

Book of abstracts

SCANDEM 2023

June 12-15, Uppsala, Sweden

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2



Conference program - Oral sessions

Monday, 12^{th} of June

- 12:00 13:30 Registration, lunch and exhibition
- 13:30 15:00 Plenary session (Eva von Bahr hall, Å100195, chair K. Leifer)
- 13:30 14:15 Emergent Phonon Phenomena at Interfaces Probed by Vibrational Electron Microscopy (Xiaoqing Pan, p.15)
- 14:15 15:00 Cryo-Electron Tomography or the Power of Seeing the Whole Picture (Wolfgang Baumeister, p.16)
- 15:00 15:30 Coffee and exhibition
- 15:30 17:00 Parallel sessions

MS1: Refinement and development of microscopy techniques

(Eva von Bahr hall, Å100195, chair J. Kotakoski)

- 15:30 16:00 (invited) Multidimensional TEM characterization of electric fields and structure based on full momentum resolution (Knut Müller-Caspary, p.17)
- 16:00 16:15 Electron beam shaping and aberration correction using optical fields (Andrea Konečná, p.18)
- 16:15 16:30 Hypermodal Data Fusion: a data-driven approach for tackling contemporary nanoscale challenges (*Thomas Thersleff*, p.20)
- 16:30 16:45 Analysis of magnetic structure in ferrimagnetic Ti-doped barium hexaferrite by analytical EMCD method (*Hitoshi Makino*, p.22)
- 16:45 17:00 Machine-Learning Assisted Exit-wave Reconstruction for Quantitative Feature Extraction (Jakob Schiatz, p.24)

LS1: Image analysis and data visualization

(Sonja Lyttkens hall, Å101121, chair I.-M. Sintorn)

- 15:30 16:00 (invited) Spatial transcriptomics (Carolina Wählby, p.26)
- 16:00 16:20 Texture Segmentation, Surface Detection, and Fast Local Thickness for Quantification of Bone Microstructure (Vedrana Andersen Dahl, p.27)
- $\begin{array}{ll} 16:20-16:40 & \mbox{Identifying bird eggshell by scanning electron microscopy (SEM)} \\ & (Kessara Anamthawat-Jonsson, p.29) \end{array}$
- 16:40 17:00 TEM Image Enhancement From Multiple Short Exposure Images (Andrea Behanova, p.31)
- 17:00 19:00 Reception & poster session (Evelyn Sokolowski hall, Å101136)
- 19:00 SCANDEM board meeting (Å101025)

Tuesday, 13th of June (morning sessions)

- 8:30 10:00 Plenary session (Eva von Bahr hall, Å100195, chair I.-M. Sintorn)
- 8:30 9:15 Spatially resolved single-cell genomics & cell atlas of the brain (Xiaowei Zhuang, p.33)
- 9:15 10:00 Electron microscopy in infectious diseases a medical perspective (Michael Laue, p.34)
- 10:00 10:30 Coffee and exhibition
- 10:30 12:00 Parallel sessions

MS2: Studies for structure and strain in materials

(Eva von Bahr hall, Å100195, chair Å. Kassman)

- 11:00 11:15 Structural mechanism of emergent magnetic properties in patterned SrRuO₃ quantum structures (Hongguang Wang, p.36)
- 11:15 11:30 Dimensionality control of Pt on CeO_2 through support interaction (Henrik Eliasson, p.38)
- 11:30 11:45 Tracking the orientation of cellulose nanofibers using Scanning Electron Diffraction (Mathias Nero, p.40)
- 11:45 12:00 Polymorph engineering and radiation tolerance in β -Ga₂O₃ (Javier García Fernández, p.42)

LS2: Beyond the resolution revolution - new challenges in cryo-EM/ET (Sonja Lyttkens hall, Å101121, chair L. Sandblad)

- 10:30 11:00 (invited) TBA (Kay Grunewald, p.44)
- 11:00 11:20 Primordial capsid and spooled ssDNA genome structures unravel ancestral events of eukaryotic viruses (Anna Munke, p.45)
- 11:20 11:40 Structurally Guided Pandemic Response: Neutralizing Antibodies for COVID-19 and Beyond (Martin Hällberg, p.46)
- 11:40 12:00 Quantitative TEM reveals novel stress response mechanism in yeast (Katharina Keuenhof, p.47)
- 12:00 13:30 Lunch and exhibition
- 12:30 13:15 SCANDEM annual assembly (Sonja Lyttkens hall, Å101121)

Tuesday, 13th of June (afternoon sessions)

13:30 – 15:00 Parallel sessions

MS3: Electron microscopy in-situ and in-operando techniques

(Eva von Bahr hall, Å100195, chair T. Willhammar)

- 13:30 14:00 (invited) Real Time Investigation of Crystal Growth and Nucleation of Low-dimensional Inorganic Nanostructures (Kimberly Dick Thelander, p.48)
- 14:00 14:15 Solid State Dynamics Visualization by In Situ Electron Microscopy (Jakob B. Wagner, p.49)
- 14:15 14:30 Revealing Surface Restraint-Induced Hexagonal Pd Nanocrystals via In Situ Transmission Electron Microscopy (You Ruiyang, p.51)
- 14:30 14:45 Emergence of ferromagnetic phase in FeRh studied by in-situ Electron Magnetic Circular Dichroism with nm-sized probe (Jan Hajduček, p.53)
- 14:45 15:00 In situ microscopy study of novel transition metal diborides (*Palisaitis Justinas*, p.55)

LS3: Correlative light and electron microscopy

(Sonja Lyttkens hall, Å101121, chair M. Hällberg)

- 13:30 14:00 (invited) Correlative light, electron and X-ray microscopy: finding the needle in the haystack (Marie-Charlotte Domart, p.57)
- 14:00 14:20 Correlative light and electron microscopy (Linda Sandblad, p.58)
- 14:40 15:00 Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) Imaging and micro-manipulation (Linda Sandblad, p.61)
- 15:00 15:30 Coffee and exhibition
- 15:30 17:00 Company presentations (Eva von Bahr hall, Å100195)
- 17:00 18:00 Early carrer session (Heinz-Otto Kreis hall, Å101195)
- 19:00 Conference Dinner (restaurant Sven Dufva)

Wednesday, 14th of June (morning sessions)

- 8:30 10:00 Plenary session (Eva von Bahr hall, Å100195, chair J. Rusz)
- 8:30 9:15 Findings from the happy marriage between low-dimensional materials and low-voltage atomically-resolved TEM (*Ute Kaiser*, p.63)
- 9:15 10:00 In situ TEM techniques for controlling structure and interfaces in 2D material (Frances M. Ross, p.64)
- 10:00 10:30 Coffee and exhibition
- 10:30 12:00 Parallel sessions

MS4: Functional materials

(Eva von Bahr hall, Å100195, chair J. Majka)

- 10:30 11:00 (invited) Unraveling the structure-property relationship of adsorbents and photocatalysts derived from 2D layered materials of natural and synthetic origin (Jakub Matusik, p.65)
- 11:00 11:15 Atomic Scale Observation of phase transition in NiFe₂O₄ (Qi Wang, p.67)
- 11:15 11:30 Practical analytical limitation of energy and wavelength dispersive spectroscopies a case of REE-Pb phosphates (Sordyl Julia, p.69)
- 11:30 11:45 Neutral Atom Microscopy: Large Area Imaging of Nanoscale Topographies (*Paul Dastoor, p.71*)
- 11:45 12:00 Domain walls properties of lead-free BiFeO3 ferroelectrics under static and dynamic conditions (*Drazic Goran*, p.73)

LS4: Ultrastrucutral pathology and disease understanding

(Sonja Lyttkens hall, Å101121, chair A. Dragomir)

- 10:30 11:00 (invited) New insights into the molecular and cellular composition of the brain barriers (Christer Betsholtz, p.75)
- 11:00 11:20 Physiological role of ATP in the inner ear (Sonal Prasad, p. 76)
- 11:20 11:40 3D optical kidney pathology (Hans Blom, p.77)
- 11:40 12:00 AuNP Loaded Liposomes: A tool for characterizing the uptake of soft nanomaterials in biological systems via TEM (Paul Kempen, p.78)
- 12:00 13:30 Lunch and exhibition

Wednesday, 14th of June (afternoon sessions)

13:30 – 15:00 Parallel sessions

MS5: Microscopy of soft and beam-sensitive materials

(Eva von Bahr hall, Å100195, chair T. Thersleff)

- 13:30 14:00 (invited) In and ex situ (S)TEM manipulation of 2D materials (Jani Kotakoski, p.80)
- 14:00 14:15 Nanofluidic liquid phase transmission electron microscopy (Murat Nulati Yesibolati, p.81)
- 14:15 14:30 3D Electron Diffraction / MicroED for Crystal Structure Determination (Hongyi Xu, p.83)
- 14:30 14:45 Imaging hard and soft materials with atoms (David Ward, p.84)
- 14:45 15:00 Zeolite intergrowth revealed using 4D-STEM (Evgeniia Ikonnikova, p.86)

LS5: Automated and AI based microscopy imaging and analysis

(Sonja Lyttkens hall, Å101121, chair C. Wählby)

- 13:30 14:00 (invited) Data-driven microscopy allows for automated context-specific acquisition of high-fidelity image data (Pontus Nordenfelt, p.88)
- 14:00 14:20 High-content imaging-based serology test for SARS-CoV-2 (Lassi Paavolainen, p.89)
- 14:20 14:40 Automated Imaging and Analysis of Pharmaceutical Particles Using a Tabletop Low Voltage TEM (Mathieu Colomb-Delsuc, p.91)
- 15:00 15:30 Coffee and exhibition

Wednesday, 14th of June (afternoon sessions, cont.)

15:30 – 17:00 Parallel sessions

MS6: Use of spectroscopic techniques in material science and geoscience (Eva von Bahr hall, Å100195, chair J. Rusz)

- 15:30 16:00 (invited) Advances in high energy and spatial resolution STEM EELS (*Demie Kepaptsoglou*, p.94)
- 16:00 16:15 Exploring Topological Features in Materials: Advancements and Challenges in Electron Microscopy Characterization (Juan-Carlos Idrobo, p.96)
- 16:15 16:30 Unravelling oxidation mechanisms in Cu nanoparticles through insitu imaging and spectroscopy and the role of the electron beam in environmental STEM (Shima Kadkhodazadeh, p.97)
- 16:30 16:45 Achromatic atomic-plane resolved imaging of electron energy-loss spectroscopy $(Bin \ Lin, \ p.99)$
- 16:45 17:00 Band gap measurements of aluminum and indium doped Ga_2O_3 multilayers (Annett Thøgersen, p.101)

LS6: In situ and live cell microscopy

(Sonja Lyttkens hall, Å101121, chair S. Barg)

- 15:30 16:00 (invited) Live-cell imaging of sub-membrane signalling and secretion (Anders Tengholm, p.103)
- 16:00 16:20 Electron Microscopy of the Human Cochlea and Why the Crocodilians Hear So well (*Helge Rask-Andersen, p.104*)
- 16:20 16:40 Dissecting the viral fusion and entry pathways of pandemic viruses, using fluorescence microscopy and reporter dyes (Steinar Mannsverk, p.106)
- 16:40 17:00 Depth and element sensitive scanning nuclear microscopy in Uppsala (Gyula Nagy, p.108)
- 17:30 Social program

Conference program - Poster contributions

- P01 Large volume high resolution 3D characterization of beam sensitive soft materials using multi-ion source PFIB technique under cryogenic conditions (Min Wu, p.110)
- P02 In situ control and observation of chemical reaction inside a reaction capsule in SEM DualBeam (Min Wu, p.111)
- PO3 Effect of decoration route on the nanomechanical, adhesive, and force response of nanocelluloses An in situ force spectroscopy study (Jing Li, p.112)
- P04 MerlinEM, Hybrid Pixel Array Counting Detector for Transmission Electron Microscopy (Klyszejko, Adriana L., p.114)
- P05 Effects of temperature for magnon detection in STEM (José Ángel Castellanos-Reyes, p.115)
- P06 Determination of radiation tolerance of crystalline ZIF-8 metal organic framework in atomic scale EM experiments. (Banerjee, Pritam, p.117)
- P07 Optical properties and structure relationship in ZnO:Fe with inversion domain boundaries and ZnFe₂O₄/ZnO heterostructures (García Fernández, Javier, p.119)
- P08 Nanochannel Liquid Phase TEM 3D ED / MicroED (Edward Broadhurst, p.121)
- P09 Atomic structure and magnetic circular dichroism of individual edge dislocation by electron magnetic circular dichroism (Zhang, Yuxuan, p.122)
- P10 Development of in situ (S)TEM cooling, biasing and heating system for atomic resolution imaging (Pivak Yevheniy, p.124)
- P11 Metal electrodeposition/stripping and 4D STEM analysis via operando liquid phase TEM (Pivak Yevheniy, p.125)
- P12 High-Resolution Electron Energy Loss Spectroscopy Mapping of Confined Electric Fields in Topology-Optimized Photonic Cavities (Seifner, Michael, p.127)

- P13 Computation of generalised oscillator strengths for the simulation and quantification of energy loss spectra (Guzzinati, Giulio, p.129)
- P14 Panta Rhei: a software framework for the acquisition of image and spectral data (Guzzinati, Giulio, p.131)
- P15 A Semiconductor-Type Segmented STEM Annular Dark-Field Detector (Pirmin Kükelhan, p.133)
- P16 A post-column imaging energy filter compatible with multiple detectors (Pirmin Kükelhan, p.135)
- P17 Optical dichroism in vortex electron energy loss spectroscopy on chiral particles (Ošmera, Martin, p.137)
- P18 Cold sprayed Aluminum-Quasicrystal Composite Coating: Bonding Mechanism Evaluation by SEM and TEM (Jafari, Reza, p.139)
- P19 Morphological changes of photothermal polymeric films during carbonization at different conditions for carbon materials production (Anna Iurchenkova, p.141)
- P20 In-situ S/TEM Visualization of Metal-to-Metal Hydride Phase Transformation of Magnesium Thin Films (Krishnan, Gopi, p.142)
- P21 Advancing phase characterization at the nanoscale by effortless multi-modal 4D-STEM applications (Nemecek, Daniel, p.144)
- P22 Interfacial structure and magnetic property at Triple-/Double- Perovskite heterojunctions (*Jie Ren*, p.145)
- P23 Unraveling the multilayer growth behavior of InGaAs nanowires using In-situ TEM (Sjökvist, Robin, p.147)
- P24 In-situ observations of size effects in GaAs nanowire growth (Marnauza, Mikelis, p.149)
- P25 Materials characterization using deep learning image analysis (Nordqvist Sammy, p.151)
- P26 Microscopic Heterogeneity of Plastic Strain and Lattice Rotation in Duplex Steel (Fengxiang Lin, p.153)
- P27 Heat Transport Properties of Au-Nanoparticles Supported by TiO₂: Insights from E(3)-Equivariant Machine Learning Potentials (Cuauhtémoc Nuñez Valencia, p.154)

- P28 Isotopic shifts of phonon bands studied by monochromated STEM simulations and experiments (Rusz, Jan, p.156)
- P29 A Comparative Study on EDX and XPS Elemental Analysis (Leskinen, Jari, p.157)
- P30 In-situ TEM study of nitrogen oxide removal (Fei Wang, p.158)
- P31 Electron Counted Spectrum Imaging Optimized for In-Situ Analysis (Twesten, Ray, p.159)
- P32 Plasmon-Enhanced Fluorescence of Site-Specifically Immobilized Single Upconversion Nanoparticles (Yupeng Yang, p.161)
- P33 Potential low-tech Zernike phase plate for soft-matter applications made from commercial carbon films (Marcus Hufe, p.163)
- P34 Progress in quantitative EMCD experiments (Ali, Hasan, p.165)
- P35 In-situ nanoscale dynamics of nanoparticles using variable temperature TEM imaging (Zulfiqar, Abid, p.167)
- P36 Measurement of EMCD signal on Fe layers with improved structural quality (Sharath Kumar Manjeshwar Sathyanath, p.169)
- P37 A new sampling paradigm for FRFPMS simulations of high energy resolution (STEM)-EELS experiments (Zeiger, Paul, p.171)
- P38 Magnetic Domain Structure of Ferromagnetic Steels Studied by Lorentz Microscopy and Magnetic Force Microscopy (Honkanen, Mari, p.172)
- P39 Colloidal gold transport in the Paleoproterozoic orogenic gold deposits: Outlining objectives of the PhD project (Tapio Soukka, p.174)
- P40 iDPC-STEM imaging as an important tool for structure determination of low dimensional nanoporous materials (Willhammar, Tom, p.175)
- P41 A Sample Preparation Methodology for In-Situ Liquid Transmission Electron Microscopy of Nanolaminated Materials (Melike Mercan Yildizhan Özyar, p.177)
- P42 Analysis of angle resolved low-loss electron energy-loss spectra of the dielectric BaTiO₃ (Ignatans, Reinis, p.179)
- P43 Impact of Electron Beam Irradiation on Carbon Black Oxidation (Wahlqvist, David, p.180)

- P44 Structure and Chemical Characterization of $\text{ZnGe}_x \text{Sn}_{1-x} \text{N}_2$ for Solar Cell Applications (Nguyen, Hao, p.182)
- P45 The effects of heavy doping on the band structure of zinc oxide observed using momentum-resolved EELS (*Elgvin*, Cana, p.184)
- P46 Recent innovation in scanning electron microscope (SEM) in-situ extreme mechanics at the micro- and nanoscale (*Pero Renato*, p.186)
- P47 Exploring micro-scale fracture behavior in brittle thin films (Mathews, Nidhin George, p.187)
- P48 Rapid in-situ magnetic imaging of artificial spin ice using STEM-DPC (Soland, Hedda Christine, p.189)
- P49 Formation of translucent nanostructured zirconia ceramics (Krisjanis Smits, p.191)
- P50 Capsid structure of a fungal dsRNA megabirnavirus reveals its unidentified structures (Wang, Han, p.192)
- P51 Uncovering functions of unique structures in a mosquito totivirus-like virus (Filipe, Diogo, p.193)
- P52 Results from the Quantum C100, a Novel CMOS Detector Optimised for 100 keV Cryo Electron Microscopy (Klyszejko, Adriana L., p.195)
- P53 3D electron diffraction of small molecules on the MerlinEM detector (Klyszejko, Adriana L., p.197)
- P54 Crystallographic Data obtained from CryoEM Imaging (Crispin Hetherington, p.198)
- P55 An Automated Method for Quantifying Actomyosin Ring Dynamics in Drosophila Cellularization (Korkiamäki, Riku, p.200)
- P56 Comparison of the fundamental cell morphology of species in genus Mycobacteroides obtained from whole-mount ice-embedded cryo-TEM examination. (Yamada, Hiroyuki, p.202)

Abstracts

Emergent Phonon Phenomena at Interfaces Probed by Vibrational Electron Microscopy

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Crystal defects and interfaces affect the thermal and heat-transport properties of materials by scattering phonons and modifying phonon spectra. Spatially resolved vibrational mapping of nanostructures and defects is indispensable to the development and understanding of thermal nanodevices, modulation of thermal transport and novel nanostructured thermoelectric materials. Through the engineering of complex structures, such as alloys, nanostructures and superlattice interfaces, one can significantly alter the propagation of phonons and suppress material thermal conductivity while maintaining electrical conductivity. There have been no correlative experiments that spatially track the modulation of phonon properties in and around individual defects and nanostructures due to spatial resolution limitations of conventional optical phonon detection techniques. In this talk, we demonstrate that space- and angle-resolved vibrational spectroscopy in a transmission electron microscope makes it possible to map the vibrational spectra of a single interface and a quantum dot. We detect a red shift of several meV in the energy of acoustic vibration modes near a single stacking fault in cubic silicon carbide, together with substantial changes in their intensity, and find that these changes are confined to within a few nanometers of the stacking fault.[1] In a two-dimensional lateral heterostructure, new phonon modes at 27.9 and 41.1 meV are observed at the interface between monolayer thick MoS₂ and WSe₂ [2]. At a Si-Ge interface, localized interfacial phonon modes at ~48 meV.[3] Simulations show that these interfacial phonon modes contribute to the total thermal interface conductance. By tracking the variation of the Si optical mode in a phonon map from a single SiGe quantum dot, the nanoscale modification of the composition-induced red shift is observed.[4] In this work we have also developed a novel technique to differentially map phonon momenta, providing direct evidence that the interplay between diffuse and specular reflection largely depends on the detailed atomistic structure. Our work unveils the non-equilibrium phonon dynamics at nanoscale interfaces and can be used to study actual nanodevices and aid in the understanding of heat dissipation near nanoscale hotspots, which is crucial for future high-performance nanoelectronics.

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Abstract

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by studying isolated and purified molecular components. This 'divide and conquer' approach has been highly successful, as evidenced by the impressive number of entries in the PDB.

However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. *Sensu stricto* the term 'structural biology *in situ*' should apply only to a scenario in which the cellular environment is preserved in its entirety.

Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible to achieve. We have used this method to study the 26S proteasome in a number of cellular settings revealing their precise location, assembly and activity status as well as their interactions with other molecular players of the cellular protein quality control machinery.

P2

Multidimensional TEM – characterization of electric fields and structure based on full momentum resolution

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In recent years, the dimensionality in transmission electron microscopy (TEM) has increased rapidly by the advent of ultrafast cameras that record at frame rates of many kHz. This development has especially paved the way for a revolution as to the versatility of scanning TEM (STEM). In particular, momentum-resolved STEM enhanced traditional Z- and phase-contrast techniques such that any conventional imaging mode is present simultaneously in a 4D data set. Most importantly, the combination of real- and reciprocal space information nowadays allows to quantify charge densities with subatomic resolution, to measure polarisation-induced electric fields, and to solve the phase problem by ptychographic techniques. This presentation demonstrates the capability of 4D-STEM by means of several examples such as the mapping of atomic electric fields in 2D materials and ptychographic reconstructions using different algorithms. Furthermore, mapping of polarization-induced electric fields in semiconductors and ferroelectrics is addressed using GaN/AlN systems and PbZrTiO. Finally, factors impacting the quantitative interpretation of 4D STEM data are worked out, namely the role of probe focus and momentum transfers due to (multiple) plasmon scattering.

Electron beam shaping and aberration correction using optical fields

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Abstract

Over the last decades, electron microscopy has become a truly powerful and versatile technique for nano- or atomic-scale imaging and spectroscopy [1]. Major advancements and outstanding capabilities have been made possible thanks to better spatial and temporal control over the amplitude and phase of the wave function that characterizes the fast electrons used as sample probes. Control over the beam shape is commonly achieved by means of complex arrangements of magneto- and electro-static electron lenses that enable sub-Ångstrom focusing and beam scanning, as well as the correction of aberrations produced by the electron optics. The phase of the electron wave function can be additionally modified by introducing static phase plates.

We envision an alternative to traditional electron-optics elements, materialized in the concept of the optically-driven electron modulator that enables dynamical shaping of electron-beam wave functions both in space and time. This approach capitalizes recent experimental demonstrations of wave function control through optical fields [2], combined with ultrafast control over the electron-light interaction [3,4]. Specifically, we propose two types of schemes to realize optical control over the electron beam shape: a photonic aberration corrector (PAC) that exploits the interaction of the electron with light scattered from a thin film; and an optical free-space electron modulator (OFEM) operating in free space (see Fig. 1). Based on realistic designs combined with detailed simulations, we demonstrate an application with high potential for improving the resolution of electron microscopes, whereby the electron-light interaction is used to correct for common aberrations introduced by electro- or magneto-static lenses in current setups [6]. In addition, we demonstrate the possibility of generating exotic electron beam shapes [5,6], with the extra advantage that fast control over such shapes is inherent to the optical elements used in our designs.



Figure 1 Optical free-space electron modulator (OFEM). (a) The proposed element is placed in the electron microscope column right before the objective lens. (b) The OFEM incorporates a parabolic mirror that focuses light with a high numerical aperture on a vacuum region that intersects the electron beam. The electric field distribution at the optical focal spot is patterned by using a far-field spatial light modulator (SLM). (c) A phase is optically imprinted on the electron wave function.

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Hypermodal Data Fusion: a data-driven approach for tackling contemporary nanoscale challenges

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While aberration-corrected STEM is most commonly associated with its ability to visualize atomic positions with sub-nanometer lateral resolution^[1], its full potential is only unlocked when the scattering cross-section arising from the highly localized interaction volume is sampled selectively by multiple independent detectors. This allows investigation of an unprecedented range of materials properties, with modern STEM detectors capable of synchronizing to the probe position at 100+ Hz and sequential scanning strategies allowing for collection of collinear scattering with disparate detector modes^[2]. With access to such enormous amounts of information, a key challenge facing modern electron microscopists now becomes distilling this glut of data into a physically meaningful understanding of a highly complex system that can drive a wider scientific inquiry.

In this contribution, I outline a data-driven approach that I am developing to tackle this challenge called "Hypermodal Data Fusion."^[3] First, unsupervised machine learning based on blind-source separation estimates a feature space tailored to reflect the scientific question. Second an arbitrary number of hyperspectral and hyperdimensional datasets are "fused" together to relate disparate materials properties to the individual recovered features. I demonstrate how to use this for many purposes, including spectral in-painting^[4–7] and correlating chemical, structural, and optical properties in photocatalytic systems^[3,8]. I subsequently focus on how to apply this to Co-free lithium ion batteries,^[9,10] vastly improving our understanding of a critical materials system that is dominated by the complex interaction of nanoscale chemical and structural features.



Figure 1 – A schematic illustrating some of the wide range of materials properties that can be studied in a single hypermodal STEM experiment^[3].

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Analysis of magnetic structure in ferrimagnetic Ti-doped barium hexaferrite by analytical EMCD method

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Introduction

Barium hexaferrite, $BaFe_{12}O_{19}$ (BFO), is a crucial material in the history of permanent magnets in terms of heat and oxidation resistance. Recently, attention has been paid to improving the magnetic properties of magnetoplumbite materials such as BFO by chemical substitution. In particular, Ti and Sb doping can increase the Curie temperature, thereby improving the high temperature stability and leading to room temperature skyrmions [1, 10]. However, a detailed understanding of the underlying atomistic mechanism is still lacking.

We analyze the chemical doping by exploiting the sensitivity of electron energy loss spectroscopy (EELS) to the chemical state of Fe sites, which changes with Ti^{4+} doping. As a reference, we use the previously identified anti-ferromagnetic coupled subsystems of BFO [2].

In this analysis, we used electron energy-loss magnetic chiral dichroism (EMCD) measurements to reveal magnetic and chemical information of BFO. EMCD allows for the element specific measurement of the orbital-to-spin magnetic moment directly in the electron microscope using special scattering geometries with up to nanometer resolution [3,11].

The chemical fingerprint of Ti doping in BFO is obtained by integrating all measurement positions in the three-beam EMCD setup.

Sample

The single crystal barium hexaferrite sample was grown by floating zone technique by melting polycrystalline sintered rods at 1350 °C in 10^{-2} atm oxygen [5]. Ti was substituted in BFO in the ratio as x = 0.2 in BaFe_{12-x}Ti_xO₁₉. A thin electron transparent sample was prepared by FIB. The lattice parameters are a = 5.9 Å and c = 23 Å as shown in Figure 1.

Experimental Method

The classical EMCD measurement was performed with a JEOL JEM F200 operating at 200 kV acceleration voltage in diffraction mode with a convergence half-angle of 0.1 mrad. An optimized 3-beam diffraction condition was selected according to theoretical calculations [6]. Recently, Haruta et al. [7] reported that GATAN High Quality Dark Reference Subtraction is not sufficient to reduce EELS detector correlation noise for ultra-low count measurements. This is true for EMCD measurements with weak magnetic signals of the order of ~1% intensity variation. To reduce the correlated noise, random energy shifts are applied during the EELS acquisition in addition to the HQ dark reference subtraction. This results in a strong noise reduction in the acquired EMCD data sets.

Analysis

The change in valence state of the Fe site due to Ti doping is resolved by decomposing the measured EELS spectra with reference spectra [8]. The decomposition is performed using Non-Negative Least Square (NNLS) fitting. In addition, Least Square fitting is applied to obtain site-specific EMCD signal using XMCD reference spectra of Fe^{3+} and Fe^{2+} cations [9], as shown in

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Figure 2. The spectral ratios (oct- Fe^{3+} : tet- Fe^{3+} : oct- Fe^{2+} : tet- Fe^{2+}) obtained from the deconvolution provide detailed information about the valence states as well as a deeper understanding of the variation of the Curie temperature upon Ti doping.

Figures Anion :0 XMCD Fe²⁺ Octahedral site Fe³ Cations : Ba²⁺ Tetrahedral site Fe³ XMCD Fe³⁺ Fe³⁺ : Octagonal Octahedral site Fe² Experimental : Tetragonal Tetrahedral site Fe² : Trigonal bipyramids 715 710 enerav loss /eV Experimenta Exp. spectrum Fitting results Residual 720 725 gyloss /eV 715 705 710 730

Figure 1 BFO unit cell. The Ti⁴⁺ ion is placed at an octagonal 4f2 site. The lattice parameters are a = 5.9 Å, c = 23 Å.

Figure 2 Results of fitting the experimental spectra to the reference spectra [8,9]. Red and bold black lines are experimental and fitting results, respectively.

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Machine-Learning Assisted Exit-wave Reconstruction for Quantitative Feature Extraction

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Reconstruction of the exit wave is a powerful tool to extract the maximal amount of information from High-resolution Transmission Electron Microscopy (HRTEM) [1,2]. In a recent publication, Chen et al. [3] used exit wave reconstruction to visualize the three-dimensional structure of cobalt doped Molybdenum Disulphide nanoparticles in a model catalyst. In addition to the three-dimensional structure of the nanoparticle, the reconstructed exit waves also contained information about the beam-stimulated vibrations of the atoms near the edge of the nanoparticle.

We have recently demonstrated that convolutional neural networks are able to reconstruct the exit wave from a focal series with a low number of images [4]. We train the neural networks on simulated images. The simulated images are produced with the multislice algorithm using the abTEM software [5], both the exit wave function and images produced with three different values of the defocus are saved. The neural network is then trained to reconstruct the exit wave from the images. The network is validated on a different set of simulated images, and if applicable applied to experimentally obtained data.

We demonstrated that it is possible to train neural networks to reconstruct the exit wave for a varied set of samples consisting of all structures in the Computational 2D Materials Database (C2DB) [6]. For a specialized dataset such as Molybdenum Disulphide (MoS_2) supported on graphene, a slightly lower error rate can be obtained (Figure 1), and realistic results can be obtained when the network is applied to experimental data [4].

In this work, we investigate how far the convolutional neural networks can be optimized towards obtaining quantitative information from experimental data, with a particular focus on the kind of data obtained by Chen et al. [3], i.e., reconstructing exit waves with sufficient accuracy to extract the three-dimensional structure and the amplitudes of the atomic vibrations. This can be realized with more flexible training sets than in our previous publication and by training the network to ignore the support when reconstructing the exit wave.

Program



Figure 1: Molybdenum Sulphide on graphene. The top row shows the structure and two of the three images used by the neural network. The second row shows the correct exit wave (the ground truth), the one predicted by the neural network, and the difference. Only the imaginary part is shown, as there is less signal in the real part. The network is partially missing a support atom which is simultaneously at the edge of the support and close to a molybdenum atom in the MoS_2 nanoparticle. Reproduced from reference 4, supplementary material.

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Spatial transcriptomics

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Abstract

Spatial transcriptomics combines microscopy with groundbreaking molecular methods enabling detection of hundreds of active genes in parallell, directly in a tissue sample at subcellular resolution. This opens up for new possibilities of linking gene expression to health, development and disease, and also to local tissue morphology. In my talk, I will focus on possibilities and recent results when it comes to understanding organ development and cancer, and showcase some of our latest tools for data visualization and computational analysis of this information-rich data.

Texture Segmentation, Surface Detection, and Fast Local Thickness for Quantification of Bone Microstructure

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Abstract

3D microscopy images acquired at synchrotron or laboratory facilities provide a high-resolution view into the internal structure of matter with contemporary scanners typically producing images containing 2048³ voxels. Analyzing such images is often performed using specialized software, requires a high level of expertise, and often involves manual intervention [1].

The choice of image processing tools is an important factor for the quantitative analysis of volumetric images. The preferred tools are freely available, reliable, easy to install and use, support the reproducibility of measures, and can process large images. We introduce three such tools: interactive texture segmentation [2], sparse layered graphs [3], and fast local thickness [4]. All tools are available as pip-installable Python modules.

To exemplify the benefits of our tools we quantify the trabecular structure from μ CT scan of mice tibia. Quantifying bone quality from high-resolution μ CT scans has become a standard in preclinical studies [5] with trabecular thickness and trabecular separation being the most used variables describing the morphology of trabecular bone.

The site of interest for our analysis is the epiphyseal plate of a growing long bone. This is a thin layer between epiphysis and metaphysis characterized by layered zones of varying microstructure. Using our three tools, we identify the epiphyseal plate, separate it into two layers, and quantify its trabecular microstructure.

First, we use interactive texture segmentation [2] to obtain probabilistic pixel classification of bone microstructure. Interactive texture segmentation allows the user to define structures of interest by marking a subset of pixels. The final result is obtained from very modest user input. Interactive texture segmentation yields a rough division into structures characterizing epiphyses and metaphysis.

Second, we used sparse layered graphs [3] to precisely separate the layered zones of the epiphyseal plate. Layered graphs allow us to constrain the geometry of the solution. We can therefore utilize the known ordering of the epiphyseal layers and their expected minimal and maximal thickness.

Finally, we used fast local thickness [4] to quantify the trabecular structure of epiphyseal layers. Fast local thickness uses an efficient approximation of the spherical structuring element which makes it suitable for processing large volumes. From local thickness, we can compute trabecular thickness and trabecular separation.

In conclusion, our processing pipeline consists of three separate steps. In the first two steps, we identify and separate the epiphyseal plate, and in the last step, we quantify the trabecular structure. By doing so we avoid selecting the volume of interest and make our results reliable and reproducible.

LS1.1

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Identifying bird eggshell by scanning electron microscopy (SEM)

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Abstract

I present here a brief overview on the use of SEM to answer archaeological questions involving the collection of eggs from wild ducks and seabirds in Iceland from the Viking Age (the settlement period), through the medieval and post-medieval periods, to the beginning of the modern time. The studies provide further insight into environmental archaeology of the regions and contribute to a better understanding of everyday life in the periods investigated. This work resulted from research collaborations between University of Iceland and City University of New York [1-2, 4-5] and within University of Iceland, between Department of Archaeology and Institute of Life and Environmental Sciences [3].

Fragments of eggshells are often left behind at sites of past human activity. In our studies we examined bird eggshell fragments in domestic wastes, such as in midden fills [1-2, 5] and under kitchen floors [3]. Large fragment or almost whole egg can be identified to bird groups, but more often, especially in old deposits, eggshells are crushed, or they are chemically eroded, thus SEM becomes necessary. We learned from numerous records that in Iceland wild duck eggs were collected [1-2], sustainably, i.e., leaving behind four to five eggs in the nest for the female to incubate. The period of harvesting wild duck eggs lasted about five weeks, not long after the migrating ducks arrived in late April to early May. Harvesting duck eggs, but not killing adult birds, has been a tradition particularly in northern Iceland, from the settlement period and well into the 20th century. Seabirds [3], on the other hand, have an aquatic lifestyle and only come to land to breed. They lay their eggs on coastal cliffs and exposed crevices. Most of the larger cliffs in the country are still visited every year for this purpose.

To be able to identify eggshell fragments in the archaeological contexts, we generated a SEM manual of species-level identification, containing micromorphology images of reference bird species [4]. We obtained eggshell samples from 25 species of birds, most of which are living, or nesting, in the northern North Atlantic region. Of these, 15 are from the family Anatidae (ducks, geese, and swans), e.g., mallard (<u>Figure 1A</u>). Other species are Galliformes (loons and ptarmigan), Podicipedidae (grebes) and Charadriiformes (waders, jaegers, auks, and razorbills).



Figure 1. A: Mallard (*Anas platyrhynchos* L.), in Reykjavík Pond, June 2022. B: SEM image of the eggshell interior of mallard at ×200 magnification.

Eggshell fragments, each about 5–10 mm in size, were prepared for SEM imaging by first removing both inner and outer membranes of eggshell with a 2% sodium hypochlorite for 10–30 min with a vortex-agitation to loosen the dissolving membranes, rinsed with distilled water and air-dried. The eggshell samples, inner side up, were scanned and imaged at ×50 magnification (for density analysis) and ×300 magnification (for qualitative analysis of the surface structure), in the SEM microscope JSM-6610LA, using BEIW (back-scattered electron imaging) with compositional contrast, low vacuum mode with pressure setting at 20 Pa, accelerating voltage of 10 kV, working distance of 8 mm, zero tilt and 45 mm spot size.

The avian eggshell is a composite structure of an organic matrix and mineral (calcium carbonate) and when fully formed it consists of morphologically distinct regions or zones. Starting from the outside, the regions are the cuticle and a thin vertical crystal layer, then the palisade layer, which makes up the bulk of the eggshell and is made of columns perpendicular to the egg surface. The lower (inner) parts of the columns have tapered ends, called mammillae, which are knob-like, and collectively create a micro-relief on the inner surface of the eggshell (Figure 1B). The SEM manual of species-level identification [4] describes mammillary density (per 0.25 mm²) and microstructure (mammillae bodies, membrane facets, fissures, and sutures). The manual [4] was used for identifying bird species from archaeological records [3, 5].

In one such study [3], we analysed eggshell samples from in-situ deposits in *Skálholt*, the southern bishopric founded in the mid-11th century CE, in the bishop's and staff living quarters dated to the 17th and 18th century. We found that half of 20 representative samples from floor layers and underfloor drain fills were from seabirds, guillemot and razorbill. Seabird eggs were likely collected on the coast and transported to the site. Uniquely to this site, eggs for consumption were also from the shore bird Arctic skua, which nests in the low-land area of the region. Two samples came from mallard, the rarity found only under the bishop's kitchen floor.

The other study is Hicks' research on the archaeofaunal assemblage from *Skútustaðir* [5], a long-term farmstead located near Lake Mývatn, northern Iceland, and occupied from the 9th century until the present. The sampling of eggshell during the excavation was from midden deposits, dated to the 10th century (the Viking Age), the early Middle Ages, and the 17th to the 18th century (early modern period). The ages were determined based mainly on known volcanic tephras. From the oldest layer, the majority of 38 samples examined showed high level of mammillary reabsorption, due to physical wear and chemical erosion, yet it was possible to identify some samples to Barrow's Goldeneye, ground-nesting duck local to the inland Mývatn wetland ecosystem. Eggs from a few other species were also present, such as from oystercatcher and seabirds. Eggshell samples (99) from the medieval layer included a large diversity of wild bird species, from Barrow's Goldeneye and horned grebe to eider duck and geese. Of 43 fragments imaged from the early modern layer, half of them were identified as Barrow's Goldeneye, again, but swan eggs were quite common in this deposit. Towards the modern period, egg harvesting seemed to be more organized, more quantity but less diversity.

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TEM Image Enhancement From Multiple Short Exposure Images

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Abstract

MiniTEMTM is a miniaturized room temperature 25keV TEM with a high degree of automation, developed with the purpose to make the technique and the information it provides more accessible and affordable for "every-day" biological applications. This miniaturization comes with some sacrifices, of which one is resolution and image quality. For inorganic samples the resolution is about 0.5 - 1nm. For biological samples it is generally somewhat lower due to the sample preparation process.

Our goal is to, by computational means, increase the image quality and enhance fine details in images acquired with MiniTEM. Registration of images, scan lines, or objects is an established approach to improve quality and reconstruct information [1]. In microscopy, variants are used in scanning instruments (electron, and fluorescence) as well as in single particle reconstruction using cryo-EM, and in certain high-end direct electron detection cameras [2-3].

Both manual and automated analysis of TEM images are negatively affected by a number of factors such as sample preparation artefacts, microscope alignment and focusing, electrons interacting with and modifying the sample (burning), noise, and motion artefacts. Noise can be reduced by increasing the exposure time, but that comes at a cost of increasing motion artefacts. The opposite action - to decrease the exposure time - results in less motion blur but more noise. In the related work, [4], different denoising strategies of short exposure images were compared.

In this work we investigate how image quality and visibility of nano-structures can be improved by registering and aggregating multiple short exposure images instead of acquiring normal (auto) exposure images degraded by fine motion (drift) and vibrations. We propose a method based on three main steps: (i) sharpening of the short-exposure images, (ii) registration, and (iii) aggregation. We consider multiple aggregation approaches; aggregation of registered images by median/mean, aggregation of point cloud representations of the registered images by median/mean, by bilateral filtering, and filtering by anisotropic diffusion. An illustration of the proposed image enhancement method is shown in Fig.1. We show that combining multiple short exposure images gives an image of higher quality both quantitatively and visually, and that the sharpening step is key for a successful result.



Stack of images

Sharpening

Output

Figure 1 Illustration of the general approach. Multiple short-exposure images are combined to obtain an enhanced image

		unsharpened			sharpened		
Registration output	Aggregation method	PSNR	SSIM	MSE	PSNR	SSIM	MSE
	Auto exposure	26.58	0.70	0.0022	16.57	0.24	0.022
Grid	mean	24.55	0.71	0.0035	27.68	0.75	0.0017
	median	26.03	0.73	0.0025	27.39	0.74	0.0018
Point Cloud	mean, k=100	25.92	0.72	0.0026	27.92	0.75	0.0016
	mean, k=1000	24.68	0.71	0.0034	28.19	0.77	0.0015
	median, k=100	25.18	0.71	0.0030	27.70	0.73	0.0017
	median, k=1000	23.65	0.70	0.0043	28.40	0.78	0.0014
	anisotropic diffusion(5 edges) mean, k=100	23.60	0.69	0.0044	27.67	0.75	0.0017
	anisotropic diffusion(5 edges) median, k=100	25.42	0.72	0.0029	28.04	0.76	0.0016
	anisotropic diffusion(10 edges) mean, k=100	24.61	0.71	0.0035	28.18	0.77	0.0015
	anisotropic diffusion(10 edges) median, k=100	24.85	0.71	0.0033	27.33	0.75	0.0018
	bilateral filter	25.49	0.72	0.0028	28.73	0.76	0.0013

Table 1 Quantitative image enhancement measured by PSNR, SSIM and MSE

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P3

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Abstract

Inside living organisms, thousands of different genes function collectively to give rise to cellular behavior and tissue function. Understanding the behaviors and functions of cells and tissues thus require imaging at the genome scale, which will advance our understanding in many areas of biology, ranging from the regulation of gene expression in cells to the development of cell fate and the organization of cell types in complex tissues. We developed a single-cell transcriptome and genome imaging method, multiplexed error-robust fluorescence in situ hybridization (MERFISH), which allows RNA, DNA, and epigenetic marks to be imaged at the genome scale. This approach enabled spatially resolved transcriptomic profiling, epigenomic profiling, and 3D-genome organization mapping in single cells. The ability to perform single-cell gene expression profiling in intact tissues further enabled the identification, spatial mapping, and functional annotation of distinct cell types in intact tissues. In this talk, I will describe the MERFISH technology and its applications, with a focus on mapping the molecular, spatial, and functional organizations of cell types in the mouse and human brain.

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Abstract

Exciting developments in light and electron microscopy over the past decades have provided better insights and literally pictures of how pathogens interact with cells at the cellular, subcellular and molecular level. However, the reaction which a pathogen causes in the human body is complex and goes beyond the interaction of the pathogen and the individual cell. Usually, it is a systemic reaction which involves a complex interplay between several systems of the human body. The presentation will highlight this aspect by showing examples from studying COVID-19 disease and the recent outbreak of the mpox. A side view will discuss the strengths and weaknesses of infectious disease models. Finally, an outlook tries to define which developments are necessary in (electron) microscopy for improving the research of infectious diseases in future.

Phase Mapping of Precipitates in Aluminium Alloys by SPED

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Heat-treatable aluminium (Al) alloys are important structural materials for construction and automotive applications due to properties like high strength/weight ratio, high corrosion resistance, and good formability. These properties are often connected to nanoscale precipitates in the Al face centered cubic (fcc) host lattice, which are given by the chemical composition and the thermomechanical treatment of the alloy [1,2]. Precipitation starts out with clustering of solute elements from a supersaturated, metastable solid solution in the Al matrix. Solving the structure, quantifying the distribution, and determining phase fractions of clusters and precipitates are important to design better alloys. In this presentation I will show how we in the TEM Gemini Centre at NTNU and SINTEF in Trondheim have utilized scanning precession electron diffraction (SPED) methods to study clusters and precipitates in heat-treatable Al alloys. In SPED we obtain a 4D dataset by acquiring a precessed electron diffraction pattern (reciprocal space) from each probe position in real space. Since the precipitates have certain orientation relationships to Al, the SPED data is acquired in a major zone axis of Al, depending on the alloy system. This gives limited numbers of unique diffraction patterns for each precipitate type and simplifies the analysis. Analysis of the 4D SPED stacks is done using the open-source python libraries hyperspy [3] and pyxem [4]. SPED has been a valuable tool for investigating the evolution of clusters and precipitate phases during artificial ageing [5,6]. We have also acquired SPED data during in-situ heating of an Al-Mg-Si-Cu alloy to study the growth, transformation, and subsequent dissolution of individual precipitate types. These and other results will be discussed in the talk [7].

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Structural mechanism of emergent magnetic properties in patterned SrRuO₃ quantum structures

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Abstract

Complex oxide heterostructures have a wealth of magnetic states stemming from the specific coupling between spin, charge, orbital, and lattice degrees of freedom, thereby leading to intriguing applications in emerging spintronic devices [1]. Tremendous efforts have been devoted to looking for novel pathways to tune the magnetic anisotropy in oxide heterostructures through controlling electron-electron correlations. Famous strategies include strain engineering, interface engineering, applying chemical or physical pressures, applying an electric field, and controlling dimensionality [2]. Among them, controlling dimensionality, a post-growth pathway, allows to alter strain field and reconstruct electronic structures, offering a playground for feasibly engineering magnetic states. Specifically, 0-dimensional nanodots have modified energy levels, presumably giving rise to unique magnetic behavior. To date, most reported nanodots are constructed from standard semiconductor materials. Patterned nanodots comprising correlated perovskite oxides have rarely been studied because of the difficulty of patterning them into small scales.

In this study, we investigate the atomic structure and magnetic behavior of $5 \times 5 \text{ mm}^2$ sized SrRuO₃ (SRO) nanodots array patterned with electron-beam lithography [3]. The SRO nanodots' size is down to 15 nm, nearly an order of magnitude smaller than most previously reported work on perovskite oxides. We find that SRO shows ferromagnetism even in the smallest dots. As the size of the nanodots decreases, their ferromagnetic behavior is investigated, and the magnetic easy-axis is found to rotate from the out-of-plane direction toward the in-plane direction. The constituent elements, particularly oxygen ions, are directly visualized via atomic resolution scanning transmission electron microscopy (STEM). Electron energy-loss spectroscopy maps evidence the unchanged elemental distribution after patterning at the atomic scale. Quantitative STEM analysis demonstrates that the magnetic anisotropy and RuO₆ octahedra distortion in SRO nanodots are both nanodot size-dependent but remain unchanged in the first 3-unit-cell interfacial SRO monolayers regardless of the dots' size. It indicates that a structural variation in the bulk part of the SRO layer gives rise to the observed alteration of the magnetic anisotropy. A unique structural mechanism behind the nanodots' size-dependent magnetic anisotropy in SRO nanodots is unraveled, suggesting that the competition between lattice anisotropy and oxygen octahedral rotation mediates anisotropic exchange interactions in SRO nanodots. These findings demonstrate a new avenue toward tuning magnetic properties of correlated perovskite oxides and imply that patterned nanodots could be a promising playground for engineering emergent functional behavior [4,5].

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Dimensionality control of Pt on CeO₂ through support interaction

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Abstract

It is difficult to control the shape of small supported nanoparticles through synthesis. By studying the atomic scale dynamics of such species, we find evidence for a strong facetdependent support interaction in Pt/CeO₂ governing the dimensionality of small platinum particles. The effect could be exploited during synthesis to promote specific structures by tuning the exposed CeO₂ support facets and Pt particle size. Among collected data of Pt structures supported on (100) and (111) ceria facets, a lone particle, estimated to Pt_{118} by atom counting and STEM simulations, is monitored as it migrates from $CeO_2(111)$ to $CeO_2(100)$. During the migration, it undergoes a dimensionality change from 3D to 2D as the supporting facet changes. The transformation mechanism shows how the 3D particle gradually disassembles by a continuous stream of atoms diffusing away from the particle base to completely wet the neighboring $CeO_2(100)$ surface. In the 100-atom size range we find that particles supported on $CeO_2(111)$ adopt truncated octahedral shapes while most observed particles on $CeO_2(100)$ are Pt(111) rafts. Density functional theory calculations confirm a thermodynamic driving force for small Pt particles to form 2D structures on $CeO_2(100)$ while 3D structures are preferred on CeO₂(111). Furthermore, the 2D structure on CeO₂(100) is energetically favorable compared to equally sized 3D particles on $CeO_2(111)$. This brings insight to the ageing behavior of the Pt/CeO2 catalyst. Knowledge of support interactions is crucial in materials like catalysts where the nanostructure is important.



Figure 1: Selected frames from a time series of a platinum particle migrating from $CeO_2(111)$ to $CeO_2(100)$. Platinum has been falsely colored yellow to improve visibility. The full sequence displays an intricate transformation mechanism as single atoms diffuse in a continuous stream from the three-dimensional particle to wet the neighboring $CeO_2(100)$ facet.

Tracking the orientation of cellulose nanofibers using Scanning Electron Diffraction

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Abstract

4D-STEM is a growing electron microscopy technique in which the regular STEM detectors are replaced by a pixelated camera in the back focal plane which records a diffraction pattern for every beam position in the scan [1]. A significant advantage of 4D-STEM is that data analysis can be performed post-acquisition e.g. by the precise positioning of virtual detectors in diffraction space. This way, various types of highly localized information from the sample, such as strain, phase, and orientation, can be determined and visualized.

Low-dose conditions, in combination with new sensitive cameras, have now enabled the characterization of very beam-sensitive materials [2]. Scanning Electron Diffraction (SED) is one segment of the 4D-STEM family where the electron beam is shaped into a highly parallel probe, unlike a convergent probe in conventional STEM, with a beam diameter of less than 10 nm. The advantage of a parallel probe is to obtain sharper Bragg spots in the recorded diffraction patterns. The ability to record diffraction patterns with sharp reflections from nano-sized areas in electron beam-sensitive samples is a unique feature of SED.

In this work, we have used SED to disclose the orientation alignment of cellulose nanofibers in a bio-composite material [3] with a nanoscale spatial resolution (fig.1). The arrangement of nanofibres is of great importance as they strongly affect the mechanical properties of cellulosebased materials [4]. From the SED data (B), we can conclude that the nanofibers stay wellaligned with each other even though their orientation changes, both within the cell as well as across the cell wall. The magnification (D) shows the orientation of cellulose nanofibers from the lumen into the next cell. The major part (the S2-layer) has a uniform orientation. Closer to the middle lamella, the fibril orientation changes with a smooth gradient. By preserving the hierarchical structure of cellulose in wood, the strength of the composite material is increased.

Figures/Tables



Figure 1. The bio-composite material, transparent wood, sectioned in the transverse direction of the cells. (A) STEM overview image of the sample area. (B) Virtual image from the processed data set with a step size of 200 nm where the colors represent the different orientations of nanofibers in the cell wall according to the color bar. Black represents a signal below the selected threshold value due to low or no nanofiber content in those areas (lumen and middle lamella). (C) Four representative binned diffraction patterns from selected positions in

which the rotation of, and lack of, Bragg spots are seen. (D) A magnified area (from the white rectangle in B) of the cell wall composed of nearly 9000 discrete diffraction patterns. In this, we can follow the alignment and direction of cellulose nanofibres, from the lumen to the left, through the cell wall, middle lamella, and into the next cell with a spatial resolution of <20 nm.

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Polymorph engineering and radiation tolerance in β-Ga₂O₃

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Abstract

 β -Ga₂O₃ is one of the wide bandgap semiconductor candidates for developing power electronics components in the next generation of ultra-high-power devices and solar-blind UV photodetectors [1]. However, other polymorphs also display attractive properties, which can be stabilized using soft chemical synthesis methods, high temperature and high-pressure solid state synthesis, strain engineering in thin films, or ion implantation [2,3].

Ion implantation produces complex changes in the microstructure and properties of the exposed material. It can lead to disorder and formation of secondary phases in the form of nanoscale inclusions. In addition, for sufficiently high fluences, amorphization commonly occurs. In this context, radiation tolerance is determined as the ability of crystalline materials to withstand the accumulation of the radiation induced disorder without losing crystallinity. In contrast to many classical semiconductors, ion implantation in β -Ga₂O₃, induced a phase transformation from β - to γ -polymorph. The metastable γ -phase, crystallizes in a defective cubic spinel structure resulting in a wide band gap (~4.7 eV). This has attracted appreciable attention due to its novel physical and chemical properties, which are of interest for diverse applications including photonics (ultraviolet photodetectors), solar devices, and photocatalysis [4].

In this work, we demonstrate that the transformation takes place independently of the ion implanted: Si, Ni, Ga and Au after a threshold dose [5,6]. The formation of γ -Ga₂O₃ is confirmed by means of electron diffraction, scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy, see Figures 1(a-e).

Motivated by this finding, we have found that the γ/β double polymorph Ga₂O₃ structures exhibit unprecedently high radiation tolerance, even at very high doses. Specifically, for room temperature experiments, they tolerate a disorder equivalent to hundreds of displacements per atom, without severe degradations of crystallinity [6]. For example, Figures 2(a-b) shows the high-angle annular dark-field (HAADF) images of the implantation with $1 \cdot 10^{17}$ Ni/cm², leading to the formation of Ni nanoparticles within the γ -Ga₂O₃, allowing the functionalization of the semiconductor in a unique way. Finally, ex-situ and in-situ heating experiments demonstrate the transformation back to the thermodynamically stable β -phase, but with different microstructure and defects depending on treatment conditions.



Figure 1. SAED pattern of the transformed layer indexed according to γ -Ga₂O₃ along the zone axes (a) [100], (b) [110], (c) [111], and (d) [112]. (e) Atomic resolution HAADF STEM image of the interface between β -Ga₂O₃ (along [102] zone axis) and γ -Ga₂O₃ (along [112] zone axis) implanted with 1 × 10¹⁵ Si/cm² sample.



Figure 2. (a) Low Magnification HAADF STEM image of 1×10^{17} Ni/cm² sample showing the whole implanted area, (b) High Resolution ADF-TEM and corresponding FFTs of the areas with (blue) and without (red) Ni precipitates. γ -Ga₂O₃ planes are indicated in yellow, metallic Ni in green and double diffraction spots are indicated with a pink arrow

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TBD

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Abstract TBD

44

Primordial capsid and spooled ssDNA genome structures unravel ancestral events of eukaryotic viruses

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Marine algae viruses are important for controlling microorganism communities in the marine ecosystem and played fundamental roles during the early events of viral evolution. Here, we have focused on one major group of marine algae viruses, the ssDNA viruses from the *Bacilladnaviridae* family. We present the capsid structure of the bacilladnavirus Chaetoceros tenuissimus DNA virus type II (CtenDNAV-II), determined at 2.3 Å resolution. A structure-based phylogenetic analysis supported the previous theory that bacilladnaviruses have acquired their capsid protein via horizontal gene transfer from a ssRNA virus. The capsid protein contains the widespread virus jelly-roll fold, but has additional unique features; a third β -sheet and a long C-terminal tail. Further, low-resolution reconstructions of the CtenDNAV-II genome revealed a partially spooled structure, an arrangement previously only described for dsRNA and dsDNA viruses. Together, these results exemplify the importance of genetic recombination for the emergence and evolution of ssDNA viruses and provide important insights into the underlying mechanisms that dictate genome organisation.

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Left: capsid reconstruction, I4 symmetry; right: genome reconstruction, C1 symmetry

Structurally Guided Pandemic Response: Neutralizing Antibodies for COVID-19 and Beyond

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Abstract

We are just out of one of the most – economically and socially – devastating pandemics in modern times. From the start of the COVID-19 pandemic, my group worked in collaboration with other groups around the world to develop therapeutic neutralizing antibodies first as a short-to-medium-term approach to tackle COVID-19 in the clinic and later as a complement to vaccination for the immunocompromised and for clinical emergency use for variants of concern for which broad communal immunity is lacking. Using cryo-EM, we were able to structurally characterize a multitude of binders that broadly neutralize SARS-CoV-2 (1,2,3,4,5). This structural information was, in some cases, fed back to develop even better neutralizing antibodies (3,5) or to guide efforts in relation to newly discovered variants of concern(5). Hence, using the recent developments of cryo-EM and new antibody development methods, our efforts provide an example of how we, with structurally guided analysis and design of biologics, can quickly respond to pandemics such as COVID-19 to minimize their devastating effects on human health and society.

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Quantitative TEM reveals novel stress response mechanism in yeast

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Abstract

Cells, the foundational stone(s) of living beings, are often exposed to different types of stressors. As a consequence, they are equipped with different stress response mechanisms. A commonly used stressor when working with the budding yeast Saccharomyces cerevisiae is heat. It causes proteins in the cell to misfold and aggregate, which simulate disease mechanisms in proteopathies, such as Parkinson's disease.

The study of cellular stress responses has been intensively studied at a molecular level, but until recently, not at an ultrastructural one. The use of quantitative transmission electron microscopy permits high-resolution analysis of changes in the cellular architecture during the stress response. We have focused specifically on stress with heat, the proline analogue azetidine-2-carboxylic acid, hydrogen peroxide, and arsenite. In the process, it was uncovered that in yeast the nuclear envelope buds out toward the cytoplasm and that material is contained between the two nuclear membranes. The proteins ubiquitin and Hsp104, both involved in disaggregation and degradation of misfolded protein, were found inside the nuclear envelope budding (NEB) events.

We are currently investigating whether NEB events have different morphology, depending on the type or stressor applied. Preliminary results indicate that differences in sizes, where NEB events in cells treated in hydrogen peroxide are larger than in the other conditions. In heatshocked cells, buds appear more electron dense than in other conditions and those cells that were treated with arsenite, have NEB events containing ribosomes. This morphological analysis will pave the way for investigations into the cargo and function of this evolutionarily conserved mechanism. As a result, we will gain more insight into cellular protein quality control and its spatial management.

Real Time Investigation of Crystal Growth and Nucleation of Lowdimensional Inorganic Nanostructures

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A major challenge to designing new, novel nanostructures with properties tailored according to specific needs is understanding at an atomic scale the nanoscale processes and size effects that lead to structures with unique properties. To address this we use atomically-resolved insitu TEM to follow the nanoscale crystal growth process in real time, which offers insights into the growth processes unparalleled by any other technique. Our approach uses an aberration-corrected environmental TEM connected to a chemical vapor deposition system designed for compound semiconductor growth with separate gas lines for supplying the different precursor gases. In this talk I will discuss recent investigations into the crystal growth of semiconductor nanostructures, including nanowires and nanoparticles.

Solid State Dynamics Visualization by In Situ Electron Microscopy

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Abstract

The development of next-generation functional materials strongly depends on the understanding of the atomic-scale structure and related structural changes under real-world conditions. Such materials can provide new solutions within fields as diverse as chemistry, electronics, and medicine. In the energy sector, advanced nanomaterials are used in energy storage, power-to-X catalysts and many other devices. State-of-the-art electron microscopy techniques serve a high spatial resolution imaging and spectroscopic solution to high-level characterization of such materials in gas or liquid environments.

Under certain conditions it is even possible to monitor evidence of adsorbed molecules on surfaces giving valuable insight into the surface dynamics of catalysts during chemical reactions. Figure 1 shows the reconstruction of a TiO_2 (001) surface under oxygen and water exposure at different pressures [1]. Surface reconstruction and indication of adsorbed molecules are visible in the micrographs.

A still existing challenge is minimizing the effect of the electron beam and reliably analyze data acquired under low dose rate conditions. Minimizing electron dose rate during data acquisition reduces the impact of imaging but at the cost of a reduced signal to noise ratio of the acquired data. The result is often that the data becomes so noisy that it is not analyzable using a manual approach. An automated approach which has gathered significant traction over the last decade is machine learning and convolutional neural networks. Well-trained networks where noisy data is included in the training set have shown promising performance, even on in situ data. Figure 2 shows a gold nanoparticle imaged at in 4.5 Pa carbon monoxide at 300°C [2, 3]. The positions of atomic columns have successfully been located using a convolutional neural network. Analyzing sequences of such frames reveal the dynamic nature and how this varies with changes in the surroundings.

In this paper, I will discuss approaches to acquiring images of dynamic phenomena in order to provide statistically relevant, reliable and user unbiased materials science conclusions.



Figure 1: TiO_2 (001) surface under exposure to O_2 and H_2O at different pressures, showing the surface reconstruction and adsorbed water molecules.



Figure 2: A gold nanoparticle on a cerium dioxide substrate acquired at 450°C in 4.5 Pa CO. Left: raw data; middle: output of a convolutional neural network; right: overlay of raw data and analysis output.

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Revealing Surface Restraint-Induced Hexagonal Pd Nanocrystals via In Situ Transmission Electron Microscopy

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Abstract

Achieving metal nanocrystals with metastable phase draws much attention due to their anticipated fascinating properties, whereas it is still challenging because their polymorphism nature and phase transition mechanism remain elusive.^[1,2] Here, phase stability of face-centered-cubic (fcc) Pd nanocrystals was studied via *in situ* spherical aberration (Cs)-corrected transmission electron microscopy (TEM). By constructing a well-defined Pd/C composite structure, Pd nanocrystals encapsulated by graphite, the dispersion process of fcc Pd was observed through a nucleation and growth process. Interestingly, Cs-corrected scanning TEM analysis demonstrated that the newly formed Pd nanocrystals could adopt a metastable hexagonal (hex) phase, which was considered challenging to obtain. Accordingly, formation mechanism of the hexagonal Pd nanocrystals was proposed, which involved the combined effect of two factors: (1) templating of graphite and (2) size effect.^[3] This work is expected to offer new insight into the polymorphism of Pd nanocrystals and pave the way for the future design of metastable metal nanomaterials.



Figures

Figure 1 Schematic illustration of the fcc-to-hex transition of Pd nanocrystals.



Figure 2 (a) HAADF-STEM image of the hex Pd nanocrystal (temperature, 20 °C; pressure, $\sim 1 \times 10^{-5}$ Pa; dose rate, $\sim 5 \times 10^4$ A/m²s). (b) The enlarged image of the white rectangle area in (a) (left) and the intensity profiles taken from the marked atomic layers (right), respectively. (c) Unit cells of hex Pd. (d) Atomic model of hex Pd with mixed unit cells, viewed along [110] direction. (e) Schematic representation of the vacancy formation in hex Pd. (f) Simulated STEM image and corresponding intensity profiles of hex Pd viewed along [110] direction.

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Emergence of ferromagnetic phase in FeRh studied by in-situ Electron Magnetic Circular Dichroism with nm-sized probe

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Abstract

Electron magnetic circular dichroism (EMCD) is an electron energy loss spectroscopy-based technique capable of resolving magnetic properties using transmission electron microscope (TEM) [1]. Analogously to X-ray magnetic circular dichroism, EMCD can resolve element-specific magnetic properties, such as spin and orbital magnetic moments but with significantly better spatial resolution. Despite the rich information embedded in the EMCD signal, accurate quantitative evaluation of magnetic properties from the EMCD signal remains challenging. Thereby, new testbed magnetic systems are required for further decoding of EMCD signals and additional extension of EMCD applications.

Magnetic phase change materials with the abrupt onset of the ferromagnetic (FM) phase have a great potential for further exploration of the EMCD technique. In this work, we focus on the FeRh alloy system that undergoes first-order magneto-structural phase transition from antiferromagnetic (AF) to ferromagnetic (FM) phase near room temperature [2].

Here, we report on the in-situ EMCD measurement with a nm-sized probe and Lorentz TEM (LTEM) analysis of magnetic phase transition in FeRh system. Temperature-dependent EMCD was correlated with LTEM analysis to reveal local properties of FeRh phase transition, such as local transition temperature and magnetic phase propagation character. The analysis was conducted on a freestanding thin film of FeRh that was epitaxially grown on MgO and chemically detached. Dynamical diffraction simulations for FeRh system were performed for optimization of experimental conditions and for quantitative investigation of magnetic properties of phase transition.

Rapid propagation of the magnetic phases upon heating and abrupt character of phase transition observed in FeRh confirm great potential of this system as a testbed for additional exploitation of EMCD. Extracted magnetic properties from localized EMCD signal upon heating are further discussed in the context of studying the rich physics behind FeRh phase transition.



Figure 1: (a) Temperature dependent magnetization of FeRh thin film showing AF to FM phase transition. (b) Electron diffraction pattern for EMCD detection with nm-sized electron probe. (c) (resp. (d)) Resulting EMCD signal detected for low-temperature AF phase (resp. high-temperature FM phase) in FeRh obtained with nm-sized probe.

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In situ microscopy study of novel transition metal diborides

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Abstract

Transition metal diborides (TMB₂) is gaining increasing attention as the future generation materials for demanding applications in extreme environments. However, the synthesis of TMB₂ films is challenging due to their line-compound nature which does not permit point defects to accommodate for slight off-stoichiometry. For example, B/TM ratio > 2 leads to the formation of a B-rich secondary phase, which is separated from the growing compound [1-3].

A recent breakthrough in achieving full control of the B/TM ratio during the deposition process has resulted in synthesis of novel coatings with ratios B/TM<2, that possess high crystal quality and hardness [4-7]. The realization of B-deficient TMB₂ films widens the compositional range and thus, design and synthesis of films with performance-tailored microstructures.

We performed an extensive aberration-corrected scanning transmission electron microscopy (STEM) analysis to reveal the atomic structure and local chemistry of novel TM films throughout a wide elemental and compositional range [8]. It was established that the stoichiometry variations of TM films play the key role for the planar defect formation.

In this contribution, we investigated TM film-metal interaction during *in situ* annealing inside STEM. The thermal stability of off-stoichiometric TM films, contacted with Pt, was studied at temperatures up to 1100°C. During *in situ* annealing the diffusion of Pt into the TM films was observed and resulted in the formation of Pt-rich regions. Figure 1 shows a plan-view high-resolution STEM image from $CrB_{1.9}$ film annealed at 1100°C for 30 min and revealed the Pt in-diffusion into the junction sites of the antiphase boundaries.



Figure 1 CrB_{1.9} film after *in situ* annealing at 1100°C for 30 min.

Atomically resolved STEM imaging and energy dispersive X-ray elemental analysis have been applied to elucidate the role of structural characteristics favoring the district Pt diffusion pathways in under-/over-stoichiometric TM films.

The study uncovered the metal diffusion phenomena into off-stoichiometric TM films and has important implications for the design of ceramic-metal interfaces/structures (e.g., thermal barriers) for high-temperatures applications.

Acknowledgments

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Correlative light, electron and X-ray microscopy: finding the needle in the haystack

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Abstract

Fluorescence microscopy is a powerful tool for localising proteins within biological samples. However, information is limited to the distribution of the tagged protein, telling us little about the ultrastructure of the surrounding cells and tissues, which may be intimately involved in the biological process under study. Electron microscopy overcomes the resolution limitation inherent in light microscopy and can reveal the ultrastructure of cells and tissues. However, protein localisation tends to be complex and is often dependent on the availability of 'EM-friendly' antibodies. Correlative light and electron microscopy (CLEM) combines the benefits of fluorescence and electron imaging, revealing protein localisation against the backdrop of cellular architecture.

To image rare events in cells, tissues and whole model organisms, we developed Correlative Light and Volume EM (CLVEM), which combines correlative workflows with microscopes that automatically collect large stacks of high resolution images. Fast automated data acquisition allowed us to move towards high throughput quantitative CLEM at a rate of one whole cell per day.

During this development work, it became clear that several technical challenges associated with CLEM are exaggerated when working in 3D. Firstly, the accuracy of the overlay between light and electron images (which is critical to successful localisation of molecules to cellular structures) becomes more difficult as sample size increases. Secondly, with data acquisition becoming more automated, the bottleneck in the workflow becomes the data analysis step. Thirdly, artifacts associated with chemical fixation, heavy metal staining, dehydration and resin embedding are exacerbated when imaging in three dimensions, and this can critically affect the outcome of high resolution imaging experiments in some biological systems.

In this talk, I will introduce several ways in which we are improving and extending CLEM so that 3D correlative imaging can be applied to a wide variety of samples and biological questions, and start to become more accessible to non-specialist laboratories.

Correlative Light and Electron Microscopy

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The Research infrastructures are associated to MIMS, NMI and SciLifeLab

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Abstract

The microscopy facilities in Umeå covers advanced technologies and supports research from sample preparation, microscopy instrumentation to image analysis. Biochemical Imaging Centre in Umeå (BICU) offering light microscopy and Umeå Centre for Electron Microscopy (UCEM) offering electron microscopy are both open-access imaging facilities providing access and training on cutting-edge imaging technologies, including a wide range of instruments and methods. BICU and UCEM closely collaborate to enable accessibility and support on various correlative light and electron microscopy (CLEM) techniques at room temperature and cryo conditions. We perform CLEM involving different sample preparation techniques such as high-pressure freezing and automated freeze substitution, Tokuyasu cryo-sectioning, vitrification of hydrated samples through plunge-freezing and focused ion beam milling, e.g. lamella preparation, for subsequent tomography acquisition (Figure 1). Light and fluorescence imaging for different CLEM workflows is performed using wide-field and confocal microscopes and electron microscopy is performed using our TEM and SEM microscopes.

Detailed information regarding our imaging centre organizations and user fees can be found on our webpages:

https://www.umu.se/en/research/infrastructure/umea-centre-for-electron-microscopy-ucem/

https://www.umu.se/en/research/infrastructure/biochemical-imaging-centre-umea-bicu/

Apart from providing microscopy services and research project support we actively take part in training programs for young researchers, in the use of microscopy techniques and basic image analysis. We offer research school courses, workshops and PhD students often obtain individual project specific microscopy training together with the facility staff.

Program

Figure 1



Figure 1. A) CLEM with Landmarks: Landmarks on substrates like MatTek dishes or coverslips are used to relocate the cells of interest from a light microscopy (LM) image in the electron microscope (EM) and perform the correlation. This CLEM method can be used to identify the region of interest (ROI) with a cellular level precision to correlate the LM and EM results. B) Cryo CLEM with Focused Ion Beam (FIB): Cryo CLEM workflow involves sample vitrification at cryo temperatures, enabling close-to-native visualization. The ROI is identified in LM followed by FIB milling to thin down the sample. The resulting lamella is imaged with TEM to correlate the fluorescently labelled structures with their ultrastructure. It is a powerful tool to investigate biological samples at nanoscale. C) CLEM with Tokuyasu: This method involves mild fixation and freezing of the sample in cryo-protectant and cryo-sectioning. Thus, fluorescence of the sample is preserved, and immune labelling is performed on the sections. LM can be performed on the sections just before the EM resulting in a higher correlation precision. D) In-resin fluorescence CLEM: High pressure freezing combined with freeze substitution and embedding in HM20 resin can be used to preserve the fluorescence of the sample until the last sample processing step. This method also allows immune labelling of the sections. LM can be performed on the sections before the EM.

Correlative light-electron microscopy of autophagosome biogenesis in photodamage-induced mitophagy

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Abstract

Autophagy is an intracellular recycling pathway for cytoplasmic components and organelles. The process starts by segregation of the cytoplasmic cargo into double-membrane bound autophagosomes, which then deliver the cargo to lysosomes for degradation. Different types of selective autophagy can specifically target e.g. mitochondria (mitophagy) or aggregate-prone proteins (aggrephagy).

In this study, we used Airyscan super-resolution microscopy and correlative lightelectron microscopy (CLEM) to characterize autophagosome biogenesis during photodamageinduced mitophagy, a form of phosphoinositide 3-kinase-independent or Type 2 mitophagy [1]. Mouse hepatocytes expressing GFP-tagged LC3, a marker protein of autophagosome membranes, were used. After photodamage with 488-nm light to small groups of mitochondria within the GFP-LC3 transgenic mouse hepatocytes, U-shaped crescents of GFP-LC3 fluorescence formed around damaged, depolarized mitochondria beginning after 31 ± 18 min after photodamage. Fluorescence of the crescents progressively intensified, but their length remained unchanged. Subsequently, bright GFP-LC3 puncta merged with the crescents containing damaged mitochondria. Finally, bright GFP-LC3 formed a continuous ring around the damaged mitochondrion. CLEM was performed using mitotracker-stained mitochondria as landmarks for registering the fluorescence and electron microscopy images. CLEM confirmed mitochondrial swelling after photodamage, leading to outer membrane rupture and herniation of the inner membrane. CLEM also revealed that bright GFP-LC3 labeling occurred at the bare surfaces of herniated inner membranes at early stages of photodamage-induced mitophagy. The bright GFP-LC3 corresponded to nascent autophagosome membrane.

The ubiquitin-ligase Parkin has been shown to function in mitophagy [2]. Adenoviral expression of mCherry-parkin showed that parkin did not associate with photodamaged mitochondria at any stage up to 2 h after photodamage. The same mitophagy pattern was observed in parkin knockout mouse hepatocytes adenovirally expressing GFP-LC3.

To summarize, mitochondrial swelling, outer membrane rupture, and inner membrane herniation after photodamage exteriorize bare surfaces of mitochondrial inner membranes that become the proximate site for LC3 but not parkin association, as well as the initiation of autophagosome biogenesis and mitophagy.

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Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) -Imaging and micro-manipulation

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Abstract

A Focused Ion Beam (FIB) is integrated in a scanning electron microscope (SEM) for micromanipulation of small volumes inside the SEM chamber. The ion source is located at an angle corresponding to the electron source in the microscope. The stage is positioned at the coincidence point for both milling and imaging. Our Scios FIB-SEM microscope is upgraded with an Aquilos cryo controlled stage, allowing operations at both room temperature and with controlled liquid nitrogen cooling. The heavy element ion source is in this system made of gallium (Ga+). The focused ion beam removes materials from the sample in a controllable fashion with nanometer precision. A gas injection system (GIS) is composed of organometallic precursor, here platinum (Pt) is used to deposit materials on the sample surface. It acts as a protective layer to prevent ion-induced (unwanted) artefacts such as preferential milling, curtaining (different materials milling rate) and milled materials re-deposition. The microscope is also equipped with a lift-out micromanipulator (cryo EasyLift) to perform *in situ* transfer of lamellae onto TEM grid for high-resolution analysis at the other microscopes of the facility. A dedicated cryo-shutter is used to further reduce water vapor contamination during cryo-lamella preparation.

At Umeå Centre for Electron Microscopy (UCEM at Umeå University) recent development of workflows for cryo-FIB and cryo-electron tomography has shown great importance for visualization of tissues, cells, subcellular structures, and organelles at near native state. We prepare electron-transparent lamellas from vitrified cells by cryo-FIB milling. Lamellas are subsequently analyzed by TEM, tomography and 3D image reconstruction using the IMOD software package, yielding low contrast but high-resolution information. We can also use FIB-SEM for 3D cellular imaging by serial sectioning of resin-embedded and contrasted samples, yielding larger (micrometer scale) reconstructed volumes with uniform resolution in x-y-z. Specimen prepared form cells and tissue become beautifully reproduced with good contrast of membranes and the intracellular organelles. In this study, we observe the morphology and cellular ultrastructure of fission yeast (Schizosaccharomyces pombe), figure 1. This FIB-SEM volume imaging of resin-embedded yeast renders spatial organization of the intracellular organelles and the full cell content in an image volume. Combining correlative imaging of fluorescence labeling and FIB-SEM of both vitrified and resin embedded cells and tissue, provides both localization and high-resolution (nanometer scale resolution) of your research question.

Figure 1



Figure 1. A) An area of a contrasted sample, here a eucaryotic cell is consecutively milled and imaged using FIB-SEM. B) Cells are vitrified on an EM grid and imaged with SEM. C) A 200 nm thick lamella of yeast cells imaged by SEM, prior to transfer for cryo-TEM for tomography. D) FIB-SEM tomogram of a yeast cell and a IMOD 3D model the intracellular organelles.

In situ TEM techniques for controlling structure and interfaces in 2D materials

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The properties of two dimensional (2D) van der Waals materials are remarkably varied, but can be expanded even further if we pattern the layers in-plane or create well-defined interfaces between the 2D layer and other types of materials. *In situ* electron microscopy helps develop and refine these strategies by providing spatially and temporally resolved information while we perform these modifications. I will first discuss interfacial control, showing how in situ deposition and annealing experiments in an ultra-high vacuum TEM enable quantification of the nucleation and growth of epitaxial nanocrystals and more complex, multi-component nanostructures on 2D surfaces. I will then discuss patterning, using 2D magnets as an example. Here, electron beam-induced atomic displacements, controlled through machine learning techniques, enable the structure to be modified at individual lattice sites, potentially tailoring the magnetic texture. Given the variety of fascinating materials problems and the exciting advances in instrumentation and data analysis that are ongoing, it seems likely that *in situ* techniques will continue to play their unique role in the development of new structures and functionalities based on 2D materials.

Findings from the happy marriage between low-dimensional materials and low-voltage atomically-resolved TEM

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We have known for about 2500 years that the world is made of atoms. But only recently have we learned that the well-defined arrangement of atoms in two dimensions leads to materials with extraordinary solid-state and quantum properties. And it is precisely in these well-defined thin materials that we are beginning to use the developments in aberration-corrected low-voltage electron microscopy that have taken place in parallel to image individual defects including their movement in these thinnest materials and to modify the material in a targeted manner. In the lecture we show selected examples.

Unraveling the structure-property relationship of adsorbents and photocatalysts derived from 2D layered materials of natural and synthetic origin

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The raw and functionalized mineral-based materials are promising for use in a wide variety of potential technological applications. Some examples include: selective adsorption, (photo)catalysis, polymer-clay nanocomposites, sensing applications, drug delivery, photovoltaics, energy harvesting and optoelectronics. The characteristics of the parent mineral material have a key effect on the properties and subsequently the functionality of the final material. These include chemical composition, structure, textural parameters and morphology.

The materials which meet the requirements of specific applications can be directly obtained from numerous geological deposits available worldwide. One example are clay minerals which are aluminum silicates having a 2D layered structure. They are traditionally used in ceramic and paper industry, foundry, drilling, as well as in adsorption technologies. Another interesting group of 2D layered structures are layered double hydroxides (LDH) which in contrast to clay minerals do not form deposits of economic value. However such structures can be easily synthesized. The clay minerals and LDH possess surface charge that results in cation and anion-exchange properties, respectively. This makes them perfect candidates in terms of adsorption of cationic and anionic species. Both the clay minerals and LDH show high diversity in terms of chemical composition, usually form plate-like micrometer- or nanometer-sized particles and easily form colloidal suspensions in water. The knowledge on their modification involving intercalation and grafting reactions as well as doping with other materials has greatly evolved giving rise to new applications.



Figure 1. TEM images of synthetic kaolinite nanotubes.

The presentation will provide selected examples of functional materials derived from clay minerals (mainly kaolin group minerals) as well as synthetic LDH structures. In particular the lecture will focus on mesoporous nanotubular particles which can be derived from natural kaolinite mineral [1, 2]. The synthesis approaches for nanotubes formation and their properties will be discussed with emphasis on characterization with microscopic techniques. Finally the potential applications of kaolinite nanotubes will be presented with focus put on their loading with semiconductors (e.g. TiO_2) for photodegradation of pollutants investigated by our research group.

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Atomic Scale Observation of phase transition in NiFe₂O₄

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Abstract

Phase transitions from spinel to rock-salt structures are of considerable interest and have practical implications across various fields, particularly in information storage and energy conversion and storage. Extensive research employing X-ray diffraction (XRD), neutron scattering, and X-ray absorption spectroscopy (XAS) has shed light on the statistical transition mechanisms and pathways.[1] However, due to the limitations of the techniques used, the transition process in localized structures—which would clarify how the transition is influenced by local lattice and chemical environments—remains largely unexplored. Factors such as local lattice symmetry and chemical bonds could be crucial determinants of phase transition behaviors, necessitating an investigation of the transition process at the atomic level.[2] Furthermore, the correlation between atomic structure and electronic structure during phase transitions has not been fully established. Transmission Electron Microscopy (TEM) combined with electron energy loss spectroscopy (EELS) provide the opportunity to observe the real-time atomic migration and electronic structure evolution during phase transitions.

In this study, we aim to investigate the phase transition of spinel-structured NiFe₂O₄ films on MgAl₂O₄ substrates, driven by an electron beam. To accomplish this, we employ the JEOL ARM 300F2 housed within our TRACE EM unit, we monitored the real-time evolution of both atomic and electronic structures during phase transitions. Using imaging techniques including High-Angle Annular Dark-Field Scanning Transmission Electron Microscopy (HAADF-STEM) and Annular Bright-Field Scanning Transmission Electron Microscopy (ABF-STEM), we observed the dynamic migration of tetrahedrally coordinated Fe atoms to adjacent octahedrally coordinated sites driven by the electron beam. Coupling with STEM image simulations and quantitative image analyses, we were able to uncover the subsequent reconstruction of octahedrally coordinated Fe/Ni atoms and corresponding lattice dynamics. This provided a comprehensive depiction of the atomic rearrangement pathways throughout the phase transition process. By acquiring EELS spectra at various stages of the transition process, we were able to monitor the real time chemical valence states of Fe, Ni, and O in NiFe₂O₄. Our observations revealed a valence shift in Fe from +3 to +2, while the valence state of Ni remained constant throughout the transition. Combing the atomic migration and chemical valence, we were able to correlate the atomic structure and electronic structure during phase transitions.

By tracking the degree of transition in various regions under identical beam conditions, we discovered site-preferred transitions, which highlighted the stability of the spinel structure under electron beam affected by lattice constraints and local chemical environment. This observation concurrently revealed the nucleation and growth of rock-salt crystals along the specific surface of NiFe₂O₄. Moreover, by fine-tuning the irradiation condition and scanning

parameters, we effectively controlled both the growth boundary and rate, showcasing potential applications in nanodevice development. Thus, The atomic and electronic dynamics elucidated in this study not only provide opportunities for investigating functional materials in the realms of energy and information technology but also presents promising in material processing and nanodevice fabrication.

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MS4.2

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Abstract

Rare earth elements (REE) are considered critical metals that are used as catalysts and magnets in low-carbon technologies and high-performance electronic sector. China leads the world in reserves and mine production of REE. Their recovery from non-traditional sources and waste is a topic of intense research of our team. Synthetic REE-enriched lead phosphates are potentially new important material in industry due to its high efficiency of REE removal (97-100%) from aqueous solutions by rapid precipitation. In such process, the obtained precipitate is a mixture of well-crystallized pyromorphite with partial REE substitutions, (Pb,REE)₅(PO₄)₃Cl, associated with a REE-Pb phosphate phase that is poorly crystalline (Figure 1), which was confirmed by powder X-ray diffraction. While secondary electron observations are excellent for detecting variation in the crystal habit of pyromorphite depending on the conditions as well as for the quantitative determination of proportion of the phases precipitated, the detailed determination of the chemical composition of the second phase remains unresolved due to technical limitations. Neither energy dispersive spectroscopy (EDS) nor wavelength dispersive spectroscopy (WDS) analysis is feasible due to the fine crystallinity of second phase. Moreover, the aggregates always contain a mixture of both phases. Physical separation of the two phases is impossible due to their fine crystallinity. This also makes it inapplicable to conduct classical wet chemical analysis. Selective dissolution also remains unsuccessful since experimental tests prove that their solubility is almost identical. Selective precipitation has so far been unsuccessful: despite changing the molar ratios of the components or pH, the product is always a mixture of two different REE-Pb phosphates formed under all conditions tested. Therefore, at this stage of the research, the chemical formula of this phase is unknown due to the difficulties of elemental microanalysis.

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Figure 1. Backscattered electron image of a mixture of a REE-enriched precipitates. Brighter crystals are REE-enriched pyromorphite.

Neutral Atom Microscopy: Large Area Imaging of Nanoscale Topographies

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Abstract

Nanoscale thin film coatings and surface treatments are ubiquitous across industry, science, and engineering; imbuing specific functional or mechanical properties (such as corrosion resistance, lubricity, catalytic activity and electronic behaviour). However, non-destructive nanoscale imaging of thin film coatings across large lateral length scales, crucial to a wide range of modern industry, remains a significant technical challenge [1].

Neutral atom microscopy is an exciting new technology that exploits the low-energy and inert nature of helium atomic beams to deliver a uniquely surface sensitive imaging instrument [2]. Moreover, by harnessing the unique nature of the helium atom–surface interaction, the scanning helium microscope (SHeM) images these surfaces without altering the sample under investigation. Since the helium atom scatters exclusively from the outermost electronic corrugation of the sample, the technique is completely surface sensitive. Furthermore, with a cross-section that is orders of magnitude larger than that of electrons, neutrons and photons, the probe particle routinely interacts with features down to the scale of surface defects and small adsorbates (including hydrogen).

Here, we highlight the capacity of neutral helium microscopy for sub-resolution contrast using an advanced facet scattering model based on nanoscale features. By replicating the observed scattered helium intensities, we demonstrate that sub-resolution contrast arises from the unique surface scattering of the incident probe. Consequently, it is now possible to extract quantitative information from the helium atom image, including localised ångström-scale variations in topography [3]. Looking ahead, once a material system is well characterised with SHeM, such analysis could readily become a routine part of quality control; a valuable tool for improving production yields and throughput.

Figures/Tables



Figure 1 SHeM micrographs and associated reconstructed height maps for gold contacts evaporated onto silicon wafer substrates. a. SHeM micrograph of gold thickness calibration bar with laterally defective (upper) and ideal (lower) gold contacts. b. Corresponding reconstructed height map from a. c. SHeM micrograph of ideal (upper) and vertically defective (lower) gold contacts. d. Corresponding reconstructed height map from c. The white arrows correspond to locations of lateral shape defects deliberately introduced into the evaporated structure.

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Domain walls properties of lead-free BiFeO₃ ferroelectrics under static and dynamic conditions

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Abstract

Perovskite ferroelectrics, materials that exhibit spontaneous polarisation at temperatures below the Curie temperature due to a phase transformation from a cubic-centrosymmetric to a non-centrosymmetric structure with lower symmetry, are of great importance in modern electronics, being an active component in capacitors, ferroelectric random access memories, sensors, transducers, or actuators. Lead zirconate titanate (Pb(Zr,Ti)O₃)-based materials have many excellent properties, but due to regulatory restrictions [1], lead-containing materials cannot be used in commercial products, so great efforts have been put into the development of more environmentally friendly lead-free materials. One such material for high temperature applications is BiFeO₃ (BFO).

Boundaries separating regions (called domains) within the ferroelectric material with different polarisation vector orientations are domain walls (DWs), which have significantly different properties than the matrix material. Since the orientation of polarisation can by definition be changed (switched) under the external electric field, the study of the dynamic properties of DWs is of high priority.

In our previous studies of BFO ceramics [2, 3], we found that defects such as Bi vacancies and Fe^{4+} accumulate in the DWs, which increase the electrical conductivity and pin the DWs. We also reported that DWs in BFO are not smooth and straight but have step-like morphology and that the thickness, strain distribution, and structure are different in uncharged and charged DWs [4].

In the present work, we report the interaction between the electric field and DWs in BFO single crystals at atomic level using in situ scanning transmission electron microscopy (STEM - Jeol ARM 200 CF). Samples were prepared in a parallel plate capacitor configuration on dedicated chips (Protochips - Aduro) with patterned electrodes processed by a focused ion beam (FIB - FEI Helios Nanolab 650) with gallium ion source. The capacitor-like device has the advantage of creating homogeneous electric field compared to other TEM voltage bias in situ configurations, such as probe techniques [5, 6]. In our studies, we used electrostatic finite element model calculations to determine the distribution of the electric field within the region of interest [7].

Information about the polarisation vector is obtained indirectly from the Fe-displacement in respect to the centre of the Bi sublattice map, which is determined from atomically resolved High Angle Annular Dark Field (HAADF) images [3]. The iron displacement is proportional

to the polarisation vector but points in the opposite direction. Alternatively, in some cases 4DSTEM using pixelate detector (Merlin - Quantum Detectors) has been used to determine the polarisation direction under static conditions.

The experimental setup and measurement methodology were extremely complex, so part of the work will be devoted to explaining the influence of various experimental parameters such as sample preparation conditions, sample thickness, acquisition of HAADF images, interpretation of 4DSTEM data, such as centre of mas and/or differential phase contrast on the final results.

The present work contributes to the fundamental understanding of the interaction of DWs with the electric field in monocrystalline BFO. We show down to the atomic level that it is a complex interaction involving movement of DWs, change of DW plane, change of unit cell distortion and strain distribution.

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New Insights into the molecular and cellular composition of the brain barriers

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Abstract

The brain is protected by both physical and biological barriers. The brain's blood vessel walls are equipped with a blood-brain barrier, and the choroid plexus and the meninges are equipped with blood-cerebrospinal fluid barriers. To ensure that the brain is reached by wanted molecules, such as glucose, at the same time as it is protected by potentially neurotoxic substances circulating in the blood, the cells that form the barriers are equipped with efficient and specific molecular transporters. To learn more about these barriers, we have analyzed the gene expression in the barriers' cell types at the level of single cells using a technique referred to as single-cell RNA sequencing (scRNAseq). We identified distinguishing markers for individual barrier-forming cells types and subtypes, as well as other neighboring cell types, and used the markers to map the position of the cells in in vivo. I will present work in which we combined scRNAseq with immunofluorescence, in situ hybridization, transmission electron microscopy and immuno-EM to arrive at highly granular molecular anatomic maps of the brain barriers. This work revealed a surprising degree of cellular and molecular complexity and sophistication of the meninges, in particular the barrier located within the arachnoid mater.

Physiological role of ATP in the inner ear

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Abstract

Painfully sustained loud sound (90-120 dB, >6h) causes protective or withdrawal responses, rather than continued listening. Purinergic P2X receptors are ligand-gated cation permeable channels which mediate adaptation to loud sounds. In the cochlea, extracellular ATP-activated P2X2 receptor channels play an important role in adaptation to elevated sound by producing a cation shunt that reduces the endolymphatic potential and the driving force for sound transduction. There has been no clear evidence that how extracellular ATP modulates the function of cochlea in regulating cochlear sensitivity in order to provide protection during loud sound exposure. In this study, we have investigated the physiological role of extracellular ATP upon activation of ATP gated cation P2X receptor channels in the inner ear by performing physiological and electrophysiological measurements, cochlear mechanics, time-resolved confocal microscopy, whole-cell patch in combination with high-resolution confocal imaging, and measurements of loud trauma. Through confocal imaging, we confirmed the localization of P2X receptor channels in the mature guinea pig hearing organ displaying a strong distribution in the Reissner's membrane and faint expression in Hensen's and hair cells (Fig.1). After application of ATP extracellularly in the organ of Corti via perfusion, a dramatic effect on sound-evoked electrical potentials and stereocilia deflection was observed in contrast to application of ATP in the scala media. After removal of ATP, the effects disappeared. Contraction of the hair cells and supporting cells were observed when ATP was injected or perfused in the organ of Corti (Fig 2, 3). Loud sounds had effects on the release of ATP inside the organ of Corti. At Hensen cells level there was a slight alteration in the vibrations after ATP application. Fast application of ATP to a whole-cell patch from Reissner's membrane epithelial cells (Fig. 4) expressing the P2X2 receptor evokes rapid currents change recorded at-60 mV holding potential. Our data shows the influence of extracellular ATP on sound-evoked responses in the perilymphatic compartment of the hearing organ. Collectively, the results strongly reveal a distinct mechanism in cochlea on how extracellular ATP plays role in protecting the hearing organ from loud sound.



Figure 1 A 3D reconstruction of organ of Corti showing localization of P2X receptor channels in the Reissner's membrane and faint expression in Hensen's and hair cells. **Figure 2** Morphological changes in the hair cells after injecting ATP in the scala media in the organ of Corti. **Figure 3** Contraction of supporting cells when ATP applied via perfusion through scala vestuibili in organ of Corti. **Figure 4** A whole cell patch of Reisnner's membrane epithelial cell in the organ of Corti.

3D Optical Kidney Pathology

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Abstract

Morphological alterations to the kidney filter, particularly to podocyte foot processes, are seen in most types of glomerular diseases. Due to the nanoscale dimensions of the filter, visualization of such alterations has historically relied on electron microscopy. However, with recent technical development, it is now possible to visualize podocyte foot processes as well as other parts of the kidney filtration barrier also with light microscopy. With developments both in sample preparation, imaging and image analysis [1,2,3,4,5], 3D optical kidney pathology is here presented with its quantitative potential of stratifying kidney diseases [6].

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AuNP Loaded Liposomes: A tool for characterizing the uptake of soft nanomaterials in biological systems via TEM

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Abstract

Liposomes and other lipid based nanoparticles are of particular interest for the treatment of a number of diseases[1]. The challenge, however, comes in characterizing the uptake of these nanomaterials *in vivo* via electron microscopy. Liposomes and lipid nanoparticles are difficult to distinguish from the surrounding biological material both *in vitro* and *in vivo*. Often, cellular vesicles are indistinguishable from liposomes within cells and tissue and as a result it is very easy to confuse the two and have both false positives and negatives.

My colleagues have developed a novel method to nucleate individual gold nanoparticles within existing liposomes using redox-gradient method[2]. This resulted in the formation of very uniform AuNPs, dependent on liposome size and reducing agent concentration. Single AuNPs were formed in approximately 35% of liposomes[2]. These AuNPs loaded liposomes are very uniform as shown in Figure 1 and easily distinguishable from the surrounding tissue as shown in Figure 2.

The ability to directly observe liposomes within tissue samples could play a major role in deciphering the uptake mechanisms for liposomes in specific disease cases and lead to improved nanoparticle design and disease treatment.

Figures/Tables



Figure 1: Cryo TEM image of AuNPs formed within unilamelar Liposomes.



Figure 2: TEM image of AuNP loaded liposomes in Kupffer cells in a mouse liver. **References**

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In and ex situ (S)TEM manipulation of 2D materials

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Structural engineering is the first step toward changing properties of materials, but has remained a challenge for 2D materials. The difficulties range from the preparation of clean and uniform samples to the sensitivity of these structures to the overwhelming task of sample-wide characterization of the subjected modifications at the atomic scale. I will demonstrate how these issues can be overcome using a near ultrahigh vacuum system comprised of an aberration-corrected scanning transmission electron microscope and setups for sample cleaning and manipulation, which are combined with automated atomic-resolution imaging of large sample areas and a convolutional neural network approach for image analysis. This combination of methods allows creating and fully characterizing atomically clean 2D materials, that can be further manipulated through introduction of defects and impurity atoms at the atomic scale.

Nanofluidic liquid phase transmission electron microscopy

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Abstract

Liquid phase transmission electron microscopy (LPTEM) has emerged as a valuable technique for investigating liquid processes at the atomic scale and with high temporal resolution in various fields such as physics, chemistry, materials science, and bioscience[1]. The use of ultrathin electron transparent membranes, particularly silicon nitride, has become widespread as a window material for LPTEM. Recent advances in bonding technology[2] have facilitated the patterning of nanochannel structures on suspended SiNx membranes, allowing for controlled liquid thickness down to tens of nanometers in a liquid cell with a membrane thickness of only 10 nm. The resulting nanofluidic LPTEM system has a well-defined flow profile that enables instant liquid mixing in the field of view and liquid exchanging. The excellent features of nanofluidic LPTEM, including its capacity to offer high-resolution, phase-contrast imaging[3], and quantitative spectroscopy analysis[4], make it a highly suitable approach for investigating liquid processes.



Figure 1. Nanofluidic LPTEM. a) a CAD drawing of the liquid cell. The cell has 4 inlet/outlets connected by two deep and wide microchannels (cyan color). The two microchannels are connected by the nanochannels (dark region in the center) with different designs. The top chip is made of silicon, and is not transparent in reality. b) Schematic drawing of the nanochannel cross-section. The liquid sample is confined in nanochannels. c) A low magnification bright-field TEM image of the nanochannels filled with liquid sample.

Quantification of water intrinsic properties in TEM using nanofluidic LPTEM:

Mean inner potential (MIP, V₀) of liquid water: The unscattered electrons' phase shift after passing through the sample carry information of the samples atomic constituents and potentials. Combining off-axis electron holography with nanofluidic LPTEM as shown in Figure 2a, b, we provide the first quantitative MIP measurement of liquid water V₀=+4.48±0.19 V [3]. This value is larger than most theoretical predictions [5] and to explain the disagreement we assess the dominant factors needed in quantum simulations of liquid water[6]. Electron λ_{IMFP} in liquid water: The electron energy-loss spectroscopy (EELS) log-ratio method is often applied in TEM to estimate the sample thickness relative to the inelastic mean free path (λ_{IMFP}) [7, 8]. We used nanofulidic LPTEM to estimate the electron λ_{IMFP} in liquid water, as shown in Figure 2c,d. We observe good agreement with the studies conducted on ice [9] but find that the widely accepted theoretical models[7, 10] significantly underestimate electron λ_{IMFP} in water.



Figure 2. (a,b) **MIP** (V₀) **of liquid water**[3] (a) Reconstructed phase image; the phase shift calibration bar unit is radian. Scale bar is 50 nm. (b) A phase profile along the *x*-direction. (c,d) **Measurement of electron** λ_{IMFP} **in water**[4]. (c) EELS spectra and (d) summed spectrum from the regions indicated in c. The signals are normalized to the maximum zero-loss peak intensity.

Soft matter and chemical reaction imaging using nanofluidic LPTEM:

Using the nanofluidic LPTEM, structures can be determined using 3D electron diffraction (Figure 3a) at room temperature in liquid. Low contrast materials can be imaged directly as shown in Figure 3b. Additionally, the nanofluidic LPTEM enables accurate control of chemical environments and mixing time (Figure 3c).



Figure 3. The nanofluidic LPTEM allows us to determine (a) the crystal structure of a novel glycine metal-cluster compound using 3D ED and (b) A virus bacteriophage T4 in liquid. (c) $CaCO_3$ nucleation occurs in the side pocket (Figure 1c) while flowing reagent in the main channels.

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3D Electron Diffraction / MicroED for Crystal Structure Determination

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Abstract

X-Ray Diffraction (XRD) has been an indispensable tool in structural chemistry, structural biology and material science. Despite the evolution in laboratory X-ray and synchrotron sources that have occurred in the last decade, a significant roadblock in the determination of molecular structures is the ability to grow crystals of sufficient size and quality which can be timeconsuming, complicated, requires a large amount of purified molecules, or even unfeasible for many biological macromolecules and pharmaceutical compounds. Three-Dimensional electron diffraction (3D ED), also known as Micro-crystal electron diffraction (MicroED), is an emerging and game-changing technique with the potential to overcome these challenges as it allows structure determination of crystals 1 billionth the size of those used for SCXRD¹. Researchers from several labs have developed different experimental set-ups for data acquisitions, for example automated diffraction tomography $(ADT)^2$ and rotation electron diffraction (RED, Stockholm University)³. In 2013, Gonen's lab successfully developed the MicroED technique for the determination of macromolecular crystal structures⁴. 3D ED/MicroED is still in its infancy. Further development is required in order to unleash the full potential of 3D ED/MicroED applications in materials science, structural chemistry and structural biology. At SCANDEM 2023, I would like to present our latest development in specimen preparation, data collection and processing, key examples, ongoing method developments and future perspective of 3D ED/MicroED.

Figures/Tables



Figure 1 – An overview of 3D ED/MicroED

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Imaging hard and soft materials with atoms

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Recent advances in atom beam microscopy are presented be discussed with a focus on a new platform developed in Cambridge, now commercially development under at Ionoptika Ltd. The novel capabilities of the instrument are shown including simultaneous multi-point detection. The new platform has enabled significant improvements in capability which are demonstrated using several applied samples, including bacteria and bacteria biofilms now accessible due to improvements in

resolution, but measured with no surface treatments required and 2D materials where diffraction from the surface is used to enhance contrast and demonstrate а particularly interesting contrast mechanism.

Atom beams of noble gasses particularly, have been used for surface science for many years but it's application to real space imaging through spatial resolving power is more recent, with the first reflection mode images reported in 2011 in Cambridge UK and Newcastle AUS. Rapid development has occurred since then in all major components of the apparatus, and here the improvements creating beams efficiently, sample in environment, scattering geometry changes and detector efficiencies are discussed. The capabilities have enabled significant growth in the application of the technique, including chemical and diffraction enhanced contrast, Figure 1 from top: Detection angle scans across reconstruction.

SHeM has been shown to re veal topographical structure completely noninvasively i.e. with no addition of a surface scattering distribution. Angularly misaligned coating or contrast agents, which has areas with the same structure appear less significant applications in studying delicate intensely.



depth profiling, real time 3D surface the full azimuthal range assembled into the inplane diffraction pattern for MoS2, c) shows an image measured with the optimum diffraction geometry highlighting the areas with the same

samples.



Figure 2 micrograph showing pseudomonas aeruginosa bacteria measured with SHeM.

In the current work there is a focus on two applied sample groups, the first is 2D materials, particularly MOS₂ where we demonstrate a capability to selectively image surface regains that are in the same phase, and thus when a phase change occurs. We also discuss some recent studies of soft samples where we address topics such as the role of an extra cellular matrix in determining bacterial biofilm formation and growth, leading to understanding on how the microenvironment mechanical regulates biological cell processes responsible for growth and transmigration. SHeM provides the unique ability to image the important interactions between surface topography and bacterial biofilm formation and growth without damaging or influencing the delicate biology of these systems.

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Zeolite intergrowth revealed using 4D-STEM

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Abstract

Zeolites are nanoporous crystalline aluminosilicates widely known due to their prominent properties, such as high catalytic activities or selectivities and high ion exchange capacities. Intergrowing zeolites material can be formed when the synthesis conditions are chosen such that more than one zeolite phase have similar energy of formation. The characterization of intergrown materials face challenges when studied by traditional techniques such as X-ray diffraction or the complementary method 3D electron diffraction, since these techniques provide average structural information. However, transmission electron microscopy can overcome these obstacles and provide local structural information of the intergrowth in the material. Using the 4D scanning transmission electron microscopy (4D-STEM) method, a small electron probe is scanned across the chosen area and acquires diffraction data for each beam position to build up a map. Thereby, 4D-STEM is a valuable technique to obtain detailed information to describe the local crystallographic arrangement in zeolites.

Here we demonstrate an intergrowth between the zeolite structures ERI and CHA investigated using 4D-STEM. The 4D-STEM data confirm the presence of the two different phases in a single crystal. The crystals aligned close to [100]_{ERI} direction studied with 4D-STEM show different types of intergrowth, see Figure 1. The CHA phase either grow as a distinct layer in the center of the ERI crystal or with a domains-like structure. Figure 1a,b shows ordered domains of CHA and ERI whereas in Figure 1c no pure CHA pattern are found since the domains of CHA are smaller than the thickness of the material. These results show that 4D-STEM is a powerful characterization technique for the analysis of complex intergrown materials.



Figure 1. 4D STEM data from three different ERI/CHA materials. Virtual dark field images (left in each panel) were constructed by filtering the map based on characteristic reflections of either ERI (middle) and CHA (bottom) respectively, marked in the corresponding diffraction pattern (right in each panel). The total intensity provides a map of the general morphology (top).

Data-driven microscopy allows for automated context-specific acquisition of high-fidelity image data

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Abstract

Light microscopy is a powerful single-cell technique that allows for quantitative spatial information at subcellular resolution. However, unlike flow cytometry and single-cell sequencing techniques, microscopy has issues achieving high-quality population-wide sample characterization while maintaining high resolution. I will present a general framework, data-driven microscopy (DDM), that uses real-time population-wide object characterization to enable data-driven high-fidelity imaging of relevant phenotypes based on the population context. DDM combines data-independent and data-dependent steps to synergistically enhance data acquired using different imaging modalities. We have developed plugins for improved high-content screening and live adaptive microscopy for cell migration and infection studies that capture events of interest, rare or common, with high precision and resolution.

High-content imaging-based serology test for SARS-CoV-2

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Abstract

Serological assays are essential for studying and controlling infectious disease outbreaks. However, widely used immunofluorescence microscopy-based serological assays often require a high biosafety level facility for handling the virus, are often low-throughput, and the images are only visually inspected by the expert. In this work [1,2], we present a high-throughput microscopy-based and machine learning-guided serology assay and demonstrate its efficacy using SARS-CoV-2 as a model pathogen (Fig. 1).

We transfected Vero E6 cells with plasmids encoding four SARS-CoV-2 antigens: spike protein (S), nucleoprotein (N), the receptor-binding domain (R), and membrane protein (M). These cells expressing viral antigens were grown in 384-well plates and fixed prior to the addition of patient sera. We used multiplexed immunostaining to label DNA (Hoechst 33342) and three immunoglobulin antibody classes: IgG, IgA, and IgM. Altogether, four plates were imaged separately for each antigen. The experiment was repeated three times, and after quality control, images from 16 plates were used to develop an automated and machine learning-based analysis pipeline in single-cell resolution. We used image data of another 16 plates prepared in a separate experiment as evaluation data.

All plates were run through an automated image analysis pipeline including nuclei segmentation using the pre-trained nucleAIzer model [3] followed by feature extraction on the single-cell level. These features were used to predict whether a cell gives a positive or negative response in the given antigen-antibody combination. We tested different classifiers and optimized the hyperparameters of these models using cross-validation. Once the hyperparameters were optimized, we trained a model for each antigen-antibody pair using the whole training dataset. These models were used to predict the evaluation dataset to output positivity ratios for each sample. We found that the models can classify individual cells accurately with specificity 0.96-0.97, 0.95-0.96, 0.96-0.97 and sensitivity 0.84-0.89, 0.79-0.84, 0.82-0.86, for IgG, IgA, and IgM, respectively. Further, six experienced virologists/cell biologists annotated 576 images as positive, negative or unclear. The models' quality was assessed to the expert consensus by measuring the area under the receiver operating characteristic curve (AUC). For IgG, the model performed at an AUC of 0.98, 0.97, and 0.98, and for IgA it performed at an AUC of 0.96, 0.96, and 0.89 for S, N, and R antigens, respectively (Fig. 2).

The assay presented can be used for serological study of 1000+ patients per day with a single microscope and minor human interaction with the automated analysis pipeline. The assay could moreover be used for other pathogens, though we recognize that the machine learning

Program



Figure 1. The assay workflow.



Antigen — N — S — R

Figure 2. IgG and IgA antibody ROC curves for N-, S-, and R-antigens.

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Abstract

The development of nanoparticle technologies has seen significant growth over the past decade, with applications in a wide range of fields including material and life sciences. As a result, the demand for particle characterization has increased substantially. Amongst the methods available for particle characterization, Electron Microscopic (EM) technologies in contrast to indirect methods like Dynamic Light Scattering and Nano Tracking Analysis result in high resolution images that provide morphological information and unambiguous particle identification. EM however requires an experienced operator and can be time and resource consuming. A method allowing to simplify the analysis process with an automated image acquisition of samples as well as an automatic image analysis on a low-voltage electron microscope is proposed herein.

Such automation capabilities prove relevant for example in the domain of the vaccine industry, where new requirements from the United States Pharmacopeial Convention (USP) and the U.S. Food and Drug Administration (FDA) put pressure for root cause analysis to understand impact on changes during process and product development. The new demands involve morphological characterization of the particles present in the formulations, typically nanoparticles like viruses, virus-like particles and adjuvants in the range of $0.01 - 0.1 \mu m$ [1]. When entering production phases of such products, the high throughput of specimen to be analyzed can be a limiting factor for electron microscopy to be used as a method of choice. We therefore introduced a process that can allow a rapid and efficient characterization of specimen, using a low voltage instrument, the MiniTEMTM (Figure 1).

The instrument consists of a low voltage (25 kV) transmission electron microscope and software including a simple control of the microscope and automated features for imaging and analysis. The instrument is small and requires only a single standard power outlet, allowing the system to be placed in any lab or office area. The simplified design of the instrument, together with its automated analysis features allowing particle detection and classification (Figure 2) while screening makes its use easier to apprehend for users with limited experience in electron microscopy.

A case study involving the characterization of biological nanoparticles in which the specimen was automatically analyzed will be presented and discussed.

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Figure 1. The MiniTEM system.



Figure 2. Representation of a typical analytical modular workflow for an adeno-associated virus particle sample with the image before and after automated analysis. The primary particles are segmented with red circles while proteasomes are segmented with blue circles. Scale bar represents 500 μ m.

Massively multiplexed single-molecule fluorescence microscopy

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Abstract

Cells critically rely on a wide range of genome-processing and nucleic acid-interacting proteins. Single-molecule fluorescence microscopy has long been appreciated as a powerful tool to study the often-complex structural dynamics that enable the biological function of these sophisticated protein machines. The sequence of their nucleic acid substrates will inevitably shape their energetic and dynamic landscapes, and massively multiplexed single-molecule observations of their complex dynamics as a function of sequence will therefore greatly facilitate an in-depth mechanistic understanding of key genome transactions. Given the inherently low throughput of single-molecule techniques, this cannot be achieved with currently existing methods. We have therefore developed a novel high-throughput platform that combines, for the first time, single-molecule measurements of complex dynamics with next-generation sequencing. This platform enables the comprehensive profiling of sequence-dependent processes at the single-molecule level. Here I will discuss examples of how we have leveraged this platform to gain new insights into the sequence-dependent mechanisms of fundamental nucleic acid transactions.

Figures/Tables



Figure 1 Single-molecule measurements of complex dynamics as a function of sequence. A FRET-labeled nucleic acid library is immobilized by hybridization to the oligonucleotides on the Illumina flow cell surface. Single-molecule fluorescence microscopy reveals dynamics and surface coordinates for individual members of the library (1). Subsequent bridge amplification and sequencing in the Illumina instrument (2) yield, upon spatial registration (3), the sequence underlying each smFRET trajectory.

Advances in high energy and spatial resolution STEM EELS

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Abstract

Engineering the structural or chemical architecture of functional materials at the nano or even atomic level enables emergent properties that rely on the interplay between fundamental properties of matter such as charge, spin and local atomic-scale chemistry. Thanks to advances in monochromators, state-of-the-art electron energy loss spectroscopy (EELS) in the scanning transmission electron microscope (STEM), offers nowadays the ability to map materials and atomic structures with an angstrom size electron beam and an energy resolution for EELS under 5meV. (Krivanek *et al.*, 2014) These capabilities have allowed to probe the spectroscopic signature of phonons down to the single atom level. (Hage *et al.*, 2020) Here, we present strategies for high spatial and energy resolution STEM-EELS experiments to interrogate proximity effects of heterostructure materials at the atomic scale and present opportunities for new experiments using monochromated electron probes.

Bi₂Se₃ is a topological insulator (TI) with topologically protected helical two-dimensional surface states and one-dimensional bulk states associated with crystal defects, in proximity with graphene. The strong spin-orbit interaction and proximity effects result in subtle and controllable electronic band structure changes at and near the interface, with exciting potential for spintronic applications. Here we probe at high energy resolution the interfaces in a system consisting of Bi₂Se₃ films grown by chemical vapor deposition on epitaxial graphene/SiC(0001), where the number of carbon layers can be carefully controlled to tune possible proximity effects between the film and the substrate. In addition to a direct interrogation of the chemical bonds (Bugnet *et al.*, 2022) between the layers via their vibrational response, these observations are linked to the interplay between the various phonon modes and the Dirac plasmons in the TI layers, whose dispersion is mapped in momentum space with nm spatial sensitivity using a recently developed methodology for nanoscale momentum-resolved spectroscopy.

Magnonics is an emergent field within spintronics utilizing the ability to generate and propagate controllably a spin-wave in nm-sized magnetic structures, with a view to build a new generation of devices for data processing and storage. It was also recently demonstrated that magnons can be utilized to convert spin to charge (or charge to spin) currents, a critical step for integration of spin and charge devices. STEM EELS is proposed to be a leading candidate technique to attempt the detection of magnons at the nanoscale, perhaps down to at the atomic scale, thanks to a recently developed theoretical calculation framework and preliminary experimental investigations (Lyon *et al.*, 2021). Here, we report on progress in

designing experiments building on these initial steps using in-situ STEM-EELS. The prototypical spin-to-charge conversion system considered for this proof of principle attempt consists of Yttrium Iron Garnet (YIG)/platinum (Pt) bilayer, a widely used materials combination where a magnon created by a thermal gradient creates spin accumulation at the YIG/Pt interface, which subsequently diffuses into the nonmagnetic Pt, and via inverse spin Hall effect (ISHE) creates a voltage signal in the Pt layer.

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Exploring Topological Features in Materials: Advancements and Challenges in Electron Microscopy Characterization

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The rapid development of materials science and engineering has led to an increasing demand for advanced characterization techniques to better comprehend the intricate microstructures and their unique properties. Over the past two decades, electron microscopy (EM) has experienced substantial progress, thanks to improvements in both hardware and software technologies. Cutting-edge developments in EM, such as aberration-corrected imaging [1,2], monochromated spectroscopy [3,4], direct electron detectors [5], and stable cryogenic stages and biasing holders [6], have empowered researchers to observe many of these properties in real-time with exceptional spatial and energy resolutions.

However, topology, an intrinsic material property, has not been fully explored and characterized using electron microscopy. Topological properties pertain to distinct features that arise or remain unaffected due to deformation, twisting, defects, or material disorder. The Hall effect and the emergence of superconductivity in twisted bilayer graphene, the later which has led to the development of Moiré materials engineering, are two examples of topological properties.

In this presentation, I will discuss the characterization of Moiré exciton bands in Moiré superlattices, created by vertically stacking two-dimensional semiconducting transition metal dichalcogenides (TMDs) with slight lattice mismatch and/or rotational alignment. I will elucidate the coupling of phonons and Moiré potentials with excitons, as well as the extent of localization of this coupling in momentum and real space and its dependence on temperature. Furthermore, I will address the experimental conditions and limitations for investigating Hall transport properties with (sub)nanometer resolution in the electron microscope [7].

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Unravelling oxidation mechanisms in Cu nanoparticles through *insitu* imaging and spectroscopy and the role of the electron beam in environmental STEM

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Abstract

Copper (Cu)-based nanoparticles in their metallic and oxide forms are of interest for several applications, including catalysis, nanoelectronics, photonics and nanomedicine [1-3]. Therefore, real-time probing of the complex processes occurring during the oxidation of Cu nanoparticles, and their implications for the properties of the particles is hugely beneficial. We have applied environmental scanning transmission electron microscopy (ESTEM) to record in situ the oxidation of hemispherical Cu nanoparticles at different temperatures and examine in detail key stages of their oxidation driven by different mechanisms. The results reveal a rich multi-mechanism process and show how the prevalence of the Cabrerra-Mott, Valensi-Carter and Kirkendall mechanisms under different conditions impact the morphology of the oxide particles; this ranges from a self-limiting few nanometer thick oxide shell at lower temperatures to the formation of a hollow nanoparticle with a double oxide shell at higher temperatures (Figure 1) [4]. Moreover, using time-resolved electron energy-loss spectroscopy (EELS), we examine and describe the signature of these oxidation mechanisms on the localized surface plasmon resonance (LSPR) signal from individual particles. Uncovering such a link between the morphology and optical properties of Cu nanoparticles is highly relevant for nanoplasmonic sensing applications.

As the electron beam can play an active role in ESTEM experiments, we have also investigated the influence of the electron beam on our observations. Comparing Cu nanoparticles oxidized at temperatures 100-200 °C in an O₂ environment while exposed to the electron beam in ESTEM, to those oxidized under the same conditions but without exposure to the beam, we find that overall, electron beam irradiation accelerates oxidation (Figure 2). However, while this is due to the enhancement of the outward diffusion of Cu⁺ ions from the particles, we find that the electron beam inhibits the inward diffusion of O⁻ ions into the particles. Our results suggest that the impact of the electron beam is mostly related to kinetic energy transfer, knock-on damage and charging and ionization effects, and the beam can both enhance and suppress reaction pathways.

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Figure 1: a) Schematic of the ESTEM – EELS experiment. b) Selected STEM images of Cu particles oxidized at 50 °C and 150 °C. c-d) EELS spectra containing the LSPR signal acquired from the same points around the particles at different times during oxidation.



Figure 2: STEM images of Cu nanoparticles oxidized to the same extent in ESTEM a) without and b) with exposure to the electron beam at different temperatures. δ denotes the volume fraction of the metal lost due to oxidation and time is the duration of oxidation.

ACHROMATIC ATOMIC-PLANE RESOLVED IMAGING OF ELECTRON ENERGY-LOSS SPECTROSCOPY

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Abstract

Energy-filtered transmission electron microscopy (EFTEM) and scanning transmission electron microscopy (STEM)-Electron Energy-loss spectroscopy (EELS) are two traditional methods to access the chemical information with high spatial resolution in the transmission electron microscope (TEM). Compared to STEM-EELS technique, the major obstacles in the application of EFTEM are related to its low dose efficiency, poor energy resolution (which is related to the slit width of energy window), poor signal to noise ratio and limited spatial resolution caused by chromatic aberration. Thanks to the development of spherical/chromatic aberration (Cs/Cc) corrector, the resolution of chemical imaging was pushed to atomic level which minimizes the delocalization of electron beams with different energy, and makes it possible to acquire atomic scale chemical information [1]. On the other hand, the roles of elastically scattered electrons should also be considered when studying the atomic scale chemical information, which was first theoretically predicted by Howie [2].

Under the parallel electron illumination mode in TEM, we combine the advantages of spatially resolved energy electron spectroscopy (SR-EELS) and Cs/Cc corrector to acquire atomic plane resolved (APR) EELS imaging [3] on SrTiO₃. Both elastic and inelastic scattering electron signals are collected simultaneously with different experiment setups and their relationships are discussed upon the topic of atomic plane resolved chemical information.

Combing with dynamic diffraction calculation, we compared the APR-EELS experiments with different focus condition, sample orientation, and thickness. The result shows that delocalized chemical information can be minimized by tuning crystal to three beam condition (3BC). Defocus -2nm and infocus situations are both good conditions for acquiring APR chemical information in which inelastic scattering signals are localized at certain atomic planes. The distributions of elastic scattering electrons are also related to the inelastic scattering signals. [4]

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Band gap measurements of aluminum and indium doped Ga₂O₃ multilayers.

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Power electronics (PE) play an important role in the collection, delivery, and storage of energy, and are a key enabler for energy efficiency, renewable energy, and smart grids. Silicon-based PE components have been optimized to the point where further improvements start to be limited by intrinsic materials properties. Wide bandgap semiconductors are therefore interesting for PE applications since they can be faster, smaller and more energy efficient than Si-based technology [1]. High-electron-mobility transistor based on novel $(M_xGa_{1-x})_2O_3$ thin film heterostructures (M = AI, In) has the potential to become faster, smaller and operate at higher power than the existing options, due to its ultra-high breakdown field [2]. Ga_2O_3 can be synthesized in several polymorphs, including the α -, β -, γ -, δ -, and ε -phases and has a bandgap of 4.5-4.9 eV depending on crystallographic orientation [3]. κ -Ga₂O₃ has shown to have a large polarization (23 μ C/cm²[4]), which suggests that the interface-localized two-dimensional electron gas (2DEG) can be achieved. This means that it can be used for so-called high-electronmobility transistors (HEMTs) [5]. Controlled alloying of $(M_xGa_{1-x})_2O_3$ by a group III metal provides the ability to tune properties such as bandgap and carrier concentrations, and therefore tailored to fit a given application. The band gap range obtained by using Al and In is spanned out by In_2O_3 (E_g= 2.9 eV) [6] and Al_2O_3 (E_g= 8.8 eV) [3], and the values can be calculated by eq1: $E_g = 4.91 + 2.10x$ for κ -(Al_xGa_{1-x})₂O₃ and eq2: $E_g = 4.90 - 1.95y$ for κ -(In_yGa_{1-y})₂O₃.

In this work we have investigated $(M_xGa_{1-x})_2O_3$ thin film heterostructures (M = AI, In) with various doping concentrations, thickness of layers, and substrate deposition, made using PLD and vertical (quasi-)continuous composition spread (VCCS) PLD. The morphology, crystal structure, element composition and band gaps have been measured by transmission electron microscopy (TEM) for three types of samples. For the analysis, we have used a FEI Titan G2 operated at 60 kV with a monochromator, DCOR Cs probe corrector, Super-X EDS detector, and Gatan GIF Quantum 965 electron energy loss (EELS) Spectrometer. The analysis of the EELS spectrum images (SI) and individual spectra (such as the band gap fitting) are performed by our own python-based EELS fitting program.

Scanning TEM (STEM) image of one type of sample is shown in Figure 1A. Where the top layer is $(Al_{0.3}Ga_{0.7})_2O_3$, second layer is $(In_{1.8}Ga_{0.82})_2O_3$, and the third layer Ga_2O_3 , where the substrate is indium tin oxide (ITO). The band gap has been fitted for each spectrum in the SI in Figure 1B. The average band gap for each row with the variations (in gray) is plotted in Figure 1C. The band gaps have been fitted to be 5.1 eV, 4.3 eV, and 4.8 eV, for $(Al_{0.3}Ga_{0.7})_2O_3$, $(In_{1.8}Ga_{0.82})_2O_3$, and Ga_2O_3 respectively. The band gap value for $(Al_{0.3}Ga_{0.7})_2O_3$ and $(In_{1.8}Ga_{0.82})_2O_3$ is lower than what can be calculated by eq1 (5.54 eV) and eq2 (4.45 eV). We also observe a decreasing band gap across the interfaces. Is this due to a decrease in band gap or because of the delocalization of the electron beam? The data has then been analyzed in detail and compared to the structural properties across the interface and compared with two other

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samples of $(M_xGa_{1-x})_2O_3$ deposited on ZnO substrates with different compositions and thinner layer thicknesses.



Figure 1: A) STEM image of (Al_{0.3}Ga_{0.7})₂O₃, (In_{1.8}Ga_{0.82})₂O₃, and Ga₂O₃ on ITO, B) Bang gap mapping of the SI, C) Average band gap value for each row with variations in band gap as gray, and D) Single spectrum with fitting of (In_{1.8}Ga_{0.82})₂O₃.

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Live-cell imaging of sub-membrane signalling and secretion

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Abstract

Critical to the understanding of cell function is availability of methods for recordings of intracellular regulators in single, living cells. Many key cellular events take place in the immediate vicinity of the plasma membrane. This presentation will highlight imaging approaches for the investigation of sub-membrane signalling of importance for the secretion of blood-glucose-regulating hormones insulin and glucagon from beta- and alpha-cells in the pancreatic islets. It will be shown how nutrients and neurohormonal stimuli induce dynamic changes of intracellular ATP, calcium ions and other second messengers and downstream effector proteins in the islet cells to shape the hormone release patterns.

Electron Microscopy of the Human Cochlea and Why the Crocodilians Hear So well

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Abstract

Novel ear surgery such as so-called cochlear implants (CI) have given opportunities to restore hearing to such an extent that children today born deaf can gain normal oral communication with substantial restoration of hearing. Likewise, adult patients with severe/profound hearing loss can benefit from this device. In Uppsala we have a long tradition to study the inner ear and in recent years also in humans. These studies have revealed compelling and unique features in the human organ of hearing that can partly explain how the electrically restored hearing can be maintained over many years. Electron microscopy has revealed that the spiral ganglion cell bodies in the human auditory nerve is unmyelinated, in opposite to other studied mammals. It explains why the central axons are preserved and why there is an incomplete retrograde degeneration after sensory hair cell loss. Out of 15 000 hair cells in each human ear, only 3 400 are so-called inner hair cells that are supplied by the great majority (95%) of the 30 000 neurons in the auditory nerve¹. Electron microscopy in combination with super resolution immunohistochemistry gave the answer. The molecular mechanisms behind this is partly unknown but satellite glial intercellular communication through connexin43 may play a major role.

Our laboratory works to better understand the degeneration process of the crucial sensory hair cells frequently causing human deafness. The terminally differentiated cells have a restricted survival causing age-related hearing loss. Novel strategies are sought among international researchers to explain the molecular background of the restricted regeneration and how it can be counteracted. Interestingly, in several non-mammalian species, auditory receptors undergo cell renewal after damage. This has raised hope of finding new options to treat human sensorineural deafness. Uncertainty remains as to the triggering mechanisms. We explored the auditory organ in the Cuban crocodile (Crocodylus rhombifer) and African Dwarf crocodile (Osteolaemus tetraspis) to validate possible ongoing natural hair cell regeneration using transmission electron microscopy and immunohistochemistry^{2,3}. The crocodile hearing organ (papilla basilaris) contains likewise two types of hair cells tightly connected by supporting cells coupled with gap junctions. Afferent neurons with and without ribbon synapses innervate both hair cell types. Supporting cells show signs of trans-differentiation into new hair cells expressing the MAFA and SOX2 transcription factors. Crocodilians seem to produce new hair cells during their life span from a range of supporting cells. Imposing efferent nerve fibers may play a role in regeneration and re-innervation of the auditory receptors, possibly triggered by apoptotic signals from wasted hair cells. Intercellular signaling may be accomplished by the elaborate gap junction and organelle systems, including neural emperipolesis. Remarkably, organ components showed signs of being replenished and its architecture sculpted by extracellular exosome-like proteolysis.



Figure 1. (A). Light microscopy of the hearing organ (papilla basilaris) in Crocodylus rhombifer. The hair cells (SHCs and THCs) have hair tufts reaching into the tectorial membrane (TM). The auditory organ contains a large population of SCs whose cell nuclei are located basally. The honeycombed TM extends from the homogene cells (HoCs) over the inner sulcus (IS). B. TEM of the framed area shown in (A). BM: basilar membrane, uMNF: unmyelinated nerve fibers, HyC: hyaline cells, CC: cubic cells.



Figure 2. (A). TEM of a short hair cell (SHC) of the *Crocodylus rhombifer*. (B). One afferent terminal is associated with ribbon synapses (RSs) (framed area magnified in D. (C). SHC with an RS. There is no halo of synaptic vesicles. (D). Framed area in B is magnified and shows two RSs at the afferent (aff) terminal.

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Abstract

Viruses with pandemic potential, like influenza, HIV, Zika and Ebola virus infect our cells through the process of membrane fusion. We employ single-virus microscopy to dissect this process in high detail, using microfluidic immobilization of virus-membrane conjugates and fluorescent reporter dyes. Our toolbox includes binding viral particles to 1. synthetic membranes with a defined membrane composition¹, 2. large plasma membrane-derived vesicles (unpublished data) or 3. isolated endosomes carrying viral particles². We label the viral membrane with a quenched fluorescent probe¹ and load the target vesicles with a nucleic-acid sensitive probe³. Upon synchronized triggering of viral fusion, we can measure hemifusion (i.e. lipid mixing between the viral and the target membrane) and genome exposure as an increase in fluorescence intensity resulting from membrane dye dequenching and nucleic acid sensitive dye binding to viral RNA, respectively (**Figure 1**.). Our data suggests that influenza fusion kinetics are independent of receptor binding and membrane curvature¹ but depends on membrane deformability⁴ and that influenza virus is sensitive to endosome-resident phospholipids for efficient genome exposure⁵.



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Figure 1. a) schematic illustrating the single-virus fusion assay where a viral particle is bound to a target tethered liposome and fusion is triggered by a rapid buffer exchange, resulting in a pH drop. Hemifusion and pore formation is measured by membrane dye dequenching and exposure of a nucleic acid-sensitive dye to the viral RNA, respectively. b) Displays sample images of pore formation events. The appearance of a white fluorescent spots after the pH drop (right image) signifies a single viral particle that has undergone pore formation and genome exposure with the target liposome. The orange box illustrates what a single white spot signifies schematically, when "zoomed in".

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Depth and element sensitive scanning nuclear microscopy in Uppsala

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Scanning nuclear microprobes use a beam of energetic ions, provided by a medium-energy particle accelerator, to induce atomic/nuclear interactions, and detect them in order to get insight into the elemental and structural composition of a sample. Several types of interaction products can be detected which results in a variety of ion-beam based microanalytical methods. All of them have their own advantages, starting from trace element sensitivity potentially down to ppb levels for Particle Induced X-ray Emission (PIXE), towards depth sensitivity potentially down to tens of micrometers for Rutherford-Backscattering Spectrometry (RBS). Ion-beam based analytical methods are providing highly accurate information on the sample composition, are considered non-destructive and usually they can be used simultaneously to combine the advantages of the different methods.

At the Tandem Laboratory of Uppsala University, there is such a scanning nuclear microscope [1], which is in extensive use primarily in collaboration with other research groups from inhouse to abroad (Figure 1). It can be used, besides many other things, in materials science [2], biomedical research [3] or materials chemistry [4].



Figure 1. Scanning nuclear microscope in the Tandem Laboratory, Uppsala University [1].

In this presentation we will present our nuclear microscope with focus on some of the most recent developments and applications. One example is to how to efficiently detect individual micro-grains in biomedical tissue sections with an effective concentration below ppm levels using μ -PIXE. Another important question that the presentation will answer is to how to quantitatively reconstruct elemental depth gradients in sensitive and highly complex materials, in particular single microcrystals of metal-organic frameworks (MOF) (Figure 2), using μ -RBS.


Figure 2. SEM micrographs of MOF single crystals (left) and Rutherford-backscattering map of the zirconium signal for a selected crystal (right) [4].

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Large volume high resolution 3D characterization of beam sensitive soft materials using multi-ion source PFIB technique under cryogenic conditions

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Abstract

Direct quantitative investigation of the inner morphology and structure of materials is of critical importance to provide profound insights for properties evaluation. The scanning electron microscope (SEM) and focused ion beam (FIB), combined known as FIB-SEM or DualBeam, are conventionally recognized as a highly effective method to acquire 3D volume information of soft materials samples. FIB-SEM together with integrated Automated Slice and View software has made the automated 3D data acquisition and analysis possible. However, slicing using Ga ion beam at room temperature has been found inducing severe damage to the beam sensitive materials, resulting in significant deterioration of the 3D data quality. Cutting edge cryogenic multi-ion source plasma FIB (PFIB) technique provides a fully automated workflow which allows large volume, damage-free ion beam slicing and high spatial resolution SEM acquisition during serial sectioning using various types of plasma ion beams under cryogenic conditions. With automated 3D reconstruction of such beam sensitive materials.

In this paper we present a series of large volume 3D imaging results of extremely beam sensitive polymer samples. The samples were sliced using oxygen plasma ion source on Thermo Scientific Helios Hydra Plasma FIB platform under cryogenic conditions. The slicing and imaging acquisition was achieved using Automated Slice and View software and the subsequent data processing was conducted using Avizo 3D analysis and visualization software. The unique technical experiment set up and comprehensive application experience will be discussed in this presentation.

In situ control and observation of chemical reaction inside a reaction capsule in SEM/DualBeam

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Abstract

It is widely acknowledged that dynamic experiments have been performed inside an environmental SEM while the entire chamber is filled with gases, however, this approach is limited by safety restrictions once an explosive or toxic gas is required. Recent developments of a novel miniature reaction capsule inside the SEM/DualBeam chamber opens new avenues for in-situ control of chemical vapor deposition, reduction and oxidation processes on a MEMS based heating stage with injection of reactive gases while maintaining the entire chamber under high vacuum. High resolution imaging of the chemical reaction process in real time is possible with in chamber or in column detectors.

In this presentation we discuss the hardware and software solutions of this novel miniature chemical reactor for in-situ dynamic experiments. The design combines both sample heating (up to 1200° C with ramping speed of 10000° C/s) on a micro heating plate and the possibilities of gas injection into the differentially pumped reaction volume (7 µl) surrounding the heated specimen. We also present application results of a series of in-situ chemical experiments such as growth of rutile (TiO₂) nanowires from titanium substrate, the reduction of oxidized polycrystalline copper surface by introducing pure H₂ to the sample surface, and growth of graphene monolayer on polycrystalline platinum bulk sample via CVD process by introducing ethylene at 1000° C.

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Abstract

Although cellulose derivatives are widely applied in high-tech materials, the relation between their force responses and their surface chemical properties in a biological environment as a function of pH is unknown. Here, interaction forces of surface modified cellulose nanocrystals (CNCs), lignin residual cellulose nanocrystals (LCNCs), and 2,2,6,6-tetramethylpiperidine-1oxyl radical (TEMPO)-oxidized cellulose nanofibres (TCNFs) with OSO3-, COO- and lignin chemical groups were measured using in situ peak force quantitative nanomechanical mapping and force spectroscopy in salt solution at two pH values. We found that the forces acting between the tip and CNC or LCNC are steric dominated showing long range and slow decay as a result of their low surface charge density. High Mw lignin contributed to the increased repulsion range for LCNCs compared to CNCs. The repulsion measured for TCNFs at the very short range was electrostatic force dominating showing a steep decay attributed to its high surface charge density. In the case of TCNFs, electrostatic double layer force was also evidenced by the attraction measured at secondary minima. In all the three cases the electro steric interactions are pH dependent. A schematic illustration of the force interaction mechanism of the chemical groups on nanocelluloses upon tip compression is shown in Fig.1. Dissipation maps verified that the force behavior for each material was related to structural conformation restriction of the groups at compression. The slow decayed repulsion of CNCs or LCNCs is related to a weak restriction of conformational change due to small surface groups or high molecular weight bound polymers forming flat layers, whereas the steep repulsion of TCNFs is attributed to a strong conformation restriction of carboxylic groups occurred by forming extended structure. Our results suggest that the force responses of the materials were dominated by surface charges and structural differences. TCNFs showed superior nanomechanical and repulsion properties over CNCs or LCNCs at neutral pH [1].



Figure 1 Approaching force-distance curve measured for the nanocelluloses. (Inserted)

dissipation maps of PFQNM results obtained in electrolyte solution at the two pH values.

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MerlinEM, Hybrid Pixel Array Counting Detector for Transmission Electron Microscopy

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At the University of Glasgow, experiments with hybrid pixel array counting detectors started with a Medipix2 detector [1]. It was clear that direct electron detection and hardware-based electron counting can offer advantageous imaging capabilities. Subsequently, a Medipix3 detector with a Merlin readout system was commercialised as a MerlinEM detector by a collaboration between the University of Glasgow and Quantum Detectors Ltd. With more than 60 systems worldwide, the detector has been applied in multiple experimental configurations.

The detector has been mainly applied in scanning transmission electron microscopy (STEM), however, diffraction and EELS applications are also wide-ranging. [2]

In STEM, the ability to collect a full distribution of electrons for each probe position with a millisecond and better timescales can be readily used in DPC [3] and ptychography [4] but it also enables a more quantitative approach to standard techniques and potential for development of new ones.

In diffraction, MerlinEM can be used to image direct probes while still retaining the ability to count single electrons for the weaker diffraction spots. This makes the detector an attractive solution for low-dose techniques in the microscopy of bio-molecules, microED and scanning precession techniques.

In EELS, the 4x1 MerlinEELS detector version, offers low noise, large dynamic range and fast framing. These characteristics are can be used to push core-loss imaging to high kV losses [5] and allow live drift corrections.



Fig. 1. Single-electron sensitivity and full diffraction pattern images collected with the same MerlinEM settings (albeit dwell time). Obtained under CC BY 4.0 from [2].



Fig. 2. Ptychography with MerlinEM. Image of single gold atoms in MoSe₂. Image courtesy Dr Shoucong Ning, NUS Singapore.

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P04

Effects of temperature for magnon detection in STEM

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Abstract

Recent technological advancements in Scanning Transmission Electron Microscopy (STEM) have opened up new possibilities for studying elementary excitations in the zero-to-few-hundreds meV range. Electron Energy Loss Spectroscopy (EELS) measurements with sub-10 meV energy resolution at high-spatial resolution [1-4] have made it possible to study phonons [3] and, potentially, magnons [5, 6] with atomic resolution. Studying magnons at both high spatial and spectral resolution in STEM has significant implications for magnetic solid-state technologies and the foundations of solid-state magnetism.

However, detecting magnon diffuse scattering (MDS) signals in STEM presents a significant technical challenge. MDS signals overlap with thermal diffuse scattering (TDS) signals, which arise from phonons and are typically orders of magnitude stronger. For example, TDS signals were found to be four orders of magnitude greater than corresponding MDS signals in simulations of bcc Fe at 300 K [5]. Moreover, the simulations of Ref. [7] for the same system showed that MDS signals comparable to or greater than TDS signals occur under the central Bragg disk, in an energy loss region currently unaccesible by STEM-EELS. Therefore, identifying conditions in which TDS and MDS signals can be distinguished from one another in diffraction plane regions outside the central Bragg disk is necessary. Temperature may be a key factor due to the distinct thermal signatures of magnon and phonon signals.

To address this question, we perform multislice simulations of TDS and MDS signals using molecular dynamics and atomistic spin dynamics [6] techniques, following the methodology described in Ref. [5]. In this work, we present an analysis of the effects of temperature on the detection of magnons in bcc Fe. Our investigation focuses on measurement conditions that allow the magnon signal to become a statistically significant contribution to the total scattering intensity outside the central Bragg disk in reasonable acquisition times. Our findings shed light on the feasibility of detecting MDS signals in STEM and provide insights into the conditions necessary to distinguish MDS from TDS signals.

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Abstract

A thorough understanding of structure-property correlations at the nm scale is essential for the development of nanomaterials' application. In general, high-resolution scanning and transmission electron microscopy (HR-S/TEM) can be used to achieve this as it can provide atomic scale resolution. On the other hand, using high-energy electrons as probe for structural characterization and for probing nanoscale properties could be invasive when nanomaterial is sensitive [1]. Therefore, it is important to study the radiation effects caused by the electrons in HR-S/TEM experiments, eg. to estimate the total critical dose the sample can tolerate in order to maintain structural integrity.

Metal-organic frameworks (MOF) are porous but chemically stable crystalline materials that already have shown their potential in a wide range of applications, e.g., in catalysis and gas separation. MOFs are one of those above-mentioned beam sensitive materials. Understanding the structure-property-relation of MOFs allows to expand their application field. This requires a sophosticated characterization of the MOF structure in HR-S/TEM experiments with atom precision [3].

Here, we study the stability of zeolitic imidazolate framework-8 (ZIF-8) in HR-S/TEM atomic scale imaging experiments, specifically the critical total electron dose tolerated. ZIFs are a subclass of MOF, where in ZIF-8 zinc (Zn) atoms are linked to 2-methylimidazolate linkers. ZIF-8 has cubic crystal structure (space group I $\overline{4}$ 3m) with a lattice parameter of 16.99 Å. The spacing between Zn triplets in unit cell along [111] direction is 3.4 Å with a pore size is 11.6 Å [2], which makes it suitable for the adsorption of small guest aggregates as well as guest gas molecules [3]. In previous study [4-6], values of critical total electron dose for ZIF-8 have been reported, however not all affecting parameters have been considered.

One method of assessing structural changes in a crystalline sample, e.g. caused by exposure to high-energy electrons, is by quantifying the drop in the intensity of specific Bragg spots in electron diffraction (ED) pattern [1]. Here, we have examined the dose-rate dependence of the stability of ZIF-8 nanoparticles (average size ~ 160 nm) with varying dose rate of 0.1, 1 and $6.6 \text{ e}^{-2} \text{ s}^{-1}$, respectively. Time series of ED pattern have been recorded in FEI Tecnai G2 microscope (LaB6, 200 keV) with exposure time of 1 sec until all the Bragg diffraction spots have completely faded out. The bright field TEM image of the ZIF-8 nanoparticles and the corresponding ED pattern, taken with a dose rate of 1 e⁻ A⁻² s⁻¹, are shown in fig. 1a and 1b, respectively. The radial average of the intensity of the Bragg rings (shown in fig. 1b) are plotted in fig. 1c. The time dependence of the relative intensity of the {431} Bragg spot, corresponding to an interplanar spacing of 3.3 Å and an indication of resolving Zn triplets in the HR-S/TEM imaging, is plotted in fig. 1d. We use this approach to estimate the effect of dose-rate on the critical dose tolerance limit in our ZIF-8 material [7].



Figure 1 (a) Bright field image of ZIF-8 MOF nanoparticles taken with an electron dose rate of 1 e⁻ A⁻² s⁻¹. (b) Ring diffraction pattern showing the {431} Bragg reflection at t =1sec (first acquisition). (c) Radial average of the Bragg reflections. Inset showing schematic of ZIF-8 unit cell along [111] direction (d) Relative intensity plot of 431 Bragg reflection with time (I₀ = intensity at t = 1sec, I = intensity at t sec).

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Optical properties and structure relationship in ZnO:Fe with inversion domain boundaries and ZnFe₂O₄/ZnO heterostructures

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Abstract

ZnO is a wide band gap semiconductor that has a broad range of potential applications, for example as a transparent conductive oxide (TCO) in photovoltaic devices [1]. Hence, ZnO film could be used as a transparent front contact to enhance the charge carrier collection from a photovoltaic cell avoiding shadowing effects. Highly conductive, but still transparent, ZnO can be obtained through doping with e.g. aluminum or gallium. A possible route to further enhance the functionality of the TCO is to modify it in such a way that it can produce charge carriers by absorbing light of shorter wavelengths, which are less efficiently used by the main photovoltaic. One way to achieve this is to embed optically absorbing nanoparticles with a suitable band gap in the TCO. The ZnFe₂O₄ spinel has a band gap of \sim 1.9 eV, and according to the ZnO-Fe₂O₃ phase diagram, it is energetically favorable at temperatures below $\sim 1200^{\circ}$ C and/or at higher Fe concentrations [2]. $ZnFe_2O4$ is therefore a well-suited candidate for the synthesis of optically absorbing nanoparticles embedded in a ZnO matrix [3]. When Fe is incorporated in ZnO, lead to the formation of periodic nanostructures known as inversion domain boundaries (IDBs), decorated with Fe [4]. The IDBs are seen to consist of a network of essentially atomic layers of Fe intergrown with ZnO wurtzite blocks which could exhibit intriguing optoelectronic properties. For example, as the IDBs are essentially two-dimensional sheets of dopants, it has been reported that while IDBs play a fundamental role in charge generation, wurtzite blocks are responsible for charge collection in photocatalytic processes [5].

In this work, we use a solid state powder synthesis route to form nanoparticles of ZnFe₂O₄ embedded in ZnO (Figure 1) or 2D structures formed by iron decorated IDBs (Figure 2) with similar cation ratio. We use a combination of (Scanning) Transmission Electron Microscopy (STEM), electron energy loss spectroscopy (EELS), and Energy Dispersive X-ray spectroscopy (EDS) to investigate the structure and chemistry of the particles and the IDBs. Thanks to the excellent spatial resolution offered by low-loss EELS measurements (Figure 3) in combination with the macroscopic data provided by X-ray photoelectron spectroscopy (XPS) and diffuse reflectance spectroscopy (DRS), we suggest that the origin of the similarities in the optical properties is due to the layers of Fe³⁺ octahedrally coordinated by O, which is present in both samples. The optical properties are measured using cathodoluminescence in a scanning electron microscope (CL). The possibility of forming such different nanostructures offers the opportunity to exploit and tune the physical properties of these systems [6].



Figure 1. High resolution high-angle annular dark-field STEM image of the interface between a ZnO and a $ZnFe_2O_4$ particle. Insets show Fourier-transforms of the image indexed according to the ZnO [100] and $ZnFe_2O_4$ [101] projection.



Figure 2. (a) Annular dark field STEM image of the sample with IDBs structure and (b-c) EDS maps corresponding to Fe and Zn confirming the high concentration of Fe at the IDBs.



Figure 3. (a) Low-loss EELS spectra from the (a) nanocomposite and (b) IDB sample, showing the EELS signal, the fitted background, the inelastic signal (background subtracted signal) and fitting of the spectra to direct and indirect transitions.

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P07

Posters Nanochannel Liquid Phase TEM 3D ED / MicroED P08 E. T. Broadhurst¹, T. Malik², E. C. S. Jensen², M. N. Yesibolati², X. Zou¹, K. S. Mølhave² & H. Xu¹

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Keywords: 3D electron diffraction, Liquid Phase electron microscopy, Polymorphism

In materials, chemistry, and medicine, small molecules can form different crystal structures of the same compound, called polymorphs.^[1] Determining the specific atomic structure of a complex molecule and the many polymorphs is challenging, often requiring X-ray synchrotron or neutron facilities.^[2] Understanding how these polymorphs behave in a variety of conditions is crucial to their potential applications. In this work, we aim to combine 3D electron diffraction methods^[3] with a novel nanofluidic microchip system^[4] and establish a foundational platform to solve new crystal structures of molecular organic crystals and their polymorphs in-situ.



Figure 1. Crystallization of the polymorphs of glycine.

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Atomic structure and magnetic circular dichroism of individual edge dislocation by electron magnetic circular dichroism

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Abstract

Electron vortex beams (EVBs) with nonzero orbital angular momentum (OAM) are expected to map magnetic images with atomic resolution via vortex beam electron magnetic circular dichroism (VBEMCD) [1], and the feasibility has been verified in simulations. In experiments, EVBs can be fabricated through topological holographic masks [2]. However, the extremely localized signal and the mismatch of coherent periodicity make the VBEMCD still a huge challenge [3, 4]. In 2019, we sculpted an electron beam into the vortex state by using an edge dislocation as an equivalent 'Y-shaped' mask [5], essentially solving the periodicity mismatch between EVBs and mask. The effect of topological defects on magnetism has always been the focus of researchers, especially in antiferromagnets [6],which are expected in the field of spintronics. The characterization of localized magnetism of an edge dislocation is important for understanding their physical origin and manipulating them in spintronic devices, but it is also facing huge challenges.

Here, we combine high angle annual dark field scanning transmission electron microscopy (HAADF STEM) and VBEMCD, experimentally synergistic probing the atomic structure and localized magnetic properties of individual topological defects in antiferromagnets. VBEMCD dynamic simulation demonstrates the ferromagnetism originates from the change of spin states near the dislocation core. Further, we trained our potential model of the system energy with respect to the atomic coordinates and magnetic moments using the Deep Learning method. Ultimately, a complex magnetic ground state of individual topological defect in NiO with 3D magnetic moment at the atomic scale was obtained. This work provides a framework for understanding the physical origin of topological defect magnetism in antiferromagnet.

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Development of in situ (S)TEM cooling, biasing and heating system for atomic resolution imaging

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Abstract

The application of cryogenic TEM (cryo-TEM) methods to Materials Science and energyrelated fields are mainly limited to beam sensitive materials like lithium-ion batteries, organic semiconductors, perovskite-based solar cells, polymers, Metal Organic Frameworks (MOFs), etc. Most low-temperature in situ experiments are carried-out using cryogenic holders based on liquid nitrogen and using 3mm sized TEM-grids. Despite their widespread use, grid-based cryogenic systems suffer from poor stability, making it challenging to obtain adequate atomicresolution imaging conditions and, are time-consuming [2]. Additionally, these systems often have the inability to set intermediate temperatures, which limits the experiments to be performed at either liquid nitrogen temperature or room temperature. Furthermore, such systems are limited in applications when understanding the structure, electronic and transport properties of materials under an applied electrical stimuli at low temperatures is of interest. For such applications, a system with low sample drift that combines liquid nitrogen cooling with an external voltage bias is needed. Such a development would enable a vast range of applications in the field of quantum materials [2], magnetic materials and nanostructures, ferroelectrics [3], topological insulators, metal-to-insulator transitions, superconductors and would be extremely valuable in the in situ TEM community.

In this talk, we will share our latest developments with respect of a combined in situ cooling, biasing and heating system. The system includes a novel double-tilt cryogenic holder which is MEMS-chips based, and uses liquid nitrogen for cooling. It has multiple electrical contacts and is compatible with our heating and biasing Lightning Nano-Chips, enabling the setting of any intermediate temperature between LN_2 and RT. Without the presence of the cooling agent, it is also possible to perform in situ heating experiments up to 1000°C. By exploiting the high stability of this system and its double tilt capability, we will demonstrate how atomic resolution imaging at various temperatures could be achieved and present several application examples, including ferroelectric focused ion beam (FIB) lamellas.

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Metal electrodeposition/stripping and 4D STEM analysis via operando liquid phase TEM

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Abstract

Aqueous metal batteries present new possibilities towards low cost and more sustainable energy storage.[1] However, one main stumbling block to this battery technology is the growth of metal dendrites, which tend to cause battery premature failure and safety issues [2,3]. Here, we use liquid cell transmission electron microscopy (TEM) [4,5] to directly visualized the electroplating and stripping of metals on micro electrodes of dedicated MEMS (MicroElectroMechanical System) chips at the nanoscale. By comparing the plating/striping under different chemical and/or electrochemical environment, including static or flow electrolyte conditions, and varying current densities, we show how metal dendrites can be effectively controlled on electrochemical cycling of the battery, as revealed by our operando liquid cell TEM and 4D STEM electron diffraction analysis. Following the experimental reesults (Figure 1), the growth of Zn dendrites can be effectively mitigated and directly minimized by flowing electrolyte into the cell and adjusting the current density, thus, providing new insights into the Aqueous metal batteries chemistry and the pathways for its optimization.



Figure 1 (a) Orientation mapped STEM image of deposited Zn in liquid by 4D STEM data analysis. (b-e) Reconstructed electron diffraction patterns corresponding to each mapped region.

P11

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High-Resolution Electron Energy Loss Spectroscopy Mapping of Confined Electric Fields in Topology-Optimized Photonic Cavities

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Abstract

Light confinement below the diffraction limit has recently been reported in topologyoptimized (TO) dielectric photonic cavities, opening new ways for integrating photonics and electronics.[1] Scattering-type scanning near-field optical microscopy (s-SNOM) was used to characterize the designed TO Si bow-tie cavities. However, the development of advanced techniques is essential due to the limitations of s-SNOM regarding spatial resolution and scanning direction. A promising approach is combining electron energy loss spectroscopy (EELS) with scanning transmission electron microscopy (STEM), which has already shown potential for imaging optical excitations with high spatial resolution.[2-4]

In this study, we used probe-corrected STEM-EELS to characterize TO Si bow-tie cavities. In particular, the electron beam's interaction with the electric field supported by the cavity caused an electron energy loss that could be measured by the addressed method. The microscope was equipped with a monochromator, essential for obtaining a sufficiently high energy resolution to resolve the mode of interest at ~0.8 eV and deconvolve the signal around the zero-loss peak. Moreover, acquiring STEM-EELS maps at various sample tilts gave detailed information about the mode's evolution in space.

High-angle annular dark-field (HAADF)-STEM images of a TO Si bow-tie cavity at 0°-(Figure 1a), 30°- (Figure 1b), and 70°-tilt (Figure 1c) reveal the structure's morphology. Moreover, the structure was investigated by EELS with a high spatial resolution to determine the mode's confinement around the bow-tie region. Advanced data processing of the EEL spectra enabled isolating peaks corresponding to the mode of interest. The electric field confinement is represented by a HAADF-STEM image and the associated relative mode intensity mapping for the structure tilted by 70° (Figures 1d-e).

The herein-presented approach highlights a promising way to obtain detailed insights into light confinement below the diffraction limit in TO dielectric photonic cavities. In particular, the generated results provide an avenue for comparing the experimentally observed interaction of electrons with the electric field supported by the cavity with corresponding electric field simulations. Moreover, developing advanced microscopy techniques for in-depth studies of dielectric photonic cavities is crucial for understanding the potential interactions of electric fields supported by such structures with their environments.

P12



Figure 1. A TO dielectric photonic structure was tilted by (a) 0° , (b) 30° , and (c) 70° . The corresponding HAADF-STEM images are presented in (a-c). The structure's active bow-tie region was investigated with a higher spatial resolution. (d) The HAADF-STEM image represents the magnified active region corresponding to (c). (e) The associated mode's relative intensity mapping reveals that the structure confines light (~0.8 eV) around the bow-tie region.

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Computation of generalised oscillator strengths for the simulation and quantification of energy loss spectra

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Abstract

The extraction of quantitative compositional data from an electron energy loss spectrum relies on the theoretical modeling and simulation of the core-loss (ionisation) edges. This allows to fit a simulated spectrum to the experimental one, using the concentrations of each element as a free parameter. Cross sections can be obtained from the so-called "generalised oscillator strengths" (GOS) which in turn depend on the wave functions of the target atoms which cannot be analytically described except in the hydrogen atom [1]. While the hydrogenic model is adequate to describe the K edges which is useful for some light elements, many elements require more complex computations in order to be quantified. While these calculations can be quite fast on a modern computer, tabulating the results is the most efficient approach for a wide deployment. Progress in methods for quantitative EELS has been limited by the existing GOS tables, which have been computed 40 years ago [2] and are not freely available.

To remedy this, we have computed a high-resolution GOS dataset covering all known EELS edges, which is now freely available [3]. We calculated the atomic wave functions self-consistently within the local density approximation, using the exchange correlation potential after Perdew [4]. For this a modified version of a program by Hamann is used [5]. Using this atomic potential, the wave function of the ejected free electron is calculated. To normalize the free wave functions they are matched to spherical Bessel and Neumann functions at large distances from the core. The remaining integral constitutes a spherical Bessel transform. Using the convolution theorem this integral is solved with a fast Fourier transformation. More details can be found in [6].

We assess the quality of the resulting data by comparing it to existing sets [2]. The agreement is good, but the new data offers better sampling, covers a larger number of edges (many minor ones), and is available over a wider range of energy and momentum. The comparison between the simulated edges, including ones simulated based on the hydrogenic model, offers good agreement across the board.

Finally, we have devised a HDF5-based file format for the distribution of GOS data which can be used in the future to ensure that new GOS data is immediately compatible with existing software.

The data, the code, and format specifications are freely available [3]. The new data can be used in CEOS Panta Rhei and in hyperspy.



Figure 1 Comparison between generalised oscillator strengths for the Ti L3 edge. Our new data is compared to an existing reference [2]. The strength is displayed as brightness in the top row, and as the y axis (1/eV) in the bottom row. The datasets show good agreement, but the new data has a finer sampling and covers a wider parameter space.

Simulated Edges, HT = 200.0 kV, β = 5.0 mrad



Figure 2 Simulated edges for the different GOS datasets, and the hydrogenic approximation. The two tabulated datasets are in good agreement, while the hydrogenic data diverges significantly already for the L edges, and is not computed for higher edges.

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130

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Panta Rhei: a software framework for the acquisition of image and spectral data

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Abstract

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. Data can be interchanged with other software using common file formats like npz, hspy, and tiff. The acquisition (via cameras or scan detectors) typically generates high volume 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 ($\pi \alpha v \tau \alpha \rho \tilde{e} \tilde{i}$ -- 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, device interfaces for the TEMs of three manufacturers, five families of detectors, two different scan generators, and the CEOS Imaging Energy Filter (CEFID [1]) exist. We expect that the number of compatible devices will continuously grow over time.

Figures/Tables



Figure 1 Software architecture of the CEOS Panta Rhei platform



Figure 2 Screenshot of CEOS Panta Rhei with Views for different imaging and spectroscopy workflows.

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A Semiconductor-Type Segmented STEM Annular Dark-Field Detector

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Spectrum imaging has developed into a very useful analytical technique. Behind the energy filter or spectrometer the EEL spectrum is recorded by a fast pixelated detector. For sample navigation and drift correction it is beneficial to simultaneously record an ADF STEM signal.

In order to capture enough signal to form a high-quality EEL spectrum a small camera length of the STEM projective has to be used. As a consequence, for many existing microscopes the inner detector angle for the available ADF detector becomes rather large leading to a small signal-to-noise ratio in the ADF images. In this situation, an additional, dedicated ADF detector inserted directly above the filter entrance aperture and adapted to its geometry can significantly improve the signal quality.

Here, we use a commercially available segmented electron detector (product of PNDetector GmbH [1]) originally developed for back-scattered electron imaging in SEM combined with a modified pre-amplifier configuration. The pneumatic insertion mechanism has been developed to be compatible with the CEOS pre-filter camera housing. The simultaneous acquisition of STEM EELS and ADF signals is fully integrated into the spectrum imaging workflows of our software CEOS Panta Rhei.

The semiconductor-type detector based on fully depleted ultra-pure silicon has a very high sensitivity up to 300 kV, a very low dark current level resulting in an excellent signal-tonoise ratio and allows for fast readout. The detector can be provided in a light insensitive version. The detector with an inner and outer diameter of Ø 5.6mm and Ø 12mm, respectively, is placed in front of the Ø 5mm entrance aperture for the CEOS Energy-Filtering and Imaging Device (CEFID [2]). While the integrated pre-amplifier provides a fixed gain, the main amplifier allows for adjustment of brightness and contrast levels. The signal from the four segments can be combined freely with a 4:1 readout using one main amplifier, while a 4:2 readout resulting in two simultaneous differential signals for orthogonal directions is possible with a second main amplifier. Additionally, the high sensitivity and discrete electron signals allow for quantitative STEM [3].

In this contribution, we exemplify different use cases and features of the detector, especially for high-resolution STEM at very small camera length and parallel acquisition of differential phase contrast and EELS signals.

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Figure 1: STEM image of Au particles on carbon film and corresponding histogram. The discrete electron events in the histogram allow for easily calibrate for quantitative STEM [3].



Figure 2: Proof of concept for differential phase contrast signals of $SrTiO_2$ using the segmented ADF detector.

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Abstract

Recent developments of detector technologies and acquisition schemes cause a demand for modular post-column energy filters with good performance for EFTEM and EELS experiments. Owing to decades of research, in academia and at Gatan company for post-column filters, and at Zeiss and JEOL companies for in-column filters, these techniques are well established [1,2]. At CEOS we re-investigated the optical design and technology and identified some incremental improvements by using approaches to design, manufacturing and alignment originally developed for aberration correctors. Complementary to established plug-and-play solutions which primarily allow for a predefined combination of microscope / spectrometer / detector, we provide an open platform with utmost freedom in the selection of components, regarding both hardware and software.

The optical design of the CEOS Energy-Filtering and Imaging Device (CEFID) is based on a highly optimized sector magnet combined with two 12-pole elements. For all other focusing elements we use pure quadrupoles supplemented by some deflectors and separate two- and three-fold stigmators. This approach allows for a 12 mm entrance aperture with a nonisochromaticity below 1 eV peak-to-peak at 200 kV (Fig. 1) and less than 0.35 % root-meansquare geometric distortion in imaging mode. In spectroscopy mode dispersions from 1 meV/channel up to 1 eV/channel are possible even at 80kV. The use of well-designed quadrupole elements made from high-quality soft iron helps to largely avoid remanence effects and improves the reproducibility of alignments. The filter design introduces a clear separation between pre- and post-slit alignments. All second- and higher-order spectrum aberrations can be measured and tuned at the slit plane by using identical methods for EFTEM and EELS modes [3]. This simplifies operation and enables to switch between imaging and spectroscopy mode with different dispersions or even different detectors with little to no retuning. The sector magnet is powered by a very stable current supply. With an entrance aperture of 5 mm an energy resolution in the sub 20 meV range should be possible, and over 12 hours a drift of less than 1 eV at 300 kV has been achieved.

Multiple pixelated detectors can be added at different distance from the final cross-over, e.g. large-field of view scintillator-coupled or direct-detection CMOS cameras or frame- or eventbased hybrid-pixel detectors with a large pixel size (Figure 1). Our Python-based software targets for open interfaces and flexibility for user-scripting, also enabling unusual experimental setups. EFTEM and (S)TEM-EELS, including STEM spectrum imaging, are readily possible [4].

P16

Figures/Tables



Figure 1. CAD Model with a selection of currently possible pre-filter and post-filter detectors. Typically only two post-filter detectors are installed on the same system.

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Optical dichroism in vortex electron energy loss spectroscopy on chiral particles

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Abstract

One of the techniques nowadays routinely used to probe optical excitations in nanostructured materials is electron energy loss spectroscopy (EELS) in scanning transmission electron microscopy (STEM). When the electron beam interacts with the sample, it can lose part of its energy (and momentum) while creating excitations in the sample. This energy loss can be measured and interpreted directly as the creation event. One kind of such excitations are localized surface plasmon polaritons (LSPP) formed in metallic nanoparticles at optical frequencies. LSPPs can be understood as collective oscillations of conduction electrons, which are accompanied by strong electromagnetic fields concentrated in the vicinity of the nanoparticles. Such plasmonic nanostructures can be thus used for manipulation of light or when strong localized fields are needed (e.g. for probing molecules).

Relativistic electrons act as a broadband source of an evanescent electromagnetic field and thus can efficiently couple to the localized fields of the sample excitations. It has been shown that EEL spectrum can be qualitatively linked to the projected local density of optical states (LDOS) [1] and thus provides relevant information on the optical properties of the plasmonic nanostructures.

The wave function of the electron beam can be nowadays tailored into different shapes with interesting properties. One example of such a tailored beam is a vortex electron beam (VEB). In VEBs there is a phase singularity in the center of the beam. The phase of the wavefunction "revolves" around the center and such a beam then carries orbital angular momentum (OAM). Thanks to this it acts both as a moving charge and a magnetic dipole in the interaction with the sample. If the mirror symmetry is broken also in the sample, i.e. the sample is, as well as the beam, chiral, different behavior of right-handed and left-handed vortices (dichroism) can be observed. [2,3]

We model dichroic EELS spectra for the interaction of extended VEBs with a chiral point-like particle, with two finite-sized metallic nanorods in chiral configuration (Born-Kuhn model, see Fig. 1), and with a plasmonic helix. We propose an experiment in which the sample is probed by Bessel-like VEBs. One concrete VEB with chosen topological charge interacts with the sample. After the interaction, an OAM sorter [4] is used to select one particular vortex state. As so we resolve the results into concrete transitions between different vortex states.

For the interaction with point-like particle, we use a semi-analytical model [3,5]. To model the finite-sized structures, the boundary element method (BEM) is used to compute the plasmonic eigenstates in the quasistatic regime numerically. From the eigenpotentials we calculate the EEL spectra [6].

We demonstrate significant dichroism for both point-like and finite-sized particles. However, we reveal a strong dependence of the dichroic signal on the relative position of the VEB and the probed particle in all studied cases. In some cases, the dichroic signal cannot be observed at all. In other relative beam-sample positions, we can on the other hand maximize the signal.

Parameters of the beam (primary energy and convergence and collection angles) also play an important role in the behavior of EEL spectra. We present our methods and an overview of results together with a hint of an intuitive explanation of observed phenomena. Our results might guide the experimental efforts leading to optimal detection of optical dichroism in the nanostructured matter.



Figure 1. Left: schematics of left-handed and right-handed VEB with topological charge l = -1 and l = +1 (blue vs. red beam) interacting with chirally-arranged metallic nanorods (Born-Kuhn model). On the rods we have labeled schematically the distribution of charges associated with the first two plasmon modes (anti-bonding and bonding charge arrangement is shown for the left-handed and right-handed beam excitation, respectively). Right: EELS spectra Γ (for beam positioned in the "center" of the nanostructure as depicted in left images) in colors corresponding to the colors of the beams. Note that the right-handed beam excites the bonding mode preferentially as opposed to the left-handed beam which excites both modes with similar probability. Both spectra can be compared in relative dichroic spectrum *D* in black. Its minimum around the antibonding peak reaches -50 %.

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Cold sprayed Aluminum-Quasicrystal Composite Coating: Bonding Mechanism Evaluation by SEM and TEM

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Abstract

Cold spraying (CS) is an emerging solid-state coating technology that has demonstrated significant industrial application potential. A wide variety of materials, such as metals and alloys, polymers, and ceramics can be effectively deposited by CS technology [1]. In addition, CS offers a promising route of composite coating formation to combine properties of several constituents and fabricate multifunctional layers, which could be challenging to obtain from single material coatings [2]. In principle, accelerating solid feedstock particles via pressurized and preheated gas, and subsequent particle impact at high speed onto a substrate lead to a coating build-up from deformed particles. Impacted particles undergo high strain rate deformation, which stimulates microscopic phenomena such as grain refinement, strain accommodation, and phase transformation [1]. In this regard, microstructural characterization is a crucial element in the development and optimization of novel coatings by revealing microscopic details.

In our previous works [3,4], CS was successfully employed to produce dense and wellintegrated aluminum-quasicrystal (Al-QC) composite coatings with superior tribological properties and increased hydrophobicity compared to Al-based coating and bulk metallurgical counterparts. As a continuation, this work aims to investigate microstructural features of CS Al-QC composite coatings with a focus on probing the bonding state and particle-particle interface. Composite coatings were sprayed with optimized parameters using a high-pressure CS system with pressurized N_2 that propels Al-QC feedstock blend toward the substrate. The process settings were also modified to accelerate a limited number of particles and obtain impact of several particles on the substrates (so-called wipe test) to simplify the deposition process. A SEM was utilized to evaluate the microstructure of the specimens. For an in-depth understanding of Al-QC interface, a (S)TEM equipped with EDS system was used to characterize a lamella extracted from the region of interest by FIB from polished surface of composite coating.

Examination of the Al-based substrate surfaces after the wipe test revealed various features as marked in **Figure 1**; Evidently, Al particles impact-induced plastic deformation could occur easier, acting as binder phase that retains the harder and more brittle QC particles inside the composite structure and provides a soft bed for the landing of subsequent QC phases. Al particles bonding to each other could be found randomly on the surface, whereas QC-QC bonding was not observed. **Figure 2a** depicts the SEM (secondary electrons) image from the region of interest for the TEM lamella prepared by FIB. TEM bright field image **Figure 2b** exhibits continuity and integrity alongside Al and QC interface. In the Al side, fine, elongated grains were formed due to heavy deformation near QC particle. Tracking the microstructure

from the interface toward the interior region of Al particle is associated with a size gradient from fine elongated grains towards larger equiaxed grains. Furthermore, the EDS line scan in **Figure 2c** confirms the presence of a concentration gradient of core QC elements at the 30 nm-thick interlayers with Al particle, implying intermixing at the interface. The observed microstructural features can endow a strong bonding between dissimilar constituents in the composite coating structure. The findings regarding the bonding state can potentially justify the enhanced mechanical and tribological properties of composite coatings, and extended retention of the reinforcing phase in the structure under load, as observed in our previous works [3,4].



Figure 1. Surface features observed after wipe test by cold spraying of Al-QC. i) craters generated at rebounded particle, ii) flattening of Al particles with random signs of viscous flow of plastically deformed particles, iii) intruded hard QC particles imposing severe deformation on substrate, iv) cracked/fragmented QCs due to their natural brittleness and v) highly deformed Al particles upon impact to/by harder QC particle.



Figure 2. (S)TEM sample preparation and observed microstructure of CS Al-QC coating; a) location of TEM sample taken in FIB-SEM, b) brightfield TEM image of the marked region in (a) depicting QC, Al and their interface, c) STEM image from the interface of QC and Al and the corresponding EDS line analysis (yellow line).

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P18

Morphological changes of photothermal polymeric films during carbonization at different conditions for carbon materials production

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Abstract

Carbon materials occupy a significant part of the world market. They are successfully used in many technological areas including catalysis, water purification, electrochemistry, component of building materials and so on. Industrial production of carbon materials is usually done through pyrolysis of biomass, wastes or artificial polymers. The majority of these precursors contains a lot of natural impurities including metal ions, sulphur, phosphorous etc. which not only affects the morphology of formed carbon materials but also can cause rapid failure of properties of produced carbon materials. Pyrolysis itself require heating of these precursors to high temperatures in harsh conditions (inert gas, aggressive chemicals) for hours. The produced carbon materials vary in morphology and microstructure (soft or hard carbon) depending on the precursor nature (graphitizable or not-graphitizable) and pyrolysis conditions. In our work we propose CO₂-laser irradiation of photothermal film as an alternative way for rapid and sustainable production of carbon materials, which requires no inert atmosphere nor any harsh conditions. The conducting polymer/Cladophora cellulose freestanding film was chosen as model system for the study due to the high absorption coefficient of the conducting polymers used and the mechanical stability of cellulose. The morphology and functional composition of carbonized films were investigated using SEM-EDX, XPS and FTIR techniques. It was shown that synthesized carbon materials have a highly disordered structure typical for hard carbon. Moreover, a number of different carbon structures, including carbon fiber net (Figure 1d), carbon globules (Figure 1e) and agglomerates (Figure 1f), all covered with small spherical particles, were formed during the carbonization process at different conditions which provides a lot of possibilities to control the microstructure and properties of the produced materials.



Figure 1. SEM images of pristine (a,b) polypyrrole/cellulose film and (c-f) carbonized polypyrrole/cellulose films.

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Abstract

In our quest for a solution to drive the decarbonization of our society and economy, hydrogen shows unique properties as a zero-emission fuel, energy storage solution, and chemical feedstock [1]. However, compact storage remains a scientific and technological challenge. Safely storing hydrogen, e.g., in the solid form of metal hydrides, has many advantages, but further improving its storage properties requires a thorough understanding of the metal-to-metal hydride phase transformation at the atomic level [1]. Although many techniques have been applied to understand these phase transformations, in-situ visualization of the transformation process is essential for a quantitative understanding [2,3]. For example, stress/strain, defects, and intermediate phase evolution during metal-to-metal hydride phase transformation are crucial to interpret the hydrogen sorption properties [4].

In this study, we use Mg thin film, as part of a model system (see stacking sequence in Figure 1), to test and understand the metal-to-metal hydride phase transformation via in-situ transmission electron microscopy (TEM). The thin films were prepared via magnetron sputtering, and a Focused Ion Beam (FIB) was used for TEM sample preparation. To make Mg film strain free, a bottom Ti buffer layer is added to prevent any substrate interaction of the Mg film with Si substrate, and a top Ti layer is added to prevent any PdMg alloy formation, as shown in Figure 1. The Pd layer is necessary to act as a catalyst for H_2 dissociation; it also prevents oxidation of the layers from the top.

During the Mg-to-MgH₂ phase transformation, the crystal structure undergoes a change from hexagonal to tetragonal, and this change is probed via in-situ S/TEM. To study this phase transformation, i.e. the hydrogenation process, we track the bulk plasmon resonance shift using electron energy loss spectroscopy (EELS). This structural transformation also leads to a change in the diffraction pattern, which we study using electron diffraction (SAED). Here, we combine these two approaches to follow and understand the process of hydrogenation.



Figure 1 shows the Mg thin film before and after hydrogenation. (a) As-prepared Mg thin film (b) The film after hydrogenation at 60 $^{\circ}$ C at 1 bar.

P20

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Abstract

Phase and structural characterization at the nanoscale play an essential role in development of new materials with improved physical and mechanical properties as well as in development and quality assessment of increasingly smaller semiconductor devices. Analytical 4D-STEM is a powerful analytical method based on nanobeam diffraction that can resolve and characterize distribution of crystalline phases and orientations of individual grains in polycrystalline materials at the nanoscale level. These analyses are critical because physicochemical, electrical, and mechanical properties strongly depend on distribution of crystalline grains and interfaces between different phases in prepared materials or annealed semiconductor devices. However, broader adoption of 4D-STEM techniques has been limited by complexity of experimental setups and challenges with synchronization of sample scanning with electron beam blanking, precession, and detector readout. In this presentation, we will show a new approach to acquisition and processing of 4D-STEM datasets quickly and with minimal user input due to full integration of all needed hardware components with high levels of system automation and optimization algorithms for on-the-fly data processing and visualization of results. The power of 4D-STEM characterization will be demonstrated on identification of phases in a semiconductor device that contained anomalies in the GST layer that could not have been revealed by using the standardly used analytical methods such as EDX elemental mapping. Additionally, improvements in accuracy and robustness of 4D-STEM phase analysis by using multi-modal analysis in combination with simultaneously acquired EDS data will be shown on an example of a polycrystalline aluminum foil with added gold nanoparticles which have very similar crystal structures (a lattice parameter difference of less than 5%).



Figure 1: Analysis of a semiconductor device by BF/DF STEM imaging and EDS mapping indicated uneven distribution of germanium in the GST layer. Phase and orientation mapping by 4D-STEM (right image) then revealed separation of germanium from the GST layer at the interface with the amorphous phase (white oval).

Acknowledgments

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Interfacial structure and magnetic property at Triple-/Double-Perovskite heterojunctions

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Perovskite interfaces that contain transition metal atoms exhibit properties that differ from their individual components, giving rise to emergent phenomena such as two-dimensional electron gas behavior, exchange bias effects, and interface magnetism [1]. A typical perovskite oxide has the chemical formula ABO₃, where A and B are metal ions. Double perovskite $A_2BB'O_6$ and triple perovskite $A_3B_2B'O_9$ can be derived from typical ABO₃ structure by adjusting B-site atoms. Previous studies have suggested that the ordering and types of B and B' atoms in double and triple perovskites strongly influence their magnetic susceptibility [2,3]. In this work, we observed and interface between the double perovskite Sr_2FeReO_6 and the triple perovskite Sr₃Fe₂ReO₉ and then determined its structure and chemical information using atomic-level experimental evidence from aberration-corrected (AC) scanning transmission electron microscopy (STEM), energy-loss spectroscopy (EELS), and nanodiffraction. Further, the first principal calculations were performed with a structure model built on the experimental results to gain insight into the magnetic and electric properties of the interface. By comparison among different magnetic coupling settings, we found different with the bulk double and triple perovskite where the antiferromagnetic (AFM) coupling is dominant due to the super exchange of Fe-O-Re, the exchange coupling of ground state near the interface is prone to be ferromagnetic (FM). The exchange parameters of every magnetic pair were calculated and confirmed the interface ferromagnetic coupling. [4]

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P22

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Unraveling the multilayer growth behavior of InGaAs nanowires using In-situ TEM

P23

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Abstract

In-situ transmission electron microscopy (TEM) has proven itself to be an important and powerful method for unraveling multiple aspects of III-V semiconductor nanowire growth, including growth dynamics[1] and compositional relationships[2]. Recently, *in-situ* TEM investigations have shown the possibility of having more than one biatomic layer nucleate and grow simultaneously at the growth front of binary compound nanowires growing in the vapor liquid solid mode[3]. The classical view has been that liquid nanoparticle mediated growth only allows the nucleation and growth of singular layers, separated by incubation periods (see schematic in Figure 1 (a)). The discovery of multiple nucleation events and subsequent growth of multilayers contradicts this previous assumption. Therefore, the investigation of multilayer growth is necessary to further the understanding of nanowire growth.

In this study, the growth of Au-seeded InGaAs nanowires was investigated using environmental TEM, revealing the occurrence of multilayers in ternary nanowires *in-situ*. The experiments were carried out in a Hitachi HF3300S environmental transmission electron microscope with an open cell configuration, integrated with a metal-organic chemical vapor deposition (MOCVD) system. The MOCVD system was used to supply metal-organic and hydride precursors to a micro-electro-mechanical system heating chip, where pre-deposited Au seed nanoparticles enabled the growth. The growth was observed and recorded using a Gatan OneView IS camera, which allows up to 100 fps 2K resolution or 25 fps 4K resolution video recording, and compositional analysis was carried out through X-ray energy dispersive spectroscopy (XEDS). The microscope is also equipped with a CEOS BCOR image aberration corrector allowing a theoretical maximum point resolution of 86 pm.

We show that multilayer growth is more prominent in InGaAs than for the binary cases previously demonstrated, and during multilayer growth, the stacks of layers growing simultaneously can reach much larger sizes[4]. The multilayer stacks are observed to form in conjunction with defects in the material, such as crystallographic twins and small compositional changes (see Figure 1 (c)), but also at sharper compositional heterostructures, as indicated by the geometric phase analysis (GPA) data and complementary scanning TEM XEDS maps shown in Figure 1 (b) and (d). Importantly, the high resolution TEM videos show that material is transported between the simultaneously growing layers, indicating that the final composition of the segment could be affected. We have also observed multilayer growth as an intermediate step to kinking, a process where the nanowire changes growth direction, which is often unwanted. Since composition and uncontrolled kinking of the nanowire greatly affects its properties and performance, the discoveries are important for device applications. This shows why multilayer growth needs to be further explored, so that it can be controlled and accounted for in conventional nanowire growth.

Figures/Tables



Figure 1: Multilayer growth in InGaAs nanowires grown and imaged *in-situ*. (a) A schematic detailing the difference in appearance between the layer-by-layer and multilayer growth modes. (b) A frame from a video recorded *in-situ*, showing a large multilayer stack and GPA treatment, indicating the axial change in lattice parameter (scale in nm). The multilayer stack shows a smaller lattice parameter, indicating an increase in Ga content (decrease in In content). (c) Image sequence of multiple nucleation events leading to the formation of a multilayer stack. The timestamp indicates the time in relation to the nucleation of the first layer, and the arrows point to the growth fronts of the individual layers. (d) Scanning TEM XEDS mapping of the nanowire shown in (b), confirming that the segment grown in the multilayer growth mode contained more Ga.

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In-situ observations of size effects in GaAs nanowire growth

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Abstract

Lateral dimensions of III-V nanowires are known to affect the growth dynamics and crystal structure. Investigations into size effects have in the past relied on theoretical models and post growth observations, which only give a limited insight into the growth process. Investigations of how nanowire dimensions affects nanowire growth rate and crystal phase selection have in the recent years been conducted as a combination of theoretical and post growth studies of ex-situ grown nanowires. The experimental works typically have used nanowire length and overall morphology obtained via electron microscopy investigations post growth. Therefore, most of the experimental studies thus far have only been able to offer limited insight into the impact of size effects on growth dynamics. However, in the recent years the use of specialized environmental transmission electron microscopes (ETEMs) have allowed to investigate the growth dynamics of III-V nanowires in real time allowing to, for example, decouple the growth process into the precursor specific incubation and step-flow processes. [1,2]

In this study we have investigated how nanowire diameter affects the growth dynamics by growing Au-seeded GaAs nanowires in-situ using an ETEM. This was done by recording videos of nanowires during growth and analysing the Ga-dependent incubation time and As-dependent step-flow time demonstrated using HRTEM images and schematic in Figure 1.

Our data shows that the incubation time is stable across the investigated diameter range aside from a sharp increase for the smallest diameter, whereas the step-flow time is observed to steadily increase across the diameter range. We show using a simple model that this can be explained by the increasing vapour pressure in the droplet. In addition to the existing understanding of nanowire growth at small dimensions being limited by nucleation this work provides experimental evidence that growth is also limited by the inability to finish the stepflow process.



Figure 1: HRTEM images and schematics demonstrating the incubation (a-b) and step-flow processes (b-d) in Au-seeded GaAs nanowire growth. Black arrow indicates the step between the growing layer and the previously flat liquid-solid interface.

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Materials characterization using deep learning image analysis

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Abstract

The quick development of materials relies on the understanding of material microstructure (size, porosity, morphology, etc.), a reliable manufacturing process, and a thorough analysis of the performance for different applications. To solve some of the research challenges, automated, reliable, and intelligent analysis techniques are needed.

Using deep learning and a powerful image analysis engine, MIPAR [1] (<u>www.mipar.us</u>) allows users to perform a fast, accurate and automated analysis of images. In three simple steps: trace, train and apply, researchers can create a model that identifies the features of interest and run personalized recipes on new images to detect complex features.

This presentation will overview the advantages of using modern analysis techniques to analyze particles, fibers and pores, droplets, defects, contaminants, and phases with real research applications.

Figures/Tables



Figure 1 Detecting grains while ignoring twins using MIPAR. Model was trained on 25 images in 40 minutes and applied to new images in 2 seconds

P25



Figure 2 Overlapping nanofiber network analysis performed using MIPAR. Model was trained on 36 images in 40 minutes and applied to new image in 1.5 seconds

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Microscopic Heterogeneity of Plastic Strain and Lattice Rotation in Duplex Steel

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Abstract

Duplex stainless steels are two-phase alloys composed of austenite and ferrite, which exhibit excellent mechanical properties and corrosion resistance, making them highly desirable for various applications in industries such as oil, chemical and power. In this study, a duplex steel with 50% ferrite and 50% austenite produced by hot isostatic pressing was investigated using in-situ electron backscatter diffraction (EBSD) during uniaxial tension deformation. Digital image correlation was employed to determine local plastic strains, while crystal lattice rotation was calculated from EBSD and correlated with local slip activities. The microscopic heterogeneity of plastic strain and lattice rotation was discussed in relation to the interactions between grains of the same and different phases.

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Abstract

Metallic nanoparticles supported by metal oxides are widely used in heterogeneous catalysis. Electron microscopy is often used to study this kind of systems, but the effect of the electron beam on the sample is difficult to quantify. Model catalysts based on Au nanoparticles on Titanium dioxide (TiO_2) actively studied, both to understand the relation between shape, size, and catalytic activity, and to study and quantify for the effects of the beam. In this connection it is essential to understand the dynamics of the Au NP on TiO_2 , specifically the heat transport properties between NP and substrate.

Due to the large size of these systems (several nanometer), calculations based on Density Functional Theory (DFT) is limited by computational capacity. Machine Learning interatomic potentials can make such simulations possible due to the capability to simulate large systems with relative low computational resources.

Understanding the nanoparticle heating due to the electron beam has been studied previously [1,2,3,4]. These studies have proven imprecise due to poor approximation to the heat flow at the nanoparticle/support interface. In this work, we studied the problem of heat flow from gold nanoparticles to substrate (specifically TiO₂) with the aim of E(3)-equivariant machine learning potentials [5], trained on a large dataset made from DFT calculations.

The heat rate between Au NP and TiO_2 was calculated using molecular dynamics simulations. Figure 1 shows how the thermalization process of 2 different nanoparticles where the initial temperature of the nanoparticle is greater than that of the substrate. We fitted a function to the thermalization of the AuNP/TiO₂ using Fourier law and conservation of energy; using this value we can estimate the temperature increase for nanoparticles under different electron dose rates. The temperature of the nanoparticle becomes stable when the heat input is equal to the heat dissipated by thermal conduction (neglecting thermal radiation), the heat input is computed as a result of the inelastic interactions between the electron beam and the sample.

Figure 2 shows the temperature increase of a nanoparticle at different wetting angles as a function of dose rate. The beam energy affects the mean free path of the electron ($\lambda = \lambda$ [E_beam]). As the beam energy increases, the mean free path becomes longer, resulting in less power input into the nanoparticles.

Figures/Tables



Figure 1: Thermalization of nanoparticles of different sizes supported by TiO₂. The value k is the thermal conductivity k at the interface AuNP/TiO₂ for the nanoparticle shown in the top panel is $4.3x10^{-6}$ eV/(Å² ps) and $7.5x10^{-6}$ eV /(Å² ps) in the bottom panel, V is the volume of the nanoparticle, A the contact area and C_p is the heat capacity of gold.



Figure 2: Temperature increase as a function of dose rate for different S/A ratio (or wetting angles). The contact area A and cross section area of the beam change depending of the wetting angle, electron beam energy is 80keV.

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Isotopic shifts of phonon bands studied by monochromated STEM – simulations and experiments

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Abstract

Monochromated electron beams with a full-width at half-maximum well below 10 meV made it possible to study phonon modes in scanning transmission electron microscopy (STEM), reaching an atomic resolution [1,2]. Careful analysis of the energy position of vibration modes even allows to distinguish between different nuclear masses of elements via isotopic shifts of phonon modes [3,4], while maintaining the high spatial resolution of STEM.

In this work we study hexagonal boron nitride (h-BN) with various isotopic compositions of the boron [5]. We have performed angle-resolved electron energy loss spectroscopy (AREELS) with a nearly parallel beam illumination, to study the dispersion of phonon modes and measure isotopic shifts of selected phonon bands.

Experiments were complemented by a detailed theoretical study. Density functional theory has confirmed a phonon band gap between optical and acoustic phonon modes. Its size sensitively depends on the isotopic composition. Notably, only the optical phonon bands shift with changing isotopic composition, while the acoustic bands remain almost unchanged. AREELS simulations utilizing frequency-resolved frozen phonon multislice method [6] give a detailed account to visibility of individual phonon modes as a function of scattering direction. Predicted isotopic shift of the longitudinal acoustic phonon band is in a detailed agreement with experimental observations.

Our results suggest a possibility of isotopic engineering of wave-guides without chemical disorder or interfaces, where waves of certain THz-range frequency could pass only via designated regions with a specific isotopic composition.

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Abstract

There are several elemental analysis techniques in world-wide use in the field of for materials characterization. One very popular method is energy dispersive x-ray spectroscopy (EDS or EDX) [1]. EDX is often integrated tool in scanning electron microscopes (SEM) and sometimes in transmission electron microscopes (TEM). Elemental composition of unknown substance can be easily analyzed using SEM-EDX and the surface of the specimen can also be chemically mapped [2]. The interaction between the material under measurement and electron bombardment, which is needed for electron microscopic imaging, produce x-ray emission. Usually, several keVs of kinetic energy is needed for characteristic x-ray production. Therefore, the electrons may penetrate several micrometers into the specimen during the analytical work. Obviously, the method has also some weaknesses [3]. EDX can not detect the lightest elements (H and He) and it is insensitive detecting low Z elements. Fortunately, there are several methods for elemental and chemical analyses. One of the surface sensitive methods is x-ray photoelectron spectroscopy (XPS). However, XPS reveals only a few atomic layers on the sample surface [4]. It can be especially practical while studying contaminations or superficial coating layers with ultra-thin characteristics.

In this study, 30 mm in diameter acid proof stainless low-carbon steel, AISI 316 L [5], cylinder was cleaned with acetone and kept in oven for 3 hours before the elemental composition of was analyzed using SEM-EDX (Zeiss SigmaHD|VP SEM with Thermo NSS EDS). Two different electron accelerations, 5 and 15 kV were applied for two measurements. To examine the EDX results, XPS (Thermo Nexsa G2 XPS) was used in steps with ion beam sputtering. The purpose of the ion beam was to etch the contamination and get information from the pure steel as pure as possible.

Both used elemental analysis methods gave practically similar identification for the composition of the alloy. However, EDX overestimates the carbon and oxygen content as usual. XPS detected the composition and could be used as function of depth stepwise with the help of ion sputtering. In addition, it also detected some traces of elements invisible to EDX. The data showed that the high carbon was due to the contaminant on the surface, and it also revealed that even with the known limitations of the EDX, it is a very useful tool in daily materials research.

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In-situ TEM study of nitrogen oxide removal

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Abstract

Due to health and environmental concerns, there is currently a strong societal demand for improved air quality, driving strict emission limits for nitrogen oxide (NO_x) ^[1]. Ammonia selective catalytic reduction (NH₃-SCR) has been developed to eliminate the NO_x in industrial exhaust gas before emission. In many cases, NO_x is accompanied by SO₂, which passivates and deactivates the catalysts at relatively low temperatures. With the assistance of *in-situ* transmission electron microscopy (*in-situ* TEM) and first-principles calculations, we designed a novel catalyst by loading MnO_x clusters on the surface of CeO₂ nanorods, which minimized the SO₂ induced catalyst deactivation in NH₃-SCR of NO_x at low-temperature through establishing a dynamic equilibrium between sulfate formation and decomposition over the CeO₂ surface during the reactions and preventing the MnO_x cluster from the steric hindrance induced by SO₂. The MnO_x/CeO₂ catalysts show almost no activity loss with an apparent NO_x reaction rate of 1800 µmol g⁻¹ h⁻¹ for 1000 h test at 523 K in the presence of 200 ppm SO₂^[2].

On the other hand, the direct decomposition of NO is regarded as the most ideal and reasonable technology for the removal of NO_x , as it does not require the addition of reductants and the gas is direct decomposed into harmless N_2 and O_2 . However, the precise identification of active sites remains a formidable challenge, especially the atomic-level dynamic evolution of active sites under NO direct decomposition conditions. Leveraging the *in-situ* TEM and first-principles calculations, we observed the atomic-level oscillatory generation and disappearance of the highly active PdO/Pd phases on the surface of Pd nanocube where the decomposition of active sites under reaction conditions. These results may have a major impact on fields such as dynamic heterogenous catalysis under reaction conditions and serve as a basis to design high-performance *de*NO_x catalysts.

Figures/Tables



Figure 1 Schematic illustration of the tracking active sites under working conditions.

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Electron Counted Spectrum Imaging Optimized for In-Situ Analysis

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The ability to record data at both high speed and high dose efficiency is critical for capturing data for in-situ analysis. Electron-counting, direct-detection cameras are the detector of choice for *in-situ* (scanning) transmission electron microscopy due to their inherently high detective quantum efficiency (DQE) and high frame rates in specific implementations [1]. Furthermore, the rejection of read noise by electron counting allows over sampling in the temporal domain during data collection which can then be rebinned as needed to balance signal-to-noise ratio against temporal resolution during data analysis without the penalty of amplifying read noise.

In a recent experiment, a prototype method for *in-situ* spectrum image (SI) acquisition was demonstrated using a GIF Continuum EELS spectrometer and Gatan DigitalMicrograph [2]. Copper (II) oxide was reduced *in-situ* by heating in a MEMS based heating holder (Wildfire, DENSSolutions) and time resolved imaging and ELNES mapping of the Cu $L_{2,3}$ edge was used to follow the Cu reduction and particle morphology changes. However, non-zero dead time between successive spectrum image passes accounted for a large fraction of total acquisition time as the SI frame rate was increased. Additionally, the requirement to keep the full SI time series in system memory in this implementation limited the temperature range and increment that could be used. Finally, a lack of native software features for processing SI time series data made processing times impractical for larger datasets including multiple ionization edges.

Here we demonstrate further advances in *in-situ* spectrum imaging capability that have been made possible by a flexible next generation scan control system (DigiScan3) and a recent expansion of the *in-situ* software feature set in DigitalMicrograph version 3.6. True continuous multiple pass scanning and spectrum imaging can now be performed, allowing successive spectrum image passes to be acquired with zero dead time between scans. Sub-pixel scanning, which lowers the dose per SI pixel while maintaining image resolution, can be performed with hardware synchronization giving an increase in scan speed of up to 90x and no increase in dead time compared to the previous generation scan system. These features combined dramatically increase the time resolution at which *in-situ* spectrum image acquisition can be performed. New software features include full *in-situ* support of all EELS and SI processing functions, allowing rapid and flexible data analysis. Finally, all *in-situ* SI data is now streamed to disk, drastically increasing the size of datasets that may be captured.

Using this new acquisition framework, EELS spectrum imaging with *in-situ* heating has been performed on an oxide nano-powders of Fe_2O_3 . H_2O as shown in figure 1. Heating cycles were performed both with and without holder synchronization and control; the benefits of both approaches are discussed. By monitoring the Fe L_{2,3} edge fine-structure change using independent standards for the Fe(III) and Fe(II) oxidations states, so called concurrent standards, a quantitative measure of the Fe(III) to Fe(II) ratio can be monitored in real time as the sample is reduced. This method gives direct feedback of the dynamics of the chemical transformation on the nanoscale.



Figure 1 - DigitalMicrograph screenshot showing *in-situ* EELS spectrum image dataset acquired during *in-situ* heating experiment performed on Fe₂O₃.H₂O from 400°C – 900°C. Data was acquired in DualEELS mode using an electron-counting detector (Gatan, K3). Live quantification using concurrent standards acquired from FeO and Fe₂O₃ references showed a reduction from majority Fe(III) to Fe(II) at elevated temperature.

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Plasmon-Enhanced Fluorescence of Site-Specifically Immobilized Single Upconversion Nanoparticles

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Abstract

Single fluorophores are essential for ultrahigh-sensitivity bioimaging and biosensing. Upconversion nanoparticles (UCNPs) have unique advantages over other fluorophores for their high signal-to-noise ratio, good biocompatibility, high penetration depth into tissues and high photostability [1]. Apart from engineering the elemental composition to attain high brightness UCNPs, plasmonic metal nanostructures can be used to significantly enhance the fluorescence intensity of single UCNPs [2]. However, it is non-trivial to position colloidal single UCNPs and integrate them onto given metal nanostructures. Here, we use electron-beam lithography to create nanodomains functionalized with biotin as well as carbon nanodomains generated by means of electron-beam induced deposition (EBID) [3] to capture colloidal streptavidinconjugated UCNPs at the electromagnetic hotspots of the metal nanostructures. Thus, the concentrated local electromagnetic field of the metal nanostructures can significantly enhance the fluorescence intensity of single UCNPs illuminated with appropriate incident light. Moreover, the streptavidin-conjugated UCNPs can capture biotin-conjugated targets for biosensing through Förster resonance energy transfer [4].

Figure



Figure 1: Two methods for site-specific immobilization of streptavidin-UCNPs at the hotspots of gold nanorod dimers (yellow bars). Selectively functionalized SiO₂ nanodomains with silane-PEG-biotin (left) and use EBID to create carbon nanodomains (right).

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P32

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P33

Potential low-tech Zernike phase plate for soft-matter applications made from commercial carbon films

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Abstract

Zernike phase plates (ZPP) for transmission electron microscopy (TEM) have been in use since 2001¹. Phase plates are of continuing interest for soft-matter applications to enhance contrast and resolution². However, they have been shown to suffer from practical drawbacks regarding their implementation, operation, and fabrication. ZPPs with carbon films as the phase-shifting material have been proven to be effective with soft-matter samples, and are generally fabricated by hand using a sputtering tool in a single-device process³. A potential low-tech alternative could be to acquire commercially available carbon films, fabricate a hole in the thin film, and then use this as a ZPP in the TEM. ZPPs function best with a phase shift of $\frac{\pi}{2}$, which for an amorphous carbon film translates to a film thickness of 25 to 30 nm, depending on the acceleration voltage of the TEM (cf. Fig. 1a). The phase shift is related to the mean inner potential V_0 and the film thickness *t* (via an electron constant C_E) in the following way⁴:

$$\Delta \varphi = C_E V_0 t. \tag{1}$$

Thin carbon films already exist in the form of single-hole TEM grids that can be purchased from a number of vendors. The carbon thin film is suspended across a circular single-hole TEM grid made from copper, with a 100 µm hole in the copper platelet, which is 3 mm in diameter, and therefore fits into standard objective aperture holders of TEMs. We propose modifying these grids by milling a hole in the center of the film as a fast and easy method to create ZPPs. The carbon thin films were purchased from *EMS Diasum* (product *CFGA100-Cu-ET*). According to the supplier, their thickness range is between 20 to 30 nm. The central hole in the carbon film was made using a *TF Helios 5 Hydra UX Plasma FIB SEM*. The hole sizes vary from 100 to 1000 nm in diameter. We then used a number of techniques to attempt to estimate the actual thickness of the carbon film: We used an SEM to measure the thickness by sandwiching the carbon film between a tungsten layer and the copper substrate, yielding a thickness of 26.4 \pm 3.9 nm (cf. Fig. 1b).

We have preliminary results and intend to do further experiments and measurements using electronenergy-loss spectroscopy (EELS) and electron holography to characterize the carbon film regarding the mean-free path of electrons (λ), the phase shift caused by the film ($\Delta \varphi$), and the mean inner potential (V_0) of the amorphous carbon. We then intend to install the ZPP in the back-focal plane of a *TF Tecnai TEM*. The ZPPs will be characterized regarding their performance and operation. We intend to investigate how they compare to conventional TEM in terms of enhancing contrast and resolution when imaging soft-matter samples. Likewise, we intend to look into their contamination during usage. Marcus F. Hufe et al.

P33

Figures



(a) Mean inner potential over film thickness.

(b) Thickness measurement with the SEM.

Figure 1: The mean inner potential of a phase shifting material is shown in dependence on thickness (a), at a phase shift of $\frac{\pi}{2}$, for two different acceleration voltages of the TEM; the mean inner potential for amorphous carbon is around 8 V⁵. The thickness measurement of the purchased carbon films is shown (b), measured with the FIB-SEM used for milling the holes.

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Progress in quantitative EMCD experiments

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In an analogy to x-ray based technique XMCD [1], it was proposed and later on experimentally demonstrated [2] that the detection of magnetic dichroic effect is feasible in transmission electron microscope by recording electron energy loss spectra (EELS) under specific diffraction conditions. Considering the spatial resolution of analysis achievable in a (scanning) TEM, electron magnetic circular dichroism (EMCD) comes with a promise to map the magnetic moments with atomic resolution.

Unlike XMCD, there are several critical challenges associated with the EMCD technique. These include experimental as well as post-processing challenges. For example, EMCD is highly sensitive to dynamical diffraction conditions and the choice of sample thickness as well as diffraction conditions greatly influence the resulting signal. Another potential challenge is the requirement to acquire two, four or more EELS spectra at various off-axis scattering angles. Keeping the experimental conditions identical between these acquisitions is demanding especially when attempting for atomic resolution maps due to spatial drift, specimen damage, contamination and other instrumental instabilities. Moreover, EMCD signal has inherently very weak signal to noise ratio (SNR) and it becomes more weaker in atomic resolution conditions. Detecting a quantifiable signal under these conditions needs a high level of optimization in experimental conditions as well as sophisticated post-processing analysis. The small SNR of the EMCD signal can lead to large quantification errors which can make the results ambiguous. Therefore, it is important to carefully determine the "true" errors in the quantification process.

Here, we report our contributions to develop the EMCD technique on experimental part as well as post-processing analysis. Using custom-made hardware apertures, we develop methodologies for simultaneous acquisition of multiple off-axis EELS spectra, making it possible to carry out the EMCD experiment in a single acquisition. We also develop a method a simultaneously map the EMCD signal and the underlying crystal orientations in a single scan in STEM mode. We extend these methodologies from the two and three beam conditions to the more complex case of zone axis and experimentally demonstrate the detection of a quantitative EMCD signal under atomic-resolution conditions. On the post-processing part, we employ a statistical resampling technique called bootstrapping to a STEM-EMCD dataset to estimate the errors of analysis under different SNR conditions.

Posters

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In-situ nanoscale dynamics of nanoparticles using variable temperature TEM imaging

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Abstract

Traditionally, TEM has been used to investigate the morphology, structures, and chemical compositions at atomic scale. However, conventional imaging does not offer opportunities to study materials dynamics under various physical parameters. In this context, in-situ TEM allows observation of the dynamic behavior of materials in response to external stimuli such as temperature, gas environment, stress, and electric or magnetic fields at nanoscale and even atomic scale. In recent years, in-situ solid-state TEM with specially designed specimen holders and MEMS chips have allowed detailed studies on agglomeration and coalescence, melting and crystallization, transformations between crystal structures, and the formation of new phases by solid-state diffusion driven by temperature, voltage, or mechanical strain. Understanding nanoscale dynamics of coalescence is a crucial process in the bottom-top strategy for the development of nano or microstructure with broad scientific and technological applications in the field of, for example, bio-and chemical sensors, protein separation, recovery of catalysts, surface-enhanced Raman scattering and data storage devices [1, 2].

In this work, we monitored the real-time, structural changes of gold nanoparticles (AuNPs) using in-situ TEM imaging. We used citrate-capped gold nanoparticles of two different sizes (5.0 nm and 10.0 nm) and studied their dynamics by in-situ heating with a temperature range between 25 °C to 1000 °C.

Figure 1 shows snapshots from in-situ TEM imaging as a function of temperature when two small nanoparticles were under close proximity with a larger nanoparticle. Upon heating the sample two small nanoparticles (NPs) approached the bigger nanoparticle (NP) (10 nm) for coalescence (Figure 1a). Our results suggest that the larger nanoparticles remain stable until 700 °C. Where the coalescence of smaller particles occurs between 400 °C to 700 °C depending on their size. The melting of NP was initiated from the shell by changing to an amorphous or liquid-like structure. At higher temperatures, the change in the orientation of the lattice plane in the NP, by 68.5°, was also observed. The data after 700 °C is not given because the NP under observation was completely changed to amorphous form with the change in the shape as shown in Figure 1k, where both are considered two different ways of NP melting [3]. Our results provide crucial insights into the effect of size, shape, and proximity of nanoparticles on thermal stability, coalescence and melting.



Figure 1 (a-h) In-situ TEM observations of coalescence and shaping changes in gold nanoparticles (AuNPs) at different temperatures. White lines in (a-e) reveal coalescence behavior of 5 and 10 nm AuNPs with the formation of neck and dumbbell-shaped structures. Blue arrow in (a) presents the complete coalescence of small nanoparticle (NP) (less than 5 nm) before 400 °C. Red arrows show the melting of gold NP from the shell. FFT patterns in (i-j) validate the change in the orientation of the lattice plane by 68.5°, which is also shown in (f-g) by orange arrows. (k) FFT pattern of amorphous NP.

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Abstract

Electron energy-loss magnetic circular dichroism (EMCD) is a versatile technique for the analysis of magnetic properties of materials at the nanoscale [1] [2] [3]. In the classical EMCD measurement, the electron beam is passed through a crystal tilted to a systematic row condition and we measure the Electron energy loss spectra (EELS) at specific points in the diffraction plane. Although classical EMCD is a straightforward technique, the main limitation is the poor signal-to-noise ratio and spatial resolution [4] [5]. The EMCD measurements to date are often limited by a weak signal to noise ratio as well as the control of acquisition parameters and the quality of the sample. We could clearly improve the signal to noise ratio in our previous work by using dedicated apertures in the entrance aperture of the EELS spectrometer [6] [7] [8] [9]. Here, we present how an improvement of the crystalline quality of an Fe film is [10], and we analyze how the Fe film with smaller rocking curve angle impacts the EMCD signal.

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A new sampling paradigm for FRFPMS simulations of high energy resolution (STEM)-EELS experiments

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Abstract

High energy resolution Electron Energy Loss Spectroscopy (EELS) in the Scanning Transmission Electron Microscope (STEM) enables the study of phonon properties of materials at the atomic scale [1,2,3]. This unprecedented capability has implications for the nano-scale design of electronic devices, in which heat management is often a limiting factor. However, due to the complicated scattering process of electrons in materials involving in principle inelastic as well as multiple elastic scattering events, so-called dynamical diffraction, the design and interpretation of such experiments calls for detailed theoretical understanding and modeling of these interactions of the electron beam with the sample.

To this end we have introduced the Frequency-Resolved Frozen Phonon Multislice (FRFPMS) method, an extension of the classic FPMS method [4,5]. The method presumes no explicit knowledge of phonon modes, but the frequency-selectivity is achieved purely through frequency-selective MD simulations. Thus far, we have used so-called δ - or hotspot-thermostats to achieve this [6,7].

In this contribution, we introduce a new sampling paradigm for FRFPMS simulations and demonstrate, that it is superior to the frequency-selective thermostats. We call this new way of sampling Fourier-filtering, since it makes heavy use of Fourier transforms in a two step process: first a standard Molecular Dynamics (MD) trajectory is Fourier transformed and then a frequency region is selected, which is back Fourier transformed. Snapshots can then be sampled from the resulting filtered trajectory. This algorithm can be implemented as a pure post-processing step of a standard MD simulation. Using the new algorithm, we show that calculations of angle-resolved EELS show lower background and sharper bands. Furthermore the new sampling technique removes spurious intensity at Γ -points observed in Ref. [8], due to the lack of low frequency tails.

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Magnetic Domain Structure of Ferromagnetic Steels Studied by Lorentz Microscopy and Magnetic Force Microscopy

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Abstract

Properties of ferromagnetic materials are determined both microstructural and magnetic features. The magnetic structure of ferromagnetic material consists of regions with internal magnetization pointing to a certain direction and these areas are called as magnetic domains. They are separated by boundaries called as domain walls (DWs) where the magnetization direction changes. The magnetic regions are formed by complicated arrangement that is determined by the energy minimization principle. [1] The domains have for example different sizes; smaller size in martensitic steels which is full of individual nucleation sites (e.g. dislocations) to them compared to simple ferritic steel structure with larger domain size. One industrially relevant technical method, where the physical principle is strongly involving the domain structure and its changes, is the non-destructive testing (NDT) method called magnetic Barkhausen noise (BN) inspection. The DW structures and their differences influence on the BN signal measured when a time-varying magnetic field is applied. The magnetic field forces the internal domain structure to change and orientate towards the applied field. The microstructural details, such as dislocations and carbides, hinder the DW motion.

The aim of this study was to compare the magnetic structure in the bulk steel sample studied by magnetic force microscopy (MFM) to the magnetic structure in the thin sample studied by Lorentz microscopy. In MFM, the contrast is produced by the magnetic interaction force between the magnetic tip and sample surface stray fields showing DWs as bright and dark lines [2]. When using Fresnel mode in Lorentz microscopy, deflected beam electrons are superposed or diverged at the domain boundary showing DWs as white and black lines. In this study, MFM (Nanoscope iCon, Bruker) was utilized for imaging of bulk samples with ferritic and ferriticpearlitic microstructures. The thin films of both microstructures were studied with TEM (JEM-F200, JEOL) by using Lorentz microscopy. Fig. 1a shows topography of the ferritic bulk sample containing a ferrite matrix with globular cementite (Fe₃C) carbides. Based on the MFM studies (Fig. 1b), the globular Fe₃C carbides have their own domain structure appearing with alternating white and black lines as presented also in [2]. Similar type of internal magnetic structure of Fe₃C carbides was also observed by Lorentz microscopy in the thin sample (Fig 1c). There are also DWs in the ferrite matrix (Fig 1b and c). More complicated domain structure in the industrially relevant ferrite-pearlite sample was studied. A topography image presented in Fig. 2a shows ferrite grains with thinner and thicker lamellas and globular carbides of cementite (Fe₃C). The MFM image (Fig. 2b) shows similar internal contrast for the thicker lamellar and globular Fe₃C than in Fig. 1b. Whereas, the thinner Fe₃C lamellas appear only as bright/dark lines (Fig. 2b). The Lorentz microscopy image (Fig. 2c) reveals similar internal domain structure in thicker lamellar and globular carbides of cementite than observed by MFM (Fig. 2b). Based on Lorentz microscopy, thinner Fe₃C lamellas have no internal domain structure. DWs in the ferrite matrix are mainly parallel and perpendicular to the Fe₃C lamellas. In addition, cross-tie DWs (Fig. 2c) were observed by Lorentz microscopy as they are related to the thin film nature of the TEM samples. To conclude, similar domain structure details were noticed and visualized in both bulk samples by MFM and thin samples by Lorentz microscopy.

P38

Posters

Both methods, however, have their unique properties for contrast occurrence [3] and therefore, we can only see those DWs oriented favorably towards the electron beam (Lorentz microscopy) and the tip (MFM).



Figure 1 (a) Topography and (b) MFM images from same ferrite bulk sample area and (c) Lorentz microscopy image (Fresnel mode, underfocus) from thin sample.



Figure 2 (a) Topography and (b) MFM images from same ferrite-pearlite bulk sample area and (c) Lorentz microscopy image (Fresnel mode, overfocus) from thin sample.

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Colloidal gold transport in the Paleoproterozoic orogenic gold deposits: Outlining objectives of the PhD project

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Abstract

Orogenic gold deposits host major part of the world's gold reserves and are the most important gold deposit type in metamorphic terrains. Deposits are strongly structurally controlled and form relatively deep in the crust (5-20 km) from metamorphic fluids. Recent studies from orogenic gold deposit in Australia show that in addition to gold transport in solution as dissolved species, hydrothermal fluids can carry colloidal particles as a suspension load [1]. These findings have enormous implications how formation of orogenic gold deposits is understanded. However, more research focusing on nanoscale processes of gold is needed globally.

In our study, nanoscale processes of gold are studied from orogenic gold deposits located within Paleoproterzoic Central Lapland belt (CLB) and Peräpohja belt (PB). Reference deposits include Ikkari Au (CLB) and Rajapalot Au-Co (PB). Analytical workflow starts with proper selection of samples containing native gold and preparation of polished thin section, Selected samples are studied under reflective light and subsequently with FE-SEM (Field Emission-Scanning Electron Microscope), followed by preparation of micron scale lamellaes from within native gold using FIB-SEM (Focused Ion Beam- Scanning Electron Microscope). These lamellaes are suitable for nanoscale research with the TEM (Transmitted Electron Microscope) instrument. Study is part of Tapio Soukka's PhD research, focusing on nanoscale processes of gold and platinum minerals in primary and secondary mineral deposits as well as developing robust workflow of nanoscale research in the Material Analytical Center in the University of Oulu.

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iDPC-STEM imaging as an important tool for structure determination of low dimensional nanoporous materials

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Abstract

Over the last decades methods for 3-dimensional electron diffraction (3D-ED), such as MicroED and cRED, have opened up a more accessible route for structure determination from sub-micrometer-sized crystals. Structure determination of low dimensional materials however still remains a challenge. The lack of 3D periodicity of the reciprocal lattice limit the possibilities to retrieve a 3D structure *ab-initio* from the diffraction data.

Recent developments in aberration-corrected electron microscopy are now enabling opportunities for real-space imaging of electron beam-sensitive porous solids. The integrated differential phase contrast (iDPC) technique is particularly attractive as a technique to image under low-dose conditions with improved signal to noise properties.

Zeolites are nanoporous aluminosilicates which have been applied widely in industry as sizeand shape-selective catalysts and adsorbents, because of their ordered microporous structure of molecular dimensions. The number of topologically different 3D periodic zeolites today exceeds 250, each with a unique pore size, shape and channel dimension. Most zeolites have 3dimensional (3D) periodic structures and some have been possible to grow into 2-dimensional sheet like morphologies.

Recently we have shown, for the first time, a 1-dimensional tubular zeolitic material combining meso- and microporosity.[1] iDPC-STEM images obtained both perpendicular to and along the nanotube direction were keys in order to determine the atomic structure of this new material, see Figure 1a. It was found that ten identical repeating units are frequently observed around the circumference of the nanotubes, and the distance between the adjacent units is about 12-13 Å. Images acquired perpendicular to the nanotube direction reveal the microporous nature of the projected wall structure in more detail. Based on the iDPC-STEM images and 3D-ED data, the atomic structure of the nanotube could be deduced, see Figure 1b. The wall of the nanotube is penetrated by micropores limited by 10 silicate tetrahedral and the structure intrinsically imposes a curvature of this atomically thin zeolitic sheet directing the formation of a nanotube.

Figure



Figure 1 (a) iDPC-STEM image of three fused zeolitic nanotubes. The circumference of the nanotube is built from a repeating building unit, which mostly repeats 10 times. (b) Structure model of the zeolitic nanotube. Each node of the net represents a Si or Al-atom, bridging oxygen atoms are omitted for clarity. Green and purple represent the 12 and 10-rings respectively.

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A Sample Preparation Methodology for In-Situ Liquid Transmission Electron Microscopy of Nanolaminated Materials

P41

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Abstract

MXenes are transition metal carbides, nitrides or carbonitrides in the form of $M_{n+1}X_nT_x$ (n=1,2,3), where M is a transition metal, X is C or N, and T represents the surface terminations. Since their discovery in 2011, MXenes attracted a lot of interest owing to their rich chemistry and tunable properties.[1–3] MXenes are excellent candidates for energy storage, catalysis, sensors, composites etc.[4] Despite growing efforts, there are still many questions remain related to their response to external stimuli and their interaction with the environment. Here, in situ techniques are key for observing and understanding the dynamics of such processes at relevant conditions. In situ TEM has strong advantages such as being able to obtain information about structure in real and reciprocal space, electronic structure, as well as composition and chemistry with nanometer resolution. Although the main application for MXenes relates to electrochemical energy storage, few TEM investigations have been conducted on material in liquids. One of the reasons for this owes to the difficulty of sample preparation that produces quantifiable, reliable results. In this study, we propose a sample preparation method for in situ Liquid Electron Microscopy, using FIB (Figure). This method enables investigations of MXenes in different electrolytes with significantly different behaviors and for different applications including supercapacitors, Lithium Ion Batteries, and, catalysts.



Figure: A partially etched MXene multilayered lamellae on the working electrode on a MEMS chip.

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Analysis of angle resolved low-loss electron energy-loss spectra of the dielectric BaTiO₃

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Low-loss electron energy loss spectra (EELS) probes information on the collective excitations of valence electrons reflecting band structure and can be used to retrieve complex dielectric functions of the material in a wide energy range. Therefore, it is a relevant method to study local charge effects associated with the ferroelectric domain walls. However, excellent dielectric properties of ferroelectric perovskite oxides, which are reflected in the high refractive indices, poses a challenge for quantitative interpretation of the low-loss EELS experimental measurements as the probing electrons pass through the material with speed greater than speed of light (i.e. $v_e > c/n$) inducing additional energy losses associated with Cherenkov light emission.

Here, we demonstrate two strategies to retrieve Cherenkov loss corrected spectra. One approach allows precise bandgap determination, but the other permits retrieval of dielectric functions. Experimental complex dielectric function is compared with theoretical one calculated by time dependent density functional theory (TDDFT) approach and shows excellent match. Additionally, TDDFT calculation of oxygen vacancy affected BaTiO₃ EELS shows promising spectral feature, which could be used to quantitatively map defects in the ferroelectric perovskite oxide materials.



Figure 1 A comparison between experimentally obtained (grey line) and DFT calculated (red line) dielectric functions of BaTiO3. (a) Dielectric loss function, (b) inverse real part of dielectric function, (c) real part and (d) imaginary part of ε at radial momentum transfer q = 0 Å⁻¹.

P42

Impact of Electron Beam Irradiation on Carbon Black Oxidation

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Abstract

The oxidation rate of carbon nanostructures, such as Carbon Black (CB) when observed in the environmental transmission electron microscope (ETEM) is strongly dependent on the electron dose rate [1]. The mechanism whereby the electron beam affects the oxidation rate needs to be known if the observations made in the ETEM are to be interpreted correctly. To date, there are few studies reporting any quantification of the effect [1,2]. To better understand the origin of the beam effect, it is noted that the unperturbed oxidation reaction primarily occurs at so called active sites. These active sites can be generated through atomic displacements resulting from the elastic interaction between primary electrons (PE) and the sample or by reaction with oxygen ionized by PE [2,3] or secondary electrons (SE) [4]. These three mechanisms for electron beam induced oxidation are distinct and will respond differently to variations in reaction and imaging conditions.

In our study, we have observed the oxidation of CB at varying electron dose rates, electron energies, sample temperatures, and gas pressure, in two ETEM setups. Data were collected using electron energy loss spectroscopy time-series for averaging over large agglomerates of particles to obtain precise oxidation rate measurements at higher pressures. Low pressure data were collected as high-resolution image series to investigate the local effect on individual particles for mechanistic determination. Both these methods of data collection show similar trends in the oxidation data with a constant oxidation rate being followed and preceded by smoothly varying oxidation rates. For both the low-pressure and high-pressure data, there is a saturation effect whereby the change in oxidation is local to where the sample has been irradiated by electrons. These findings contradict the common claim that oxygen ionization by PE is the driving force behind the increased oxidation rate in TEM. The saturation effect also precludes ionization by SE as an important process unless the sample is located over a SiN_x support, in which case there are so many more SEs that they have a noticeable but minor impact. Atomic displacement induced active sites can explain our observations well.

Therefore, we have shown that CB oxidation is mainly affected by elastic high energy electronsample interactions, with a smaller contribution from inelastic electron-gas processes from secondary electrons emitted from the sample and sample support. Figure 1 also shows the presence of some more or less safe imaging conditions. If a safe dose rate is determined as a dose rate where the intrinsic reaction rate is more prevalent than the electron irradiation induced rate, then only the lowest measured datapoints with 19000 and 37000 e/nm² s could be considered safe. This is far below what is conventionally used in high resolution imaging, where 500000 e/nm^2 s is not uncommon signifying the importance of considering the electron dose rate in in-situ experiments.
Figures/Tables



Figure 1: Data showing the rate of oxidation of CB as a function of electron energy and dose rate. A shows aggregate data collected with electron energy loss spectroscopy and B shows data from individually followed CB particles. Note the apparent saturation of the effect of changing the dose rate, illustrated by the dotted line in A as a guide for the eye, and the linear interpolation of the mean value of the oxidation rate at each 300 kV dose rate. The green region in B corresponds to a zero-dose oxidation observation.

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Abstract

Nitride materials possess a variety of properties, such as a high dielectric breakdown voltage and bandgap energies ranging from ultraviolet to infrared, favorable for scientific development and application in efficient tandem solar cells, where the ideal bandgap for top cells is ~ 1.75 eV in combination with Si bottom cells.[1][2] However, some challenges related to materials phase separation and lattice mismatch, as well as cost barriers related to the choice of rare materials and initial growth process, pose problems for consumer implementation.

Thin films of $ZnGe_xSn_{1-x}N_2$ (where x=0, 0.428 or 0.736) were grown on P:ZnO by reactive co-sputtering of metallic Zn, Sn and Ge targets by high-power impulse magnetron sputtering (HiPIMS). These materials were selected due to their earth-abundance, non-toxicity. Structural properties were characterized by electron diffraction (ED) and chemical properties were analyzed with energy dispersive X-ray spectroscopy (EDS), both in transmission electron microscopy (TEM).

In the present work, high resolution TEM and ED were used in combination to indicate that thin films grow in the (0002) axis, perpendicular to the surface of the substrate, and a higher applied Ge target power predictably yields a higher Ge incorporation. A higher Ge incorporation is found to produce a more polycrystalline structure, in comparison to pristine ZnSnN₂. Additionally, ED indicates that the film structure without Ge has a significant lattice mismatch with the ZnO substrate ($a = (0.336 \pm 0.004)$ nm for ZnSnN₂, $a = (0.319 \pm 0.001)$ nm for ZnO, mismatch ~5.3%) and the induced strain is relaxed through formation of misfit dislocations. In contrast, films with higher Ge incorporation ($a = (0.328 \pm 0.005)$ nm for x=0.74, $a = (0.326 \pm 0.001)$ nm for ZnO, mismatch ~0.6% and ~1.2% respectively).



Figure 1. DF-TEM images (left), SAED patterns (middle) and EDS linescan of Zn, Ge and Sn atomic percentage across cross-section (right). Thin films deposited with a higher/lower applied Ge target power (sample A/B) and no Ge target (sample C).

Short bio

Hao Nguyen is a Master's student in materials science from the University of Oslo (UiO), mainly using transmission electron microscopy (TEM) to study structure and lattice strain in thin films in order to describe optical and electrical properties. He obtained a Bachelor's degree in Nanotechnology from the University of Bergen (UiB).

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The effects of heavy doping on the band structure of zinc oxide observed using momentum-resolved EELS

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Abstract

The ability to control materials' optical and electronic properties by modifying their structure with e.g. doping, is crucial for developing novel electronic devices. Zinc oxide (ZnO) is used in optoelectronic devices due to its direct band gap ($\sim 3.3 \text{ eV}$), affinity for n-type doping and strong excitonic properties, such as a high binding energy of $\sim 60 \text{ meV}$ [1]. Upon n-type doping, ZnO can be used as a transparent conductive oxide (TCO) in optoelectronic devices. A high doping level substantially fills the conduction band, resulting in a blueshift of the energy of the optical band gap. This is known as the Burstein-Moss shift [2-5] which has been studied using optical techniques and electron energy loss spectroscopy (EELS), dominated by contributions from transitions with small momentum transfer [4,5]. Low loss EELS in the Scanning Transmission Electron Microscope (STEM) is a powerful technique for characterizing the electronic and plasmonic response at high spatial resolution [6,7]. However, it provides only limited information about the momentum dependence of the electronic response. Doping a crystalline solid not only shifts the Fermi level but can also have dramatic effects on the electronic band structure [8]. By using momentum-resolved EELS, we measure the momentumdependent dielectric response [9], which provides insight into how electronic excitations depend on momentum transfer. This allows us to track spectral signatures of the dopant-induced alteration of the band structure, and further elucidate details behind the Burstein-Moss shift.

Ga-doped ZnO (GZO) was grown as a wurtzite thin film on a sapphire substrate using molecular beam epitaxy [10]. The GZO was heavily doped with a measured charge carrier concentration of $1 \cdot 10^{21}$ cm⁻³. Loss spectra in Fig. 1a shows a clear blueshift in the spectrum onset of the GZO compared to undoped ZnO, which is assigned to a Burstein-Moss shift of the band gap energy. Momentum-resolved EELS was acquired in parallel using the ω q-map technique [11]. Fig. 1b shows a recorded ωq -map of ZnO along ΓM direction in momentum space. The experimental results are complemented by *ab initio* calculations of the full momentum-dependent dielectric response function. Processed EELS data and the modeled loss function of ZnO along the ΓM direction is shown in Fig. 1c. The results are in excellent agreement, where the dispersion of the band gap edge is most prominent in the figure. Furthermore, by using different levels of model complexity, from density functional theory (DFT) and the random phase approximation (RPA) to the more advanced and Green's function methods such as GW and BSE, one can deduce information about the nature of excitations in the EEL spectra. Such as, identifying spectral features as having a single electron transition character or as arising from collective excitations. In this way, we explore the use of the momentum-resolved EELS combined with modeling for a detailed analysis of electronic excitations and their relationship to band structure in doped and undoped systems.

P45

Posters



Figure 1. (a) EELS at q = 0 for Ga-doped ZnO shows a blueshift of the spectrum onset (that is related to the band gap energy E_g) compared to undoped ZnO. The shift is designated to the Burstein-Moss shift. The peaks at 9.4 eV are assigned to interband transitions. (b) ω -q map of ZnO along the Γ M direction. (c) ZnO intensity maps of zero loss peak-subtracted EELS (left) and the loss function Im[$-\varepsilon^{-1}$] (right) calculated using DFT + RPA with PBE0, from Γ (q=0) along the M direction. The gap energy of ZnO with a minimum at q = 0 increases with momentum transfer towards the M point.

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Recent Innovation in Scanning Electron Microscope (Sem) In-Situ Extreme Mechanics at The Micro- and Nanoscale

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Abstract

Micro- and nanomechanical experiments are moving beyond the basic measurement of hardness and elastic modulus thanks to advantages of testing in situ in SEMs with EBSD. For example, until only recently, high strain rate mechanical testing of materials, at strain rates from $100 \text{ s}^{-1} - 10,000 \text{ s}^{-1}$, has only been possible using macroscale techniques, such as split Hopkinson bar, Kolsky bars, and plate impact testers. At the microscale, strain rates have typically been limited to approximately 0.1 s^{-1} or less, owing to limitations in instrumentation, insufficient data acquisition rates and elastic wave propagation conflicts during testing. The latest developments in nanoindentation instruments now allow access to extremely high strain rates up to 10^4 s^{-1} and high oscillation frequencies up to 10 kHz at the nanoscale. These state-of-the-art instruments have unlocked access to a wide range of previously unattainable micromechanical properties such as strain-rate sensitivity, fracture-toughness, or high cycle fatigue, by taking advantage of novel piezo-based nanoindentation methods.

This talk will focus on the most recent developments in instrumentation for in situ extreme mechanics testing at the micro and nanoscales. In the focus is a testing platform capable of strain rate testing over the range 0.0001 s⁻¹ up to 10,000 s⁻¹ (8 orders of magnitude [1]) with simultaneous high-speed actuation and sensing capabilities with nanometre and microNewton resolution, respectively. Additionally, the challenges and solutions to performing mechanical testing and imaging over the temperature range from -150 °C to 1,000 °C (Fig. 1), and the inherent advantages of using small volumes of sample material will be discussed, alongside the presentations of some examples of test data. Finally, future research directions in the field of extreme micromechanics will be discussed.

Figures/Tables



Figure 1 Load and displacement vs time curves under (a) 0.05 μ m/s (0.0025 s⁻¹) and (b) 6000 μ m/s (1425 s⁻¹). (c) Comparison of stress-strain curves at different strain rates. Test performed on a fused silica micropillar with 5 μ m height.

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Exploring micro-scale fracture behavior in brittle thin films

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Abstract

Fracture is the catastrophic failure event which limits the efficiency and lifetime of all materials in their applications. Functional materials used in different miniaturized applications like microelectromechanical (MEMS) systems are used in the form of thin films, and these materials (mainly oxides) are inherently brittle in nature. During service, they develop mechanical stresses due to incompatibility between substrate and film, which leads to its failure due to film cracking or interface delamination. It is important to know the behavior of fracture in these thin film systems and design strategies to improve the service life of the system. Since the thin film thickness are in the order of few micrometers (or even hundreds of nanometers), size effects play a prominent role in their mechanical behavior which is very much different from the bulk material behavior.

In this study, we report *in situ* microcantilever bending and fracture tests using an Alemnis insitu nanoindenter inside a scanning electron microscope to study the deformation and fracture behavior of brittle thin film systems like barium titanate (BaTiO₃) [1] and aluminum oxide (Al₂O₃) [2]. Micro-cantilevers were milled using a Ga⁺ ion focused ion beam (FIB). Microfracture experiments were carried out on free-standing and bi-layered film-substrate combinations with stiff and compliant substrates to study the effect of interfaces, film thickness and elastic modulus mismatch on fracture resistance. Fracture toughness enhancement was seen in the presence of stiffer substrates, while interface delamination induced failure was observed in systems with flexible substrates. *In-situ* tensile test based on the shear lag model in combination with digital image correlation was used to understand the cracking behavior of these films when deposited on flexible substrates. Various aspects affecting the fracture behavior of thin film systems will be discussed in detail.



Fig 1: a) *In situ* microcantilever fracture experiments and b) *in situ* micro-tensile shear lag tests on BaTiO₃ thin films [1].

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Rapid in-situ magnetic imaging of artificial spin ice using STEM-DPC

P48

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With the increasing demand for more energy-efficient computing devices, there is a need to move beyond traditional silicon-based semiconductors. One possibility is artificial spin ice (ASI), where a large number of nanometer-scale magnets are arranged in a lattice close enough to interact with each other through their stray fields (Fig 1a) [1]. By tuning the size, shape, and distance between the magnets, the large-scale behavior changes, possibly allowing them to be used as in-materio computational devices by applying external stimuli [2].

However, to properly study the magnetic properties of these systems, both the full lattice and individual nanomagnets, there is a need for techniques that can study the magnetic properties at nanometer length scales while applying external stimuli such as magnetic fields. Scanning Transmission Electron Microscopy (STEM) is a well-established technique predominantly used to study the nanoscale structure and composition of materials. However, by turning off the objective lens of the microscope, internal magnetic fields in materials can be imaged by utilizing the Lorentz force. This force leads to a deflection of the electron beam, which can be detected on any standard STEM detector. This technique is called STEM – Differential Phase Contrast (DPC) [3]. By combining this with weakly exciting the objective lens and tilting the sample, it is possible to image the flipping of ferromagnetic domains in materials down to the nanoscale.

In this work, we utilize a JEOL 2100F TEM to study nanostructured permalloy ASI using STEM-DPC while applying an external magnetic field via the objective lens. The beam deflection is measured using a standard Annular Dark Field (ADF) detector, which is available on the vast majority of STEMs. We demonstrate that this approach works for ASI elements down to 225x75nm, which is comparable to the dimensions typically used in the ASI community [1]. Figure 1 shows an example of this, where the collective behavior of such an ASI into stable or metastable states at various tilt angles. In summary, this works shows that an uncorrected STEM with a standard ADF detector can be used to study ferromagnetic domains in ASI structures and might contribute to more accessible, flexible, and efficient studies of nanomagnetism in the future.



Figure 1 STEM-DPC data of a pinwheel ASI structure composed of 225x75nm islands. The structures were FIB milled into 20nm of Permalloy. Subfigures (a-c) display the STEM-DPC signal at zero tilt magnetization, where the decomposed DPC signals are shown in (b) for x-direction and (c) for y-direction. Subfigures (d-f) illustrate the collective behavior of the

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Abstract

Our study aims to investigate the mechanisms that affect the optical transparency of nanostructured translucent ZrO2 ceramic formation [1]. The production of advanced ceramics with desirable properties for various applications, such as 3D printed ceramics, has been a subject of extensive research. Achieving optimal microstructure and properties of ceramics requires a deep understanding of their nanocrystal growth and pore formation. Our study has demonstrated the essential role of electron microscopy in providing a detailed analysis of the microstructure of ceramics and identifying changes resulting from different processing methods. These findings can inform the development of more effective techniques for producing high-quality ceramics with desirable properties.

To achieve translucent ceramic samples, we obtained a low agglomeration nanosized powder and sintered it at low pressure and low temperature. Even low pressures caused structural changes and defect creation in the nanocrystals, which we studied through annealing. Our findings highlight the critical role of electron microscopy in examining the microstructure of nano ceramic samples. Specifically, cross-section TEM images show a low quantity of pores in the pellets, with grain growth observed during annealing and the fusion of smaller nanocrystals into edge arrangements. Additionally, SEM images reveal changes in pore abundance and size with varying annealing temperatures. By analysing the SEM images, we determined the percentage and volume of pores and crystalline growth. Significant changes in translucency were observed with an increase in pore size.

Our study provides insights into the mechanisms that affect the optical transparency of nanostructured ceramics and highlights the critical role of electron microscopy in examining the microstructure of these materials.

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Abstract

White root rot is a disease of fruit trees caused by a fungus, *Rosellinia necatrix*. This fungus lives in humid soil and attacks the plant's roots, eventually killing them if left untreated. However, this disease is difficult to control with fungicides. Therefore, we isolated a dsRNA virus, then named it from a fungus field strain, W779, as Rosellinia necatrix megabirnavirus 1-W779 (RnMBV1). RnMBV1 is a candidate for controlling white root rot disease; however, more molecular and structural investigations are needed. Here, we have performed cryo-electron microscopy (cryo-EM) single-particle analysis, to obtain the first atomic models of RnMBV1 particles. Based on the structures, three main features were identified: 1) Extra-long arms on the C-terminus of major capsid proteins (MCPs); these are hypothesized to facilitate viral particle assembly. 2) MCP protrusion domain and extra surface Crown Proteins (CrPs); these may be required for viral transmissions. 3)5-fold obstructed pores; these may be required for cellular nucleotide triphosphates (NTPs) intake and synthesized genome release or both. These structural features might play important roles in the RnMBV1 viral life cycles. Understanding these roles would encourage us to apply this virus as a fungal control.

Figures



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Uncovering functions of unique structures in a mosquito totiviruslike virus

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Totivirus-like viruses are an unclassified taxon of double-stranded (ds)RNA viruses and are responsible for diverse problems in the fishery industries such shrimp cultivations in tropical countries and salmon cultivations in Norway. However, very few studies have been published regarding their structures or molecular mechanisms both of which are essential for transmission and replication in the host cells. We are therefore gravely lacking important concepts necessary to inhibit these viruses' proliferation and identify possible vaccine targets.

We have, therefore, studied structure-functions of totivirus-like viruses hypothesized to be involved in the mechanisms involved in transmission and replication. The atomic model of the totivirus-like Omono River virus (OmRV) capsid (Figure 1A) was previously determined through cryo-EM single particle analysis (3.2 Å) [1, 2, 3] and this atomic model allowed us to point out that totivirus-like virus have acquired surface crown protein (CrP) pentamers on top of pores obstructed by a spherical plug (Figure 1B). Both are located on the 5-fold vertices of the capsid.

In order to assess the function of these acquired structural features, we have developed the first totivirus-like OmRV infectious DNA clone [4] and generated a mutant (R925A) that has an impaired function concerning the obstructed pores. This OmRV mutant displays a significant reduction in its propagation efficiency in host cells, which allows us to conclude that these acquired structural features in the totivirus-like viruses are indeed important for their replication. We have determined the atomic model (2.98 Å) of this OmRV mutant which revealed unobstructed pores in the 5-fold vertices (Figure 2). We hypothesize that these pores are involved in nucleotide uptake necessary for genome synthesis inside the viral capsid and we are currently developing an assay to confirm and quantify the replication of the viral genome.



Figure 1 - Volume of the Omono River Virus and its distinct structural features. (A) Volume of the Omono River Virus (OmRV) atomic model (3.2 Å) with a subunit marked by the red triangle. Each of the two proteins that compose a subunit are in different colors. (B) 5-fold volume of OmRV with a crown protein (CrP) pentamer on top of a pore obstructed by a spherical plug. Each of the two proteins that compose a capsid subunit are in different colors.



Figure 2 – Volume of the Omono River Virus (OmRV) R925A mutant atomic model (2.98 Å). It is possible to see the unobstructed pore in the 5-fold vertex. Each of the two proteins that compose a capsid subunit are in different colors.

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Results from the Quantum C100, a Novel CMOS Detector Optimised for 100 keV Cryo Electron Microscopy

P52

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Recent developments in direct electron detection technology are opening new research avenues in cryo–Electron Microscopy (cryo–EM). Faster and more efficient detectors significantly expand insights into the structure and function of biological molecules. Cryo–EM presents exciting opportunities to study proteins, protein complexes, organelles, drug delivery systems, cells and tissues in great detail.

A study by Peet et al. quantifies the beam induced sample damage depending on the accelerating voltage of the microscope. It is evident that reducing the operating voltage from 300 keV to 100 keV significantly reduces sample damage and simultaneously allows for recovery of 25 % more structural information¹. To date, the majority of structures deposited in the Electron Microscopy Data Bank (EMDB) with resolution better than 3 Å were determined using single particle approach at 300 keV accelerating voltage^{2,3}. Importantly, transmission electron microscopes (TEMs) operating at 300 keV are expensive to purchase and maintain, therefore, putting them out of reach for many researchers and present a significant barrier to discovery⁴.

100 keV TEMs offer a solution for democratic access to structure determination. Until last year, there was a paucity of suitable detectors optimised for 100 keV operation⁵. Here, we present first data from our newest direct electron detector optimised for 100 keV work, the Quantum C100.

The Quantum C100 was specifically optimised with 100 keV accelerating voltage in mind. Main characteristics of the ideal detector are high detective quantum efficiency (DQE) and modulation transfer function (MTF). These critical parameters are greatly improved by de novo design of the pixel itself.

It contains a wafer scale CMOS sensor with a gapless array of 2048 x 2048 pixels and 54 um pitch. The large area is combined with a 2000 fps frame rate, resolution of 12 bits and

P52

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3D electron diffraction of small molecules on the MerlinEM detector

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The MerlinEM/Medipix3 is a versatile detector widely used for scanned electron diffraction, precession, dynamic transmission electron microscopy (TEM) and scanning transmission electron microscopy (4D STEM). It can be used to image direct probes (up to 300kV) while still retaining the ability to count single electrons for the weaker diffraction spots (1). It contains two 12-bit counters, enabling zero dead time acquisitions and noiseless readout. This makes MerlinEM an attractive solution for low-dose techniques in many areas of electron microscopy.

We are particularly interested in the application of micro electron diffraction (3DED, microED) techniques for structure determination of small molecules of biological and pharmaceutical importance (2). 3DED has been growing in popularity in recent years thanks to the development of more sensitive detectors and stable automated diffraction setups. Electron diffraction has the essential advantage over X-ray crystallography when crystal size is concerned. It produced multiple medium to high resolution structures from nano crystals and from artificially thinned (by focused ion beam milling) crystals (3 and 4). Furthermore, electrons have an advantage over X-rays as they interact orders of magnitude more strongly with samples. This allows hydrogen atoms to be located within the structure to gain insights into the chemical properties of small compounds. This is essential in chemical research, pharmaceutical drug discovery and other industries (5). Furthermore, performing electron diffraction tomography on multiple crystals in an automated manner enables efficient collection of large datasets. For this reason, we worked closely with the team at Quantum Detectors. A recently available plugin for MerlinEM to the academic SerialEM software environment (6) has enabled low-dose 3DED data collections on the MerlinEM Quad 4S installed on the FEI Tecnai F30 cryo electron microscope at the Rosalind Franklin institute. We present data obtained on small compounds of pharmaceutical interest by HeXI at Diamond Light Source using 3DED with MerlinEM.

- 1. Medipix3 has been commercialised as a MerlinEM detector by a collaboration between the University of Glasgow and Quantum Detectors Ltd.
- 2. Shi et al., 2013
- 3. Duyvesteyn et al., 2018
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- 5. Palatinus et al., 2015
- 6. Mastronarde, 2005

Crystallographic Data obtained from CryoEM Imaging

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Abstract

Much cryoEM imaging of soft materials employs magnifications of between 10,000x and 80,000x. The pixel sizes in this magnification range are around 1nm to 0.15nm and hence the images do not record lattice spacings of typical crystalline materials present in the sample.

For the large defocus values typically used in cryoEM, however, "ghost images" of reflecting crystals may be observed. These are "dark field images" and they would be superimposed on the "bright field image" at focus. (And with small objective apertures, they would be removed from the image anyway.) But for larger apertures, images will be formed by both the unscattered beam and the diffracted beam, and at large defocus values, there will be a measurable displacement between these two images. The defocus is generally available from the FFT of the image and this value, combined with displacement and the wavelength, allows the spacing of the lattice planes can be calculated.

We note here that the standard routes for obtaining information about the lattice are a) to record at higher magnifications - but then the electron beam dose is an issue, or b) to switch to diffraction mode - but that is awkward and rarely done in any standard cryoEM workflow. Hence it is thought this straightforward method will allow some crystallographic data to be obtained that adds to the standard data in the image about size, shape etc.

The expression for the displacement of the images (often referred to as delocalization) is given by

$$R = \lambda g f + C_s \lambda^3 g^3$$

where λ is the electron wavelength, g is the spatial frequency of the lattice planes, f is the defocus, C_s is the coefficient of spherical aberration [1]. For the case of the large defocus values mentioned above, e.g -10 μ m (contrast this to a typical value for Scherzer defocus of around -0.1 μ m), the contribution from the defocus term at left is much larger than the spherical aberration term at right and we can simplify to

$$R = \lambda g f$$

As an example of the effect, we show in Figure 1 an image taken on the Lund JEM 2200FS. The cryo polepiece has a C_s of 2 mm, the accelerating voltage is 200 kV ($\lambda = 0.0025$ nm). The sample is held in a Fischione cryoholder at around -170 C. The magnification is 20kX (and pixel size 0.58 nm), and the defocus measured from the position of the first dark ring in the FFT is -11 µm. The sample includes nanoparticles of TiO₂ which are expected to take the rutile or anatase structure. The displacements between images of two particles are measured at 53 and 57 nm. We assume they are the same diffracting lattice planes responsible for the "dark field" images and feeding in the average value of 53 nm into the above expression, we find the lattice spacing responsible is around 0.5 nm.



Figure 1. An image showing two nanoparticles which each have a "bright field image" and corresponding "dark field image". The white lines indicate the estimated displacements between these bright and dark images (53 and 57 nm) in each case. The nanoparticles are held in vitrous ice, from whose image the FFT is used to estimate image defocus. Some of the (rather damaged) carbon support is also visible.

The presentation will discuss the accuracy that might be possible when making these measurements and provide other cases apart from inorganic particles, for example, layered structures in fibres. We will also compare this procedure to measurements of interplanar spacings using other diffraction or imaging methods.

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References

An Automated Method for Quantifying Actomyosin Ring Dynamics in Drosophila Cellularization

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Abstract

Cell skeleton dynamics are regulated by numerous proteins, with actin and myosin forming the basis of the dynamic structure. Proper regulation of cell shape is crucial in various biological processes such as cell migration, cell division, tissue morphogenesis, and cell signalling¹. The fruit fly (Drosophila *melanogaster*) cellularization serves as a widely used model system to study protein interactions and the role of cytoskeletal dynamics during tissue development. Cellularization occurs following 14 consecutive nuclear divisions². After the final division, membrane furrows ingress around the nuclei (Figure 1A),

driven by an actomyosin ring at the leading edge. The dynamics of the 10 min actomyosin ring, including its shape and contractility, are tightly regulated by proteins like the actin cross-linker Filamin. Incorrectly timed or shaped changes can often result in developmental defects or lethality, highlighting the importance of proper regulation during these processes².





Figure 1 Cellularization of *Drosophila Melanogaster* during early embryo development visualized with Filamin-GFP. A) Vertical progression of the cellularization front along the Z-axis. B) Top view depicting the cellularization front, illustrating how the initial hexagonal structure transitions to a more circular arrangement during cellularization. Scale bar $5 \mu m$.

Initially, the actomyosin ring assembles into a hexagonal array and as cellularization progresses, it becomes more circular (Figure 1B). Concurrently, the rings undergo contraction in two distinct phases: slow contraction during Phase I, and faster contraction during Phase II, as the leading-edge approaches completion.



Figure 2 Workflow of the automated actomyosin ring segmentation process. 1) A single actomyosin ring is selected using the oval selection tool. 2-3) Noise is eliminated from the isolated ring with the Noise2Void plugin. 4) The ring is thresholded. 5-6) A selection is created that traces the inner contour of the ring.

When segmentation of actomyosin ring dynamics is done by hand, the analysis can lose contrast, be time-consuming, and carry the risk of introducing bias towards more favorable or expected outcomes. To address these limitations, we developed our own automated method by creating a custom ImageJ macro (Figure 2) for quantifying actomyosin ring circularity and perimeter changes. This automation process uses the Noise2Void machine learning ImageJ plugin for noise removal. After this step, other ImageJ plugins enable more accurate thresholding, leading to precise shape segmentation. Our method accelerates the analysis process, eliminates potential bias, and enhances the overall quality of the analysis while detecting subtle changes in circularization and perimeter during cellularization.

Our method enables the investigation of protein interactions related to myosin activation. In our ongoing study, we are examining the relationship between the myosin-activating protein kinase Drak and cross-linker Filamin the actin during cellularization. The current working model is that Drak is activated when it binds to the mechanosensory region of Filamin, which is exposed by contractile forces within the actomyosin ring. Activated Drak subsequently induces myosin contraction,

leading to further ring contraction. Preliminary results indicate that defects in the mechanosensory region produce changes similar to those observed in Drak knockout fly models. Specifically, the actomyosin rings in defected Filamin mechanosensory flies and Drak knockout flies don't become round and contract as well, suggesting defected myosin activity. These findings further emphasize the importance of understanding the interactions between Drak and Filamin in the regulation of actomyosin ring dynamics during cellularization.

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Comparison of the fundamental cell morphology of species in genus *Mycobacteroides* obtained from whole-mount ice-embedded cryo-TEM examination.

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Abstract

In 2018, new classification was proposed for the family *Mycobacteriaceae*, which has contained nearly 200 species in a single genus *Mycobacterium*, and novel genera have been emended (1, 2). In 2019, this new classification was restored to the previous single genus although new names for genera and species emended in 2018 were allowed to use as heterotypic synonyms. In 2020, we have reported the fundamental cell morphology of 31 species (34 strains) in five genera in the family *Mycobacteriaceae* examined with whole-mount ice-embedded cryo-TEM (3).

One of the genera *Mycobacteriodes* contains 6 species with 3 subspecies. These species share 24 conserved signature peptides and 27 conserved signature indels, and, therefore, are closely related in the phyletic affinity to each other. We have reported the results of the cryo-TEM examination in 4 species with 3 subspecies. In this study, the fundamental cell morphologies of all 6 species of genus *Mycobacteroides* containing 853 cells were examined with whole-mount cryo-TEM, and cell diameter, cell length, cell perimeter, circularity, and aspect ratio were measured with ImageJ/Fiji software and compared between species or subspecies.

35 comparisons were performed in each species with 140 comparisons in total. There were no significant differences between *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* in all comparisons. *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *massiliense*, *M. franklinii* showed 18, 12, 15, and 17 non-significant differences between other species, respectively. On the other hand, *M. chelonae*, *M. immunogenum*, and *M. salmoniphilum* showed 28, 26, and 30 significant differences between other species.



Figure 1. Representative cell morphologies (the average, the shortest, and the longest) of the species or subspecies in the genus *Mycobacteroides*.



Figure 2. Scatter chart defined by cell diameter and length of each species or subspecies. Each spot indicates a single cell. Bar charts indicate the average, standard deviation, the shortest, the longest values of the cell length, and the longest / shortest ratio in the species or subspecies.

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