Invited Speaker

419 Unsupervised Quantification of large EELS datasets

Prof. Dr. Jo Verbeeck[1,2], Dr. Daen Jannis[1,2], Dr. Wouter Van den Broek[3], Arno Annys[1,2], Dr. Zezhong Zhang[1,2,4], Prof. Dr. Sandra Van Aert[1,2]

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Oral Presentation

327 Automated feedback correction of the specimen drift at the atomic scale

Dr Christophe Gatel[1]

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Loïc Grossetête[1], Cécile Marcelot[3], Christophe Gatel[1], Sylvain Pauchet[2], Martin Hytch[1]

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Dr. Giulio Guzzinati[1], Pirmin Kükelhan[1], Martin Linck[1], Angelika Leibscher[1], Dominique Lörks[1], Volker Gerheim[1], Heiko Müller[1]

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Dr. Gabriel Dehnez L'Hôte[1], Dr. Joël Lachambre[1], M. Thierry Douillard[1], Dr. Hdr Cyril Langlois[1]

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[1]JEOL Ltd, 3-1-2 Musashino Akishima, Japan, [2]Kyushu University, 744 Motooka NIshi-ku Fukuoka, Japan

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480 Active focus stabilisation using astigmatism with universal objective lens compatibility and sub-10 nm precision
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[1]School of Physics and Astronomy, University of Leeds, Leeds, UK, [2]School of Food Science and Nutrition, University of Leeds, Leeds, UK

918 Time-resolved TEM observation of CeO2 surface with electrostatic sub-framing system
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924 Development of chopped scan control for beam blanking
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[1]Department of Physics, Norwegian University of Science and Technology, Trondheim, Norway

1057 Accessible low-cost, long range, optical autofocus module for open-source multiwell plate and slide scanning microscopy
Miss Sara Habte[1], Mr Miguel Boland[2], Ms Sara Habte[1], Dr Jonathan Lightley[1], Dr Edwin Garcia[1], Professor Christopher Dunsby[1], Dr Edward Cohen[2], Professor Paul French[1]
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1065 Innovative Microfluidic chip for Raman spectroscopy and advanced electron microscopy techniques

Ing. Tomas Laznicka[1], Jan Jezek[1], Kamila Hrubanova[1], Vladislav Krzyzanek[1]
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1104 Ultra-low-cost, high-dynamic-range, additively manufactured CMOS spectrometers with UV, visible, and NIR sensing functionality

Mr. Hironori Kondo[1,2]
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Late Poster Presentation

1330 Pulse counting in the SEM

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Unsupervised Quantification of large EELS datasets

Prof. Dr. Jo Verbeeck[1,2], Dr. Daen Jannis[1,2], Dr. Wouter Van den Broek[3], Arno Annys[1,2], Dr. Zezhong Zhang[1,2,4], Prof. Dr. Sandra Van Aert[1,2]
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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims
Electron energy loss spectroscopy (EELS) has experienced substantial steps forward over the last decades in terms of energy resolution from monochromators, signal to noise and speed from improved detectors, vastly improved optics and stability in spectrometers and advances in the statistical treatment of the recorded data.
Yet, despite these efforts, EELS relies heavily on the interpretation skills of expert operators. This limits the widespread adoption of EELS and leaves room for experimenters' bias posing a significant reproducibility risk.
In this talk, I will give an overview of recent efforts towards the goal of an entirely autonomous data processing workflow, which could significantly improve the quantification of EELS spectra in terms of ease of use, precision and accuracy.
Methods
The method relies on model-based quantification as was e.g. implemented in EELSMODEL [1] but improves on several important aspects:

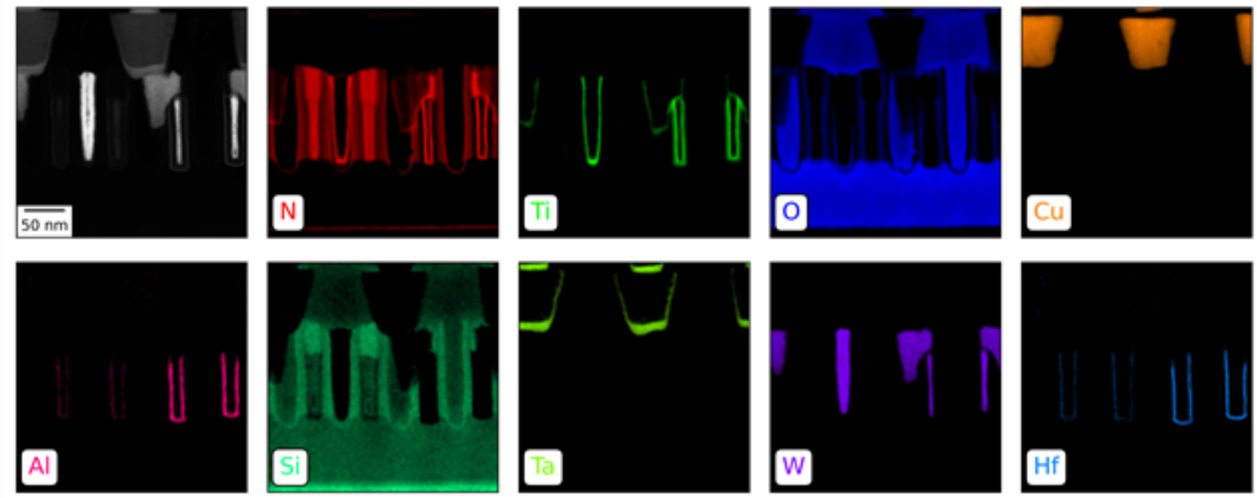
- The physical model is made entirely linear. This results in a single solution without the need for user provided initial parameter estimates.
- The background modelling process is significantly improved through a combination of a linearized model and constrained quadratic programming methods [2].
- The fine structure is described with a piecewise linear function with a nonlinear energy sampling to express that most significant ELNES features occur near the edge onset
- A complete Dirac-based database of open generalized oscillator strengths significantly improves the fit with experiments and provides a way to improve accuracy and precision. [3]

We investigate automatic identification of all EELS edges present in a dataset making use of neural network approach [4]. This allows building a complete model without any user input thereby completely removing the experimenter's preferences and opinions from the process. We discuss achieved precision approaching the statistical limit and show attempts to evaluate the accuracy with reference samples.

Results
The result provides a reliable quantification with highest possible precision and excellent accuracy and seamlessly provides background subtracted and deconvolved ELNES features without any user intervention. This is particularly interesting for high energy K-edges as they provide a direct lab-based alternative for XAS measurements even for edges that are traditionally considered to be far outside the achievable EELS energy range.

Conclusion
In summary, we believe that the days of manual processing of EELS data are over and fast and fully unsupervised data interpretation is available. Together with advances in EELS spectrometers, this

widens the scope of EELS significantly and confirms its position as an indispensable analytical tool, also for non-expert users. It also satisfies the need for high reproducibility, essential in particular for industry applications.



Keywords:

EELS, model based fitting, signalprocessing

Reference:

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[5] This project has received funding from the ECSEL Joint Undertaking (JU) under grant agreement No 875999. The JU receives support from the European Union’s Horizon 2020 research and innovation programme and Netherlands, Belgium, Germany, France, Austria, Hungary, United Kingdom, Romania, Israel.

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Automated feedback correction of the specimen drift at the atomic scale

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background

Transmission electron microscopy (TEM) has always been an active subject of research, in particular with the advent of new instrumentation like brighter electrons sources, sensitive cameras (direct electron detection), phase plate and vortex beam, aberration corrector, high collection angle EDX detector. Such new advanced capabilities have improved conventional TEM techniques to higher resolution (spatial and in energy) and better signal–to-noise ratios (SNR).

In parallel to these instrumental and methodological developments, automation of the electron microscope has become an essential tool in cryo-microscopy for recording images of many specimen areas and defocus values for single-particle analysis [1], or in the acquisition of tomographic tilt series, diffraction tomography, and holographic tomography [2]. Further refinements include object displacement and focus prediction to accelerate acquisition of tilt series for shorter acquisition and less beam induced damage, online reconstruction of tilt series for preliminary inspection of data at the microscope, alignment of individual particles in images to correct for beam-induced movement. More recently, automation has been developed for 4D-STEM data acquisition, and pipeline acquisition and analysis for routine sample studies.

In these examples of automation, the specific sequence of images is recorded with predetermined experimental conditions. High numbers of images can thus be easily acquired without requiring any operator interaction, leaving human errors and fatigue out of the acquisition process. The computer-controlled execution can thus reduce the time the specimen is exposed to electrons and improves the studies of beam-sensitive materials. In addition, series of images acquired on the same area with a short exposure time to minimize the effect of instabilities, mainly the specimen drift, can be realigned using sophisticated post-processing and summing to improve the SNR. However, the latter approach generates large volumes of data, requires significant calculation time and reduces the field of view of the final image by keeping only the common area of all the images making up the series. The field of view is all the more reduced if the drift is significant. It is therefore interesting to consider whether the specimen drift could be carried out continually and autonomously during image acquisition, or experiments in general, and to investigate the precision with which dynamic drift correction can be achieved [3].

Methods

For several years now, we have been developing software tools to dynamically stabilize experiments. The position of the region-of-interest is determined continuously by analyzing the live flow of images from the camera coupled with feedback control to the specimen stage, or image deflection coils, to stabilize the position mechanically or optically, respectively [4]. This plugin can be installed on any platform (microscope + computer) equipped Digital Micrograph and any type of STEM camera or detector.

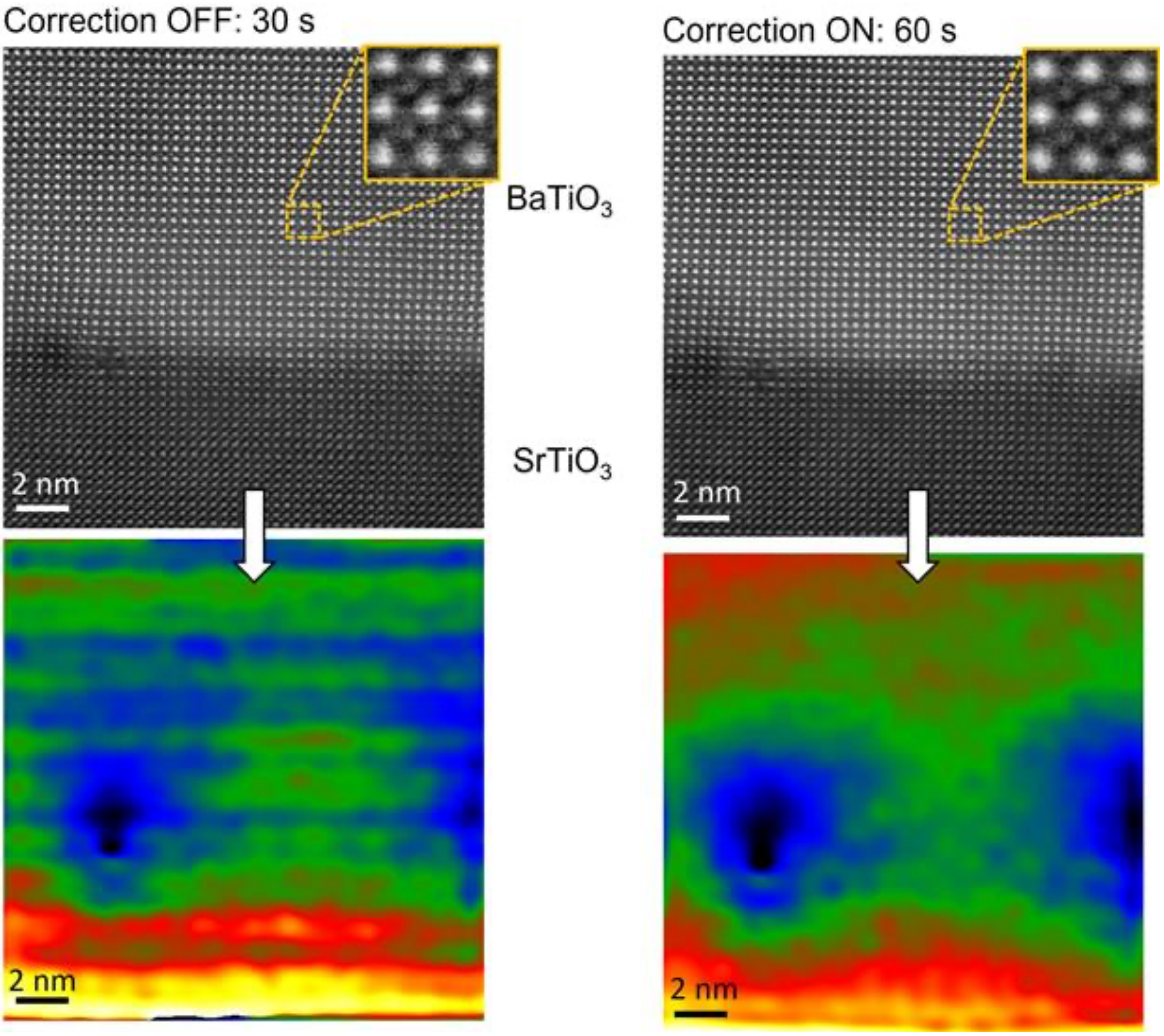
The specimen drift measured between two successive images can be corrected on a time scale of less than a second by the use of speed-optimized routines. Whilst the image deflector coils can be used for optical correction, we have mainly worked on mechanical stabilization of the stage in order to preserve the optical alignment and to maintain identical conditions during observations.

Results

We tested our approach on several different microscopes from different manufacturers. In addition, different modes of observation (Lorentz mode, high-resolution TEM or STEM) have been used, combined with in-situ experiments (biasing, temperature). We will show that stabilization at the atomic scale can be obtained for an unlimited time if the stage is equipped with piezoelectric movement.

The figure shows HAADF high-resolution STEM images acquired on a BaTiO3 thin film epitaxially grown on a SrTiO3 substrate. On the left are presented an image recorded in normal conditions during 30 s with the corresponding phase image extracted using the geometrical phase: scan error and distortions are clearly visible. On the right the same images recorded during 60 s using automated feedback of the piezostage allowing accurate correction at the atomic scale.

We will thus discuss the precision of the correction and the perspectives for HR-STEM studies for which scan errors and distortions are eliminated, or for studies of change at the atomic scale as a function of temperature.



Keywords:

Specimen drift, automated feedback, control

Reference:

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AI Automation for Transmission Electron Microscope Alignment

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims

Transmission electron microscopes, like other scientific instruments, are becoming increasingly complicated. Consider the I2TEM in Toulouse, a dedicated TEM for electron holography and in-situ research (HF-3300 C from Hitachi) which has a cold-field emission gun, 9 lenses, 4 apertures, 3 biprisms, 18 pivot points to align, and nearly as many elements in the corrector. The operation involves more than one hundred configurable parameters, but with approximately 10^300 theoretically possible configurations, one wonders if the instrument is used to its full potential. Furthermore, appropriate microscope alignment takes between 20 minutes and an hour each day, depending on the experiment and the microscopist's effectiveness. We propose to explore the use of artificial intelligence to automate the alignment, therefore tackling the complexity and reducing the time taken by the task.

Methods

To address this complexity, we first developed full computer control of the microscope. Hitachi provided access to every single element (including aperture positions, deflector currents and alignment) as well as details about the communication protocol. This enabled us to develop dynamic automation of the microscope to stabilize the specimen and hologram alignment through traditional control and feedback loops in real-time [1]. But to go further, we wondered if the computer could take complete control of the microscope according to the user's needs using artificial intelligence (AI).

Machine Learning, such as Convolutional Neural Networks (CNN) [2] is gradually replacing older forms of automation in various industries. We developed an Application Programming Interface (API) to automatically change the microscope parameters while acquiring images (Figure 1). This allowed us to create training datasets for matching the configuration to the images produced by the microscope. Because most configurations would not create an image on the screen, we first aligned the TEM before randomly shifting the parameters around their respective values. This ensures that we stay close to a configuration producing an image and by knowing the difference between the aligned value and the shift we can form a dataset linking the image to the shift. This allowed us to predict image characteristics based on the microscope configuration, as well as configurations that met specific image characteristics. In parallel, we have developed a realistic simulation of the I2TEM to produce a dataset of virtual experiments [3] which allows us to test models on various scenarios without the associated cost of directly using the microscope.

Results

This allowed us to train models for each step of the alignment process on the I2TEM (aperture alignment, condenser astigmatism correction, pivot points correction, eucentric position correction and focus correction). The only remaining step is to correct the objective lens astigmatism, which is handled by the corrector using a different API than the rest of the microscope. Each step of the correction, while not as good as human performance yet, takes up to twenty seconds at most, reducing the correction time to less than two minutes. Figure 1 depicts the first step in the alignment: aperture correction.

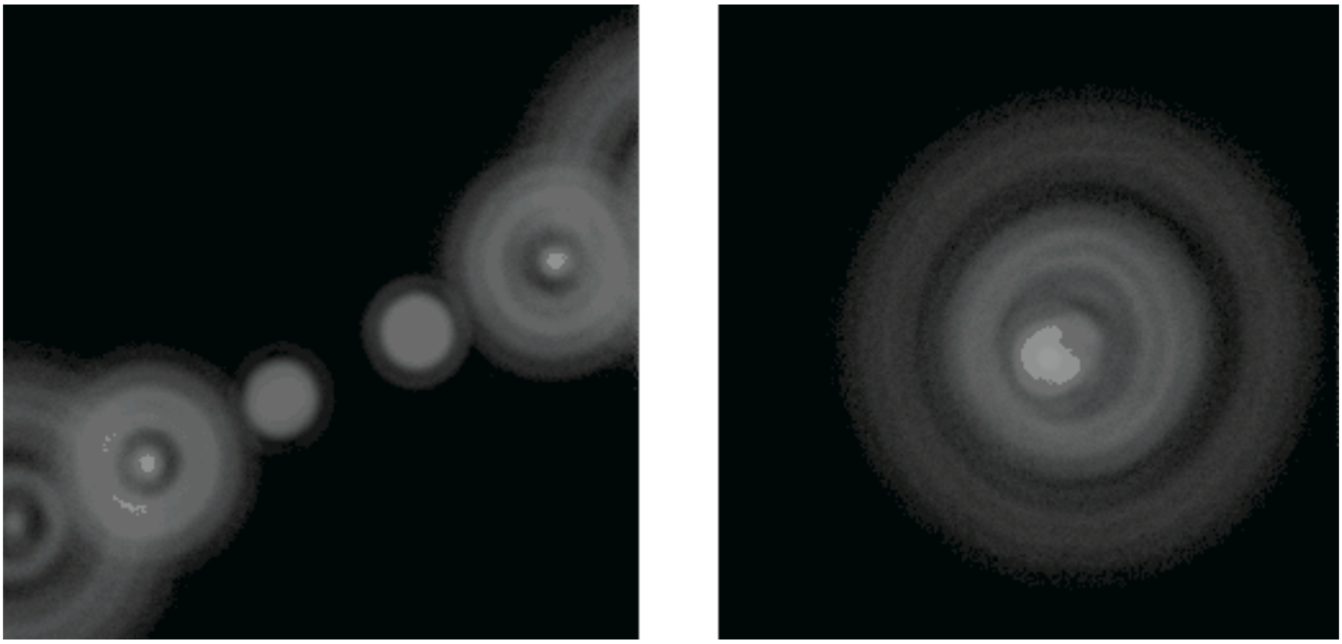
Conclusion

The results show that AI allows for TEM alignment automation, and while precision is still not on par with human levels, the complex task of finding a good alignment is met, allowing for much faster alignment overall. The method could be extended to other microscopes if they can be controlled via an API and the captured images can be processed in real-time. Additionally, the method used could be modified to address issues like object stabilization, or the calibration of new components.

The aim is to integrate the entire solution into an application that allows microscopists to use the automation functions seamlessly while also allowing them to easily generate or strengthen new automation routines. We are also considering developing a reinforcement learning [4] model that can achieve or maintain a set of meta-parameters according to the user's needs. The meta-parameters are the microscope's parameters that users are most interested in, such as beam size, beam position, focus and magnification, rather than the microscope configuration itself. We intend to encode the image in the meta-parameter space using a constrained variational auto-encoder [5], with limitations to ensure that they either coincide with a previously identified parameter, such as the focus or are at least intelligible to humans. The user can then manipulate the encoded image, and the model will change the configuration accordingly for the microscope to produce an image meeting the user's needs.

Acknowledgements

The authors acknowledge funding from the European Union under grant agreement no. 101094299 (IMPRESS). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.



Keywords:

TEM, AI, CNN, Automation

Reference:

[1] C. Gatel, J. Dupuy, F. Houdellier, M.J. Hÿtch, Appl. Phys. Lett. 113, 133102 (2018). Unlimited acquisition time in electron holography by automated feedback control of transmission electron microscope. 10.1063/1.5050906, hal-01884057v1

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CEFID: A flexible platform for spectroscopic experiments

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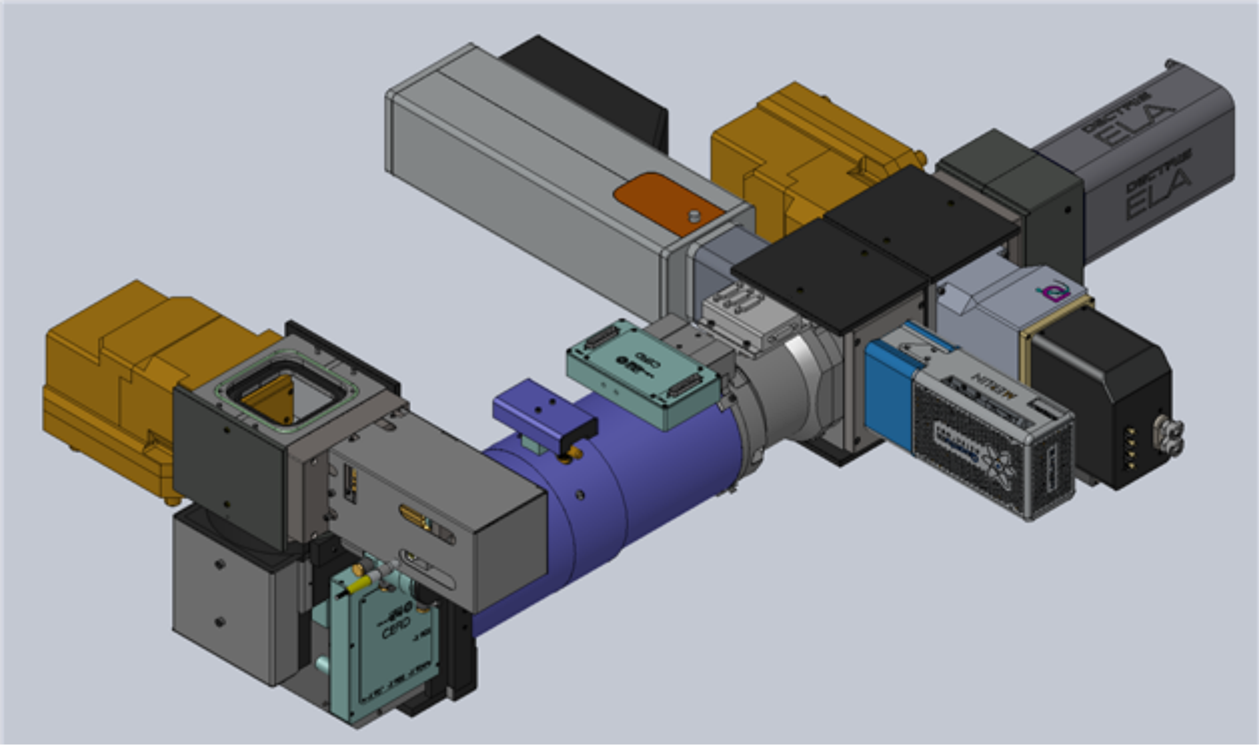
IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

TEM-related instrumentation has been developing rapidly in the last few years and a state-of-the-art experiment often involves a variety of third-party components ranging from advanced in-situ specimen holders and new generation detectors to pulsed lasers and programmable phase plates.
The CEOS Energy Filtering and Imaging Device (CEFID) is an energy filter and spectrometer offering state-of-the-art specifications and the flexibility to implement ambitious and unprecedented experiments.
Under a concept of separation of function that mirrors the design of a TEM itself, the filter design comprises highly optimised and stable optics up to the energy-selecting slit, and a flexible and minimalist projective stage [1]. This gives high performance and stability, while allowing to hop between modes (imaging, spectroscopic dispersions) with little to no re-tuning. The Python/Qt-based graphical software used for the filter operation, implements interactive and automated procedures for alignments, common workflows ranging from EFTEM to 4D-STEM, and tools for on-the-fly analysis such as live DFT, EELS maps computation/quantification, Center-of-Mass, etc.
The software is highly extendable and offers a scripting and plug-in API in python and a remote control interface for the integration into third-party software.
A wide range of detectors and scan generators from different manufacturers has already been integrated and can be used for both the tuning and data acquisition, and the filter can be installed on TEM columns from the three major manufacturers. The capability and versatility of the system make it well suited for rapidly acquiring and evaluating spectroscopic information on samples of interest [2], while its flexibility and compatibility make it a platform for complex experiments where different tools need to work in unison, such as the synchronization of acquisition with in-situ stimuli, or photon-induced near-field electron microscopy [3].
Recent developments on the CEFID will also be shown. This includes the recently introduced Dual Range EELS, a solution to rapidly switch energy range so that two different losses can be recorded concurrently, as well as methodological developments in energy-momentum mapping, and in the measurement of high-loss edges.



Keywords:

EELS, Spectrometer, Energy Filter

Reference:

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[3] Henke, JW. et al, Integrated photonics enables continuous-beam electron phase modulation, Nature 600, 653–658 (2021).

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acquiCHORD : track the rotating ROI !

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background and aims:
Acquiring images automatically in a SEM opens up a multitude of possibilities: temporal tracking of surface evolution (mechanical tests, environmental SEM...), image mosaics to cover a large field of view, drift correction, 3D acquisition with FIB... This is not new, but often this aspect of microscopy has been the preserve of microscope suppliers or equipment manufacturers (EDX, EBSD...). With the progressive opening of control APIs, and more particularly the advent of the Python language as an interface with the microscope, it is becoming increasingly easy to take full control of the machine. We present here the software acquiCHORD dedicated to the acquisition of images of a sample rotating in the microscope around an axis perpendicular to the stage. Such series of images are used in the laboratory for two main subjects: 1/ the 3D reconstruction of the surface of objects in the micrometric range [1], and 2/ the use of channeling contrast in crystalline materials for the study of crystalline defects by ECCI [2], for the obtaining of crystallographic orientation maps by the eCHORD approach [3], and for the automatic determination of grain size distributions by Machine Learning [4].

Methods:
Despite the relative simplicity of the idea (rotation - image acquisition - rotation, etc.), several locks must be released to allow this type of acquisition. We present in this work how the different solutions have been implemented in the acquiCHORD software to manage i/ the significant geometric offset between the area of interest and the rotation axis used, ii) the tilt between the optical axis and the rotation axis, which is necessary to obtain usable contrast variations, and iii) the mechanical imprecision of the rotation itself. Moreover, in order to be able to implement the acquiCHORD software on different brands of scanning electron microscopes, the program was designed to place on one side the computational calculations and drift correction, and on the other side the application of movement commands to the microscope. Indeed, the geometric references and the senses of displacement are not always the same from one brand of microscope to another. The program, coded in Python language, has been successfully implemented on ZEISS and ThermoFisher brand SEMs, with automatic machine recognition and notably of the APIs available (old OCX for ZEISS on a SUPRA model; full Python API for ThermoFisher QUATTRO SEM).

Results:
Two examples are presented. The first concerns the surface reconstruction of an OstraCode from the collection of the Laboratory of Geology of Lyon (Ecole Normale Supérieure). The particularity is the micrometric size of the object which makes impossible a 3D reconstruction of the surface by classical methods of optical photogrammetry. The second example concerns the characterization of the microstructure of a copper film for microelectronics with twins of a few tens of nanometers. Obtaining series of images in rotation on objects as small as these adds experimental challenges that have been overcome thanks to the use of a piezo-electric control stage fixed in the microscope, also controlled by the acquiCHORD software. Two examples of the use of the corresponding image series (orientation mapping and grain size determination by clustering) are explained

Conclusion:

The acquiCHORD software now makes it possible to obtain series of images in rotation in a SEM that can be used for several objectives, ranging from 3D surface reconstruction to fine characterization of the microstructure of polycrystalline materials. The software is under CeCILL license (from CEA CNRS INRIA Free Software) which is a free software license adapted to both international and French legal matters, in the spirit of and retaining compatibility with the GNU General Public License (GPL). AcquiCHORD is available on request.

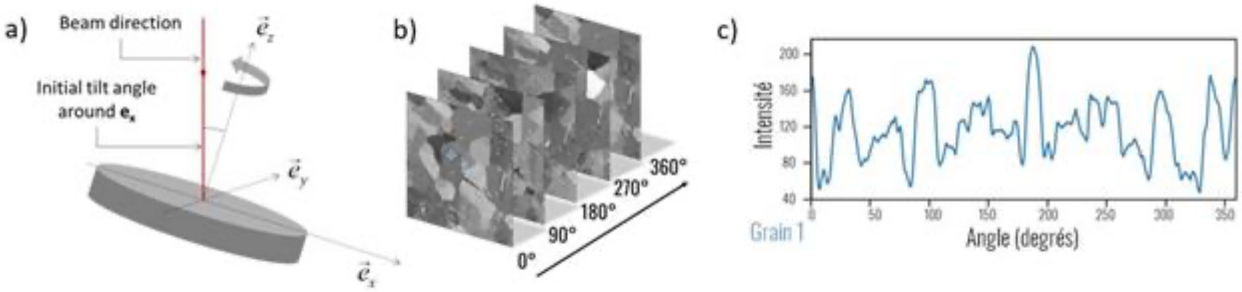

Figure 1: Geometrical setup for acquiring image series during sample rotation, b) image series of the region of interest with one image for each rotation step, c) In the case of a polycrystalline sample and backscattered electron images, this intensity profile corresponds to the evolution of the channeling contrast during the rotation, which is a unique signature of the crystalline orientation at this position

Keywords:

API, Python, SEM, channeling contrast

Reference:

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Advancing Ultrafast Transmission Electron Microscopy with Dielectric Metalenses

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims

In 2005, Ahmed H. Zewail merged ultrafast femtosecond laser with femtosecond precision with TEM's sub-nanometer spatial resolution developing Ultrafast Transmission Electron Microscopy (UTEM). This technique combines the sub-nm spatial resolution of TEMs with the fs temporal resolution of ultrafast lasers and is paving the way to the study of ultrafast fundamental processes at the nanoscale. In the last years, a new paradigm for the arbitrary modulation of the probing-electron wave function phase through quantized photon-electron interaction was put forward and has the potential for enhanced microscopic sensitivities to material properties [1]. The quality and the extent of the electron beam shaping strongly depend on matching the transverse coherence of the electron beam (~ 1 µm) with the spot size of the laser beam, which generally exceeds the former by more than one order of magnitude. This mismatch prevents the modulation of the electron wave function phase, which is crucial for the ability to probe specific materials degree of freedom (such as chirality of materials when adopting a vortex electron beam). In order to address this issue, we propose the integration of a crystallin silicon metalens in the platform that enables electron-light interaction.

Methods

The electron beam shaping inside the UTEM is facilitated by a Photonic free-ELectron Modulator (PELM). An external Spatial Light Modulator (SLM) imprints an arbitrary amplitude and phase pattern on an ultrafast optical field. The light is then projected on a flat electron-transparent metallic thin film on the PELM platform, where it interacts with the electron beam. The integration of a dielectric metalens into the PELM enables the focusing of incident light down to a few micrometers before its interaction with the electron beam. We fabricated a metalens on a free-standing silicon membrane kept in place by a silicon window. Then, we integrated the silicon windows to the PELM platelet positioned at 45° with respect to the metallic film. The focal distance of the metalens is on the order of several hundreds of µm's. This geometry allows the focusing of the light arriving horizontally from the side of the microscope on the metallic film, where the electron-photon interaction will occur. A metalens is composed of properly arranged nano antennas (also called meta-atoms) [2]. We designed the meta-atoms geometric parameters by performing extensive finite element method (FEM) simulations through the wave optics module of the COMSOL Multiphysics® software. The position and orientation of the meta-atoms on the metalens surface are then arranged to obtain the geometric phase spatial variation needed to focalize the incident light beam.

Results

From the Comsol simulations we obtained the meta-atoms dimension that optimizes the transmission and the phase difference condition, as well as mechanical constraints. The obtained theoretical transmission is equal to 44%. The metalens-integrated PELM geometry requires a focal length of 0.7 mm to let the electron beam interact with the focused laser spot and then proceed along the microscope. For that focal length and a 500 µm metalens diameter, the wave propagation simulations provide the focusing of the incident laser down to 2.5 µm.

We have also computationally characterized the performance of the designed dielectric metalens by simulating the propagation of a gaussian plane. Moreover, we explored the metalens robustness to non-idealities by applying a random noise on the phase and we verified that a transversely-patterned light beam, such as a Hermite-Gaussian profile, is correctly focused without distortions.

Conclusions

This setup is a compact solution that can be inserted in the UTEM to allow matching the laser spot size with the transverse coherence length of the electron beam, providing access to a wide range of excitation schemes and pump-probe geometries. Currently, we are working on the optical characterization of the metalens, in order to experimentally confirm its performances. The next step will be to mount the metalens windows in the PELM and characterize its performance inside the UTEM.

Funding

This work is part of the SMART-electron Project that has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 964591.

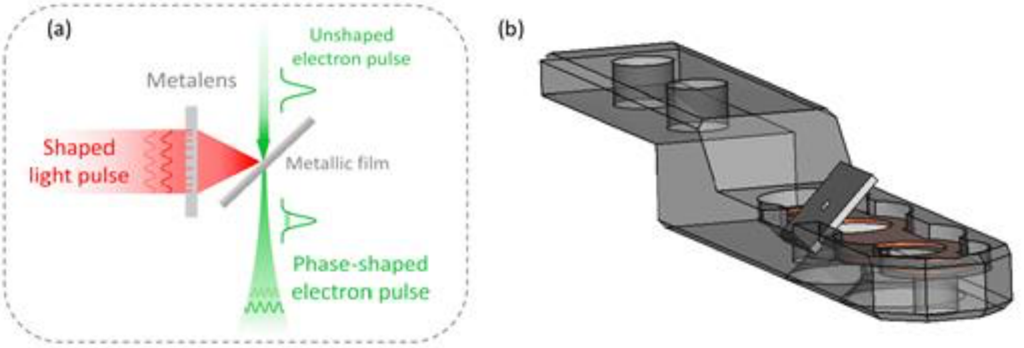

Figure 1| Electron beam shaping setup. (a) schematics of electron-photon interaction using a dielectric metalens. (b) Metalens-integrated Photonic free-ELectron Modulator (PELM). A silicon window is angled at 45° relative to the metallic mirror on the PELM platform.

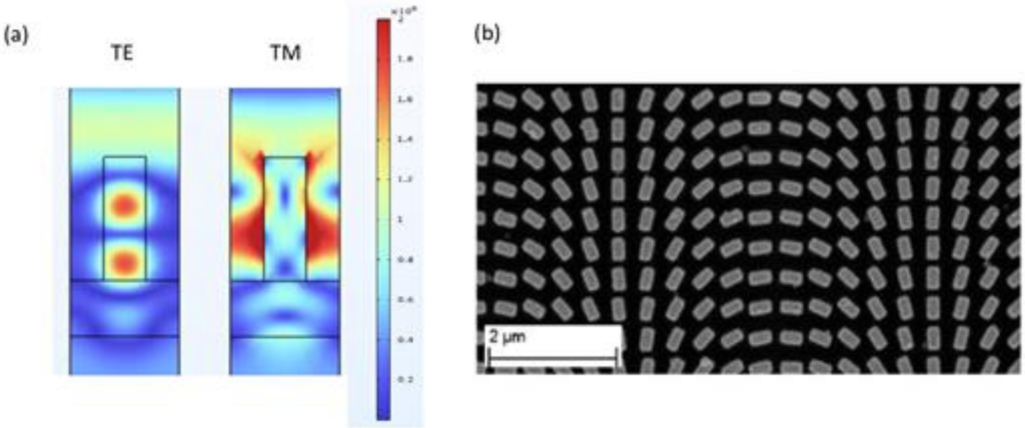

Figure 2| Metalens design. (a) Electric field [V/m] maps of TE and TM mode in a meta-atom from wave optics module of COMSOL Multiphysics® simulations (xy view). (b) SEM image of meta-atoms orientation in the metalens surface.

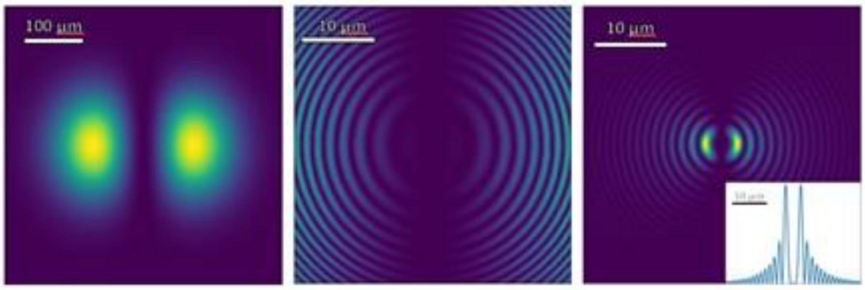

Figure 3| Wave propagation simulation of a Hermite-Gaussian beam through a 12-level discretized focalizing phase profile. (Left) Initial beam wavefront. (Middle) Wavefront after the interaction with the focalizing phase profile. (Right) Beam spot in the focal plane and normalized intensity.

Keywords:

Metalens, electron-photon interaction, Ultrafast TEM

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Charge Particle Optics Simulation Utilizing Hamiltonian Mechanics Perturbation Expansion and Boundary Elements Field Computation

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IM-02, Lecture Theater 3, august 27, 2024, 14:00 - 16:00

Background incl. aims

Advanced charge particle optics (CPO) requires fast and accurate computational tools for calculating (relativistic) particle trajectories including aberrations that, ideally, handle both arbitrary electrics and magnetic field sources, handle arbitrarily bend optical axis, allow incorporation of symmetries (e.g., rotational or mirror), incorporate optimization of design parameters such as pole piece diameter or pole distances. Ideally such tools should also be available under open source licenses in order to facilitate widespread use as well as distributed and sustainable development. Despite the enormous level of development and usefulness of commercial (e.g. Simion, EOD, Comsol) and open source packages, they often lack a subset of the above functionalities, somewhat hampering a wide spread development and use of advanced CPO for, e.g., Transmission Electron Microscopy, Secondary Ion Mass Spectroscopy, Photo Electron Spectroscopy, in academia, industry, and also teaching. The CPO software development described below intends to address that need.

Methods

Here we report on the development of an open source computational CPO framework incorporating the following principles to allow for an accurate, fast and flexible trajectory calculation: (A) We use boundary element method (BEM) computation of electric and magnetic fields, yielding smooth and accurate potentials, fields and higher-order derivatives at optical axis at arbitrary sampling, while reducing the meshing effort to surfaces (e.g., electrodes and pole pieces) of the CPO device. Herein, single layer representations of both electric and magnetic scalar potential are most efficient, while Green's representation with Calderon preconditioning allows stable single step solution of magnetic field distributions in the presence of high μr materials. (B) We employ semianalytical hierarchical solution of perturbation series of Hamiltonian equations of motion around an optical axis[1] in order to provide computationally effective, fast and accurate built-up of aberrations along particle trajectories. While not implemented yet the Hamiltonian perturbation expansion also facilitates straight forward extension to curved axis and the eikonal representation of aberrations. (C) We integrate the fast field and particle trajectory computation with non-linear optimization routines facilitating automatic optimization of design parameters (e.g., multipole sizes, pole piece gap) with respect to certain target functionalities. This tool chain is written in Python and makes use of advanced open source libraries (namely OpenCascade for CAD, gmsh for meshing, BEMPP for BEM field computation, sympy for semianalytic Hamiltonian mechanics perturbation expansion including automatic code generation, scipy for solving equations of motion, nlopt for geometry optimization) in a modular way, which are partly adapted to the specifics of CPO. Notably, BEMPP was extended by parallel just-in-time compiled numba and opencl kernels for field derivative computations on optical axis as required for computation of paraxial trajectories and aberrations.

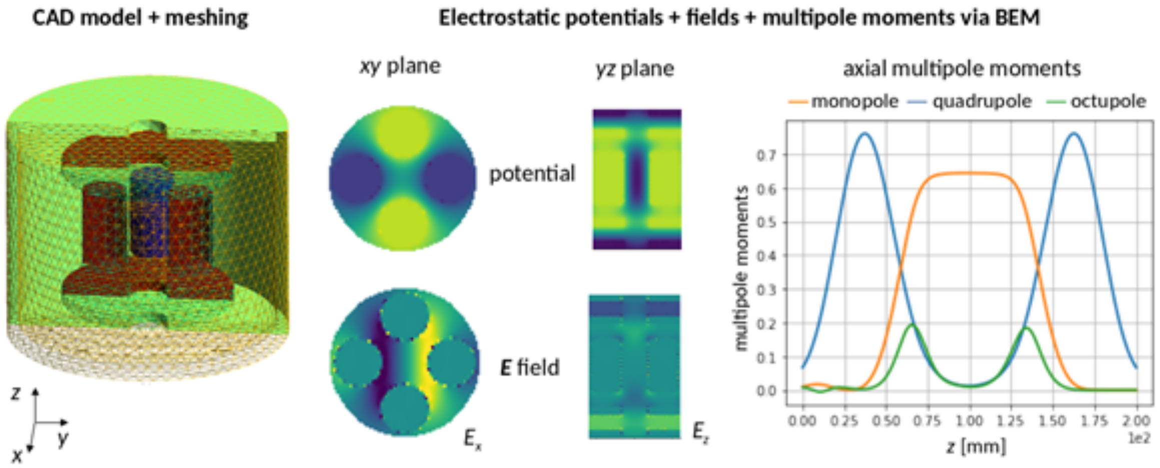
Results

We demonstrate and discuss the above tool chain with the help several electrostatic and magnetostatic CPO / building blocks of CPO, notably electrostatic einzellens, electrostatic quadrupole – round aperture assembly (see Fig. 1) and magnetostatic quadrupole, touching implementation (e.g., CAD import, defining boundary conditions, vector potential gauge), numerical (e.g., mesh size, precision of paraxial solution) and CPO (e.g., chromatic and geometric aberrations) aspects.

Conclusion

A modular combination of adapted BEM field computations and semianalytical perturbation series expansion of Hamiltonian equations of motion admits a computationally efficient modeling of CPO utilizing a combination of powerful and freely available open source software packages. Further development aims at incorporation of curved optical axis and general enhancement of functionality and user friendliness in order to support development of advanced CPO across the community.

Fig. 1: CPO of electrostatic quadrupole – aperture assembly (building block of low-voltage aberration corrector[2]): Computer aided design (CAD) modeling, meshing, and BEM field computation including axial multipoles. The octupole utilized for spherical aberration correction has been multiplied by 10 for visualization purposes.



Keywords:

Charge Particle Optics, Boundary Elements

Reference:

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[3] We acknowledge financial support by the European Union's Horizon Europe framework program for research and innovation under grant agreement n. 101094299 (IMPRESS project).

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

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Poster Group 1

Background incl. aims

Efforts in the miniaturization of magnetic electron optics exist for at least 10 years now, because smaller electron optics not only require less material and are potentially easier to build, but also result in other favorable scaling effects. Firstly, the small pole distances allow for magnetic flux densities in the hundreds of millitesla with a significantly reduced energy consumption and lower overall complexity regarding vacuum and cooling. Secondly, the small size translates to a low inductivity, which in turn enables switching of the optics on short time scales/with high frequencies (HF). The aim of this work is to utilize those favorable scaling effects by building miniaturized, magnetic, planar micro optics that are capable of manipulating a fast electron beam like in TEMs on sub nanosecond timescales/with gigahertz frequencies, allowing for stroboscopic, time resolved measurements in that regime.

Methods

The micro optics are produced using lithographic structuring techniques. The first layer consists of the conducting copper paths with a thickness of several hundred nanometers. The second layer consists of sputtered permalloy with a thickness of 1 µm. Through this process, many different optics e.g. dipole deflectors, quadrupoles, and higher order multipoles with various pole geometries can be produced in an easily scalable way. In order to supply the optics, a custom TEM holder capable of transmitting HF signals was engineered. The holder carries a small circuit board on which the optics are mounted and that allows for impedance matching in close proximity. To characterize the electromagnetic fields, differential phase contrast (DPC) measurements were conducted. The HF capabilities of the dipole optics were characterized in diffraction mode by measuring the amplitude of the oscillation of the diffraction disc.

Results

The optics show deflections in the single to double digit µrad range at 300 kV acceleration voltage and no major drop off in deflection up to an AC excitation frequency of 2 GHz. This corresponds to a maximum magnetic flux density of 84 mT and a possible focal length of a quadrupole of 15 cm at an acceleration voltage of 80 kV.

Conclusion

Miniaturized planar electron optics can be produced such that their diffraction capabilities especially in the HF domain are powerful enough to make them interesting for novel applications like stroboscopic, time resolved measurements on sub nanosecond time scales, imaging phenomena like the motion of domain walls with time resolved TEM. Furthermore, the miniaturization of entire CPO instruments e.g. SEMs might become possible in the future.

Keywords:

TEM, Ultrafast Microscopy, MEMS

Reference:

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The Environmental Impact of Large Scientific Infrastructure

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Poster Group 1

Standardised energy labelling is routinely used on many of the consumer goods we interact with daily. These aim to inform customer purchasing and usage behaviours and are used by regulators to incentivise the replacement of older equipment with more environmentally-conscious choices. Currently, there is limited sustainability data available for large scientific infrastructures (such as magnetic resonance imagers, electron microscopes, and research furnaces, among many others). As a result, no scheme currently exists to fairly compare energy usage across different devices or between families of devices.

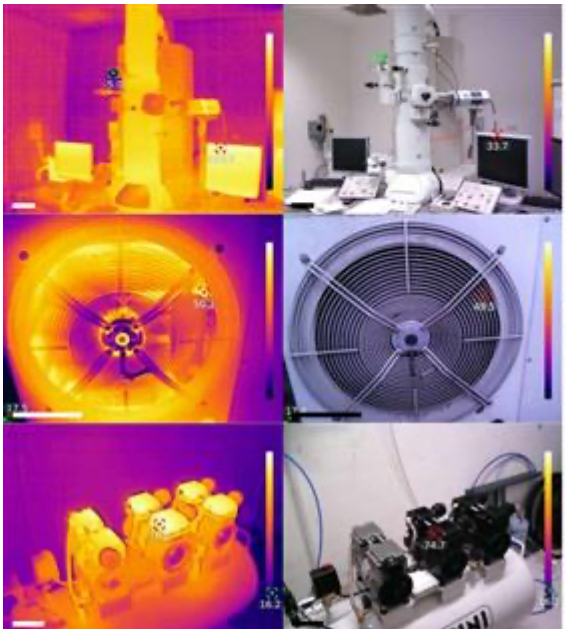
Energy usage is often not easily characterised for such infrastructure due to their diverse and multi-faceted needs; aspects may include but are not limited to, electrical draw, air compressors, water cooling, laboratory air conditioning, cryogenic gas and liquid purification/storage, as well as compute power. Usage patterns must also be considered, and optimised for equipment that is required to always be on. Some effort has been made in characterising energy consumption of hospital equipment such as MRI and CT scanners that show some parallels with scientific equipment [1-3].

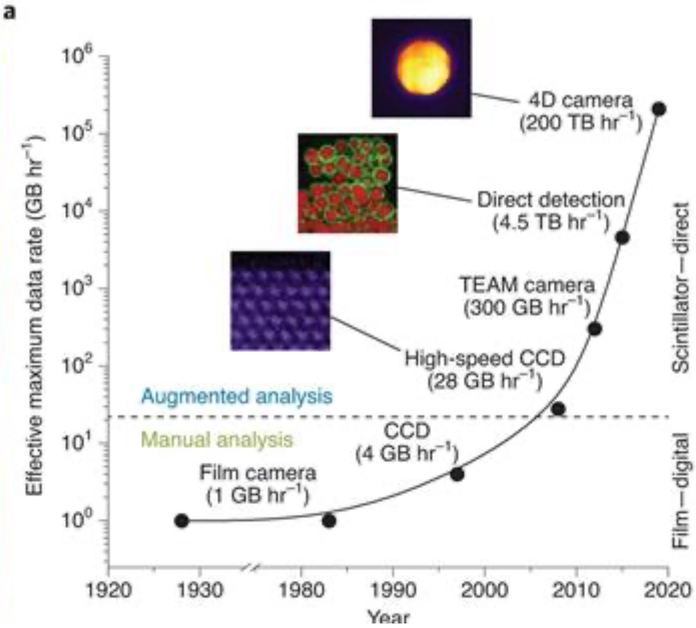
The left of Figure 1 shows thermal images taken in the Advanced Microscopy Lab, Dublin of a JEOL-2100 transmission electron microscope, the ventilation output for one of the building's chillers, and a building air compressor. In the latter two, this energy is currently vented directly to the environment with no heat recovery implemented. In a cooler country such as Ireland, this means waste heat is being exhausted from instruments while central heating systems are used to heat the building's working spaces.

Datacentres occupy an increasing slice of global energy demand and associated CO2 emissions, and this is expected to grow significantly [4]. While much of this consumption is due to sectors unrelated to scientific research, modern science is driven by huge quantities of data generation and the storage and processing of this data can still result in significant energy usage. In microscopy, the data generated by modern imaging techniques can reach 200TB per hour (Figure 1, right), and will likely increase further in the future [5].

The scientific community has increasingly moved towards open data initiatives, and to support this we will present and share a fully open access tool for calculating the complex energy usage of large scientific infrastructure. Through collaboration with facility managers on a global scale, we hope to provide an open database from which a sustainability labelling system can be created, sourced entirely from community input.

With increasing global concern both with energy usage and energy efficiency, it is vital that the footprint of modern scientific research equipment be assessed and quantified to enable new auditing and labelling to be carried out. This can inform decisions made by facility managers to reduce energy consumption, leading to both lower environmental impact and financial cost. Eventually, we hope this will lead to enhanced policy direction and apply pressure to manufacturers to be more mindful in the future design and manufacture of what is today wholly unlabelled hardware.





Keywords:

Research infrastructure, energy labelling, sustainability

Reference:

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Glovebox coupled TEM – a new method for versatile investigations of air-sensitive samples

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Poster Group 1

Background incl. aims

Transmission electron microscopy is a versatile and indispensable technique when investigating catalysts. When studying metal containing materials, it is of utmost importance to treat the samples under inert conditions in order to preserve the status of air-sensitive samples and their oxidation states as they get easily destroyed or transformed upon contact with air. Usually this is performed using vacuum-transfer sample holders. However, using such holders poses limitations to the investigations that can be performed (e.g., limited tilting range, shadowing effects, etc.). We therefore developed a unique system that allows for the use of all available sample holders without limitations under inert conditions.

Methods

Our novel setup consists of a glovebox system that is directly connected to the TEM. The interface is designed such that it can keep the inert atmosphere while operating and transferring the sample from the glovebox to the TEM. At the same time its construction avoids the transfer of vibrations to the TEM while investigating the sample. The experimental workflow includes the final preparation steps of the sample into the sample holder being performed in the glovebox after inert transfer of the catalyst material to the box. The sample holder is subsequently transferred to the TEM. For high-resolution (S)TEM investigations the glovebox is put in a special operating mode which includes reduced circulation and shutdown of the vacuum pump.

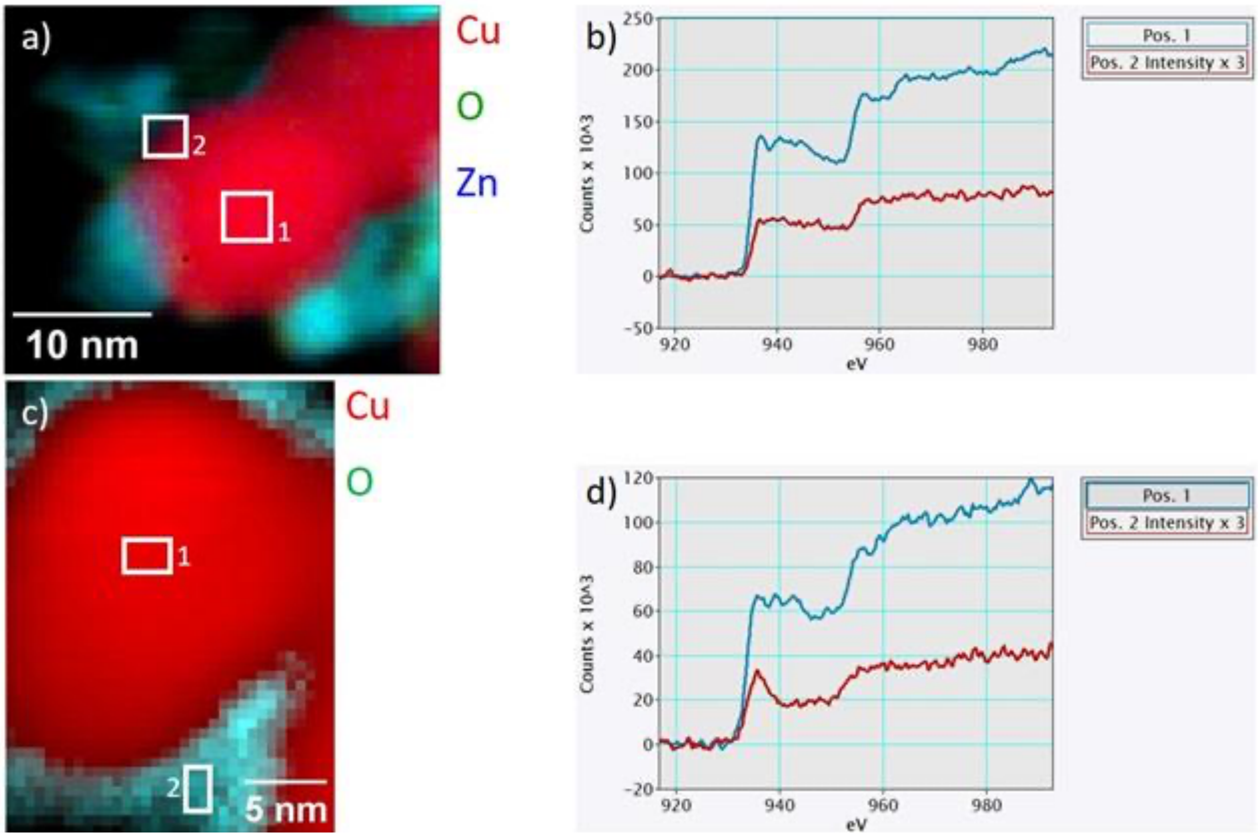
Results

In order to test the specifications of the system we measured the HR-TEM information limit and STEM resolution after connecting the glovebox and compared the results with the tests performed prior to the modifications. The unique modified system is able to operate within the same specifications as the non-modified TEM, in our case even exceeding the specification limits of the vendor. To test whether the samples can be handled under inert condition from inserting to the sample-holder until insertion to the TEM, we investigated two showcase materials. A reduced Cu-metal based catalyst [1] was transferred via the glovebox-system to the TEM and kept under inert conditions at all times. Additionally, a commercially available metallic Cu sample was investigated without inert treatment. Figure 1 a) and b) show an EELS map and extracted energy-loss spectra from the inertly treated sample. It can be seen that the Cu metal is present in its reduced form in the bulk and on the surface. For comparison, figure 1 c) and d) show the same experiments performed on the sample which was not treated under inert conditions exhibiting surface oxidation which can be seen from the Cu_2O spectrum in figure 1 d).

Conclusion

As it is of utmost importance to investigate metal-containing catalyst materials under inert conditions, we designed a modified TEM-system with a glovebox directly attached. We showed that the system is able to function within specifications of the non-modified TEM and allows the investigation of sample while keeping them under inert atmosphere during preparation and insertion to the TEM. This modified glovebox-TEM is currently successfully employed for investigations of different air sensitive catalyst materials as it allows us to use all available sample holders and perform the investigations without the limitations of a vacuum-transfer sample holder.

Figure 1: a) EELS map of a reduced Cu based catalyst [1] investigated in the described glovebox-TEM under inert conditions. b) Extracted EEL spectra from the regions marked in a). The presence of only metal Cu on the surface and in the bulk of the investigated nanoparticle is visible by inspection of the Cu-$L_{2,3}$ edge. c) EELS map of a metallic Cu sample without inert treatment. d) Extracted EEL spectra of the Cu-$L_{2,3}$ edge from the regions marked in c). The presence of Cu_2O in the surface region is clear from inspection of the spectra.



Keywords:

Instrumentation, inert-transfer, catalysis, oxidation-state, EELS

Reference:

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Characterization of Aberration-Corrected Lorentz TEM Applying a Magnetic Field with Objective Lens

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Poster Group 1

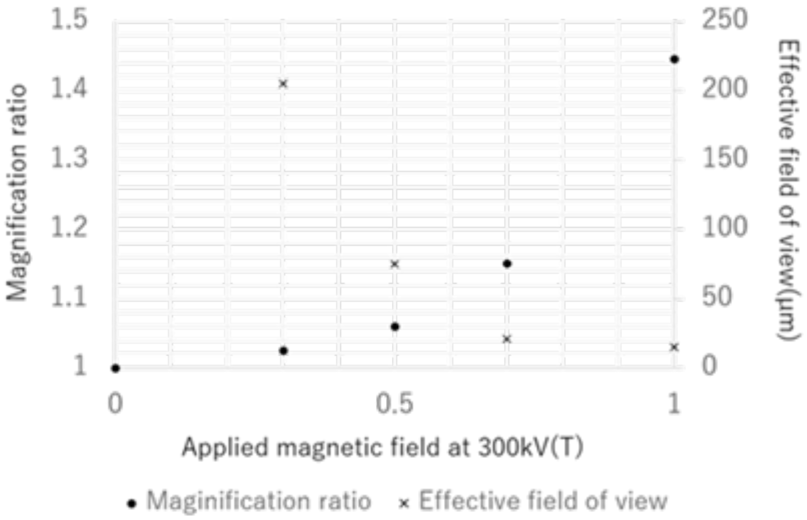
Lorentz microscopy is one of the powerful observation methods without the influence of magnetic field from the objective lens (OL) of a transmission electron microscope (TEM). Normally, the magnetic field around the TEM specimen is about 2T. This field could affect the observation of magnetic material. To avoid the influence, OL is turned off and the lens under the OL is used as focus lens, where it is called an objective mini-lens (OM) in JEOL instrument. We can use the OL to apply some amount of magnetic field to samples in Lorentz microscopy [1]. Recently, the spherical aberration correction was applied to Lorentz microscopy, the resolution was improved [2]. In addition, the applied magnetic field with OL is possible with spherical aberration correction. In this research, we characterized the detailed capabilities of spherical aberration-corrected Lorentz TEM while applying magnetic field by the OL.

In this time, JEM-ARM300F2 with TEM spherical aberration corrector was used. The pole-piece was wide gap pole-piece. The TEM resolutions with / without spherical aberration correction were investigated in 300kV. The varied resolution with applying the magnetic field and the field of view were also measured at that time. The aberration measurement was done by diffractogram tableau method. The correction area was about 4mrad and the third order spherical aberration coefficient of OM was under 10mm.

The Young's fringe test was performed to check the TEM resolution. The resolution without spherical aberration correction was 1.83nm without applying the magnetic field, 1.75nm with 0.5T and 1.61nm with 1T. On the other hand, they were improved with spherical aberration correction. 0.76nm, 0.67nm and 0.58nm with 0T, 0.5T and 1T were achieved. The effective field of view (FOV) was changed as applying magnetic field with objective lens. FOV was decreased as a function of the applied magnetic field. They were measured to be 200µm with 0.3T, and 20µm with 1T. This limitation was occurred by replicating of electron beam. The replicating was caused by the aberration of imaging system. On the other hand, the ratios of magnification were increased 6% with 0.5T and 45% with 1T.

The principal plane of complex lens was getting closer to the sample plane as applying magnetic field. The complex lens was formed by OM and OL. This lens improved the spatial resolution, and this result showed the resolution was limited by the chromatic aberration.

We have addressed making a setting of aberration-corrected Lorentz TEM imaging mode. The TEM resolution was improved by spherical aberration correction of OM. Additionally, applying the magnetic field by the OL, the resolution was also slightly improved. The magnification and FOV were changed as a function of applied magnetic field. In the presentation, which condition could be the better for imaging a magnetic sample will be discussed.



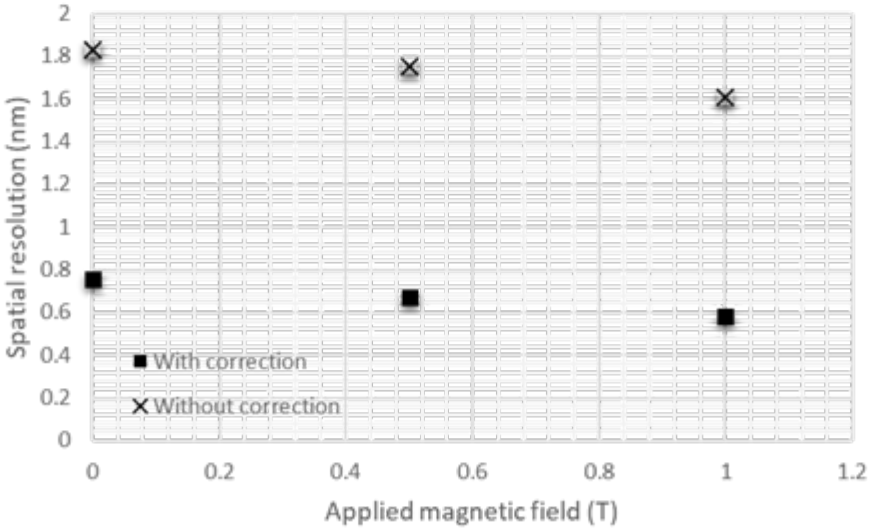


Figure 2. Variation of spatial resolution applying the magnetic field. Acceleration voltage was 300kV. The resolution was improved as applying OL in the both cases of with correction and without correction.

Keywords:

Lorentz, Aberration correction, Magnetic field

Reference:

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[2] Takuro Nagai et al, PHYSICAL REVIEW B 96,100405(2017)

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

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Poster Group 1

Recent developments of add-on equipment in transmission electron microscopy cause a demand for modular and flexible data acquisition software. Fully integrated commercial solutions exist, but these are typically tailored towards a certain combination of proprietary hardware components from one manufacturer. For off-line data analysis and very specific high-throughput workflows open source software packages became available during the last years. Nevertheless, a lack of highly interactive software, directly usable during the operation of the instrument with its different components, is obvious.

To close this gap, CEOS Panta Rhei is designed as an interactive platform for data acquisition, processing and visualization in electron microscopy written in Python using the Qt, ZMQ and numpy libraries.

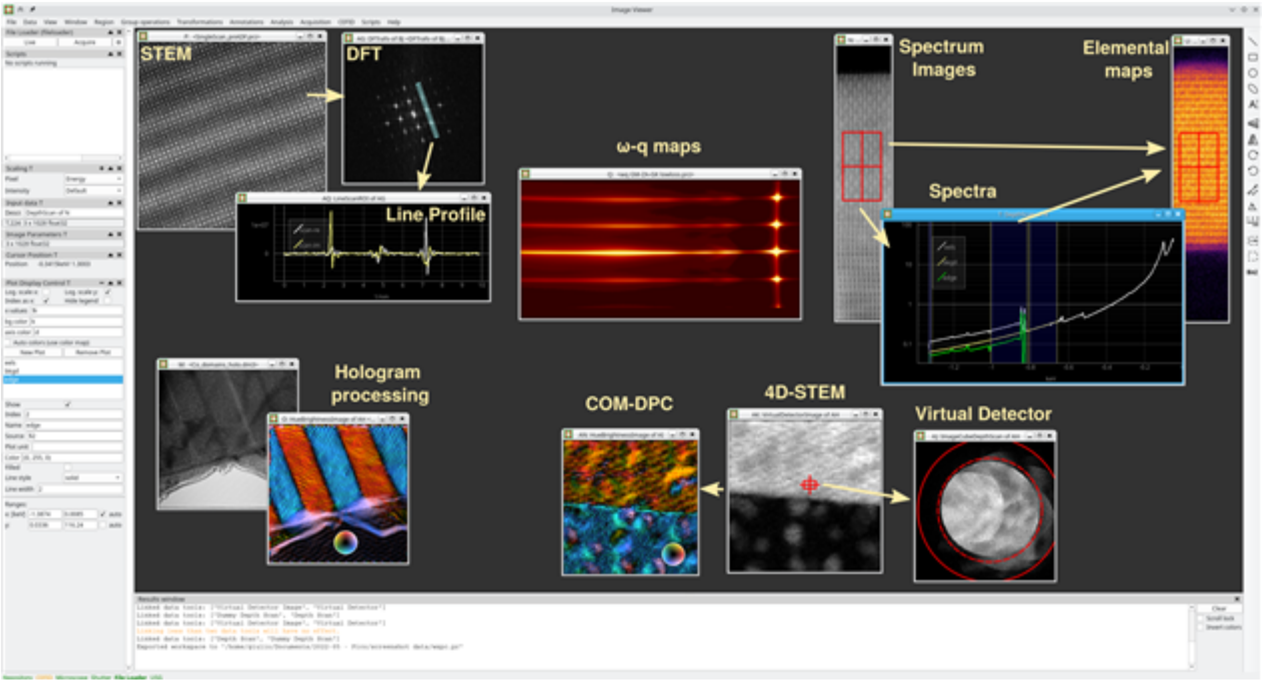
The primary purpose of Panta Rhei is to enable simple steering and supervision of workflows combining the control of the microscope and its accessories with data acquisition, online data analysis, and direct visual feedback. The capabilities for online data evaluation are progressing. We currently concentrate on functionality for quick and meaningful assessment of data quality and online session planning like image filters, data statistics, diffractograms, live 4D-STEM evaluation, and elemental mapping and quantification for EELS as well as EFTEM. Recently, the fitting of EELS spectra (and maps) using a library of precomputed generalised oscillator strengths (GOS) was also added [1]. Data can be interchanged with other software using common file formats like npz, hspy, mrc, msa, and tiff.

Interactive dialogs are provided to control the supported hardware, and perform interactive data acquisition from the separate device (e.g. camera images or STEM images), or combining multiple hardware components (e.g. STEM-EELS mapping).

The acquisition (via cameras or scan detectors) can generate a high volume of data which has to be transferred and processed efficiently. Therefore, a central component of Panta Rhei is a separate server process called Repository providing access to a managed shared memory. As soon as an acquisition device stores data in the Repository other clients are notified and can directly access the data with minimal CPU load and memory consumption. Clients themselves may also use the Repository to store processed data.

The Panta Rhei GUI is an application to acquire, display and process data and control hardware components which connects as client to the Repository. It displays Views of data from the Repository via a multiple document interface (MDI). Views are used to live display certain aspects of the data and the numerous available DataTools continuously calculate dependent data from updated input. The name Panta Rhei (πάντα ῥεῖ -- everything flows) is motivated by these chains of transformations that may even run in separate processes.

For custom extensions, a scripting interface provides control of data processing and display tools as well as hardware devices. For easy external access to hardware control, an RPC-interface is available. Currently, Panta Rhei has interfaces for the TEMs of 3 manufacturers, 5 families of detectors, 4 different scan generators, and the CEOS Imaging Energy Filter (CEFID [2]). We expect that the number of compatible devices will continuously grow over time.



Keywords:

Software, data evaluation, image processing

Reference:

[1] Generalised Oscillator Strengths for the simulation of EELS spectra, with a broader coverage of high energy and minor edges, https://zenodo.org/records/7645765
[2] F. Kahl et al. (2019) AIEP 212 including Proceedings CPO-10.

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M-SIS Software: Automatic tilted series acquisition for environmental (gas, liquid and temperature) multi-scale electron tomography

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Poster Group 1

Background incl. aims

Electron tomography is a technique that allows 3D data analysis at the nanometer scale. It has always been considered a difficult and time-consuming technique, from tilt series acquisition to volume reconstruction and data analysis. In the beginning, the tilt series acquisition was performed in several tens of minutes in bright field mode to several hours in scanning transmission mode (STEM). Huge technical developments have been made to make tilt series acquisition faster and faster, and to enable fast electron tomography compatible with environmental electron microscopy. [1,2]
Environmental electron tomography in gas, and even more in liquid state, is very sensitive to the electron beam, therefore recording fast tilt series in STEM mode with a very low electron dose received by the sample is a real challenge even for the most experienced users. One solution to ensure the recording of fast tilt series with low electron dose at a constant rate is to automate the recording process.
This presentation will introduce the M-SIS software. Its purpose is to take advantage of the power and robustness of the computer code to assist the operator and improve his/her skills while acquiring a series of tilts in electron microscopy. The automation minimizes the electron dose received by the sample by reducing its exposure time to the electron beam.

Methods

The M-SIS code (Figure1) is written in Python and is compatible with different environments and machines. It can be installed on an Environmental Scanning Electron Microscope (ESEM), which is compatible with Thermo Scientific™ Autoscript, as well as on an Environmental Transmission Electron Microscope (ETEM), as a plug-in of DigitalMicrograph™. In addition, built-in libraries enable hardware control, such as the Smaract MCS-3D piezo-inertial stages used to control the stage and detector in the ESEM.

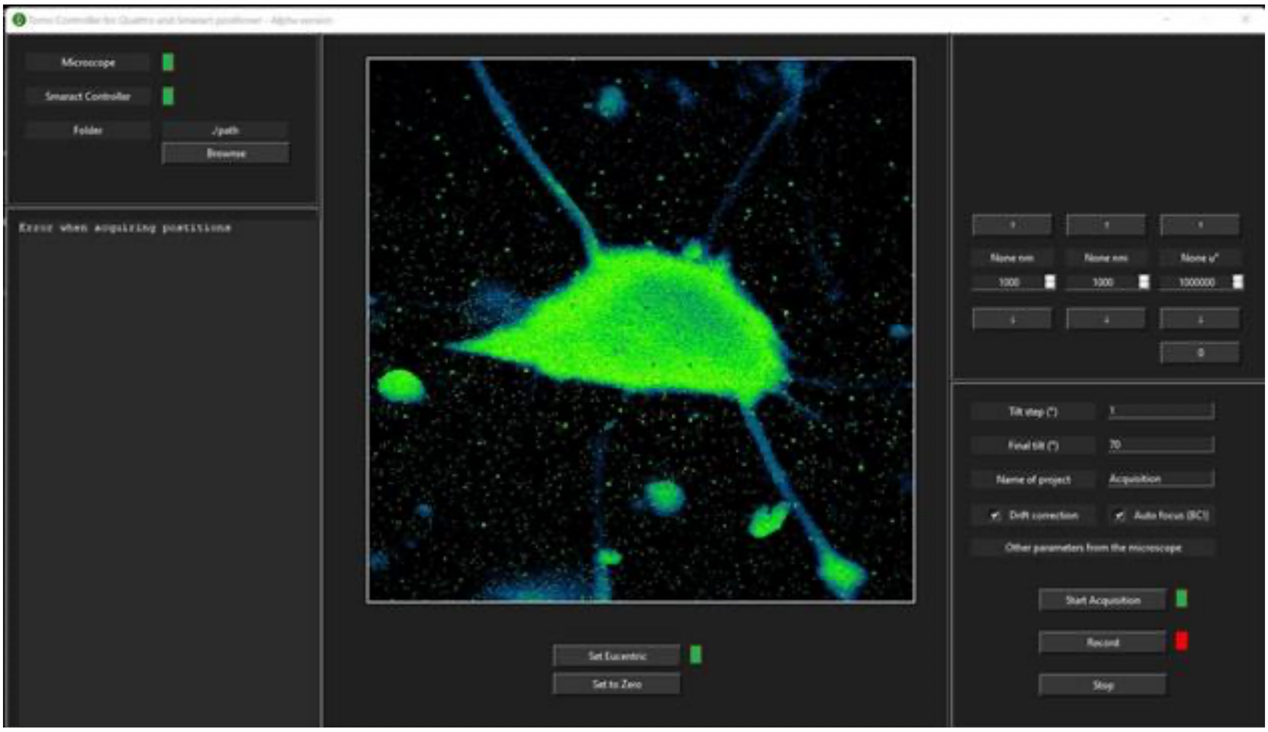
Results

The M-SIS software is compatible with ESEM and ETEM, enabling multi-scale electron tomography in environmental mode at very low electron dose in STEM (scanning transmission) imaging mode. It is able to automatically set the sample at the eucentric position. The brightness and contrast of the images are automatically adjusted, and then fast tilt series are acquired. One of the problems of fast tilting is that the sample drifts out of the field of view due to mechanical imperfections while tilting. The M-SIS software automatically corrects the drift of the specimen during image acquisition and keeps it in the field of view during the tilt. The code allows the acquisition of multiple imaging modes simultaneously, i.e. SE, BF, DF and HAADF, all while protecting the sample from beam damage. During validation tests, the total electron dose received by the sample ranged from 1,042 e-/nm2 in ESEM at 30 keV to 16,000 e-/nm2 in ETEM at 300 keV both recorded in liquid mode, for the acquisition of more than 100 images per imaging mode, in about 10 min. Volumes have been reconstructed on very sensitive samples from materials science and biology, at different hydration states, and quantitative data could be reliably extracted.

Conclusion

We have developed a software based on Python language, which is compatible with ESEM and ETEM electron microscopes, allowing the recording of low electron dose tilt series in STEM mode in environmental mode at multi-scale. The software is user independent, automatically sets the sample to the eucentric position, corrects brightness and contrast, and records fast tilt series with controlled electron dose, while tracking the sample as it rotates. [3]

Figure 1: M-SIS software, a Python based software for electron tomography in environmental conditions i.e. gas, liquid and temperature, conceived for beam sensitive samples investigation.



```
Error when acquiring positions
```

1000 | 1000 | 1000000

Tilt step (°) 1

Final tilt (°) 70

Name of project Acquisition

☑ Drift correction ☑ Auto focus (BC)

Keywords:

tomography automation in-situ

Reference:

[1] S. Koneti et al, Fast electron tomography: Applications to beam sensitive samples and in situ TEM or operando environmental TEM studies. Materials Characterization 151, 2019.
[2] X. Jiao et al, Electron tomography on latex particles suspended in water using environmental scanning electron microscopy, Micron, 2019, 117.
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Active focus stabilisation using astigmatism with universal objective lens compatibility and sub-10 nm precision

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Poster Group 1

Background incl. aims

Super-resolution microscopy techniques can resolve biological samples with nanoscale resolution that surpasses the diffraction limit. This, however, requires focus stabilisation to correct for axial drift, which is particularly important for high-throughput automated imaging. Many solutions have been developed to tackle this problem. Marker-based methods are highly accurate, but the introduction of fiducials during sample preparation can give rise to additional challenges. With a beam-based approach, it is common to monitor the reflection of an infrared laser from the coverslip-sample interface. Nevertheless, there are limitations of current beam-based approaches that can be summarised as: (i) relatively low sampling rates; (ii): only being compatible with high numerical aperture (NA); (iii) electronic components requiring soldering and custom circuit boards and (iv): the need to put a focus stabilisation module close to the objective lens as the performance is limited by laser pointing stability.

Methods

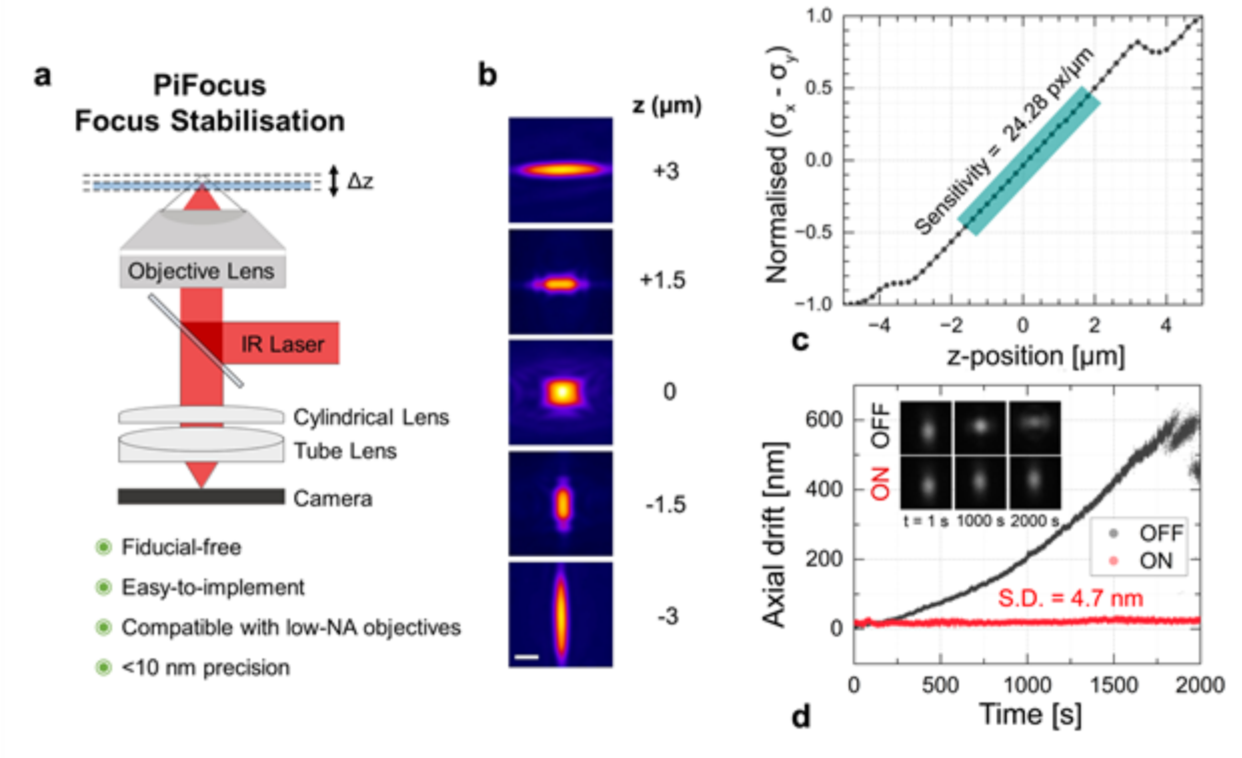
Here, we present a standalone, cost-effective, and fiducial-free focus stabilisation system that operates over a long axial range with nanoscale precision and can be implemented using off-the-shelf components. In our focus stabilisation system, an infrared laser beam is focused by the objective lens, back-reflected from the coverslip-sample interface, and then imaged onto a camera (Fig. 1a). Introducing a cylindrical lens in front of the camera creates an astigmatic point-spread-function (Fig. 1b). Real-time monitoring of variations in the shape (Fig. 1c) of this astigmatic intensity profile as a response to focal drift allows for the transmission of a control signal to a piezo z-stage, consequently facilitating the stabilisation of the sample.

Results

We characterise the performance of our astigmatism-based drift correction system and find that it is much less sensitive to optical component stability, unlike TIR-based systems that need to be close to the coverslip. We achieve sub-10 nm axial drift estimation and correction. We then show the astigmatism leads to one of the advantages of our system – its ability to operate within a large axial range, extended over 20 µm by employing cylindrical lenses with different focal length. This trade-off between precision and axial range makes it possible to achieve 20 nm precision with low numerical aperture 10x objective lenses. We have implemented our solution on a Raspberry Pi platform that can perform stabilisation at 100 Hz (Fig. 1d), which is suitable for drift correction on most super-resolution methods.

Conclusion

With compatibility across different objective lenses, we have created a straightforward and low-cost optical device that can seamlessly integrate into most microscopy setups, offering advantages such as ease of implementation, universality, and robustness.



Keywords:

Autofocus, Focus stabilisation, Drift correction

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

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Poster Group 1

Time-resolved high speed TEM imaging technique is of great importance for in-situ observations such as heating/cooling, biasing, stressing, and liquid-cell experiments. Recently camera's frame rate is getting faster, but to dynamically visualize a structural change process of materials happen at a high speed, it is necessary to record TEM micrographs with a higher frame rate in the order of sub-millisecond.
Electrostatic type deflector can deflect the electron beam in tens of nanoseconds to microseconds. To using this property, there is a possibility to realize a high-speed imaging technique. In this study, we demonstrate a result of feasibility test for this system, electrostatic sub-flaming system developed by IDES Inc. This method improves temporal resolution by effectively increasing the frame rate while maintaining the same performance of the existing camera.
Fig. 1 shows schematic illustration of experimental setup. Electrostatic electron beam deflectors were installed beneath a projector lens cross-over in TEM column with a CMOS camera. The deflector itself acts as an aperture, limiting the area of the camera's sensor to which the electron beam is exposed, thus producing a small TEM image (sub-framed TEM image). By electrostatically deflecting the sub-framed TEM image at high speed in front of the camera sensor during exposure time, the images are laid out like 5x5 or 7x7 tiles. By doing this, each sub-framed image having a different time stamp. The difference between the timestamps of each sub-frame image is the time resolution.
Using this system, we observed time-series changes in (111) surface facet structure of CeO2 nanoparticles [1]. The data was recorded in 7x7 sub-frames. Since 49 sub-frames are recorded in one frame (CMOS Camera's exposure time is set to 40 ms), the exposure time per single sub-frame image is ~0.82 ms. The frame rate increased 25 fps to 1,225 fps. The 1st (0 ms) and 6th (4.08 ms) sub-frame TEM images are shown in Fig. 2. The atomic columns indicated by the arrows can be clearly seen in the 1st image, but are obscured in the 6th image. Motion of the atomic column of CeO2 (111) surface ware successfully observed in atomic level with a time scale of 4 ms by enhancing an effective frame rate of camera acquisition.

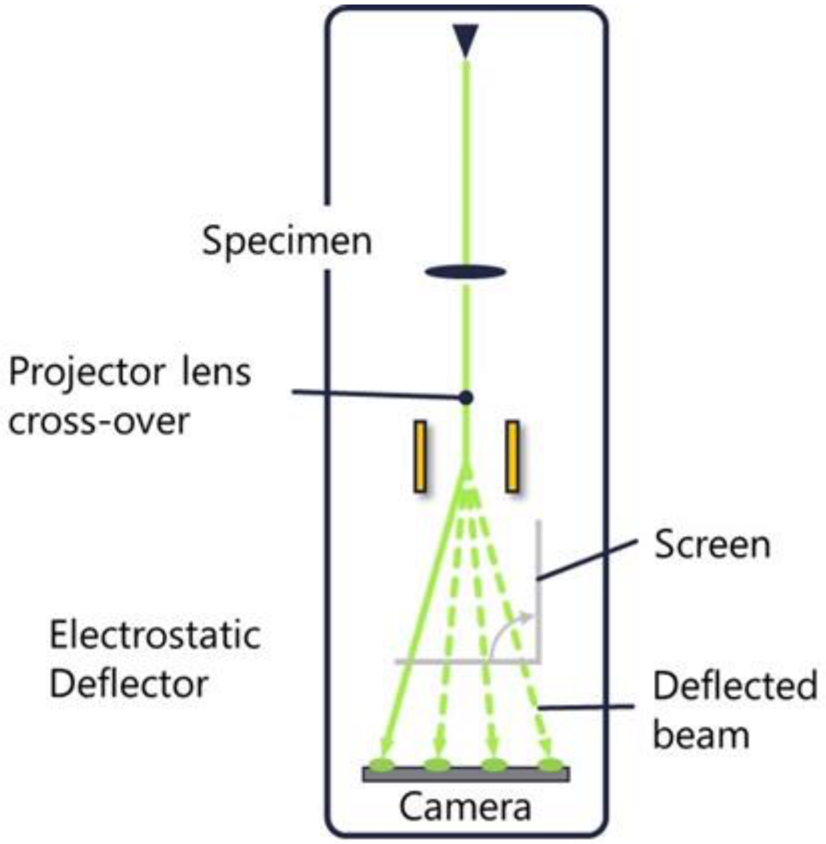

Fig. 1: Schematic illustration of electrostatic sub-flaming system installed on JEM-ARM200F

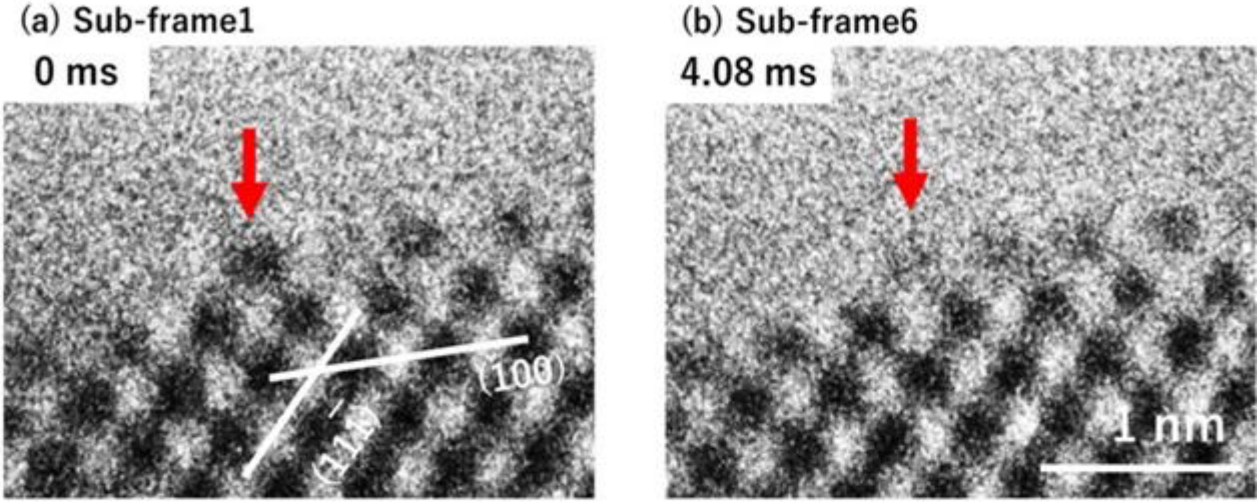

Fig. 2: Time-resolved TEM images of the surface of CeO_2 nanoparticle.
An atomic column of arrow-headed in (a) became obscured (b) after 4 ms.

Keywords:

High-speed-imaging, High frame-rate, Sub-framed imaging

Reference:

[1] CeO2 Nano particles specimen: Courtesy of Johnson Matthey

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Development of chopped scan control for beam blanking

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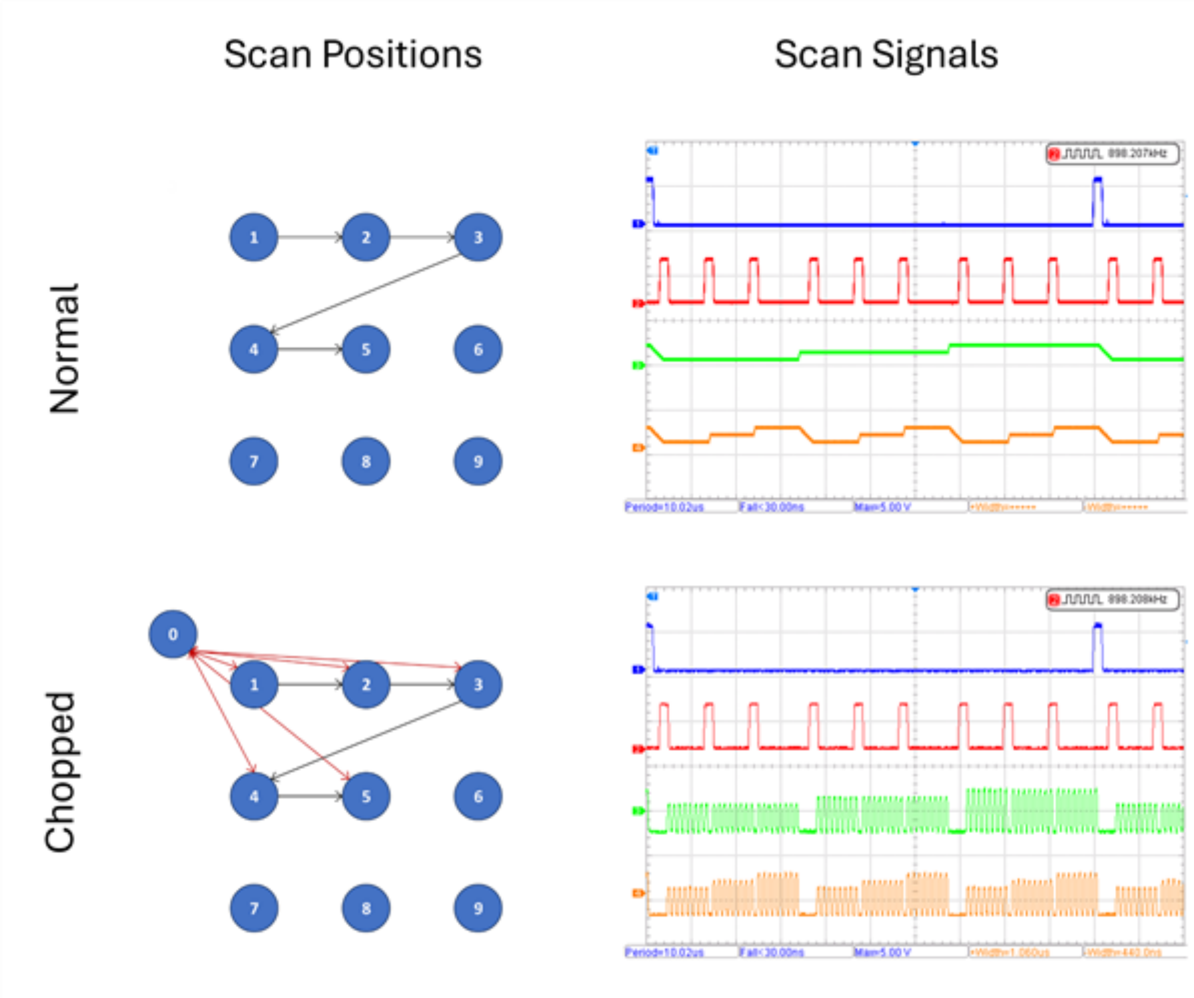
Poster Group 1

Beam blanking is a technique in electron microscopy whereby the electron beam is interrupted from reaching the sample at high speeds or frequencies. This is required for a range of experiments, including lock-in amplification of minute signals, close control of electron dose/fluence, or capture of fast events with high temporal resolution or in synchronization with external clocks. Beam blanking is typically achieved by deflecting the beam from the optical axis with added blanking plates into a purposely designed blanking aperture. However, this approach requires installation of additional hardware into the microscope column. Development of an alternative approach is presented here, where the beam is deflected using the normal scan control coils into a vacuum or sacrificial point in the sample plane. This removes the need of additional components in the microscope column and places the added complexity of beam blanking into the scan controller only.

Whilst blanking with the scan coils can be thought of as adding a second blanking pattern on top of the regular scan pattern, such a hardware configuration does not provide means to choose the position of the vacuum or sacrificial beam position, or to synchronize the two patterns, therefore the normal scan controller must take on the task of beam blanking in addition to beam scanning. This enables the user to freely define a position for blanking, which may be from a pre-scanned image, or from preplanned coordinates. Generation of both patterns from the same controller also enables the freedom to choose if blanking and scanning are synchronised to the same clock or are free running. Use of the scan controller in this fashion requires complex scan algorithms, as established scan patterns in electron microscopy already include advanced flyback strategies at end of line and end of frame.

Attached Figure presents such a scan pattern with blanking – a very small 3x3 pixel scan is shown here to simplify presentation, but resolution in this scan mode is only limited by the scan resolution of 16-bit corresponding to 65,536 x 65,536 pixels. A 1 µs acquisition time was used, with a line start wait of 10 ns, a beam return time of 320 ns, without any pre-scan, pixel settling or additional holding time. Digital frame, line and pixel trigger outputs typically used for detector or camera synchronization are shown here to guide the presentation and were set to a duration of 200 ns. A blanking reference frequency of 10MHz was selected for this example in order to give a visible blanking duration comparable to the pixel acquisition time. The blanking position was set to 0,0 (top left) and the image scan was set to begin at 32768,32768 (middle) of the scan space. Note in the analog scan outputs for column and row signals how the beam is encoded to jump between the blanking pixel and the image pixels, which gives a chopped appearance to the analog traces.

Practical application of this chopped scan mode for beam blanking will be shown in TEM and SEM, including the limitations to be expected from using the scan coils for such fast beam motion. It will be shown that hysteresis and amplifier bandwidth limitations restrict the maximum blanking speed possible to a range of approximatively 100kHz, which could be overcome with further development of distortion removal algorithms, however all this is achieved without modifications to the microscope column.



Keywords:

Scan control, Beam blanking

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Towards Automation of the Transmission Electron Microscope

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[1]Department of Physics, Norwegian University of Science and Technology, Trondheim, Norway

Poster Group 1

The Transmission Electron Microscope is a powerful tool investigating samples at the nano-Ångström scale. Despite its increasing popularity due to its unmatched spatial resolution, operation of the microscope is time-consuming and tedious. At times, even trivial tasks may require hours of manual operation. Automation has thus become attractive in the field, as it would lower the threshold and workload for advanced material investigations.

Scanning TEM (STEM) differential phase contrast (DPC) is a technique utilizing movement of the centre beam to determine electric and magnetic domains. For a magnetic sample with domain formation, the beam will be deflected in different directions depending on the in-plane magnetic alignment in the sample, see graphic. This slight change can be measured using e.g. a pixelated detector. Nanomagnets with dimensions 225-75 nm are shown to have interesting monodomain properties, being currently widely studied for reservoir computing [1,2].

Problems arise when using the TEM as the magnetic field is typically too big for domain-formation. For this reason, the objective lens must be turned off, reducing the spatial resolution. With the objective lens turned off (so-called Low-Mag), the magnetic field can be estimated to be of the order of tens of millitesla. This is sufficiently low for ASI-samples to show hysteretic behaviour, and in-situ studies of the dynamics can be performed by either tilting the sample, increasing the magnetic field from the objective lens or rotating the sample.

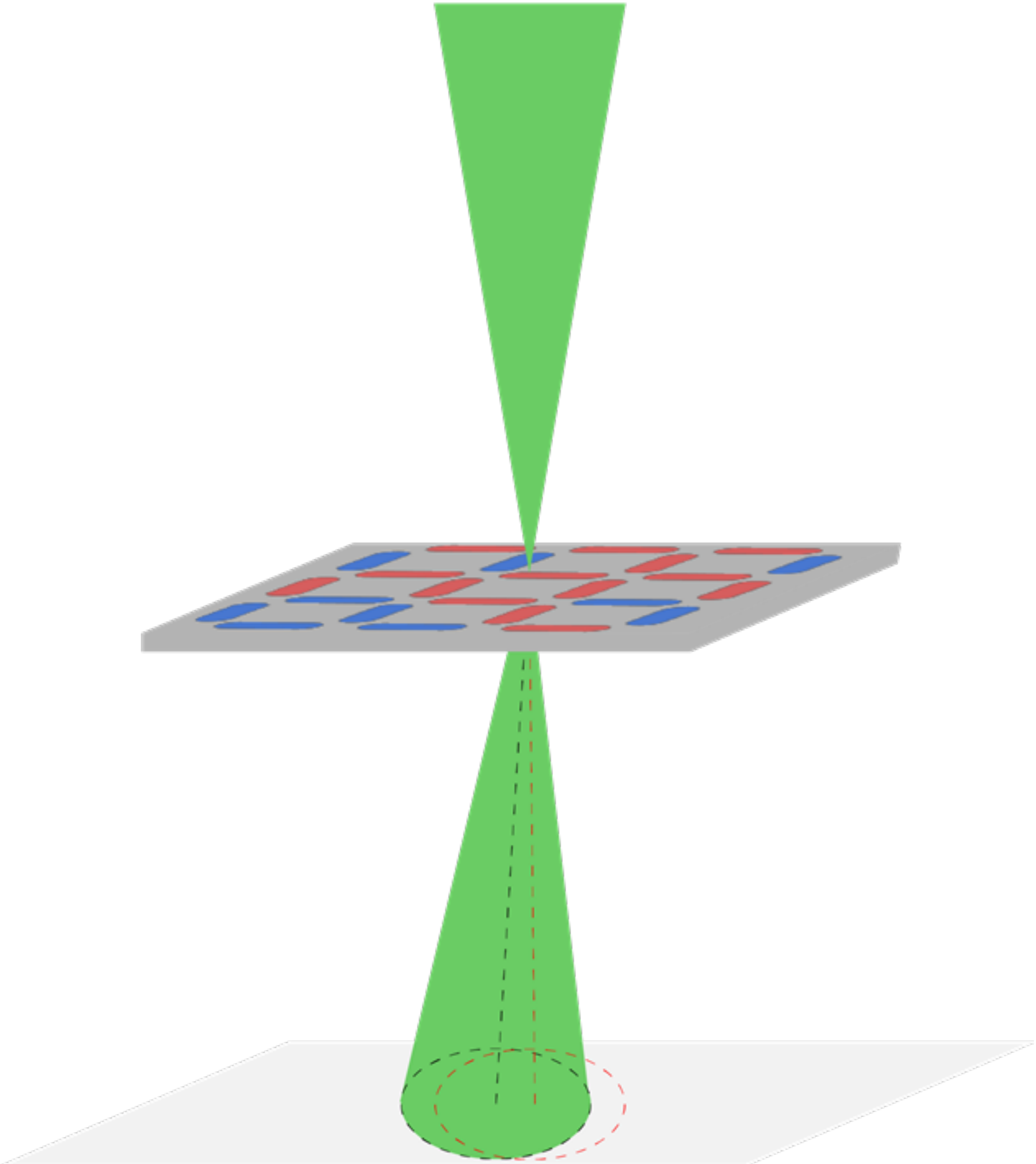
Once the TEM is aligned, the STEM-DPC technique is rather trivial, that is: acquire data, then change the magnetic field, repeat. This experiment is typically referred to as “continuous-tilt STEM-DPC” and can take hours to complete. The task however, is trivial and using human operating time is both inefficient and prone to human errors. This procedure is easy to automate, and allows other in-situ experiments such as varying the temperature over critical regions.

ASI structures will be prepared from TEM samples with 20-50 nm Si windows with top layers of permalloy and aluminium using FIB, creating nano-magnets with mono-domain behaviour.

Continuous-tilt STEM-DPC will then be performed with either a MerlinEM direct electron detector or a conventional ADF detector followed by procedural post-processing. This experiment will be a proof-of-concept, and the choice of detector will depend on access to the Merlin detector.

The project aims to gain complete control of both the microscope and the detector- and scanning systems and build a python-library allowing experienced microscopists to automate their own experiments. Creating possibility for feedback-controlled automatic decision-making will also be done. Even further, programmable input-output devices for in-situ experiments will allow scripted exploration of the parameter space of all TEM samples using custom in-situ chips.

Further, given control of the scanning system, random and quasi-random sequences for data-acquisition will be implemented, reducing the necessary acquisition time for sufficient statistic. This procedure will also in particular benefit samples prone to beam damage.



Keywords:

TEM, Automation, in-situ

Reference:

[1] Skjærvø, S.H., Marrows, C.H., Stamps, R.L. et al. Advances in artificial spin ice. Nat Rev Phys 2, 13–28 (2020). https://doi.org/10.1038/s42254-019-0118-3
[2] Johannes H. Jensen, Erik Folven, Gunnar Tufte; July 23–27, 2018. "Computation in artificial spin ice." Proceedings of the ALIFE 2018: The 2018 Conference on Artificial Life. ALIFE 2018: The 2018 Conference on Artificial Life. Tokyo, Japan. (pp. pp. 15-22). ASME.

1057

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

Miss Sara Habte[1], Mr Miguel Boland[2], Ms Sara Habte[1], Dr Jonathan Lightley[1], Dr Edwin Garcia[1], Professor Christopher Dunsby[1], Dr Edward Cohen[2], Professor Paul French[1]
[1]Physics Department, Imperial College London, London, UK, [2]Mathematics Department, Imperial College London, London, UK, [3]Francis Crick Institute, London, UK

Poster Group 1

Background incl. aims

We have previously presented novel optical autofocus modules that simultaneously provide extended range of operation (>100 µm) and high precision (<600 nm) using machine learning [1] in a 2-step approach or providing closed loop “single-shot” operation over up to ±37 µm with <50 nm accuracy [2]. These levels of performance are realised by focusing an infrared laser beam onto the microscope coverslip with the back reflection being imaged on a dedicated autofocus camera. We derive a metric that quantifies defocus from the size of the light distribution at the autofocus camera that can be independent of laser power and insensitive to drift in the optical alignment. The operating range and precision depend on the confocal parameter of the autofocus laser beam after being focused by the objective lens. This can be adjusted by changing the diameter of the autofocus laser beam incident at the objective lens. By contriving a different beam diameter in two orthogonal planes using either a rectangular aperture [1] in the collimated autofocus laser beam, or using different orthogonal cylindrical lenses [2] to collimate the autofocus laser beam emerging from the single mode fibre that delivers it to the autofocus module, we can maximise precision (with maximum beam diameter) and extend operating range (reducing orthogonal beam diameter), making both measurements simultaneously by resolving the autofocus camera image along orthogonal directions.

While these two approaches can provide months of stable operation, they each have their drawbacks. The machine learning approach with the rectangular apertured beam [1] requires a convolutional neural network to be trained to determine magnitude and sign of defocus from the autofocus camera image and we found it necessary to train it over ~10 days to make it independent of any system variations impacting the autofocus camera image. For the second approach [2], we slightly offset the collimation of the cylindrical lenses such that the measured defocus is different for the two planes defined by the orthogonal cylindrical lenses, and this enables the magnitude and sign of the defocus to be calculated from a single autofocus camera image following calibration of the system. However, while the system reported in [1] utilised a low-cost single-mode fibre (SMF)-coupled laser diode, we used a superluminescent diode (SLD) in the system reported in [2] since its performance was impacted by interference between the autofocus laser beam reflected from the coverslip and unwanted beam(s) reflected from other surfaces in the optical system. Using the SLD removed this interference. Unfortunately, SLDs are significantly more expensive than laser diodes, and availability can be intermittent. Accordingly, we are redesigning the optical system and analysis method to enable the closed-loop approach of [2] to be used with a simple fibre-coupled diode laser for implementation in slide scanning and automated multiwell plate microscopy.

Methods

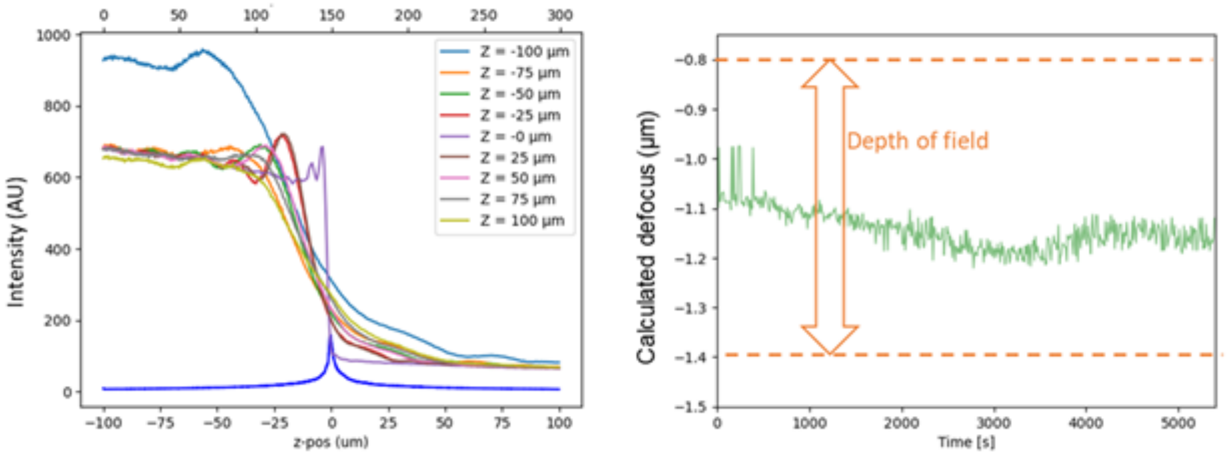
We determined that the primary source of unwanted back reflections of the autofocus laser beam were from the microscope objective lens, and we modified the optical system such that the curvatures of the unwanted back-reflected wavefronts are different from the desired autofocus beam reflected from the coverslip. Utilizing a modified background subtraction and signal processing algorithm we were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens that was controlled using MicroManager [3]. To independently measure the performance of the autofocus system, we configured the microscope for brightfield transillumination imaging of a USAF test chart and imaged the edge of a bar to derive a metric of defocus from the steepness of the gradient of this edge. We are also working on a fluorescent bead image-based approach utilising machine learning to determine defocus from a single bead image for real-time monitoring.

Results

We were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens. When imaging a test chart in transillumination, focus was maintained in closed loop within 200 nm over 5000 seconds – and within < 50nm over 500 seconds. The autofocus system can recover focus with single-shot operation within a range of ~60 µm and up to ~80 µm in a multi-step mode [2]. We are working to improve the autofocus precision and range and are cross-validating the measurement of defocus between the autofocus readout, the transillumination edge measurement and the machine learning approach applied to bead images. We will also explore using higher power multimode laser diodes that exhibit shorter coherence lengths [4] although these present additional laser safety considerations.

Conclusions

We have demonstrated that we can implement an optical autofocus using a low-cost diode laser that can provide closed loop “single-shot” operation and is suitable for multiwell plate imaging and slide scanning. This is important for the development of cost-effective instrumentation, including modular openFrame-based instruments for pathology and high content analysis. We will present the latest design together with methods to independently validate the correction of defocus.



(a) (b)

Figure caption: (a) line profiles though edge of a test chart bar in z-stack imaged in transillumination (b) defocus calculated from gradient of test chart bar edge measured as a function of time.

Keywords:

Optical microscopy, autofocus, slide-scanning, characterisation

Reference:

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[2] J. Lightley et al., J Microsc, 292 (2023) 64, https://doi.org/10.1111/jmi.13219
[3] A. D Edelstein et al., J. Biological Methods 1 (2014) e10, https://doi.org/10.14440/jbm.2014.36
[4] A. Rahmani et al., Opt. Expr. 32 (2024) 13331, https://doi.org/10.1364/OE.520845

1065

Innovative Microfluidic chip for Raman spectroscopy and advanced electron microscopy techniques

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Poster Group 1

Background

In microbiology research, there is a growing need for equipment that allows the study of biological samples with high resolution and preservation of their structural integrity. In this article, we present a new device, a microfluidic chip, designed for high-pressure freezing (HPF) of biological samples, freeze-fracture and their subsequent analysis using a combination of Raman spectroscopy and focused ion beam scanning electron microscopy at cryogenic temperatures (cryo-FIB-SEM).
Microfluidic chips, created by microfabrication techniques, manipulate with a small amount of fluid volumes using intricately designed channels and chambers. Their precision and efficiency offer advantages over traditional methods, leading to the applications in bioanalysis, medicine, and environmental monitoring. Ongoing research focuses on enhancing chip design and integration with complementary technologies to extend their capabilities further [1]. Microfluidic chip technology has revolutionized fluid handling and analysis at a miniature scale, offering unprecedented control over fluidic processes through intricate networks of microchannels, chambers, valves, and pumps fabricated using advanced microfabrication techniques. This miniaturization enables precise manipulation of small fluid volumes, leading to applications in bioanalytical chemistry, biotechnology, medicine, and environmental monitoring.
The cryo-fixation of the microfluidic chips is very limited by a thickness of the material (sample) that must be frozen [2]. The only known microfluidics freezing system used for cryo-fixation of cells was published in 2014 using direct freezing in the optical microscope. The use of HPF presents the possibility of rapid freezing of even thicker samples (up to 600 nm) with subsequent observation by electron microscope under cryogenic conditions.
In our study, we present the application of a microfluidic chip in the research of microorganisms producing Polyhydroxyalkanoates (PHAs), which have the potential to serve as an alternative option to petrochemically produced plastics [3].

Methods

The microfluidic chip is designed primarily for frozen biological samples using a combination of Raman spectroscopy and focused beam scanning electron microscopy (cryo-FIB-SEM). This advanced chip allows the manipulation of biological samples that are either dissolved or mixed with liquid, providing the ability to perform detailed analysis and manipulation at the microscopic level. Biological samples are fixed using (HPF) and stored in liquid nitrogen. HPF is the only method used for freezing thick biological samples without the effect of ice crystalization. Fabrication of the structures that make up the microfluidic chip is done by soft lithography followed by the use of oxygen plasma to fuse the structures.

Results

The structure of the microfluidic chip consists of two thin layers of polydimethylsiloxane (PDMS). One of these layers contains channels for the flow of the sample liquid and a chamber for higher sample concentration (the chamber passes through the entire layer), while the other layer does not contain these structures. These PDMS layers are then bonded using oxygen plasma, which provides a tight bond and allows the sample to move only within the channels. The microfluidic chip also includes a sapphire disk that allows the sample to be observed inside the chip and serves as a heat transfer medium for fast freezing processes. Ports are also attached to the inlets of the channels for easier introduction of liquid samples, which also allows for various manipulations such as mixing liquids. Once the chip is filled with the sample liquid, analysis is performed using Raman spectroscopy and the possible use of Raman tweezers to manipulate cells or particles. Subsequently, the chip is modified using a unique punch device to be inserted into the HPF. The sample is rapidly frozen using HPF and preserved in liquid nitrogen, allowing the sample to be studied in the state in which the biological material was at the time of freezing. The sample can then be inserted into a scanning cryo-electron microscope and analyzed using a focused ion beam.

Conclusion

Overall, this microfluidic chip represents an innovative tool for studying biological samples and using high-pressure freezing; high resolution is achieved by preserving their structural integrity. The combination of Raman spectroscopy and scanning cryo-electron microscopy enables detailed analysis and manipulation of biological samples, which has the potential to push the boundaries in microbiological research and biomedical science.

Keywords:

Microfluidic chip, electron microscopy, HPF

Reference:

[1] Whitesides, G. M., et al.: Nature, 442(2006), 368-373
[2] Mejia, Y. X., et al.: Lab on a chip, 14(2014), 3281-3284
[3] Anjum A., et al.: International Journal of Biological Macromolecules, 89(2016), 161-174

Acknowledgement: The research was supported by the Czech Science Foundation (GA23-07962S), the Technology Agency of the Czech Republic (TN02000020) and the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

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Ultra-low-cost, high-dynamic-range, additively manufactured CMOS spectrometers with UV, visible, and NIR sensing functionality

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[2]Department of Chemistry and Chemical Biology, Cambridge, USA

Poster Group 1

Background incl. aims

Ultraviolet (UV)-Visible and infrared (IR) spectroscopy are widely used to characterize material and chemical samples. Inexpensive and high-performance spectrometers would broaden the impact of these tools. Educational spectrometers often lack accuracy, resolution, and repeatability, whereas industrial spectrometers are often expensive and hard to customize. We used consumer microprocessor development boards, image processing, and additive manufacturing (AM) to create an ultra-low-cost spectrometer platform, capable of modularly converting between UV, visible, and near-IR (NIR) measurements. The system can also track reaction progress via tandem temperature monitoring, with the possibility of further General Purpose Input/Output (GPIO) channels. This device offers high precision and flexible customization within a simple and inexpensive package.

Methods

This work comprises 1) hardware, 2) electronics, 3) firmware and software. The hardware architecture uses design for AM (DfAM) principles to minimize the part count, while achieving high performance, and low cost. We explored Fused Deposition Modeling (FDM) and stereolithography (SLA) manufacturing strategies. The electronics are based around an ESP32S3 development board from Seeed Studio, which has a high pseudostatic random-access memory (PSRAM) capacity that enables long-term data-gathering and GPIO expansion. The board is paired with complementary metal oxide semiconductor (CMOS) OV2640 and OV5640 camera modules from Seeed Studio, with the former offering low costs and the latter's backside-illuminated (BSI) construction enabling UV sensing. Temperature sensing is via a thermistor. The firmware is written in C/C++ using ESP32 camera libraries. The control software and user interface are written in Python using standard serial communication, image processing, time series analysis, and graphical user interface libraries. Features include automatic wavelength calibration using curve registration algorithms and an option for manual exposure control. Debvec's algorithm constructs a camera response curve from the bracketed exposures to produce a high-dynamic-range (HDR) reading.

Results

Using these inexpensive CMOS arrays, rapid data collection with high-precision sensing is achieved. Camera module and development board costs are less than USD 15 combined, and each scan takes on the order of 10 milliseconds (excluding HDR processing, on the order of seconds). For a 500-nanometer detection range, the 1600-pixel CMOS array width corresponds to approximately three pixels of data per nanometer. Practical resolution is limited by the choice of diffraction grating. NIR functionality relies on the intrinsic NIR sensitivity of CMOS arrays. UV functionality requires the removal of the Bayer filter from a BSI sensor, increasing fabrication cost and complexity. Software calibrations correct for color casts (i.e., intrinsic tints from the sensor) and uneven black levels. Raw RGB sensor data is not accessible due to poor library and hardware support, but raw grayscale data yields low noise (70dB peak signal-to-noise ratio for non-HDR data). JPEG compression is available given resource limitations. Tandem temperature monitoring successfully tracks reaction progress. SLA manufacturing requires fewer parts, while FDM achieves similar performance with simpler post-processing. Further quantification of usable resolution, noise performance, dynamic range, and wavelength limits is ongoing.

Conclusions

Desktop AM systems, low-cost development boards, and library support for CMOS sensors enable the design of ultra-low-cost and compact spectrometry systems that can be tailored to specific experimental needs. With appropriate image processing and hardware, these systems achieve the repeatability and precision of commercial solutions that are more expensive and less modular. The approaches presented here enable low-cost, high-performance, agile spectrometry solutions that experimentalists can tailor to their specific needs, including in distributed IoT and other non-laboratory systems.

Keywords:

Spectroscopy, Additive manufacturing, CMOS

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Pulse counting in the SEM

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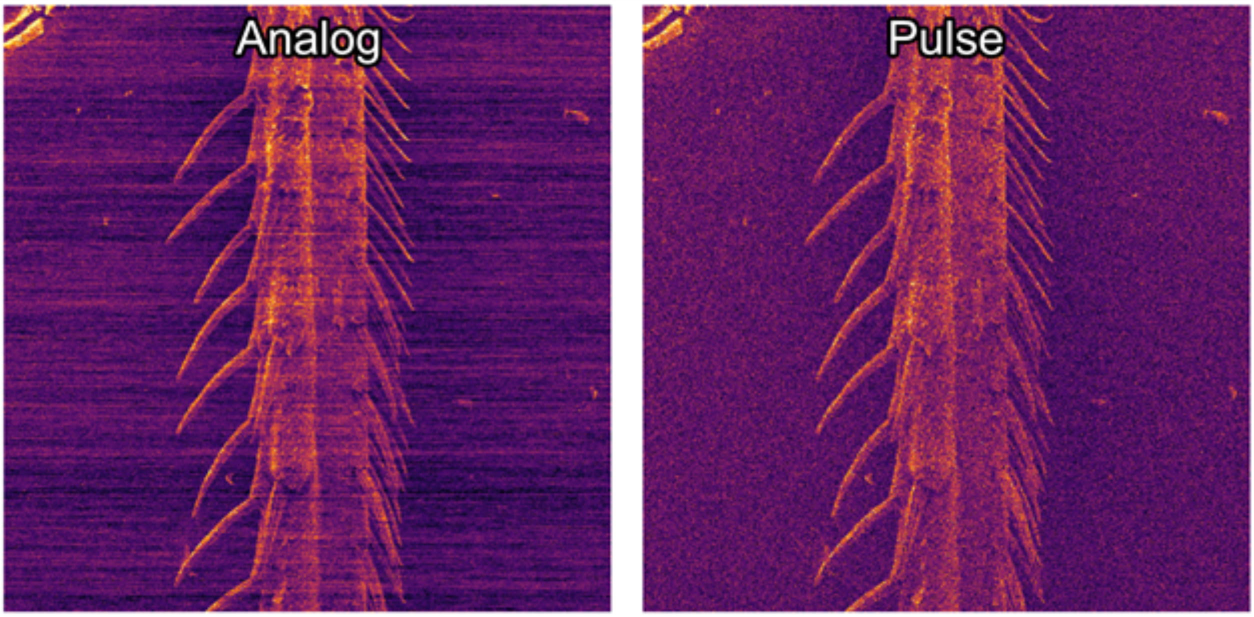
Single electron pulse counting has revolutionised low-dose TEM and STEM imaging by providing zero background noise, quantitative information, and fast detector response times. This allows the uses of lower doses with faster image acquisition times whilst maintaining image fidelity and signal-to-noise ratio. Whilst this technology is common in the context of (S)TEM, the benefits of electron pulse detection are not obvious in the context of SEM and similar techniques such as Helium ion microscopy. Beam-related modification of samples can still occur (for example in battery anode materials), particularly for insulating samples where a low dose-rate may allow charging effects to dissipate. Scanning at high speeds can achieve this but can run into problems with slow detector response times, particularly scintillator-based technologies such as Everhart–Thornley detectors. Pulse counting can mitigate this, though the effect on image contrast is not as simple as the case of (S)TEM. For example, in secondary electron imaging, each signal pulse on the detector may represent multiple electrons, with the number (and therefore intensity) determined by the secondary electron yield from the sample. In this work, we present and discuss preliminary data using the turboTEM Pulse counter combined with SEM detectors to show the benefits and potential use cases for SEM imaging, from mitigating noise for low signals, increasing detector speed, and exploring the possibilities of new imaging modes.



Keywords:

SEM, Electron Counting

Reference:

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