PROGRESS OF PHASE I OF PROJECT ER-C 2.0: INSTALLATION AND PERFORMANCE OF THE INSTRUMENTS BIO AND TOMO

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Within the framework of the National Roadmap for Research Infrastructures of the German Federal Ministry of Education and Research (BMBF), the team of the Ernst Ruska-Centre at Forschungszentrum Jülich together with partners at RWTH Aachen University and Heinrich-Heine-University Düsseldorf has proposed to establish a national infrastructure for ultra-high-spatial-resolution characterization using the most advanced electron microscopy techniques, spanning from physical sciences and materials science to life science. While the initial proposal was already submitted in January of 2016, the rigorous evaluation process lasted until fall of 2019, when the project ER-C 2.0 was accepted and incorporated in the National Roadmap for Research Infrastructures. Funding of the project was split in two phases, in Phase I the instruments BIO and TOMO and the first part of the building were funded. In Phase II (funded in 2021) funding for the instruments SPECTRO, FEMTO and OPERANDO and the remaining building costs was obtained. Here we report on the progress of the installation of the BIO and TOMO instruments, their unique features and the results of initial performance tests and applications.

The BIO instrument combines three novel developments in a dedicated instrument for cryo-electron microscopy: helium temperature stage, double Cs/Cc corrector and a dedicated STEM probe corrector. In this combination, the BIO instrument has been designed to improve the visualization of thick specimens in the crowded cellular environments. Imaging of vitrified single-particle specimens at helium temperatures has recently been demonstrated to reduce radiation damage effects and improve information transfer at high resolution[1]. While chromatic aberration correction extends the information limit for microscopes with large pole piece gaps, it also enables the contribution of inelastically scattered electrons towards image formation[2]. Spherical aberrations of convergent beam probes limit resolution for STEM methods such as center-of-mass imaging. Correction of this aberration has been shown to improve optical resolution and contrast for radiation sensitive specimens. In this microscope, the beneficial effects of probe aberration correction will be applied to vitrified biological specimens with state-of-the-art 4D-STEM detectors. We will report on the practical aspects of the operation of the BIO instrument and present first results obtained from vitrified biological test specimens.

The TOMO instrument is based on a combination of two well-known but fundamentally different materials analysis techniques in one device: a state-of-the-art atom probe integrated into a high-performance TEM. The TOMO instrument represents a novel approach towards atomic-precision imaging and analysis, coined 'Atomic-Scale Analytical Tomography' (ASAT) by Kelly, Gorman and Ringer[3], first realized in proof of concept instruments by Gorman[4] and Lefebvre[5]. The most important advantage results from the essential complementarity of the two techniques. In a correlative approach, the local elemental composition at a defect or any other microstructural feature can most sensitively be analysed with the atom probe technique, while the atomistic structure and the local bonding situation in a complimentary way can be obtained by HRTEM/STEM and EELS. We will present the design and novel features of the instrument and the first results of applications of the combined APT/HRSTEM instrument.

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Advances in 3D reconstruction of analytical signals for elemental mapping in cryo-preserved biological samples

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Protein complexes depend on a variety of bound species to mediate and regulate their activity, including metals and other ions, organic cofactors, substrates, products and inhibitors, and lipids. However, identifying these species within the experimental maps produced by cryo-EM single-particle analysis (SPA) is challenging, since no methods exist for elemental mapping in such samples. Existing analytical modes of operation, including scanning transmission electron microscopy-electron energy loss spectroscopy (STEM-EELS) mode, can produce atomically-detailed elemental maps, but only at much higher electron doses than are compatible with cryo-preserved biological material. We have shown that 3D reconstruction of such analytical signals, using a correlative image processing pipeline, allows STEM-EELS to produce meaningful elemental maps for cryopreserved biological samples imaged with less than 100 $e^-/Å^2$ [1]. Two major obstacles were identified to reaching single-atom sensitivity with this approach: the size (number of images, and therefore cumulative dose) of the reconstructed dataset, which is limited by the speed of image acquisition; and the quality of the reference image used to calculate reconstruction poses. I will discuss our recent progress in addressing these two limitations.

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Cryo-EM reveals naturally occurring dimeric photosystem II lacking the Mn_4CaO_5 cluster

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Photosystem II (PSII) is a crucial membrane complex in cyanobacteria and chloroplasts, driving the water-splitting reaction and reducing plastoquinone during photosynthesis. PSII assembly is a stepwise process involving several accessory factors that regulate its formation. Recent cryo-EM studies have shed light on various PSII assembly intermediates by deleting PSII subunits to accumulate protein level of PSII intermediates. In this study, we used cryo-EM to determine the structure of a naturally occurring less active dimeric PSII complex from *Thermosynechococcus elongatus* at 2.2 Å resolution. This species consists of active dimers, semi-active dimers with extrinsic subunits at one monomer, and inactive dimers lacking extrinsic subunits. Our findings revealed structural differences in the inactive monomers, particularly around the Mn_4CaO_5 cluster, providing insights into the final stages of PSII assembly and potential photodamage mechanisms.

Advancing Quantum Material Characterization via the JEOL Atomic-Resolution Multi-Dimensional TEM: From Sub-15 meV Excitations to Dynamic Polarization Mapping

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The rapid development of quantum-materials and reconfigurable-electronics research demands instruments that combine sub-Å spatial resolution with meV-scale energy resolution. The JEOL Atomic-Resolution Multi-Dimensional Transmission Electron Microscope (ARMD-TEM) was expressly engineered for such multidimensional electron-optical studies [1]. Integrated with the CEOS Energy-Filtering and Imaging Device (CEFID) [2,3], it pushes the limits of high-resolution electron energy-loss spectroscopy (EELS) and related imaging.

Operating at 60 or 200 kV, the ARMD-TEM features a wide-gap objective pole piece for *in-situ* and tomography experiments, a C_s-probe corrector, and bright-field, annular-dark-field and 8-segment SAAF detectors, delivering sub-Å STEM resolution [1,4]. Its data-acquisition suite comprises a pre-filter MerlinEM direct-electron detector, pre- and post-filter TVIPS XF416R CMOS cameras, a post-filter Dectris ELA hybrid-pixel detector, and a dual EDX detector, enabling sophisticated mapping. A Schottky FEG, double-octopole Wien-filter monochromator and post-column CEFID provide ultrahigh-resolution spectroscopy. Long-term stability is demonstrated by <200 meV peak drift over 11 h. Coupled with the Dectris ELA detector, routine energy resolution is <25 meV. After Gaussian drift correction of 10 000 spectra (0.1 ms / frame), we achieve a best-case FWHM of 14.3 meV (60 kV) and 17.1 meV (200 kV). A dedicated condenZero liquid-helium side-entry holder enables cryogenic operation at ~5 K (±2 mK) [1], allowing temperature-dependent studies of low-energy excitations with unprecedented clarity. Ongoing installation of a cold-field-emission gun targets sub-10 meV, ultimately 5 meV, resolution, expanding access to even lower-energy phenomena.

Complementary in-situ biasing experiments were performed on a second JEOL platform using a Protochips biasing holder. 4D-STEM of lithographically defined, FIB-refined Au nanostructures yielded quantitative maps of local electric fields and polarization dynamics within nanoscale junctions [6]. This dual-platform strategy, anchored by JEOL's technical excellence, offers a comprehensive framework for correlating charge redistribution and field confinement, thereby facilitating rational design of quantum sensors and ultra-low-power memory elements. The presentation will first showcase ARMD-TEM performance, then illustrate selected applications [7].

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Stroboscopic single electron Cryo-EM and 4D STEM to study Parkinson's Disease

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Short Abstract

Cryo-transmission electron microscopy (cryo-EM) or tomography (cryo-ET) of frozen hydrated specimens is an efficient technique for analyzing the structure of proteins or tissue sections. We study human brain tissue from patients who died from Parkinson's disease or Alzheimer's disease, using high-pressure freezing, sectioning and cryo-ET, as well as correlative light and electron microscopy (CLEM). We also study alpha-synuclein fibrils, which is the protein underlying Parkinson's disease, multiple system atrophy (MSA) and Dementia with Lewy bodies (DLB). Different fibril polymorphs will be presented and their potential impact on intracellular neurodegeneration mechanisms discussed.

Cryo-EM and cryo-ET suffer from a signal-to-noise ratio or recorded images. We evaluated, if stroboscopic single electron illumination, generated with the help of a 2.4 GHz RF Cavity, could be used to improve SNR. We also employ a 4D-scanning transmission electron microscopy with parallax, shadow image, or ptychography data processing, using an aberration-corrected Titan Krios, to study neurodegeneration. This 4D-STEM approach shows improved contrast of cryo-EM images.

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RADIOLYSIS OF LIQUID WATER AND DYES IN THE STEM

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Radiolysis is a major barrier in liquid phase - (scanning) transmission electron microscopy, LP-(S)TEM, introducing artefacts in the study of nanomaterials in solution and directly impacting the reliability and reproducibility of experiments. The radiolytic products formed by the electron beam change the chemistry of the solutions and of the nanomaterials of interest. While the radiolysis of liquid water by high-energy electrons has been extensively studied by conventional radiation chemistry methods, the radiolysis of water and of organic molecules at the very high fluxes and fluences of the electron microscope is poorly understood. Electron energy loss spectroscopy (EELS) can be performed inside the STEM, and thus, can allow for in situ analysis of radiation damage effects. In frozen hydrated aqueous specimens, cryo-EELS has already been used to study radiation damage.[1-3] Using monochromated sources[4] and increased peak(signal)-to-background (S/B) ratio, new insights on the radiation damage of materials have been made possible. Recently, we have shown that by using monochromated EELS at the oxygen K-edge and at cryogenic temperatures on thin films of ice, all radiolysis products (radicals and molecules) of water ice can be resolved (except for H2 and •H)[5]. In liquid water, however, the concentrations of radiolytic species in the probed area decrease sharply due to higher diffusion and reactions, thus requiring much higher exposures to obtain enough S/B for species such as the hydroxyl radical (OH•). In this presentation, we will introduce an alternative methodology allowing to experimentally track the evolution of the radiolytic species production in aqueous solutions and in the electron microscope. We will also discuss the decomposition of dyes by the electron beam. In molecules with cyclic compounds, ionization of molecules by high-energetic electron beams is followed by very rapid ion recombination reactions which lead to a high production of excited triplet and singlet states.[6] These excited electronic states give rise to molecular and radical products and are thus behind the degradation of aromatic compounds and dyes in electron microscopy. We will demonstrate the ability of STEM-EELS to probe the excited electronic states of Rhodamine B (RhB) dye molecules as a solid and in a liquid solution. Finally, we will present predicting tools for the production of radiolysis species in STEM.[7]

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Microanalytical Sensitivity of X-ray Energy Dispersive Spectroscopy in the Analytical Electron Microscope

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Microanalytical resolution and sensitivity are two of the key parameters to be considered when planning experiments in modern transmission/scanning transmission (TEM/STEM) analytical electron microscopes (AEMs). The configurations of these instruments commercially available today is diverse and while a consensus on exists on maximizing high resolution TEM/STEM imaging, the same does not always apply for discussions on microanalytical sensitivity. In this experimental and theoretical study, a systematic experimental validation of conditions for optimizing both minimum detectable mass (MDM) and minimum mass fraction (MMF) using x-ray energy dispersive spectroscopy (XEDS) has been explored from 60 to 300 kV. The experimental work was conducted on the Analytical PicoProbe Electron Optical Beam Line equipped with the ANL XPAD [1] using a custom built low background specimen holder. Uniformly thick TEM film specimens of Ge/SiNx, Cr, and MoO_3 were examined, all of which were cleaned using Ar/O plasmas to mitigate organic hydrocarbon contamination. All measurements were conducted over the operating range of 60-300 kV using probe currents of 100-105 pA, with the specimen untilted to maximize the collection solid angle and thus the measured signal. Figure 1 plots the relative experimental variation in MDM and MMF for Si, Cr, Ge, Mo K shell signals as a function of accelerating voltage, normalized to 300 kV. In Figure 2 we show the theoretical variation of both MDM and MMF. While the trends for MDM show, not surprisingly, an improvement with decreasing accelerating voltage the same does not apply to MMF, which is strongly atomic number dependent and related to the variation of the relative Intensity and Bremsstrahlung cross-sections as both a function of Eo and Z.

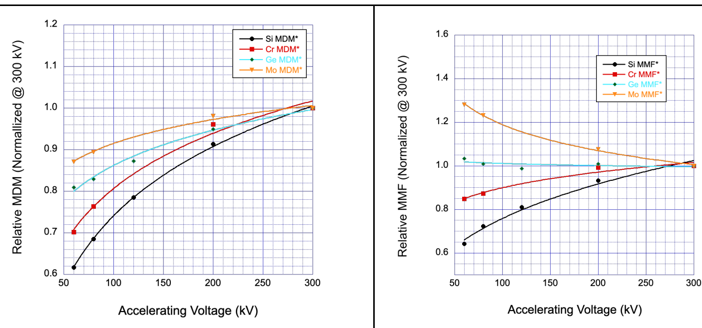

Figure 1. *Normalized experimental variation of the MDM and MMF for Si, Cr, Ge, Mo K shell signals and as a function of accelerating voltage from 60-300 kV*

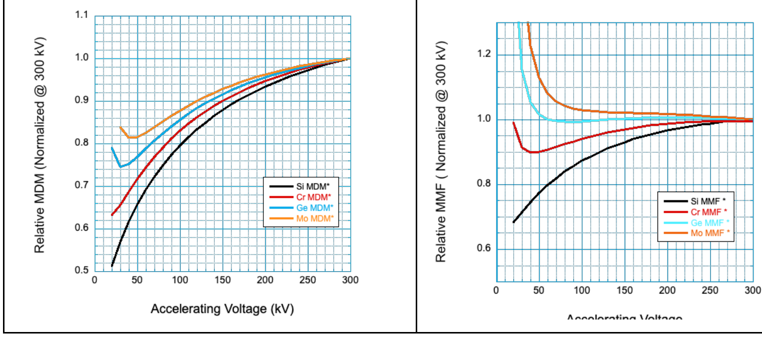

Figure 2. *Normalized theoretical variation of the MDM and MMF for Si, Cr, Ge, Mo K shell signals and as a function of accelerating voltage from 20-300 kV*

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High resolution and high throughput on the JEOL CryoARM300-II with narrow-gap optics

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The new JEOL CryoARM300-II cryo-TEM recently installed at Okinawa Institute of Science and Technology (OIST) is the first of its kind equipped with narrow-gap polepiece. In combination with cold field emitter, energy filter and direct electron detection, the instrument delivers sub-Ångstrom resolution without image corrector. The extended contrast transfer envelope is chiefly due to the reduced chromatic aberration (Cc=1.5 mm). We are furthermore developing high-throughput single particle imaging by combining multiple samples on a single grid.

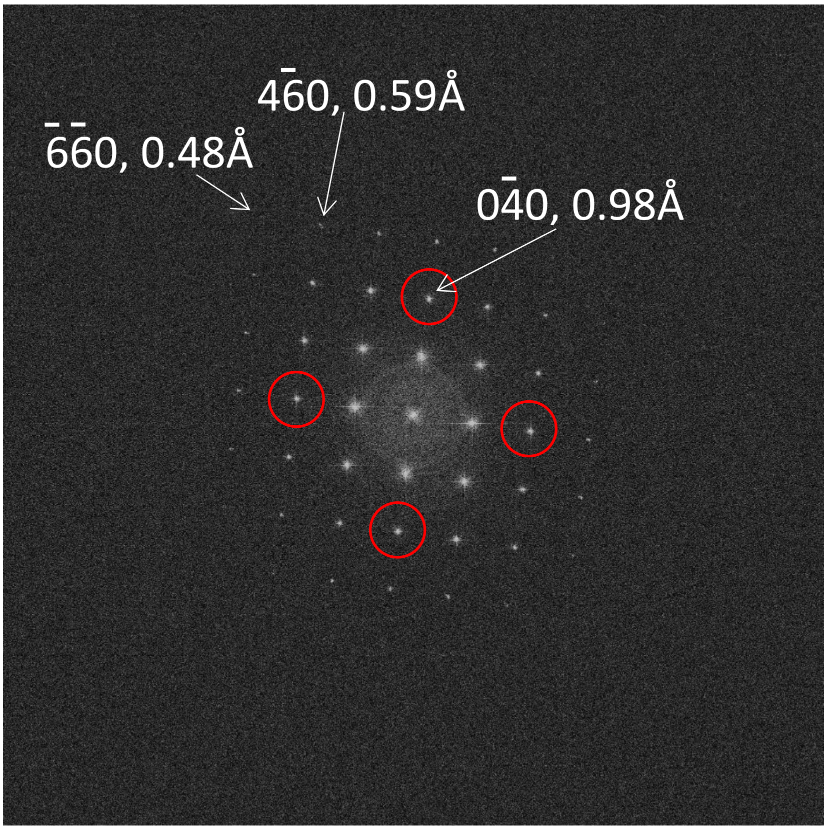

Fig. 1: Power spectrum of image of single crystal gold film.
Acc. Voltage: 300 kV. MAG: 500kx. Detector: DirectElectron Apollo. Exp time 3.0 sec, Dose rate 4.7 e-/pix/s. Sample temperature: 89K

On the Computation of Electron Trajectories with Bohmian Mechanics and their Measurement with Electron Holography

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One of the many outstanding achievements of David Bohm was the pilot wave theory, a reformulation of de Broglie's proposal in 1927, which is known as Bohmian mechanics. This framework explains quantum mechanics and posits that electrons are actual point particles moving along defined trajectories governed by specific equations, which are a reformulation of the Schrödinger equation with the introduction of the Quantum Potential, leading to a quantum force [1]. This paper will present the computation of electron trajectories using Bohmian mechanics, as performed by Rudinsky and Gauvin (2020) (an example is shown in Figure 1) and preliminary results for the measurement of electron trajectories in the double-slit experiment using electron holography.

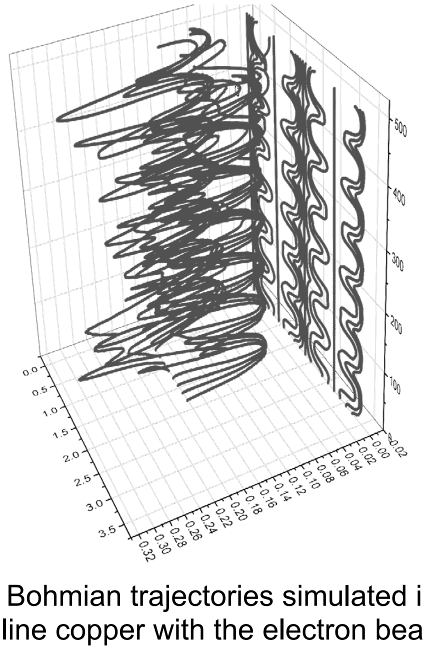

Fig. 1: Bohmian trajectories simulated in crystalline copper with the electron beam parallel to the [001] direction. Units are in A. Electron beam energy is 30 keV, and specimen thickness is 500 A.

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