Poster Presentation

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853 All-Micro: a Network for Sustainable Innovation in Slovenia- Italy Area
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1144 IMG Electron Microscopy Core Facility
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Late Poster Presentation

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1269 Understanding of electronic structure by combination of soft X-ray spectrometer with electron energy loss spectrometer

Dr. Cheng Sun[1,2], Dr. Anton Orekhov[2], Dr. Agata Plucienik[2], Dr. Ali Rinaldi[2], M.Sc. Nico Radde[2], Dr. Elena Willinger[2], Prof. Marc-Georg Willinger[2]
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1298 CELS-3D – Cutting edge light source for exciting fluorescence in microtome-based 3D microscopy

Vladyslav Denyshchenko[1], PhD Christopher Evans[1], Ms Tiina O'Neill[1], PhD Silas O'Toole[1], Clara Scholz, PhD Vladimir Lobaskin[1], Phd Dimitri Scholz[1]
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1350 Global BioImage Analysts' society: CZI funded initiative and future association

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Asbestos Analytics In Relation To Changes Of The European Directive

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Poster Group 1

In 2023, the EU Parliament passed a resolution to amend the Asbestos Directive. Within a transitional period of 6 years, laboratories must either implement a detection limit of 2000 F/cm³ for the evaluation of workplace measurements and or determine fibres thinner than 200 nm (lower limit has not been defined) using electron microscopic methods (phase contrast microscopy is no longer applicable) in order to be able to apply a limit value of 10,000 F/m³.
This poses challenges for analysis. On the one hand, not every laboratory has suitable equipment to fulfil the requirements, on the other hand, the users of the equipment are usually not scientists who are fully familiar with the equipment, but users who follow laboratory instructions. The lecture or poster presents the pitfalls of SEM/EDS analysis in relation to air samples that are to be expected when detecting thin asbestos fibres.
If the detection limit is lowered, at least a semi-automatic evaluation is necessary, as otherwise the analysis times will be unacceptable.
When evaluating asbestos fibres with a diameter of less than 200 nm, two fundamental distinctions must be made. These are the visibility of fibres in the SEM and identification using EDS. The former is certainly dependent on the choice of device (device with Wolfram cathode or field emitter), but also on the choice of detector (SE or BSE detector), primary energy, working distance, brightness, contrast and other parameters. Settings at which fibres with a diameter of 200 nm or larger can be displayed may not be optically recognisable for fibres that are significantly thinner. If an acceleration voltage that is too low is selected, this can make it impossible to identify the fibres, depending on the EDS system used.

Keywords:

Asbestos, Fibres Directive

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

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AllMicro, ALLiance to boost cross-border innovation through MICROscopy, project born from the core idea of an open and inclusive innovation process, allowing a free flow of knowledge and technological collaboration. This leads to the creation of technological novelty, which in turn opens up new markets and encourages entrepreneurship. Research and innovation not only improve products and services but also create investment opportunities, ultimately boosting competitiveness and generating employment [1]. Understanding cutting-edge methods in optical and electron microscopy is frequently confined to highly specialized organizations and individuals, posing a challenge for those seeking to utilize these tools to enhance their creativity and competitiveness on a global scale. Additionally, the significant expenses associated with equipment and ongoing technological advancements hinder the transition of these techniques from academic or fundamental research settings to practical applications in industries and services. This project establishes an international network focused on advanced optical and electron microscopy methods, connecting academic and research institutions with technology parks.
Technology parks Friuli Innovazione (FINN/TEC4I.FVG, Italy) and Primorski Tehnoloski Park (PTP, Slovenia) are vital hubs for fostering innovation, offering their facilities and training services to further bolster collaboration and skill development. They leverage extensive networks to identify potential partners from academia and the private sector, provide essential physical infrastructure and administrative support, cultivate vibrant innovation ecosystems to encourage knowledge exchange and idea generation, and support commercialization efforts through services like intellectual property management and access to funding [3]. Their crucial role is to identify and engage potential partners interested in exploring the opportunities presented by the new microscopy techniques.
In this joint project between Slovenia and Italy, the Universities of Trieste (Italy) and Nova Gorica (Slovenia), along with the research centers of the Istituto Officina dei Materiali (National Research Council, Italy) and the Nanocenter (Slovenia), will contribute to the network's goals. State-of-the-art facilities ranging from electron to optical to atomic force microscopy will be accessible within the target area. These facilities will be available for training and utilization across a broad spectrum of applications in materials science, biology, and various academic and industrial fields. This valuable expertise covers a wide range of knowledge areas and complements one another.
Here, the main objective is to share insights into the capabilities of optical and electron microscopy in driving technological progress and innovation, particularly within the designated region. This area, encompassing numerous small labs with limited resources, holds immense potential. By uniting these labs, a critical mass can be achieved, leading to a formidable presence for economic and innovative development within the region. Additionally, this initiative aims to enhance the competitiveness of all businesses across the larger region by harnessing the collective strength of these united labs.

First, the project focuses on creating and maintaining the ALL-MICRO network to develop synergies among partners. It involves establishing project management infrastructure and conducting meetings for information exchange and collaboration. The next step is to outreach to stakeholders interested in advanced microscopy methodologies, facilitated by technology hub partners. Pilot projects are conducted to demonstrate the potential of advanced microscopy techniques and initiate synergistic partnerships. In this way, by forming a unified network that operates in harmony, spreading the word, and sharing information this project creates a positive atmosphere that encourages other labs to join in or fosters fruitful collaborations with companies. Moreover, the ALL-Micro network will organize and maintain a series of high-specialization courses aiming at the formation of a future class of experts able to take full advantage of advanced microscopy methodology in R&D.
In conclusion, the ALL-MICRO project not only presents a promising opportunity to bridge the gap between academia, industry, and research in advanced microscopy but also aligns with an environmental sustainability perspective. By establishing a cross-border network and leveraging expertise from academic institutions, research centers, and technology hubs in both Italy and Slovenia, the project aims to disseminate knowledge and drive innovation in microscopy techniques. Through collaborative efforts and strategic partnerships, it seeks to enhance regional competitiveness while promoting environmental sustainability. This objective necessitates the crossing of national borders to share resources and avoid duplication of costly and environmentally impactful instrumentation, ensuring a more sustainable approach to research and development. With structured management and active stakeholder participation, the project has the potential to deliver significant benefits and make a lasting impact on microscopy techniques and innovation in the region. With the success of this collaborative project, we are planning to extend the network partners and the target area to nearby countries such as Croatia, Austria, or Balkan countries. This will provide a useful precedent for potential similar approaches in regions different from ours.

Keywords:

Electron Microscopy, networking, All-Micro, Italy-Slovenia

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IMG Electron Microscopy Core Facility

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Poster Group 1

Background and objectives
Imaging techniques are essential for modern biomedical research. State-of-the-art microscopy technologies are becoming increasingly sophisticated and require not only expensive equipment, but also highly qualified personnel to operate the instruments, collect high-quality data and analyse the results. This is why imaging core facilities are becoming increasingly important, helping scientists to use advanced imaging techniques in a time and cost efficient manner. The Electron Microscopy Core Facility at the Institute of Molecular Genetics in Prague provides open access to a wide range of imaging, analysis and sample preparation methods in biological electron microscopy.
Methods and results
Our team's expertise is supported by state-of-the-art equipment for sample preparation and ultrastructural imaging. A high-end transmission electron microscope (TEM) operates at up to 200kV and offers high resolution TEM, imaging in STEM mode, 3D analysis by TEM or STEM tomography, cryo-electron microscopy and STEM-EDS elemental analysis. A standard 120kV TEM with the user-friendly Limitless Panorama application is used for routine observation. Recently we have incorporated a cryoFIB-SEM microscope and set up cryo workflows for cryoTEM tomography or diffraction analysis on FIB-milled frozen-hydrated lamella. For precise targeting of the region of interest within the sample, light fluorescent imaging in a specialized cryoCLEM microscope can be used.
Standard and advanced techniques are also used in sample preparation. Starting from routine chemical fixation and resin embedding or negative staining of weakly stained specimens, we can progress to better preservation of natural specimen appearance by cryofixation using plunge freezing or high pressure freezing, followed by freeze substitution, cryosectioning or freeze fracture replica labelling. Detection of specific moleculles within the cells or tissues can be achieved using pre- or post-embedding immunolabeling techniques using gold nanoparticles of different sizes.
The facility can accommodate a wide range of biological samples for processing and imaging, including human and animal cell cultures, plant and animal tissues, worms, microorganisms, lipid micelles, isolated DNA, and purified proteins. Furthermore, the facility offers tailored development and optimization of sample preparation, based on a longstanding expertise and fruitful collaborations with companies manufacturing equipment for electron microscopy.
Conclusions
The Electron Microscopy Core Facility is part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node. It offers open access to its technologies and expertise, and is prepared to welcome users from all fields.

Keywords:

Electron microscopy, open-access, Czech-BioImaging, Euro-BioImaging

Reference:

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Vacuum ultra violet emission spectra observed by newly designed grating with improved diffraction efficiency

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Poster Group 1

Introduction

In the research and development of new functional materials, obtaining information on their chemical bonding states is one of the most important subjects for understanding the physical properties of the materials. In the soft X-ray and vacuum ultra-violet (VUV) energy regions, various characteristic X-ray emissions owing to electronic transitions from the valence bands (bonding electron states) to the inner-shell electron levels are observed. In other words, the analysis of these emission spectra will lead to an understanding of the chemical bonding states and the physical properties of the materials. For the purpose described above, Terauchi et al. fabricated a soft X-ray emission spectrometer (SXES) accommodated to a transmission electron microscope (TEM) and demonstrated a high energy resolution of 0.22 eV for Al L-emission spectrum [1]. Based on their work, JEOL Ltd. commercialized a SXES, which can be mounted on a general-purpose scanning electron microscope (SEM) / electron probe micro analyzer (EPMA) using newly developed varied-line-spacing laminar-type gratings in 2013. This model, named SS-94000SXES, accepted the energy range of 50–210 eV. SS-94000SXES has been applied successfully to analyze battery materials and contributed to a trendy discussion of Li bonding with Si anode materials [2]. On the other hand, in Li oxides, the Li spectrum is predicted to shift significantly to the lower energy side compared with that of Li metal. However, it has not been accessed with SS-94000SXES due to its limitation of acceptable energy range.
To overcome this difficulty, a new diffraction grating which covers an energy range of 35–100 eV and a larger reflection efficiency with a coating was developed [3]. This grating system allows the observation of the whole band-spectrum of Li in the compounds with high sensitivity [4]. In addition, this energy range accepts to measure whole Mg L-emission spectrum. In this paper, some preliminary results obtained with the new grating system are presented.

Experiments

The modified SXES with the new grating was installed to an EPMA (JEOL JXA-8230 and JXA-iSP100). Samples used for the present experiments were selected for emphasizing the fascinating detection of lower-energy emission, The energy axes of these spectra shown in this paper are calibrated by C K-emission (277.0 eV) [5] and its higher order diffracted lines of HOPG.

Results

Figure 1 shows Al L-emission spectra of aluminum metal obtained by using the new grating (hereafter called as NEW) and SS-94000SXES system (hereafter called as CONVENTIONAL). Labels of L_3-M, L_2-M and L_1-$L_{2,3}$ are the assignments of related electronic transitions between shells. This shows that the intensity of NEW is more than two times larger than that of CONVENTIONAL. Besides, an intensity enhancement of approximately 5-times is obtained for Mg L-emission [4]. Inset (a) is the enlargement of Fermi-edge region of Al L-emission, where intensities of the two spectra are normalized by L_3-M peak for comparison. It is seen that the energy resolutions of NEW and CONVENTIONAL are the same. The L_2-M intensity, which is separated from L_3-M by 0.4 eV, is clearly

observed. Inset (b) is the enlargement of Al L_1-$L_{2,3}$ emission region, which is out of the energy range of SS-94000SXES. Furthermore, the spectrum of NEW shows the second-order Al $L_{2,3}$-M spectrum because of the extended performance of NEW down to 35 eV. This means that NEW covers the energy region of shifted Li K-emission intensities of compounds.
Figure 2 shows $M_{2,3}$-$M_{4,5}$ emission spectra of Manganese and Cobalt. These transition metals were selected to show the applicability to battery cathode materials. Spectral intensities of Mn and Co are normalized by peak intensities at 46.2 eV and 58.1 eV, respectively. The vertical dotted line is the lower limit of the detection energy of CONVENTIONAL. Then, the Mn $M_{2,3}$-$M_{4,5}$ emission spectrum shown here can only be measured by using NEW. Furthermore, this Mn $M_{2,3}$-$M_{4,5}$ emission spectrum shows asymmetric intensity profile with an additional structure in the high energy side (49.0 eV) indicated by a red vertical line, which reflecting the chemical bonding state of the material.

Conclusion

VUV region emission from 35 eV could be observed by using the NEW grating system. Compared to CONVENTIONAL, the intensity of Al L-emission has improved more than 2 times (more than 5 times for Mg L-emission [4]). Furthermore, Mn $M_{2,3}$-$M_{4,5}$ emission spectrum, which could not be observed with CONVENTIONAL, reflecting the chemical bonding state was observed by NEW.

Graphic

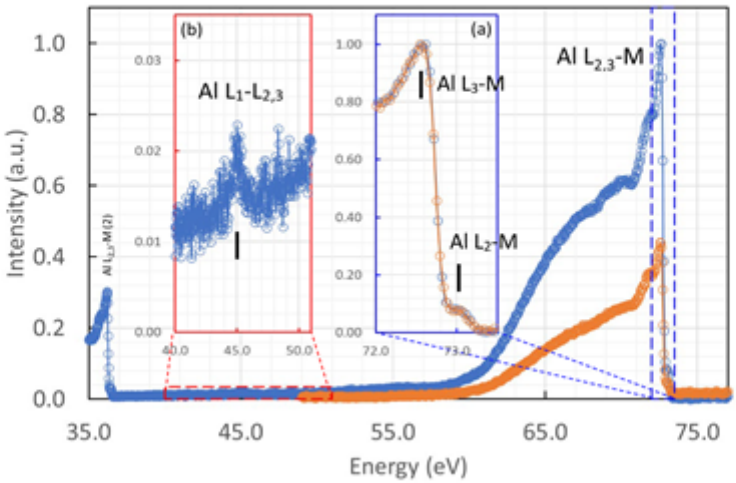

Fig. 1. $L_{2,3}$-M emission spectra of aluminium obtained by using CONVENTIONAL (orange curve) and NEW (blue curve). The NEW achieved more than two times enhancement in the intensity of Al L-emission and an extention of the lower detection limit down to 35 eV compared with those of CONVENTIONAL. Inset (a) shows almosnt the same energy resolution and a cleare observation of L_3-M and L_2-M for both grating systems. Inset (b) shows Al L_1-$L_{2,3}$ intensity by NEW, which is out of the energy range of CONVENTIONAL.

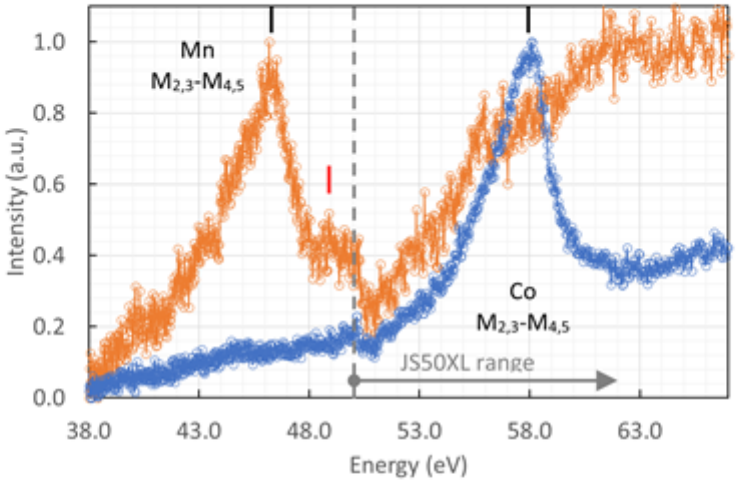

Fig. 2. $M_{2,3}$-$M_{4,5}$ emission spectra of Mn (orange) and Co (blue) metal. The vertical dotted line at 50 eV is the lower detection limit of CONVENTIONAL system. Mn $M_{2,3}$-$M_{4,5}$ emission shows an asymmetric peak intensity profile with an additonal structure in the high energy side reflecting the chemical bonding state of the material.

Keywords:

SXES, VUV, detection efficiency

Reference:

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Cathodoluminescent and Characteristic X-ray-emissive Rare-Earth-doped Core/Shell Protein Labels for Spectromicroscopic Analysis of Cell Surface Receptors

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Poster Group 1

By unlocking previously unattainable scales, microscopy proved pivotal for our today's understanding of the fundamental processes in living systems and enabled future-shaping discoveries. However, understanding the interactions of biomolecules at the nanoscale and putting them in a cellular context remains a major challenge as light diffraction events limit the achievable resolutions. Electron microscopy (EM), unlike light-based techniques, gives access to the cellular ultrastructure yet results in grey-scale images and averts unambiguous (co-)localization of biological targets without electron-dense labels or correlative approaches.
This work presents multimodal nanoparticle-based protein labels for correlative cathodoluminescence electron microscopy (CCLEM) and energy-dispersive X-ray spectromicroscopy (EDX-SM). By incorporating rare-earth dopants in a low phonon host lattice and shielding them from their environment using an inert shell, the characteristic cathodoluminescence (CL) and characteristic X-ray emissivity of sub-20 nm nanoparticles were utilized as unique spectral fingerprints for precise label identification and localization. The core/shell nanoparticles were decorated with either folic (terbium-doped) or caffeic acid (europium-doped), bridging the single-particle response to a biological entity. Their potential for (protein-)labelling was examined using HeLa cells expressing different surface receptors that bind to folic or caffeic acid, respectively.
Single-particle cathodoluminescence along with a distinctive energy-dispersive X-ray signal was successfully presented for both populations, with the latter outcompeting CL as response for ColorEM. EDX-SM persuaded with swift imaging times well below 2 mins per µm² while offering high resolution with a pixel size of 2.78 nm, enabling the observation of biological relevant areas.
Taken together, these results pave the way for multi-color labelling based on electron spectromicroscopy and show its unleashed potential for the study of structure-function relationships.

Keywords:

Nanoparticle, Ultrastructure, Protein Targeting, Multi-Color

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ExpertPI: A Comprehensive Tool for Automated 4D-STEM Multimodal Analysis and Method Development

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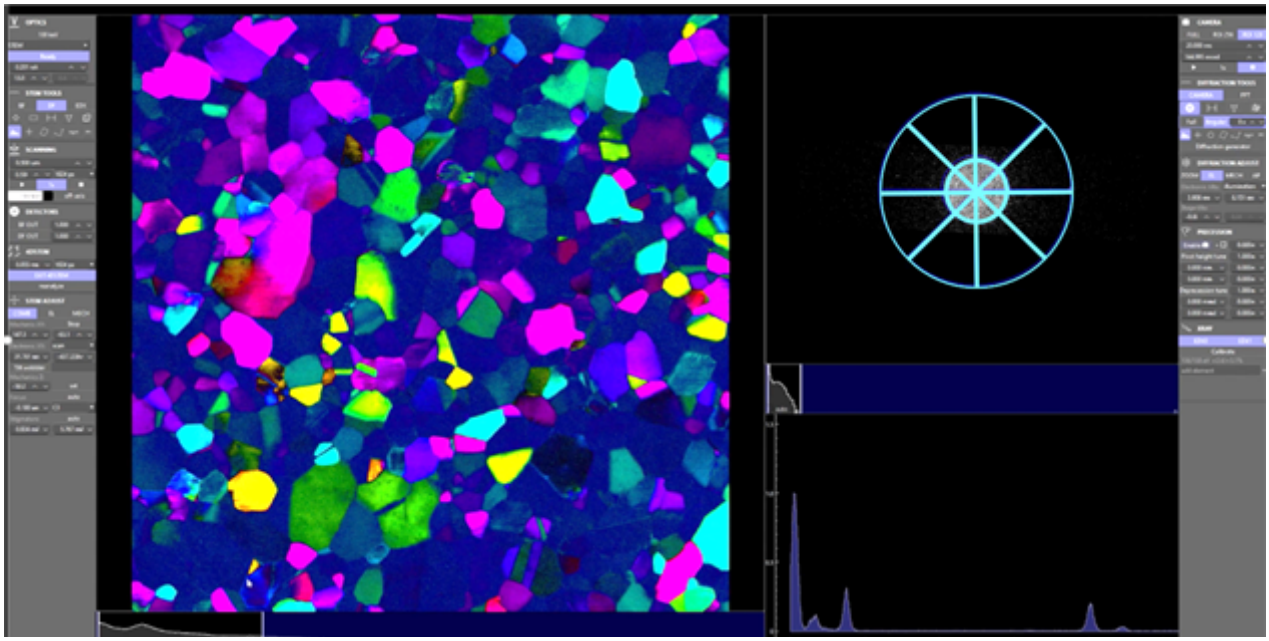
Poster Group 1

Background including aims
The advancement of analytical techniques, particularly in scanning transmission electron microscopy (STEM), necessitates sophisticated tools that can balance ease of use for routine applications and flexibility for method development. The TESCAN TENSOR is a new analytical scanning transmission electron microscope (STEM) that is optimized for electron diffraction experiments, both by unique hardware and software. Specifically, the Expert PI package is an innovative solution designed for development and implementation of new methods and approaches in (multimodal) analytical STEM, 4D-STEM, and 3D-ED techniques.

Methods
ExpertPI is a Python-based library integrated into the TESCAN TENSOR platform, enabling full access to the microscope's functionalities. Unlike the traditional user interfaces that simplify the microscope control and operations at the cost of limited advanced capabilities, the ExpertPI enables direct and unconstrained utilization of all components and modules to their full potential. The core functions of ExpertPI include: i) full instrument control including EM optics, stage, detectors, etc., ii) customizable User Interface with powerful access to all functions via Python console, iii) possibility of integration with third-party libraries, iv) automated data acquisition and processing, v) advanced analytical techniques (such as DPC), vi) customized method development environment, and vii) AI-assisted workflows.

Results
Consequently, such comprehensive control over all electron-optical parameters, beam scanning and acquisition settings, sample stage movements, and synchronized readout of all detectors facilitates unparalleled experimental control, thereby empowering researchers to innovate and refine their techniques, thereby driving forward the capabilities of STEM analysis in material sciences and semiconductor research.

Conclusions
The TESCAN TENSOR therefore represents a highly versatile platform for both routine and advanced 4D-STEM applications. By providing full control over the microscope functionalities and integration with third-party analytical tools, ExpertPI bridges the gap between ease-of-use of routine measurements and the need for advanced method development. This dual capability ensures that the TESCAN TENSOR remains at the forefront of STEM technology, meeting the evolving demands of both application-focused users and method developers.



Keywords:

Analytical STEM, 4D-STEM, precession, development

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Understanding of electronic structure by combination of soft X-ray spectrometer with electron energy loss spectrometer

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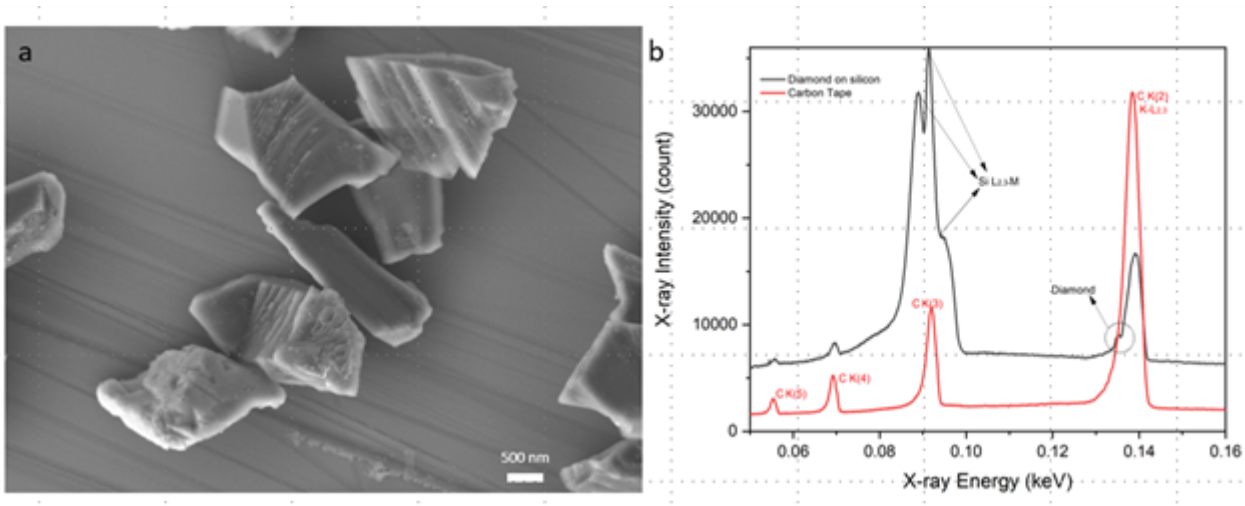
Poster Group 1

The development of soft X-ray emission spectrometer (SXES) significantly enhances the capabilities of scanning electron microscopy (SEM) in elucidating the electronic structure of material [1]. In SEM, the primary electron beam excites inner electrons of the sample, resulting in the emission of X-rays due to energy transitions as electrons move from higher to lower orbitals to stabilize the sample. For detecting soft X-rays, a mirror and diffraction grating are employed to focus and guide the X-rays to a CCD camera within the SEM, enabling subsequent signal analysis [2]. Soft X-rays, originating from electron transitions from the valence band to the core level, provide valuable information about the bonding energy states, such as the 2s, 2p electrons from carbon. This makes SXES a promising tool for analyzing energy materials. In 2024, the installation of a new JEOL IT-800 SEM in Tumint-Energy Research GmbH, equipped with SXES, will support our efforts to characterization electronic structure of various materials. With an energy resolution 0.3 eV and the non-destructive nature of the technique, SXES holds potential for analyzing a wide range of energy materials.
Electron energy loss spectroscopy (EELS) is well-established for detecting the density of states (DOS) from the conduction band. The combination of SXES with EELS provides electron microscopists with a comprehensive approach to analyzing the total electronic structure of materials. Our goal is to integrate these two methods to gain a deeper understanding of the electronic structure of different materials.
We utilized two samples for the preliminary assessment of SXES determination. Fig.1(a) shows the SEM image of diamond crystals on a silicon wafer. Fig.1(b) presents the soft X-ray results: the black curve responds to the diamond sample, while the red curve represents soft X-ray obtained from carbon tape. A notable small peak in the circled area of Fig.1(b) distinguishes the diamond sample from the carbon tape successfully.



Keywords:

soft X-ray, electronic structure, EELS

Reference:

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CELS-3D – Cutting edge light source for exciting fluorescence in microtome-based 3D microscopy

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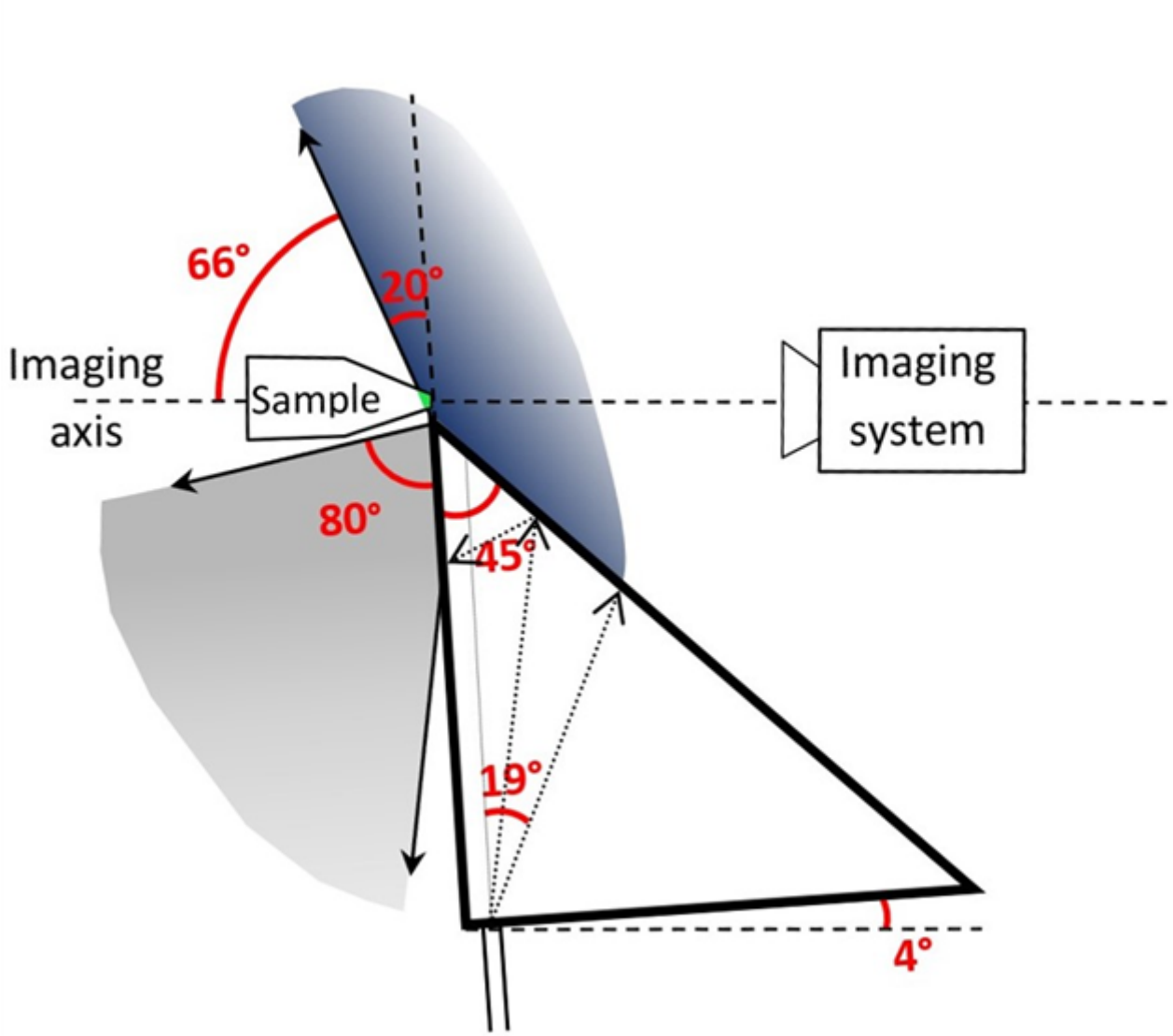
Poster Group 1

It was discovered that the light entering a triangle ultramicrotome glass knife from the bottom exits the knife through its cutting edge, forming an oblique light sheet-like illumination that is suitable for side-illumination of the sample block and 3D imaging. Several challenges were successfully managed, and a working prototype of a novel serial sectioning block-face imaging microscope was constructed. The CELS-3D (Cutting Edge Light Source, Three-Dimensional) is a microscope mounted on a commercial ultramicrotome. The CELS-3D has been characterised and applied for three-dimensional imaging of human liver spheroids with a diameter of approximately 500 micrometres. The structure of nuclei and tight junctions has been successfully reconstructed over the full spheroid volume. The formation of bile sinusoids in the central region of the spheroid was identified, which is crucial for modelling hepatitis viral infection in vitro. In comparison, the confocal microscope was unable to image spheroids to a depth exceeding approximately 50 micrometres and failed to detect the sinusoids.
The CELS-3D can be utilised for the three-dimensional reconstruction of fluorescent biological and/or artificial materials, irrespective of whether they are transparent or not. This encompasses a range of applications, including operation biopsies, experimental organoids/spheroids, artificial cartilage, and bone, among others. The CELS-3D can be effortlessly mounted on the top of any commercially available ultramicrotome, and its operation is straightforward and intuitive. It is anticipated that this technology will find applications in the expanding market for 3D microscopy, which is seeking a compromise between sample size and resolution.



Keywords:

3D microscopy, Serial sectioning, CLEM

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

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Poster Group 1

GloBIAS is a non-profit association to be officially constituted by 2025. GloBIAS aims to develop, discuss and establish the role of BioImage Analysts worldwide by expanding the NEUBIAS activities globally in the same spirit and disciplines, to provide the community with a sustainable base (educationally and financially) through a society and welcome a larger number of scientists involved in bioimage analysis.

Keywords:

Bioimage Analysis, Society, Association, Training