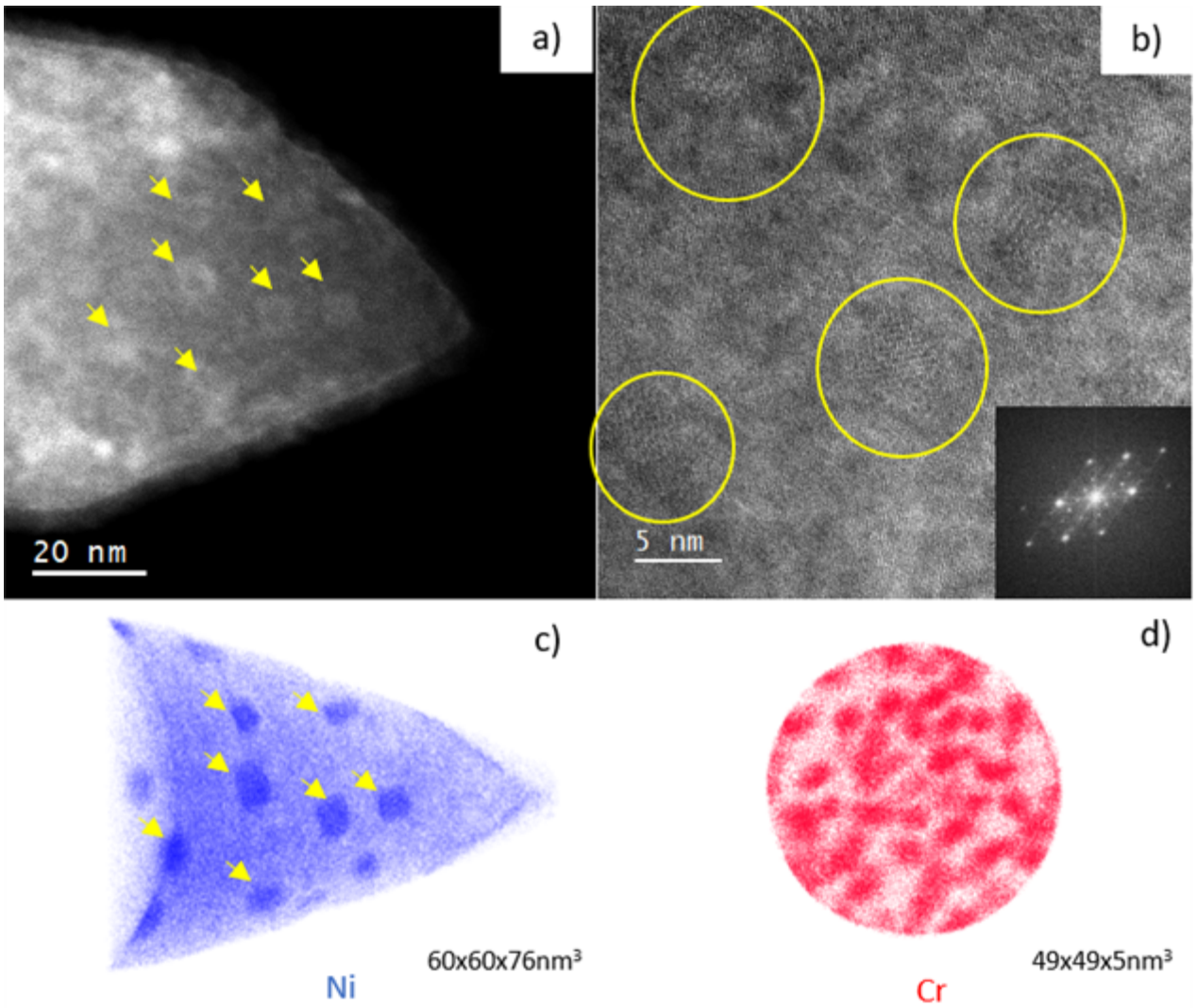
The second part of the study is to use TEM images as additional information to obtain reliable 3D reconstructions with atom probe data, even without the presence of crystallographic poles. Through the comparison of different 3D reconstruction methods that exist on an experimental dataset from TEM-APT 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:

[1] Herbig, M., Choi, P., & Raabe, D. (2015). Combining structural and chemical information at the nanometer scale by correlative transmission electron microscopy and atom probe tomography. Ultramicroscopy, 153, 32-39.

[2] Legras, L., Lescoat, M. L., Jublot-Leclerc, S., & Gentils, A. (2016, November). Optimisation of TEM preparation in metallic materials using low voltage ions. In European Microscopy Congress 2016: Proceedings (pp. 443-444). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

[3] Hatzoglou, C., Da Costa, G., & Vurpillot, F. (2019). Enhanced dynamic reconstruction for atom probe tomography. Ultramicroscopy, 197, 72-82

[4] Li, Yao, et al. "Flash electropolishing of BCC Fe and Fe-based alloys." Journal of Nuclear Materials 586 (2023): 154672.

851

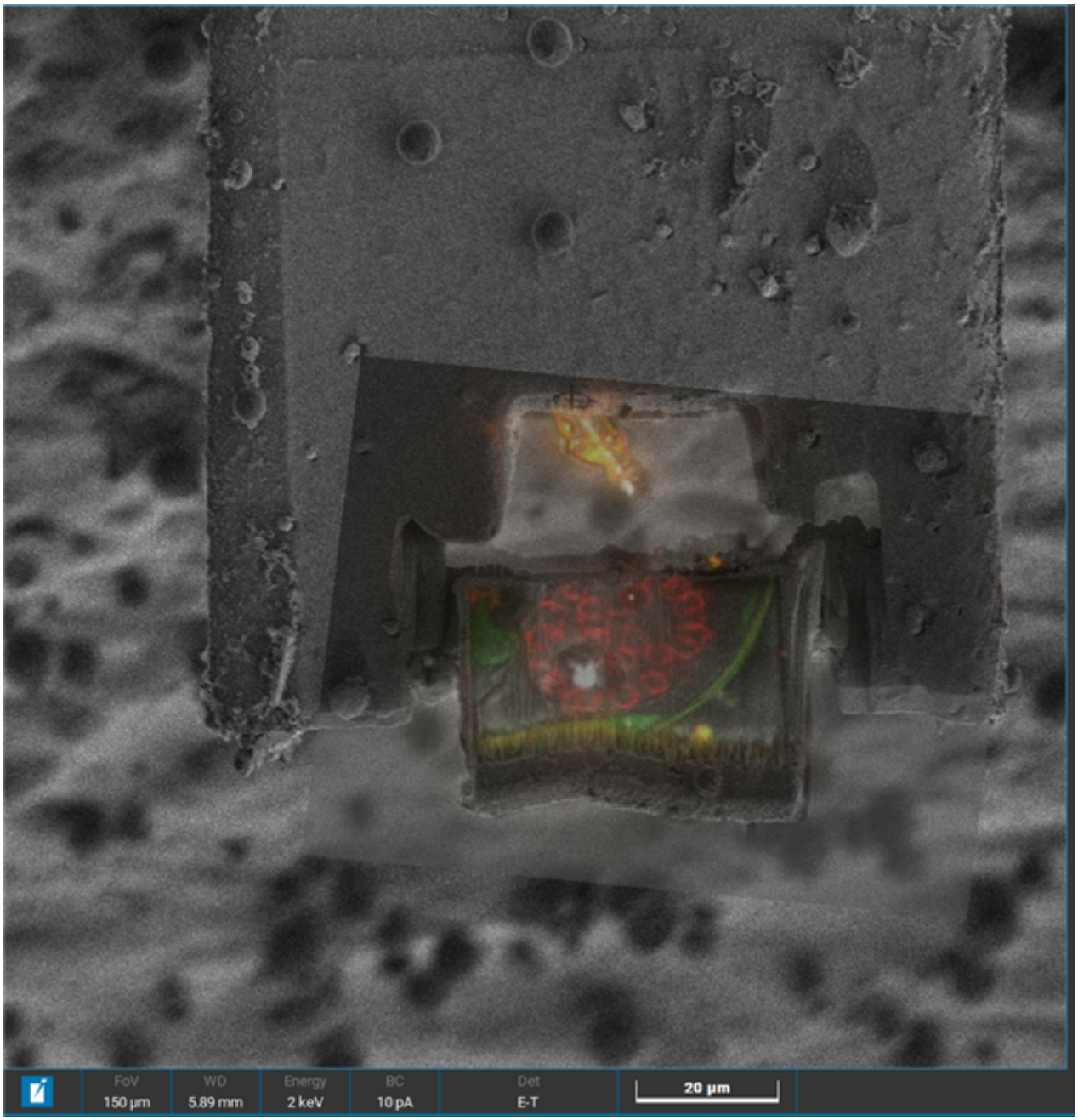
Precise light and fluorescent microscopy guided sequential cryo FIB lift-out

Dominik Pinkas[1], Samuel Záchej[2], Martina Zanova[2], Prof. Pavel Hozak[3], Vlada Filimonenko[1,3]
[1]Electron Microscopy Core Facility, Institute of Molecular Genetics ASCR, Prague, Czech republic, [2]TESCAN GROUP, Brno, Czech republic, [3]Department of Biology of the Cell Nucleus, Institute of Molecular Genetics ASCR, Prague, Czech republic

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
Electron Microscopy Core Facility, IMG ASCR, Prague, CR, is supported by MEYS CR (LM2023050 Czech-BioImaging) and ,OP RDE (CZ.02.1.01/0.0/0.0/18_046/0016045, CZ.02.1.01/0.0/0.0/16_013/0001775). and IMG grant (RVO: 68378050).



Keywords:

CLEM, CryoFIB lift-out, cryo ET

899

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

Msc. Jan Čermák[1], PhD. Jiří Materna[2], MSc. Ondřej Ambrož[1], BSc. Patrik Jozefovič[1], PhD. Šárka Mikmeková[1]
[1]Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic, [2]Machine Learning College, Brno, Czech Republic

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.

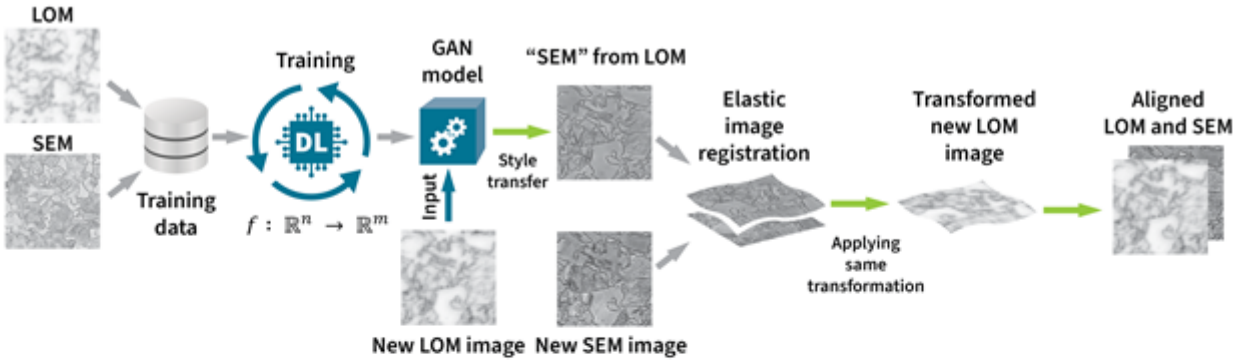


Figure 1: A schematic of image registration utilizing deep learning style transfer.

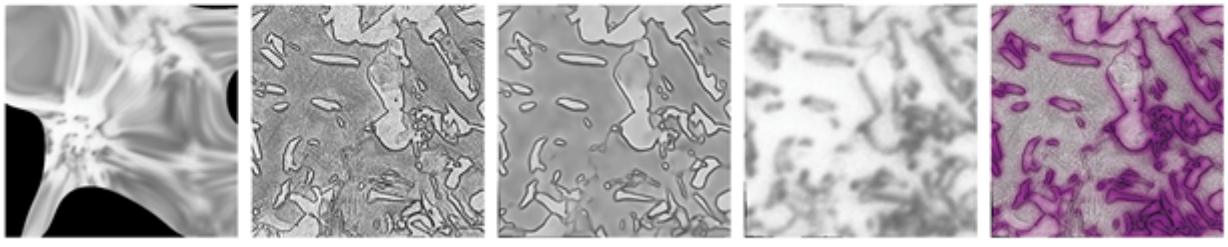


Figure 2: Depiction of image registration failure or success depending on the incorporation of style transfer.

Keywords:

Correlative-microscopy, image-registration, deep-learning, style-transfer

Reference:

[1] NenoVision. "Correlative Microscopy." NenoVision, 2021. Available at: https://www.nenovision.com/technology/correlative-microscopy.
[2] Müller, M., Britz, D. and Mücklich, F.. "Scale-bridging Microstructural Analysis – A Correlative Approach to Microstructure Quantification Combining Microscopic Images and EBSD Data" Practical Metallography, vol. 58, no. 7, 2021, pp. 408-426. https://doi.org/10.1515/pm-2021-0032
[3] Microscopy and Microanalysis, Volume 29, Issue Supplement_1, 1 August 2023, Pages 2016–2018, https://doi.org/10.1093/micmic/ozad067.1044
[4] I. Arganda-Carreras, C. O. S. Sorzano, R. Marabini, J.-M. Carazo, C. Ortiz-de Solorzano, and J. Kybic, “Consistent and Elastic Registration of Histological Sections using Vector-Spline Regularization,” Lecture Notes in Computer Science, Springer Berlin / Heidelberg, volume 4241/2006, CVAMIA: Computer Vision Approaches to Medical Image Analysis, pages 85-95, 2006.

955

Localisation of nanoparticles in whole cells using correlative cryo soft x-ray tomography and fluorescent microscopy

Mr Kenneth Fahy[1], Sergey Kapishnikov[1], Paul Sheridan[1], William Fyans[1], Fergal O'Reilly[1,2], Tony McEnroe[1]
[1]SiriusXT, Dublin , Ireland , [2]UCD Dublin, Dublin 4, Ireland

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).

84

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

Dr Jorge Diniz[1], Miss Jennifer Ferguson[1], Mr Pete Johnson[1], Mr Tim Smith[1]
[1]Renishaw plc, New Mills, Wotton-under-Edge, UK

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.

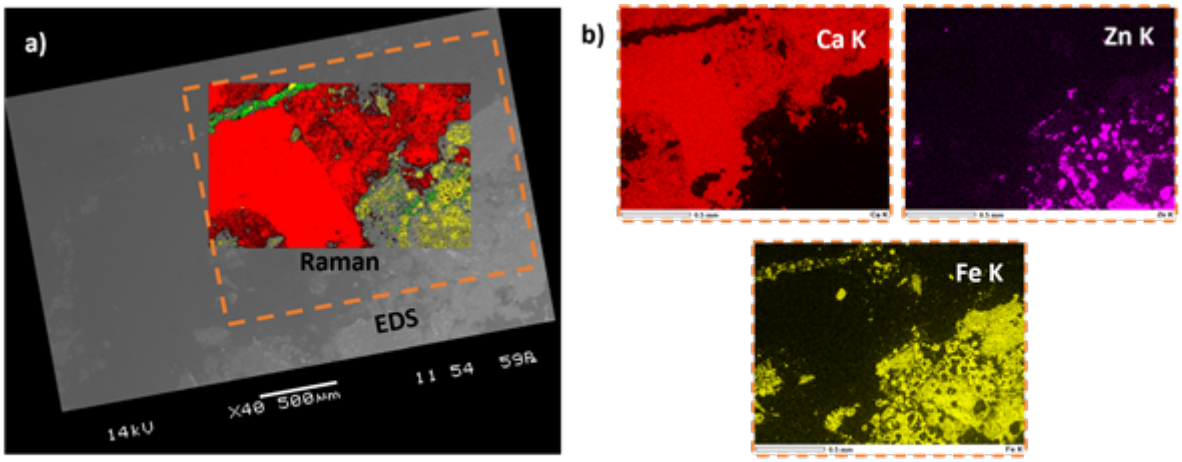


Figure 1. a) BSE image of a mineral section with overlayed Raman image illustrating the distribution of calcite (red), pyrite (yellow), and anatase (green); b) complementary EDS information agrees with the Raman data, and in addition highlights Zn due to the presence of sphalerite.

Keywords:

Raman imaging, Correlative analysis, SEM

90

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

Marit Smeets[1], Deniz Daviran[1], Wessel Teunisse[1]
[1]Delmic B.V., Delft, The Netherlands

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:

1. M. Smeets et al. (2021). Micros Today, vol. 29, no. 6, pp. 20–25.
2. D. Boltje et al. (2022). eLife, vol. 11
3. J. Yaeng et al. (2023) Microscopy and Microanalysis, vol. 29, no. 1, pp-1055-1057

133

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

Senior Researcher Alba Garzon Manjon[1], Mr Miquel Vega Paredes[2], Miss Raquel Aymerich Armengol[2], Mr Daniel Arenas Esteban[3], Miss Sara Martí Sánchez[1], Miss Sara Bals[3], Miss Christina Scheu[2]
[1]Institut Català de Nanociència i Nanotecnologia, Bellaterra, Barcelona, [2]Max Planck Institut für Eisenforschung GmbH, Düsseldorf, Germany, [3]University of Antwerpen - Electron microscopy for materials science, Amtwerpen, Belgium

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:

[1] Garzón Manjón, A§.; et al. (2022). Nanoscale. 14, 18060-18069. [2] Vega Paredes, M.; Aymerich Armengol, R.; Arenas Esteban, D.; Martí Sánchez, S.; Bals, S.; Scheu, C.; Garzón Manjón, A§. (2023) ACS Nano. 17, 17, 16943–16951.

323

4D STEM and EELS Acquired Simultaneously with a Fast Pixelated Direct Detector with Center Hole

Dr. Martin Huth[1], Valentin Kroner[1], Yassine El Imari[1], Dr. Stefan Aschauer[1], Dr. Ryusuke Sagawa[3], Dr. Hiroki Hashiguchi[3], Dr. Akiho Nakamura[3], Prof. Lothar Strüder[2], Dr. Heike Soltau[1]
[1]PNDetector GmbH, Munich, Germany, [2]PNSensor GmbH, Munich, Germany, [3]JEOL Ltd., Tokyo, Japan

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

366

CL-EBSD-TUNA Correlative Multi-microscopy Study of Grain Boundaries in Pseudo-symmetric Cu(In,Ga)S2 Solar Cell Absorber

Yucheng Hu[1], Dr Gunnar Kusch[1], Miss Damilola Adeleye[2], Prof Susanne Siebentritt[2], Prof Rachel Oliver[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Luxembourg, Esch-sur-Alzette, Luxembourg

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

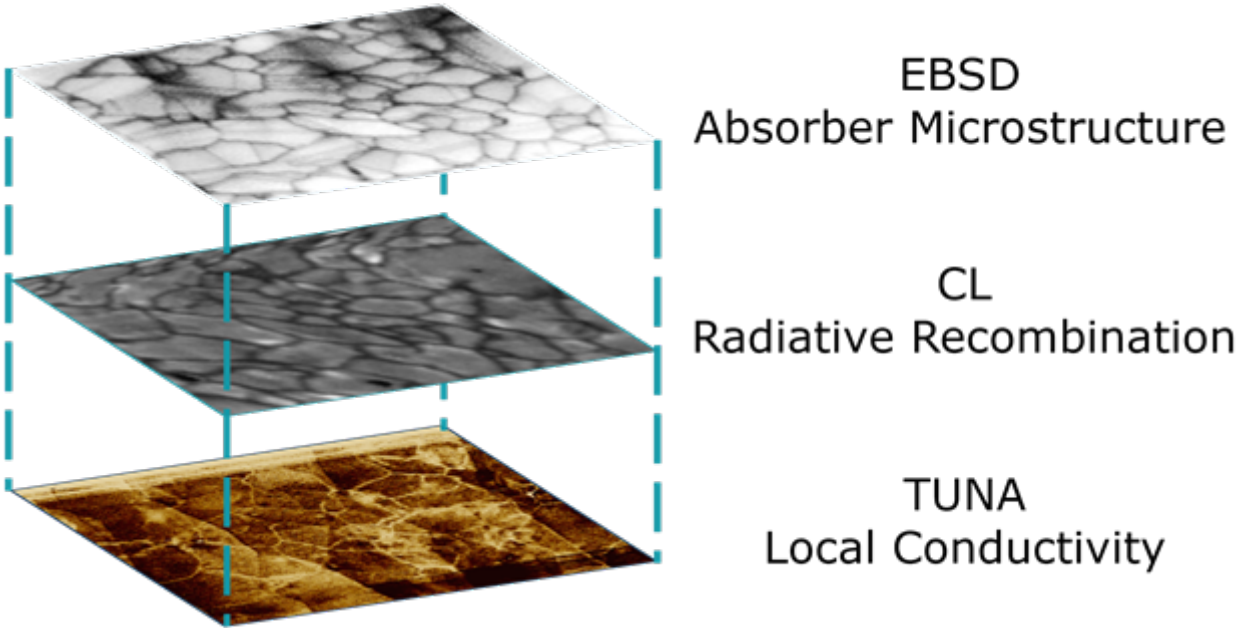
Analysing the EBSD maps we obtained structural information about the CIGS samples, including grain structure, phase orientation, and GB misorientation. We divided the GBs in CIGS into two categories, twin boundaries (TBs) and random high angle grain boundaries (RHAGBs). TBs had a high relative frequency 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:

1. Krause, M. et al. Microscopic origins of performance losses in highly efficient Cu(In,Ga)Se2 thin-film solar cells. Nat Commun 11, 4189 (2020).
2. Shukla, S. et al. Over 15% efficient wide-band-gap Cu(In,Ga)S2 solar cell: Suppressing bulk and interface recombination through composition engineering. Joule 5, 1816–1831 (2021).

472

Illuminating the Microscopic Realm: Application of Resin R221 for CLEM in microbes and plant tissues

Andrea Elizabeth Babu Dominic Cheradil[1], Dr Julia Buchner[1], Dr Matthias Ostermeier[2], Isabella Gantner[1], Jennifer Grünert[1], Prof. Dr. Andreas Klingl[1]
[1]Plant Development & Electron Microscopy, Planegg-Martinsried, Munich, Germany, [2]Department of Molecular Plant Science, Planegg-Martinsried, Munich, Germany

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:

Heiligenstein, X., & Lucas, M. S. (2022). One for all, all for one: a close look at in-resin fluorescence protocols for CLEM. Frontiers in Cell and Developmental Biology, 10, 866472. https://doi.org/10.3389/fcell.2022.866472

Tanida, I., Yamaguchi, J., Kakuta, S., Uchiyama, Y. (2023). Osmium-Resistant Fluorescent Proteins and In-Resin Correlative Light-Electron Microscopy of Epon-Embedded Mammalian Cultured Cells. In: Sharma, M. (eds) Fluorescent Proteins. Methods in Molecular Biology, vol 2564. Humana, New York, NY. https://doi.org/10.3389/fcell.2022.866472

Tanida, I., Yamaguchi, J., Kakuta, S., & Uchiyama, Y. (2022). Osmium-resistant fluorescent proteins and in-resin correlative light-electron microscopy of Epon-embedded mammalian cultured cells. In Fluorescent Proteins: Methods and Protocols (pp. 287-297). New York, NY: Springer US. doi: 10.1007/978-1-0716-2667-2_15. PMID: 36107349.

680

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

Mgr. Jan Dobeš[1], Jaromír Bačovský[1], Radka Martínková[1], Jan Vysloužil[2], Zuzana Bytešníková[3]
[1]Delong Instruments, Brno, Czech Republic, [2]Pragolab, Brno, Czech Republic, [3]Department of Chemistry and Biochemistry, Mendel University, Brno, Czech Republic

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.

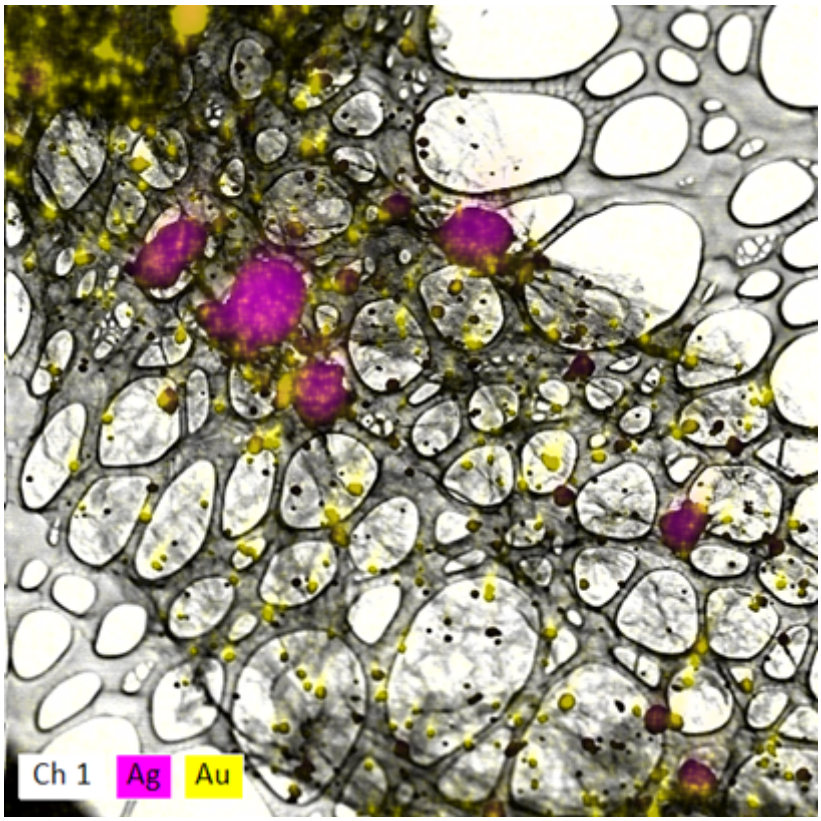
Methods
LVEMs are versatile in imaging (TEM, SEM, STEM) and analytical (ED, EDS) modes. In TEM mode, LVEMs enable the users to observe and explore thin specimens' internal structures from low magnification for a quick overview to high magnifications with high resolution. While STEM mode enhances the 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

781

The development of multimodal imaging with functional silica nanoparticle

Dr. Tomohiro Haruta[1], Prof. Michihiro Nakamura[2]
[1]JEOL Ltd., Akishima, Japan, [2]Graduate school of medicine,Yamaguchi university, Ube, Japan

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

861

Correlative microscopy and spectroscopy of nanophotonic materials

Michael Foltýn[1], Petr Liška[1], Peter Kepič[1], Rastislav Motúz[2], Jaroslav Jiruše[2], Andrea Konečná[1], Vlastimil Křápek[1], Tomáš Šikola[1], Michal Horák[1]
[1]Brno University of Technology, Brno, Czech Republic, [2]TESCAN Group, R&D Department, Brno, Czech Republic

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

Reference:

1. P Kepič et al., ACS Photonics 8 (2021), p. 1048. https://doi.org/10.1021/acsphotonics.1c00222
2. P Liška et al., J. Phys. Chem. C 127 (2023), p. 12404. https://doi.org/10.1021/acs.jpcc.3c03056
3. This research is supported by TAČR (FW06010396) and GAČR (22-04859S).

868

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

Mr William Bowles[1,2,3], Dr Abner Velazco-Torrejon[1], Dr Siva Ramadurai[1], Dr Marcus Gallagher-Jones[1], Prof Angus Kirkland[1,4], Dr Alistair Siebert[2], Prof James Naismith[5], Dr Maud Dumoux[1]
[1]Structural Biology, Rosalind Franklin Institute, Didcot, United Kingdom, [2]Electron Biology Imaging Centre, Diamond Light Source, Didcot, United Kingdom, [3]Doctoral Training Centre, University of Oxford, Oxford, United Kingdom, [4]Department of Materials, University of Oxford, Oxford, United Kingdom, [5]Division of Structural Biology, Wellcome Trust Centre of Human Genomics, Oxford, United Kingdom

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

974

Strategies for Multimodal Image Data Transformation to a Common Format for Cloud Integration and Visualization

Phd Rafael Camacho[1], PhD Jens Berndtsson[1], PhD Massimo Micaroni[1], PhD Karl Zhang[1], PhD Julia Fernandez-Rodriguez[1]
[1]Center for Cellular Imaging, Core Facilities, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, Gothenburg, Sweden

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:

[1] Moore, Josh et al. “OME-Zarr: a cloud-optimized bioimaging file format with international community support.” Histochemistry and cell biology vol. 160,3 (2023): 223-251. doi:10.1007/s00418-023-02209-1
[2] Ahlers, J., et al. Napari: A Multi-dimensional Image Viewer for Python. v0.4.18, Zenodo, 5 July 2023, doi:10.5281/zenodo.8115575.

[3] Boergens, Kevin M et al. “webKnossos: efficient online 3D data annotation for connectomics.” Nature methods vol. 14,7 (2017): 691-694. doi:10.1038/nmeth.4331
[4] https://www.zeiss.com/microscopy/en/products/sem-fib-sem/sem/multisem.html
[5] Kay, Emma et al. “NanoSIMS Imaging Reveals the Impact of Ligand-ASO Conjugate Stability on ASO Subcellular Distribution.” Pharmaceutics vol. 14,2 463. 21 Feb. 2022, doi:10.3390/pharmaceutics14020463

1167

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

Dr Sangjun Kang[1], Prof. Xiaoke Mu[1,3], Dr Di Wang[1], Prof Christian Kübel[1]
[1]Tu Darmstadt, Darmstadt, Germany, [2]Karlsruhe Institute of Technology , Karlsruhe , Germany, [3]Lanzhou University, Lanzhou , China

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.

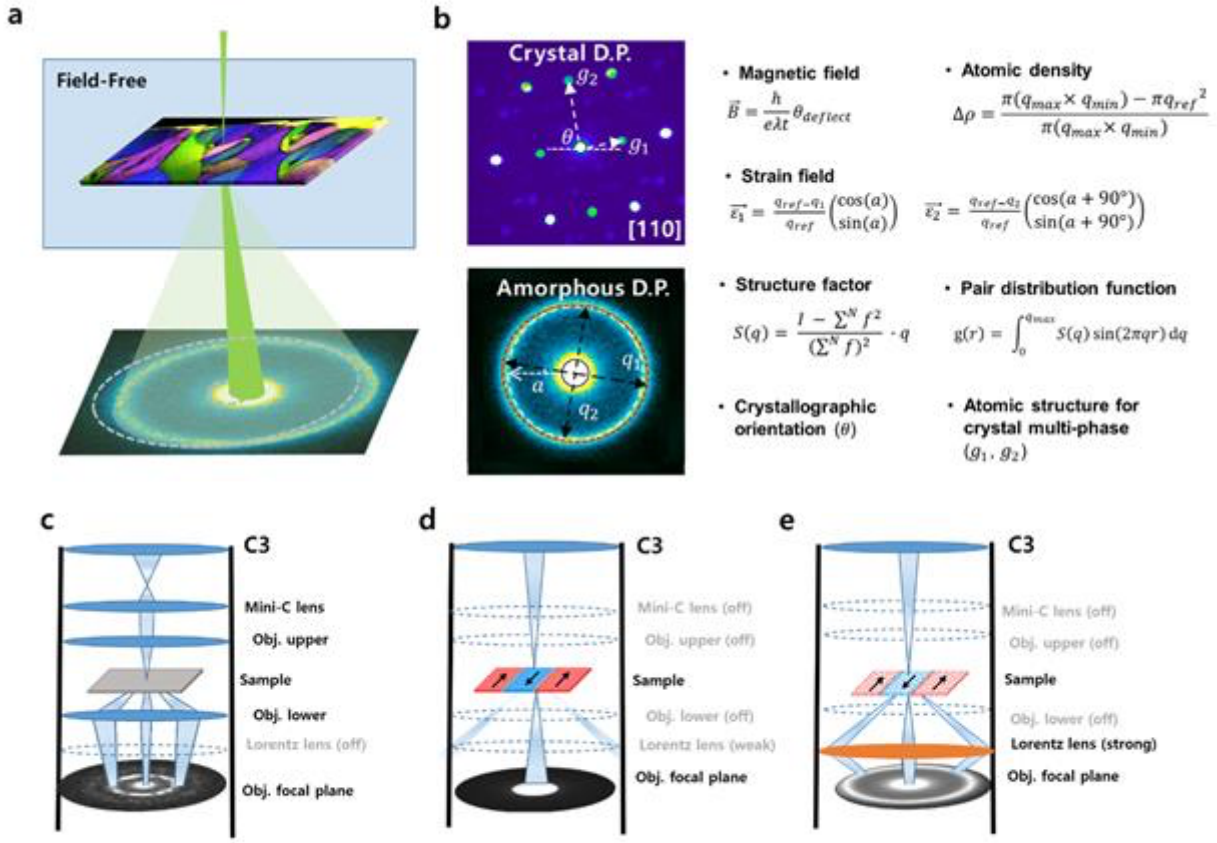


Fig. 1: Schematic illustration of LA-Ltz-4D-STEM and its optical setup. **a** The electron probe is focused on the TEM sample in a free-field condition (Lorentz mode). Spatially-resolved diffraction patterns are collected during scanning over an area of interest using 4D-STEM. **b** Exemplary data analysis: The position of the direct beam measures the momentum transferred by the Lorentz force, reflecting the local magnetic field of the sample. The relative atomic density can be calculated by quantifying the area encircled by the 1st diffraction ring. The 1st and 2nd principal strain ($\vec{\varepsilon}_1$ and $\vec{\varepsilon}_2$) can be calculated from the elliptical distortion of the diffraction ring. The diffraction patterns can be processed to determine the structure factor $S(q)$ by azimuthal integration into intensity profiles $I(q)$ and background subtraction. A PDF can be obtained by Fourier sine transformation of $S(q)$ at every scan position. Moreover, Ltz-4D-STEM of crystalline materials can provide information on crystal symmetry, orientation, lattice parameters, etc. **c-e** Illustrations of the optical setup using in conventional nanobeam 4D-STEM, conventional field-free 4D-STEM, and the LA-Ltz-4D-STEM.

Keywords:

LA-Ltz-4D-STEM, magnetic imaging, magnetic structure

Reference:

[1] S. Kang et al., Advanced Materials (2023) DOI: 10.1002/adma.202212086
[2] Silveyra et al., Science 362, 418 (2018)

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]
[1]Technical University of Darmstadt, Department of Electrical Engineering and Information Technology, Darmstadt, Germany, [2]Technical University of Darmstadt, Department of Biology, Darmstadt, Germany

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
This project has received funding from the European Research Council under the European Union's Horizon 2020 research and innovation programme (No. 772441) and the Deutsche Forschungs-gemeinschaft (DFG, German Research Council) grant CA 198/20-1 Project ID 529989072.

Keywords:

Cryo-immersion-microscopy, Cryo-fluorescence-microscopy, Correlative-microscopy, Super-resolution-microscopy, Photobleaching

Reference:

[1] P. D. Dahlberg und W. E. Moerner, Annual Review of Physical Chemistry, Bd. 72, Nr. 1, S. 1–26, 2021, doi: 10.1146/annurev-physchem-090319-051546.
[2] R. Faoro et al., Proceedings of the National Academy of Sciences, Bd. 115, Nr. 6, S. 1204–1209, Feb. 2018, doi: 10.1073/pnas.1717282115.

1302

Correlative microscopy of graphene with SEM, Raman spectroscopy and AFM

Dr Lok Yi Lee[1], Juliet Biard[1]
[1]Paragraf Ltd, Somersham, United Kingdom

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

Reference:

[1] Song, G. et al. "Operation of graphene magnetic field sensors near the charge neutrality point." Communications Physics 2.1 (2019): 1-8
[2] Prattis I, Hui E, Gubeljak P, Kaminski Schierle GS, Lombardo A, Occhipinti LG. Graphene for Biosensing Applications in Point-of-Care Testing. Trends Biotechnol. 2021 Oct;39(10):1065-1077. doi: 10.1016/j.tibtech.2021.01.005. Epub 2021 Feb 8. PMID: 33573848.
[3] Thomas, S. “A method of producing a two-dimensional material”, WO2017029470
[4] Massabuau, F. & Trình, Linh & Lodie, D. & Thrush, Edward & Zhu, Dandan & Oehler, F. & Zhu, Tongtong & Kappers, M. & Humphreys, Colin & Oliver, R.. (2013). Correlations between the morphology and emission properties of trench defects in InGaN/GaN quantum wells. Journal of Applied Physics. 113. 073505. 10.1063/1.4792505.

1335

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

Dr. Purvesh Soni[1], Dr. Anna Mogilatenko[2,3], Dr. Meiken Falke[1]
[1]Bruker Nano GmbH, Berlin, Germany, [2]Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik , Berlin, Germany, [3]Institute of Physics, Humboldt-University of Berlin, Berlin, Germany

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.

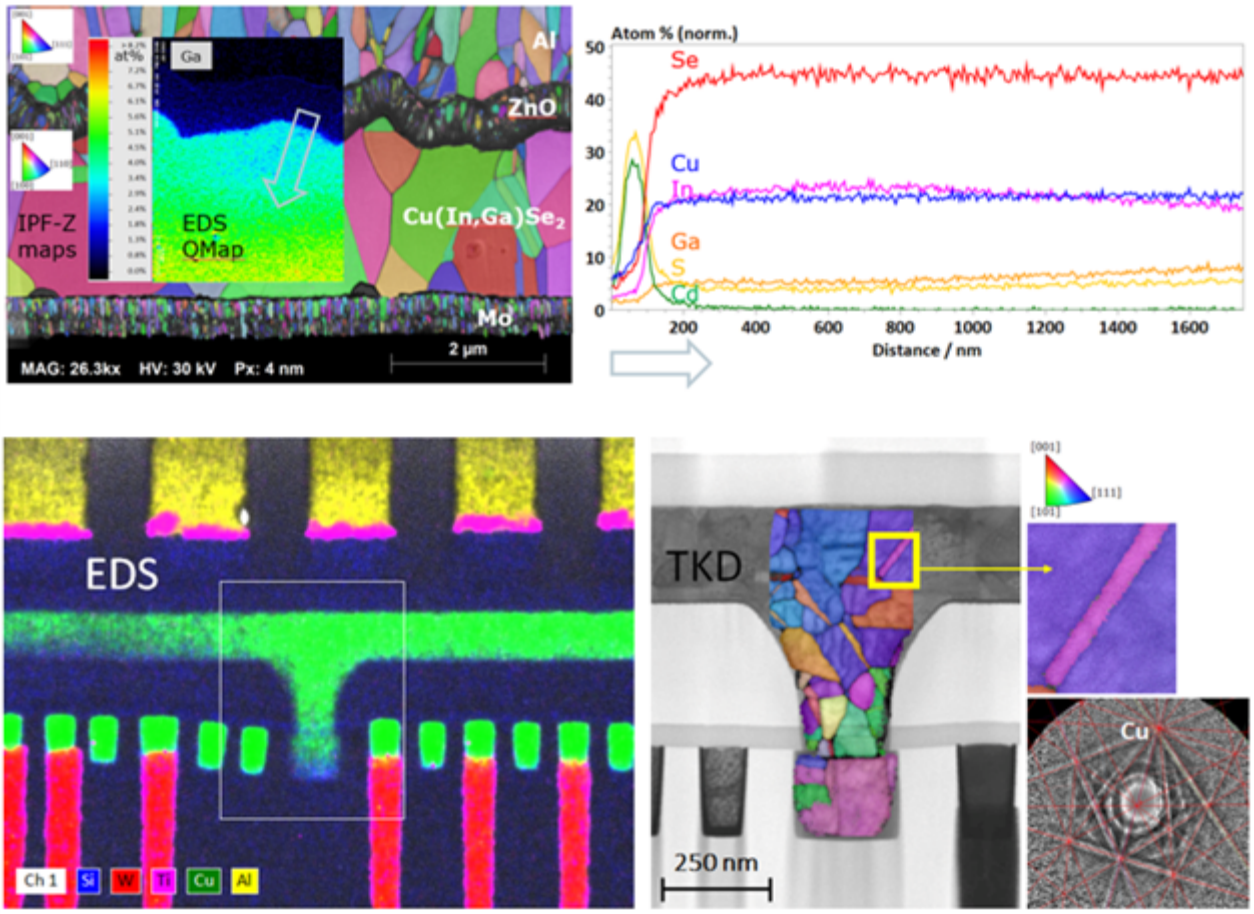
Methods
For the experiments we used an annular EDS detector [2] placed horizontally between the specimen and the SEM pole piece, thus providing a high solid angle of around 1 sr for X-ray collection. This geometry allows high count rates in case of low X-ray yield from thin lamellae, light element containing 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:

[1] Brodu E, Bouzy E and Fundenberger J 2017 Microsc. Microanal. 23 (Suppl. 1) 530-531.
[2] Terborg R and Rohde M 2008 in: EMC 2008 14th European Microscopy Congress. (Luysberg M, Tillmann K and Weirich T; Eds.) 1-5 September 2008, Aachen, Germany. (Berlin, Heidelberg: Springer). https://doi.org/10.1007/978-3-540-85156-1_317.
[3] Watanabe M and Williams D B 2006 J. Microscopy 221 89-109.