CSMS

CONFERENCE

MICROSCOPY2023

5 - 7 JUNE 2023

Faculty of Law
Palacký University in Olomouc
Olomouc

Czechoslovak Microscopy Society

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ČESKOSLOVENSKÁ MIKROSKOPICKÁ SPOLEČNOST

Československá mikroskopická společnost je občanské sdružení vědeckých, pedagogických, technických a odborných pracovníků, kteří se zabývají a zajímají o jakýkoliv typ mikroskopie. Cílem tohoto sdružení je zvyšovat úroveň mikroskopických oborů, rozvíjet je a vzdělávat především nastupující vědeckou generaci. Společnost vytváří prostředí a podmínky pro vzájemnou odbornou spolupráci a výměnu znalostí mezi členy působícími ve vědě, školství, praxi a spolupracuje za tímto účelem také s firmami, vyvíjejícími a vyrábějícími mikroskopickou techniku. Společnost každoročně pořádá česko-slovenskou konferenci a vícero odborných kurzů a uděluje výroční cenu CSMS významné osobnosti mikroskopie. Z mezinárodních aktivit společnost pravidelně spolupořádá Multinational Congres on Microscopy a je členem European Microscopy Society a International Federation of Societies for Microscopy..

CSMS in short

The Czechoslovak Microscopy Society is a voluntary organisation gathering of scientists, pedagogical, technical and other specialists in the area of electron, optical, and other types of microscopy. Its purpose is to develop and advance standards in the field, to provide conceptual prognosis/assessment, and to promote the results of the research in the field. The Society fulfils the following objectives:

Support of the progress in all branches and applications of microscopy, promotion of science and popularization of science to the public.

Advancement and prognosis of achievements, encouragement and coordination of collaboration between members in research, education and praxis.

Contribution to the growth of the academic level of members, particularly those who are novices in the field.

Promotion of the relevant associated bodies related to scientific work to stimulate development in the field.

Maintainance of the specialist expertise in projects involving microscopy techniques and equipment.

The Society is a member of the International Federation of Societies for Microscopy (IFSM) and the European Microscopy Society (EMS). It collaborates also with other international organisations of similar goals.

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Exhibitors & Sponsors

CSMS Conference - **MICROSCOPY 2023**

5 - 7 June 2023, Olomouc

Auditorium of the Faculty of Law, Palacký University
(17. listopadu 8, Olomouc)

Monday 5th June 2023

14:00	Meeting of the CSMS board
15:00	**Registration of participants** *(until 19:00)*
15:30	**Member meeting of CSMS including election of new CSMS board**
17:30	**Conference opening**
17:40	**Announcement of the winner of the scholarship competition by Thermo Fisher / CSMS** Peter Kepič *(talk in Session II)*
17:45	**Announcement of the winner of the scholarship competition by CSMS** Michal Franek *(talk in Session I)*
17:50	**Announcement of the winners of the best CSMS dissertation sponsored by ZEISS**
17:50	Martina Grajciarová "Histological evaluation of surgical experiments in animal models"
18:10	Šárka Křížová "Chemical and physical properties of impact glasses: Traces of extraterrestrial components or an imprint of source rocks?"
18:30	**Announcement of CSMS award for merit in microscopy** Vladislav Krzyžánek "Lecture of the laureate"
19:00	**Welcome party**

Tuesday 6th June 2023

9:00	**Plenary lecture** Martin Setvín "Combined AFM/STM: Challenges and Opportunities in Studying Complex Materials"
9:45	**Session I. - Biomedical sciences** (chairs: Marie Vancová and Pavel Hozák)
9:45	**Invited talk** Peter Mojzeš "Raman Microscopy of Cells and Tissues: Bioimaging Based on Inherent Molecular Contrast"
11:15	Eva Ďurinová "Formation and maturation of avian reovirus viral factories"
10:30	Michal Franek "Correlative super-resolution and electron microscopy analysis of cell replication and nucleolar structure in A. thaliana" *(winner of the scholarship competition by CSMS)*
10:45	Tereza Juříková "Aspergillus fumigatus and Pseudomonas aeruginosa: friends or enemies?
11:00	Aneta Křížová "Time-lapse Quantitative Phase Imaging for the Study of Cell Migration"
11:15	**Poster session + coffee break**

11:45	**Technology news I** (chairs: Jana Nebesářová and Vladislav Krzyžánek)
11:45	TESCAN / Michal Svoboda "New Generation TESCAN CLARA UHR SEM for fast, accurate and comprehensive nanoscale surface analysis of any material"
12:15	Carl Zeiss / Joerg Lindenau "Scanning Electron Microscopy in Live Sciences - a widening field of applications"
12:30	JEOL / Guillaume Brunetti "New FIB-SEM by JEOL"
12:45	Thermo Fisher Scientific / Daniela Slamková "Processing of life-science cryo-samples in Helios Hydra"
13:00	**Lunch**
14:00	**Session II. - Material sciences** (chairs: Petr Svora and Miroslav Šlouf)
14:00	**Invited talk** Miloslav Klinger "Electron crystallography with CrysTBox: Low barriers for newcomers, advanced features for experts and high precision for everyone"
14:30	Peter Kepič "Observation of the crystalline phase transition and characterization of individual crystals in Sb2S3 thin films and nanostructures for tunable metasurfaces" *(winner of the scholarship competition by Thermo Fisher / CSMS)*
14:45	Martin Kalbáč "2D materials for application in devices"
15:00	Ivo Kuběna "The influence of powder modification on printed microstructure of the VDM 699XA"
15:15	Josef Skopalík "Detection of Gd-Rhod liposomes diffusion and stability in model of dermal tissue and wound structure"
15:30	**Poster session + coffee break**
16:00	**Czech-BioImaging session** (chair: Pavel Hozák)
16:00	Pavel Hozák "News from Czech-BioImaging"
16:20	**Invited talk** Martin Mistrík ""Laser wars" at the cellular and subcellular level"
16:50	**Czech optical cluster session** (chair: Petr Přikryl)
16:50	Petr Přikryl "Introduction of the Czech optical cluster"
17:10	**Invited talk** Josef Kapitán "50 years of Raman optical activity - a rich history and a look into the future"
17:40	Break
19:00	**Social evening with banquet** (in Pevnost poznání)

Wednesday 7th June 2023

9:00	**Session III. – Optics and Instrumentation** (chairs: Kamila Hrubanová and Dušan Chorvát)
9:00	**Invited talk** Michal Horák "Electron energy loss spectroscopy and its applications in nanophotonics illustrated mostly on gallium nanoparticles"
9:30	Dušan Chorvát "Advanced optical microscopy as a tool for visualisation of micro-plastics"
9:45	Miroslav Šlouf "4D-STEM/PNBD: Powder electron diffraction in SEM made fast and easy"
10:00	František Kitzberger "Utilization of advanced software features for better, faster, and less laborious segmentation of data from TEM and 3D-SEM"
10:15	Radek Dao "AFM-in-FIB/SEM: Advancements for in-situ sample analysis in batteries"
10:30	**Poster session + coffee break**

11:00	**Technology news II** (chairs: Jana Nebesářová and Vladislav Krzyžánek)
11:00	Carl Zeiss / Pavel Krist "The Fastest and Most Sensitive Confocal Microscopes with Superresolution for Life Cell Imaging"
11:15	HPST / Barbora Kobidová "Igniting new confocal Imaging Potential of Nikon AX/AX-R in a Combination with new Super-resolution Module NSPARC"
11:30	KRD / Jiří Vašák "IBIDI: 3D Cell Culture"
11:45	Pragolab / Martin Kopecký "Pushing the Boundaries: Latest Advances in High-End Microscopy Technology for Leica-Microsytems, Delong Instruments and Telight"
12:00	Specion, Leica Microsystems / Andreas Nowak "Discover how to use Freeze Fracture to Explore the Subcellular World"
12:15	SVEN BioLabs / Tomáš Pop "Live cell super resolution imaging based on spinning-disc and optical photon reassignment"
12:30	**Conclusion**
12:40	**Lunch**
13:45	**Excursions to local laboratories**
	• Czech Advanced Technology and Research Institute (CATRIN) • Department of Optics, Faculty of Science, Palacký University • Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University

Plenary session

Type of presentation: Plenary

PL-PL-3165 Combined AFM/STM: Challenges and Opportunities in Studying Complex Materials

Setvin M.[1]

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Email of the presenting author: martin.setvin@mff.cuni.cz

Scanning probe microscopies represent a prominent tool for mapping the surface properties with single-atom precision. Since the invention of STM in 1982, the field has gone a long path towards its maturity and many techniques from the SPM family have successfully reached their limits. This talk will focus on recent developments and opportunities offered by the noncontact atomic force microscopy (nc-AFM), a technique that has shown dramatic advances in the last decade. The main emphasis will be put on the research of transition metal oxides, such as copper oxides, iron oxides and perovskites. The possibilities of structural analysis will be discussed, as well as the options of imaging and manipulating charge states of lattice atoms and adsorbed molecules.

Acknowledgement: Support from projects GACR EXPRO 20-21727X and GAUK Primus/20/SCI/009 is acknowledged.

Technology news

Type of presentation: Oral

TN-O-3182 Scanning Electron Microscopy in Live Sciences - a widening field of applications

Lindenau J.[1]

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Recent technical advances made modern Scanning Electron Microscopes become the standard tools in biomedical EM imaging. Today's SEMs provide better resolution, more sensitive detectors and faster image acquisition, which makes them applicable for a broad range of applications in life sciences and biomed routine applications.
By implementation of automated workflows for serial images acquisition, SEM based imaging became the method of choice for 3D ultrastructure analysis of biological samples. Accordingly, we offer dedicated solutions for all major approaches in 3D SEM imaging; Array Tomography, Serial Block Face imaging and Focused Ion Beam Milling.
As a manufacturer of Light-, Laser Scanning-, X-Ray-, Electron- and Ion-Microscopes, Zeiss offers a wide range of microscope systems which enable the user to capture a whole bunch of various image modalities for in depth sample analysis. It is obvious, that the correlation of these different image information is of major importance for research. Easy to use correlative workflows allow to utilize images of various sources for sample navigation in SEM or to combine image modalities like Fluorescence and Back Scattered Electron contrast in 3D.

Type of presentation: Oral

TN-O-3186 New FIB-SEM by JEOL

Brunetti G.[1]

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Email of the presenting author: brunetti@jeol.fr

Since 1949, JEOL's legacy has been one of the most remarkable innovations in the development of instruments used to advance scientific research and technology. JEOL has 60 years of expertise in the field of electron.

This year, JEOL introduces a new generation of FIB-SEM the JIB-PS500i.

The JIB-PS500i has been designed to prepare various specimens of high quality for superior atomic-resolution transmission electron microscopy (TEM) observations. This instrument provides three solutions to assist TEM specimen preparation.

- TEM-LINKAGE: The use of JEOL’s double tilt cartridge and TEM holder* facilitates linkage between the TEM and the FIB. The cartridge can be attached to the dedicated TEM specimen holder with a single touch.
- CHECK-AND-GO: To precisely and efficiently prepare a TEM specimen, it is essential to quickly check the preparation progress. With its high-tilt stage and detector scheme, the JIB-PS500i allows for seamless transition from FIB milling to scanning transmission electron microscope (STEM) imaging. Fast transitions between lamella processing and STEM imaging lead to efficient specimen preparation.
- AUTOMATIC PREPARATION: The JIB-PS500i automates specimen preparation using the STEMPLING2* automatic TEM specimen preparation system. This automatic system enables any operator to smoothly prepare specimens for TEM.

Other features will be also introduced during the presentation:

- High-resolution & High-contrast SEM Imaging
- High-power & High-quality FIB processing
- New Chamber & Stage Design

Fig. 1: JEOL JIB-PS500i

Type of presentation: Oral

TN-O-3143 Processing of life-science cryo-samples in Helios Hydra

Slamkova D.[1], Hovorka M.[1]

[1]Thermo Fisher Scientific Brno, Vlastimila Pecha 1282/12, Černovice, 627 00 Brno

Email of the presenting author: daniela.slamkova@thermofisher.com

The Thermo Scientific™ Helios Hydra™ Plasma FIB-SEM with the unique capability to deliver four ion species (Xe, Ar, O, or N) as the ion beam source is a novel technology in the life sciences for multiscale analysis of biological samples. The Helios Hydra with integrated cryo stage (Cryo Hydra) and correlative light-electron microscopy for targeting features of interest is a powerful tool for analysis of cryo-immobilized, hydrated samples where different ions can be used for automated volume data acquisition and cryo-lamella preparation.

Cryo-PFIB-SEM volume imaging with Auto Slice & View (ASV) can deliver high-quality cryo-3D data. This can be used either as a targeting method to approach the region of interest for subsequent lamellae preparation for cryo electron tomography in TEM, or to gain the contextual information required for the scientific study at the histological level. Hydra offers a real step forward in cryo-applications as it enables practical work with large high-pressure frozen samples of up to 200 µm thickness. Having access to tissues, biopsies or small organisms in their native state is the only path to study complex and dynamic biological processes.

The contribution will introduce the system capability and discuss selected applications examples.

Acknowledgement: We would like to thank MRC Laboratory of Molecular Biology of Cambridge for collaboration on the cryo-experiments.

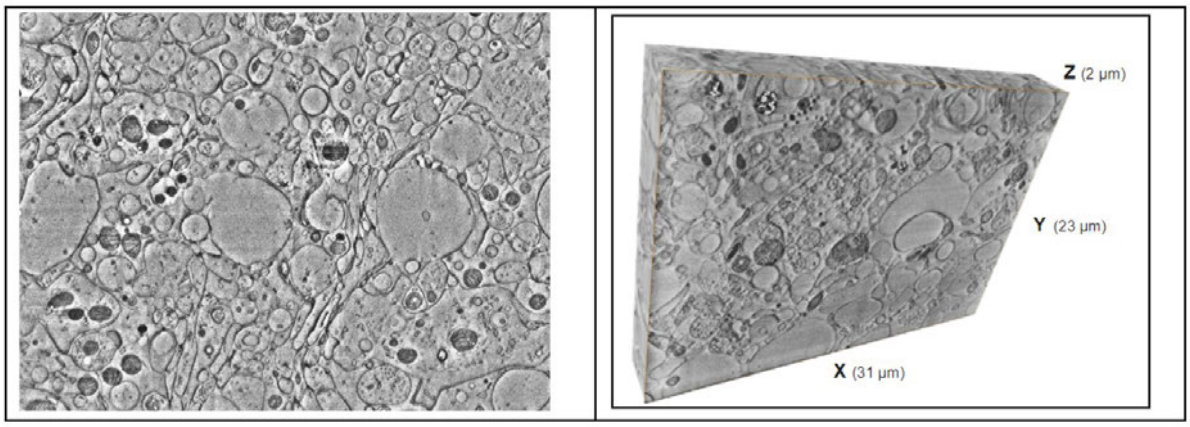

Fig. 1: Cryo ASV of high-pressure frozen mouse-brain tissue acquired with Cryo Hydra, using Nitrogen as a primary ion. Left: x-section image, right: acquired sample volume.

Type of presentation: Oral

TN-O-3181 The Fastest and Most Sensitive Confocal Microscopes with Superresolution for Life Cell Imaging

Krist P.[1]

[1]Carl Zeiss spol. s r.o.

Email of the presenting author: pavel.krist@zeiss.com

Confocal microscope is the “Swiss knife” in high-end microscopy. ZEISS LSM systems offer the best parameters which will help you in your research and routine. ZEISS LSM offer the best sensitivity, speed (precise linear scanners with up to 47,5 fps at 51x512), resolution (very easy with no limitations down to 90nm), spectral flexibility (from 380 nm to 900 nm), easy sample navigation (unique AI Sample Finder), large field of view, multiphoton imaging (NLO), Cryo imaging for correlative microscopy (with unrivaled high resolution), easy to use software, advanced imaging and processing methods including unmixing, manipulation (FRAP etc.), correlation methods (FCS, FCCS, RICS), best lifetime (FLIM), Image analysis (including AI, Machine Learning, Deep Machine Learning), etc. For more information visit our web pages: www.zeiss.com/microscopy

Type of presentation: Oral

TN-O-3153 Igniting new confocal Imaging Potential of Nikon AX/AX-R in a Combination with new Super-resolution Module NSPARC

Kobidová B.[1]

[1]HPST, s.r.o., Prague, Czech Republic

Email of the presenting author: barbora.kobidova@hpst.cz

The newly developed Nikon Spatial Array Confocal (NSPARC) detector utilizes an ultra-low noise detector array to collect a two-dimensional image at each scanned point. This method of image scanning microscopy (ISM) improves signal-to-noise ratio by increasing the available signal level while simultaneously allowing imaging with lower excitation power.
Single-photon sensitivity and array detection extend the capabilities of the AX system by revealing unseen details in every image, while array detection pushes the boundaries of resolution beyond the theoretical limits.

Type of presentation: Oral

TN-O-3196 IBIDI: 3D Cell Culture

Vašák J.[1], Egermajerová D.[1]

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Email of the presenting author: jiri.v@krd.cz

Ibidi is reacting to the booming trend of 3D cell cultures. For these applications, they have developed microscopic chambers with special surfaces and several options of different geometries.
Thanks to the complete solution offered by Ibidi, ideal conditions for your 3D cell cultures will be ensured during cultivation and live cell imaging.

Type of presentation: Oral

TN-O-3194 Pushing the Boundaries: Latest Advances in High-End Microscopy Technology for Leica-Microsytems, Delong Instruments and Telight

Kopecký M.[1]

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New Autonomous Microscopy powered by Aivia!

Artificial intelligence (AI) is on its way to becoming an integral part of life science research. Integrated in the right way into a microscopy workflow, AI can enable researchers to gather data in a highly efficient way and conduct experiments that were previously not accessible. The brand-new Rare Event Detection Workflow for the STELLARIS confocal platform is such an AI-based tool. The synergetic combination of data acquisition by STELLARIS and AI-powered image analysis by Aivia results in the attainment of high-quality information fast and with reduced effort.

Leica tauSTED is a cutting-edge super-resolution microscopy technique developed by Leica Microsystems that allows for imaging of biological structures at a resolution of up to 20 nanometers. It employs a technique called stimulated emission depletion (STED) combined with time-gated detection to enable imaging of structures at very high speed and resolution. The system is particularly well-suited for studying cellular structures and processes that are too small to be seen with conventional light microscopes, such as synaptic vesicles, protein aggregates, and the cytoskeleton. In addition, the system allows for imaging of live cells, making it a powerful tool for studying dynamic processes in living cells.

Delong Instruments LVEM25E is a cutting-edge electron microscope developed by Delong Instruments that allows for high-resolution imaging of biological and material samples. The LVEM25E is a benchtop transmission electron microscope that operates at 25 kV and can achieve a resolution of up to 1 nanometer. One of the key advantages of the LVEM25E is its compact size, which makes it easy to install and operate in a variety of laboratory settings. In addition, the LVEM25E features a unique sample preparation system that allows for quick and easy sample preparation, minimizing the time and effort required to prepare samples for imaging.

Type of presentation: Oral

TN-O-3193 Discover how to use Freeze Fracture to Explore the Subcellular World

Nowak A.[1]

[1]Specion, Leica Microsystems

Email of the presenting author: bacik@specion.cz

Freeze Fracture is a powerful technique that allows researchers to study the internal structures of cells and tissues at the nanoscale level. It involves freezing a sample at ultra-low temperatures and then fracturing it to reveal the internal structures. By using Cryo SEM, researchers can image these structures dynamically, providing high-resolution images with great clarity.

Type of presentation: Oral

TN-O-3174 Live cell super resolution imaging based on spinning-disc and optical photon reassignment

Pop T.[1]

[1]EVIDENT Europe GmbH

Email of the presenting author: office@svenbiolabs.cz

In the decades following the discovery of fluorescent proteins, live cell imaging has become an indispensable tool for the investigation of intracellular functions inside tissues and cells in the field of life science research. Today, various super resolution microscopy techniques are advancing fluorescence imaging even further. Super resolution imaging is attracting attention as a revolutionary tool in the life science field because it enables the observation of fine structures that are less than 200 nm (nm=10-9 m) in size, an imaging performance that is unattainable using a conventional optical microscope. However, applying super resolution microscopy techniques to live cell imaging is limited because of time resolution and phototoxicity issues. In addition, the reliability (quantitative performance) of imaging data is also a significant concern because image processing including estimation or spatial frequency shift can form artifacts that corrupt results.
In response, Olympus has developed the super resolution IXplore SpinSR system for live cell super resolution imaging. This system utilizes super resolution components as well as the optical sectioning capability of confocal fluorescence microscopes. The IXplore SpinSR system simultaneously achieves high reliability, fast image acquisition, low phototoxicity, and deep tissue observation, which cannot be done using conventional methods. Data reliability is ensured owing to our Olympus Super Resolution (OSR) technology. Based on optical theory, our OSR processing algorithms are capable of obtaining a spatial resolution of 120 nm, providing highly reliable results below the diffraction limit. Fast image acquisition is achieved using the spinning disk confocal microscope technique. Using super resolution SoRa disk technology, phototoxicity is also reduced to 30% of conventional techniques. Deep tissue observation is achieved using silicone immersion objectives and through the optical sectioning capability of confocal microscopes.

Biomedical sciences

Type of presentation: Invited

LS-IN-3160 Raman Microscopy of Cells and Tissues: Bioimaging Based on Inherent Molecular Contrast

Mojzeš P.[1]

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Confocal Raman microscopy (micro-Raman) combines the molecular specificity of vibrational spectroscopy with the simplicity and technical undemandingness of confocal optical microscopy. Confocal micro-Raman provides images of micro-objects with diffraction-limited spatial resolution in the chemical contrast, simultaneously for all chemical compounds present in the specimen if they provide sufficiently intense Raman spectra (Fig. 1). Micro-Raman may be a method of choice for label-free, non-destructive, and easy-to-be-applied chemical imaging of biological objects ranging from single-cell microorganisms up to tissues, often alive and without complicated preparation of the specimen. Although it is already used in structural and metabolic studies of bacteria, yeast, and tissues, its potential for solving a wider range of problems is not fully exploited.

Besides the simultaneous detection, visualization, and quantification of the compounds such as lipids, polysaccharides, polyphosphates, and proteins, conventional micro-Raman based on commercially available Raman microscopes can be of great help for identifying the real chemical nature of various intracellular structures, frequently noticed by advanced methods of optical microscopy or visualized with the incomparably better spatial resolution by electron microscopy but still of unknown or questionable molecular composition. In such a way, we have recently identified enigmatic inclusions found in various protists as crystalline purines. As Raman spectra are sensitive to isotopic substitution, Raman microscopy can be useful for metabolic studies at a single-cell level, representing thus a less laborious alternative to bioimaging by nanoscale mass spectrometry. Recent progress in the application of confocal micro-Raman in microbial research will be presented and demonstrated using our original research results. Advantages and perspectives, but also limitations and pitfalls of the method will be pointed out and discussed.

Acknowledgement: This work was supported by the Czech Science Foundation (grants 21-26115S and 22-25396S).

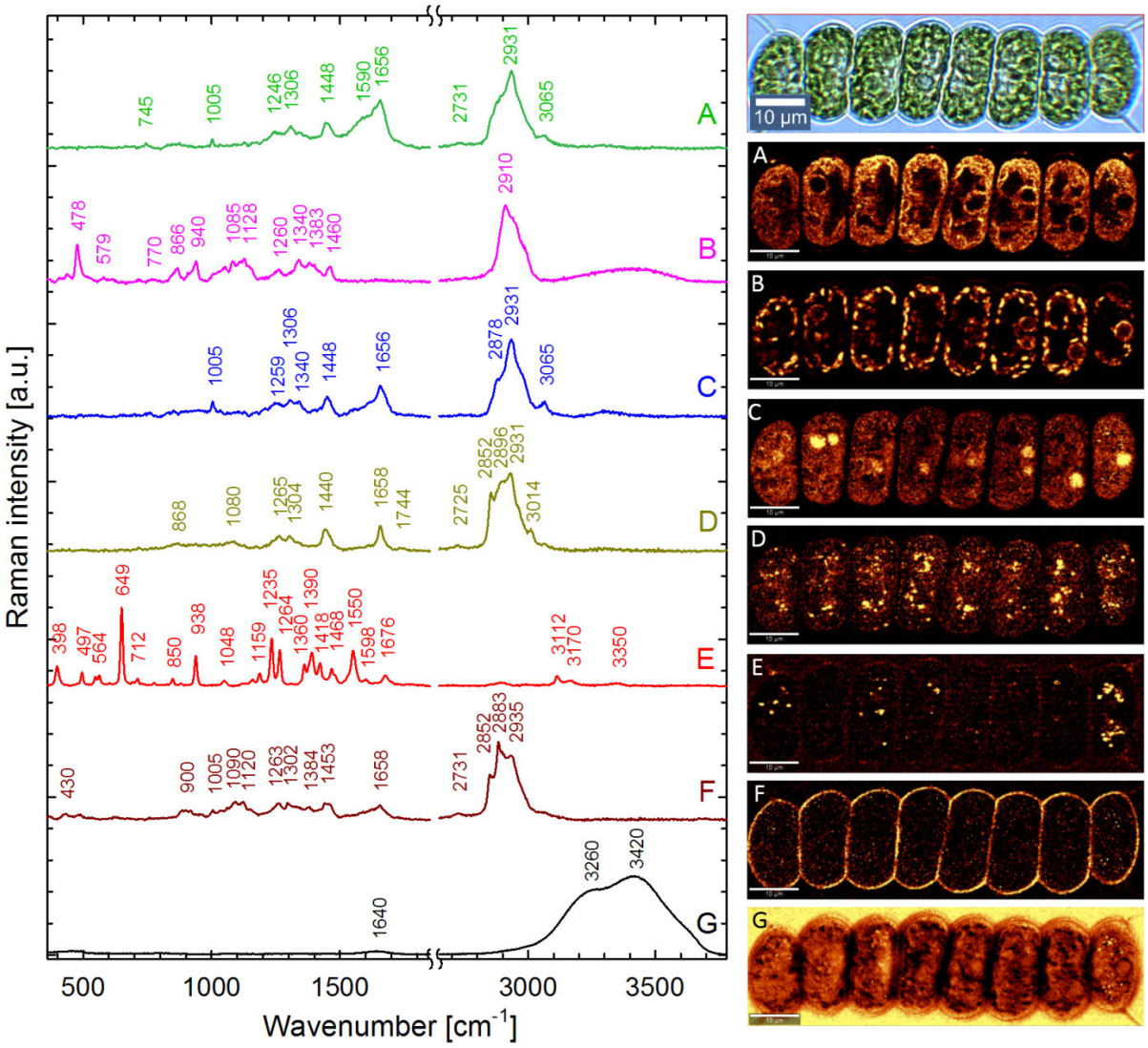

Fig. 1: Bright-field image and Raman chemical maps of microalgae *Desmodesmus quadricauda*. The hyperspectral dataset was decomposed into seven spectral components assigned to plastids (A), starch (B), protein component coinciding with pyrenoids (C), lipid droplets (D), crystalline guanine (E), cell wall containing cellulose (F), and water (G).

Type of presentation: Oral

LS-O-3140 Formation and maturation of avian reovirus viral factories

Ďurinová E.[1,2], Bílý T.[1,2], Kaščáková B.[1], Franta Z.[1], Mojzeš P.[3], Tuma R.[1]

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Email of the presenting author: evadurinova12@gmail.com

Avian reovirus (ARV), a dsRNA virus of Reoviridae family, significantly affects the world poultry industry. The available vaccine is not economical and often inefficient due to new emerging ARV strains. ARV replication and assembly is a promising target for viral inhibition by antiviral drugs. However, ARV replication and assembly mechanisms are poorly understood and seem to differ from the more thoroughly studied mammalian reovirus.
Viruses of Reoviridae family, including ARV, replicate and assemble in viral factories (VFs) which are dense cytoplasmic inclusions. Here we combine different visualization methods (TEM, fluorescence, Raman) to obtain insight into their formation, maturation, and events during virus assembly.
First, we established a stable mammalian cell lines (BHK-21) expressing non-structural proteins (μNS and σNS) which were fused to fluorescent proteins (eGFP and mCherry). The cells were infected by an ARV S1133 vaccine strain. Formation and maturation of VFs were followed by live-cell imaging combined with fluorescence recovery after photobleaching (FRAP) to characterize their fluidity. These time-resolved studies were corelated with ultrastructure (TEM) and chemical composition (Raman) snapshots during infection.
By fluorescence imaging, we first observed formation of spherical liquid condensates which in the later stages of infection progressed towards more solid, dense, and irregularly shaped inclusions with ARV cores inside (Fig. 1, VF). Large 3D arrays of newly assembled virions (VA in Fig. 1) were found emerging in the vicinity and eventually filling most of the cytoplasmic space in regular arrays. We described the viral arrays by electron tomography.

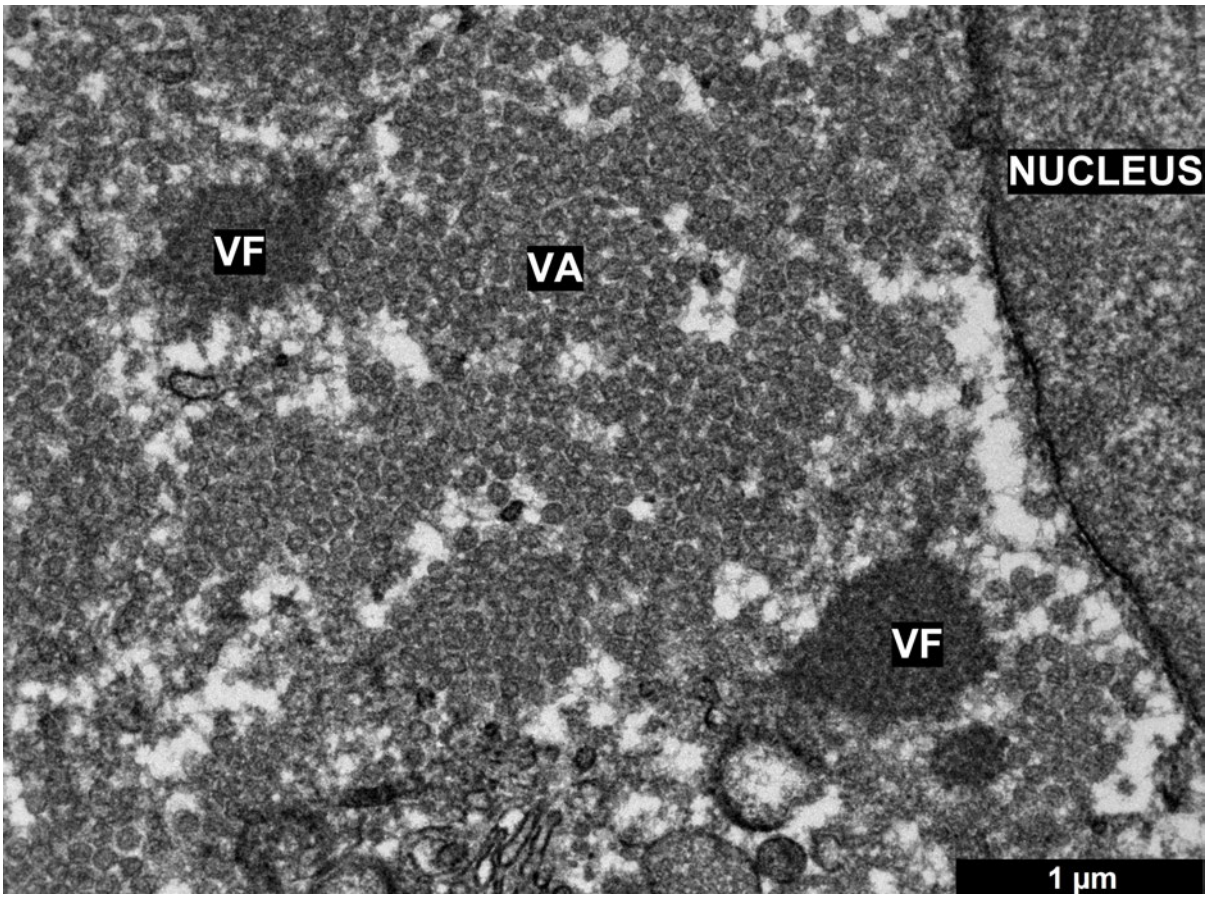

Fig. 1: Infected BHK-21 cells were fixed 24 hours post infection and sectioned into 80 nm thin sections, contrasted with uranyl acetate and lead citrate (on section) and imaged by transmission electron microscopy (120kV Jeol JEM-1400f), scale bar 1 µm.

Type of presentation: Oral

LS-O-3175 Correlative super-resolution and electron microscopy analysis of cell replication and nucleolar structure in A. thaliana

Franek M.[1,2], Koptasikova L.[3], Miksatko J.[3], Pospisil J.[4], Liebl D.[3], Esner M.[4], Dvorackova M.[1,2], Fajkus J.[1,2]

[1]Mendel Centre for Plant Genomics and Proteomics, CEITEC, Brno, Czech Republic , [2]Laboratory of Functional Genomics and Proteomics, National Centre for Biomolecular Research Brno, Czech Republic, [3]Charles University, Faculty of Science, Imaging methods core facility at BIOCEV, Vestec, Czech Republic , [4]Cellular Imaging Core Facility CELLIM, CEITEC, Brno, Czech Republic

Email of the presenting author: michalfranek20@gmail.com

Correlative light and electron microscopy (CLEM) is an essential tool that allows us to localize particular target molecules on the ultrastructural map of subcellular features at the nanometer scale. Adoption of these advanced imaging methods has been limited in plant biology, due to challenges with plant tissue permeability during embedding in resin, issues with labeling efficiency and with indexing (and re-localization) of features of interest throughout the complex 3D volume. We were able to implement an imaging approach based on plant tissue processing and embedding into methacrylate resin followed by imaging of serial sections by both single-molecule localization microscopy and transmission electron microscopy for correlative analysis. Importantly, we show that the use of a particular type of embedding resin is not only compatible with dSTORM super-resolution microscopy, but shows a dramatic improvement in fluorophore blinking behavior relative to the whole-mount approaches. Here we used DNA replication labeling by click-IT detection to visualize the replication of wild-type Arabidopsis thaliana seedlings, as well as FASCIATA1 and NUCLEOLIN1 mutants (Fig. 1). As a result, we identified discrepancies between the size of intranucleolar replication clusters in plants with damaged nucleolar structure and wild-type plants, as well as observed changes in the nucleolar architecture itself provided by electron microscopy. As an extension of the embedding approach, we tested the possibility of introducing a novel labeling approach for nucleic acids based on di-amino-benzidine polymerization, catalyzed by Alexa Fluor 647 photochemistry. Our preliminary data suggest that this method can enhance contrast for nucleic acids labeling in EM for plant tissue sections, which would open the possibility to study nuclear architecture in plant tissues.

Acknowledgement: „We acknowledge the CEITEC core facility CELLIM and the Imaging Methods Core Facility in BioCEV, supported by MEYS CR (LM2023050 and LM2018129 Czech-BioImaging, respectively).“

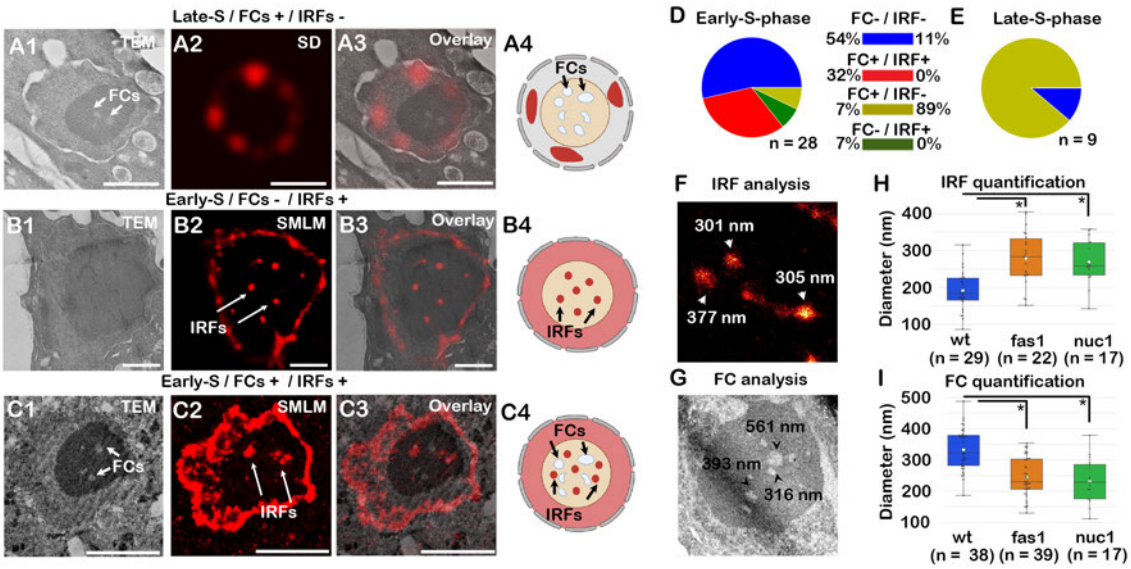

Fig. 1: Left – Correlative imaging of replication and nucleolar ultrastructure. Middle – Schematic depiction of nucleolar replication foci (IRF) and fibrillar centers (FCs). Right – Quantification of the size of IRFs and FCs in wild-type and mutant Arabidopsis thaliana plants.

Type of presentation: Oral

LS-O-3150 Aspergillus fumigatus and Pseudomonas aeruginosa: friends or enemies?

Juříková T.[1,2], Vydrová R.[3], Patil R. H.[1], Marešová H.[1], Palyzová A.[1], Havlíček V.[1], Benada O.[1]

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People affected by chronic diseases are often prone to persistent infections by multiple pathogenic microbes. The growth of the microbes in the host is limited by oxygen and carbon sources, as well as micronutrients such as iron. These limitations may result in various interspecies interactions. Our study focuses on the coexistence of two opportunistic pathogenic microbes, the bacterium *Pseudomonas aeruginosa*, and the fungus *Aspergillus fumigatus*, in liquid cultures. Both microbes affect patients with chronic respiratory diseases and can be life-threatening. Previous studies already showed their mutual interactions at different levels depend on the growth form, fitness, and growth conditions. However, a detailed morphological study of their physical interactions in mixed cultures is unavailable. Here, we present *in vitro* study of co-cultures of both microbes under specific growth conditions mimicking the iron limitation in the host. The mainly proposed method is scanning electron microscopy. *P. aeruginosa* and *A. fumigatus* could coexist in our cultivation set-up for several days. Although the growth of both organisms in mixed culture was affected, fungal spore germination was not inhibited, nor was bacterial growth suppressed. We observe bacterial colonization of fungal hyphae in the forming pellets. After 24 hours of co-cultivation, the bacteria covered almost all the fungal pellets' surfaces with a characteristic layer suggesting biofilm formation. After 48 hours, we observed significant damage to the fungal cell wall in both single and mixed culture types. That is characteristic, and the natural aging of cells primarily causes it. The bacterial cells in mixed cultures were already released from the hyphae surface as biofilm plates. Our results correspond with other works. In contrast, some studies describe stronger competition and growth inhibition of one of the microbes. A comprehensive knowledge of the *in vitro* interactions of these opportunistic pathogenic microbes can contribute to understanding chronic respiratory infections and possibly their treatment.

Acknowledgement: The authors gratefully acknowledge the support from the Czech Science Foundation (21-17044S).

Type of presentation: Oral

LS-O-3145 Time-lapse Quantitative Phase Imaging for the Study of Cell Migration

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Quantitative Phase Imaging (QPI) is a powerful label-free technique widely used to study the dynamic processes of living cells in real-time. QPI in time-lapse microscopy offers several benefits, including high sensitivity, low phototoxicity, high spatial and temporal resolution, and quantitative measurements of cellular morphology and mechanical properties.
In this presentation, we will discuss using of QPI to study cell migration, a fundamental biological process that is critical for tissue development and repair. We will demonstrate how QPI can provide detailed insights into the dynamics of cell migration, including the speed of cell movement, the direction of migration, and the evaluation of the migrastatic drugs, or the way of migration in extracellular matrices. Additionally, we will show how QPI can be used to quantify cellular parameters such as cell dry mass, area, perimeter, migration index, or Euclidean distance.
Our results demonstrate the potential of QPI in time-lapse microscopy as a powerful tool for studying cell migration and other dynamic processes in living cells.

Acknowledgement: 1. Slabý, Tomáš, et al. Optics Express 21.12 (2013): 14747-14762.
2. Tolde, Ondřej, et al. Scientific reports 8.1 (2018): 1-13.

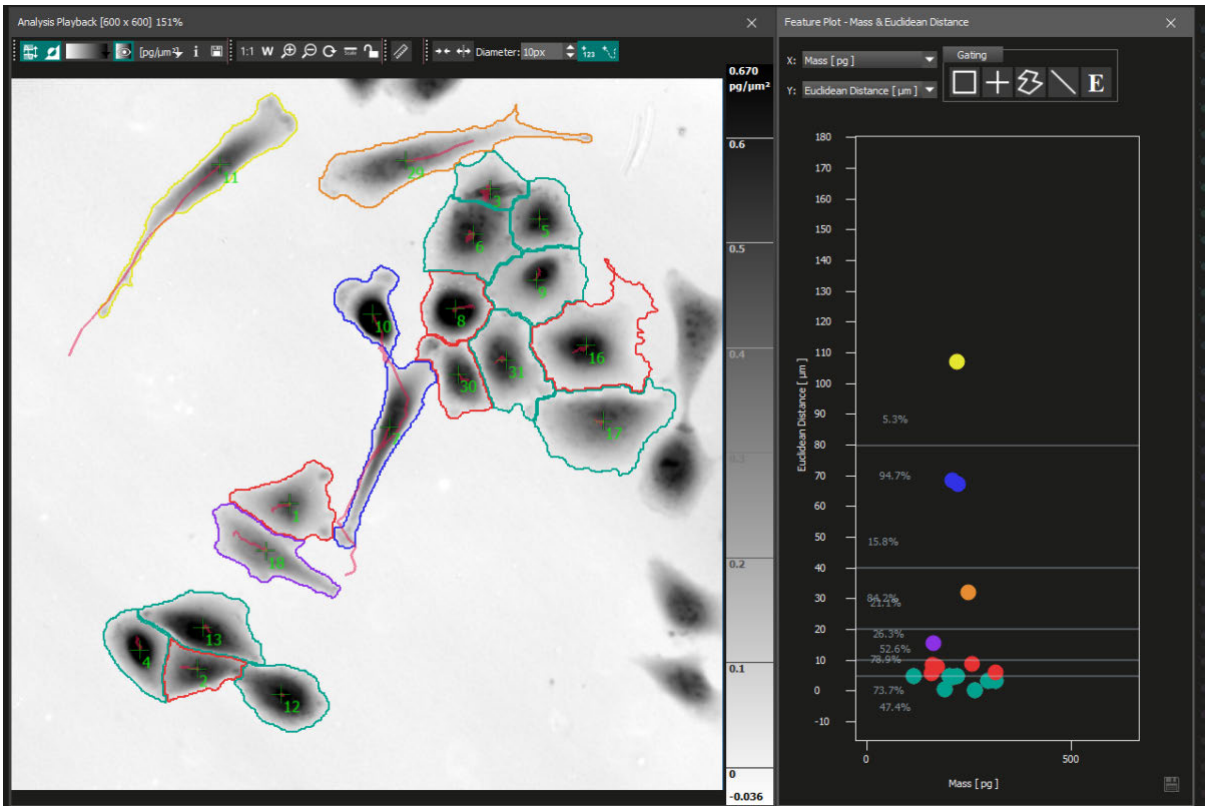

Fig. 1: Quantitative phase imaging and cell tracking, cell line A549, objective 10x/0,25, analysed in SophiQ.

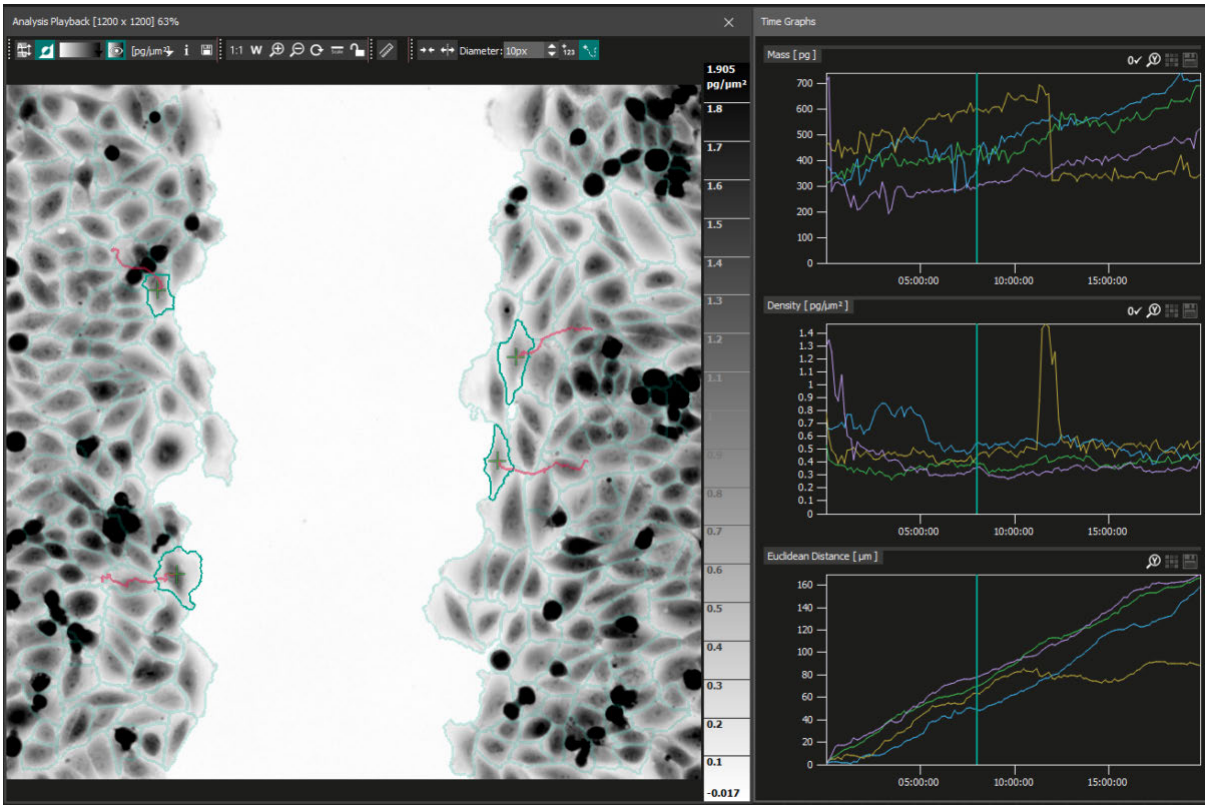

Fig. 2: Quantitative phase imaging, cell tracking and morphological analysis in wound healing experiment, cell line LW13K2, objective 10x/0,3, analysed in SophiQ.

Type of presentation: Invited

LS-IN-3136 Histological evaluation of surgical experiments in animal models

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Introduction: The subject of investigation is based on six studies that focus on the application of quantitative histology. It includes a presentation of virtual microscopy procedures and image field sampling strategies; mapping changes in the microscopic structure of ovine and porcine common carotid arteries (CCAs) and their comparison with human coronary arteries (CAs) and internal thoracic arteries (ITAs); vascularization assessment in a mouse model of lymphoma xenografts (PDX); the effect of hyperbaric oxygen therapy on type III collagen production and vascularization in a skin wound in diabetic rats.
Methods: The review article about virtual microscopy was focused on an example of sampling images of quantitative histology. In other studies, we quantified the area fraction of microscopic components using a stereological grid point and two-dimensional density using a counting frame.
Results: The differences in microscopic composition between the left and right CCA of the same individual were significantly greater in sheep than in pigs. In both animal models, the area fraction of elastin and chondroitin sulfate decreased in the proximodistal direction, while the area fraction of smooth muscle actin increased. The CCAs of both animal models are not equivalent to human CAs and the ITA.
By comparing the use of Doppler ultrasonography and quantitative histology to determine the area fraction of microvessels in lymphomas, significant differences were found. In quantitative histology, the area fraction of small PDX models was smaller and large PDX models were greater than in Doppler ultrasonography.
Density and area fraction of microvessels were significantly lower in mouse xenografts than in primary human lymphomas.
Hyperbaric oxygen therapy of skin wounds in diabetic rats II. increased the volume fraction of type III collagen in the healing skin.
Conclusion: The individual studies and their common denominator include practical recommendations for optimizing study design with respect to quantitative histological assessment.

Acknowledgement: The project FIND No. CZ.02.1.01/ 0.0/0.0/16_019/0000787; NPU I Nr. LO1503; Progress Q39; Cooperatio Program (MED/DIAG); GAUK No. 1313420; SVV 260 536.

Material sciences

Type of presentation: Invited

MS-IN-3204 Electron crystallography with CrysTBox: Low barriers for newcomers, advanced features for experts and high precision for everyone

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Thanks to its automation and interactivity, CrysTBox (Crystallographic Tool Box) software suite offers 21st century tools for everyday interpretation of TEM images. Highly accurate analysis combined with illustrative visualization gained popularity from pole to pole: It makes crystallographic analysis and interpretation accessible to non-crystallographers paving the road for interdisciplinary research. At the same time, expert users can enjoy effortless routine analyses or instrumental support for advanced experimental techniques. Both rely on tandem of analytical and visualization tools.

Analytical tools employ artificial intelligence and computer vision to perform the analysis faster and more accurately than what is achieved manually. They can determine crystal orientation, interplanar distances and angles using various types of diffraction patterns (SAED, CBED, nanodiffraction) and HRTEM. They can improve readability of ring diffraction, identify the rings and quantify them. Strain maps can be generated using geometric phase analysis (GPA).

Visualization tools can help all the way through the experiment from a simulation-based exploration of the sample material, through planning the experiment to understanding the results. The tools can interactively depict a single material as well as an intergrowth of two different materials in direct or reciprocal space or using stereographic projection.

Though it brought new and innovative aspects to diffraction analysis eight years ago, CrysTBox became widely adopted by researchers all around the world. By tackling the work they need to do, CrysTBox allows them to focus on the tasks they want to do.

Acknowledgement: CzechNanoLab project LM2023051 funded by MEYS CR is gratefully acknowledged for the financial support of the software development and maintenance at LNSM Research Infrastructure.

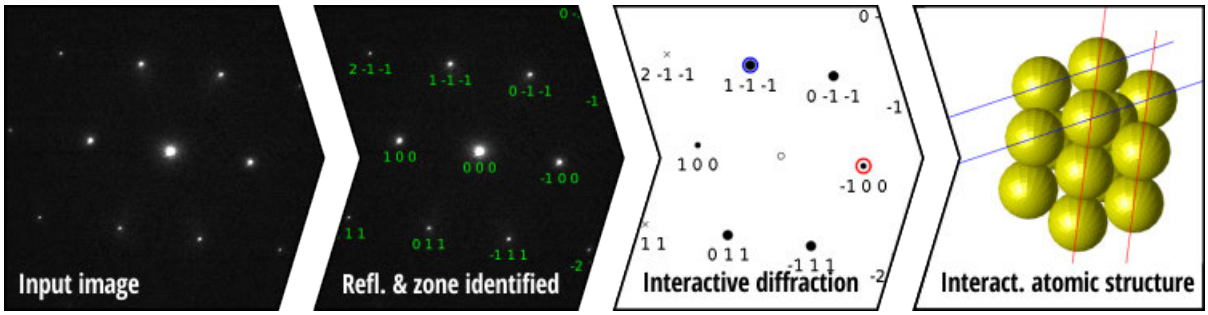

Fig. 1: In simple cases, you are 30 seconds and two clicks from an interactive digital twin of your input image.

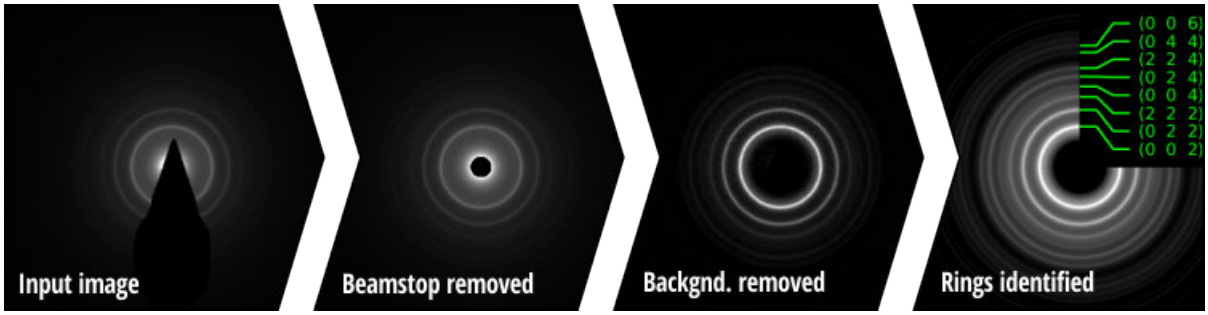

Fig. 2: Apart from automated interpretation, the tools can significantly improve the image readability.

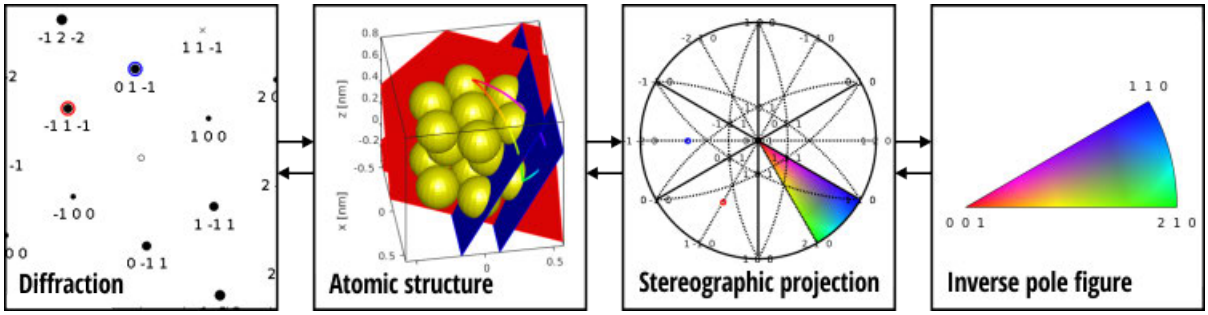

Fig. 3: Interactive visualization is helpful from planning the experiment to understanding its results.

Type of presentation: Oral

MS-O-3152 Observation of the crystalline phase transition and characterization of individual crystals in Sb2S3 thin films and nanostructures for tunable metasurfaces

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Metasurfaces represent an evolution in optics, where regular bulky components are replaced by ultrathin nanostructured surfaces that can shape the beam of light. This shaping is achieved through precisely aligned nanostructures, which control light amplitude, phase, and polarization at the nanoscale. Fabricating the nanostructure from a phase-change material allows tuning the function of the already created metasurface. The tuning is possible by changing the nanostructure's material's crystal structure and, therefore, optical properties.

One such phase-change material is antimony trisulfide (Sb2S3). It is a non-volatile phase-change material that goes through an amorphous–crystalline transition (~250°C) that can be achieved by simple heating and crystalline–amorphous transition (~600°C) that is based on a very fast (~10°C/ns) melt-quench process achieved by high-energy pulses [1]. Its most significant advantage lies in its lowest absorption ($k \approx 0$ for $\lambda \geq 633$ nm) and relatively large refractive index modulation ($\Delta n \approx 1$) in the visible and near-infrared spectral region. While several articles report applications of Sb2S3 into tunable metasurfaces [1, 2], the characterization of individual crystals and their formation within films and nanostructures are missing.

Here we present the observation of the gradual crystallization of Sb2S3 films and nanostructures prepared by pulsed laser deposition and electron beam lithography. The process is recorded and analyzed in situ at the atomic resolution by transmission electron microscopy (Fig. 1a) and at the microscale (grain level) by scanning electron microscopy (Fig. 1b). Grains within films and nanostructures are also characterized ex situ by electron and optical microscopy techniques. These results will help us understand the crystalline phase transition of Sb2S3 and utilize it to gradually tune the light properties at the nanoscale.

[1] ACS Nano 15, 12 (2021), p. 19722-19732
[2] Sci. Adv. 6, 51 (2020), p. 2375-2548

Acknowledgement: Supported by Czech Science Foundation (21-29468S), Brno Ph.D. talent, Thermo Fisher Scientific and CSMS scholarship, and MEYS CR (LM2018110).

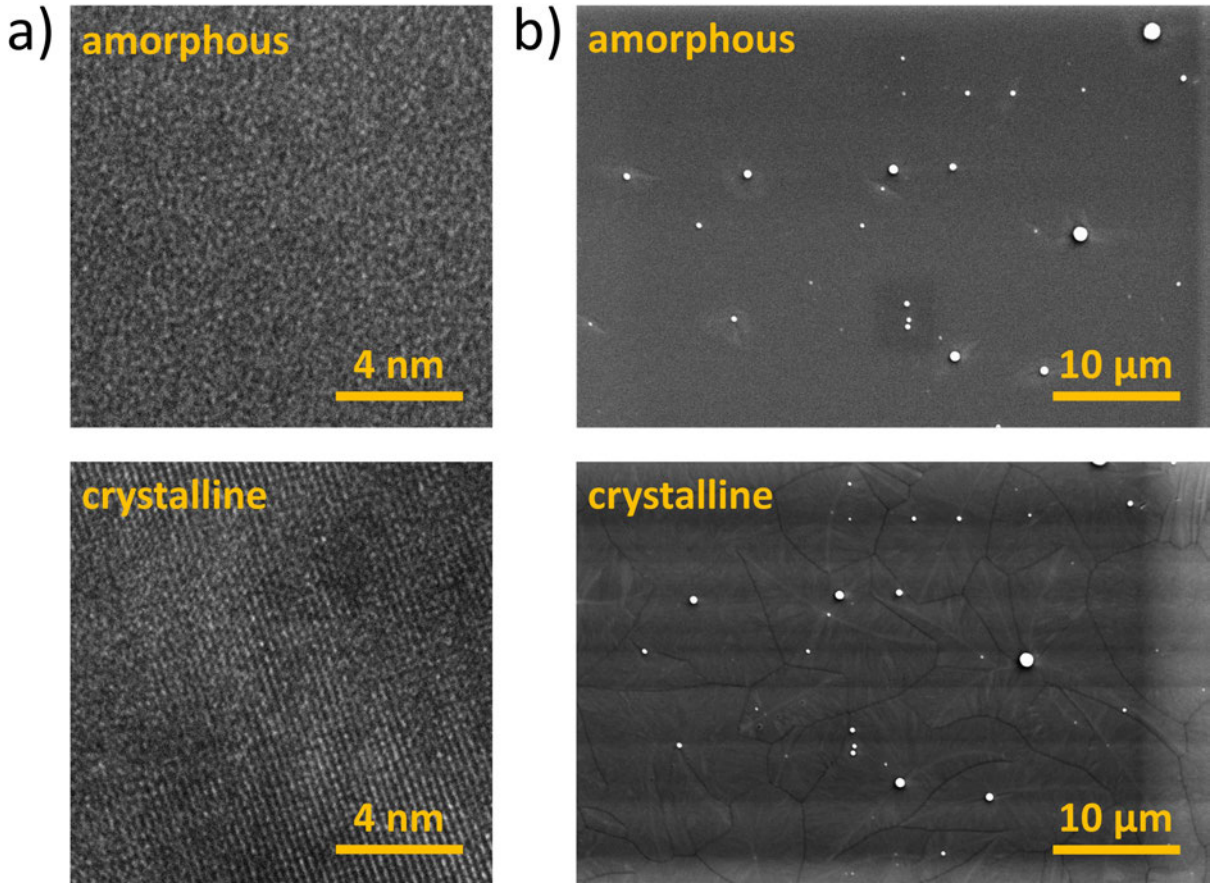

Fig. 1: a) Transmission electron microscopy and (b) scanning electron microscopy micrographs of amorphous and crystalline Sb2S3 thin films on SiN membrane and silicon, respectively, captured before and after the phase transition.

Type of presentation: Oral

MS-O-3177 2D materials for application in devices

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Recently, it was clearly demonstrated, that functionalization of graphene dramatically enhance potential for application of this material. However, so far realized strategies do not typically allow to control the functionalization process in terms of localization of the functional groups and mutual interactions with the graphene. Mastering of these processes will pave the way to precisely control electronic structure of graphene and thus enable more advanced applications. It will be also shown how tailored functionalization can be applied to optimize function of supercapacitor and to realize fast, ultrasensitive and broadband 2D detectors.

Acknowledgement: We acknowledge the support of Czech Science Foundation Project No. GX20-08633X.

Type of presentation: Oral

MS-O-3148 The influence of powder modification on printed microstructure of the VDM 699XA

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The microstructure of the powder particles of the VDM 699XA with unmodified chemical composition was analyzed by the means of the electron microscopy. The nominal chemical composition of the powder consists of 28% Cr, 2.5% Al, 1% Fe and Ni for balance (weight %). The electron backscattered diffraction (EBSD) image is shown in Fig. 1. It is obvious, that powder particles are polycrystals with no preferential crystallographic orientation. The chemical mapping showed that elements are homogenously distributed in the particles. The three different modifications of VDM 699XA were produced. Namely addition of Al, Y and/or Ti was introduce into standard alloy composition. Modified powders were investigated by the same routine as unmodified powder. Some differences such as segregation at grain boundaries (Fig. 2) or precipitation of new phases were detected. The bulk materials were prepared from powders by selective laser melting and the microstructural features, with respect to the particular powder, were analyzed.

Acknowledgement: Authors would like to acknowledge the financial support from European Union's Horizon 2020 research and Innovation programme under grant agreement No. 958192.

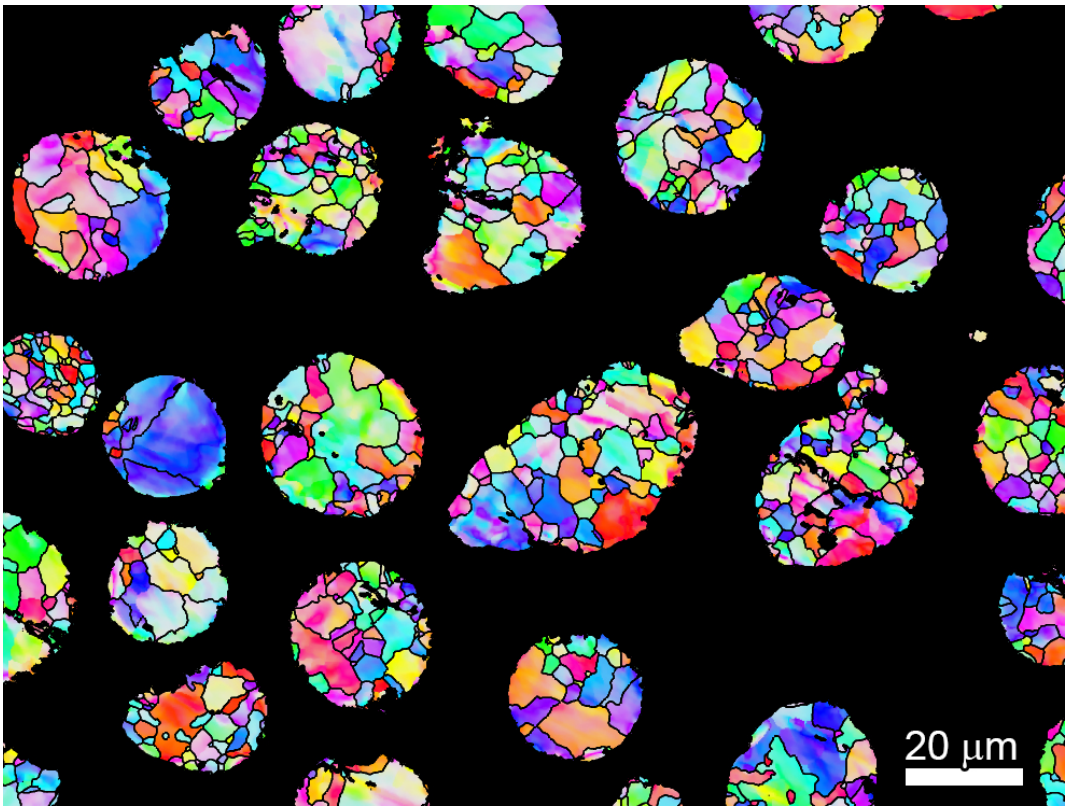

Fig. 1: The microstructure of unmodified powder of VDM 699XA as revealed by EBSD observation.

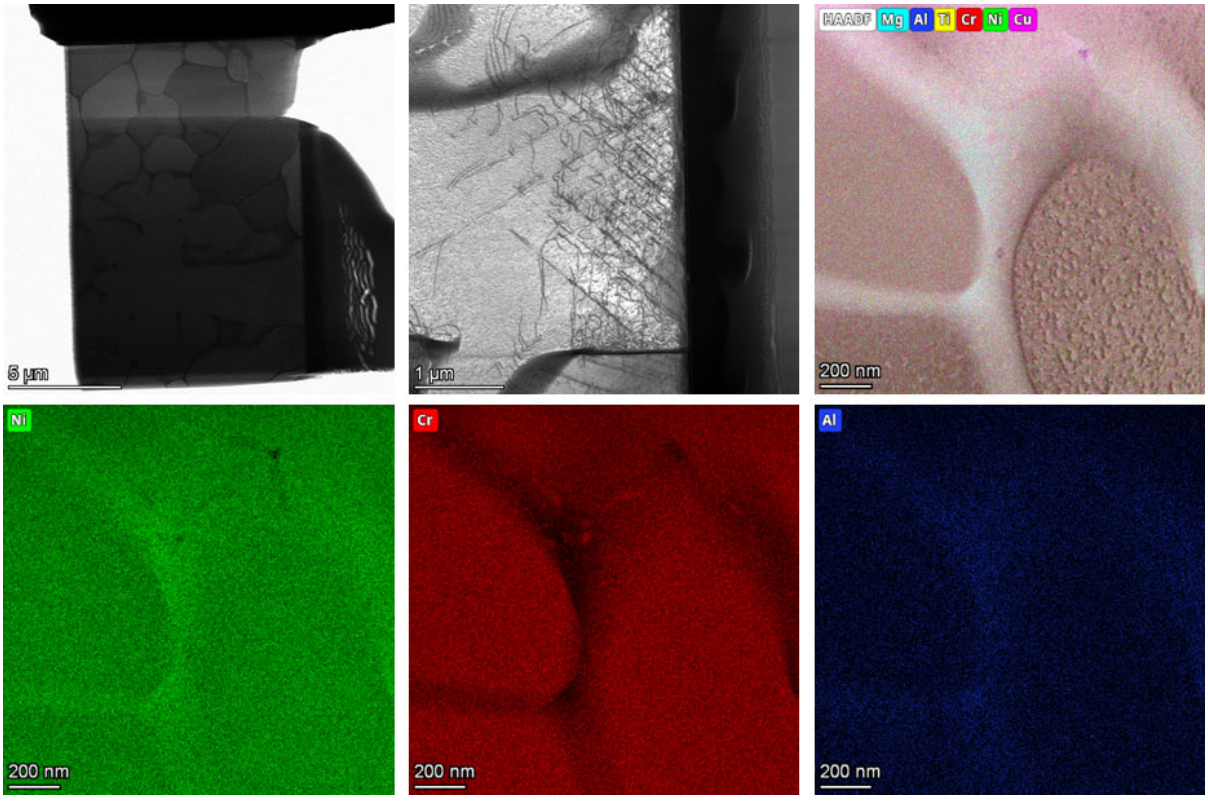

Fig. 2: TEM analysis of powder with modified powder of VDM 699XA alloy (2.9% Al; 0.7% Y).

Type of presentation: Oral

MS-O-3170 Detection of Gd-Rhod liposomes diffusion and stability in model of dermal tissue and wound structure

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Introduction: Liposomes as nano-carriers migrating to different semisolid materials are still challenge, which need deep material and microscopic characterisation. The effective and low-cost delivery of liposomes is still one of the never-ending problem of medical care too. Pilot test of new liposome formulation with additives of Gadolinium and Rhodamine contrast agents was prepared, kinetic of diffusion under external force (sono-pulse) was tested on model of diabetic rats subdermal structure. The kinetic were characterised by fluorescence and electron microscopy, additionally by MRI imaging based on Gd-contrast.
Methods: Synthetic liposomes were prepared using structure components and additive lipids with Rhodamine and Gadolinium components, the source solution was: DSPC/Cholesterol/PE-DTPA(Gd)/Lisamine Rhodamine B. The folding of liposomes from the source solution was prepared by two methods: TF - thin-film hydration methods (using home made Arduino temperature controlled heating system based on Heidolph Instruments heat block) and MF - microfluids mixture methods (using Blaze - NanoAssemblr fluidic system). The liposomes from both methods was characterised by spectrometer and by electron microscopy. Liposomes were mixed into the semi–liquid collagen hydrogel, applied to the surface od dermal sample. The kinetic of liposome diffusion into the sample material and their incorporation to keratinocytes were characterised by microscopy and by MRI with high resolution.
Results: Both type of liposomes (TF vs. MF) was compared. 220 nm vs. 130 nm was average liposomes size from the methods, the average result of Z-potential was -4mV and -22mV. The migration kinetic characterised by time lapse and electronmicroscopy was better for MF derived liposomes. Migration was significantly increased by sono-pulses.
Conclusion: The effective liposomes diffusion into subdermal structure and incorporation into keratinocytes bring possibility of future miRNA and plasmid delivery to specific region in diabetic wound and monitoring of delivery by fluorescence and Gd-contrast.

Acknowledgement: Supported by: Technology Agency of Czech Republic – project FW01010106 and FV30393.

Type of presentation: Invited

MS-IN-3144 Chemical and physical properties of impact glasses: Traces of extraterrestrial components or an imprint of source rocks?

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Tektites are natural silicate glasses formed by melting the topmost, most commonly sedimentary rocks at the target area during the initial stages of hypervelocity impacts of extraterrestrial bodies on the Earth's surface. Tektites may display contamination derived from a projectile. The potential presence of a meteoritic component was tested by a thorough study of microstructural features using different microscopic techniques such as, e.g., SEM/BSE/EDS/WDS/EBSD as well as the contents of highly siderophile (HSE) and platinum group elements (PGE), the Re–Os, oxygen, and chromium isotope systematics in three types of impact glasses (moldavites (CET), Zhamanshin impact glasses (ZIG), Australasian tektites (AAT)). Microscopic methods revealed the presence and nature of mineral inclusions. However, they did not provide an unambiguous answer to whether projectile material is included in these glasses. Unusual inclusions with high Fe, Cr, Ni, and Ti content were found in ZIG. Further, sulfide inclusions composed of minerals shenzhuangite (a chalcopyrite-structured $NiFeS_2$ yet found only in meteorites) and troilite (hexagonal stoichiometric FeS) were found for the first time in Muong Nong-type AAT. The results of a detailed chemical study obtained for moldavites and Australasian tektites indicate a possible minor addition of a meteoritic component from the projectile. However, the nature of the projectile cannot be unequivocally identified. Traces of the extraterrestrial component and the type of projectile were determined only in irghizites (tektite-like glasses from Kazakhstan, ZIG). The chromium isotope data identified the meteoritical contamination to represent a projectile of the Ivuna-type carbonaceous chondrite (CI) character.

Acknowledgement: This research was funded by the GAČR projects 13-22351S and 17-27099S and co-financed by institutional support RVO 67985831.

Optics and Instrumentation

Type of presentation: Invited

OI-IN-3166 Electron energy loss spectroscopy and its applications in nanophotonics illustrated mostly on gallium nanoparticles

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In this invited talk, I will present a wide field of electron energy loss spectroscopy (EELS) applications in nanophotonics. EELS is based on inelastic scattering and measures the energy transferred from the primary electron beam to the sample to induce any excitation. The power of EELS will be mostly illustrated on gallium nanoparticles presenting results from my recent publication "Plasmonic properties of individual gallium nanoparticles" [1], which was recently awarded by prestigious Fritz-Grasenick Award 2023 by Austrian Society for Electron Microscopy [2].

The EEL spectrum contains the zero-loss peak (ZLP), low-loss region covering mainly excitations of valence electrons, and the core-loss region covering excitations of core electrons. ZLP can be used for thickness mapping. In the low-loss region (energy loss below 50 eV) we observe volume plasmon peaks, surface plasmon peak, localized surface plasmon peaks, band gap, intra- and inter-band transitions, and even phonon excitations. In the core-loss region we observe characteristic material edges and their fine structure. The chemical composition can be determined from volume plasmon energy, material edges, and from the near-edge structure.

In the case of localized surface plasmons, EELS is sensitive to the electric near-field component parallel with the electron beam and cannot be directly associated with the magnetic near field. However, by a trick with Babinet's principle we might access the magnetic near-field indirectly, too [3-5]. Moreover, I will also discuss optimal experimental conditions for achieving the best signal-to-background ratio [6].

[1] M. Horák et al., J. Phys. Chem. Lett. 14 (2023) 2012-2019.
[2] https://asem.at/fritz-grasenik-preis/
[3] M. Horák et al., Microsc. Microanal. 26(S2) (2020) 2628-2630.
[4] M. Horák et al., Sci. Rep. 9 (2019) 4004.
[5] V. Křápek et al., Nanophotonics 9 (2020) 623-632.
[6] M. Horák, T. Šikola, Ultramicroscopy 216 (2020) 113044.

Acknowledgement: Supported by Czech Science Foundation (22-04859S) and MEYS CR (CzechNanoLab, LM2018110).

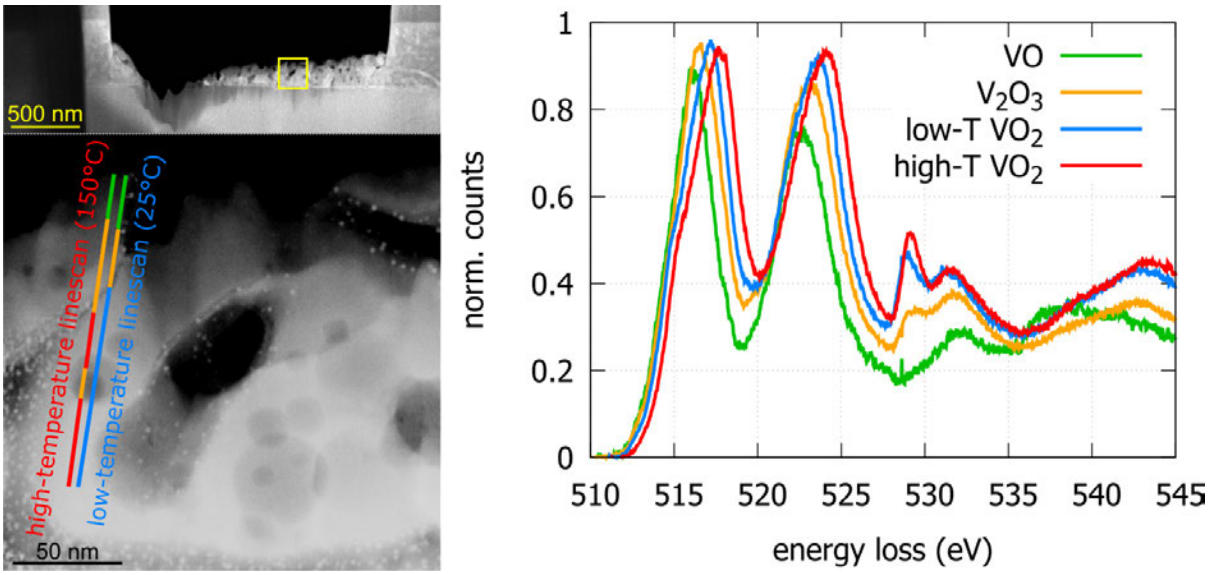

Fig. 1: Determination of local stoichiometry of vanadium (di)oxide lamella by EELS: near-edge fine structure of VO, V_2O_3, dielectric low-temperature VO_2, and metallic high-temperature VO_2.

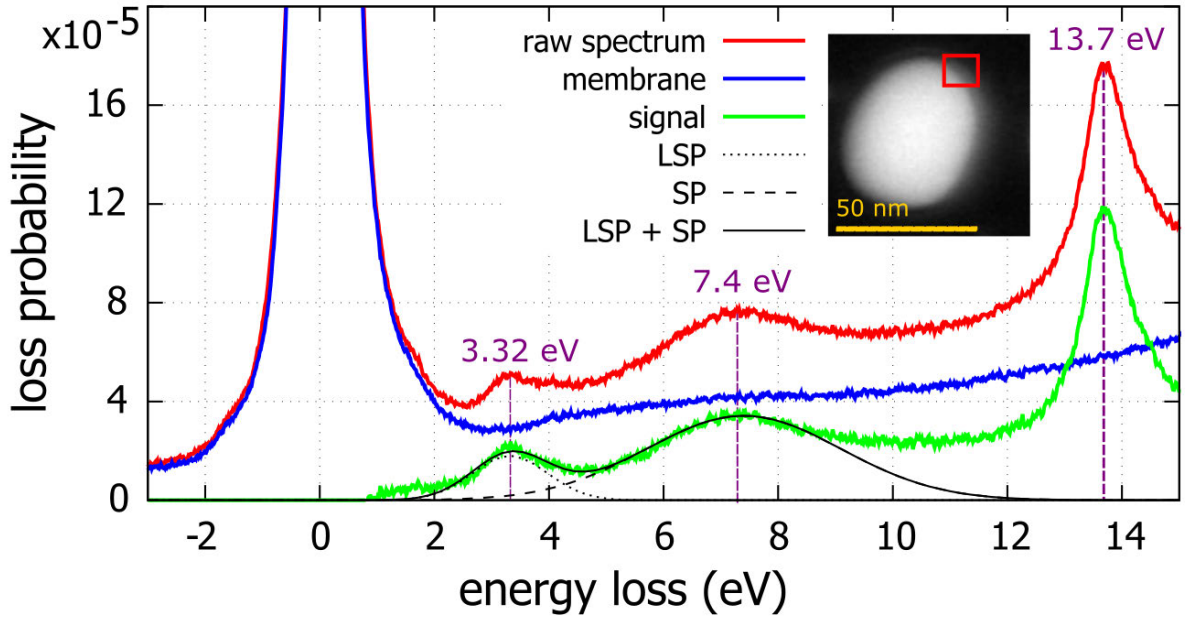

Fig. 2: Processing of the EEL spectrum in the case of a gallium nanoparticle with the diameter of 56 nm. The low-loss EEL spectrum contains three peaks (volume plasmon at 13.7 eV, surface plasmon at 7.4 eV, and dipole mode of localized surface plasmon resonance at 3.32 eV).

Type of presentation: Oral

OI-O-3103 Advanced optical microscopy as a tool for visualisation of micro-plastics

Marcek Chorvatova A.[1,2], Mateasik A.[1], Ariese F.[3], Chorvat D.[1]

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Environmental pollution by microplastics (MPs) is becoming a serious environmental burden of the 21st century. Our aim is to test advanced optical microscopy methods in order to identify various types of MPs from samples taken directly in the environment, as well as to evaluate interactions of MPs with aquatic photosynthetic organisms based on the study of their endogenous fluorescence derived from chlorophylls [1-3]. In this work, we present advantages of fluorescence lifetime imaging microscopy (FLIM) and spectrally-resolved fluorescence microscopy methods in monitoring the MPs properties and distribution, and to monitor the interaction of algae Chlorella sp with model MPs, namely the effect of MPs size, ranging from nanometers (20-500 nm) to micrometers (up to 2 μm).

Using these imaging techniques, we examined the responsiveness of the endogenous chlorophyll fluorescence of sweet water algae Chlorella sp. [2]. Our results uncovered differential interaction of algae to polystyrene spheres in the nano- vs. in the micro-metric sizes. We are convinced that advanced optical microscopy methods has a potential for evaluation of pollution in the aquatic environment and thus for improvement of our understanding of the interaction of living systems with pollutants, such as microplastics.

References

1. Marcek Chorvatova A., et al., Biosensing the presence of metal nanoparticles by spectrally- and time-resolved endogenous fluorescence in water moss Fontinalis antipyretica. Frontiers in Physics 9 (2021): 634324: 1-9. doi: 10.3389/fphy.2021.634324.

2. Marcek Chorvatova A., et al., Time-resolved endogenous chlorophyll fluorescence sensitivity to pH: study on Chlorella sp. algae. Methods and Applications in Fluorescence 8 (2020): 024007, doi 10.1088/2050-6120/ab77f4.

3. Z. Pavlinska, et al., Fluorescence responsiveness of unicellular marine algae Dunaliella to stressors under laboratory conditions. Journal of Biotechnology 324S (2020): 100018, doi 10.1016/j.btecx.2020.100018.

Acknowledgement: Supported by LASERLAB-EUROPE (grant agreement no. 871124, European Union's Horizon 2020 research and innovation programme) and by VEGA No. 2/0070/21.

Type of presentation: Oral

OI-O-3137 4D-STEM/PNBD: Powder electron diffraction in SEM made fast and easy

Slouf M.[1], Skoupy R.[2,3], Pavlova E.[1], Vancova M.[4], Pilatova J.[4,5], Krzyzanek V.[2]

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We have introduced a novel SEM method, named 4D-STEM/PNBD, which yields powder electron diffraction patterns [1]. The quality of 4D-STEM/PNBD patterns can be improved to that of standard TEM/SAED powder diffractograms [2]. Recent results have demonstrated that our method can analyze nanocrystals in biological tissues, where classical TEM/SAED techniques may fail [3].

4D-STEM/PNBD method requires a modern SEM microscope equipped with a pixelated detector of transmitted electrons. The pixelated detectors (2D-array detectors) for SEM have been commercialized recently [4]. They enable fast collection of nanobeam electron diffraction patterns (NBD) from selected locations. This is called 4D-STEM-in-SEM, as we obtain a 2D-NBD for each point of the 2D-scanning array. The 4D-STEM datasets are easy to collect, but the individual NBD's are difficult to analyze due to the random orientation of nanocrystalline material (Fig 1a). In our method, all individual spotty NBD patterns are combined into one composite powder diffraction pattern (PNBD; Fig. 1b-e). That is why the method was called 4D-STEM/PNBD. The conversion of 4D-STEM datasets to a 2D-powder diffractogram is performed by our freeware package STEMDIFF [5]. The final 2D diffractograms can be analyzed easily with standard programs for TEM/SAED, such as ProcessDiffraction [6], CrystTBox [7], or our package EDIFF (output in Fig. 2; ref. [8]).

This contribution summarizes recent development of 4D-STEM/PNBD method. Analyses of nanocrystals in biological samples will be shown. Recent improvements of STEMDIFF [5] and EDIFF [8] programs will be demonstrated.

References:

[1] *Nanomaterials* 11 (2021) 962; doi: 10.3390/nano11040962
[2] *Materials* 14 (2021) 7550; doi: 10.3390/ma14247550
[3] *Small Methods* (2023); submitted.
[4] *Microsc Microanal* 23 (2017) 554; doi: 10.1017/s1431927617003452
[5] *https://pypi.org/project/stemdiff*
[6] *https://public.ek-cer.hu/~labar/ProcDif.htm*
[7] *https://www.fzu.cz/en/crystbox*
[8] *https://pypi.org/project/ediff*

Acknowledgement: Projects TN02000020 (TACR), 21-13541S (GACR) and 21-26115S (GACR); Thermo Fisher Scientific company for a high-resolution SEM with pixelated detector installed at ISI CAS.

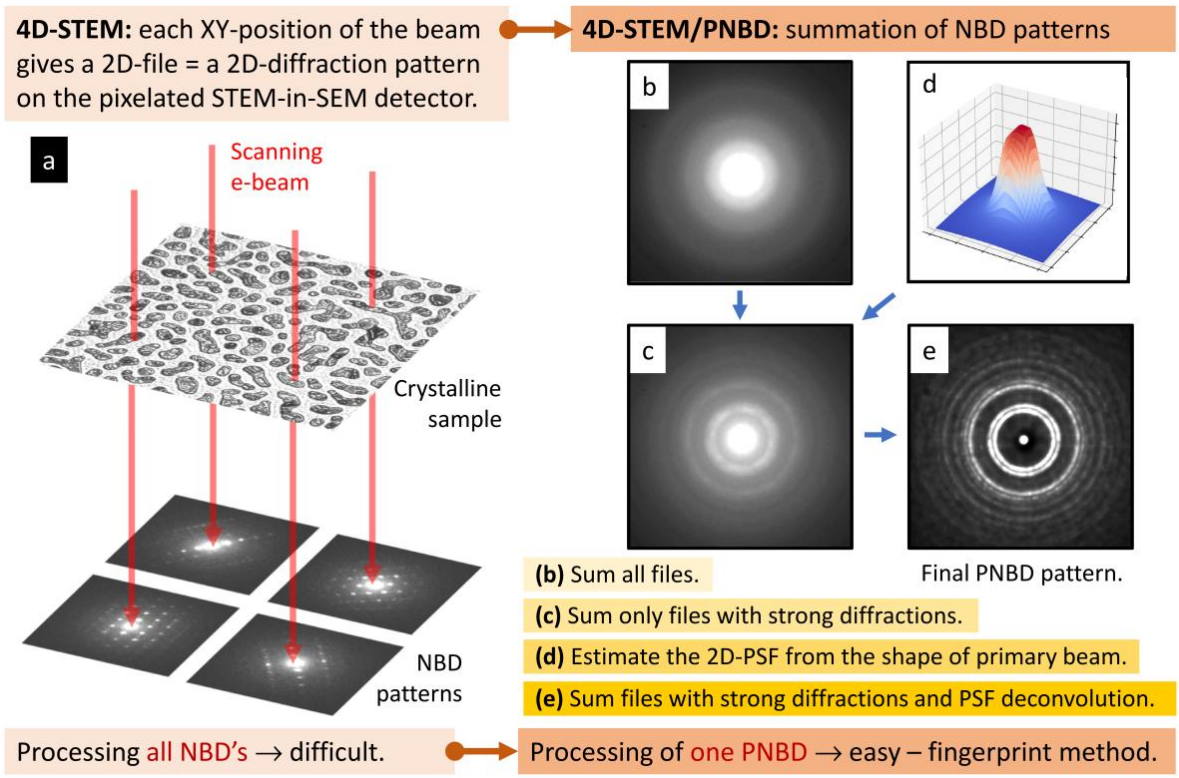

Fig. 1: Principle of 4D-STEM/PNBD method: (a) 4D-STEM dataset contains 2D-array of 2D-nanobeam diffraction patterns, which can be (b) summed, (c) summed after filtering out files with weak diffractions, (d) employed in estimation of primary beam shape (2D-PSF function), and (e) summed after filtering and 2D-PSF deconvolution.

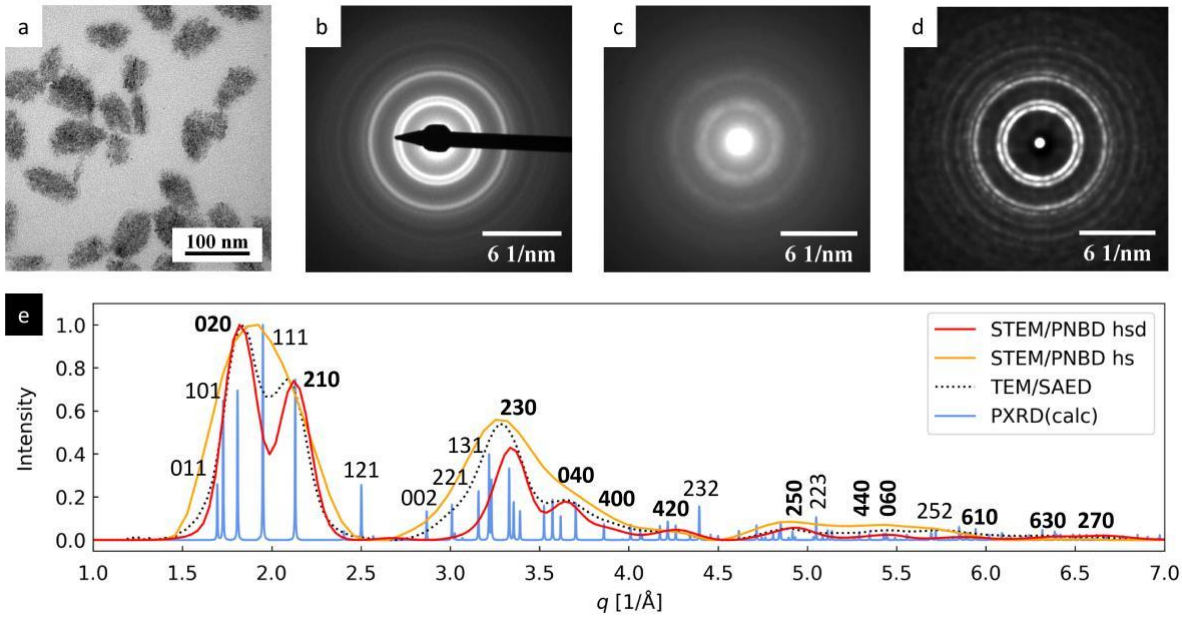

Fig. 2: TEM and 4D-STEM/PNBD results for TbF_3 nanocrystals: (a) TEM/BF, (b) TEM/SAED, (c) 4D-STEM/PNBD based on filtered files, (d) 4D-STEM/PNBD based on filtered and deconvoluted files, and (e) the comparison of radially averaged results from TEM/SAED (blue), 4D-STEM/PNBD with/without deconvolution (red/orange), and theoretically calculated PXRD (blue).

Type of presentation: Oral

OI-O-3142 Utilization of advanced software features for better, faster, and less laborious segmentation of data from TEM and 3D-SEM

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The segmentation is nowadays an inseparable part of the electron microscopy – especially the 3D-SEM. It is a tool that allows us to label certain parts of imaged object with the corresponding name and material. The segmented image then allows us to extract the data such as areas, lengths and volumes directly from the image. However, with the never-ending development and improvement of the image acquisition techniques the quantity of obtained data grows. This also means, that the simple segmentation tools are becoming highly insufficient, as they are too slow and may be imprecise for the big quantities of data.
Fortunately, the image segmentation software is constantly being improved to keep up and advanced segmentation tool are being added to speed the segmentation up and make it as precise as possible. From the simpler tools we can nowadays utilize the super-pixel clustering that allows us to segment the areas of interest faster and more precisely. Another tool using the super-pixel/super-voxel clustering to segment various parts of an image just by 2-3 clicks.
The utilization of straightforward Deep Learning is one of the most powerful “weapons” on the segmentation field. It allows us to process even very big datasets and reconstruct very big volumes.
In our laboratory we now utilize the Microscopy Image Browser (MIB). The utilization of all the advanced features of this software sped up our data processing immensely and allowed us to do the segmentation of the huge dataset of tick intestine (0.018 mm3 – the segmented part is approximately 0.006 mm3), that would require months of consecutive work in 2 weeks of actual work (Fig. 1).
Another example of utilization of advanced functions was in the reconstruction immune cells in COVID-19 infected lungs.

Acknowledgement: Acknowledgement: This work was supported by the LM2023050, CZ.02.1.01/0.0/0.0/18_046/0016045, andTN02000020.

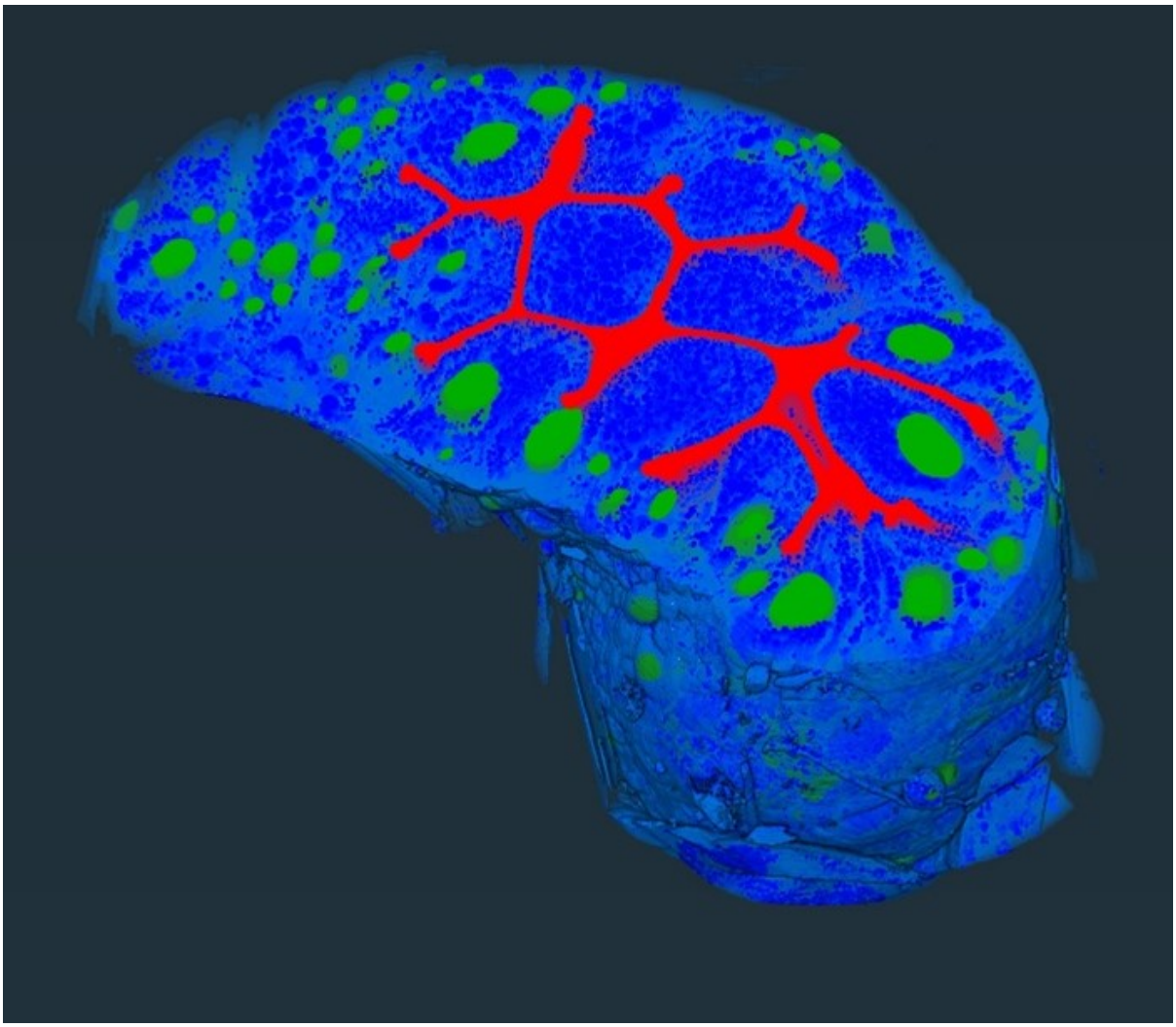

Fig. 1: Fig. 1: Tick intestine reconstruction.

Type of presentation: Oral

OI-O-3149 AFM-in-FIB/SEM: Advancements for in-situ sample analysis in batteries

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Highly air-sensitive samples from the battery industry are difficult to work with, especially when the analysis requires multiple instruments. Such is the case with the cathode active material (NCM) dispersed within the solid electrolyte, provided by BASF SE. While Atomic Force Microscopy (AFM) can map the conductivity of the grains in electrolyte, it is not feasible to perform such measurement on a sample degraded by exposure to air and humidity. On the other hand, Scanning Electron Microscopy (SEM) does not provide enough information about the battery's condition but allows precise surface modification in a vacuum. We present a way to combine SEM and AFM analysis, achieving virtually no air exposure in the target area.

The setup relies on using the AFM LiteScope inside a FIB-SEM system equipped with a gas injection system (GIS or MultiChem). Gallium contamination of the battery sample can be avoided by using Xe or Ar plasma FIB (e.g. Thermo Scientific Helios 5 Hydra DualBeam). A wall of protective material is deposited perpendicular to the surface of the air-sensitive sample using GIS. The whole AFM with the sample is then tilted away from the FIB to achieve as shallow etching angle as possible, see Fig. 1. By etching along the edge of the protective wall, a smooth, shallow trench with minimal curtaining effects can be milled into the surface. The freshly exposed surface of the trench can be subsequently scanned with Conductive AFM (C-AFM) without leaving the vacuum, as visible in Fig. 2. This way, we found out that certain parts of the surrounding material are more conductive than others and the conductivity map shows how the grains are composed of coagulates.

In-situ approach significantly reduces the complexity of the experimental setup. A secondary benefit is a prolonged sample lifetime. Because a new fresh surface can be exposed before each measurement, the sample can be used multiple times, even if exposed to air for a short time between sessions.

Acknowledgement: This work is co-financed from the state budget by the Technology Agency of the Czech Republic within the NCK Programme with project number TN02000020.

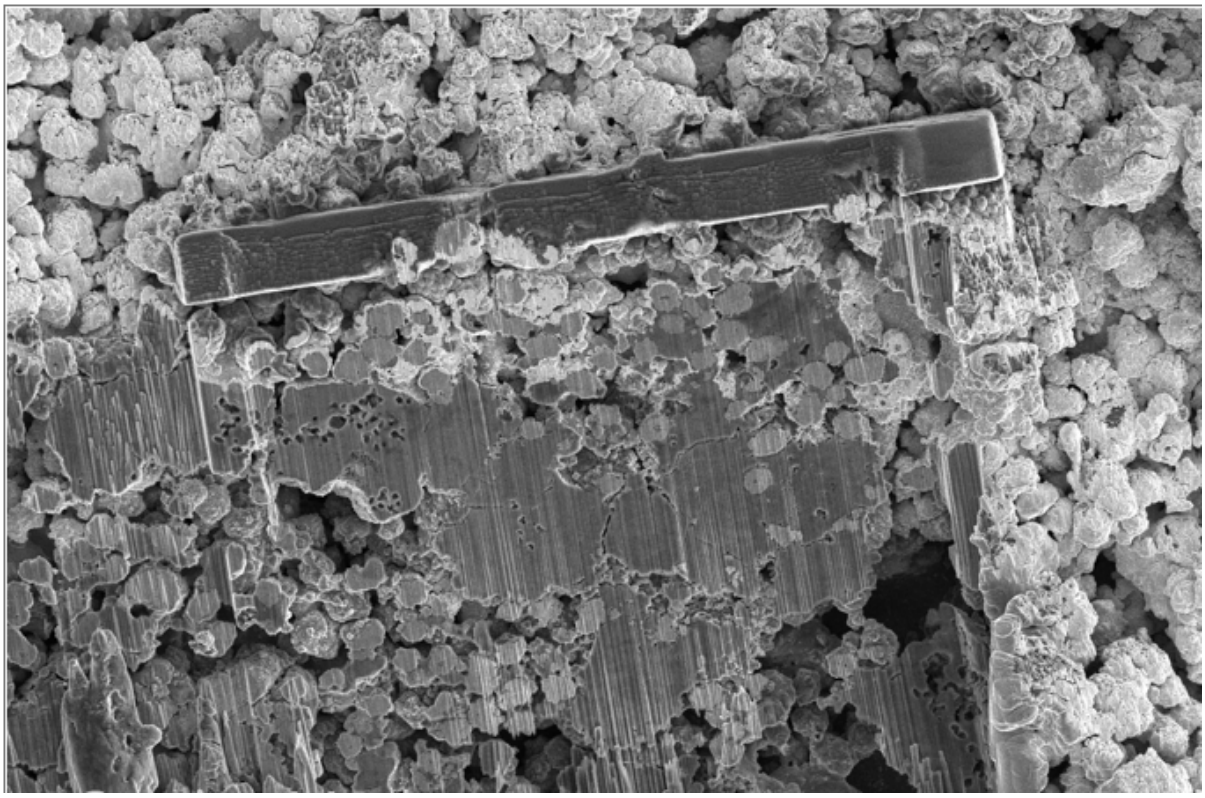

Fig. 1: In-situ battery surface preparation for AFM-in-SEM measurement.

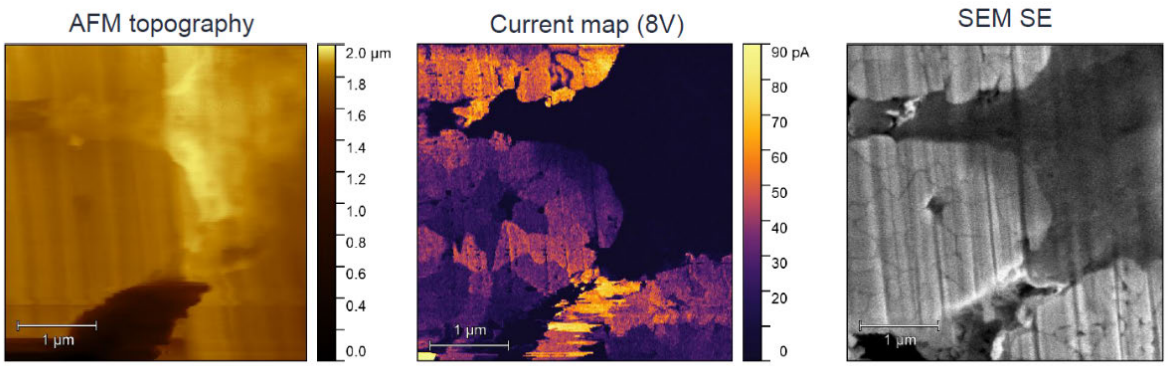

Fig. 2: AFM-in-SEM measurement (AFM, C-AFM, and SE) of NCM grains prepared in-situ using GIS and FIB.

Czech-BioImaging

Type of presentation: Oral

CzBI-O-3213 Czech-BioImaging: a national imaging platform open to all scientists

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Innovative imaging technologies allow us to study hidden biological processes in cells, tissues, and whole organisms thanks to technological development. Imaging became one of the crucial elements of research in biological and medical fields.

Czech-BioImaging is a national research infrastructure for biological and medical imaging. It has been in operation since 2016 and is the only advanced research platform performing cell and molecular imaging for research in the Czech Republic. It also represents three National Euro-BioImaging ERIC Nodes providing technical and scientific excellence to international researchers by the integration of multiple imaging technologies together with image processing and analysis tools in an open-access mode.

Sixteen core facilities involved in the Czech-BioImaging are providing open access to most advanced imaging technologies to users from both academic, medical and private sectors. The distributed character allows access to a portfolio of technologies to many users at different locations covering the major cities in the Czech Republic.

Czech-BioImaging provides users with the technical background and necessary expertise and guidance from experiment design to analysis and interpretation of acquired data for project solutions, especially in cell and molecular biology, genetics, physiology, parasitology, tumor biology, neuroscience, developmental biology and pathology. Czech-BioImaging portfolio includes advanced light and fluorescence microscopy, super-resolution microscopy, electron microscopy, correlative light and electron microscopy, sample preparation, various anatomical and molecular methods in preclinical and medical imaging, and image data analysis.

The technological and methodological foundation of Czech-BioImaging significantly improves biomedical research in the Czech Republic. Due to the extensive expertise and long-time experience, Czech-BioImaging is also heavily involved in organization of various hands-on courses, workshops, annual conference, and many other awareness-raising activities. Owing to many methodological publications, it has become a partner of instrumentation manufacturers.

Acknowledgement: National Infrastructure for Biological and Medical Imaging – Czech-BioImaging is funded by the Ministry of Education, Youth and Sports of the Czech Republic (project No. LM 2023050) and supported from European Regional Development Fund (project No. CZ.02.1.01/0.0/0.0/18_046/0016045).

Type of presentation: Invited

CzBI-IN-3156 "Laser wars" at the cellular and subcellular level

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Laser scanning microscopes (LSM) are not only for visualization. The embedded LSM lasers can be precisely controlled and used for various photomanipulation techniques in live cells. The presentation will introduce approaches to how LSM lasers can induce specific cellular stresses involving nuclear DNA damage and cellular or subcellular heat damage. Our latest publications and unpublished data will demonstrate the application of such methods, including detailed qualitative and quantitative readouts we develop for monitoring cellular responses in live and fixed cells.

Acknowledgement: Ssupported by MEYS CR: (Large RI Project LM2018129 - Czech□BioImaging)

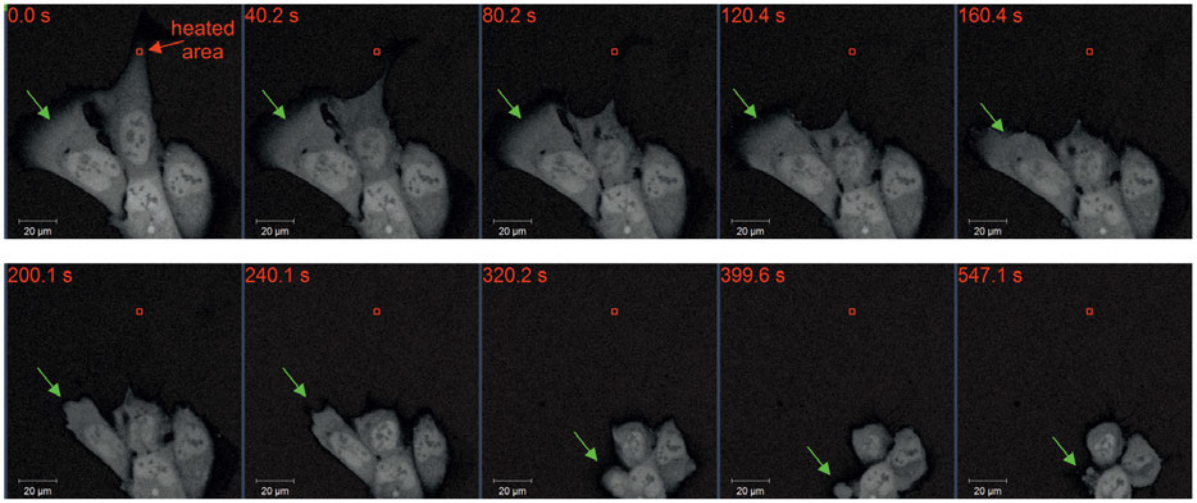

Fig. 1: Image depicting cellular reactions on local subcellular burning. The tip of one of the cells (red rectangle) was heated using the plasmon resonance principle. The cell actively escapes from the burned area and spreads the „I was burned“ information to bystanders, which also move, shrink, and even die (see arrow) despite not being heat damaged.

Czech optical cluster

Type of presentation: Oral

COC-O-3214 Czech optical cluster introduction

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Czech Optical Cluster (COC) is a voluntary non-profit organization founded in 2017, which brings together and represents representatives of science and industry operating in the field of Optics and related fields.

Czech Optical Cluster (COC) carries out its activities mainly in these pillars:

- Industrial and Consumer Optics
- Military Optics
- Lighting Technology
- Laser Technology and Applications
- Microscopy
- Optical quantum technologies

Czech Optical Cluster was created with the aim to improve the conditions for optical industry development in the Czech Republic through cooperation of companies, public sector and educational sector in the entire value chain in the field of optics, optomechatronics, photonics, optoelectronics and fine mechanics, including related production, technology and service development in supplier's and customer's domain.

The main objective of the COC is to build a platform for collaboration and knowledge sharing among actors of the science and industry in the field of Optics, to interconnect cluster members to strengthen their influence, innovations, and competitiveness, to promote the field of Optics and improve conditions for its development in the Czech Republic.

Acknowledgement: Czech optical cluster is funded by the Ministry of industry of the Czech Republic (project CZ 01.1.02/0.0/0.0/20_333-0023636 Český optický klastr: Tvoříme optiku pro budoucnost).

Type of presentation: Invited

COC-IN-3178 50 years of Raman optical activity - a rich history and a look into the future

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Raman optical activity (ROA) is a special phenomenon that measures the small difference in Raman scattering of chiral molecules between right- and left-handed circularly polarised radiation. This phenomenon was predicted theoretically by L.D. Barron in the late 1960s, and the first successful experimental confirmation of Raman optical activity was published in 1973, just 50 years ago. Since then, ROA has evolved from an obscure technique to a very promising analytical method that can be applied to a wide range of molecules - from determining the absolute configuration of organic molecules to investigating the conformation and dynamic behaviour of complex biological polymers (such as nucleic acids in the capsid of viruses). In this review lecture, in addition to describing important results obtained in recent years, we will focus on further possible extensions of the technique and its potential application in the field of microscopy.

Acknowledgement: The work was supported by the Czech Science Foundation (22-04669S).

Poster session

Type of presentation: Poster

PO-P-3086 Morphological Study of PHA Producing Bacteria

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Cupriavidus necator is considered as one of the biggest PHA producers. Unfortunately, the production of PHA using heterotrophic microorganisms requires large amounts of carbon substrates, such as glucose or fructose, which makes the production quite expensive compared to PP. Carbon substrates could make up to 50% of all production costs. One of the possibilities, how to reduce these expenses, could be the use of waste material (eg. frying oils) as a carbon source or production of PHA by photoautotrophic microorganisms. [1,3]
The surface of the block of resin-embedded sample is imaged by detection of back-scattered electrons. Following imaging the ultramicrotome is used to cut a thin section from the face of the block. After the section is cut, the sample block is raised back to the focal plane and imaged again.

After SFBSEM imaging we improved the image quality for a segmentation of cells and PHA granules using filtration via anisotropic diffusion in ImageJ. The contrast and brightness were adjusted to emphasize the differences between image background and regions of interest. Image segmentation was performed using the AMIRA software.

Our comprehensive morphological study indicates that the method involving cryo-SEM imaging in combination with high-pressure HPF freezing and perpendicular freeze-fracturing, is an excellent technique for analysing highly hydrated samples. Cells containing polymer particles, which are elastic even at very low temperatures, were visualized using this method. By TEM it is able to monitor the distribution of PHB granules inside the microbial cells. SBFSEM provides spatial information. A comparison of the morphology of the internal and external cell structure shows that the Cupriavidus necator H16 contains significant amounts of PHA granules, which are distributed throughout the whole cell volume.

Acknowledgement: We acknowledge the core facility CF Electron microscopy and Raman spectroscopy (ISI) and LEM (BC) supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

Type of presentation: Poster

PO-P-3132 Effect of near-infrared photobiomodulation on cellular cytoskeleton and mitochondria

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Photobiomodulation (PBM) is a modern trend to improve the metabolism of cells. In particular, mitochondria are affected by near-infrared radiation, which is believed to play a crucial role in wound healing. In our work, we focused on the organisation of mitochondria and cytoskeleton in two different regimes. In one regime, light with a wavelength of 590 nm was used to stimulate light damage in cells, known as photodynamic therapy, induced by externally administered photosensitizers. In the second regime, light with a wavelength of 808 nm was used to modulate metabolic activity in the mitochondria of cells. By fluorescence immunostaining, we detected differences in beta-actin fibre bundles. Co-staining with mitochondrial complex V revealed the distribution of fused mitochondria along these fibres. Our results suggest that the cytoskeleton plays an important role in the response of cells to near-infrared PBM.

Acknowledgement: This research was supported by APVV-20-0340, VEGA 1/0187/23, BioPickmol ITMS2014+: 313011AUW and OPENMED ITMS2014+: 313011V455.

Type of presentation: Poster

PO-P-3133 Identification of hypericin fluorescence in protein corona of nanoporous silica particles

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In this work, we present preliminary results of the interaction of photodynamically active molecule – hypericin with nanoporous silica and cells. The architecture of nanoporous silica, has been exploited in the last decades for numerous applications, and allows them to bind and fill with various molecules. Silica nanoporous particles are known for their biocompatibility and porous character, which allows to adsorb hydrophobic and hydrophilic fluorophores. Our aim was to investigate the capability of these particles to adsorb proteins and hypericin. The ability to release hypericin from particles was studied fluorescence spectroscopy and confocal fluorescence microscopy. We have observed that protein corona enhances hypericin redistribution from nanoporous silica particles towards cancer cells. Singlet oxygen production was detected thanks to monomeric hypericin form in protein corona. It suggests that this complex silica-hypericin possesses promising properties in photodynamic therapy (PDT). PDT efficacy of silica-hypericin complex was comparable to hypericin-PDT without silica particles. Thus, this formulation was shown to be promising delivery system for hydrophobic molecules to cancer cells in PDT.

Acknowledgement: This work was supported by vvgs-2022-2184, OPENMED, ITMS2014+: 313011V455 and BioPickmol ITMS2014+: 313011AUW6 projects.

Type of presentation: Poster

PO-P-3151 Visualisation of endothelial glycocalyx in light and transmission electron microscopy

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The endothelial glycocalyx represents a network of membrane-bound proteoglycans and glycoproteins, covering the endothelium luminally, with many functions in vascular physiology and pathology. This dynamic and fragile structure is usually disturbed during routine histological processing and in conventional transmission electron microscopy, its components are indistinguishable from the environment. Thus, more sophisticated methods have been developed to visualize the endothelial glycocalyx. The aim of this work was to implement methods of imaging the endothelial glycocalyx in situ and in vitro. Lectin histochemistry was applied to visualise the endothelial glycocalyx in situ. Wheat Germ Agglutinin and Lycopersicon Esculentum lectins conjugated to fluorochromes were used to image the glycocalyx of the vascular endothelial cells of the human kidney and the porcine liver and mesentery in light microscopy. The method based on the bond of negatively charged glycocalyx components to positively charged cationized ferritin was used to visualise the endothelial glycocalyx in transmission electron microscopy. The cationized ferritin particles determined presence of the glycocalyx on the surface of the human umbilical vein endothelial cells (HUVEC cell line) cultured in vitro, and moreover, on the surface of the human erythrocytes from the peripheral blood. These imaging methods will be used in studies investigating the impact of infusion therapy, general anaesthesia and cardiopulmonary resuscitation on the endothelial glycocalyx.

Acknowledgement: This work was supported by the Cooperatio Program, research areas DIAG, from the Charles University.

Type of presentation: Poster

PO-P-3162 Bacteriophage φ8 protein P4 - an RNA binding molecular motor.

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The hexameric protein P4 plays multiple roles in bacteriophages of the Cystoviridae family. It functions both as a channel and a translocating motor with RNA helicase activity, while also having ATPase activity and playing a critical role in packaging genomic precursors and regulating their replication. While the structure of P4 has been solved to a resolution of 2.79 Å using X-ray crystallography, the details of its RNA binding mechanism remain elusive due to the lack of P4-RNA complex structures.

To address this issue, we utilized cryo-electron microscopy (cryo-EM) to obtain high-resolution maps of P4. Our samples were prepared by plunge freezing on glow-discharged holey carbon grids and analyzed using a JEOL 2100F transmission electron microscope equipped with a Gatan K2 Summit direct electron detector. For higher resolution mapping, we used a ThermoFisher Krios TEM equipped with a K3 DED and a Bioquantum K3 imaging filter at CEITEC, Brno. Cryo-EM data were processed using cryoSPARC or RELION, and we obtained electron density maps of at least two conformers. Based on these maps, we are currently building structural models of the P4-RNA complex using both manual and automated modelling tools, as well as molecular dynamics flexible fitting.

In summary, cryo-EM has provided a valuable tool for understanding the structure and function of P4, and our work has the potential to shed light on the mechanism by which P4 interacts with RNA.

Type of presentation: Poster

PO-P-3201 Morphological characterization of the gram-negative bacteria of the genus Aeromonas by scanning electron microscopy

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Aeromonas sp., free-living Gram-negative bacteria, are ubiquitous in water ecosystems. They can colonize aquatic organisms asymptomatically or cause mass mortality in farmed fish. Bacteria are emerging human pathogens, causing gastrointestinal, systemic, and wound infections in both immunocompetent and immunocompromised individuals. These rod-shaped bacteria produce numerous virulence factors allowing them to survive in the environment, evade the host immune response, and cause disease, including flagella and type IV pili, which facilitate motility, attachment to host cells, and biofilm formation. To our knowledge, no systematic study has investigated *Aeromonas* planktonic or sessile cells.
We focused on *A. veronii* strain Hm21, a symbiotic isolate from the medicinal leech *Hirudo verbena*, and *A. schubertii* strain 43700, a human isolate from a forehead abscess. We performed an in-depth analysis that elucidated how *Aeromonas* behaves at all stages of its growth in M9 minimal and tryptic soy broth (TSB) rich media, with or without orbital shaking, for 48 h. Cultures taken at specific times during their growth, fixed in aldehyde solution, were processed for high-resolution scanning electron microscopy.
We observed several structures facilitating cell-to-cell attachments: unique lateral single-point connections between cells and complex intercellular networks composed of filaments of different thicknesses. The presence of flagella was largely independent of growth conditions and growth phase, with cells appearing consistently monotrichous in the TSB medium and, in some cases, peritrichous in the M9 medium. Cultures from minimal medium contained more varied structures, including the outer membrane vesicles. Filamental growth, typical for bacteria starvation, was also detected. Overall, bacterial populations remained visibly heterogeneous throughout the cultivation period. Aside from cell shape, we didn't notice any significant differences between the two analyzed strains.

Acknowledgement: The authors gratefully acknowledge the access to the electron microscopy facility of IMIC, supported by the Czech Academy of Sciences (RVO CZ61388971).

Type of presentation: Poster

PO-P-3211 Low Voltage Electron Microscopy as Uranyless Imaging Technique Employed in the Study of Cyanobacterial Cells

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Uranyl acetate as a contrasting solution for biological samples plays a crucial role in transmission electron microscopy since the 1960s. However, because of its mild radioactive properties and high toxicity, the legal restrictions for its usage and waste disposal are rising. Several studies, therefore, searched for a suitable replacement for the conventional staining agent focusing on lanthanoid compounds as well as more exotic substances such as oolong tea extracts. [1,2] A different approach, how to image biological specimens composed of light elements, is to change the imaging technique itself.

Low voltage transmission electron microscopy (VL-TEM) was proven to enable imaging of carbon-based samples without the necessity of staining them using salts of heavy metals as it was possible to even distinguish regions of samples with different chemical compositions of polymers at the voltage of 5kV. [3] Unfortunately, using such low voltage requires the sample to be even thinner than conventional ultrathin sections, making the sample preparation procedure rather challenging. However, if the voltage is increased to 25kV, primary electrons have sufficient energy to penetrate common sections of 70nm thickness. [4]

In our work, we have focused on the usage of LV-EM for the study of cyanobacterial cells. Samples were imaged using both low-voltage TEM (25kV) as well as STEM (15kV) to explore the possibility of reducing the effect of chromatic aberration and the results were compared with the standard procedure of heavy metal staining followed by high-voltage TEM (200kV). [5]

References

[1] Moscardini, A. et al.: Scientific Reports, 2020, 10, 11540.
[2] He, X. et al.: Journal of Microscopy, 2017, 267, 27–33.
[3] Drummy, L.F. et al.: Ultramicroscopy, 2004, 99, 247–256.
[4] Möller, L. et al.: Journal of Histochemistry and Cytochemistry, 2020, 68, 389–402
[5] Mrazova, K. et al.: Microorganisms, 2023, 11, 888.

Acknowledgement: The authors acknowledge funding from the GACR project GF19-29651L and support from CF CryoEM of CIISB project by MEYS CR (LM2018127). We acknowledge the core facility CF Electron microscopy and Raman spectroscopy (ISI) supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

Type of presentation: Poster

PO-P-3146 BONE BREAKAGE EXPERIMENTS UNDER MICROSCOPE

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Bone fragmentation represents direct evidence of deliberate human activity connected to the exploitation of animal resources in the Upper Paleolithic Specific diagnostic traces enable us to distinguish between different fragmentation agents of the biotic and abiotic origin However, they are oriented mainly on macroscopic features such as angle, outline or surface of fracture. Since fractures arise at a micro level and the final differential characteristics on the macroscale result from various mechanic forces spreading in bone structure, we used the scanning electron microscopy (SEM) to observe irregularities in bone fracture surface on a microscale. Transmitted light microscopy was also used to verify presence and characteristics of micro cracking. Both selected methods were applied on experimental material with known fragmentation history, using either fresh, frozen or dried long limb bones of cattle and of red and fallow deer. These were subsequently subjected to rockfall experiments or to intentional fragmentation by human with unmodified hammer stone on an anvil. Microscopic microstructural differences of these samples are discussed, as is the potential application in archeology.

Acknowledgement: Author Z.P. wishes to thank the program Strategie AV21 for support.

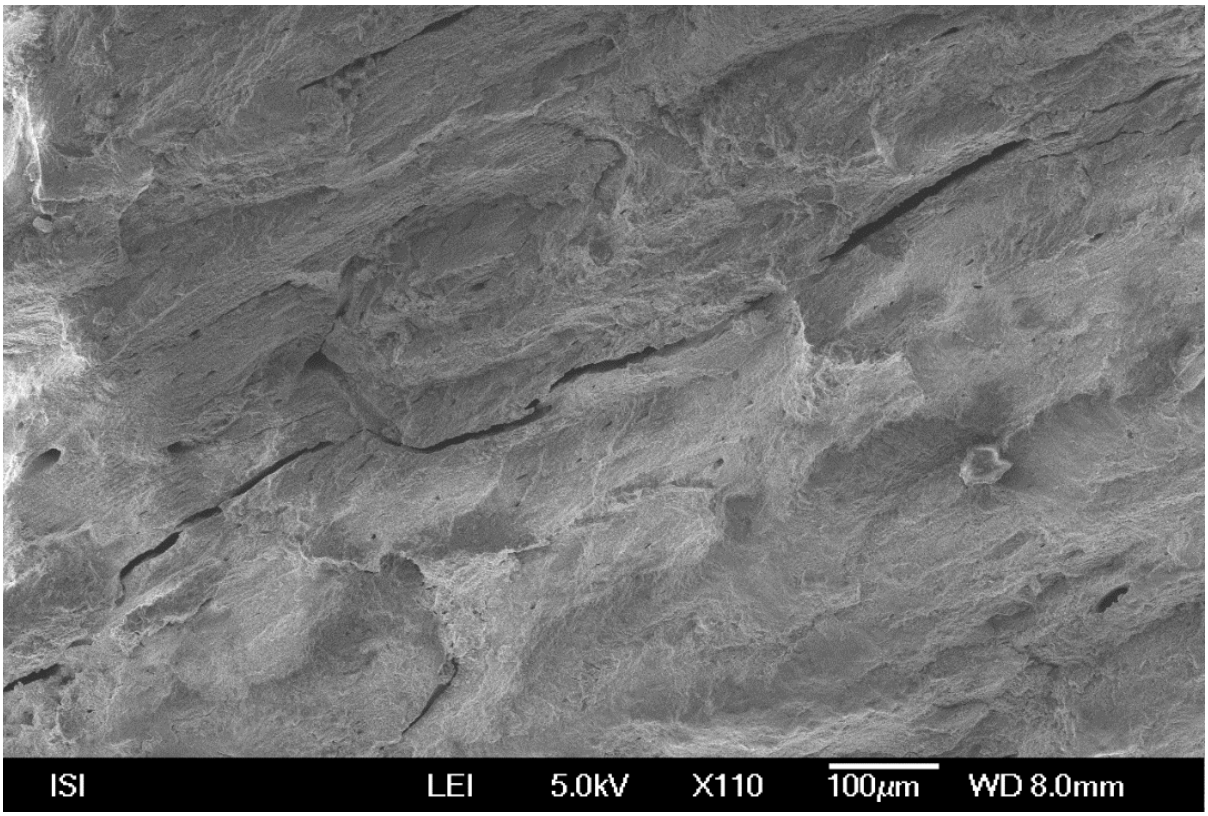

Fig. 1: Micro-crack on a fresh bone, following the lamellar arrangement.

Type of presentation: Poster

PO-P-3138 The effect of low concentrations of dimethylsulfoxide on the morphology of keratinocytes during their short-term cryopreservation observed by the scanning electron microscope

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For their successful cryopreservation of cells, choosing a cryoprotectant in an appropriate concentration is important. Dimethyl sulfoxide (DMSO) is used as a cryoprotectant at a standard concentration of 10% in the freezing medium, but it shows some toxicity to cells. Previous studies have shown that DMSO protects cells during cryopreservation even at lower concentrations (2.2%), but the effectiveness depends on the cell type and the protocol used. In the study, we monitored the effect of low concentrations of DMSO on the morphology of human keratinocytes after thawing using scanning electron microscopy (SEM). The morphological characteristics of the cells were evaluated in terms of cell adhesion, ability to form a monolayer, and changes in morphology. Cells were observed by SEM at magnifications of 63×, 500×, 1000×, 2000×, and 5000× (Fig. 1). Control cells were interconnected and had two distinct regions - the cell body and the lamella. At magnification (5000×), the structure of the connection by intercellular fibers was revealed. A monolayer formed by keratinocytes frozen in 1.8% DMSO showed signs of damage, it was formed from cells without projections. The layer of cells frozen in 2.2% DMSO was without obvious damage, the cells formed projections. At magnification (5000×), we observed small spherical vesicles (~200-800 nm) on the surface of cells frozen in 1.8% and 2.2% DMSO (Fig. 2). A layer of cells frozen in 5% DMSO had a surface with visible protrusions. The layer of cells frozen in 10% DMSO was intact and very smooth. Due to the thin structure, the centrally located cores were visible. Control cells were ~ 9 μm in size, cells frozen with DMSO concentrations (1.8% and 2.2%) were smaller (~ 20-30 μm), and cells frozen with DMSO concentration (10%) were larger (~62 μm) (Fig. 3). Analysis of cellular ultrastructure showed that the concentration of DMSO during cryopreservation can be reduced (≤ 2.2% v/v) in short-term freezing of keratinocytes. However, a further decrease in DMSO concentration leads to a limited ability to form a monolayer after thawing.

Acknowledgement: This research was funded by the Slovak Research and Development Agency (APVV) (SK-BY-RD-19-0019 and SK-PL-21-0073).

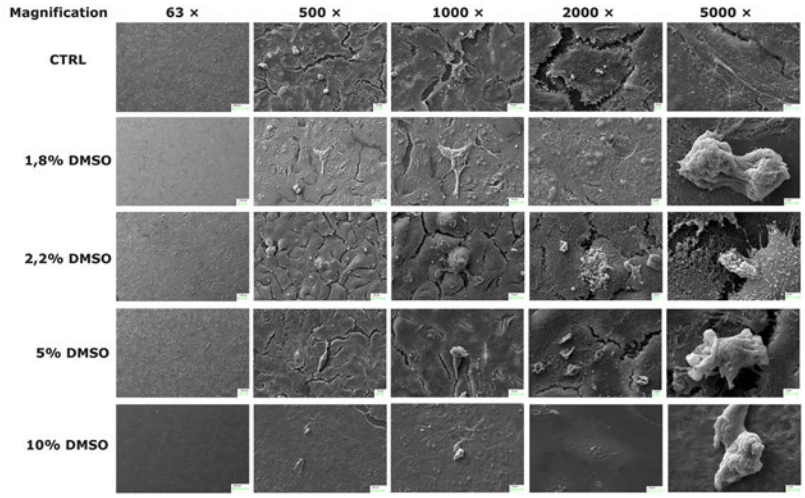

Fig. 1: Effect of different low concentrations of DMSO on the morphology of the cell structure of human keratinocytes observed by SEM. The scale on the SEM microcraphs at magnification 63× is 100 µm, at 500×, 1000×, 2000× and 5000× is 2 µm.

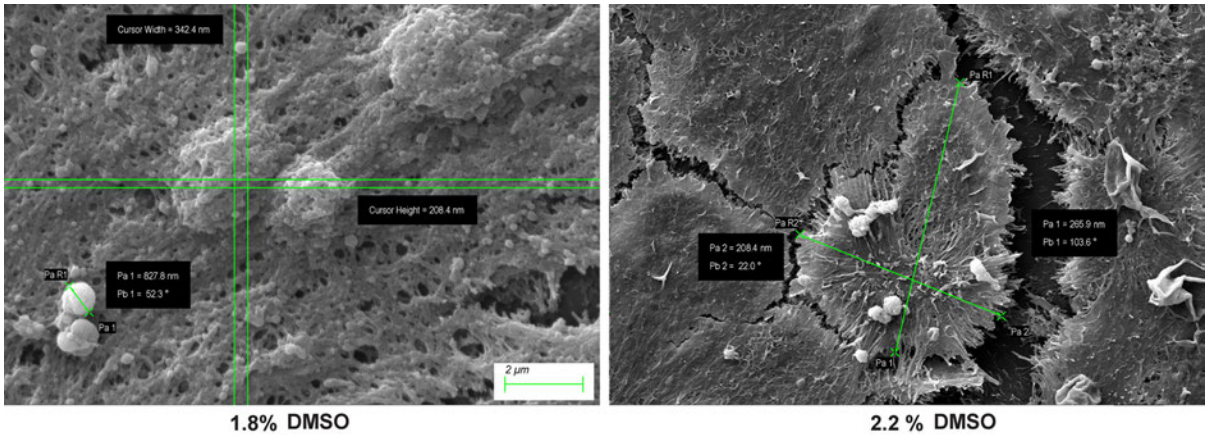

Fig. 2: Small spherical vesicles ~200-800 nm in size on the surface of cells frozen in 1.8% and 2.2% Me2SO were observed at high magnification (5000×). The scale on the SEM microcraphs is 2 µm.

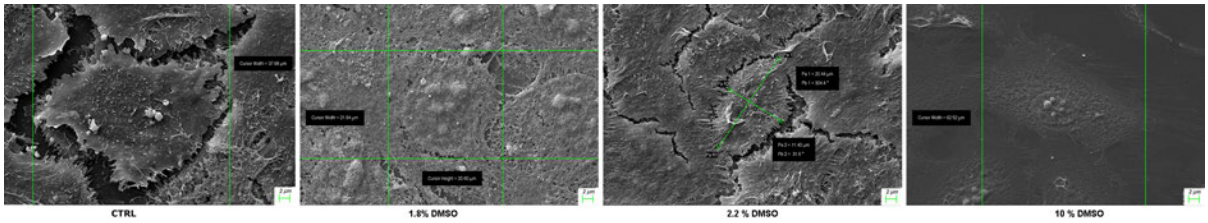

Fig. 3: Size of a control cell (~ 9 µm) compared to the size of a cell frozen with a higher concentration of DMSO (10%; size of cell ~ 62 µm) and cells frozen with lower concentrations of DMSO (1.8% and 2.2%; size of cells ~ 20-30 µm). Cell size was measured by SEM. Scale bar on micrographs is 2 µm.

Type of presentation: Poster

PO-P-3135 Minimal resin embedding of SBF-SEM samples reduces charging and facilitates finding the region of interest

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On the examples of small arthropods and fish roe, we describe a workflow to prepare samples for SBF-SEM using the minimal resin (MR) embedding method.
We show that for imaging of surface structures, this simple approach tackles conveniently at once both two major problems of SBF-SEM samples, normally embedded in an excess of overlying resin. As the surface ROI is not masked by the resin samples can be precisely trimmed before they are placed into the imaging chamber. The initial approaching step is fast and easy. No extra trimming inside the microscope is necessary. Importantly, charging is absent or greatly reduced meaning that imaging can be undertaken under good vacuum conditions, typically at the optimal high vacuum. This leads to better resolution, better signal to noise ratio, and faster image acquisition.
In MR embedded samples charging is minimized and ROI is easily targeted. MR embedding does not require any special equipment or skills. It saves effort, microscope time and eventually leads to good quality data. Studies on surface-linked ROIs, or any samples normally surrounded by the excess of a resin, would benefit from adopting the technique.

Acknowledgement: CZ.02.1.01/0.0/0.0/16_013/0001775
LM2023050
TN02000020

Type of presentation: Poster

PO-P-3155 Further development and optimization of the novel embedding resins for Volume EM with higher resistance to e-beam damage.

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Electron microscopy is a widely used technique for characterization of biological samples. A common step in EM preparation of bio-samples consists in protecting their structure by embedding them in synthetic polymer matrices (embedding resins). Commonly used embedding resins are optimized for TEM, which detects high-energy transmitted electrons.
Recently introduced and increasingly popular volume EM visualization techniques employ lower energies, backscattered electrons, and higher electron doses. The traditional embedding resins used for TEM suffer from charging and e-beam damage in volume EM. Therefore, we have developed two new types of polymer resins with expected higher resistance to e-beam damage: (i) the commercial resins with different types of chemical stabilizing agents marked temporarily as A, B and C (novel resins type I) and (ii) the resins based on more e-beam resistant polymer (novel resins type II).
In this contribution, we compare mouse brain tissue embedded in commercial epoxy resin (HARD PLUS resin 812), resin with chemical stabilizing agents (resins type I), and several types of novel resins with different chemical composition (resins type II). All prepared resins were characterized in detail and compared from the point of view of their homogeneity, cuttability, mechanical properties, contrast in SEM, and stability under electron beam (charging at specific electron doses). Prepared samples were tested on three different SEM microscopes in order to assess the resin properties reliably. The novel resins type I exhibited lower charging and almost identical properties like non-modified commercial resins. The novel resins type II exhibited lower charging, overall quality of the micrographs was very good, and contrast was higher in comparison with commercial resins. Nevertheless, their mechanical properties, adhesion to biological specimens, cuttability and volume contraction upon cure are still being optimized.

Acknowledgement: Financial support through grant TN02000020 (TA CR) is gratefully acknowledged.

Type of presentation: Poster

PO-P-3184 IMG Electron Microscopy Core Facility

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The Electron Microscopy Core Facility provides expertise and cutting-edge equipment for a broad range of biological sample preparation and ultrastructural imaging techniques. The core facility deals with various biological samples: human and animal cell cultures, plant and animal tissues, worms, microorganisms, lipid micelles, isolated DNA, or purified proteins. The sample preparation techniques include both standard and advanced techniques, such as routine chemical fixation and resin embedding, negative staining, cryofixation using plunge-freezing or high-pressure freezing, freeze-substitution, cryosectioning, freeze fracture replica labeling, pre- and post-embedding immunolabeling, the list being lately extended by cryoCLEM using specialized microscope.
Transmission electron microscopes (TEM) installed at the core facility include a standard 120 kV instrument for routine observation and an advanced 200 kV S/TEM providing the possibility of high-resolution TEM, 3D analysis by TEM- or STEM tomography, cryo-electron microscopy and STEM-EDS elemental analysis. High-pressure freezer with light stimulation module, two automatic freeze-substitution machines, cryo-ultramicrotomes, automated plunge-freezer, freeze-fracture replica making device, cryoCLEM microscope, as well as additional wet lab equipment are available.
The team has a long expertise in the development and optimization of sample preparation techniques, including fruitful collaborations with companies providing equipment for electron microscopy. The Electron Microscopy Core Facility is part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node. We provide open access to our technologies and expertise and are ready to welcome users from all fields.

Acknowledgement: MEYS CR (LM2018129, LM2023050), ERDF (No. CZ.02.1.01/0.0/0.0/18_046/0016045), TACR (TN02000122), IMG grant (RVO: 68378050)

Type of presentation: Poster

PO-P-3185 Solutions for preparation and visualization of vitrified biological samples at IMG Electron Microscopy Core Facility

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Continual advancement in a broad range of interconnected cryo-electron microscopy pipelines has made the preservation and subsequent visualization of sensitive biological samples in close-to-native state more reliable and convenient than ever before. Cryo-electron microscopy has therefore become the new standard for dependable analysis of biological objects. Our facility has been continually updating the cryo-workflows to stay up-to-date with these advancements and to provide professional solutions for current scientific demands.
A 200 kV Jeol JEM-F200 TEM, cold FEG, sensitive 4k CMOS camera TVIPS XF-416, and volta phase plate provides optimal configuration for a broad range of cryoTEM applications including observation of morphology of small objects sensitive to dehydration, such as e.g. small organisms or DNA origami, quality check of purified protein samples prior to SPA analysis, collection of diffraction patterns of frozen protein crystals, and cryo-electron tomography of subcellular structures. We are also equipped with state of the art cryo FIB-SEM Tescan Amber Cryo for cryo FIB lamella preparation with integrated nanomanipulator for lamella lift-out and Leica THUNDER cryo-CLEM for navigation with light and fluorescent microscopy.
Several other skill-demanding workflows utilizing sample vitrification, such as freeze-fracture replica immunolabeling using multi-purpose sputter coater Leica EM ACE900, cryo-CLEM imaging with reliable correlation of images from both modalities, or cryo-sectioning followed by immunolabeling after Tokuyasu are established at the core facility. Samples vitrified by high-pressure freezing in Leica EM ICE HPF machine with light-stimulation module can be alternatively processed to resin blocks upon freeze-substitution in Leica EM AFS2.
The EM CF, being a part of large imaging infrastructures Czech-BioImaging and Euro-BioImaging, provides open access to all described technologies with professional support on all steps of your project.

Acknowledgement: Acknowledgements: MEYS CR (LM2018129, LM2023050), ERDF (No. CZ.02.1.01/0.0/0.0/18_046/0016045), TACR (TN02000122), IMG grant (RVO: 68378050)

Type of presentation: Poster

PO-P-3147 Characterization of the microgel particles by the light microscopy and cryo-SEM

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Polymer hydrogels and/or microgels can be studied by several microscopic techniques. Light-microscopy (LM) visualizes samples in their natural state – submerged in water, but suffers from lower resolution. Cryo-TEM microscopy exhibits the best resolution, but the method is not suitable for particle with sizes >300 nm. Cryo-SEM offers sufficient resolution and, at the same time, it allows observing even large particles.

In this contribution, we summarize LM and Cryo-SEM study of synthetic microgel particles with potential medical applications. Polymer precursor for the microgel preparation was Poly[N^5-(2-hydroxyethyl)-L-glutamine-ran-N^5-propargyl-L-glutamine-ran-N^5-(6-aminohexyl)-L-glutamine]-ran-N^5-[2-(4-hydroxyphenyl)ethyl)-L-glutamine] (PHEG-Tyr). The PHEG-Tyr microgel particles were prepared by the Horseradish peroxidase/H_2O_2-mediated crosslinking in an inverse suspension method.

The PHEG-Tyr microgels were first observed by LM microscopy (Fig. 1). LM displayed microgel particles with average size 20 μm. Nevertheless, internal morphology and possible surface details were beyond LM resolution. The cryo-SEM technique was used to visualize possible internal morphology of frozen microgel particles. Several method of sample preparations were used such as fracture of flash-frozen microparticle suspension in a capillary, fracture of a free-standing drop, and thin layer sublimation. It was proved that even freezing at low temperatures in a large layer of water can cause artifacts as demonstrates Fig. 2A. The wrong condition of sublimation during the sample preparation connected with the sample preparation also affected the final morphology as demonstrate Fig. 2B. The proper way of the sample preparation (in our case the sublimation from the very thin solution layer) led to the visualization of the smooth surface morphology without artifacts.

Acknowledgement: Technology Agency of the Czech Republic Project (TACR; project TN02000020) is gratefully acknowledged.

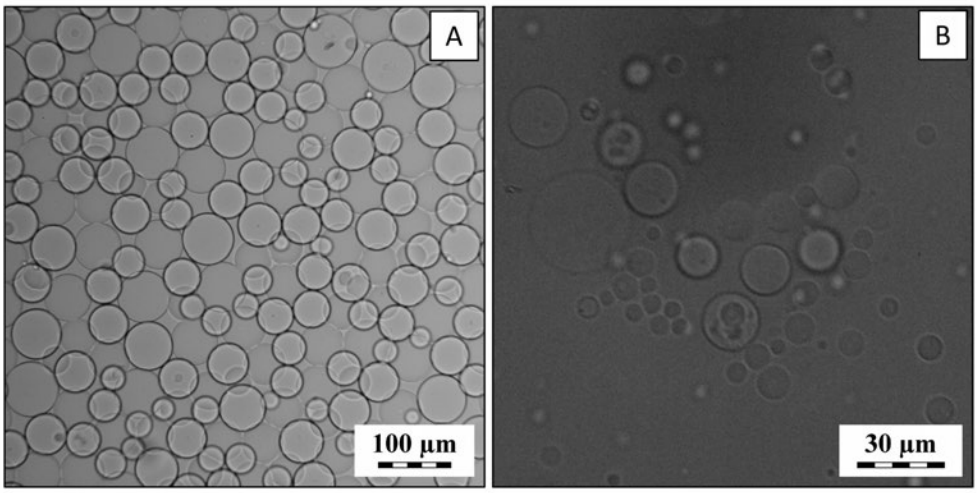

Fig. 1: Light micrographs showing the PHEG-Tyr microgel particles in different magnification.

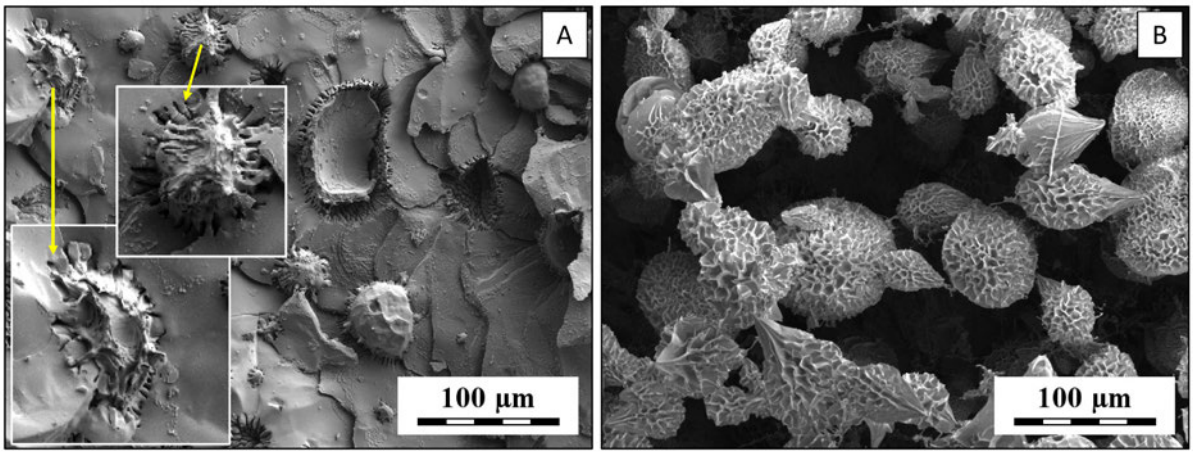

Fig. 2: SEM micrographs of the artefacts resulting from improper sample preparation (A) and inappropriate sublimation conditions (B).

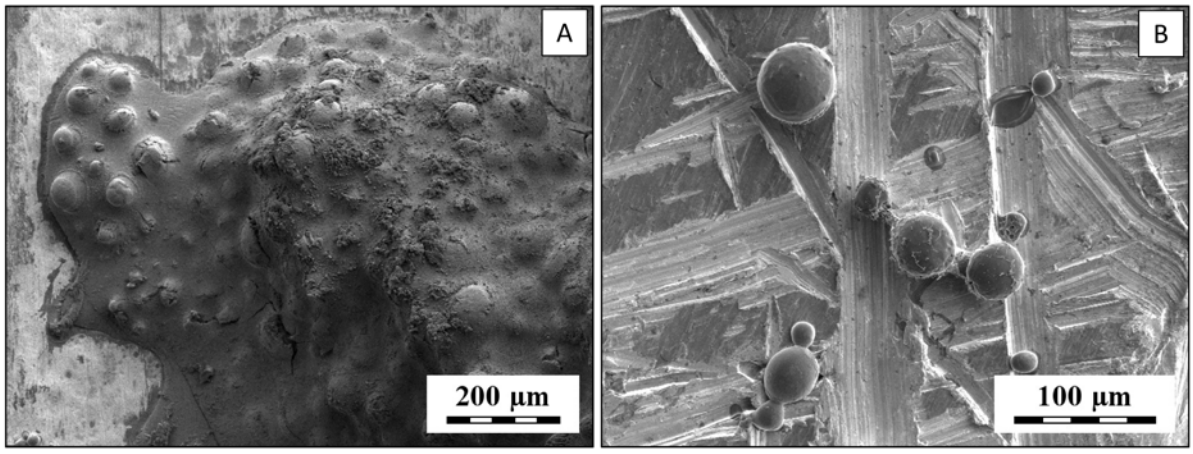

Fig. 3: SEM micrographs of the PHEG-Tyr microgel particles in a layer of the ice (A) and after complete sublimation of ice (B).

Type of presentation: Poster

PO-P-3141 Metal-insulator transition in vanadium dioxide nanoparticles studied by in-situ transmission electron microscopy

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Vanadium dioxide is a reversible phase-changing material with a low transition temperature (around 67°C) with a dielectric low-temperature and metallic high-temperature phase. Due to the proximity of its transition temperature to the room temperature, VO2 is a promising candidate for applications in photonics including fast optical switching or tunable optical metasurfaces [1]. However, the nature of the metal-insulator transition in VO2 is not yet fully understood. In this contribution, we present a comprehensive study of VO2 using analytical transmission electron microscopy combined with in-situ heating. A combination of imaging and spectroscopy with nanometer spatial resolution allows us to locally correlate the optical properties of VO2 with applied temperature. In the presentation, I will present slides from a video showing that we can visualize the insulating-to-metallic and back metallic-to-insulating switching by contrast changes in annular dark-field scanning electron micrographs (STEM-ADF) during an in-situ heating experiment. Selected slides from this video are shown in Fig. 1a. Fig. 1b then presents a single-particle analysis by temperature-dependent low-loss electron energy loss spectroscopy. The spectra in blueish colors represent the dielectric low-temperature phase while the spectra in reddish colors prove the metallic high-temperature phase in which the plasmon peak around 1.6 eV arises.

[1] ACS Photonics **8** (2021), p. 1048

Acknowledgement: Supported by Czech Science Foundation (22-04859S) and MEYS CR (CzechNanoLab, LM2018110).

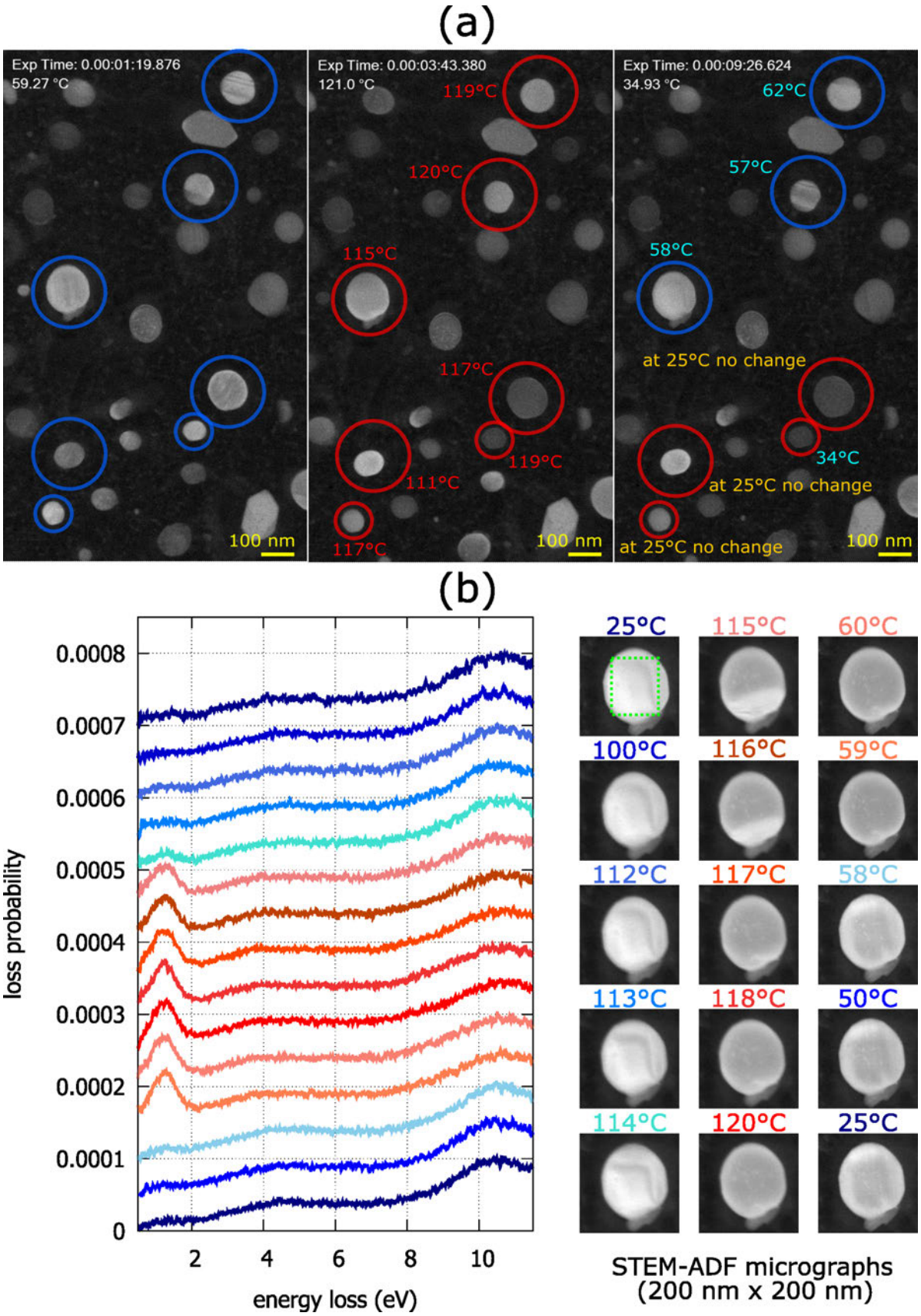

Fig. 1: Insulator to metallic switching of vanadium dioxide nanoparticles on SiN_x heating chip studied by analytical transmission electron microscopy: (a) Selected slides from in-situ STEM-ADF, (b) temperature series of low-loss EELS and STEM-ADF of one VO_2 nanoparticle.

Type of presentation: Poster

PO-P-3206 Metal nanoparticles prepared in-batch and on-chip: comparative electron microscopy study

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Current research supported by analyzes of nanoparticle morphology demonstrates the importance of microfluidic syntheses in the preparation of nanoparticles from precursors by the wet chemical route. Microfluidic reactors can be designed according to the needs of the synthesis, scaled-up and are increasingly used due to the advantages that flow technology offers compared to the classic batch process. The most significant advantages are optimized reaction kinetics, economy of reactants and higher safety and better process control.
In the case of nanosilver, which is very attractive from the point of application view, the use of microfluidic synthesis makes sense, because nanoparticles in a colloidal environment meet the requirements for the feasibility of on-chip reaction. 3D-printed microfluidic chips with more complex channel geometry enable more pronounced mixing of the metal precursor with the reducing agent resulting in smaller and more stable colloidal nanoparticles. Moreover, on-chip nanoparticles may often have different morphology compared to the particles prepared in-batch.
Microfluidics is shifting basic research in the green preparation of metal nanoparticles more forward again. Nanoparticles prepared on-chip with optimal size, shape and dispersion have a high application potential, which is the same important, as a well-tuned and environmentally friendly preparation technology.

Acknowledgement: Financial support from the project MEYS SP 2023/026 and grant programme „Support for Science and Research in the Moravia-Silesia Region 2022" (RRC/12/2022).

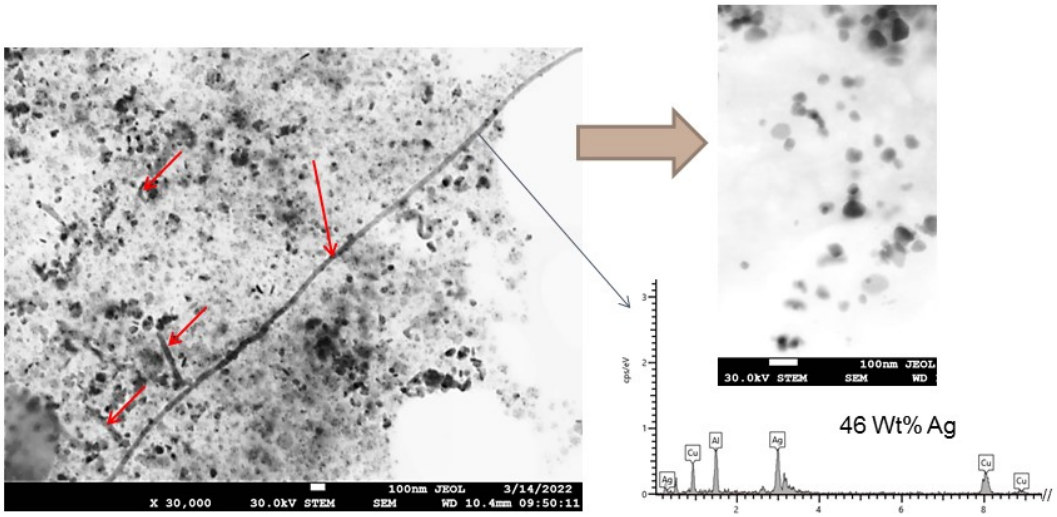

Fig. 1: On-chip biosynthesis of silver nanowires promoted by phyto-chemicals in high concentrated silver precursor.

Type of presentation: Poster

PO-P-3161 Phase transformations during formation of elemental 2D materials monitored with low energy electron microscope

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Besides graphene, the family of elemental 2D materials comprises other attractive members - silicene, germanene, borophene etc. These materials exhibit exceptional electronic and optoelectronic properties as well as graphene, however, their preparation is still in its infancy. We will first report our results on preparation of silicene mono- and multilayers on silver, and follow-up with description of phosphorene formation on copper.

Unlike many other 2D materials, a monolayer of black phosphorus is strongly buckled and, thus, is not flat as e.g., graphene. 2D materials are preferably grown on non-interacting substrates, as the interaction with the substrate may potentially result in spoiling of the desired properties and, potentially, prevent exfoliation. From this reason, the growth of (blue)phosphorene has been so far intensively studied on substrates predicted as non-interacting (hBN) or moderately-interacting (Au and Ag). It has been predicted by DFT simulation of phosphorene on different substrates that the weak (or non-existent) interaction leads to phosphorene stabilization, while the strong interaction results in breaking the phosphorene layer into separate nanodomains/clusters of phosphorus or metal phosphides.

On contrary, here we show that 2D phosphorus can be stabilized even on highly interacting substrate (Cu(111)) via series of unexpected phase transformations. Our data show that the copper phosphide phase is indeed formed, but it serves as a buffer layer for phosphorene formation. The related phase transformations are very rapid and point to the existence of a large “sea” of adatoms on the surface during deposition. We have been able to observe and monitor these transformations by concerted use of in-situ techniques, namely low energy electron microscopy (LEEM) and scanning tunnelling microscopy (STM). Our experimental results thus support recent theoretical predictions of these phase transformations playing significant role in formation of elemental buckled 2D materials.

Type of presentation: Poster

PO-P-3164 Maximizing Cathodoluminescence Efficiency with Graphene Coatings: A Breakthrough in Low-Energy Imaging

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Low-energy electron imaging has become increasingly popular due to its higher surface resolution and lower sample damage depth compared to higher-energy imaging. However, detecting backscattered electrons (BSE) can be problematic as they cause lower cathodoluminescence (CL) response of the detector due to their lower energy [1]. Therefore, finding the ideal combination of coating and scintillator for maximum CL efficiency is necessary.
In this study, samples of CRY018™ were provided by CRYTUR, Ltd., and were coated with Al, Indium Tin Oxide (ITO), and graphene. The details of the graphene preparation method and the transfer of graphene to the substrate have been described in detail elsewhere [2]. The graphene was transferred onto a 5.7 mm diameter and 0.5 mm thick CRY018™ sample covering the whole sample.
Raman spectroscopy confirmed (Fig. 1) that the graphene coating had a 2D peak (2660 cm^{-1}) at least 2× larger than the G peak (1583 cm^{-1}), indicating a single carbon layer.
The CL efficiency of the scintillators was measured with a specialized apparatus [3]. It was found that the CRY018™ sample with a 50 nm Al coating had the highest CL efficiency for primary energy ≥ 5 keV (Fig. 2). However, BSE slower than 1.9 keV could not pass through this coating. Graphene was found to be the best coating for slow BSE and could detect BSE down to 400 eV. Although large-scale deposition of graphene on scintillators is not yet possible in large quantities, it is a promising method for effectively dissipating charge from the scintillator surface while maintaining maximum CL efficiency.

[1] Yacobi B. G., *Cathodoluminescence microscopy of inorganic solids*, New York, Plenum Press (1990).
[2] Průcha L., et al., *Mater. Today Commun.* **35**, 105921 (2023).
[3] Bok J., Schauer P., *Meas. Sci. Technol.* **25**, 7 (2014).

Acknowledgement: The research was supported by TACR (TN02000020) and MEYS of CR. We thank the company CRYTUR™ for supplying with the samples of bulk single-crystals.

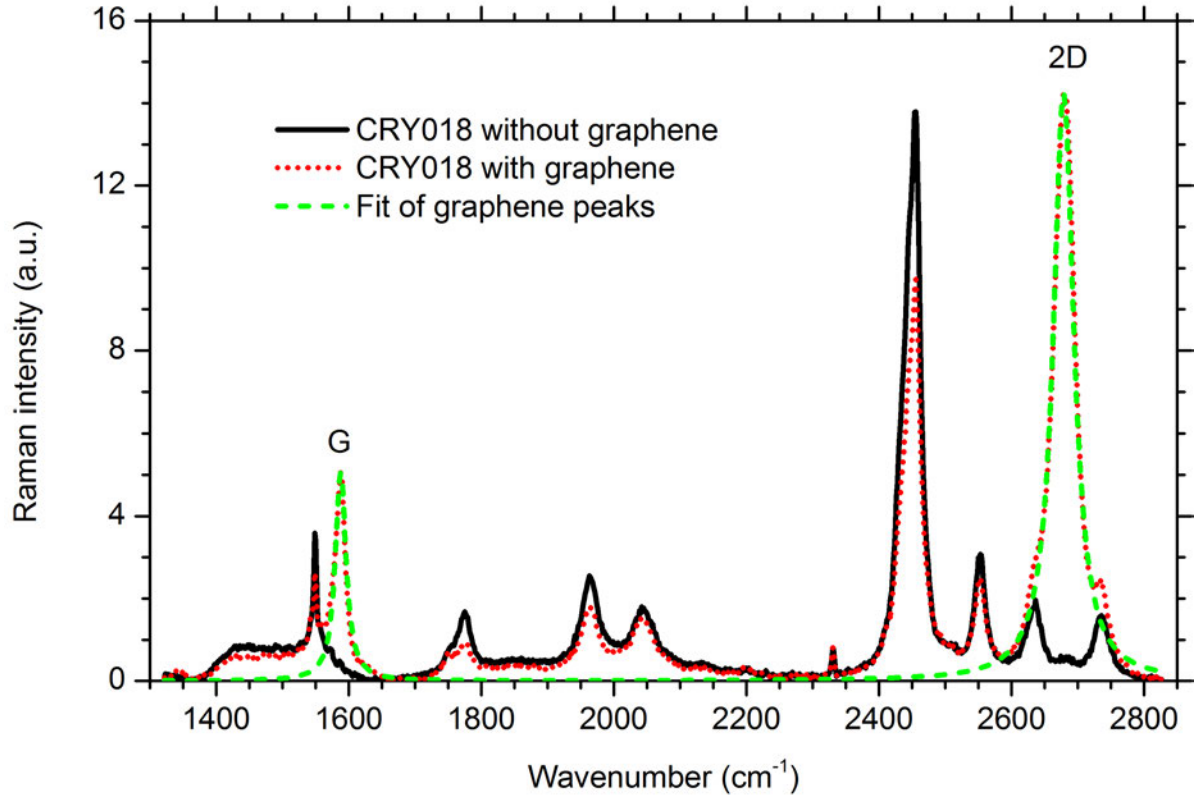

Fig. 1: Raman spectra of CRY018™ before and after graphene deposition. Significant peaks to demonstrate the existence of graphene (G and 2D) are highlighted by fit.

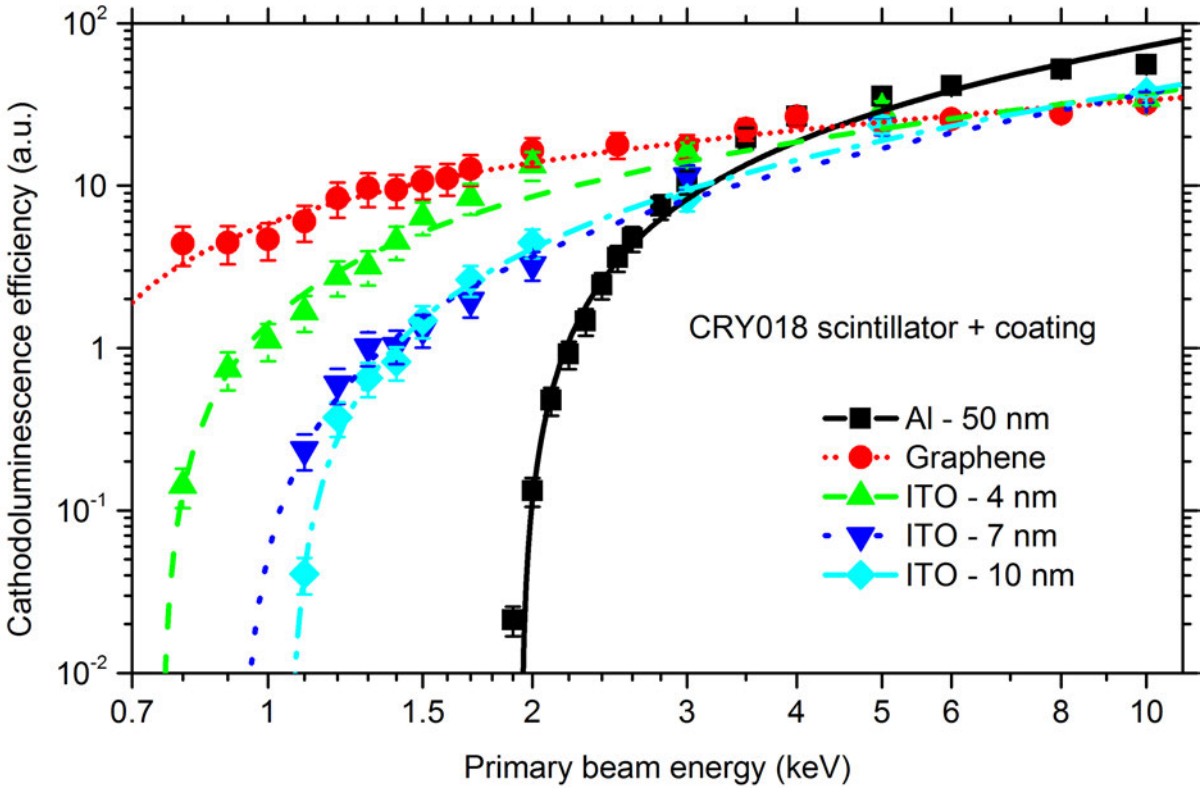

Fig. 2: CL efficiency of CRY018™ scintillation material coated by various coatings.

Type of presentation: Poster

PO-P-3200 Absorption of thermal radiation by contaminated metallic surfaces at cryogenic temperatures

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Cryo-electron microscopes (cryo-EM) operate at low temperatures determined often by the boiling temperatures of liquid nitrogen (77 K) or liquid helium (4.2 K). Low thermal absorptivity values of metallic surfaces of cold cryo-EM components, such as sample holders or thermal and anti-contamination shields, reduce the heat load on their cryogenic cooling systems. Metallic materials with a clean surface and high electrical conductivity (copper, aluminium, etc.) typically exhibit a very low thermal absorptivity. However, contamination from the vacuum system of the microscope can significantly increase the thermal absorptivity and thus the heat load on the cooling systems. Another negative effect can be, for example, an increase in temperature gradients around the sample. In our laboratory, we use a dedicated apparatus (Fig. 1a), designed to determine the total hemispherical absorptivities (or emissivities) of various materials, tested in the form of circular discs 40 mm in diameter and 1–5 mm thick (Fig. 1b). These thermal properties can be measured in dependence on the temperature of thermal radiation starting at 20 K and ending at 320 K [1]. Here, we focused on the effect of the aluminium disc contamination by condensed water vapour [2], vacuum grease [3], and several other agents that can typically occur in vacuum parts of cryogenic systems. As a special case, we investigated the effect of contamination by the mineral dust [3]. In all these measurements, the absorptivities of an original clean surface of aluminium samples were significantly increased. The highest absorptivity increase, more than ten times, was observed in the case of the silicon grease for high vacuum (approx. 0.234 mg/cm2; thickness of 2.3 μm) (Fig. 1c). Our findings can be valuable for improving the thermal design of cryo-EM and their cryogenic cooling systems.

References

[1] doi.org/10.1088/0026-1394/53/2/743
[2] doi.org/10.1016/j.cryogenics.2010.01.007
[3] doi: 10.18462/iir.cryo.2023.142

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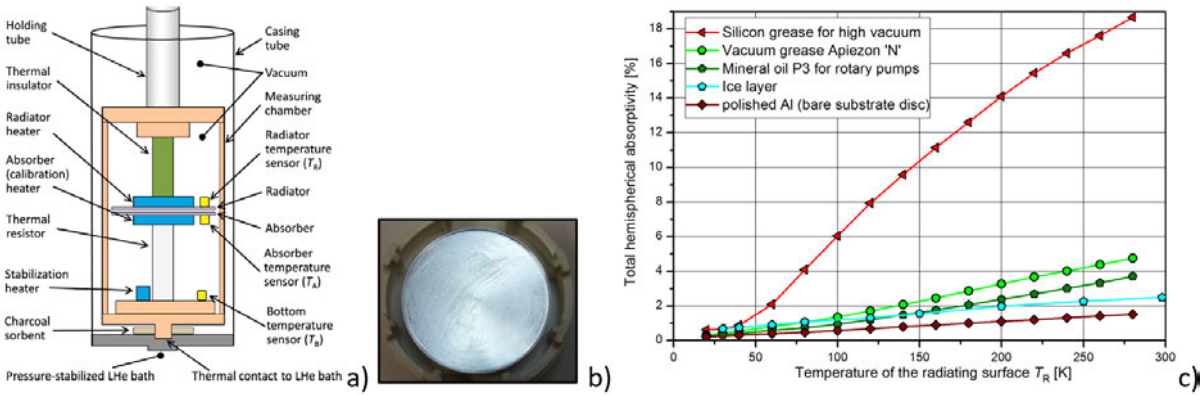

Fig. 1: a) Apparatus scheme [1], b) Aluminium surface contaminated by vacuum grease [3], c) Total hemispherical absorptivity of the contaminated surfaces [2, 3].

Type of presentation: Poster

PO-P-3191 A soft touch with electron beams: Digging out structural in-formation of nanomaterials with advanced scanning low energy electron microscopy coupled with deep learning

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Nanostructured materials continue to find applications in various electronic and sensing devices, chromatography, separations, drug delivery, renewable energy, and catalysis. While major advancements on the synthesis and characterization of these materials have already been made, getting information about their structures at sub-nanometer resolution remains challenging. It is also unfortunate to find that many emerging or already available powerful analytical methods take time to be fully adopted for characterization of various nanomaterials. The scanning low energy electron microscopy (SLEEM) is a good example to this. In this report, we show how clearer structural and surface information at nanoscale can be obtained by SLEEM, coupled with deep-learning. The method is demonstrated using Au nanoparticles-loaded mesoporous silica as a model system. Moreover, unlike conventional SEM, SLEEM does not require the samples to be coated with conductive films for analysis; thus, it is both convenient to use and does not give artifacts. The results further reveal that SLEEM and deep-learning can serve as great tools to analyse materials at nanoscale well. The biggest advantage of the presented method is its availability, as most modern SEMs are able to operate at low energies and deep learning methods are already being widely used in many fields.

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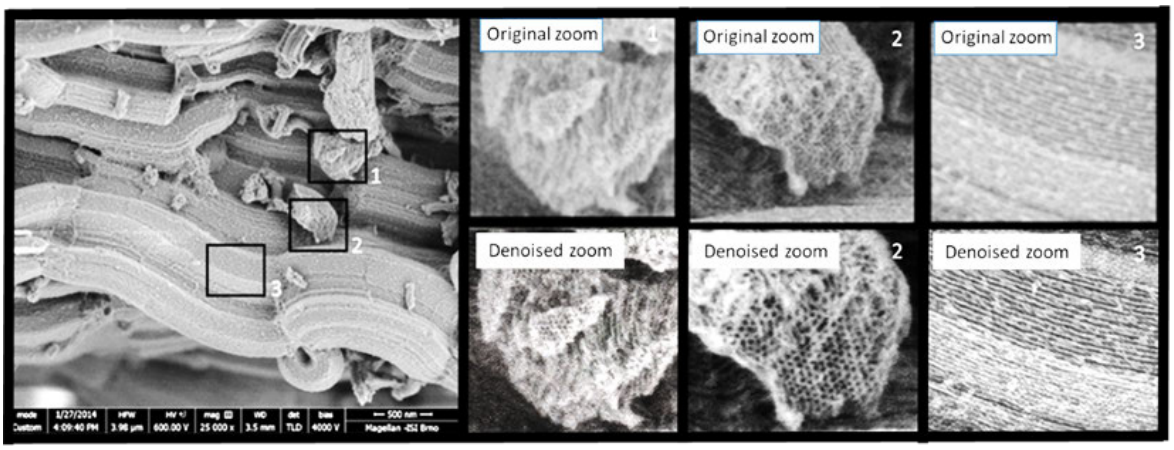

Fig. 1: SLEEM images supported by deep learning, showing selected details before and after “de-noising”, are displayed.

Type of presentation: Poster

PO-P-3159 Low-energy scanning electron microscopy and spectroscopy for the study of advanced 2D materials and thin films

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Scanning low energy electron microscopy and time-of-flight spectroscopy are suitable methods for the study of advanced 2D materials and thin films. In recent years, there has been rapid progress in the technological development of advanced 2D materials. This places high demands on analytical techniques for the study and analysis of these materials. Theoretical and experimental studies of low-energy electron transport near solid surfaces are important for surface-sensitive electron spectroscopy and microscopy. In particular, a reliable knowledge of electron transport in and across the sample interface is necessary to obtain quantitative information.
A typical representative of 2D materials is graphene, whose unique properties such as electrical conductivity and transparency make graphene an ideal candidate for study at very low energies in the transmission mode of the electron microscope. We use commercial samples as well as our CVD-grown graphene, which was produced in our in-house CVD furnace [1].
We have designed and assembled a device for experiments with 2D materials that allows us to obtain electron energy loss spectra (EELS) for low landing energies [2]. To validate our experiments, we simulated the EELS using density functional theory and many-body perturbation theory.
References:
[1] Průcha, L.; et al. In-situ thermal Raman spectroscopy of single-layer graphene on different substrates. Mater. Today Commun. 2023, 35, 105921, doi.org/10.1016/j.mtcomm.2023.105921.
[2] Konvalina, I.; et al. Low-Energy Electron Inelastic Mean Free Path of Graphene Measured by a Time-of-Flight Spectrometer. Nanomaterials 2021, 11, 2435, doi.org/10.3390/nano11092435.

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Type of presentation: Poster

PO-P-3163 Users guide for 4D-STEM and electron ptychography

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4D-STEM allows the electron microscopist to choose a wide variety of imaging techniques in order to get the required information from an investigated sample. The acquisition of a full-frame diffraction pattern per probe position provides high freedom in data processing. As an example, those can be analysed in terms of virtual detectors, mapping em. fields via differential phase contrast (DPC) or centre of mass (CoM), or mapping crystallographic orientation and strains via diffraction pattern identification. Moreover, dose efficient ptychographic reconstructions may take place to get high contrast images visualzing both heavy and light atoms.
Depending on the sample type and composition, sensitivity to radiation damage and required structural information, the ultimate acquisition strategies would differ. The variation can be in terms of beam convergence angle, scan position spacing, angular range read out by the detector, applied dose or beam focus position [1]. Some methods, like virtual DPC or CoM can run with on-the-fly data acquisition providing valuable information about microscope alignments and sample structure, other methods are more suitable for post-acquisition processing (i.e. defocused beam ptychography).
We compare different methods (STEM with virtual detectors, CoM, ptychography: SSB, WDD, PIE) for high-resolution imaging of crystalline samples (with SmB6 and PrBaCo2O5+δ perovskites as examples) and in terms of data processing strategies, required beam settings and microscope calibrations, achieved contrast and processing time. In our case, the real-time processing capability is enabled by a fast hybrid-pixel direct electron detector DECTRIS ARINA, with maximum frame rate of 120 kHz using binning (96x96 effective pixels) [2] and integrated data processing of the LiberTEM platform [3]. With this work, we suggest a set of “recipes” for efficient experimental setup and processing strategies of 4D-STEM datasets.
1. Ophus, C. 2019 Micr. Microan. 25(3), 563-582
2. Zambon P. 2023 Nuclear Inst. Met. Phys. Res. A, 1048
3. Clausen A. 2020 J. Open Source Softw. 5(50)

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Type of presentation: Poster

PO-P-3179 Assembly for sample cooling in cryo-FIB/SEM

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Cryo methods visualize biological samples in a scanning electron microscope in a state closest to the native state. That gives a good opportunity to examine samples in a state in which they are difficult to observe with the help of other methods or their preparation. Samples are kept at cryogenic temperatures, often using liquid nitrogen (LN2, ~ 77 K). The scanning electron microscope (SEM) equipped with a source of high-energy ions (FIB) together with a cryogenic cooling system of samples, forms the so-called Cryo-FIB-SEM. The cryogenic cooling system is typically consisting of a cryogenic sample holder and anticontamination shields thermally connected to the cooled copper finger of an LN2 bath Dewar vessel connected to the microscope chamber. We have designed an assembly for sample cooling that we built-in a classical room temperature FIB-SEM Helios G4 (Thermo Fisher Scientific). By this relatively simple upgrade, we obtained Cryo-FIB-SEM working at cryogenic temperatures from ~ 90 K to 300 K.
The Dewar vessel (double-shell for LN2 with a separated vacuum [1]) is connected to the stage inside the microscope ensuring sufficient cooling during observation. A conductive finger leads the heat from the cryogenic sample holder of the microscope to the bottom part of the vessel. Two anti-contamination shields are attached to the end of the finger (one directly, the second via cooling braids). One shield is located slightly above the bottom of the pole piece; the second shield is attached to the cryo-holder and is placed just above the sample to minimize sample contamination. The sample holder is compatible with the Leica Microsystems system, is thermally isolated from the microscope stage, and is flexibly connected to the conductive finger by braids, which allows the stage to move in all three axes, limited rotation, and tilt for the needs of surface treatment using ions. Finger, shields, and sample holder are equipped with temperature sensors (sample holder also with a heater) to control the temperature of the sample.
[1] Brauner T. et. al, in Microscopy 2023

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Type of presentation: Poster

PO-P-3199 Design of a nitrogen Dewar vessel for cryo-electron microscopes

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Observation of biological samples in electron microscopes in a state that is closest to the native one requires reaching cryogenic temperatures, most often with the use of liquid nitrogen (LN_2). We have designed a nitrogen Dewar vessel (Fig. 1) for cooling a sample holder and anti-contamination shields inside a vacuum chamber of cryo-electron microscopes (cryo-EM). The Dewar vessel includes a cooled copper finger (length of 410 mm) thermally anchored to the bottom of the liquid nitrogen (LN_2) vessel. The end of the finger allows thermal coupling of the sample holder [1, 2] and the anti-contamination shields to the finger via solid or flexible copper couplings. The Dewar vessel can be operated in two versions, the simpler version has a thermally-insulating vacuum space connected with the microscope chamber. In the second version, the vacuum space of the Dewar vessel can be separated, which prevents contamination of the microscope chamber with evaporated condensates when the Dewar vessel is warmed up. The design of the removable bottom of the outer shell, with the connecting flange and the removable finger, makes possible to adapt the Dewar vessel to different types of microscopes by adaptation of the removable parts. The outer cylindrical vacuum shell has a diameter of 102 mm and a height of 208 mm. The volume of the internal LN_2 vessel is 0.91 l. The performance of the Dewar vessel is characterized by three basic parameters, which are dependent on the heat load at the end of the finger and were verified experimentally. Namely, the evaporation rate, the refilling interval of LN_2, and the ultimate temperature at the end of the finger were determined as follows:

- Without heat load: 0.04 l/h, 23.7 h, 80.8 K.
- Heat load of 1 W: 0.06 l/h, 15.2 h, 85.4 K.
- Heat load of 2 W: 0.08 l/h, 11.1 h, 90.6 K.

References

[1] doi: 10.18462/iir.cryo.2019.0031.

[2] P. Hanzelka et al., International Journal of Refrigeration 132 (2021) 92–99.

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Fig. 1: The top view of the nitrogen Dewar vessel with the cooled copper finger.

Type of presentation: Poster

PO-P-3183 Practical tweaks in building STED microscope

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The STED microscopy is a technique that directly breaks the diffraction limit in far-field microscopy. There are STED microscopes that can be purchased commercially, however, the price is usually a limiting factor. In addition to that, such machines are frequently difficult to modify. IPHYS Bioimaging facility possesses a dual output femtosecond laser source that theoretically can serve as a 2P excitation and depletion laser source. There was a request from a user to implement 2P STED into our current customized Bruker Ultima microscope. Although the system is developed based on several similar published articles, the reality of implementing STED introduces a number of practical challenges. Therefore, this session will introduce some practical experience with building a STED system. Such information can help potential lab developers or start a possible cooperation.

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