CSMS

CONFERENCE

# MICROSCOPY2025

19 - 21 MAY 2025

**Grandhotel Clarion Zlatý Lev**
**Liberec**
**Czech Republic**


# Microscopy 2025

## Book of abstracts

Vladislav Krzyžánek, Kamila Hrubanová
(eds.)

Czechoslovak Microscopy Society

Brno, 2025

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Annual CSMS conference Microscopy 2025, 19.-21.5.2025, Liberec, Czech Republic

# Content

# Welcome Letter

Microscopy 2025 – Czechoslovak Microscopy Society (CSMS) Annual Conference

May 19–21, 2025, Liberec, Czech Republic

Dear Colleagues and Friends,

On behalf of the organizing committee and Czechoslovak microscopy society (CSMS), it is my great pleasure to welcome you to Microscopy 2025, held this year in the vibrant city of Liberec.

Our meeting continues a proud tradition of bringing together researchers, students, and professionals from across the fields of life sciences, materials science, and instrumentation, united by a common passion for microscopy and imaging technologies. The conference offers a rich scientific program that includes invited lectures, oral and poster sessions, company presentations, and practical workshops. We are also excited to support the next generation of microscopists through student sessions, the yCSMS LiftOff event, and hands-on learning opportunities. Beyond the scientific content, we hope you will enjoy the accompanying social program, excursions, and the beautiful surroundings of Liberec. These events provide a great opportunity to reconnect with colleagues, meet new collaborators, and engage in informal discussions that often spark future research.

I would like to extend my sincere thanks to all presenters, session chairs, sponsors, and the organizing committee for their efforts in putting together this year's conference.

Thank you for being part of Microscopy 2025. I wish you an inspiring, productive, and enjoyable time in Liberec!

Kamila Hrubanová

President of CSMS

# 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.

# Exhibitors, Sponsors and Partners





















# CSMS Annual Conference - **MICROSCOPY 2025**

***19 - 21 May 2025, Liberec, Czechia***
*Grandhotel Clarion Zlatý Lev*

## Monday 19th May 2025

| | |
|---|---|
| **8:00** | **Trip to the mountains / Glassworks** *(expected arrival time 13:00)* |
| **14:00** | **Registration of participants** *(until 19:00)* |
| **14:30** | **Member meeting of CSMS** |
| **16:00** | **Conference opening** |
| **16:05** | **Greeting from the Regional Representative – Vladimír Richter** |
| **16:10** | **ARR Liberec, CEO of Crystal Valley** David Pastva: Welcome to Crystal Valley |
| **16:20** | **Announcement of the winner of scholarship competition by CSMS** |
| **16:25** | Hana Polášek-Sedláčková: Quantitative AI-based DNA fiber workflow to study replication stress |
| **16:40** | **Announcement of the winner of scholarship competition by Thermo Fisher / CSMS** |
| **16:45** | Petr Liška: Selective Anion Exchange in CsPbBr3 Low-Dimensional Systems via Focused Electron/Ion Beam Induced Deposition |
| **17:00** | **Short Break** |
| **17:15** | **News in microscopy** |
| **17:15** | **European Microscopy Society (EMS)** Vladislav Krzyžánek: News in EMS |
| **17:25** | **yCSMS** Eva Ďurinová: yCSMS News and Events You Shouldn't Miss! |
| **17:30** | **CEITEC MUNI** Jiří Nováček: CIISB - Czech Infrastructure for Integrative Structural Biology |
| **17:40** | **UFI BUT** Michal Horák: Physical Engineering and Nanotechnology – the way to understanding and application of state-of-the-art technologies |
| **17:50** | **Czech-BioImaging** Pavel Hozák: 10 Years of Building the National Imaging Infrastructure Czech-BioImaging |
| **18:05** | **Czech Optical Cluster** Petr Přikryl: Czech-European Photonics Cooperation: Opportunities and Challenges |
| **18:15** | **JIC** Radka Novak: News from Brnoregion Microscopy |
| **18:25** | **MUNI** Petr Mikulík: The study program "Mikroskopie" at the Masaryk University |
| **18:40** | **Microscopy elevator pitch** |
| **18:40** | **Innovative Bioimaging, s.r.o.** Josef Lazar: Ultrafast Confocal Microscopy for Biological Imaging |
| **18:45** | **ISI CAS** Pavel Urban: Cryogenic technologies for conversion of room-temperature EM to cryo-EM |
| **19:00** | **Welcome party** |

## Tuesday 20th May 2025

| | |
|---|---|
| **9:00** | **Plenary lecture**<br>Rainer Heintzmann: Structured Illumination Microscopy |
| **9:50** | **Session I – Life sciences** (chairs: Marie Vancová and Josef Lazar) |
| **9:50** | Markéta Benešová: Surface-Enhanced Raman Spectroscopy (SERS) for Detection, Characterization and Identification of Microorganisms |
| **10:05** | **Thermo Fisher Scientific** Lukáš Kejík: Hydra Bio for advanced life science applications |
| **10:20** | **Poster session + coffee break** |
| **11:00** | **Invited lecture** Vladimíra Petráková: Detecting molecules in single-molecule microscopy images using probabilistic thresholding |
| **11:30** | **Specion** Nikodem Szymanski: Applying the power of science and technology |
| **11:45** | Kateřina Mrázová: Approach to Imaging Bacterial Cells Encapsulated in Alginate Hydrogel Using LV-STEM |
| **12:00** | **Altium** Kateřina Lónová: Nanolive |
| **12:15** | **Miriam Unger:** Multimodal Submicron O-PTIR: Advancing Life Science Applications with Simultaneous IR, Raman, and Fluorescence Microscopy |
| **12:30** | **Lunch** |
| **13:30** | **Session II - Interdisciplinary sciences** (chairs: Eliška Materna Mikmeková and Aleš Benda) |
| **13:30** | **Invited lecture** Filip Šroubek: Image Analysis in the Age of Deep Learning |
| **14:00** | **AdvaScope** Pavel Stejskal: High-Speed Hybrid-Pixel Detectors with Low-Energy Sensitivity |
| **14:15** | Jan Přibyl: Correlative Microscopy Techniques Serving as Core Facility |
| **14:30** | Jan Schäfer: Microscopy, Characterization, and Mechanistic Studies at VSB-TUO: From Graphene to Fusion Plasmas |
| **14:45** | **ZEISS** Ivana Burianová: The Next Generation of Serial Block-Face Imaging |
| **15:00** | **Poster session + coffee break** |
| **15:40** | **Session III: Material sciences** (chairs: Mariana Klementová and Miroslav Šlouf) |
| **15:40** | **Invited lecture** Pavel Kejzlar: Application of Scanning Electron Microscopy for the Material Research at Technical University of Liberec |
| **16:10** | **JEOL** Laurent Vasse: SXES analyzer - new possibilities of analytical methods on SEM |
| **16:25** | Ivo Kuběna: Structural changes in 3D printed L-PBF IN939 due to thermomechanical fatigue |
| **16:40** | **Short break** |
| **17:00** | **Invited lecture** Miroslav Cieslar: In-situ electron microscopy of nanoparticles |
| **17:30** | Lucia Bajtošová: In Situ and Ex Situ TEM Analysis of Al-Al2Cu Heterogeneous Nanostructures: Insights into Phase Stability and Diffusion |
| **17:45** | Jan Michalička: Atomic resolution DPC-STEM imaging of magnetic signal in antiferromagnets and beyond |
| **18:00** | **Break** |
| **19:00** | **Conference dinner and yCSMS welcome drink** |
| **21:30** | **Dance party with Dj – music and yCSMS social activities (until 01:00)** |

## Wednesday 21th May 2025

| | |
|---|---|
| **9:00** | **Workshops**<br>**SVEN BioLabs & Hana Polášek-Sedláčková**: ScanR workshop: Basics of high-content imaging<br>**yCSMS session** Eva Ďurinová: LiftOff: Launching Early Careers in Microscopy and Science |
| | Lukáš Malčický: Platelet endothelial cell adhesion molecule PECAM-1 could be detected in Reissner's fibre of brain ventricular system in rats |
| | František Kitzberger: Why manual segmentation is not always bad, but never should be used as the only one? |
| | Jakub Opelka: High-Throughput Oocyte Detection in Histological Slides Using YOLOv5 Neural Network |
| | Vladimíra Petráková – special guest |
| **10:00** | **Announcement of the CSMS Award** |
| **10:05** | Tomáš Čižmár: Exploring brain with fibre optics |
| **10:45** | **Announcement of the Best PhD thesis award - ZEISS** |
| **10:50** | Miroslav Stiburek: Confocal microscopy through a multimode optical fibre |
| **11:05** | **Session IV– Instrumentation and optics** (chairs: Dušan Chorvát and Vladislav Krzyžánek) |
| **11:05** | **Renishaw** Josef Sedlmeier: Multi-modal correlative microscopy: simultaneous and colocalised Raman & SEM imaging |
| **11:20** | **ZEISS** Pavel Krist: LSM Lightfield 4D - Instant Volumetric High-Speed Imaging of Living Organisms |
| **11:35** | **Poster session + coffee break** |
| **12:00** | **Invited lecture** Ondrej Krivanek: Atomic-resolution SE imaging and other developments in instrumentation for electron microscopy |
| **12:30** | **TESCAN** Tomáš Borůvka: TESCAN MIRA XR: Accessible Ultra-High-Resolution SEM for Advanced Research |
| **12:45** | Miroslav Šlouf: Semi-automated processing of 4D-STEM diffraction data with open-source Python packages STEMDIFF and EDIFF |
| **13:00** | Michal Horák: Characterization of perovskite single-photon emitters and solar cells by 4D-STEM in an FIB-SEM |
| **13:15** | **Conclusion** |
| **13:20** | **Lunch** |
| **14:00** | **Transfer to excursions and workshop** |
| **14:30** | **Excursions and workshop (until 16:30)** |
| | **CXI TUL (Liberec)** |
| | **TESCAN workshop: at the Technical University of Liberec (Liberec)** |
| | **CRYTUR, spol. s r.o. (Turnov)** |
| | **Institute of Plasma Physics of the Czech Academy of Sciences – toptec (Turnov)** |

# MICROSCOPY 2025
# yCSMS EVENTS


## JOIN US ON OUR ACTIVITIES

### PHOTOBOOTH

READY TO ZOOM IN ON FUN? GRAB A PROP, STRIKE A POSE, AND SHOW YOUR LOVE FOR MICROSCOPIC WORLDS.

WHEN? MONDAY, WELCOME RECEPTION

### FIND YOUR SCIENTIFIC SOULMATE!

DRAW A NAME AND STEP INTO THE SHOES OF A LEGENDARY SCIENTIST, GROUNDBREAKING DISCOVERY OR ESSENTIAL TECHNIQUE AND GO FIND YOUR PERFECT MATCH! ARE YOU WATSON LOOKING FOR CRICK? OR AN ELECTRON IN SEARCH OF A MICROSCOPE? CHAT, ASK CLEVER QUESTIONS, AND TRACK DOWN YOUR SCIENTIFIC PARTNER (OR PARTNERS). A FUN NETWORKING GAME FOR CURIOUS MINDS AND SCIENCE LOVERS. JOIN THE HUNT AND MEET YOUR SCIENCE SOULMATE!

WHEN? TUESDAY, CONFERENCE DINNER

### LIFTOFF: LAUNCHING EARLY CAREERS IN MICROSCOPY AND SCIENCE

YOUNG SCIENTISTS TAKE THE STAGE TO SHARE THEIR RESEARCH AND STORIES, FOLLOWED BY AN INSPIRING TALK FROM A WELL-KNOWN SCIENTIST ABOUT HER LIFE IN SCIENCE. EXPECT GOOD TALKS, HONEST CAREER MOMENTS, AND OPEN CHATS IN A RELAXED, FRIENDLY SPACE. COME JOIN US, MEET NEW FACES, AND LIFT OFF YOUR SCIENCE JOURNEY TOGETHER! BOTH EARLY-CAREER AND EXPERIENCED SCIENTISTS ARE WELCOME TO JOIN THE SESSION, SHARE PERSPECTIVES, AND ENGAGE IN AN OPEN DIALOGUE!

WHEN? WEDNESDAY, WORKSHOPS
FEATURING: VLADIMÍRA PETRÁKOVÁ

YCSMS.ORG

young CSMS microscopists

SCAN ME!

# Plenary session

*Chairs: Kamila Hrubanová and Dušan Chorvát*

# Structured Illumination Microscopy

Heintzmann R.[1,2]

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[2] Leibniz Institute of Photonic Technology, Jena, Germany

Email of the presenting author: heintzmann@gmail.com

In the past two decades revolutionary advances have been made in the field of microscopy imaging, some of which have been honoured by the Nobel prize in Chemistry 2014. One high-resolution method is based on transforming conventionally unresolvable details into measurable patterns with the help of an effect most people have already personally experienced: the Moiré effect. If two fine periodic patterns overlap, coarse patterns emerge. This is typically seen on a finely weaved curtain folding back onto itself. Another example is fast moving coarse patterns on both fences of a bridge above a motorway, when approaching it with the car. The microscopy method of structured illumination utilizes this effect by projecting a fine grating onto the sample and imaging the resulting coarser Moiré patterns containing the information about invisibly fine sample detail. With the help of computer reconstruction based on several such Moiré images, a high-resolution image of the sample can then be assembled. The presentation will provide insight into the method and its recent extensions.

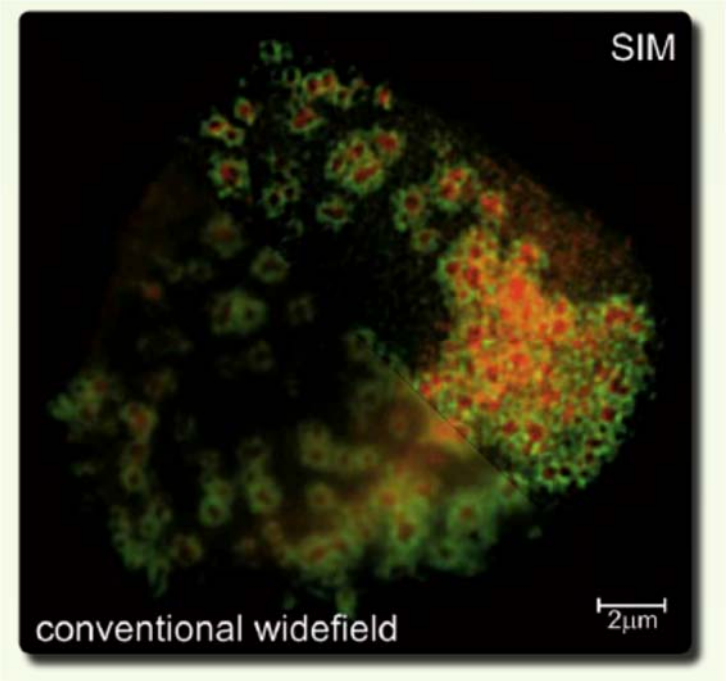


*Fig. 1: Podosomes of a cell image with structured illumination (SIM). F-actin shown in red and vinculin in green. Image courtesy of Marie Walde, Gareth Jones and James Moneypenny*

# Exhibitor talks and posters

*Talks are included within the corresponding scientific sessions*

# Hydra Bio for advanced life science applications

Kejík L.[1], Slamková D.[1], Hovorka M.[1], Wandrol P.[1], Ošťádal R.[1]

[1] Thermo Fisher Scientific, Brno, Czech Republic

Email of the presenting author: lukas.kejik@thermofisher.com

Plasma focused ion beam (PFIB) technology offers several advantages over conventional gallium-based FIB systems, including significantly higher material removal rates and the absence of implanted gallium ions that can react with the milled material. The Helios Hydra PFIB DualBeam platform exemplifies these advantages by combining an innovative multi-ion species PFIB column with a monochromated Elstar SEM Column. This versatile platform supports the use of four ion species (Xe, Ar, O, N) to find a best match to the analyzed material, with fast and automated switching capabilities. The SEM column provides a wide range of working conditions for imaging nanoscale details. Additionally, the Cryo Stage enables work with vitrified biological samples or unstable material science samples like polymers.

The Hydra Bio PFIB system is the life sciences-dedicated representative of the Helios Hydra platform. It features several standout modules, including the integrated micro-sputter coater that permits the in situ metal coating to reduce the sample charging, eliminating the need for external devices, and the integrated fluorescence light microscope (iFLM) module which allows targeting a specific region of interest inside the cell or tissue based on the fluorescence data. All the unique capabilities of the Hydra Bio allow a full coverage of the two main applications – lamella preparation for the TEM and high-resolution volume imaging. The automated on-grid lamella preparation is facilitated by the AutoTEM cryo software while lift-out lamella preparation can be done with EasyLift cryo.

However, the main feature brought about by the high-resolution Elstar column is the volume imaging with the Auto Slice and View (ASV) application. It facilitates automated serial milling and imaging of cryogenic and resin-embedded samples, enabling the acquisition of high-resolution 2D image stacks that can be reconstructed into 3D volumes (tens of microns in all dimensions). To study even larger volumes of resin-embedded samples, the Spin Mill Bio method (controlled by the ASV) can be used as it allows for the preparation and analysis of horizontal surface areas up to 1 mm in diameter at a near-glancing angle (Fig. 1). This approach is particularly advantageous for accessing large areas and imaging sparse regions of interest, enabling the collection of 3D data from multiple areas. Additionally, novel functionalities powered by artificial intelligence will be introduced to increase throughput and enhance automatic data collection quality.

In conclusion, the Hydra Bio PFIB system offers a unique versatility and a broad range of life science applications which make it an invaluable tool for researchers aiming to study the smallest details as well as much larger structures to obtain the information in a larger context.

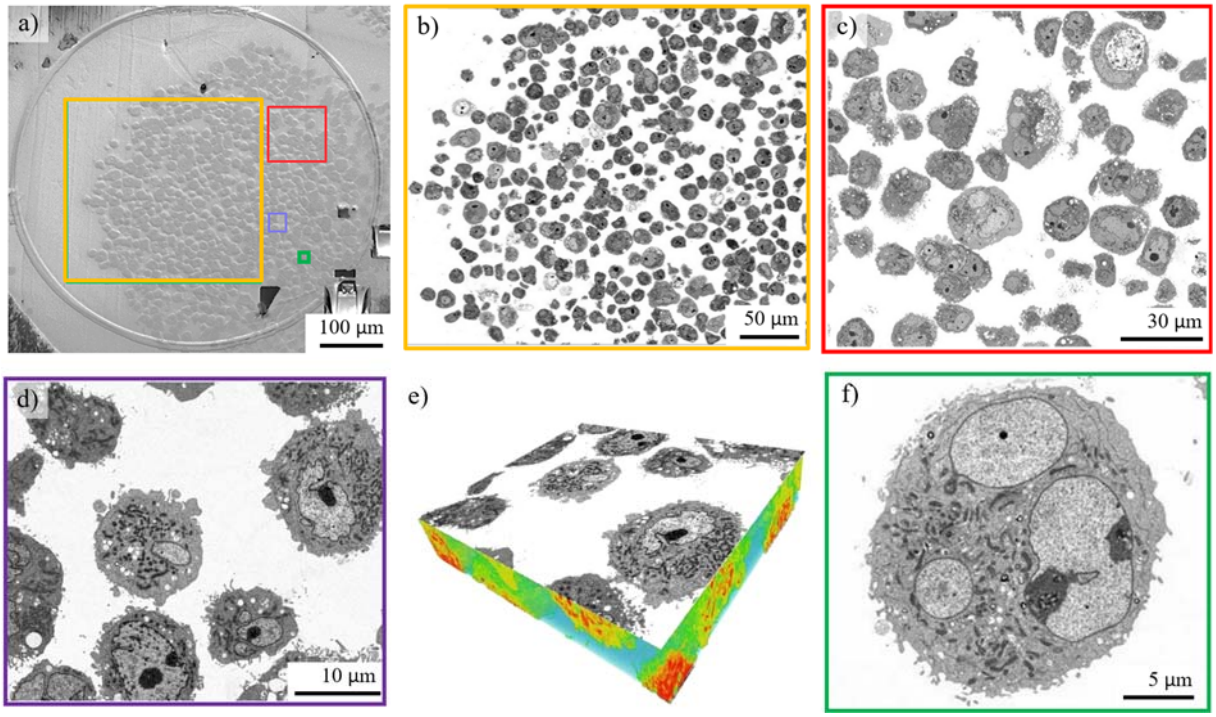


*Fig.1: a) A detail of a Spin Mill Bio area of interest marked by a circular marker with several regions selected for image collection highlighted. CBS overview images taken with the pixel size of b) 80 nm and c) 30 nm, higher-resolution image with d) 10 nm and e) corresponding reconstructed 3D volume of images, f) high-resolution image with 3 nm pixel size. Sample of Chinese hamster ovarian cells embedded in DURCUPAN resin.*

# Applying the power of science and technology

Szymanski N.[1]

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

Email of the presenting author: info@specion.cz

In this talk, we will present the latest technologies from Leica Microsystems designed to meet the growing demands of advanced life science imaging. The spotlight will be on the Leica STELLARIS confocal platform, offering enhanced sensitivity, spectral flexibility, and integrated lifetime-based imaging. We will also introduce XTEND STED for super-resolution imaging beyond the diffraction limit, as well as the powerful Multiplexing capabilities on STELLARIS, enabling simultaneous detection of multiple targets with exceptional clarity.

Furthermore, we will discuss Leica AIVIA – an intuitive, AI-powered software solution for 2D, 3D, and 4D image analysis – and how it complements Leica's hardware to streamline and accelerate data interpretation. This session is ideal for researchers seeking high-performance imaging solutions and efficient workflows from acquisition to analysis.

# Nanolive

Lonova K.[1]

[1] Altium International s.r.o., Prague, Czech Republic

Email of the presenting author: katerina.lonova@altium.net

Cell metabolism plays a crucial role in health and disease, yet traditional fluorescence-based methods pose challenges such as phototoxicity, photobleaching and complex sample preparation. Nanolive's label-free holotomographic technology enables high-resolution, real-time quantification of metabolic activity without disrupting live cells.

The Smart Lipid Droplet Assay and Smart Mitochondrial Assay provide quantitative insights into lipid droplets morphology and distribution and assess critical mitochondrial dynamics. By eliminating dyes and ensuring prolonged real-time observation, Nanolive's technology represents a transformative tool for biomedical research, accelerating discoveries in drug response and disease mechanisms.

# Enhancing Diffraction Analysis: High-Speed Hybrid-Pixel Detectors with Low-Energy Sensitivity

Stejskal P.[1]

[1] AdvaScope s.r.o., Brno, Czech Republic

Email of the presenting author: pavel.stejskal@advascope.cz

Advances in electron microscopy are reshaping how clearly scientists can observe materials at the microscopic level. This contribution introduces a cutting-edge detector designed specifically for scanning electron microscopes (SEMs), significantly enhancing their ability to perform sophisticated diffraction analyses, such as EBSD and/or 4D STEM.

The new detector leverages advanced hybrid-pixel technology (Timepix2/3/4) capable of capturing very low-energy signals, traditionally challenging for standard detectors. With this innovative solution, SEMs can now rapidly capture extremely detailed datasets, revealing more accurate structural details with improved resolution and clarity. Unlike conventional methods, this technology digitizes electron signals instantly, filtering out noise and unwanted interference to deliver cleaner, sharper images. Thanks to the unique data-driven readout of Timepix3/4, experiments that previously took extensive time can now be completed in mere seconds, making it ideal for applications requiring high precision and speed, such as strain mapping or material orientation studies. This breakthrough provides researchers and industries with a powerful, user-friendly method for obtaining detailed microscopic data quickly and effectively, opening new possibilities in quality control, material science, and electronics.

# The Next Generation of Serial Block-Face Imaging

Burianová I.[1]

[1] Carl Zeiss spol. s.r.o., Radlická 3201/14, Prague, Czech Republic

Email of the presenting author: ivana.burianova@zeiss.com

With extensive experience in volume electron microscopy and serial block-face imaging, ZEISS introduces its own serial block-face solution: Volutome. This in-chamber ultramicrotome, designed for field emission scanning electron microscopes (FE-SEM), enables high-resolution 3D imaging of the ultrastructure of biological, resin-embedded samples over large areas. Developed from research technology pioneered by the Max-Planck Institute for Neurobiology, ZEISS Volutome is a comprehensive solution integrating both hardware and software, including image processing, segmentation, and visualization.

A key advantage of Volutome is its adaptability—users can seamlessly replace the ultramicrotome with a conventional SEM stage, transforming their 3D FE-SEM into a standard, multipurpose FE-SEM. Imaging resin-embedded samples presents challenges, particularly in balancing contrast and sample integrity. While high acceleration voltages enhance contrast, they risk damaging sensitive samples, whereas low kV imaging preserves sample integrity but reduces contrast. To address this, ZEISS introduces the Volume BSD detector, optimized for Volutome, providing high-contrast images even at low kV. Combined with Focal Charge Compensation, this system enables effective charge neutralization for charge-prone samples, ensuring high-quality imaging.

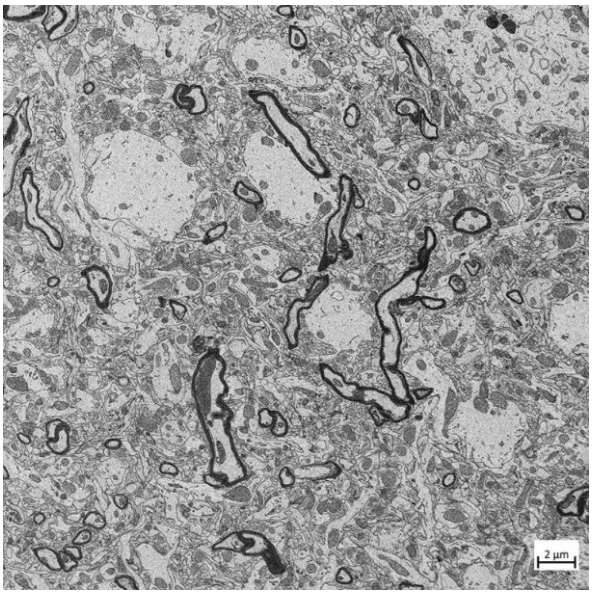


*Fig. 1: Mouse brain tissue acquired with ZEISS Volutome and GeminiSEM; pixel size: 3 nm. Sample courtesy of Christel Genoud, Université de Lausanne, Switzerland*

# Multi-modal correlative microscopy: simultaneous and colocalised Raman & SEM imaging

Diniz J.[1], Ferguson J.[1], Johnson P.[1], Smith T.[1], Sedlmeier J.[2]

[1] Renishaw plc, New Mills, UK
[2] Renishaw s.r.o., Czech Republic

Email of the presenting author: josef.sedlmeier@renishaw.com

Multi-modal sample analysis is essential to thoroughly characterise and understand our samples and materials and ultimately conduct cutting edge science. Here we focus on four different modalities: Raman spectroscopy, optical imaging, SEM (scanning electron microscopy) imaging and EDS (energy dispersive X-ray spectroscopy). These techniques allow us to determine chemical composition, molecular and crystalline structure (Raman spectroscopy), collect optical images, image samples at high resolution (SEM imaging) and determine elemental composition (EDS). When combined, these techniques provide an excellent toolbox for sample characterisation. Measurements were conducted using an inLux™ SEM Raman interface attached directly to the SEM. This enabled colocalised and simultaneous SEM, Raman, and optical imaging inside the SEM chamber, making correlation between the three techniques trivial. Figure 1 illustrates SEM, EDS and Raman images taken from a mineral section, demonstrating a clear and accurate overlay between minerals found with three techniques. Additionally, the complementary potential of the multi-modal analysis showed species detected only with Raman (anatase) or EDS (Zn, sphalerite). The presence of anatase also shows that Raman spectroscopy is sensitive to the many polymorphs of TiO2. The application potential of correlative SEM and Raman imaging will also be demonstrated on batteries, polymeric and biological samples. With these examples, we illustrate how Raman and SEM can increase understanding of materials, and the power and ease of use when the techniques are combined inside a SEM.

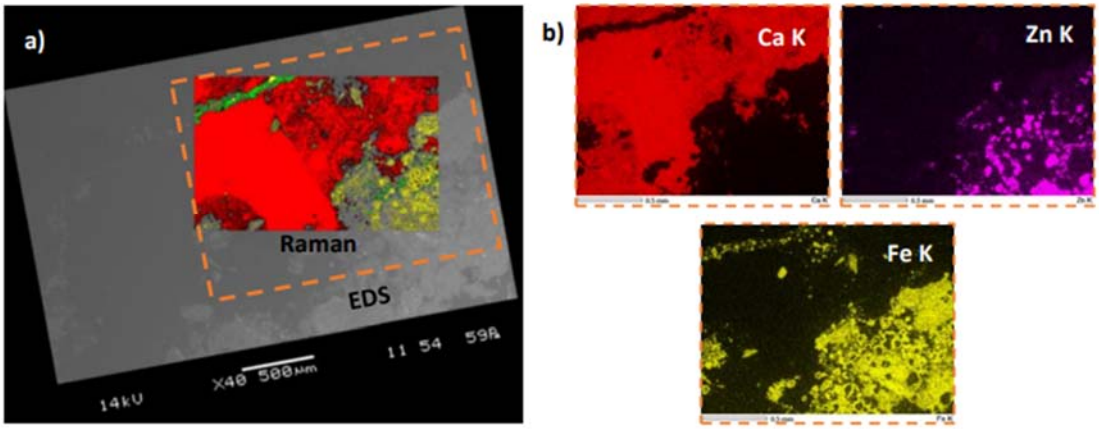


*Fig. 1: 1. a) BSE image of a mineral section with overlayed Raman image illustrating the distribution of calcite (red), pyrite (yellow), and anatase (green); b) complementary EDS information agrees with the Raman data, and in addition highlights Zn due to the presence of sphalerite*

# LSTM Lightfield 4D–Instant Volumetric High-Speed Imaging of Living Organisms

Krist P.[1]

[1] ZEISS Research Microscopy Solutions, Prague, Czech Republic

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Lightfield 4D is instant volumetric imaging at high speed. Acquire comprehensive 3D information with a single snap and say goodbye to any time delay within an imaged volume. For the first time, capture the fastest movements within whole organisms at up to 80 volumes per second – with all spatiotemporal information intact. Crawling larvae, beating hearts, flowing blood, and firing neurons can be studied in 3D at unprecedented speed to unravel the secrets of life. More information at [1].

References:

[1] https://www.zeiss.com/microscopy/en/products/light-microscopes/confocal-microscopes/lightfield-4d.html

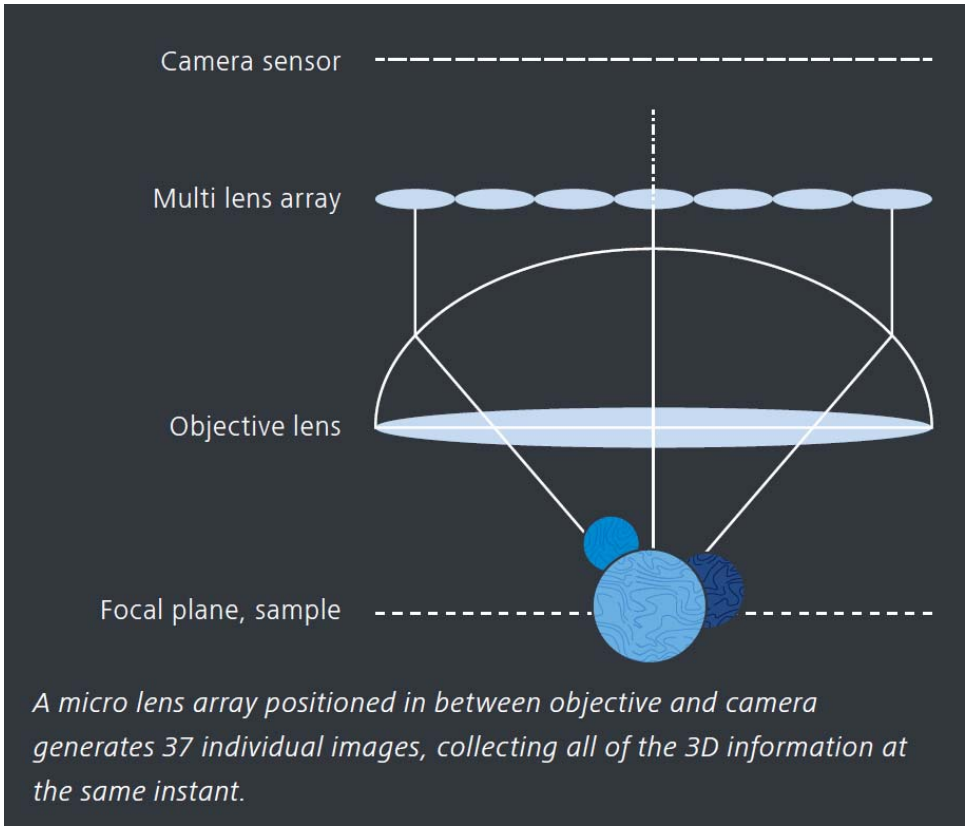


*Fig. 1: Principle of LSTM Lightfield 4D–Instant Volumetric High-Speed Imaging*

# TESCAN MIRA XR: Accessible Ultra-High-Resolution SEM for Advanced Research

Boruvka T.[1], Rosikova K.[1], Klimek P.[1]

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TESCAN MIRA XR is a newly introduced scanning electron microscope (SEM) designed to deliver ultra-high-resolution imaging combined with intuitive operation. Its goal is to expand access to advanced SEM capabilities across a broad range of scientific applications–enabling even non-expert users to achieve high-quality results with ease.

Built on TESCAN's BrightBeam™ technology, MIRA XR provides detailed topographic imaging and compositional contrast at low accelerating voltages. This makes it an ideal tool for studying beam-sensitive and nanostructured materials, as well as metallic systems.

Beyond its imaging performance, MIRA XR includes integrated tools such as VisualCoder (a block-based workflow builder) and SEM Expert PI (a Python scripting interface), empowering both novice and experienced users to automate complex routines or fully customize their data acquisition workflows.

We present several use cases from materials science. In nanomaterials research, MIRA XR has been successfully applied to image non-conductive materials such as mesoporous silica and nanofibers without conductive coatings, preserving nanoscale surface features and ensuring reproducibility. In nanoparticle synthesis and process development, automated imaging using MIRA XR and SEM ExpertPI allowed consistent data collection across varying synthesis parameters.

A further example demonstrates the use of MIRA XR for automated *in situ* testing, combining heating and EBSD analysis in an additively manufactured steel sample. The system enabled real-time observation of intermediate phase transformations during thermal cycling, revealing a "memory effect" and recrystallization behavior. With automated control of stage movement, signal settings, and detector parameters, EBSD maps were collected at precise 2 °C intervals—offering valuable insights into microstructural evolution.

In conclusion, TESCAN MIRA XR is a versatile and high-performance SEM that unites ultra-high-resolution imaging with accessibility and open integration. It supports both routine accessible characterization and advanced experimental workflows across a wide range of scientific fields.

References:

[1] Taylor M. et al.: IOP Conf. Ser.:Mater. Sci. Eng. 1310 (2024) 012001
[2] Kedronova E. et al: Ostrava, Nanocon 2016 - Proceedings, 490-496,

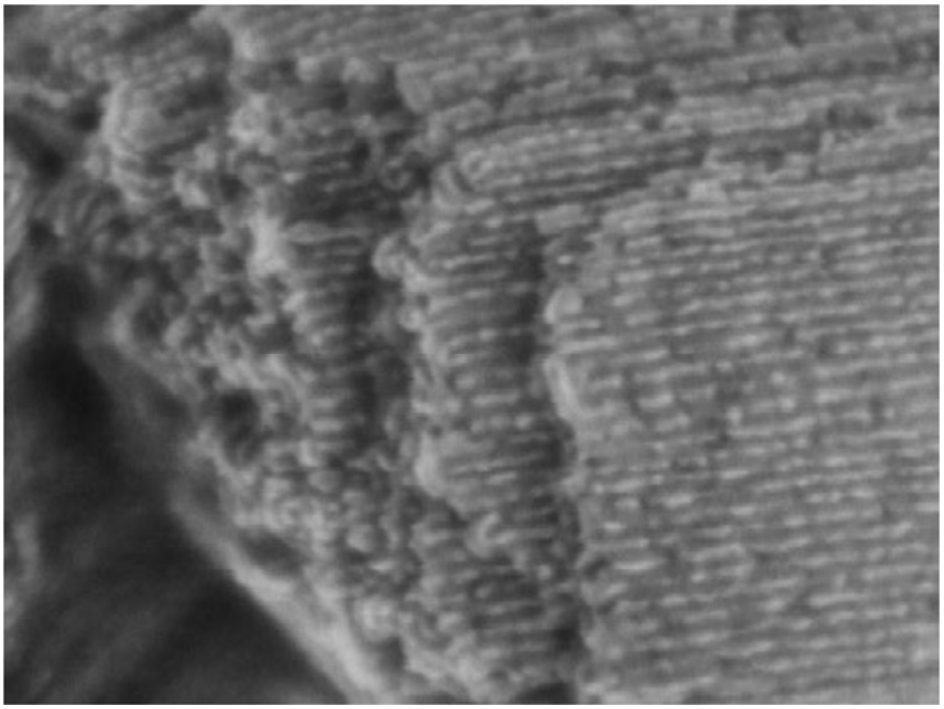

*Fig. 1: Detailed SEM image of SBA-15 particles showing nanorod-like substructures on the surface.*

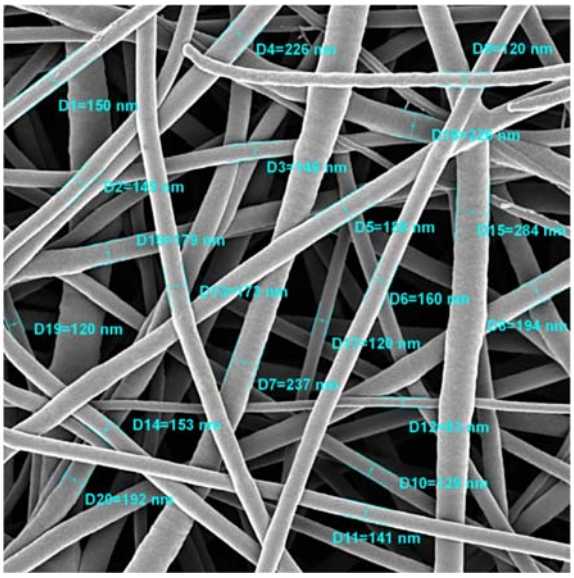


*Fig. 2: Measurement of nanofiber diameters on PCL nanofibrous mesh after optimization of electrospinning process parameters*

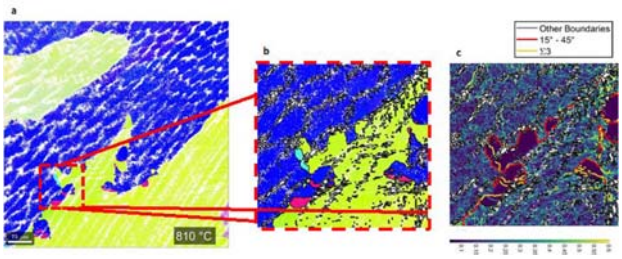


*Fig. 3: a) EBSD map of ROI at 810°C; b) inlay to highlight bulging of recrystallised grains into grains undergoing transformation by memory and c) the KAM map for the inlay displayed in b)*

# Minimally invasive holographic microendoscope for subcellular deep brain imaging

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Neurobiologists face a challenge in light-based structural and functional in-vivo microscopic imaging inside sensitive brain tissue. Non-invasive multiphoton microscopes are limited to an imaging depth of about 1mm. Reaching beyond this point, the tissue's light-scattering and absorbing properties make implanting a gradient index rod lens necessary. The physical dimensions of the lens and the implantation process require the removal of tissue, thus affecting the brain function under study.

We present NeuroDeep, a compact design of a holographic endoscope that enables laser-scanning fluorescent imaging at the tip of a 110µm fibre probe. The device is based on recent advances in holographic light control in individual multimode glass fibres. A single glass fibre, functioning as the endoscopic probe, can be inserted into the tissue at arbitrary depths without a lengthy implantation procedure. As the fibre enters the brain tissue through a 200µm cranial window, the operator receives immediate, real-time volumetric images from a 100µm x 100µm wide field of view at the fibre tip. Offering submicrometric lateral resolution and on-the-fly focal distance adjustment, the probe can reach as deep as the amygdala and brain stem. The holographic approach allows for a scanning speed of up to 1kHz, allowing the recording of high-speed electrical signals between neuron cells using voltage sensors.

We demonstrate in-vivo imaging of fluorescently labelled neurons in anaesthetized and awake animal models acquired through an acute insertion up to a depth of 5 mm. We present high-resolution imaging of subcellular structures like dendritic spines and recording of neuronal activity with calcium and voltage indicators in deep structures like the amygdala, ventral tegmental area, and ventral hippocampus. Postmortem analysis of insertion-induced inflammation indicates lower tissue damage compared to state-of-the-art endoscopic solutions.

Holographic endoscopy offers less traumatic deep tissue imaging with high speed and high resolution. As a research-driven company, DeepEn is committed to providing users with this innovation in an easy-to-use, robust and compact plug-and-play solution.

References:

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[2] Vasquez-Lopez, S.A. et al. "Subcellular spatial resolution achieved for deep-brain imaging in vivo using a minimally invasive multimode fiber". Light Sci Appl 7, 110 (2018).

[3] Turtaev, S., Leite, I.T., Altwegg-Boussac, T. et al. High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging. Light Sci Appl 7, 92 (2018).

Acknowledgement:

DeepEn GmbH, is a spin-off of Leibniz Institute of Photonic Technology in Jena, Germany. The technology has been advanced by research groups at Leibniz Institute of Photonic Technology Jena, Germany and Institute of Scientific Instruments of the CAS, Brno, Czechia.

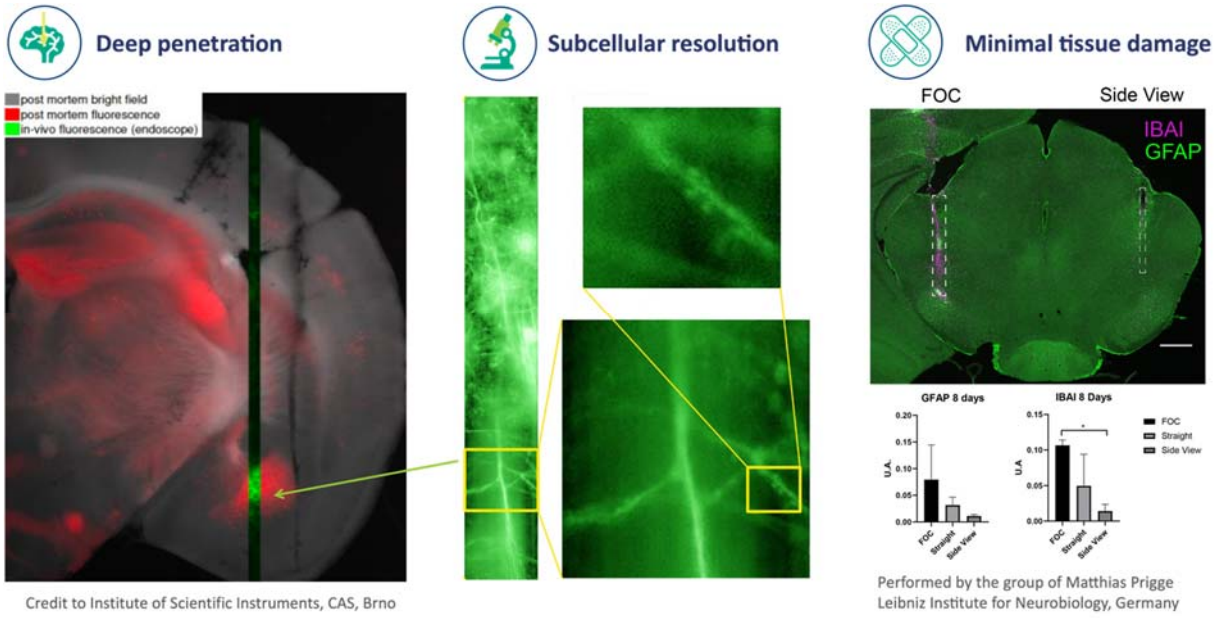


*Fig. 1: Advantages of imaging with the NeuroDeep*

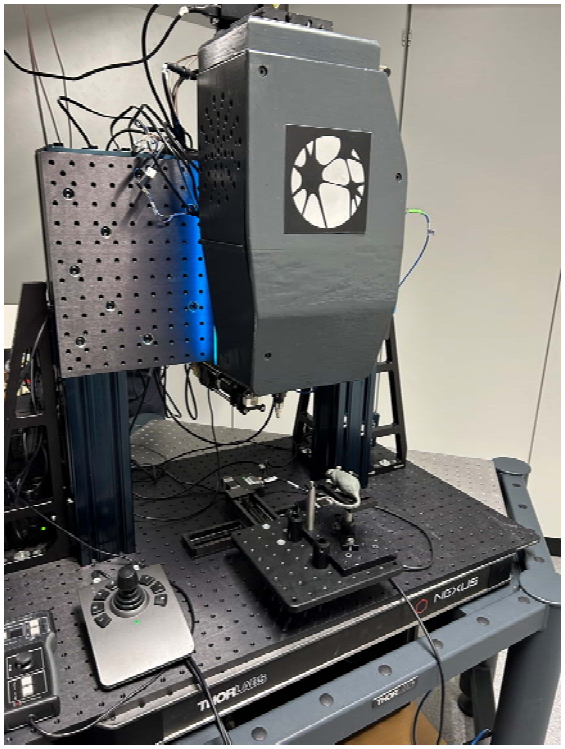

*Fig. 2: Fully functional prototype of NeuroDeep*

# CSMS awards

*Chairs: Kamila Hrubanová and Dušan Chorvát*

# Quantitative AI-based DNA fiber workflow to study replication stress

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Replication stress (RS) is a prominent source of genome instability and human diseases, including developmental defects, premature aging, and cancer. Understanding the molecular mechanism of RS has great potential for early diagnosis and development of effective strategies for human diseases characterized by unstable genomes. One of the most powerful methods to study DNA replication dynamics and its alterations at the single-molecule resolution is the DNA fiber assay. However, this method relies exclusively on manual image acquisition and analysis, which can be time-consuming and prone to user bias, potentially affecting the interpretation and reproducibility of the collected data. Here, we developed an automated workflow for the acquisition and analysis of DNA fibers using the scanR high-content imaging system and TruAI deep learning module. Our quantitative AI-based DNA fiber (qAID) workflow enables imaging and real-time multiparameter analysis of thousands of DNA fibers within several dozen minutes. The multiparameter analysis classifies fibers into five standard classes and measures properties such as fiber length and symmetry of two-color labeling. qAID multiparameter analysis is complemented by visual inspection of individual DNA fibers using unbiased image galleries. In summary, qAID workflow provides a fast and effective examination of replication dynamics and its alterations at the single-molecule resolution.

Acknowledgement:

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# Selective Anion Exchange in $CsPbBr_3$ Low-Dimensional Systems via Focused Electron/Ion Beam Induced Deposition

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Lead halide perovskites (LHPs) have gained significant attention due to their outstanding optoelectronic properties, numerous applications (solar cells, quantum communication, etc.), and easily tunable band gap [1, 2]. The band gap tuning in LHPs is facilitated through halide anion exchange (HAE), resulting in a band gap tuning over the whole visible spectrum. So far, the HAE has been achieved chemically through introducing different halides into the LHPs synthesis. However, the resulting compounds have suffered from halide phase segregation and lacked any kind of control over the reaction, spatial selectivity in particular [3].

In this project, we propose a precise and delicate method for handling the halide anion-exchange in $CsPbBr_3$ nanocrystals/thin films to address the stability and precise reaction site selection issues in mixed-halide perovskites by incorporating post-synthesis chemical treatment via halide gas inlet system (GIS) in scanning electron microscope (SEM) system (Fig. 1). Furthermore, by implementing subsequent focused ion beam (FIB) nanoprocessing, we aim to precisely engineer the optical properties of such systems through localised resonances. The resulting nanostructures could potentially serve as a low-dimensional tunable light source, ideal e.g. for design of high-resolution colourful displays or colorimetric scintillation detectors.

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[2] Raíno G. et al.: Nat Comunn 13(2022), 1-8.
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Acknowledgement:

The financial support from ThermoFisher Scientific is gratefully acknowledged. The authors acknowledge financial support from the Czech Science Foundation (grant No. 25-17500S) and CzechNanoLab (project LM2023051, MEYS CR).

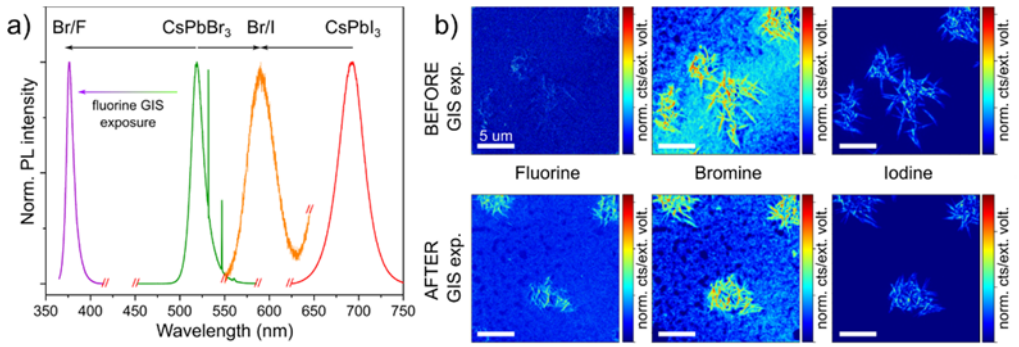


*Fig. 1: a) Photoluminescence (PL) spectrum of CsPb(Br/I) low-dimensional systems after the fluorine GIS exposure indicate a change in the internal electron structure visible in the changed emission shifted towards UV range. b) This is supported by time-of-flight secondary ion mass spectrometry analysis prior to the fluorine deposition and after the fluorine deposition, where the fluorine concentration is strongly elevated.*

# Exploring brain with fibre optics

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Light-based in-vivo brain imaging relies on light transport through highly scattering tissues over long distances. As scattering gradually reduces imaging contrast and resolution, visualising structures at greater depths becomes challenging, even when using multi-photon techniques. To overcome this limitation, minimally invasive endo-microscopy techniques have been developed that typically use graded-index rod lenses. A recently proposed alternative involves the exploitation of holographic control of light transport through multimode optical fibres [1], which promises superior imaging performance with less traumatic application [2]. Following the review of the fundamental and technological bases, the talk will introduce a 110µm thin laser-scanning endo-microscope, which enables volumetric imaging of the entire depth of the mouse brain in vivo [3]. The system is equipped with multi-wavelength detection and three-dimensional random-access options, and it has a lateral resolution of less than 1µm. Various modes of its application will be presented including the observations of fluorescently labelled neurons, their processes, and blood vessels. Finally, the use of the instrument for monitoring calcium signalling and measurements of blood flow in individual vessels at high speeds will be discussed

References:

[1] Čižmár T. & Dholakia K.: Nature Communications 3(2012), 1027
[2] Turtaev S. et al.: Light-Science & Applications 7(2018), 92
[3] Stibůrek M. et al.: Nature Communcations 14(2023), 1897

# Confocal microscopy through a multimode optical fibre

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High-resolution optical microscopy of thick biological samples requires minimizing out-of-focus light. Traditional techniques such as confocal and multi-photon microscopy encounter limitations beyond 2 mm in depth due to scattering and absorption. Multi-mode fibre (MMF)-based holographic endoscopes enable minimally invasive imaging at greater depths with sub-micron resolution but often suffer from out-of-focus fluorescence. This work presents a fluorescence confocal imaging approach using an MMF-based endoscope. The proposed fibre probe integrates a graded-index MMF with a step-index MMF to selectively filter fluorescence, effectively distinguishing in-focus signals from background noise. Experimental results demonstrate a significant reduction in background fluorescence, leading to enhanced image contrast. The method's effectiveness is showcased by imaging neurons, revealing improved clarity and structural details.

# Life sciences

*Chairs: Marie Vancová and Josef Lazar*

# Surface-Enhanced Raman Spectroscopy (SERS) for Detection, Characterization and Identification of Microorganisms

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Raman spectroscopy is an analytical technique known for its non-destructive nature and based on Raman scattering. Raman scattering is the inelastic scattering of photons that occurs when they interact with electrons in chemical bonds. This technique enables the rapid analysis of chemical compounds, compound mixtures, and biological samples, including living organisms, in a non-contact, non-destructive manner. The obtained spectra can be compared with spectral databases for more precise identification. However, a common challenge of Raman spectroscopy is the weak signal from certain samples such as biological samples, which is often overshadowed by intense fluorescence. A micro-Raman spectrometer is used to obtain Raman spectra, see *Figure 1*.

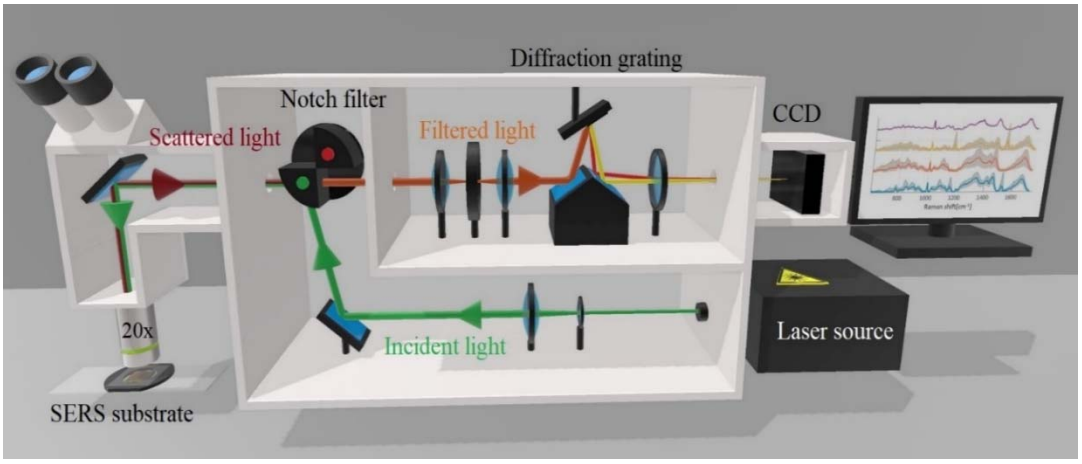


*Figure 1: Micro-Raman spectrometer – the experimental setup diagram. The light from the laser passes through the optical elements to the microscope objective and hits the sample. The scattered light is collected by the objective and separated from the excitation radiation on a notch filter. It then passes into the spectrometer and onto the CCD detector.*

Metallic nanoparticles can be used to create so-called Surface-enhanced Raman spectroscopy (SERS) to amplify the Raman signal. Metallic nanostructures exhibit plasmon resonance, a coherent oscillation of conduction electrons induced by the interaction of visible radiation with those structures. The Raman signal is enhanced when the analyte, often gold or silver, is adsorbed onto the surface of metallic nanoparticles. This approach can achieve Raman signal enhancement by several orders of magnitude, commonly around $10^3$, but in some cases as high as $10^{11}$–$10^{14}$.
SERS substrates exist in various forms, including nanoparticles (e.g., gold nanorods, AuNRs) or nanostructured planar layers. The SERS method allows the rapid identification and differentiation of a wide range of chemical and biological samples, including various types of microorganisms. However, variations in the chemical composition of bacteria may be too insignificant to reliably distinguish closely related species.
To achieve species-specific identification, diagnostics can be combined with so-called SERS-tags, a technique that involves using specific antibodies. SERS-tags are gold nanoparticles with a surface modified with a Raman reporter, which provides a strong and distinct spectral signal enhanced by the

SERS effect. Additionally, the nanoparticle surface is functionalized with an antibody that selectively binds to a specific bacterial strain based so the SERS-tags using is based on antigen-antibody affinity. The presence of the Raman reporter signal in the sample spectrum validates the presence of the target bacteria. These SERS-tags can be modified for labelling any bacterial species by modifying their surface with strain-specific antibodies [1].
SERS can be directly used to analyze bacterial metabolism under stress conditions. Bacteria can be applied directly onto gold nanoparticles and afterward measured. The obtained signal may originate from the bacterial surface or external metabolites. For example, one of the stress markers of *Staphylococcus aureus* is adenine (see *Figure 2* and *Figure 3*) [2].

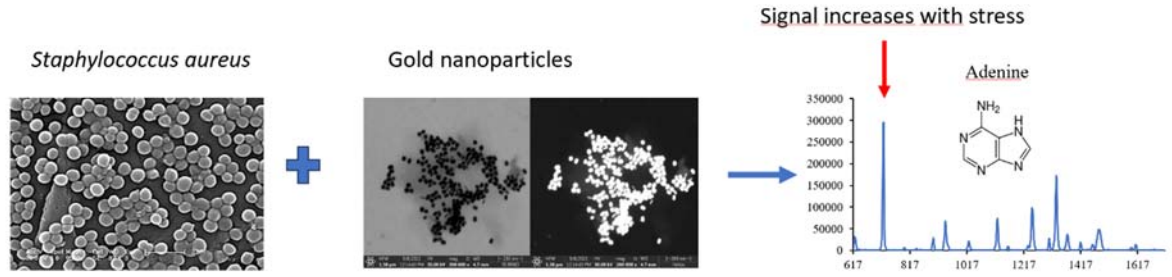


*Figure 2: The SERS measurement of the Staphylococcus aureus using gold nanoparticles to enhance signal of the external metabolites such as adenine.*

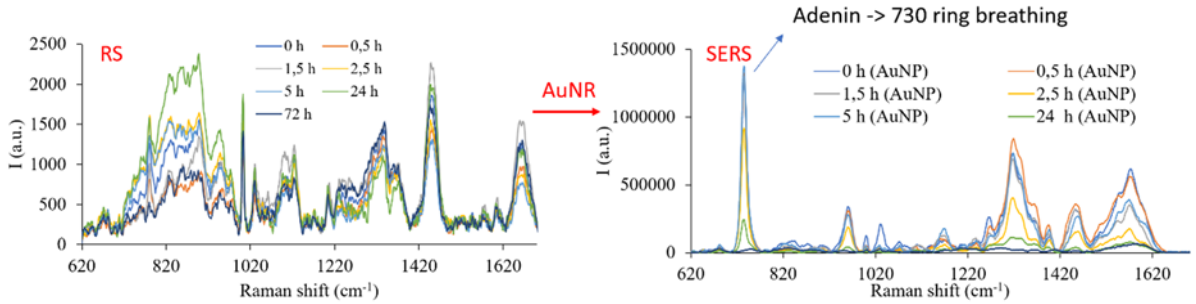


*Figure 3: The comparison of the Raman and SERS spectra of the Staphylococcus aureus.*

The presented real-time (RT) setup also has the potential to be adapted into a portable version, enabling on-site analysis of microbial processes (MPs).

Acknowledgment:

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# Detecting molecules in single-molecule microscopy images using probabilistic thresholding

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Single-molecule localization microscopy (SMLM) allows imaging beyond the diffraction limit. Detection of molecules is a crucial initial step in SMLM. False positive detections, which are not quantitatively controlled in current methods, are a source of artifacts that affect the entire SMLM analysis pipeline. Furthermore, current methods lack standardization, which hinders reproducibility. Here, we present an optimized molecule detection method which combines probabilistic thresholding with theoretically optimal filtering [1]. The probabilistic thresholding enables control over false positive detections while optimal filtering minimizes false negatives. A theoretically optimal Poisson Matched Filter is used as a performance benchmark to evaluate existing filtering methods. Overall, our approach allows the detection of molecules in a robust, single-parameter and user-unbiased manner. This will minimize artifacts and enable data reproducibility in SMLM.

References:

[1] Hekrdla M. et al.: Nat Commun 16(2025), 601.

Acknowledgement:

This work was supported by Czech Science Foundation, grant number 21-17847M (MH, NH, SF, VP) and Czech Academy of Sciences, Premie lumina quaeruntur LQ200402101 (KU, DR, VP).

# Approach to Imaging Bacterial Cells Encapsulated in Alginate Hydrogel Using LV-STEM

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The imaging of hydrogel-based samples presents a challenge for electron microscopy. These polymeric structures, capable of binding significant amounts of water, find applications in a wide range of industries (medicine, food science, agriculture, etc.)[1]. It is the high water content and the sensitive fine structure that make these samples difficult to observe with an electron microscope. Different preparation techniques combined with different types of scanning electron microscopy are most commonly used to describe hydrogel materials [2]. However, if we want to study complex samples where, for example, bacterial cells are encapsulated in a hydrogel network, we are also interested in their internal structures. In our work, we focused on designing a protocol for the chemical preparation of plant growth-promoting rhizobacterium *Azotobacter vinelandii* encapsulated in an alginate hydrogel for imaging in a low-voltage scanning transmission electron microscope (LV-STEM) so that not only the ultrastructure of the cells but also of the hydrogel is preserved.

*Azotobacter vinelandii* cells encapsulated in alginate hydrogel were cut to approx. 1mm$^3$ cubes and fixed using 2.5% glutaraldehyde and 2% formaldehyde solution. To stabilize the hydrogel ultrastructure, 1% solution of $CaCl_2$ was added to the following protocol steps. Samples were postfixed using 1% $OsO_4$ with 1% $K_3[Fe(CN)]_6$, and then stained by a commercial uranium-free staining reagent UA-zero. Methanol was used for the dehydration of the samples since one of the most common dehydrating agents acetone and ethanol do not provide either sufficient solubility of $CaCl_2$ or volatility for this type of porous specimen. Samples were embedded in Spurr's resin and cured for 48h at 62°C. Ultrathin sections were briefly contrasted with Reynold's lead citrate and imaged using a scanning electron microscope (Magellan 400/L, FEI) equipped with a STEM3+ detector.

The LV-STEM images showed cells of *A. vinelandii* surrounded by a polymer capsule, which consists of two parts. An electron-dense exine forms a filamentous structure and an electron-transparent intine surrounding the immediate vicinity of the cell. The polymeric alginate net was also observed, not only close to the cells but also in the outer areas of the sample. The results we obtained suggest that further efforts to optimize the preparation protocol for such complex samples are relevant. The focus of the following experiments could be given to exploring the effect of different $CaCl_2$ concentrations.

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Acknowledgement:

The authors acknowledge funding from GACR (project GA23-06757S). Microscopic analysis was provided by CF ISI EM which is supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

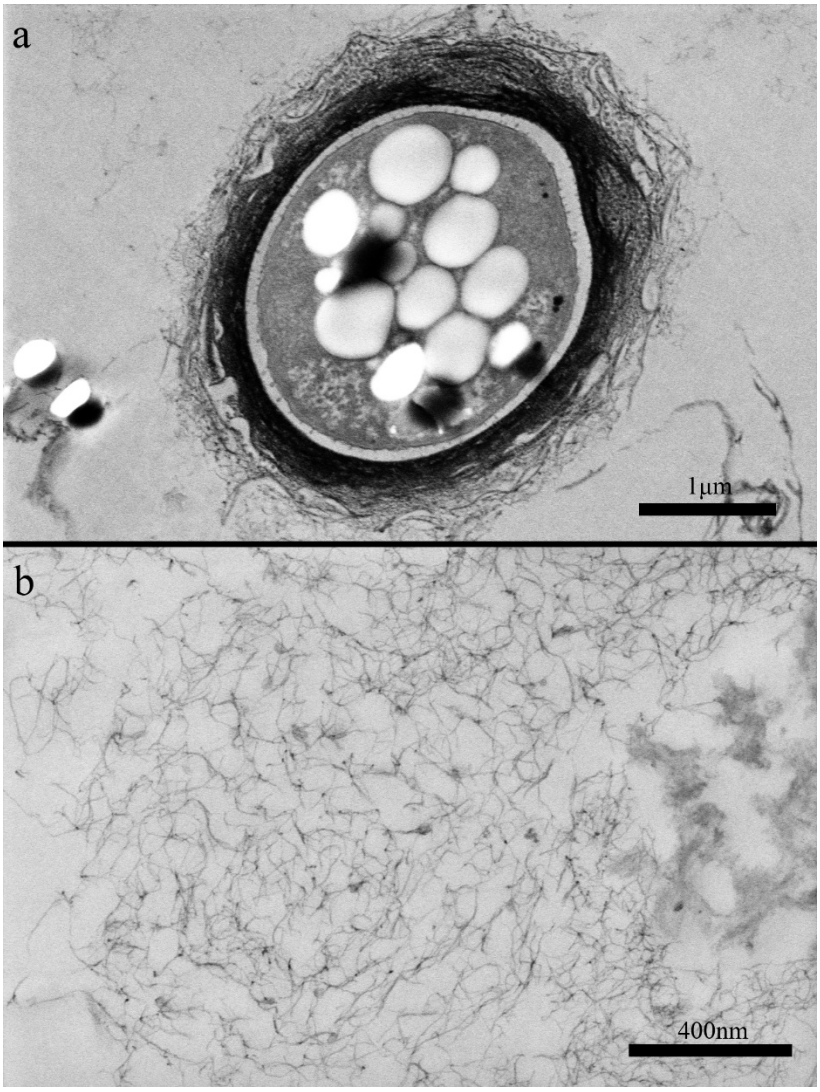


*Fig. 1: LV-STEM images of a) A. vinelandii cells encapsulated in the alginate-based hydrogel, b) detail of crosslinked alginate polymer net*

# Multimodal Submicron O-PTIR: Advancing Life Science Applications with Simultaneous IR, Raman, and Fluorescence Microscopy

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Introduction:

Traditional infrared (IR) microscopy has been limited by spatial resolution constraints and the requirement for contact-based techniques such as attenuated total reflection (ATR). Optical Photothermal IR (O-PTIR) spectroscopy overcomes these challenges, enabling true submicron spatial resolution in a non-contact reflection mode while maintaining FTIR transmission/ATR-like data quality. Additionally, it bridges the gap between conventional IR microspectroscopy and nanoscale IR spectroscopy. A groundbreaking advancement now allows for simultaneous IR and Raman measurements from the same sample location, offering complementary chemical information with identical spatial resolution. [1]

Materials and Methods:

This study utilizes an advanced O-PTIR system equipped with a counter-propagating modality that enhances spatial resolution (~300 nm) and sensitivity through high numerical aperture (NA) refractive objectives. Widefield epifluorescence was integrated to enable fluorescence-guided O-PTIR microspectroscopy. The latest development, Fluorescence Enhanced O-PTIR, utilizes fluorescence signals to facilitate widefield IR imaging, further improving IR sensitivity. Various biological samples, including live cells, hydrated tissues, and bacterial specimens, were analyzed using simultaneous IR, Raman, and fluorescence imaging.

Results:

O-PTIR demonstrated superior spatial resolution and chemical specificity across various life science applications. The technology enabled high-resolution imaging of lipid metabolism in live cells in aqueous environments, protein misfolding analysis in hydrated tissues, and stable isotope-labeled bacterial studies. Furthermore, intracellular polymer deposits in single bacterial cells were successfully characterized, showcasing the technique’s ability to provide molecular insights at the subcellular level.

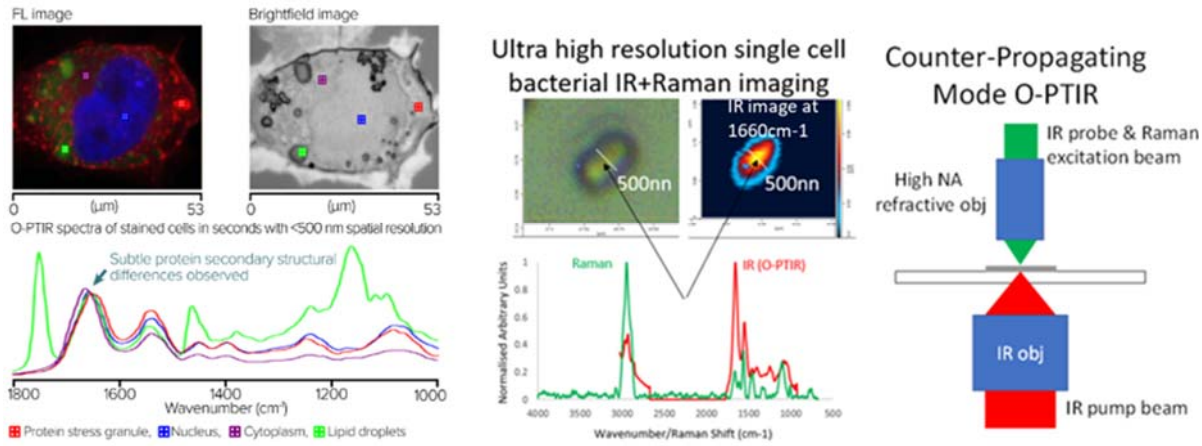


*Fig. 1: Multimodal High-Resolution Imaging with O-PTIR. (Left) Fluorescence and brightfield images of a stained cell analyzed with O-PTIR, providing chemical spectra at sub-500 nm resolution. (Middle) High-resolution IR and Raman imaging of a single bacterial cell, demonstrating the technique's ability to achieve submicron spatial resolution. (Right) Schematic of the counter-propagating mode O-PTIR, employing high NA refractive optics for improved resolution and sensitivity.*

Conclusion:

The integration of multimodal submicron IR, simultaneous Raman, and fluorescence microscopy represents a new paradigm in vibrational spectroscopy. O-PTIR's ability to achieve high spatial resolution, sensitivity, and multimodal imaging expands its potential applications in biological and biomedical research, providing unprecedented insights into cellular and molecular processes.

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[1] Prater C. et al.: APL Photonics 9 (2024), 091101.

# Interdisciplinary sciences

*Chairs: Eliška Materna Mikmeková and Aleš Benda*

# Image Analysis in the Age of Deep Learning

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Recent advances in deep learning are reshaping image analysis, particularly in time-lapse microscopy. Key challenges include image restoration, automatic segmentation, object detection, and tracking. Microscopy images often suffer from low signal-to-noise ratios and insufficient resolution, making restoration methods such as deconvolution and super-resolution essential preprocessing steps. Traditional model-based restoration is gradually being replaced by hybrid approaches that integrate pretrained neural networks to better encode prior knowledge.

Object detection and instance segmentation are now primarily performed using supervised deep learning. However, supervised methods require extensive training data, which can be difficult to obtain, especially for rare samples and unique microscopy setups. Recent developments in self-supervised learning offer promising alternatives that reduce reliance on large, labeled datasets.

Tracking dynamic processes remains a significant challenge due to the large number of small, similar objects, making multi-object tracking particularly difficult. We propose a globally optimal linking algorithm that combines independent detections into coherent tracks while respecting biological constraints.

We demonstrate our methods in the study of sub-cellular processes using super-resolution Structured Illumination Microscopy (Fig. 1) and highlight freely available tools that facilitate these analyses efficiently.

Acknowledgement:

The author acknowledges funding from the Czech Science Foundation (GAČR), project No. GA25-15933S.

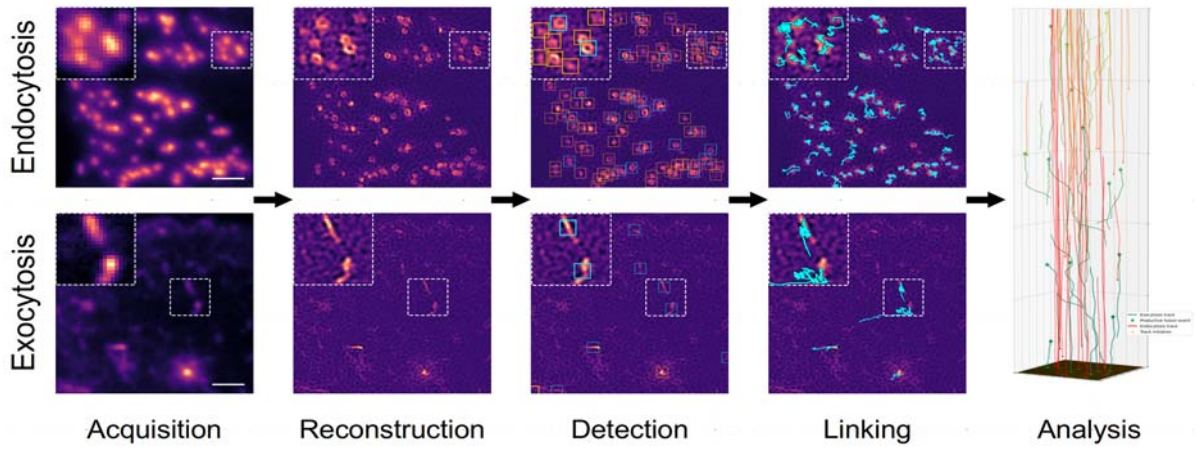


*Fig. 1: Super-Resolution High-accuracy Analysis of Plasma membrane Events*

# Correlative Microscopy Techniques Serving as Core Facility

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Scientific Core Facilities are essential hubs within research institutions, offering access to cutting-edge technologies, equipment, and expertise. They empower researchers and students through training, technical support, education, and consultation. Our facility specializes in bioAFM, i.e., atomic force microscopy applied on bio-samples, combined with advanced correlative microscopy techniques, such as optical and Raman microscopy, uniquely complementing the more common and established techniques.

By fostering interdisciplinary collaborations and emphasizing meticulous experimental planning, we have successfully applied force microscopy in diverse modes to achieve significant results in bio-related projects. Notable applications include imaging single molecules to elucidate their structure and interactions [1], exploring the mechanical properties of microenvironments on cellular behavior [2], assessing the effects of nanoparticles on cell growth and function [3], and investigating pathological phenomena such as liver fibrosis formation and treatment [4]. These studies have provided valuable insights into molecular mechanisms and cellular dynamics.

Our facility also integrates correlative approaches to combine complementary data from different modalities. For instance, hybrid techniques like AFM coupled with optical or Raman microscopy allow simultaneous acquisition of mechanical, structural, and chemical information. This multimodal strategy enhances our ability to analyze complex biological systems at multiple scales.

Figure 1 highlights selected applications: (A) imaging long RNA structures using AFM, (B) studying cell morphology changes due to substrate stiffness, and (C) visualizing collagen fiber formation in liver tissue slices. These examples underscore the versatility of our methods in addressing a wide range of scientific challenges.

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Acknowledgement:

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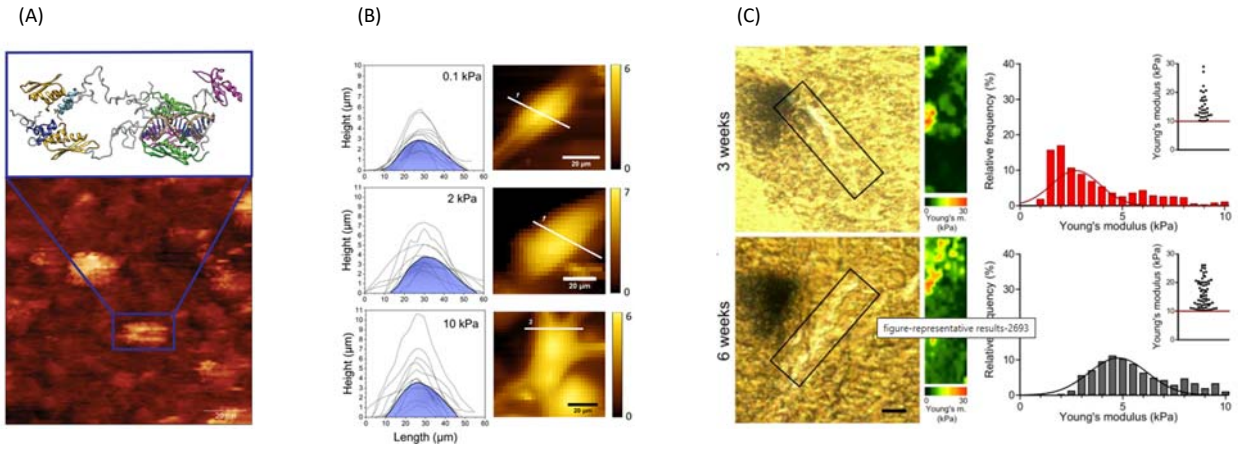


*Fig. 1. An example of the AFM application in the investigation of long RNA structure (A, adopted from [1]) is cell morphology changes affected by the different substrate stiffness (B, adopted from [2]) and collagen fibers formation in liver tissue slices (C, adopted from [4]).*

# Microscopy, Characterization, and Mechanistic Studies at VSB-TUO: From Graphene to Fusion Plasmas

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Advanced microscopic methods play a pivotal role in interdisciplinary research. The ability to correlate spatial information from multiple analytical methods is crucial for understanding material formation mechanisms and functional properties across diverse applications – including catalysis, energy storage, and/or photonic materials.

The Characterization and Mechanistic Studies (CMS) group operates as a core facility within the Materials-Envi Lab (MEL) at CEET, VSB-TUO, addresses a broad spectrum of scientific challenges, spanning from energy and catalysis to biomedicine and environmental technology [1-6]. In particular, the CMS infrastructure houses two advanced electron microscopes.

The first instrument is the Verios 5 UC (Thermo Fisher Scientific), an ultrahigh-resolution scanning electron microscope equipped with a monochromated field emission gun (UC+), delivering sub-nanometer resolution over an accelerating voltage range of 1–30 kV. It is equipped with an energy-dispersive X-ray spectroscopy (EDS) detector, enabling high spatial resolution compositional analysis.

The second instrument, the Helios 5 CX DualBeam (Thermo Fisher Scientific), was installed at VSB-TUO on March 17, 2025 (Fig. 1). This cutting-edge focused ion beam – scanning electron microscope (FIB-SEM) integrates an Elstar electron column for high-resolution imaging and a Tomahawk HT ion column for precision-focused ion beam milling. It is further enhanced with EDS and electron backscatter diffraction (EBSD) detectors (Oxford Instruments), facilitating simultaneous structural and crystallographic analysis.

The capabilities of this instrumentation are exemplified through the analysis of graphite samples coated with a protective tungsten layer; a material relevant for divertor components in fusion reactors. Changes induced by plasma exposure in the Wendelstein 7-X stellarator [7] were characterized using a combination of different electron detectors and EDS analysis on the Helios 5 CX system. The results highlight the power of correlative electron microscopy in understanding material transformations in a complex reactive environment (Fig. 2). The micrograph shows a vertical cross-section of the tungsten layer and the graphite substrate created with a gallium ion beam. The cross-section was made before the experiment in W7-X. After the experiment, the image captures the precipitation of residual gallium from the cross-section surface due to plasma exposure. The tungsten coating remained microscopically stabile [8].

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Acknowledgement:

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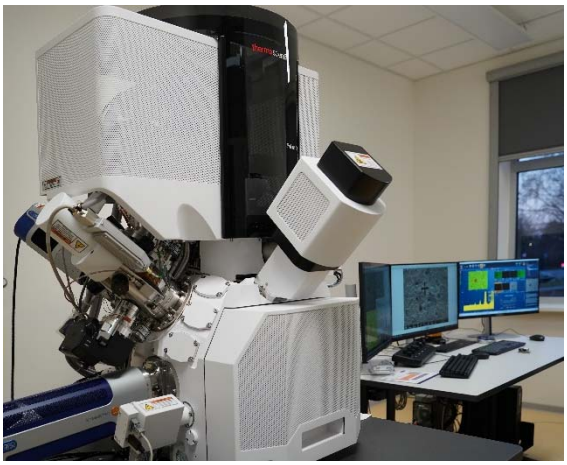

*Fig. 1: FIB-SEM Helios 5CX DualBeam at VSB-TUO.*

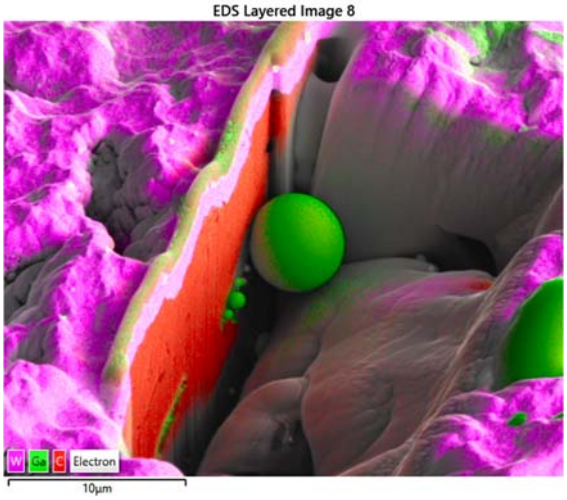


*Fig. 2: EDS chemical mapping of graphite-tungsten system analyzed by means of FIB-SEM Helios 5CX DualBeam.*

# Material sciences

*Chairs: Mariana Klementová and Miroslav Šlouf*

# Application of Scanning Electron Microscopy for Material Research at the Technical University of Liberec

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In 2004, Professors Oldrich Jirsak and David Lukas from the Technical University of Liberec made a groundbreaking contribution to the field of nanotechnology by patenting a method of electrostatic spinning of polymer solutions, widely known as electrospinning. This innovation led to the development of the Nanospider™ device, enabling the industrial production of nanofibers. Its successful transfer into commercial practice had a significant impact on the development of the textile industry, medical materials, and advanced filtration systems. The subsequent boom in nanomaterials research culminated in the establishment of the Institute for Nanomaterials, Advanced Technologies and Innovation (CxI) in 2009, which today serves as one of the university's key research centers.

One of CxI's fundamental research pillars is material characterization, where scanning electron microscopy (SEM) plays an indispensable role. At TUL, SEM is employed not only for routine morphological investigations but also for advanced characterization of complex nanostructures and interfaces. The microscopy laboratory at CxI is equipped with state-of-the-art instruments, providing high spatial resolution imaging, detection of secondary backscattered and transmitted electrons, analytical mapping using energy-dispersive X-ray spectroscopy (EDS), wavelength-dispersive spectroscopy (WDS), electron backscattered diffraction (EBSD), and other advanced techniques like focused plasma ion beam milling (PFIB).

SEM significantly contributes to research in areas such as:

- **Nanofibrous structures** – optimization of fiber morphology, diameter, surface modifications, and layered composites.
- **Thin films and coatings** – analysis of layer continuity, homogeneity, and adhesion in coatings prepared by methods such as magnetron sputtering and ALD.
- **Porous and 3D structures** – characterization of aerogels, metallic foams, and other ultra-low-density materials designed for thermal insulation or acoustic applications.
- **Nanoparticles and functional surfaces** – evaluation of particle dispersion, shape, and size, essential for the development of catalysts, sensors, and antibacterial surfaces.

Through the integration of SEM with complementary techniques (such as EDS, WDS, and EBSD), it is possible to not only visualize morphology but also perform detailed analysis of chemical composition and crystallography. This comprehensive characterization provides researchers with the feedback necessary to optimize the preparation and modification processes of nanomaterials.

The presentation introduces selected applications of SEM techniques in research projects conducted at TUL. It demonstrates morphological and analytical studies that have served as a foundation for the development of new materials, including characterization of nanoparticles, composite nanofiber membranes for filtration, bioactive surfaces for tissue engineering, thin layers enhancing tribological and mechanical properties and porous structures with unique mechanical and thermal insulation properties.

Scanning electron microscopy represents a key tool without which it would not be possible to efficiently guide and advance the development of next-generation materials within the research activities at the Technical University of Liberec.

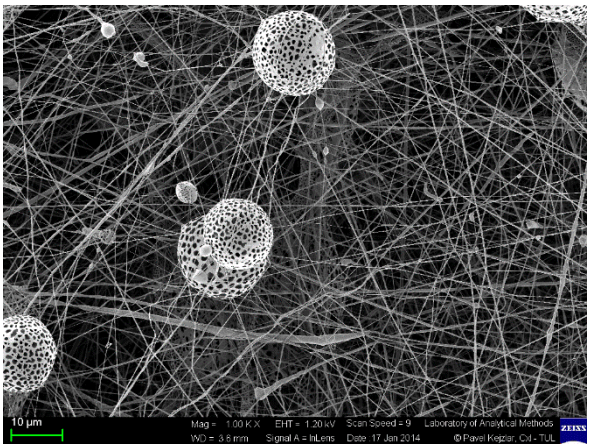


*Fig. 1: Electrospunned nanofibrous membrain with defects caused by low viscosity of the polymer solution*

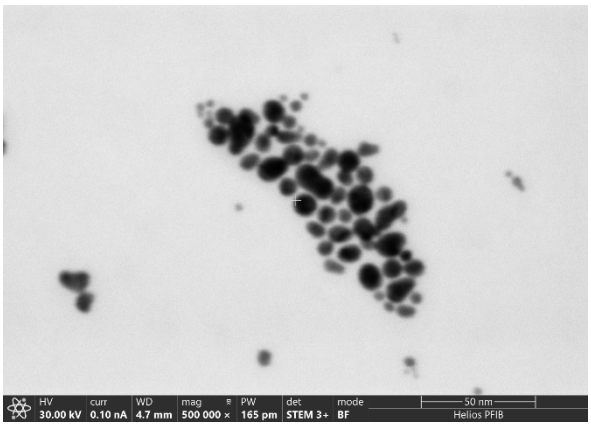


*Fig. 2: Gold nanoparticles produced by femtosecond laser beam*

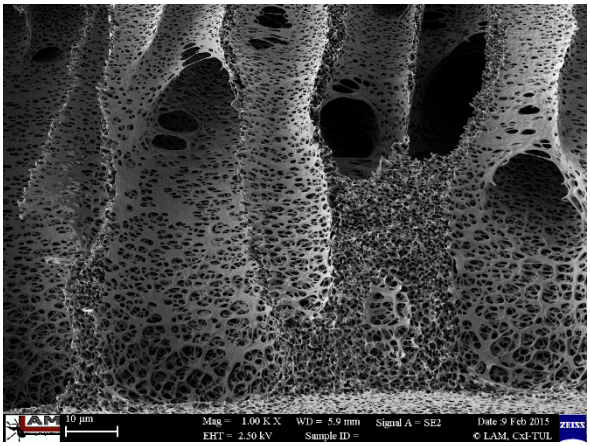


*Fig. 3: Structure of nanoporous membraine used for water filtration*

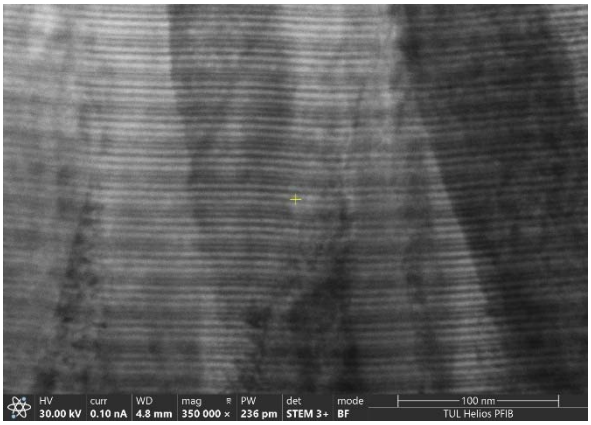


*Fig. 4: STEM image of lamella prepared by PFIB showing AlTiCrN multilayer*

Acknowledgement:

The authors wish to express their gratitude to the bees for tirelessly producing honey, and to all the coffee growers whose work keeps our research alive and the authors functional.

# Structural changes in 3D printed L-PBF IN939 due to thermomechanical fatigue

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Materials outstanding high temperature applications including mechanical loading are a specific group of materials characteristic by their complex structure and quite high price. Methods of additive manufacturing allow preparation of specific material with tailored properties via varying mechanical composition and internal structure as a result of processing parameters while controlling the costs as a waste free approaches. Reached specific structure, however, has usually a different response to the loading compared to the conventionally prepared materials.

Inconel 939 powder with a particle size of 20 – 55 μm was processed via laser powder bed fusion (L-PBF) technique using EOSM 400 printing machine with Yb-fiber laser adopting optimized parameters of printing. The printed material was subjected to a three-step heat treatment procedure that triggered the gamma prime (γ') strengthening phase precipitation with bimodal size distribution. The heat treatment consisted of solution annealing at 1175 °C/45 min and precipitation hardening at 1000 °C/6 h + 800 °C/4 h. The typical microstructure of the experimental material can be seen in Fig. 1. TEM micrograph can be seen in Fig. 2, and a diffraction pattern of the present structure providing crystallographic information about the face-centred cubic (fcc) γ matrix and fcc γ' strengthening precipitates. MC carbides and eutectics were present in the microstructure of the material as well as can be seen in Fig. 1. Preferential crystallographic orientation of the 3D printed elongated microstructure was <001> (parallel to the printing direction). The characteristic grain size was 14.8 ± 13.3 μm in a section parallel to the building direction with an aspect ratio of around 8, while the average size of the γ′ precipitates is 16.3 ± 7.99 nm for the fine particles and 159.44 ± 62.37 nm for the large precipitate particles. The TEM analysis showed a γ' precipitate area fraction of 50.2 ± 3.5%.

Cylindrical specimens with a gauge length of 16 mm and a diameter of 7 mm were used for thermomechanical fatigue testing. In-phase (synchronized mechanical and thermal loading) and out-of-phase (opposite mechanical and thermal loading) testing loops were used considering the relation between the thermal and mechanical loading. Tests were performed at temperatures ranging from 400 °C to 800 °C with a constant heating and cooling rate of 10 °C/s, giving a cycle time of 80 s. MTS 880 servo-hydraulic system was used for material testing, measuring the total strain by a high-temperature extensometer. During the cycle, the specimens were heated by a copper coil induction system and the cooling was realised by thermal conduction into the water-cooled grips and air nozzles for the specimens.

To compare the material response to the loading at several levels of strain amplitude $\varepsilon_a$, both stress amplitude $\sigma_a$ and mean stress $\sigma_m$ were plotted into one graph against the number of cycles $N$. The results can be seen in Fig. 3 for the in-phase and in Fig. 4 for the out-of-phase loading. From the plots, different characters of the material response can be seen. While the cyclic softening can be seen for the in-phase loading, the increase in the stress indicating cyclic hardening for the out-of-phase can be seen.

Different loading condition and alternation of mechanical and thermal loading has a different impact on material structure. Cyclic softening observed for in-phase loading is a result of slip activity across to primary cell structure and coarsening of the γ' strengthening precipitates. Usually, the MC carbides remained intact. The particle cutting was rarely observed and is usually associated with high strain amplitudes. On the other hand, the cyclic hardening observed for the out-of-phase loading can be

attributed to the higher stability of the microstructure at this condition (the highest stress at the lowest temperature during the cycle), where the dislocation climbing can be neglected.

Acknowledgement:

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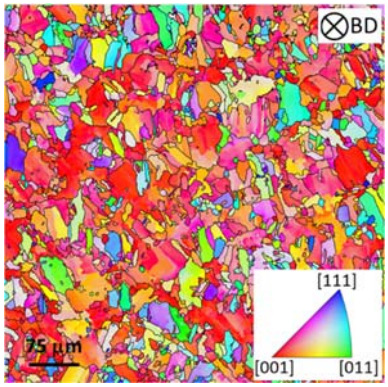


*Fig. 1: Typical structure of the material with preferential crystallographic orientation, SEM EBSD*

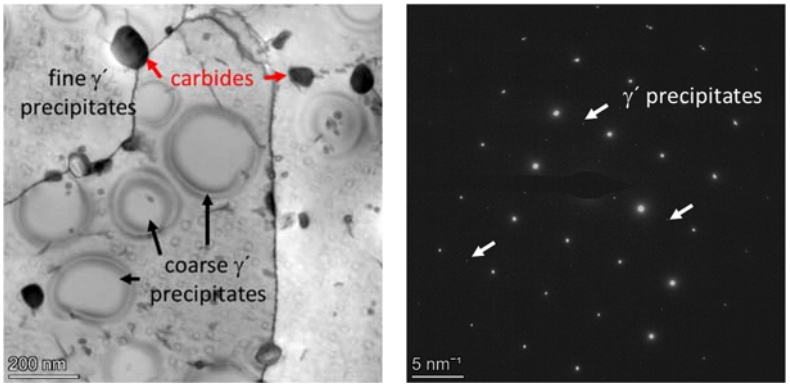


*Fig. 2: Structure of the material before loading, STEM-BF and diffraction pattern matrix and of γ´ precipitates*

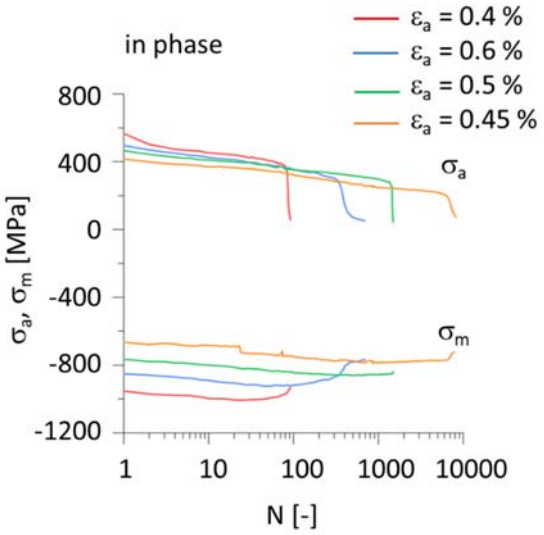


*Fig. 3: In-phase loading hardening/softening curves*

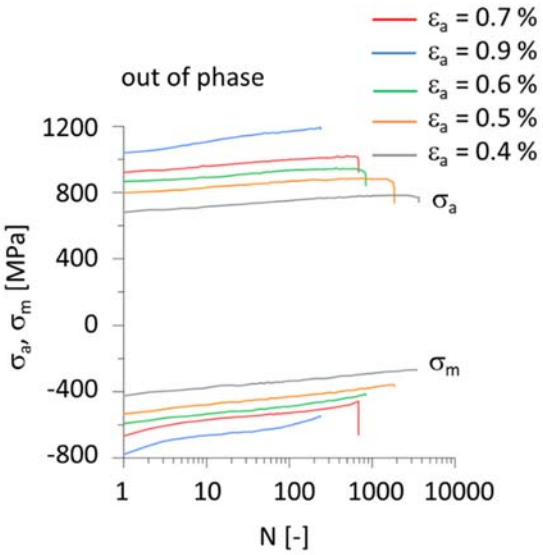


*Fig. 4: Out-of-phase loading softening/hardening curves*

# In-situ electron microscopy of nanoparticles

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Self-propagating High-temperature Synthesis (SHS) is a relatively novel and straightforward method for producing advanced ceramics, composites, and intermetallic compounds [1]. SHS reactions have also been observed in Ni-Ti multilayer films [2], possessing a great potential as an external heat source in a wide area of applications, including joining, ignitors, and intermetallic synthesis on the nanoscale. A novel approach uses nanoparticles to achieve similar self-propagating reactions on a nanoscale. This concept is up-and-coming due to the ease of application of nanoparticles on various surfaces. Core-shell nanoparticles, especially those with a titanium (Ti) core and a nickel (Ni) shell, are of significant interest. This configuration ensures a stable Ni to Ti ratio while the Ni shell protects the Ti core from oxidation, preserving its reactive properties. However, several studies on bulk Ni-Ti systems show that preheating and rapid ignition temperature increases are necessary to start the combustion [3]. Low heating rates, on the other hand, limit the SHS and inhomogeneous structure with separated Ni and Ti forms. The thermal stability of Ti-Ni core-shell nanoparticles produced using a gas aggregation source were studied. The thermal behavior of the composite was examined through slow in-situ heating within the transmission electron microscope (TEM), allowing for real-time observation of morphological and phase composition changes and identification of mechanisms controlling the separation of Ni and Ti.

DC magnetron sputtering with two planar magnetrons was used to prepare Ti@Ni core-shell nanoparticles and a mixture of Ni and Ti nanoparticles. The sputtering system consists of a primary gas aggregation cluster source (GAS) for producing Ti nanoparticles (NPs) and a secondary chamber for coating them with a Ni film. The samples were prepared for TEM analysis by depositing the nanoparticles, suspended in methanol, onto a grid with a SiN support film. A Jeol 2200FS transmission electron microscope operated at 200 kV was employed in STEM mode, equipped with bright field (BF), high-angle annular dark field (HAADF), and secondary electrons (SEI) detectors and energy dispersive spectroscopy (EDS).

The formation of core-shell nanoparticles with Ni shell and Ti core was achieved by adjusting the current on both magnetrons. This core-shell structure was confirmed by STEM and EDS analysis (Figure 1). Ti@Ni core-shell nanoparticles were annealed up to 900 ˚C (Figure 2). Sintering and formation of large particles occur at 700 ˚C. The sintering further continues with increasing temperature. EDS maps (Figure 3) show that the largest particles contain only Ni. The initially core-shell nanoparticles lose their Ni shells, which sinter into new, above 100 nm large Ni particles. Meanwhile, the first signs of Ti cores sintering could be found.

During the annealing process, the sintering of Ni nanoparticles via surface diffusion occurs as they seek to minimize surface energy, resulting in the formation of larger Ni nanoparticles. This aggregation leads to the separation of Ni and Ti, thereby preventing the occurrence of the SHS reaction.

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Acknowledgement:

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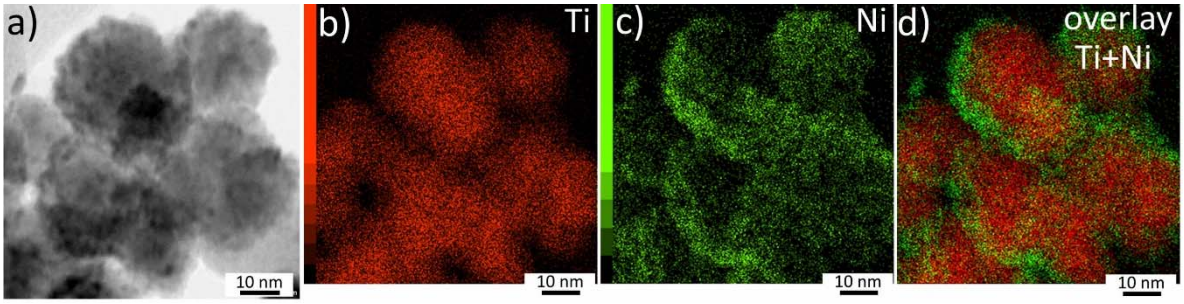


*Fig. 1: Ti@Ni core-shell nanoparticles: a) STEM BF, b-d) EDS maps.*

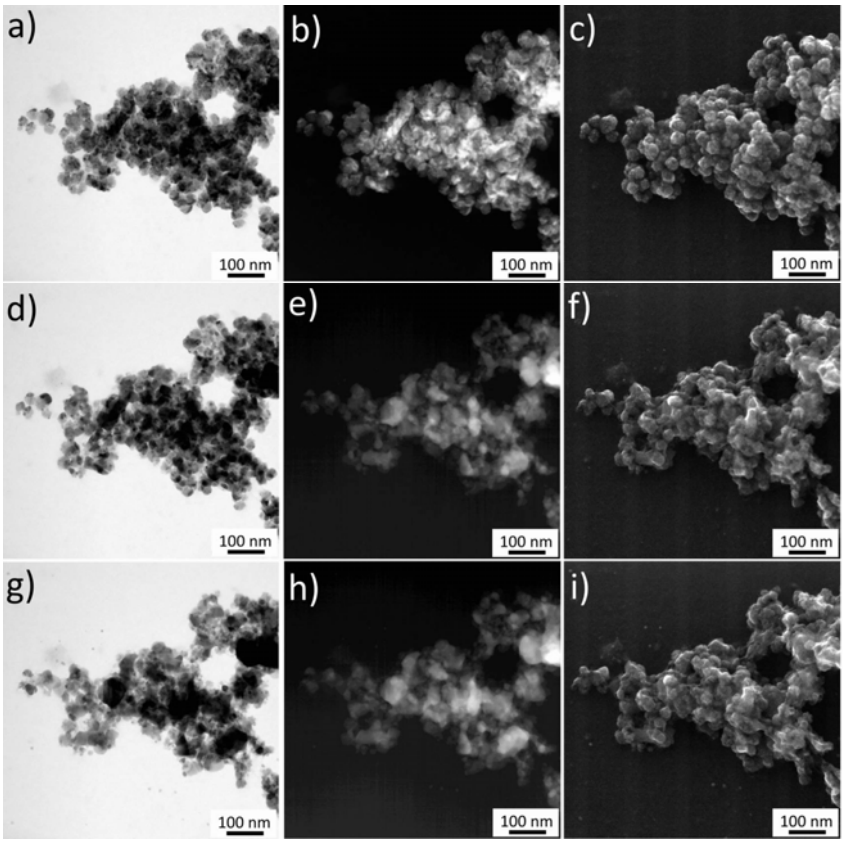


*Fig. 2: Annealing of Ti@Ni core-shell nanoparticles: a-c) RT, d-f) 700 ˚C, g-i) 900 ˚C, a,d,g) STEM BF, b,e,h) STEM HAADF, c,f,i) STEM SEI.*

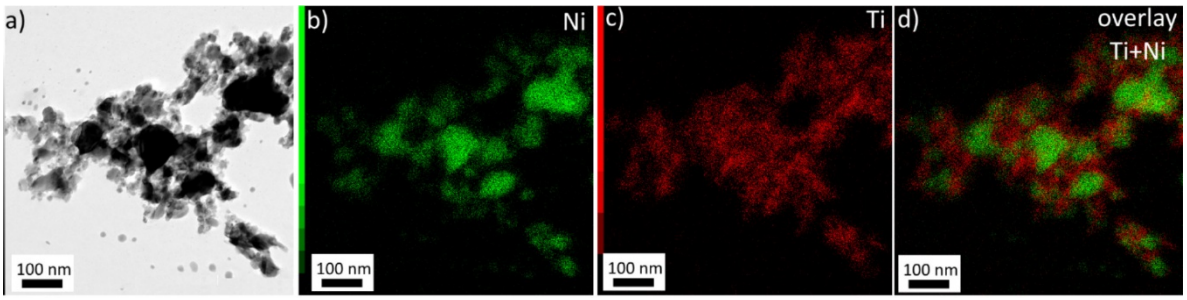


*Fig. 3: Annealed Ti@Ni core-shell nanoparticles after cooling to room temperature: a) STEM BF, b-d) EDS maps*

# In Situ and Ex Situ TEM Analysis of Al-Al2Cu Heterogeneous Nanostructures: Insights into Phase Stability and Diffusion

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Nanofabrication enables the precise design and manipulation of materials at the nanoscale, allowing for the controlled synthesis of structures with tailored properties for advanced technological applications [1]. Fabricating Al-$Al_2Cu$ nanostructures is crucial for advancing aluminum-based energy storage systems, particularly aqueous aluminum batteries [2]. These batteries offer a safer and more environmentally friendly alternative to lithium-ion batteries due to their use of water-based electrolytes. However, challenges such as the formation of oxides on aluminum anodes, hydrogen evolution reactions, and dendrite growth limit their efficiency and lifespan. The incorporation of $Al_2Cu$ nanolamellae within an aluminum matrix can mitigate these issues by providing a more stable and less reactive anode material. The geometry of $Al_2Cu$ nanolamellae plays a crucial role in battery performance, and precisely controlled nanostructures can be achieved through a combination of magnetron sputtering and controlled annealing.

In situ transmission electron microscopy (TEM) is a powerful tool for understanding the thermal stability and phase evolution of Al-$Al_2Cu$ nanostructures during annealing. By enabling real-time observation of structural changes at the nanoscale, in situ TEM provides crucial insights into diffusion processes, phase transformations, and the formation of intermetallic compounds. This technique allows researchers to fine-tune annealing conditions, such as temperature and duration, to achieve the desired phase composition and microstructure.

In in-situ TEM studies, the thickness of the sample can significantly impact diffusion behavior, leading to variations in observed phase transformations and microstructural changes [3]. Thinner samples generally exhibit higher diffusion coefficients due to the increased surface-to-volume ratio, which facilitates faster atomic migration. This can lead to accelerated phase formation or grain growth compared to bulk materials. As a result, the annealing conditions optimized for thin samples may not directly translate to bulk structures. Understanding these effects is crucial for accurately interpreting experimental results.

The Al-Cu nanostructures were produced using a dual-chamber system. A primary gas aggregation cluster source (GAS) was employed to generate Cu nanoparticles (NPs), while an arrow-shaped secondary chamber was used to coat the Cu NPs with a thin Al film. The specimens were produced by alternating deposition of pure Al and Al together with Cu nanoparticles (Fig. 1).

The specimens were characterized by a JEOL 2200FS TEM operated at 200 kV using scanning TEM (STEM) mode with bright field (BF), high angle annular dark field (HAADF) and secondary electron (SE) detectors (Fig. 2a) combined with energy-dispersive X-ray spectroscopy (EDX) mapping. The annealing of the specimen was done in situ in the same microscope using Gatan heating holder. Moreover, high resolution (HRTEM) images were captured as well as phase orientation maps using automated orientation phase mapping (ACOM-TEM) with the JEOL 2200FS TEM equipped with "Spinning Star" electron precession with an ASTAR software package. Cross sectional lamellae were prepared by a focused ion beam in a Zeiss Auriga scanning electron microscope (Fig. 2b).

In situ and ex situ annealing experiments were performed on thin films and lamellae with varying Al-Cu compositions to study phase formation and stability. In thin films, both methods yielded similar results, leading to the formation of the $Al_2Cu$ phase inside Al matrix (Fig. 3). Other phases can be formed depending on the initial Al:Cu ratio, the heterogeneous nanostructures are then reached by deposition of additional Al layers. However, for lamellae, ex situ annealing followed by TEM analysis proved to be a more reliable approach, as it minimized aluminum loss and better preserved the intended layer structure.

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Acknowledgement:

The authors would like to thank the support from the Grant Agency of Charles University under project 280223.

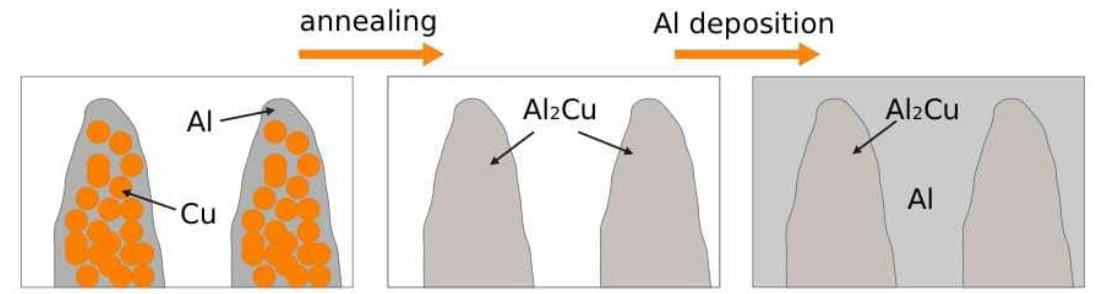


*Fig. 1: Schematics of the deposition and annealing process.*

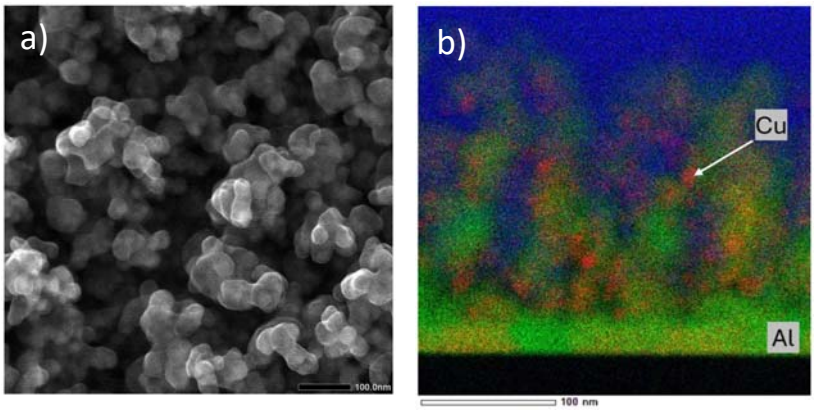


*Fig. 2: (a) EDS of the deposited lamella, (b) STEM SE image of the deposited film*

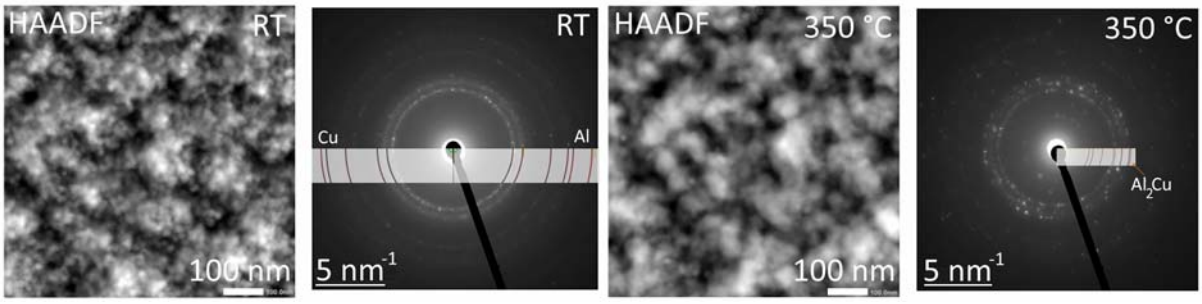


*Fig. 3: Phase transformation between room temperature and 350 C, STEM HAADF and SAED diffraction.*

# Atomic resolution DPC-STEM imaging of magnetic signal in antiferromagnets and beyond

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Efficient manipulation of antiferromagnetic domains (AFD) and domain walls has opened up new avenues of research towards ultrafast, high-density spintronic devices [1,2]. AF domain structures are known to be sensitive to magnetoelastic effects, but the microscopic interplay of crystalline defects, strain, and magnetic ordering remained largely unknown. The previous study of antiferromagnetic CuMnAs epilayers by X-ray photoemission electron microscopy (XPEEM) imaging revealed that its AFD structure is dominated by nanoscale crystalline defects [3]. The results emphasized the crucial role of these defects in determining the AFDs and domain walls and provided a route to optimizing device performance in terms of scaling limits for the data density in the bulk of the antiferromagnet. However, even smaller magnetic objects were indirectly observed in the material, but they remained below the detection limits of XPEEM.

Here, atomic resolution imaging of abrupt AFD walls in CuMnAs epilayers is achieved by utilizing scanning transmission electron microscopy (STEM), differential phase contrast (DPC), and 4D-STEM techniques [4]. The magnetic domain DPC signal was identified based on the CuMnAs crystal's specific symmetry, where the opposite magnetic Mn sublattices occupy crystallographically distinct noncentrosymmetric sites. With a focus on small field-of-view high-resolution imaging, the DPC-STEM signals could be associated with two types of abrupt Néel vector reversals: The first type occurs at a crystallographic antiphase boundary defect, while the second type forms in a part of the epilayer with no crystallographic perturbation detectable by STEM.

The DPC-STEM magnetic signal was reconstructed by calculating the centre of mass (COM) shifts of ronchigrams recorded with a pixelated or 4-segment detector. The signal intensities were plotted as a function of the integrated [001] component of the COM shifts corresponding to the direction of the Lorentz force due to sublattice magnetic Mn moments pointing in the (001) easy plane of antiferromagnetic CuMnAs aligned to the [100] zonal axis. This qualitative assessment of the magnetic order in Mn sublattices was paradoxically possible in substantially thick TEM lamellae, above 50 nm, and with small collection angles, below 10 mrad, since dynamical diffraction effects can be advantageous for magnetic imaging, as revealed by DFT Pauli multislice STEM-DPC simulations [4].

The observation of AFD walls in CuMnAs sheds light on the physics and engineering of spintronic devices with potential in neuromorphic and ultrafast optical applications. The novel application of STEM-DPC can play a crucial role in atomically resolved magnetic signal imaging in studies of other materials with similar symmetries, including α-MnTe, in which altermagnetism has recently been imaged by XPEEM [5]. Here, the applied DPC technique is additionally utilized to detect magnetic DPC signals from the collinear AF magnetic moments of Mn sublattices in the α-MnTe epilayer using the segmented DPC detector.

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# Instrumentation and optics

*Chairs: Dušan Chorvát and Vladislav Krzyžánek*

# Atomic-resolution SE imaging and other developments in instrumentation for electron microscopy

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Instrumentation for electron microscopy has progressed remarkably in the last two decades.

Aberration correction has become commonplace, allowing the atomic arrangements and the bonding properties in many materials to be explored in great detail, both by conventional transmission electron microscopy (CTEM) and especially by scanning transmission electron microscopy (STEM).

Fast 2-dimensional (2D) detectors that register single incoming electrons with DQE > 0.8 and allow a dynamic range greater than $10^7$:1 have become widely available, and have revolutionized electron microscopy, diffraction and spectroscopy in both physical and biological sciences.

Electron Energy Loss Spectroscopy (EELS) in the electron microscope has progressed to 3 meV energy resolution, i.e. no longer orders of magnitude worse than the closely related infrared (IR) absorption spectroscopy, and surpassing IR by orders of magnitude in spatial resolution. It has allowed the vibrations of single atoms to be studied in ultrathin materials, and the vibrational properties of inclusions and defects such as grain boundaries to be explored with unprecedented spatial resolution.

After decades of being seen as a "medium resolution" technique, scanning electron microscopy (SEM) has achieved atomic resolution, first at 200 keV primary energy [1], and more recently at 30-100 keV [2]. It has resolved carbon atoms separated by 1.42 Å in graphene, shed light on the origin of the secondary electron (SE) signal, and allowed the atomic structure of entrance and exit faces of monolayer $MoS_2$ to be imaged simultaneously (Fig. 1). Imaging twisted bilayer $MoS_2$ has allowed the escape probability of SE electrons originating in sub-surface layers to be quantified [2].

These capabilities have been complemented by stable sample stages that can reach temperatures below 10 K [3] while attaining better than 1 Å spatial resolution. They open new vistas on low temperature properties of quantum materials, and may lead to reduced radiation damage for biological samples [4].

My presentation will review the broad progress, and then focus on atomic resolution SE imaging, plus STEM and EELS carried out at <10 K, which have all been recently realized in our laboratory.

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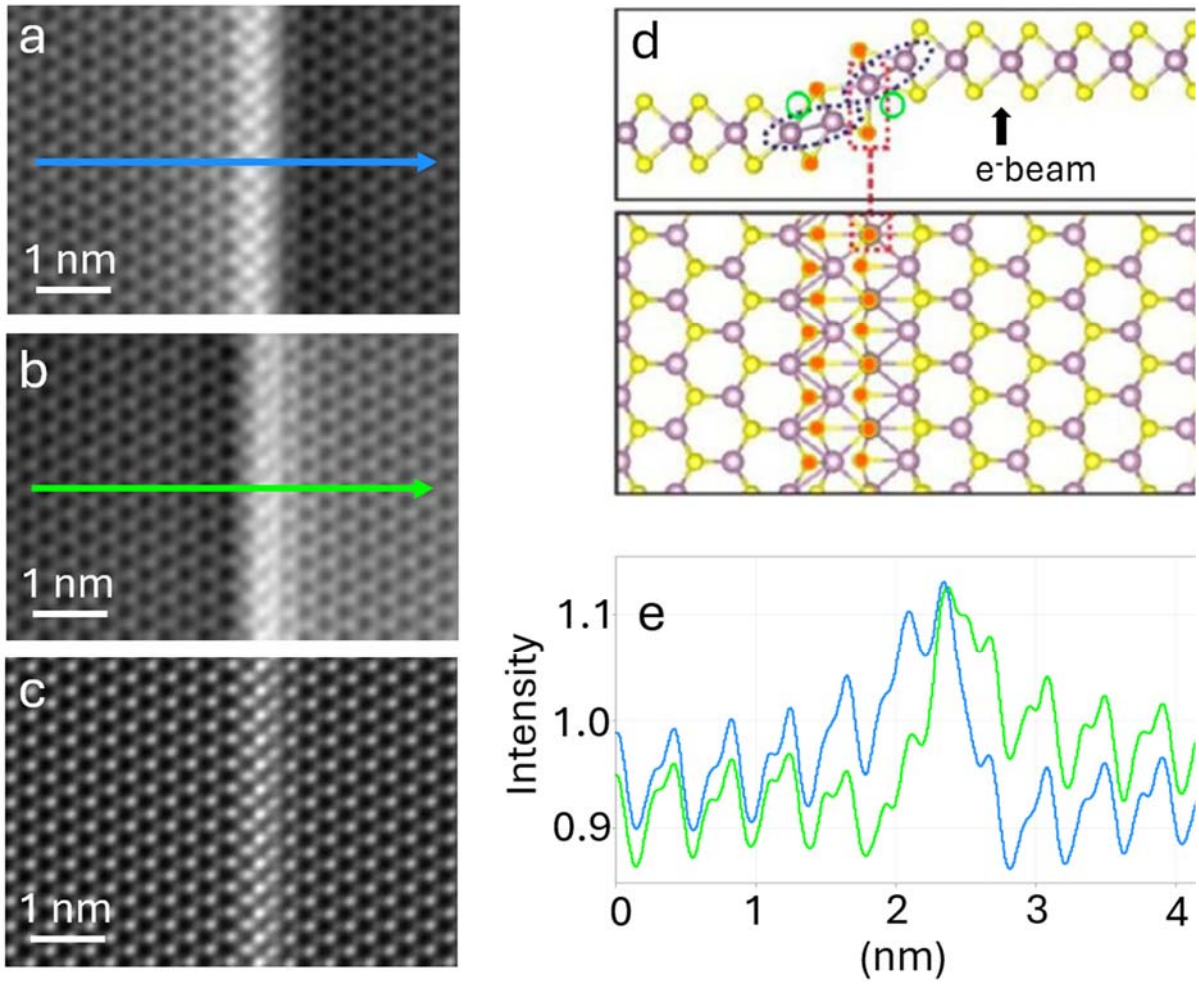


*Fig. 1. A line defect in $MoS_2$ imaged with entrance-face and exit-face secondary electron (SE) detectors simultaneously. a) entrance face SE image, b) exit face SE image, and c) medium-angle annular dark field (MAADF) image. The line defect is believed to have an out-of-plane "buckled" structure as shown in d), which was adapted from reference [5]. e) Line profiles through the entrance (blue) and exit face (green) SE images. Courtesy EPJAP [2] and my colleagues at Bruker.*

# Semi-automated processing of 4D-STEM diffraction data with open-source Python packages STEMDIFF and EDIFF

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We describe recent improvements of our method named 4D-STEM/PNBD (powder nanobeam diffraction in four-dimensional scanning transmission electron microscopy). The method can change an SEM microscope equipped with a 2D-array STEM detector to a user-friendly powder electron diffractometer. The whole process of obtaining and interpreting powder electron diffraction pattern in SEM consists of three steps, which are illustrated in Fig. 1 and described below:

1. Collection of 4D-STEM dataset with a quasi-parallel scanning beam from a region of interest in the specimen. This step is easy with modern hardware (SEM equipped with an array detector) and software (graphical user interface of modern microscopes). The resulting dataset may be huge (several gigabytes of data), but its collection is easy and reasonably fast (from tens of seconds to a few minutes).
2. Conversion of the huge and complex 4D-STEM dataset to a single 2D-powder electron diffraction pattern, by combining the individual nanobeam diffraction patterns (NBD). This step is surprisingly challenging, as we have to maximize signal-to-noise ratio by clever algorithms such as multiparameter filtering of the individual NBDs. However, the 4D-STEM → 2D-diffractogram conversion can be automated by means of our open-source program package STEMDIFF (https://pypi.org/project/stemdiff).
3. Final processing of 2D powder electron diffraction pattern, which is quite easy even for non-crystallographers, comprising three sub-steps: (i) conversion of the experimental 2D-difractogram to 1D-diffraction profile, (ii) calculation of theoretical powder X-ray diffraction pattern of the expected crystal structure, and (iii) comparison of the 1D experimental and theoretical diffraction profiles. If the two profiles overlap, we have successfully identified the investigated nanocrystalline material. The 2D-diffractogram → 1D-profile conversion and processing are automated in our open-source EDIFF package (https://pypi.org/project/ediff).

This contribution includes a live demo, demonstrating that the recent improvements of both packages (STEMDIFF and EDIFF) enable fast, user-friendly, semi-automated processing of 4D-STEM datasets. Thanks to some behind-the-scenes processing tricks, such as estimating the shape of primary beam followed by 2D-PSF deconvolution of the individual NBDs, the 4D-STEM/PNBD results can be even better than those from standard TEM/SAED experiments.

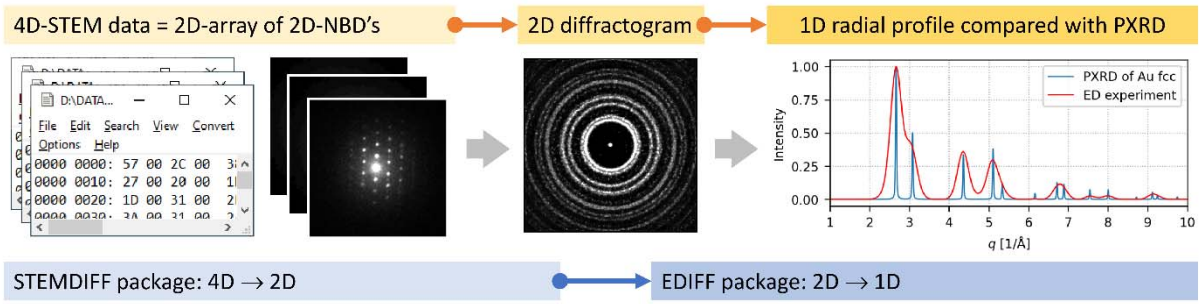


*Fig. 1. Principle of 4D-STEM/PNBD method: the conversion of a 4D-STEM dataset to a powder electron diffraction pattern and its comparison with theoretically calculated X-ray diffractogram.*

Acknowledgement:

Technology agency of the Czech Republic, program NCK2, project TN02000020.

# Characterization of perovskite single-photon emitters and solar cells by 4D-STEM in an FIB-SEM

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Four-dimensional scanning transmission electron microscopy (4D-STEM) is a characterization technique that allows high-resolution imaging of thin samples while providing structural and crystallographic information. We have developed a 4D-STEM solution for scanning electron microscopes based on the Tescan Amber dual beam microscope, that enables lamella fabrication and analysis without exposing the lamella to air. This technique has been used to analyze low-dimensional lead halide perovskites and perovskite solar cells [1].

We resolved individual $CsPbBr_3$ nanocrystals with high contrast and determined their lattice constant reading $(0.57 \pm 0.02)$ nm, which is in perfect agreement with previous measurements by high-resolution transmission electron microscopy [2,3]. Moreover, we mapped the crystallographic orientation of individual nanocrystals (see Figure 1) to locate well-ordered superlattice ensembles of nanocrystals, which are considered to be a platform for single-photon emission.

In the case of perovskite solar cells, we observed a nondegenerate tetragonal methylammonium lead iodide ($MAPbI_3$) photoactive layer and evaluated the quality of its interface with the electrodes. Moreover, we documented the amorphization and subsequent degradation of the methylammonium bismuth iodide ($MA_3Bi_2I_9$) photoactive layer when exposed to atmospheric conditions.

To conclude, our results obtained in a scanning electron microscope are comparable to those achievable with a high-tech transmission electron microscope, which is a much more sophisticated and expensive instrument. The application of 4D-STEM in the FIB-SEM enables the expeditious identification and exhaustive characterization of lead halide perovskite nanocrystal ensembles and perovskite photoactive layers, thereby reducing the time required for these processes. This efficiency is generally valuable for large-scale production and quality control of materials manufactured on an industrial scale.

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Acknowledgement:

This research has been supported by the Technology Agency of the Czech Republic (FW06010396) and the Czech Science Foundation (25-17500S).

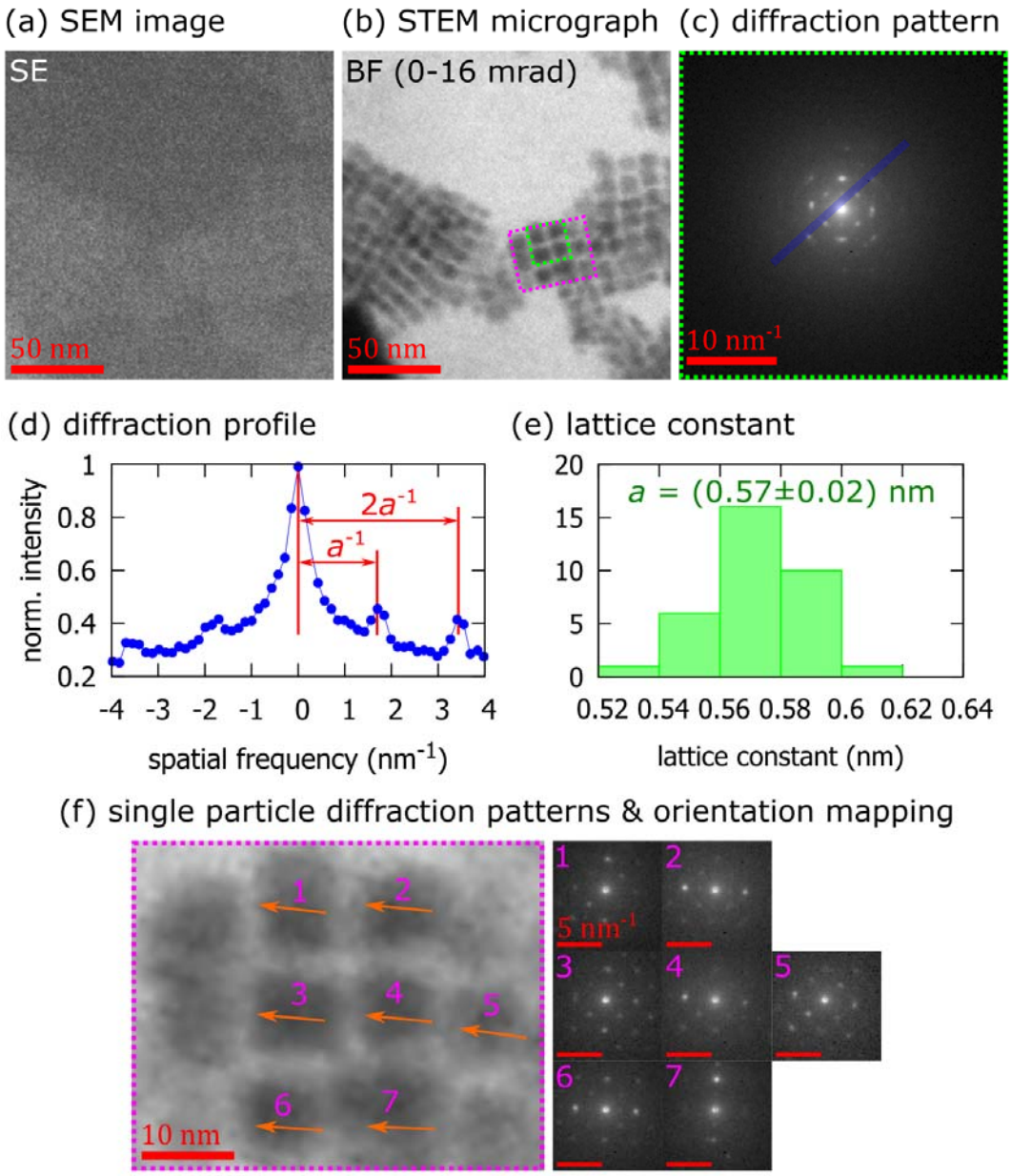


*Fig. 1: Analysis of $CsPbBr_3$ nanocrystals: (a) conventional SEM image using secondary electrons, (b) STEM bright field image reconstructed out of the 4D-STEM data set using a virtual detector, (c) diffraction pattern integrated over the green area in (b) out of the 4D-STEM data set, (d) line profile from the diffraction pattern in (c) used to determine the lattice constant, (e) histogram showing the statistics of determined lattice constants of $CsPbBr_3$, (f) BF STEM micrograph of the purple area in (b) overlaid with a schematic map of the crystallographic orientations of these nanocrystals and their diffraction patterns.*

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# News in microscopy community

*Chairs: Jana Nebesářová and Vladislav Krzyžánek*

# Young Microscopists at MICROSCOPY 2025: yCSMS News and Events You Shouldn't Miss!

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The Young Czechoslovak Microscopy Society (yCSMS) serves as a vibrant community for students and early-career scientists passionate about microscopy. Our goal is to foster connections among like-minded individuals, creating a platform for networking, knowledge sharing, and skill development. Operating under the guidance of the Czechoslovak Microscopy Society (CSMS), we organize various events, such as community meetings, industry and academic visits, and opportunities for collaborative research. Whether you're just starting out in microscopy or you're an experienced microscopist, yCSMS provides a welcoming space to expand your knowledge and engage with a supportive community that shares your enthusiasm for microscopy.

For the MICROSCOPY 2025 conference, we have prepared a special program that combines both fun and educational elements. We warmly invite all attendees to join us, and the details of our program will be shared in this brief talk.

We also invite you to visit our webpage [1] and follow us on social media [2, 3] to stay connected and up-to-date with our latest activities.

References:

[1] https://ycsms.org/
[2] Facebook @YCSMS.org
[3] LinkedIn @YCSMS.org

*Fig. 1: Link to the yCSMS registration form.*

# CIISB – Czech Infrastructure for Integrative Structural Biology

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The goal of the CIISB research infrastructure is to offer access to technologies for a variety of users from both the academic and the industrial community (pharmaceutical, biotechnology and agricultural companies) from the Czech Republic and other countries. CIISB expertise covers a wide range of topics in cellular and molecular structural biology and secures necessary support for external users in all stages of project implementation, from sample preparation, data acquisition, up to data processing and evaluation.

# Physical Engineering and Nanotechnology – the way to understanding and application of state-of-the-art technologies

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The talk will inform on the BSc. and MSc. study programme of Physical Engineering and Nanotechnology guaranteed by the Institute of Physical Engineering and Nanotechnology (IPE) at the Faculty of Mechanical Engineering, Brno University of Technology. This study programme combines principles of applied physics and engineering and trains students to solve practical problems of the interdisciplinary nature of science and technology. The students are taught to understand principles of physics through practical problems and projects and apply them to state-of-the-art technologies of surfaces, thin films and micro/nanostructures, and/or to design scientific instruments and devices. This is aimed at application domains suitable for nanoelectronics, nanophotonics, semiconductor technologies, electron and optical microscopy, biophotonics, etc. The students benefit from an individual approach to teachers and mentors of IPE, participation in research projects of the institute, and the international character of the study.

In the talk the structure and organization of the study will be discussed, and the practice of 6-month internships in research groups at foreign universities as a regular part of the MSc. study programme outlined. Thanks to this project-oriented study and international experience, the graduates of the Physical Engineering and Nanotechnology study programme comfortably find their jobs at renowned academic institutions and high-tech companies, both in the Czech Republic and abroad.

More info: https://www.fme.vutbr.cz/fakulta/struktura/pracoviste/ufi

# 10 Years of Building the National Imaging Infrastructure Czech-BioImaging

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Czech-BioImaging is the national research infrastructure dedicated to advancing biological and medical imaging in the Czech Republic. Since its launch in 2015, Czech-BioImaging has evolved into a crucial European infrastructure, significantly expanding its facilities and services. It now encompasses 16 core facilities and hosts three Euro-BioImaging Nodes, making it an integral part of the European imaging landscape. The establishment of Czech-BioImaging is closely linked to the activities of the Czechoslovak Microscopy Society (ČSMS), reflecting a history of close collaboration. In 2025, Czech-BioImaging celebrates 10 years of providing open access to imaging services for the scientific community.

The infrastructure offers a wide range of cutting-edge imaging technologies and expert support, continuously expanding its portfolio in consultation with specialists. This ensures that the latest advancements are available to users from academic, medical, and private sectors. Researchers are guided throughout the entire workflow, from experimental design to data interpretation, across fields such as cell and molecular biology, genetics, physiology, parasitology, tumor biology, neuroscience, developmental biology, and pathology. Czech-BioImaging places a strong emphasis on user satisfaction, regularly gathering feedback to improve its services and address the needs of its diverse user base.

In addition to providing access to advanced imaging technologies, Czech-BioImaging supports the scientific community through training courses, workshops, and user project support. Its annual conference fosters collaboration and innovation, contributing to the education and advancement of imaging sciences.

Czech-BioImaging's commitment to scientific excellence and its role in fostering international collaboration underscore its importance as a leader in the field of biological and medical imaging.

Acknowledgement:

National Infrastructure for Biological and Medical Imaging is co-funded by the Ministry of Education, Youth and Sports of the Czech Republic (project LM 2023050 and OP JAK infrastructure project CZ.02.01.01/00/23_015/0008205).

# Czech-European Photonics Cooperation: Opportunities and Challenges

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Czech optical cluster plays a vital role in European photonics through participation in initiatives like PhotonQBoost, LASER-PRO, and the Photonics21 platform. These projects foster collaboration, drive innovation, and enhance competitiveness in photonics.

PhotonQBoost supports SMEs in adopting photonic and quantum technologies. The funding will be distributed over a four-year period. LASER-PRO connects academia and industry to develop laser-based solutions for manufacturing, semiconductors, and healthcare. Photonics21 unites over 3,800 members to set Europe's photonics innovation agenda.

Opportunities include knowledge sharing, funding access, and strengthened innovation. Challenges involve coordination, administrative hurdles, and long-term sustainability. Success depends on effective collaboration and adaptability in a rapidly evolving technological landscape.

# News from Brnoregion Microscopy

Novák R.[1], Neuman J., Martínková R., and many other collaborators :)

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BRNOREGION MICROSCOPY is a community of companies, research and educational institutions developing the field of electron microscopy in cooperation with the city of Brno and the South Moravian Region, working together on the vision of BM - "whenever the world talks about (electron) microscopy, the first association is #brnoregion".

BM's mission is to push the boundaries of microscopy and science, to deliver the best equipment in the world, to support talent and education in electron microscopy.

We are working together on core activities:

1. SUPPLIER NETWORK DEVELOPMENT - Supporting the growth in the number and competence of microscopy suppliers in the region.

2. COMMERCIALISATION SUPPORT - Transferring research results into practice, supporting the creation of spin-offs.

3. COMMUNITY DEVELOPMENT AND EDUCATION - Promoting sharing, attracting new talent, training and development within the ecosystem.

4. PR AND MARKETING - Coordinated awareness raising of (electron) microscopy in the region and globally.

For the MICROSCOPY 2025 conference, we have prepared a pitch about first year of operation of the Brnoregion Microscopy, news from the present and views for the future.

We warmly invite all attendees to join us and visit our webpage [1] and follow us on social media [2] to stay connected and up-to-date with our latest activities.

References:

[1] https://brnoregion.com/en/landing-pages/brnoregion-microscopy

[2] https://www.linkedin.com/company/brnoregion-microscopy/

Acknowledgement:

We thank all the people, institutions and companies that support Brnoregion Microscopy.

BRNOREGION MICROSCOPY

*Fig. 1: logo Brnoregion Microscopy*

*Fig. 2: LinkedIn with all the news from BM*

"120 Seconds: Meet Brnoregion Microscopy"

The key networking event of the Brnoregion Microscopy community – biannual meeting to meet old friends, get new contacts, inspiration and introduce your work to partners.

- ✓ May 29, 2025, from 15:00 to 18:30 in the atrium of the CEITEC MUNI, Brno-Bohunice.
- ✓ What can you expect?
  - o An overview of news and upcoming events in Brnoregion Microscopy and how to get involved.
  - o 20 short presentations of interesting microscopy related projects followed by networking.
  - o Opportunity to present your work, offer your unique solutions and request collaborations.

For more information and registration, please visit https://brnoregion.com/en/events/120-seconds-meet-brnoregion-microscopy-2025_05

*Fig. 3: web 120" with more information and registration*

# The study program “Mikroskopie” at the Masaryk University

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The new study programme “Mikroskopie” (Microscopy) has started in the Autumn semester 2024, thus this summer the first study year is to be finished by the involved students. It is a two-year follow-up master's degree study which is accredited at Masaryk University, Faculty of Science [1]. In addition to academic staff from this faculty and CEITEC MU, experts from the Institute of Scientific Instruments and the Institute of Physics of Materials of the Academy of Sciences and companies participate in courses as well. These institutions are also discussing their broad activities as members of Brnoregion Microscopy. The programme “Mikroskopie” starts in Czech language, and an English version “Microscopy” is supposed to follow in the near future.

The aim of the study programme is to provide students the professional qualification of an expert in the field of microscopic methods. It focuses on electron and light microscopy and other imaging methods useful for research, development and applications. The study is aimed both at consolidating and deepening the education obtained by students in their previous bachelor's programme with physical, mathematical-physical or biophysical focus. The programme deals with areas of light and particle optics, properties of materials and the structure of biological samples, physics related to the properties and construction of imaging devices. The students obtain particular knowledge and skills in the field of physics, structural biophysics and biology required for the profession of development and application specialists. The aim is to prepare qualified experts theoretically, with an emphasis on the ability to follow the development of related physics methods, as well as practically through practical training at standard and development workplaces of manufacturing companies and at academy.

References:

[1] https://www.sci.muni.cz/pro-uchazece/navazujici-magisterske-studium/26987-mikroskopie

Acknowledgement:

We thank all the people, institutions and companies supporting this new study programme.


*Fig. 1: Flyer of the Microscopy programme*

# Microscopy elevator pitch

*Chair: Dušan Chorvát*

# Ultra-fast, ultra-sensitive confocal microscope for biological imaging

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Innovative Bioimaging, s.r.o. is a small Czech manufacturer of equipment for advanced optical microscopy. Traditionally, we provide solutions (microscope modules, software, molecular probes) for polarization-resolved confocal microscopy [1, 2]. However, in collaboration with the laboratory of P. Dedecker at KU Leuven, we have recently developed a novel design of a very fast, sensitive laser-scanning confocal fluorescence microscope for biological imaging [2, 3]. With frame rates up to 1 kHz, the microscope (TriScan Ultra) is particularly suitable for rapid 3D imaging of biological samples (cells, organoids, worms). Furthermore, the TriScan Ultra is ideal for real-time imaging of dynamic molecular processes of cell signaling (calcium imaging, other molecular biosensors). We believe the TriScan Ultra will soon find uses in many biological imaging facilities and microscopy laboratories.

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Acknowledgement:

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# Cryogenic technologies for conversion of room-temperature EM to cryo-EM

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Cryogenic electron microscopy (cryo-EM) plays a key role in both life sciences and materials science by enabling the observation of samples in a near-native state under the high-vacuum conditions of an electron microscope (EM) [1]. At cryogenic temperatures, samples exhibit significantly higher resistance to radiation damage caused by electron or ion beam exposure compared to those maintained at room temperature [2]. The most basic cryo-EM cooling systems typically use either bath cooling or flow cooling techniques. These systems often rely on liquid nitrogen ($LN_2$) with a normal boiling point of 77 K and liquid helium (LHe) with a boiling point of 4.2 K to cool the samples. Cryo-EM has become a central focus for global manufacturers of electron microscopes (such as Thermo Fisher Scientific and JEOL), who produce cryogenic transmission electron microscopes (cryo-TEM) and scanning electron microscopes (cryo-SEM). However, purchasing new cryo-EM systems is prohibitively expensive (hundreds of thousands of Euro). An alternative approach, which is far more cost-effective, involves converting existing room-temperature EMs to function at cryogenic temperatures. This offers a more affordable solution. Converting different types of EMs to cryo-EMs requires designing a custom cryogenic cooling system, which must be adapted to the specific model of EM.

We provide an overview of the cryogenic technologies we have developed for both cooling systems, enabling the conversion of existing room-temperature EMs into cryo-EMs [3]. These technologies include a $LN_2$ Dewar vessel with a cooled copper finger, an LHe/$LN_2$ flow cryostat equipped with two heat exchangers, a LHe/$LN_2$ low-loss vacuum-insulated transfer line with controlled flow rate, a cryogenic sample holder equipped with ten spring-loaded electrical contacts and a thermally insulating pad (InBallPad) with high mechanical stiffness for sample holder mounting [4].

We used these technologies for reference conversions of EM located in the laboratory of the Microscopy for Biomedicine group at the Institute of Scientific Instruments in Brno (ISI Brno) [3]:

- Conversion of the Helios G4 SEM/FIB (Thermo Fisher Scientific) to Cryo-SEM/FIB using an $LN_2$ bath cooling system: The converted SEM is equipped with a focused ion beam (FIB) source. The $LN_2$ Dewar vessel is used to cool the sample holder and an anti-contamination shield that partially surrounds the sample holder (Figure 1b). The Dewar vessel is installed on the high-vacuum chamber ($10^{-5}$ Pa) of the SEM (Figure 1a). The cooled components are connected to the copper finger via copper braids with high thermal conductivity. These anchors allow X-Y-Z positioning of the sample holder, rotation around the Z-axis within ±30°, and tilting of the sample holder relative to the SEM and FIB columns. An ultimate sample holder temperature of 112 K was reached in approximately 90 minutes.
- Conversion of the Magellan 400 L SEM (Thermo Fisher Scientific) to Cryo-SEM using an LHe flow cooling system: To convert the Magellan 400 L SEM into a cryo-SEM, we employed a liquid helium (LHe) flow cooling system. This system incorporated the LHe/$LN_2$ flow cryostat and the LHe/$LN_2$ low-loss vacuum-insulated transfer line, along with a simplified version of the sample holder thermally insulated by the InBallPad. The cooled sample holder was connected to the heat exchanger of the flow cryostat using a copper braid. The system achieved a sample holder temperature as low as 31 K, while the heat exchanger itself reached approximately 4.2 K.
- Conversion of the EM ACE 600 system (Leica Microsystems) for sample preparation from $LN_2$ to LHe temperatures: The EM ACE 600, originally designed for high-vacuum film deposition and sample preparation at $LN_2$ temperatures, was modified to operate at LHe temperatures. It was initially equipped with a cooled, gold-coated copper sample holder and a sharp blade, enabling the freeze-fracturing of samples by cutting off protruding parts or lightly scratching the surface. In this conversion, the original $LN_2$ Dewar vessel of the bath cooling system was removed and replaced with an LHe/$LN_2$ flow cryostat. The original thermal insulators, consisting of four PEEK plastic columns between the sample holder and the surrounding vacuum chamber at room temperature, were insufficient to maintain

the required LHe temperatures. Therefore, we designed new thermal insulators composed of three fiberglass laminate pillar tubes characterized by significantly higher thermal resistivity [3]. The temperature of approximately 30 K was reached at 50 minutes on both the sample holder and the blade.

These conversions open new research avenues for the Microscopy for Biomedicine group, such as investigations into novel ecological biodegradable materials and biotechnologically important microorganisms (Figure 1c). Last but not least, the high-quality and valuable outputs from cryo-EM will serve as a foundation for promotional materials showcasing the presented technologies and supporting the evaluation of their potential commercial applications.

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Acknowledgement:

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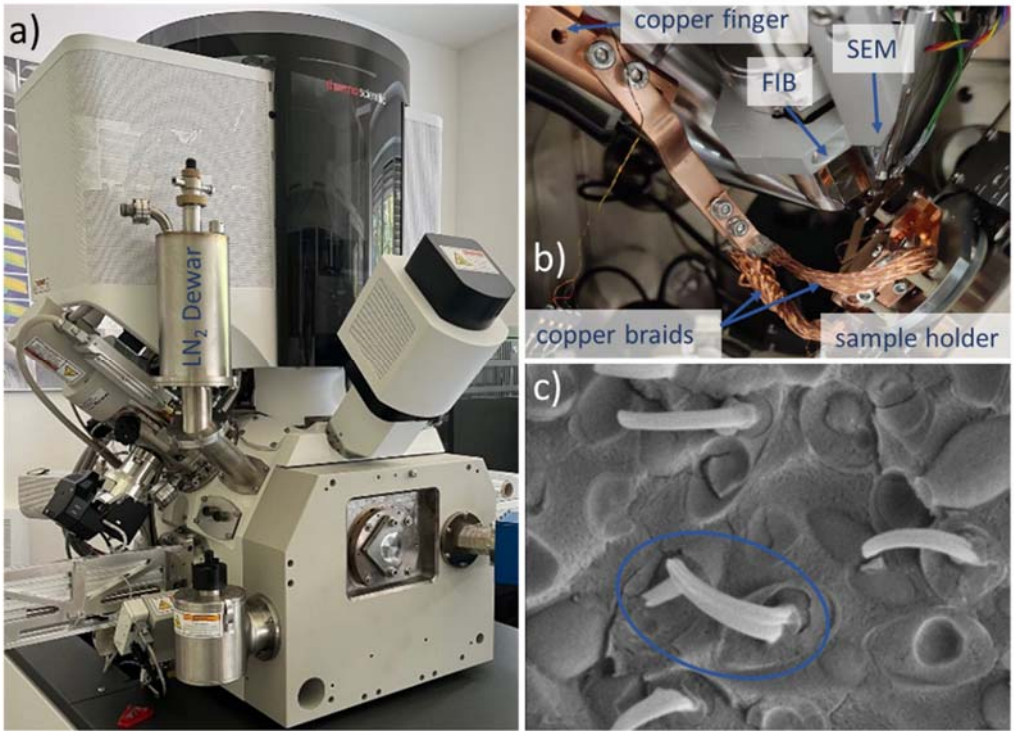


*Fig. 1: Conversion of the Helios G4 SEM/FIB (Thermo Fisher Scientific) to a cryo-SEM/FIB with the installation of the $LN_2$ Dewar vessel; b) View of the installation of the sample holder inside the SEM chamber; c) Biopolymer granules produced by bacteria, exhibiting elastic fracture at 30 K (blue ellipse).*

# yCSMS session

## LiftOff: Launching Early Careers in Microscopy and Science

*Chairs: Eva Ďurinová and Martin Strnad*

# Platelet endothelial cell adhesion molecule PECAM-1 could be detected in Reissner's fibre of brain ventricular system in rats

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Introduction:

Reissner's fibre (RF) is an extracellular structure that passes through the brain ventricular system and the central canal of the spinal cord. It is formed by the aggregation of products released by cells of the subcommissural organ (SCO) located in the wall of the third brain ventricle. Although the SCO and RF are formed in the CNS of various mammals, their exact role remains elusive[1]. There is evidence that early embryonic abnormalities in the SCO and RF lead to severe conditions, such as hydrocephalus and body axis deformities[2,3]. The best-known component of RF is a large glycoprotein SCO-spondin, which is continuously synthesised and released into the CSF[4]. In addition to SCO-spondin, other proteins are associated with the RF structure. In lampreys, the SCO and RF have been characterized as structures that are immunoreactive to anti-GAP43 antibody, which detects the growth-associated protein 43 in axonal growth cones[5]. In rats, galectin-1 glycoprotein has been identified as another component of the RF, which is likely involved in the aggregation of SCO-spondin[1].

The aim of our study was the characterisation of the SCO and RF in Wistar rats and BALB/c mice using electron and fluorescence microscopy. Interestingly, while studying CNS vasculature in rats, we found that the RF is immunoreactive to anti-PECAM-1 antibody detecting platelet endothelial cell adhesion molecule (PECAM-1).

Materials and methods:

Adult Wistar rats at the age 90-120 postnatal days and BALB/c mice at the age 30 postnatal days were transcardially perfused with heparinized saline and fixatives according to the standard protocols for TEM and immunolabelling. For ultrastructural analyses, 200 µm thick vibratome sections of brain and spinal cord were processed according to TEM protocol (postfixation in $OsO_4$, dehydration and embedding in Epon). Ultrathin sections were cut using ultramicrotome (Leica EM UC7, *Leica Microsystems*), transferred on formvar coated slot or mesh copper grids, contrasted with uranyl acetate and lead citrate and analysed using TEM (JEOL JEM 1230, *Jeol*). For immunogold labelling, ultrathin sections of the brain and the spinal cord embedded in LRWhite were used and immunolabelled using goat anti-PECAM-1 primary antibody (*R&D systems*, code no. AF3628) diluted at 1:20. For immunofluorescent labelling, the same primary antibody (diluted at 1:500) was used on 40 µm thick frozen sections. Samples were incubated with AF488 donkey anti-goat secondary antibody (*Abcam*, code no. ab150129; diluted at 1:500) or secondary antibody conjugated with 12 nm gold nanoparticles (*Jackson ImmunoResearch*, code no. 705-205-147; diluted at 1:50). Samples were analysed using a confocal microscope (Leica TCS SP5 X, *Leica Microsystems*) or TEM.

To study the surface of SCO and RF using SEM (JEOL JSM-6510 LA, *Jeol*), hemispheres of brain and spinal cord were separated by sagittal section, exposing the ependymal lining of the 3$^{rd}$ and 4$^{th}$ cerebral ventricles as well as the central canal. The tissues were then fixed, dehydrated, and immersed in hexamethyldisilazane and sputter coated using gold.

Results:

In both studied rodent species, the SCO has been identified in the postero-dorsal wall of the third brain ventricle as a structure composed of polarized cells with basally located nuclei, close to the posterior commissure. We observed sparsely distributed cilia at the apical pole of the SCO cells, which luminal surface was covered with filamentous material. In the vicinity of the cerebral aqueduct, the observed material formed the RF, which continued as a thread-like structure into the central canal. However, unlike in mice, PECAM-1 glycoprotein was identified as a structural component of the RF in brain and spinal cord of rats. Using immunogold labelling, we identified that PECAM-1 was present in the vesicles located in the apical cytoplasm of the SCO cells. We also observed PECAM-1 in the extracellular material present on the surface of SCO in the brain ventricular system of rats, but not in mice. In contrast to rats, in mouse nervous system, PECAM-1 was observed only in the vasculature of the brain and spinal cord.

Conclusions:

Based on our observations, we suggest that the SCO cells of rats could produce PECAM-1 glycoprotein, which is subsequently incorporated into the RF in the similar manner as its other components, such as SCO-spondin. Since we did not observe PECAM-1 in the SCO and RF of mice using immunolabelling, potential interspecies differences may exist. However, our results should be verified using other methods, such as gene expression analyses and *in situ* hybridization.

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# Why manual segmentation is not always bad, but never should be used as the only one?

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Segmentation is an inevitable part of the analysis process in microscopy and imaging data. Different imaging modalities push specific segmentation strategies—while light microscopy often demands full automation, techniques like electron microscopy (EM) still rely heavily on manual segmentation. In this lift-off talk, I will highlight why manual segmentation remains important—especially when high accuracy and expert interpretation are required—and how it complements automated methods. I will argue that hybrid approaches are essential for achieving both reliable and efficient analysis. In the end, it's not manual versus automatic—it's manual with automatic that unlocks the full potential of imaging data.

Acknowledgement:

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# High-Throughput Oocyte Detection in Histological Slides Using YOLOv5 Neural Network

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Oocyte aging is a complex biological process that differs significantly across species, with the case of mole-rats being particularly unique. Traditionally, the quantification of primordial oocytes on histological slides has relied on manual examination—an approach that is both time-consuming and inherently subjective [1]. The emergence of whole-slide imaging (WSI), which enables high-resolution digitization of entire tissue sections, combined with rapid advancements in deep learning, has significantly improved the efficiency and accuracy of histopathological image analysis—offering a promising solution to this challenge [2]. However, despite the biological relevance of oocyte quantification, there remains no standardized or automated method for calculating oocyte numbers from histological samples.

In this study, we introduce an automated workflow based on a customized YOLOv5 (You Only Look Once) object detection model [3], trained specifically to identify oocytes in histological images (*Fig.1*). We validated the model across three different mole-rat species, demonstrating high reliability and consistency in detection performance. Furthermore, when compared with human-annotated datasets, the model achieved comparable accuracy while significantly reducing analysis time (*Fig.2*). The proposed approach not only standardizes oocyte quantification but also improves detection precision, offering a scalable and objective alternative to manual assessment for histological studies involving mole-rat species.

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Acknowledgement:

We thank the developers and contributors of YOLOv5 by Ultralytics for providing an efficient and open-source platform for object detection tasks.

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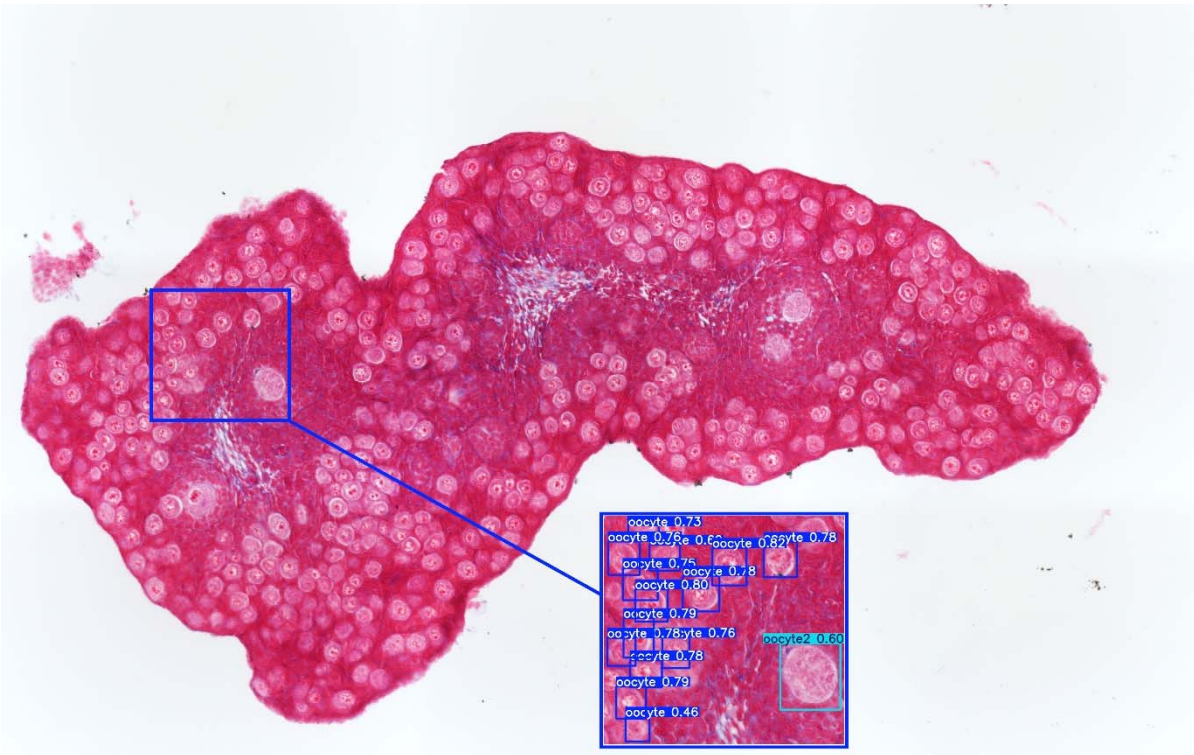


*Fig. 1: Whole histological section of a mole-rat ovary.: The image shows a complete stained ovary slice used for oocyte detection. The blue inset highlights the output of a YOLO-based model trained to detect oocytes. Within the inset, small blue ROIs indicate primordial oocytes, while turquoise ROIs represent primary oocytes.*

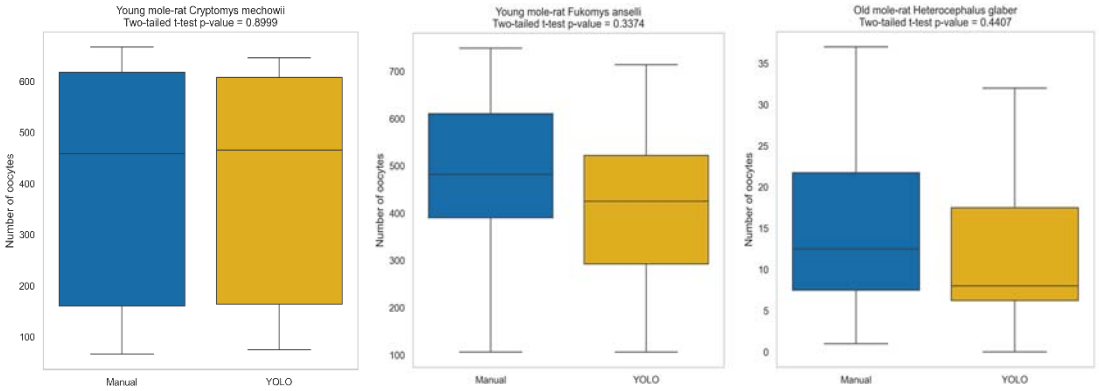


*Fig. 2: Comparison of YOLO-predicted and manually annotated oocyte counts across three mole-rat species.: Boxplots show the number of detected oocytes using a YOLO model and human annotation for each species. Each pair of boxplots represents one species, allowing visual comparison between automated and manual methods.*

# Posters

# UiO-66-$NH_2$ nanoparticles designed for bioimaging and anticancer treatment of 3D organoids

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Nanotechnology is a rapidly developing field with applications in medicine, including the diagnosis, treatment and targeting of tumours in a safer and more effective way. It makes it possible to create functional materials, devices and systems at the atomic and molecular level, utilising novel properties and phenomena. Nanoscale drug delivery systems have many advantages in cancer therapy, such as good pharmacokinetics, precise targeting of tumour cells, reducing side effects and overcoming drug resistance. Attention for targeted drug delivery is focused on micro- and mesoporous nanoparticles, especially metal-organic frameworks. A large capacity for drug entrapment in the pores, kinetic and thermodynamic stability, good biocompatibility, low cytotoxicity and the possibility of surface functionalisation are the main features of these materials.

UiO-66-$NH_2$ is a porous material that allows the entrapment of a large number of hydrophilic drug molecules. In the present study, 5-fluorouracil (5FU) was entrapped in UiO-66-$NH_2$ with the aim of transporting it into cancer cells. The UiO-66-$NH_2$ material is based on zirconium (IV) ions forming hexanuclear clusters $[Zr_6O_6(OH)_6]^{6-}$ bridged by a derivative of terephthalic acid containing amine functional group and is characterised by an extensive surface area, a large pore volume and a high thermal stability (up to 500 °C). The compound is characterised by high resistance to many chemicals and remains in crystalline form even under extremely acidic conditions. The UiO-66-$NH_2$ nanoparticles also show *pH*-dependent stability and are resistant to degradation under extracellular conditions and disintegration in intracellular environments.

The prepared nanocarriers showed high biocompatibility and low cytotoxicity *in vitro* (cell culture) and are well tolerated *in vivo*. The subcellular localisation of the particles was confirmed by various techniques, including fluorescence microscopy, Raman microspectroscopy, multiphoton imaging of metabolic activity and optical coherence tomography of 3D cancer cell organoids. This multimodality enables the identification of cell populations signalling cell death after treatment with 5-FU. In addition, the UiO-66-$NH_2$ nanoparticles have promising properties for antibacterial activity and potential for photodynamic therapy.

Acknowledgement:

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# Imaging of designed protein photosensitizers in targeted tumor therapy

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Photodynamic therapy is a modern form of targeted cancer treatment. Its principle consists of utilising the property of a photosensitive substance – a photosensitizer (PS), the excitation of which by light of a specific wavelength can generate singlet oxygen. When PS is successfully delivered to tumour cells, it is possible to trigger oxidative stress in the cell, which leads to premature programmed cell death - apoptosis. The question arises as to how PS can most efficiently reach the target cells and trigger programmed cell death without negatively affecting healthy cells. This would solve the problem of the undesirable side effects of current treatments such as chemotherapy, radiotherapy or treatment with monoclonal antibodies. One possibility is to create an efficient transport system for PS that can be established through protein engineering.

The AsLOV2 domain is one of the phototropic proteins that naturally contain a binding site for a photosensitive substance, namely flavin mononucleotide (FMN), in their structure. Targeted irradiation of the protein with blue light leads to a change in the conformation of the complex, which results in the release of FMN from its structure [1]. To increase the specificity of transport to the target cells, specifically designed proteins – DARPins - are used that can bind to different epitopes. Their unique structure contains repetitive building units that increase their stability and resistance to environmental changes [2]. By conjugating a specific DARPin to the FMN-binding AsLOV2 domain, two-step photodynamic therapy (PDT) could be effectively delivered to breast tumour cells with elevated HER2 receptor (HER2r) expression. The initial irradiation step would induce the release of FMN from the carrier domain, while the subsequent irradiation would facilitate the generation of singlet oxygen and thus enable a targeted therapeutic effect.

We used the conjugate of the AsLOV2 domain with the specific bivalent DARPin with high affinity to HER2r [3] as a transport system for delivery of PS to target cells, with another conjugate with the non-specific DARPin OFF7 serving as a control. Targeted binding of the conjugate to HER2r was confirmed by confocal fluorescence microscopy (Fig.1) using mitochondrial autofluorescence, FMN and selective fluorescent labelling of HER2r. By re-irradiating the cells with blue light (400-500 nm), we triggered the generation of singlet oxygen. We monitored the effects of oxidative stress on the cells by changing the morphology of mitochondria and lysosomes using confocal microscopy with fluorescent markers: Rhodamine 123 for mitochondria and LysoTracker for lysosomes.

This innovative approach, in which proteins are used as carriers for PS and their specific design targets breast cancer cells that conclusively induce oxidative stress, could represent a promising strategy for cancer treatment.

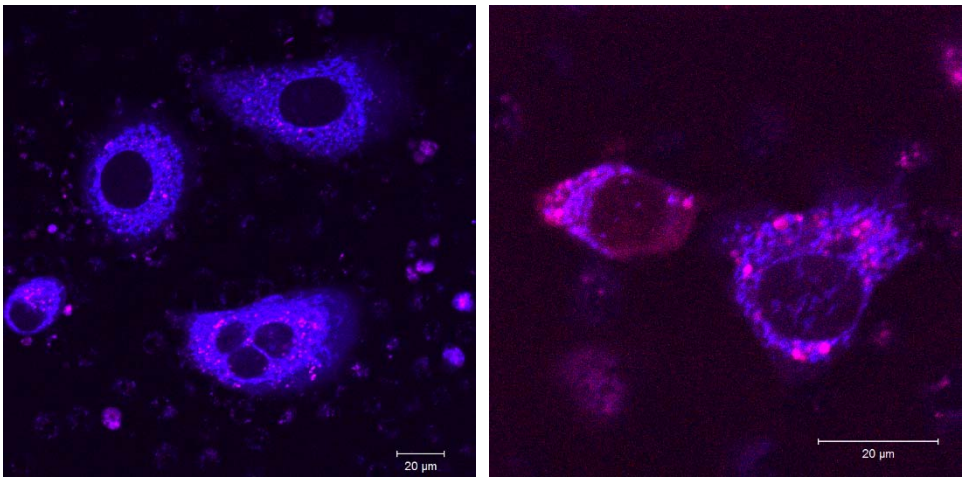


*Fig. 1: Visualizing of conjugate DARPin-HER2-AsLOV2 on HER2r on SKBR3 cell line by confocal fluorescence microscopy under excitation at 405 nm. Left: Treatment with DARPin-HER2-AsLOV2. Right: Treatment with DARPin-HER2-AsLOV2 in addition of 50µM FMN. Colour code: blue – autofluorescence of mitochondria, pink/violet – free and bound FMN to AsLOV2.*

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Acknowledgement:

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# Electron Microscopy and Ultrastructural Analysis at IMCF-BIOCEV

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Electron microscopy (EM) is a powerful experimental tool for the analysis of composition, ultrastructure, and function of biological, bio-composite, and material samples down to nanometer resolution. From tissues and cells to subcellular features and macromolecular complexes, Transmission and Scanning electron microscopy (TEM and SEM) provides 2D and 3D ultrastructural analysis with optional detection and localization of specific antigens by immuno-gold labeling or specific elements by EDS analysis. The Electron Microscopy division of the Imaging Methods Core Facility (IMCF-EM) supports research and development projects by providing advanced instrumentation, methods, and expertise in TEM and SEM and their application to a broad range of sample types. The IMCF-EM houses one FIB-SEM system Helios Nanolab 660 G3 UC (Thermo Fisher Scientific), one 200kV cryo-TEM system JEM2100-Plus (JEOL) and outstanding (cryo-)preparation instrumentation from LEICA Microsystems including Plunge Freezer (LEICA EM-GP2), Critical Point Drier (Leica EM CPD300), High-Pressure Freezer (Leica EM HPM 100) with Freeze Substitution units (Leica EM AFS2) and Ultramicrotomes (Leica UC7 FC). The facility supports all steps of the analytical workflow from consultation on proper sampling and sample preparation to a full assistance with sample processing, TEM/SEM imaging, data processing and qualitative or quantitative data analysis. Each step of the workflow is carefully customized to accommodate sample-specific and objective-specific needs of even the most challenging project. The poster we present is an overview of sample processing workflows and methods of TEM, SEM, and CLEM imaging available to users of this open-access core facility.

Acknowledgement:

IMCF at BIOCEV is supported by the MEYS CR (LM2023050 Czech-BioImaging).

# Morphological Study of *Dermacentor marginatus* Using Scanning Electron Microscopy

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Fixation protocols play a fundamental role in determining the quality and reliability of scanning electron microscopy (SEM) results. In this study, we utilized *Dermacentor marginatus* as a model organism to compare standard fixation methods applied to fresh specimens and archived material preserved in 70% ethanol over several months.

We analyzed live female ticks fixed overnight in 3% glutaraldehyde (0.1 M cacodylate buffer) and ticks archived for nine months in 70% ethanol. Both groups were dehydrated through ascending ethanol concentrations and dried using a Quorum K850 system. Samples were sputter-coated with gold (20 nm) or platinum (3 nm) and imaged on a Nova NanoSEM 450 at 3-5 kV.

SEM analysis revealed clear morphological structures in both sample types. Low-magnification images provided an overview of the tick's idiosoma and gnathosoma, while high magnification allowed detailed visualization of sensory organs and cuticular structures. Internal organ examination displayed characteristic features, such as acinar-arranged salivary glands, midgut cleavages containing digestive material, and branching tracheal networks. All observed structures showed characteristics typical for the family Ixodidae.

Our findings demonstrate that SEM analysis of freshly fixed samples results in high-resolution images, revealing fine morphological details essential for tick morphology research. Although ethanol-archived specimens retain diagnostic characteristics, optimal fixation with glutaraldehyde enhances image clarity and structural integrity. This methodological insight supports future SEM-based studies of archived arthropod material and comparative morphological analyses.

Acknowledgement:

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# Exploring silk gland morphology in lepidopteran caterpillars using 3D micro-computed tomography

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## Introduction

Lepidopteran caterpillars are one of the most significant insect silk producers. Depending on diverse life strategies of the two sister families of Bombycoidea, Saturniidae and Sphingidae, the caterpillars have evolved various ways of using silk. Most of the sphingiid larvae use silk exclusively in their first instars to produce rescue silk fibres that prevent the tiny caterpillars from falling off the host plant. In contrast, their closest relatives, the Saturniidae, save all of their silk for the end of larval development to construct complex silk cocoons that serve as a protective outer shell for the individual during its pupal stage. In general, silk is secreted by specialised ectodermal cells that form a silk gland. The silk glands of individual lepidopteran families differ significantly in their overall morphology. This research aims to analyse the in situ localisation of silk glands of several representatives of the Saturniidae and Sphingidae using micro-computed tomography (microCT). The data obtained allow the generation of detailed 3D projections of a caterpillar body, including its internal organs. Here we compare the advantages of the Surface module for segmentation and surface rendering in three different versions of the Imaris software (Imaris 9.3, Imaris 10.0 and Imaris 10.1).

## Material and methods

Last instar caterpillars of the Saturniidae and Sphingidae representatives were submerged in Bouin-Hollande's fixative and contrasted in Lugol's solution. X-ray microCT SkyScan, model 1272 (Bruker microCT, Belgium) was used to visualise iodine-contrasted specimens. High-resolution 3D output tomography data were reconstructed in SkyScan's volumetric NRecon software version 2.2.0.6 (Bruker microCT, Belgium). 3D models were created in Imaris software versions 9.3, 10.0 and 10.1 (Oxford Instruments, UK) using the Surface module: Surpass - Contour Surface, and Machine learning segmentation based on Artificial Intelligence.

## Results

Non-invasive microCT is the ideal technology to project internal structures and organs in situ without destroying the specimens. Using Imaris analysis software, we found that Imaris 9.3 is excellent at creating semi-transparent surfaces with adjustable transparency, while the latest Imaris 10.1 does not allow this. On the other hand, Imaris 10.1 uses Machine learning segmentation based on Artificial Intelligence, which makes surface creation much faster and easier.

## Conclusion

The procedure for preparing caterpillars for microCT has been optimised. Detection of silk glands in members of the Saturniidae and Sphingidae families based on microCT scanning reveals their exact location in the larval body. This approach allows detailed comparison of the silk gland morphology in relation to the location of other internal organs. Imaris 9.3 and Imaris 10.1 are a suitable combination for complex 3D renderings of biological objects.

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Acknowledgement:

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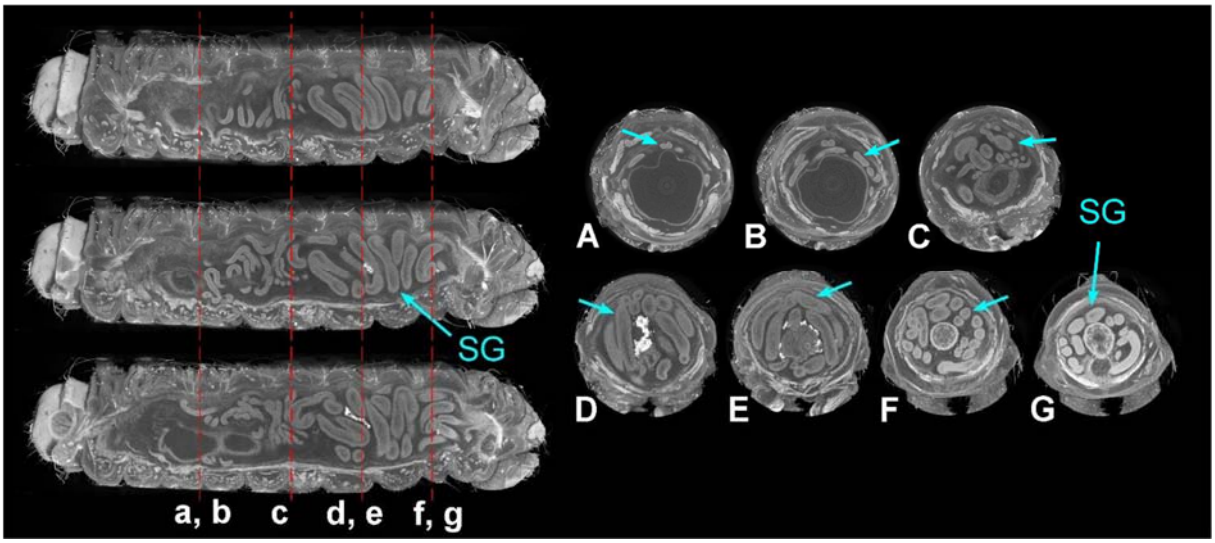


*Fig. 1: microCT images of longitudinal and transverse sections of the last larval instar of Antheraea pernyi showing the position of the silk glands (SG)*

# A Macroscope in the Microscopy Facility: To Be or Not to Be?

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We are introducing a new acquisition in our Laboratory of Microscopy and Histology at the Institute of Entomology, Biology Centre CAS in Ceske Budejovice – the **Macro Zoom Fluorescence Microscope System Olympus MVX10**.

The MVX10 Macroscope features a single-zoom, large diameter optical path that is optimized to collect light with high efficiency and resolution at all magnifications. In addition, the MVX10 has a unique pupil splitting mechanism in the optical path that mimics the effect of a stereo microscope.

The customized configuration of the macroscope enables observation and high-resolution photo documentation of large 3D objects (e.g. whole insects) as well as **fluorescence** observation of whole organisms at low magnification through to detailed observation of gene expression at cellular level at high magnification.

**Technical specifications:**

***Objectives:*** 0.63x, 1x, 2x; Zoom 10:1 (0.63x – 6.3x)

***Illumination:*** Transmitted light, “Sunflower” light source, "Goose necks", Ring light

***Fluorescence:*** LED light source, Filter sets: DAPI, GFP, TRITC, RFP

***Automated:*** XY stage, Z-axis movement

***Setup capabilities:*** Extended focal imaging, Imaging multiple regions of interest, Stitching multiple fields of view, Timelapse imaging

Acknowledgement:

The authors acknowledge funding from the Czech Academy of Sciences


*Fig. 1: Macro Zoom Fluorescence Microscope System Olympus MVX10*

# FLIPs: Genetically encoded biosensors for functional imaging of cell signaling at endogenous protein expression levels

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FLIPs are genetically encoded fluorescent probes that take advantage of directionality of optical properties of fluorescent proteins. The probes design offer capabilities for high sensitivity, multiplexing, ratiometric output, resilience to bleaching artifacts, without requiring any modifications to the proteins of interest. Their performance was demonstrated on real-time single-cell imaging of activation of G protein-coupled receptors (GPCRs), G proteins, arrestins, small GTPases, as well as receptor tyrosine kinases. However, non-physiological protein expression levels can potentially lead to imaging artefacts (ectopic sub-cellular localization, erroneous formation of protein complexes, aberrant organelle morphology). We continued our efforts and demonstrated FLIPs efficiency in detection of physiologically relevant signaling processes involving non-modified proteins at endogenous expression levels.

# Determinations of orientations of fluorescent protein molecules in living cells by two-photon polarization microscopy

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Non-linear optical properties of fluorescent molecules can often provide molecular information that is not accessible through more conventional, linear optical properties. In biological microscopy, the most commonly used non-linear optical process is two-photon excitation of fluorescent molecules. The quadratic dependence of two-photon excitation on illuminating light intensity enables facile optical sectioning, reduces out-of-focus photobleaching, eliminates fluorescence excitation by scattered illuminating light, and allows using even scattered fluorescence as signal. Thus, two-photon fluorescence microscopy is particularly suitable for imaging deep in tissues. Apart from these advantages, two-photon excitation also typically exhibits high sensitivity to orientation of the absorbing molecule. In our recent work, we determined the directionality of two-photon light absorption in the most commonly fluorescent proteins [1]. We have now combined this information with previously acquired knowledge of directionality of single-photon light absorption [2] in order to determine orientational distributions of fluorescent protein moieties in several genetically encoded biosensors [3]. Our results validate the ability to carry out such determinations and yield insights applicable to development of genetically encoded biosensors.

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Acknowledgement:

The authors acknowledge funding from the Czech Science Foundation grant 23-05983S (J.M)

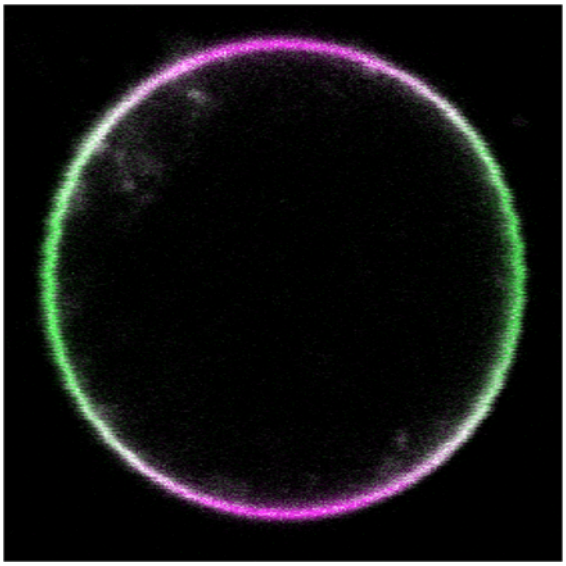

*Fig. 1: Image of an HEK293 cell expressing a genetically encoded fluorescent probe of activity of G protein signaling (KB1753-cpeGFP-hRas), circularized by hypotonic environment, imaged by two-photon polarization microscopy. Magenta/green colors indicate fluorescence excited by light polarized horizontally/vertically, respectively. Images such as this now allow determining orientational distributions of molecular orientations of the fluorescent proteins present.*

# The structure of chromatin in selected lower model organisms

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Introduction:

The analysis of chromatin structure in lower model organisms is important for advancing our understanding of fundamental cellular processes, including gene regulation, chromatin remodeling, and cellular development. These organisms often possess simpler genomes, making them ideal for studying the basic mechanisms Investigating chromatin organization in these models not only sheds light on conserved biological pathways but also provides valuable insights into how chromatin structure influences gene expression, replication, and repair, which can be translated to more complex organisms, including humans.

The aim of our research is to analyse the ultrastructural distribution of DNA/ chromatin in the nuclear space in the context of its large-scale organization and functional status in animal models as *Caenorhabditis elegans* and planktic Foraminifera (*Ammonia tepida, Haynesina germanica*).

Materials and methods:

The *C. elegans* strain N2 var Bristol was used as wild type and maintained according to standard protocols. Strains OD139 and JH2108 were obtained from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN, USA). These strains were crossed to generate a strain expressing three fluorescent markers (GFP-PGL-1; YFP-LMN-1; mCherry-H2B) and a strain expressing only GFP-PGL-1 and mCherry-H2B. For light microscopy the eggshell of embryos was freeze-cracked and samples were fixed in methanol and formaldehyde. For electron microscopy, samples were frozen under high pressure, cryo-substituted and embedded in Lowicryl HM20 (1).

*Ammonia tepida* and *Haynesina germanica* were sampled on the intertidal mudflat in the bay of Bourgneuf at la Couplasse station (Loire-Atlantique, France). The top 2 cm of sediment was collected at low tide, transferred to plastic jars, and immediately chemically fixed. The samples were then dehydrated and embedded in LRWhite resin (2). Some samples were stained for specific chromatin visualization and/or immunolabeled with primary antibodies and secondary antibodies coupled with colloidal gold nanoparticles.

For fluorescence microscopy, images of fixed samples were acquired on a Leica SP5 confocal microscope with a HCX Plan Apochromat ×100 oil immersion objective. Electron micrographs were acquired on a FEI Morgagni 80-kV transmission electron microscope equipped with an Olympus SiS Megaview III CCD camera (Olympus, Tokyo, Japan) and JEOL JEM – 1011 equipped with Veleta camera.

Brief results and conclusion:

Using light and electron microscopy we reported that the chromatin organization in the germ line founder cell of the early *C. elegans* embryo is distinct from that in the neighboring somatic cells. This unique organization is characterized by a greater chromatin compaction and an expansion of the interchromatin compartment. This higher order reorganization of chromatin occurs during the establishment of definitive germ cell identity (1).

Although *Ammonia tepida* and *Haynesina germanica* are unicellular organisms, their calcareous shell and size of around 0.5 mm made it challenging to locate the nucleus during microtomy sectioning. We identified a single nucleus, approximately 25 μm in size, containing several smaller and larger nucleoli. Additionally, a few lobate structures were observed, which may indicate an expansion of the nuclear

material and envelope prior to reproduction. Our current focus is on a more detailed analysis of chromatin distribution.

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Acknowledgement:

This research was funded by Charles University (program Cooperatio—Oncology and Hematology).

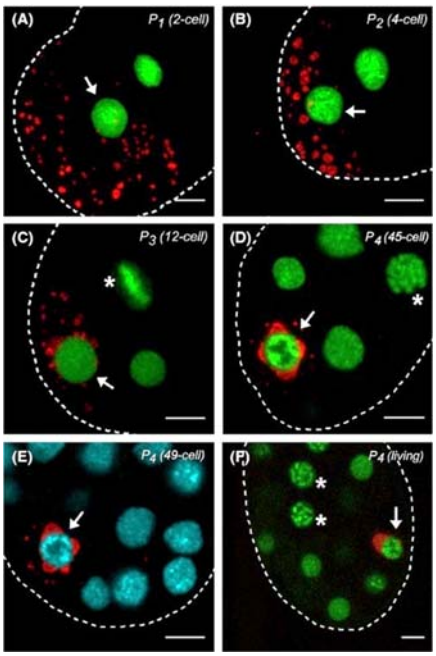


*Fig. 1: Chromatin organization in the germ lineage of the C. elegans embryo. DNA (DAPI, green or TO-PRO-3, cyan) and PGL-1 (red), a marker of the germ lineage.*

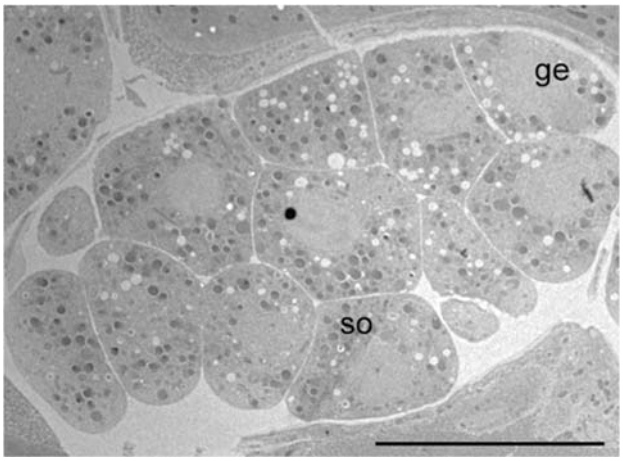


*Fig. 2: Ultrastructure of an ~30-cell C. elegans embryo. Germ line (ge) and soma (so) cells.*

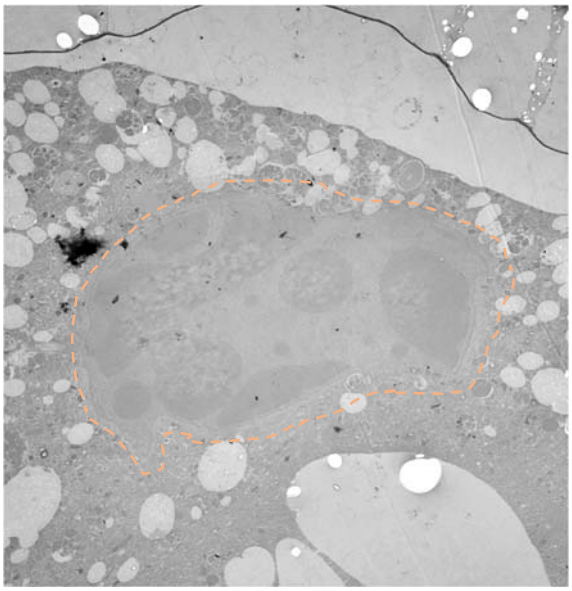

*Fig. 3: Ammonia tepida, nucleus with several nucleoli.*

# Raman tweezers for the analysis of secondary microplastics generated by degradation of surgical masks in water

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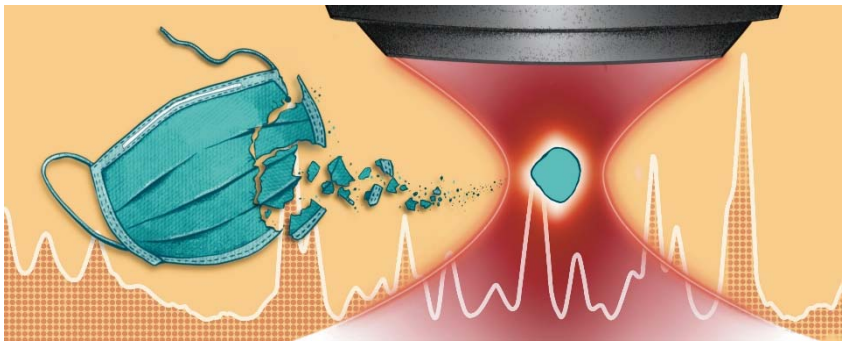

The COVID-19 pandemic has underlined the role of plastics as an irreplaceable material to society, proving an inexpensive and widely available feedstock for single-use medical equipment, groceries and commerce packaging[1]. In 2021, an estimated 89 million surgical masks were required each month[2]. These masks are non-recyclable and, as the most of single-use plastic items, either end up in landfills or are released directly into the environment, including oceans, freshwater, soil and air[3]. The transport from land to oceans is usually caused by aeolian processes and aquatic runoffs of mismanaged plastic waste[4]. Weathering of plastics causes break down into microplastics (MPs)[5] and nanoplastics (NPs)[6]. MPs range from 1 μm to 5 mm in diameter. NPs are generally addressed as objects smaller than 1 μm[7].

This study presents a combination of optical tweezers and Raman spectroscopy – Raman Tweezers (RT) – for the detection and chemical identification of secondary MPs. Tightly focused laser passed through a microscope objective allows for simultaneous micromanipulation and chemical analysis of MPs in aquatic environment[8]. The origin of the MPs in this study was from mechanical degradation of surgical face masks followed by UV-induced photodegradation.

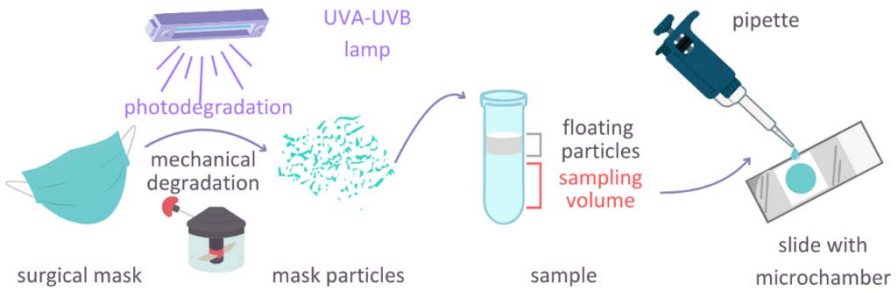


*Fig. 1: Process of the MPs preparation.*

RT enabled to optically trap and acquire Raman spectra of freely floating fragments down to approximately 760 nm inside a glass microchamber. Also, fragments of up to tens of µm deposited at the bottom of the microcell were identified. The size and the Raman response of the generated MPs was correlated. The main chemical compound of the MPs from surgical masks was identified as polypropylene. More interestingly, several of the analyzed fragments also contained a pigment, phthalocyanine, that serves as a UV and antimicrobial protective substance.

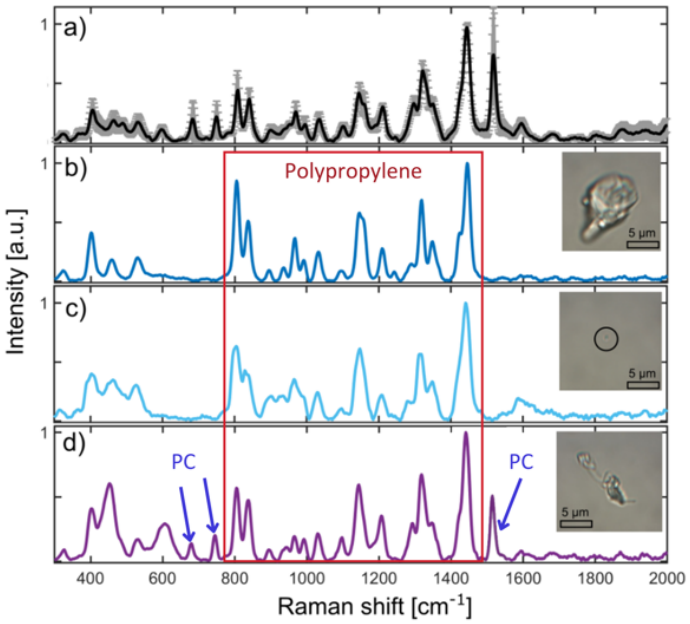


*Fig. 2: Raman spectra of the analyzed MPs.*

This study introduces RT as a tool to analyze size and chemical composition of sub-20 µm secondary MPs, directly in water, at the single particle level, with a minimal or no requirement for the pretreatment of the samples. The presented RT setup also offers the potential to be modified into a portable version, which would enable on-site analyses of MPs.

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Acknowledgement:

This project is financed from the state budget by the Technology Agency of the Czech Republic in the frame of the National Centres of Competence (TN02000020/Centre of Advanced Electron and Photonic Optics), and by the Czech Academy of Sciences (RVO: 68081731). Electron microscopy and Raman spectroscopy analysis provided at Core Facility Electron microscopy and Raman spectroscopy, ISI CAS, Brno, CR, is supported by MEYS CR (LM2023050 Czech-BioImaging). This study is based upon work from COST Action PRIORITY (CA20101), supported by COST (European Cooperation in Science and Technology).

# Use of ultrastructural expansion microscopy to determine the number of cells in the corpora allata of *Aedes aegypti*

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The corpora allata (CA) are a pair of endocrine insect organs that produce juvenile hormones and typically form a neurohemal complex with the corpora cardiaca (CC) [1], which synthesize the adipokinetic hormone [2]. In the larval stage, this complex is often accompanied by the ecdysteroids-producing cells of the prothoracic glands, which are later eliminated [3]. The neurohemal complex in the larvae of the mosquito *Aedes aegypti* has a special morphology: The cells of the CC usually remain at the basal end of the complex, where they mix with the A-cells, which are presumably the cells of the prothoracic gland [1]. In comparison, the cells of the CA form a compact cluster in the apical part of the complex, which is surrounded by a sheath of A- cells [1].

The compactness of the CA poses a challenge for the quantification of its cells, even when confocal microscopy is used. The overlap of nuclear signals makes it difficult for the software to distinguish individual cells. Although it is possible to estimate the cell number by a calculation with a normalization for the number of layers per nucleus, it is almost impossible to determine the exact cell number with standard confocal microscopy. However, the implementation of the ultrastructure expansion microscopy provides images with greatly improved resolution, making it possible to create 3D models with clearly distinguishable individual cell nuclei and thus count them accurately. Besides that, this methods also provides much more detailed structure of the tissue.

References:

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Acknowledgement:

The authors acknowledge funding from Czech science foundation project 25-15453S to Marcela Nouzova.

# Analysis of Alginate Hydrogel Structure as a Function of the Crosslinking Agent in *Azotobacter vinelandii* Encapsulation

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Alginate is a linear polysaccharide composed of β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G) [1]. Its crosslinking is most commonly achieved using divalent cations such as $Ca^{2+}$. This process is known as the "egg-box" model, where the chains are linked by the cation between the G-blocks of the alginate chains. Perhaps the most frequently used crosslinking agent is $CaCl_2$, however its application leads to the rapid formation of an inhomogeneous hydrogel due to the high solubility of this compound in water at neutral pH [2, 3]. To achieve a more homogeneous hydrogel, almost water insoluble $CaCO_3$ can be used in combination with glucono-δ-lactone (GDL), which gradually lowers the pH, dissolves the $CaCO_3$, and releases $Ca^{2+}$ ions for crosslinking [3, 4]. Protonation ($H^+$) can also induce crosslinking, resulting in the formation of hydrogen bonds between alginate chains [2].

This study investigates the bacterium *Azotobacter vinelandii*, a producer of extracellular alginate. To examine its encapsulation, selected crosslinking agents (2% (w/w) $CaCl_2$, 0.5 M malic acid, 1 M GDL and a mixture of 1 M GDL with 0.5 M $CaCO_3$) were added to cultured bacterial samples. The encapsulated *A. vinelandii* cells were analysed using cryogenic scanning electron microscopy (cryo-SEM) and low-voltage scanning transmission electron microscopy (LV-STEM). Cryo-SEM sample preparation consisted of high-pressure freezing (HPF), freeze fracture and freeze etching. LV-STEM sample preparation also included HPF, however, it was followed by freeze substitution, resin embedding, ultrathin sectioning, and contrasting with lead citrate [5].

Encystation of *A. vinelandii* under unfavourable conditions leads to rounding of the cell's central body and the formation of an outer capsule. The outer capsule consists of two layers, the exine, which is composed of polysaccharides, proteins and lipids in roughly equal proportions, and the intine, which also contains these macromolecules, but polysaccharides and lipids as the predominant components, while proteins are present in smaller amounts. The main polysaccharide in the capsule is the alginate, with the M/G ratio differing between the exine and intine. G-blocks predominate in the exine, whereas M-blocks are more abundant in the intine. This is supported by our research, as the exine appeared well crosslinked and highly contrasted, while the intine was observed as an electron-transparent layer. In addition to the capsule, alginate is secreted into the surrounding environment. When $CaCl_2$ or malic acid was added, a similar hydrogel structure was formed, with GDL, the hydrogel was denser and more precise. An even denser and precise structure was observed for the samples with addition of GDL and $CaCO_3$.

These findings demonstrate the potential of cryogenic preparation and electron microscopy techniques for the ultrastructural analysis of encapsulated bacteria in hydrogel. Further research aims to deepen our understanding of the effect of different crosslinking reagents on the alginate structure.

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Acknowledgements:

This research was supported by the projects 25-18143S provided by The Czech Science Foundation (GACR) and GA23-06757S (GACR). We also acknowledge the Core Facility Electron Microscopy

and Raman Spectroscopy, supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR).

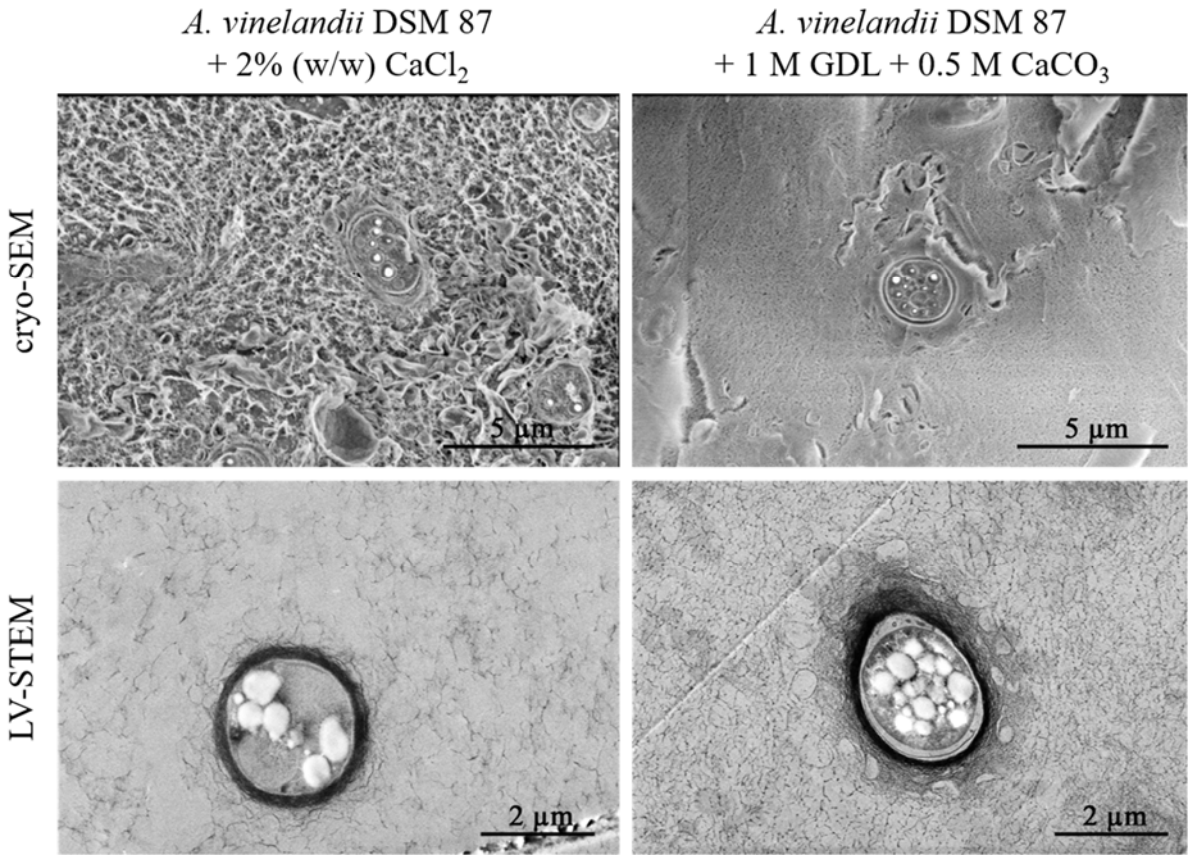


*Figure 1: Cryo-SEM and LV-STEM of Azotobacter vinelandii DSM 87 encapsulated in an alginate hydrogel crosslinked with either 2% (w/w) $CaCl_2$ or 1 M GDL + 0.5 M $CaCO_3$.*

# Volume electron microscopy reveals rare host–pathogen interactions in skin: An efficient targeted imaging approach

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Although the contours of the dissemination pathways of human pathogenic spirochetes in the vertebrate hosts are known, detailed high-resolution data on these processes remain lacking. In this study, we established an efficient large-volume electron microscopy workflow incorporating semi-automatic AI-driven segmentation to investigate the architecture of early events following the deposition of *Borrelia burgdorferi* at the tick bite site. We visualized the infiltration of immune cells in the mouse skin and their interactions with invading bacteria. We captured evidence of *Borrelia* penetrating lymphatic endothelium via both transcellular and paracellular routes, and observed its early presence within the lumen of lymphatic vessels. Our findings reveal that the initial contact of *B. burgdorferi* and blood capillaries is not random, but frequently involves close interactions with pericytes. These observations suggest that *Borrelia* strategically targets vascular regions with lower mechanical resistance to breach the endothelial barrier, thereby enhancing its dissemination.

Acknowledgement:

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# Biogenesis of extracellular vesicles from tapeworm Hymenolepis diminuta investigated by electron tomography

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Extracellular vesicles (EVs) derived from parasites have been identified as key regulators of host-parasite interactions (Marcilla et al., 2014). However, the mechanisms underlying their formation, release, and secretory activity remain poorly understood. In this study, we present preliminary findings on the formation of EVs by the adult tapeworm Hymenolepis diminuta using electron tomography.

Adult Hymenolepis diminuta (Cestoda: Cyclophyllidea) specimens were obtained from experimentally infected Wistar rats (Rattus norvegicus, Berkenhout) provided by the Laboratory of Parasitic Therapy, BC CAS, Czech Republic (see Jirků-Pomajblíková et al., 2018). The tapeworms were processed for electron tomography using high-pressure freezing (HPF) and freeze substitution (FS) methods, then sectioned into 100 nm thick slices. A complete map of the tapeworm's surface was generated, and 22 tomograms were reconstructed from five selected worm surface areas. Electron tomography was performed using a JEOL 2100F transmission electron microscope equipped with a Gatan K2 Summit direct electron detector. Tomogram reconstruction was carried out using IMOD software package.

These findings provide insights into the structural basis of EV biogenesis in H. diminuta, offering a foundation for future studies on the role of EVs in host-parasite communication.

Acknowledgement:

This work was supported by the Czech-BioImaging large RI project (LM2023050),
OP VVV CZ.02.1.01/0.0/0.0/18_046/0016045 funded by MEYS CR and TACR (TN02000020).

# High-Resolution Scanning Electron Microscopy (HRSEM) in the Taxonomic Identification of *Penicillium* sp: A Comparative Evaluation of Sample Preparation Techniques

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High-resolution scanning electron microscopy (HRSEM) is widely employed for the taxonomic identification of fungal species, primarily based on the morphological characteristics of their spores [1]. As the resolution capabilities of HRSEM continue to improve, increasing demands are placed on sample preparation techniques to minimize artifacts.

Filamentous fungi of the *Penicillium* genus possess a complex structural organization that is particularly prone to deformation during specimen processing. Before HRSEM analysis, samples must be completely dehydrated to withstand the high vacuum environment within the microscope chamber. The most commonly used preparation protocol involves chemical fixation with glutaraldehyde and osmium tetroxide, followed by dehydration using organic solvents and critical point drying. However, this method frequently induces structural distortions, including shrinkage, shape deformation, and collapse of fragile fungal elements.

To mitigate these issues, modified protocols employing osmium tetroxide vapor fixation followed by air-drying or freeze-drying have been proposed as superior alternatives [2]. These approaches better preserve native morphology, although some degree of cell shrinkage still occurs.

Currently, the direct observation of frozen samples via cryo-HRSEM is considered the most effective approach for visualizing fungal spores in a state closely resembling their natural environment. However, this technique requires, when needed, the long-term storage of samples in liquid nitrogen. In response to these limitations, we explored the feasibility of using the freeze substitution method, which integrates the benefits of cryofixation with those of conventional chemical processing.

In this study, *Penicillium* mold samples were prepared using four distinct protocols—two based on chemical fixation and two employing cryofixation. Evaluation of the resulting samples, including spore size measurements and conidial morphology assessments, revealed that only cryofixation-based methods yielded specimens free from preparation-induced artifacts such as shrinkage, shape distortion, and collapse of delicate structures.

References:

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Acknowledgement:

This work was supported by TACR (TN0100008) and MEYS CR (Czech BioImaging LM2018129).

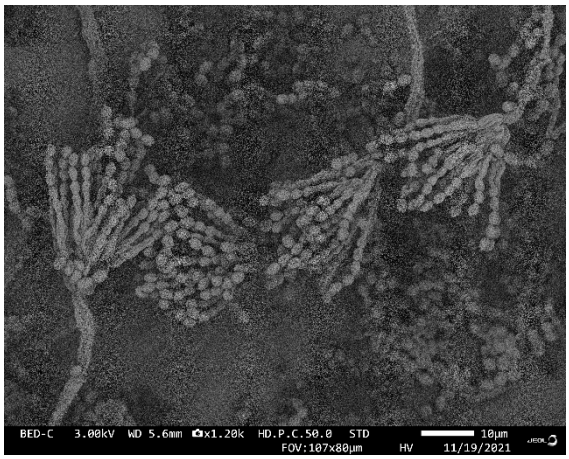


*Fig. 1: Preserved structure of conidia and conidiophore in a sample of Penicillium mold prepared by freeze substitution method.*

# IMG Electron Microscopy Core Facility

Filimoněnko V. V.[1,2], Pinkas D.[1], Vlčák E.[1], Dalecká M.[1], Raabová H.[1]

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The Electron Microscopy Core Facility offers services in a wide range of both advanced and routine techniques, focused mainly on biological samples. The team's expertise is supported by state-of-the-art equipment for sample preparation and ultrastructural imaging. Our high-end transmission electron microscope (TEM) operates at up to 200 kV and offers high-resolution TEM, imaging in STEM mode, 3D analysis by TEM- or STEM tomography, cryo-electron microscopy and STEM-EDS elemental analysis. For routine observation, a standard 120kV TEM with a user-friendly Limitless panorama application is used. Recently we have incorporated FIB-SEM instrument and developed a cryo-lamella lift-out workflow.

Standard and advanced techniques are tied up also with the sample preparation. Starting from routine chemical fixation and resin embedding, or negative staining of weakly observable samples, we can proceed to better preservation of natural sample appearance by cryofixation using plunge-freezing or high-pressure freezing, followed by freeze-substitution, cryosectioning or freeze fracture replica labeling. For even more advanced applications we can provide cryoCLEM technique using a specialized microscope. Another field forms pre- and post-embedding immunolabeling techniques using gold nanoparticles of different sizes.

Users as well as potential applicants can think of service processing and imaging of various biological samples – in the core facility we can deal with human and animal cell cultures, plant and animal tissues, worms, microorganisms, lipid micelles, isolated DNA, or purified proteins. We provide development and optimization of sample preparation, based on a long expertise and 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:

We acknowledge funding from MEYS CR (LM2023050), ERDF (CZ.02.1.01/0.0/16_013/0001775, CZ.02.1.01/0.0/0.0/18_046/0016045, and CZ.02.01.01/00/23_015/0008205), and IMG grant (RVO: 68378050).

# Drawings versus micrographs: Kidney and pancreas in the histology classroom

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Medical students' compulsory curriculum has traditionally included histology since times immemorial, as it is essential to understand the function of tissues and organs in health and disease. Typically, a textbook (or "*Lehrbuch*" in German) and an atlas were expected to be used in concert [1]. While the textbook typically featured only schematic drawings of the relevant structures, it contained references to *camera lucida* drawings or micrographs shown in the atlas. The schematic drawings were produced by professional illustrators, the *camera lucida* ones by the authors themselves. In both cases, the important/insignificant features could be emphasized/omitted (much like e.g. in drawings of animals for children), with the ultimate aim to facilitate navigation in micrographs. Indeed, some authors [2] rightly claim that a good histological illustration should be able to 'speak' to the reader, which the micrograph alone often does not.

With the advent of molecular biology, morphological subjects started to be sidelined in the curriculum, and as the histology atlas naturally could not be completely eliminated, it was the traditional textbook rich in high-quality drawings that became the 'endangered species'. Indeed, the one-volume model combining the textbook and the atlas has ultimately prevailed [3], often at the expense of the schematic drawings instrumental in understanding the cell/tissue architecture. Junqueira's histology originally published in Portuguese in early 1970s represents a rare exception from this trend; the state of São Paolo provided funding for a professional illustrator (Gonçalves) to help Junqueira.

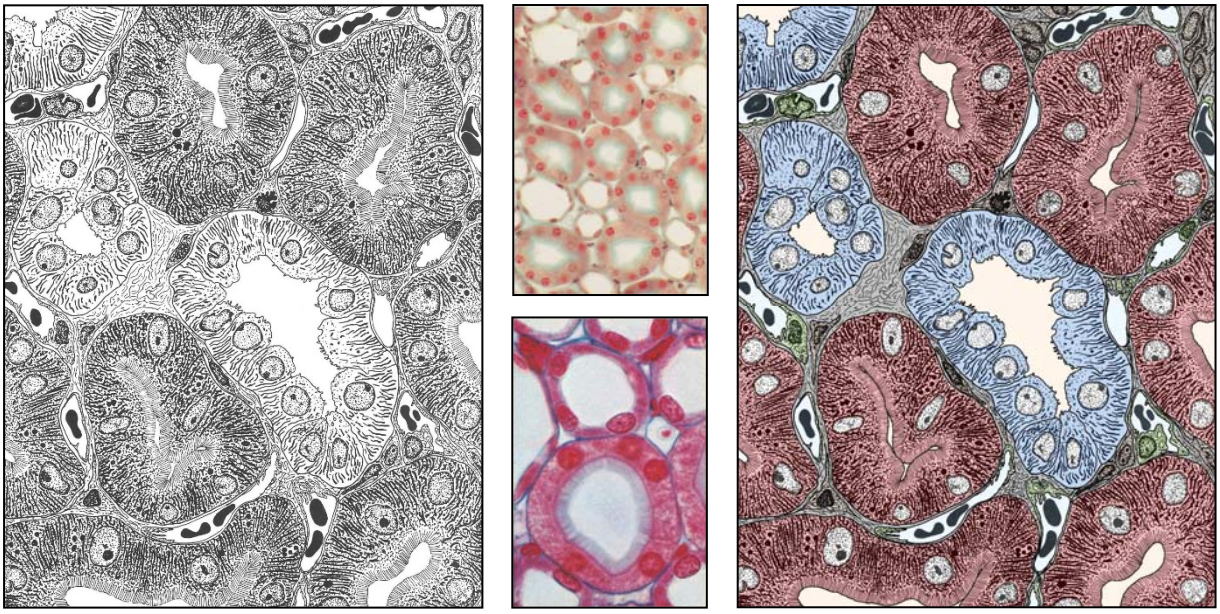

***Fig. 1**. Kidney tubules. **LEFT**: Original drawing reprinted from Ref. [4]. **RIGHT**: Updated version (by Shuvangini Ranjitkar, using the ProCreate software) rendering the proximal/distal tubules (pars convoluta) in red/blue. Endothelial cells of blood capillaries are highlighted in green. **MIDDLE**: Matching light micrographs (reprinted from Ref. [5]) of the proximal/distal tubules (pars recta). The brush border of the proximal tubules is stained either green by Masson-Goldner trichrome (**top**), or pale blue by azan (**bottom**)*

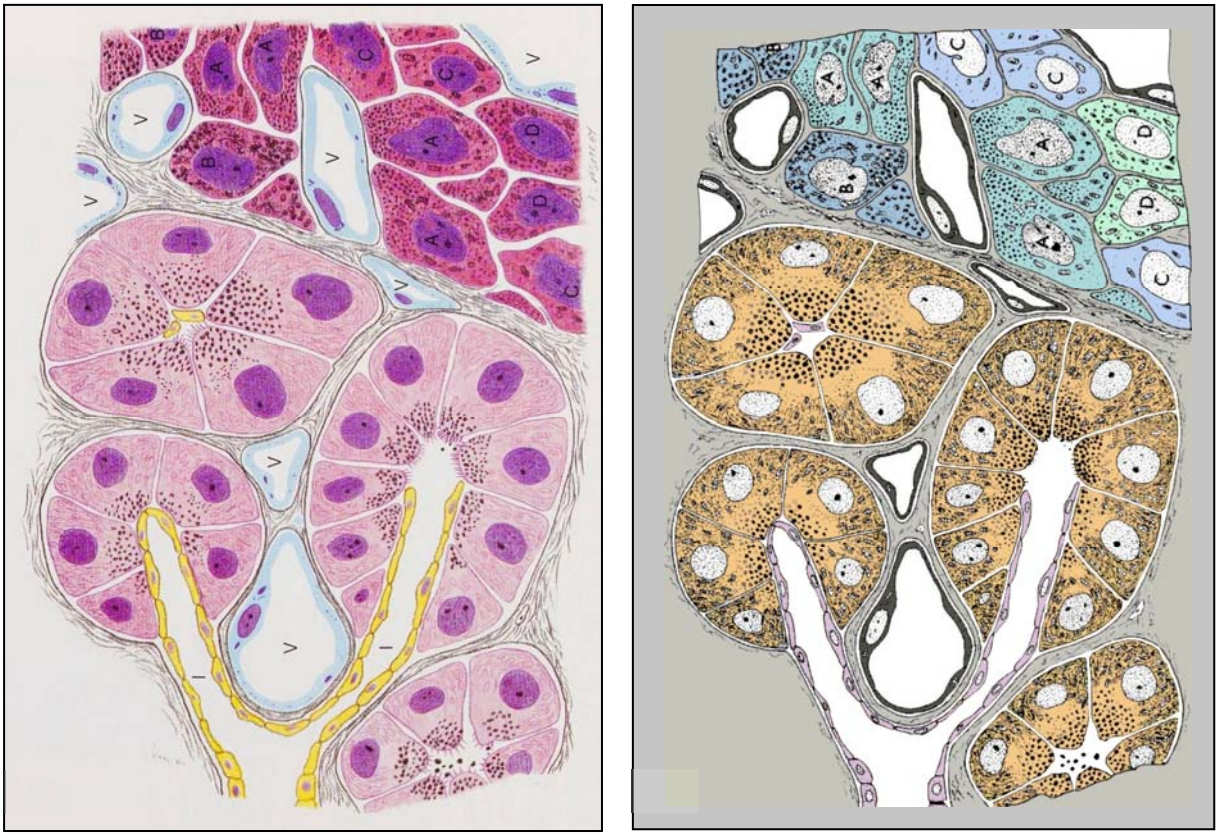

***Fig. 2**. Ultrastructure of the pancreas. **LEFT**: Original drawing reprinted from Ref. [3]. **I**, intercalated ducts. **V**, blood vessels. **RIGHT**: Updated version (by Ada Syed, using the GIMP software) rendering the endocrine/exocrine part in cold/warm colours. **A**, **B**, **C** and **D** denote the α-, β-, δ- and PP-cells, respectively*

While Radivoj Krstić (Lausanne) and Gerhard Spitzer (Kiel) are well known medically-qualified illustrators, Sylvia Collard Keene (Boston), Gordon/Irvin/Watkinson (Toronto) and many others are also prominent. Frequently, their artwork is found in German literature published in the pre-digital age [4,6], i.e., nowadays practically 'invisible'.

As the drawings are typically published in black & white, we enhanced them by applying suitable colour schemes using ImageJ (National Institutes of Health, Bethesda, MD, USA), GIMP, ProCreate and other software tools. In some cases, the colour schemes were optimized on student volunteers.

Cells of the convoluted tubules in the kidney are presented (**Fig. 1**), some of them in drawings extracted from electron micrographs by morphometric analysis (poster). The endocrine/exocrine pancreas is rendered in pastel (high-value, low-saturation) colours, and individual cell types of its endocrine part (α, β, δ and PP) in shades of blue/green (**Fig. 2**). Matching colour-enhanced mesoscopic-scale drawing and electron micrographs are also included (poster).

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# Snapshots of the glucose metabolism studied by electron cryo-microscopy *in vitro* and *in situ*

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Insulin is a key hormone responsible for maintaining glucose homeostasis. It is stored in pancreatic cells in a form of dense granules. We have used FIB/SEM microscopy and correlative light-electron microscopy (CLEM) to quantify the granule presence in different cell lines and studied their structure. Insulin receptor (IR) is a receptor tyrosine kinase which upon insulin binding on the extracellular receptor domain induces autophoshorylation reaction on its cytoplasmic domains. Misregulation in the insulin signalling is a cause of Diabetes melitus I and II. We have studied the mechanism of IR inhibition with insulin non-related peptidomimetics capable to fully antagonise insulin action to prevent aberrant IR signalling.

# The study of a chromatin-associated protein using Single-molecule FRET

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Transcription is controlled by epigenetic regulators that modify histones via deposition of epigenetic marks, which are in turn 'read' by epigenetic readers. Lens epithelium-derived growth factor (LEDGF) is an essential epigenetic reader and a transcriptional co-activator. LEDGF is therapeutically important as a host cell co-factor in HIV-1 integration and a key factor in MLL (Mixed lineage leukemia) transformation. It interacts with HIV-1 Integrase and chimeric MLL/menin, among others, via its C-terminal protein-binding domain. It then 'reads' the mark of active genes and tethers the complexes to active chromatin via its N-terminal PWWP domain. These two domains are linked by the central intrinsically disordered region (IDR). Due to the protein's disorder and flexibility, traditional structural biology methods were only able to resolve the N-terminal PWWP domain. Single-molecule (sm)FRET offers to 'view' the structural heterogeneities and dynamics of biomolecules. Experiments with fluorescently labelled nucleosomes revealed that binding of LEDGF/p75 leads to a more unwound nucleosome. Analysis of fluorescently labelled LEDGF shows distinct conformational shifts in different protein regions upon binding to DNA and nucleosomes. Fast and transient interactions and conformational changes, otherwise masked by ensemble averaging, are revealed thanks to single-molecule methods. Analyses performed validated smFRET, with Pulsed interleaved excitation (PIE), as an excellent tool to study the protein-nucleosome interactions. smFRET, in combination with other methods from the Dynamic structural biology toolbox, can allow a full picture of the protein's conformational states coupled to the biological functions to emerge.

Acknowledgement:

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# Simple ultrafast confocal microscopy for imaging fluorescent molecular biosensors

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Here we present a simplified design of our tri-scanning confocal microscope[1], along with examples of applications in biological imaging. By focusing the illuminating laser into a line, scanning with a single galvanometric mirror, and detecting the fluorescence by a sensitive camera, the microscope achieves biological imaging with up to 1 kiloHertz image acquisition rates. We demonstrate uses of the microscope in fast imaging of fluorescent biosensors of molecular events of cell signaling [2], as well as in fast 3D imaging of organoids.

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Acknowledgement:

The authors acknowledge funding from European Innovation Council PathFinder project UniSens (J.L) and European Research Council grant 101113439 (P.D.)

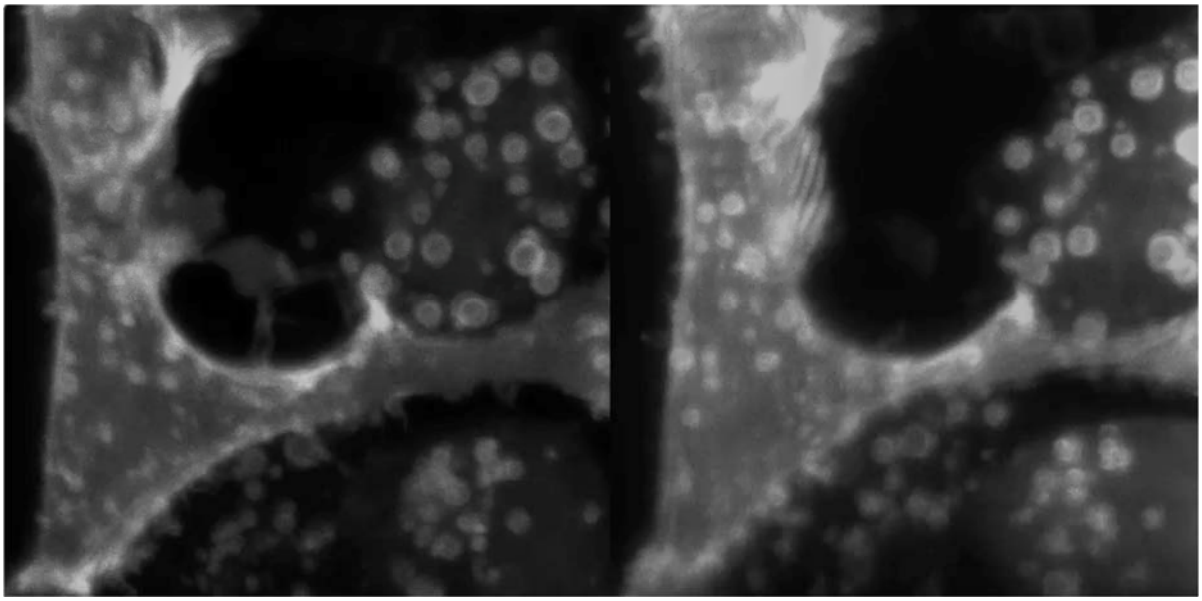

*Fig. 1: Images of an HEK293 cell expressing a genetically encoded fluorescent probe of activity of β-arrestin, imaged by a commercial point-scanning confocal microscope (left, image acquisition time 3 seconds) and by the new tri-scanning confocal microscope (right, image acquisition time 150 milliseconds).*

# Detection and Characterisation of Nanoparticles Using LVEM equipped with EDS

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Nanotechnology is becoming increasingly important in various industries, particularly in the cosmetics and food sectors. Despite its widespread application, ensuring the safety and accurate quantification of both inorganic and organic nanoparticles remains a challenge, especially with the introduction of stricter regulations governing their use in food products. As these regulatory measures come into effect, the demand for effective monitoring grows, necessitating the development of reliable analytical techniques for nanoparticle detection and control [1], [2].

This study explores the application of low-voltage electron microscopy (LVEM) combined with energy-dispersive spectroscopy (EDS) for detecting and characterizing inorganic nanoparticles in food products. Although LVEM is widely recognized for its high-contrast imaging of light-element samples, the introduction of the LVEM 25E—an "all-in-one" device—has significantly broadened its capabilities. With support for multiple imaging and analytical modes, including transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM), scanning electron microscopy (SEM) with backscattered electron detection (BSE), electron diffraction, dark-field imaging, and elemental analysis via EDS, this system provides a versatile and user-friendly platform for routine nanoparticle characterization.

The combination of LVEM and EDS offers a powerful tool for detecting and analyzing nanoparticles in food products, a field of growing scientific and regulatory interest. Ensuring the presence—or absence—of nanoparticles in food is crucial for health safety, regulatory compliance, and quality control. Our research focused on the analysis of selected food products, including Silver Sugar Pearls (Cake), Shimmering Deco Colored Lustre Dust, and Sugarflair Extra White Semi-Liquid Coloring, which were chosen based on their ingredient lists suggesting the potential presence of nanoparticles.

To optimize the analytical workflow, a sample preparation protocol was experimentally refined. In the case of silver sugar pearls, silver was detected as expected. However, titanium was also identified, despite not being listed among the ingredients and its use no longer being permitted. These findings underscore the reliability of LVEM 25E equipped with EDS for detecting nanoparticles in food additives and demonstrate its potential as a routine analytical method for food safety and regulatory enforcement.

Given the increasing importance of microscopy techniques in nanomaterial analysis, the integration of LVEM and EDS presents a valuable advancement for more accurate nanoparticle characterization in food products. This approach not only enhances food safety monitoring but also supports compliance with evolving regulations, making it a promising tool for routine quality control and regulatory oversight.

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Acknowledgement:

The authors gratefully acknowledge the Brno University of Technology for funding this research. We also extend our sincere gratitude to Delong Instruments a. s. for providing high-quality instruments that significantly contributed to this study.

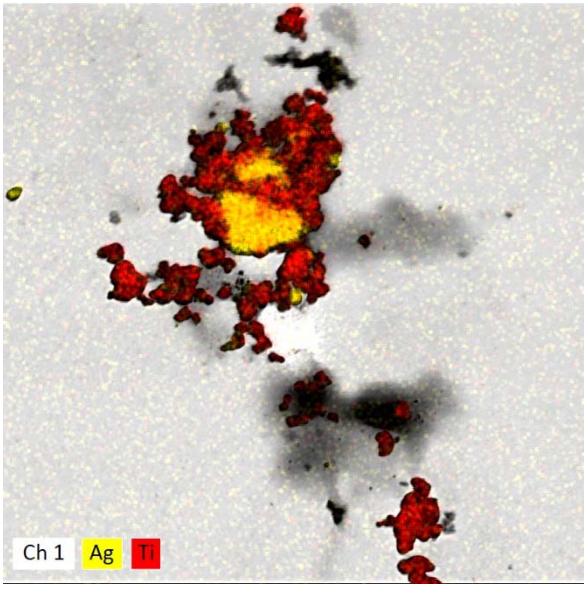


*Fig. 1: EDS mapping of titanium and silver nanoparticles in Silver Sugar Pearls*

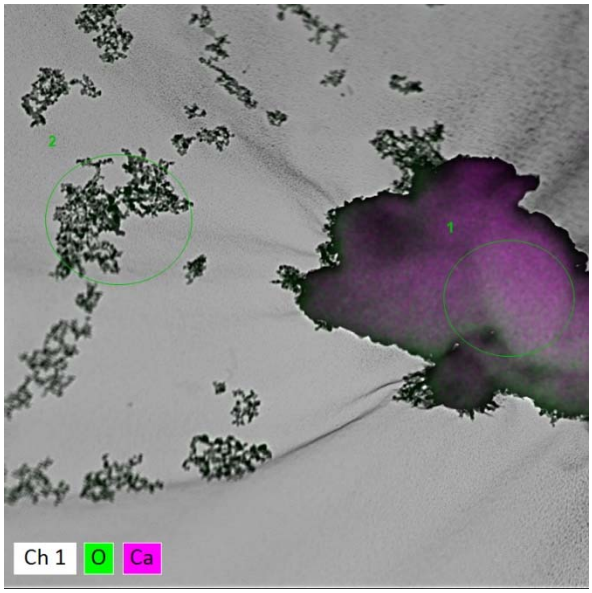


*Fig. 2: EDS mapping of oxygen and calcium nanoparticles in Sugarflair Extra White Semi-Liquid Coloring*

# Microstructure of NiAl/FeSi intermetallic composites

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The NiAl/FeSi intermetallic composites are developed as a replacement of common tooling materials containing critical elements like W and Co. The material is formed from NiAl matrix with reinforcement FeSi particles. In this work, detailed microstructure of dual phase material will be described. The composite materials were observed by TEM with special focus paid to the grain boundaries. Subsequently, the samples were observed by SEM and the crystallographic orientation of phases was estimated by the EBSD technique. As the materials exhibited preferential orientation between phases, the obtained result were key factor for suggesting of post-processing of NiAl/FeSi intermetallic composites.

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Acknowledgement:

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# The problem of asbestos identification in building materials

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Asbestos materials are products in which asbestos was used as a construction or industrial material, taking advantage of its unique properties, such as resistance to high temperatures, strength, flexibility and insulation properties. Asbestos was widely used in the construction, automotive, electrical and other industries until its harmful health properties were discovered. Today, products containing asbestos are mostly banned in many countries, including all EU countries. However, they can still be found in older buildings that were built before the ban on asbestos was introduced [1]. Identification of asbestos in building materials is a key process that aims to ensure the health safety of people staying in buildings where asbestos-containing materials may be present.

Asbestos was widely used in construction, especially in producing building materials (boards, pipes, roof tiles). It was also used in the production of facades, floors, windows, roofs, fences and in various ventilation systems. In addition, identifying the type of asbestos present in the material is important in the context of its disposal and potential recycling methods, e.g. thermal. Asbestos occurs in nature in several varieties, each with different physical and chemical properties. There are six main varieties of asbestos, which are divided into two groups: amphibole asbestos and serpentine asbestos (Fig. 1). The diversity of asbestos results from different crystalline structures and chemical compositions, although generally speaking, chemically asbestos is a group of hydrated silicate minerals of various metals [2,3]. Identification of asbestos type is very important due to the fact, that amphibole asbestos minerals are considered as more dangerous for health in comparison to serpentine asbestos i.e. chrysotile [2].

Laboratories use various methods to test and identify samples with asbestos. There is no universal method - each of them has its advantages and limitations. One of the possible methods for identifying asbestos in materials, especially construction materials, is scanning microscopy with X-ray spectrometry (SEM-EDS). Asbestos identification is based on the evaluation of the sample image and finding fibrous forms in the sample. In the next step, the chemical composition of the observed fibres should be determined (Fig. 2 and Fig. 3) and compared with the chemical composition of individual varieties of asbestos minerals [4]. Identification of asbestos varieties is crucial in the context of health hazards, first and foremost. However, it may also be significant in terms of possible thermal treatment of asbestos waste, as each mineral behaves differently at high temperatures. Amphibole asbestos requires higher temperatures for its thermal decomposition to occur. The presence of amphibole fibres will be a key aspect influencing the minimum temperature of thermal treatment of the waste.

References:

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Acknowledgement:

This research was funded in whole by the National Science Centre, Poland under “Sonata 17” grant number UMO-2021/43/D/ST5/00356.

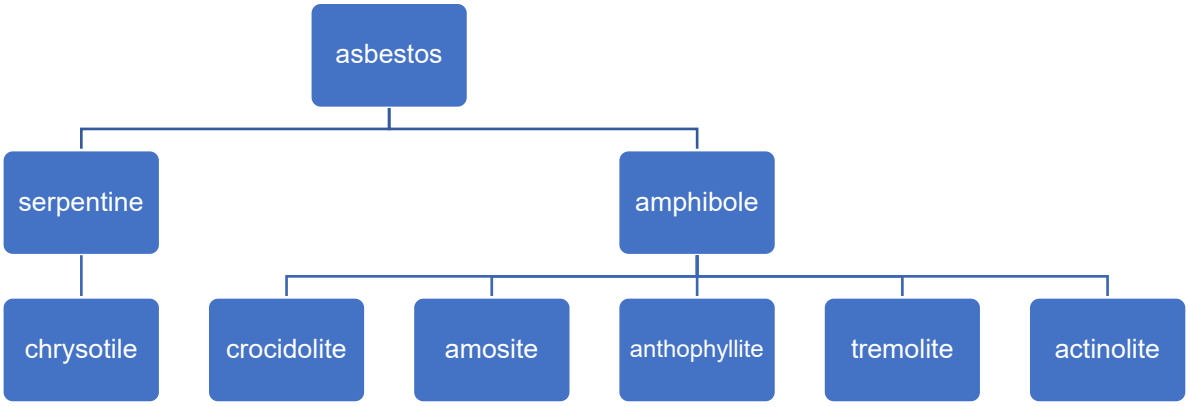


*Fig. 1: Types of asbestos minerals*

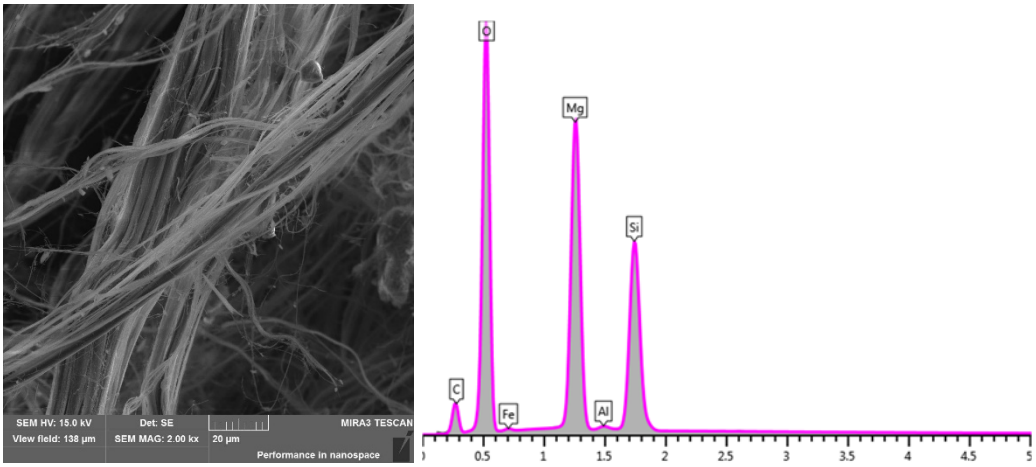


*Fig. 2: Chrysotile asbestos during identification by SEM-EDS*

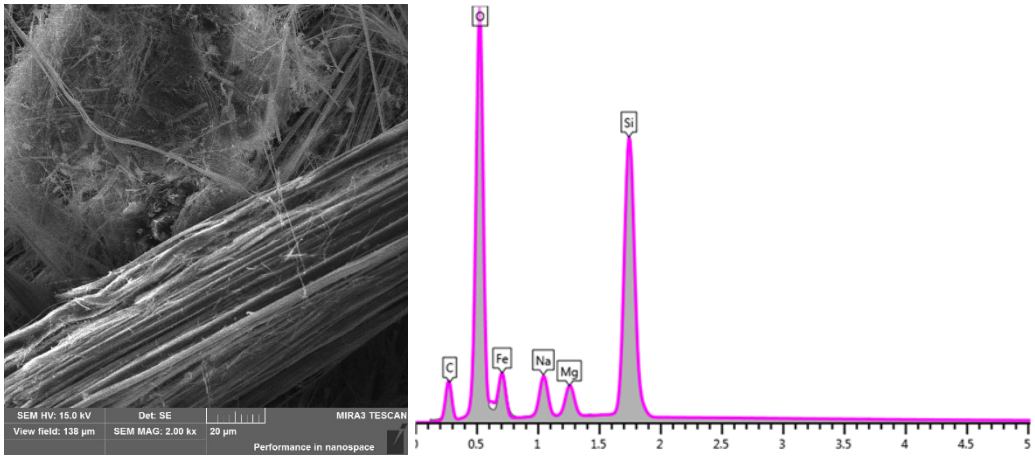


*Fig. 3: Amphibole asbestos (crocidolite) during identification by SEM-EDS*

# Degradation processes in polyolefins with phenolic stabilizers

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Phenolic stabilizers are employed in the stabilization of polymers during the processing to prevent thermooxidation [1]. They are also applied in medical applications to protect the polymers against biooxidation (in vivo oxidation) [1,2]. We investigated photodegradation processes in polymer plaques made of bulk polyolefin (HDPE or UHMWPE or COC) prepared by melt-mixing with or without phenolic natural (α-tocopherol) or synthetic (Irganox®1010) stabilizers and spin trapping agent (TTBNB; 2,4,6-Tri-tert-butylnitrosobenzene). The photodegradation was initiated either by non-ionizing radiation (wavelengths corresponding to terrestrial range of solar UV radiation) or ionizing radiation (high-energy electron beam).

HDPE powder and COC granulate were first homogenized by melt-mixing in the W 50 EH chamber of a twin-screw laboratory mixer (Brabender Plasti-Corder; Germany). The UHMWPE´s plaques were prepared by the compression molding. The UHWMPE powder was dry-mixed with additives and filled into the 6 mm thick frame, which was covered with aluminum foil and a metal plate from each side. Non-ionizing radiation was carried out d in an Atlas Ci 3000+ Weather-Ometer (WOM; Atlas, USA) to simulate solar UV radiation. For ionizing radiation was used an electron beam accelerator ELV-2 (Budker Institute of Nuclear Physics, Novosibirsk, Russia).

In this contribution, we summarize our results concerning the comparison of the degradation processes in selected polyolefin after non-ionizing and ionizing radiation. The samples were characterized IR microspectroscopy, ESR spectroscopy including ESRI imaging, LM and SEM microscopy, and microindentation hardness testing. Both non-ionizing and ionizing radiation have generated polymer radicals in the whole volume of the irradiated samples, albeit by different mechanisms. The low-energy non-ionizing radiation is expected to split C-C bonds indirectly, through the formation of hypothetical chromophores, while high-energy ionizing radiation splits C-C bonds directly. Nevertheless, the resulting alkyl radicals might be assumed to undergo analogous reactions, regardless of their origin. However, our study has demonstrated clearly that the stability/reactivity of the generated radicals (and the concentration of the subsequently generated radical-induced oxidation products) depended on the mechanism of the radical generation, in addition to the dynamics of polymer chains inside the studied polymers, which is closely related to their glass transition temperature. Moreover, the results have documented that the natural phenolic stabilizer α-tocopherol exhibited antioxidant activity during thermooxidation, prooxidant activity during exposure to non-ionizing radiation, and antioxidant activity during exposure to ionizing radiation. Finally, the addition of TTBNB spin trapping agent enabled us to catch short-living and unstable radicals in HDPE, which could not be detected in the previous studies.

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# Commercial Alternatives to Novel Synthesized Embedding Resins with Higher Resistance to E-Beam Damage

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Electron microscopy (EM) is a widely used technique for characterizing biological samples, such as tissues, cells, or their organelles, from both plants and animals. A crucial step in preparing such samples for EM observation is to assure the respective specimen's structural integrity. This can be done by the embedding of the specimen into a synthetic polymer matrix (embedding resin). That preparation method provides efficient protection and fixation of the specimen, and hence embedding is one of the preferred preparation approaches for EM.

Modern volume visualization techniques in 3D-SEM employ lower electron energies, backscattered electrons, and higher electron doses, in contrast to 'classical' TEM techniques. The conventional embedding resins, however, are optimized for the classical 2D-TEM imaging at high accelerating voltages, and they were repeatedly reported as unsuitable for the modern volume visualization methods: These conventional resins often suffer from charging and electron beam damage, if volume EM is performed.

To address the above-described problem, we have developed two new types of embedding polymer resins with enhanced resistance to e-beam damage:
a) Commercial resins incorporating different chemical stabilizing agents, temporarily designated as A, B, and C (already presented; patent application pending).
b) Siloxane-based resins, which are inherently more resistant to e-beam exposure.

Concerning the siloxane resins, the first family of these products was synthesized directly in our laboratory, and it has been gradually optimized via small chemical modifications, in order to improve the resins' adhesion to biological samples, as well as their cuttability in microtomes and ultramicrotomes. Additionally, efforts have been made to simplify the resins' preparation protocol, for the sake of facilitating its use in biological laboratories.

While the above-mentioned siloxane-type embedding resins yielded promising results, their synthesis remains time-consuming and complex, at least for a common biological laboratory. Moreover, the properties of the above materials may exhibit slight variations between individual laboratory-prepared batches. Therefore, we sought to find a commercially available siloxane-based alternative material, which would maintain a comparable EM performance like the laboratory-prepared resins, but would be easier to handle.

The present study focuses on testing a commercially available siloxane resins with high resistance to e-beam damage, for embedding various biological samples, such as mouse brain, cerebellum, lungs, and plant specimens. Additionally, we tested also 'difficult' specimens of non-biological type, such as magnetic polymer microspheres, which normally present a considerable challenge for the SEM analysis, due to their negligible conductivity and high susceptibility to e-beam induced charging.

Our results have demonstrated that the commercially available siloxane resins exhibit promising potential as e-beam resistant embedding resins, which offer lower charging and improved contrast than the common, traditional, commercial carbon-based resins. A further optimization and perfection of the sample preparation protocol still is the subject of our ongoing research.

Acknowledgement:

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# Lowering thermoplastic starch processing temperature by means of acid hydrolysis

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**Introduction.** Thermoplastic starch (TPS) is a promising biodegradable material; however, its processing typically requires high temperatures, which can be energy-intensive and environmentally impactful1. To address this challenge, we explore the potential of acid hydrolysis to reduce the molecular weight of starch, thereby lowering the processing temperature while maintaining the homogeneity of the final product. In this study, starch was subjected to acid hydrolysis at three different times to evaluate its effect on the morphology, mechanical performance, and processing properties.

**Experimental.** Starch was subjected to acid hydrolysis for various times (0, 15, 30, and 60 minutes), followed by neutralization and drying in an oven for 24 hours at 45°C. The dried starch was milled into a fine powder and processed using the previously established two-step preparation protocol2. The homogeneity of the resulting TPS samples was assessed using polarized light microscopy (PLM) and scanning electron microscopy (SEM). Furthermore, the influence of starch modification on the macro- and micromechanical properties of TPS was evaluated through rheological measurements including dynamic mechanical thermal analysis (DMTA) and instrumented microindentation hardness testing (MHI).

**Results and conclusion.** Our study highlights the structural, morphological, and crystallinity changes that occur with the increasing time of acid-hydrolysis treatment of the starch powder, both before and after processing. Despite small differences in mechanical properties—such as higher values of G', G", EIT, and HIT observed for the longest acid hydrolysis time—we establish a clear correlation between stiffness and hydrolysis duration. This aligns with WAXS data, which confirm an increase in crystallinity with the increasing hydrolysis time, indicating that the stiffness-related properties increase alongside crystallinity. Additionally, homogeneity evaluation via PLM further supports these findings, as partially destroyed granules can be seen in the 60-minute treated starch, even after processing through SC+MM (Fig. 1d). This reinforces the beneficial role of controlled crystallinity in optimizing material properties.

The slight crystallinity-induced stiffening of the acid-hydrolyzed starch might be seen as a negative effect, but the molecular splitting via acid hydrolysis reduced the viscosity and processing temperature despite this phenomenon, as evidenced by in-situ measurements during the starch processing (Fig. 2). The decrease in the processing temperature could be attributed to the fact that the acid hydrolysis increased the number of short molecules, which acted as a lubricant during the melt mixing of TPS.

**Acknowledgement:** Technology agency of the Czech Republic, program NCK2, project TN02000020.

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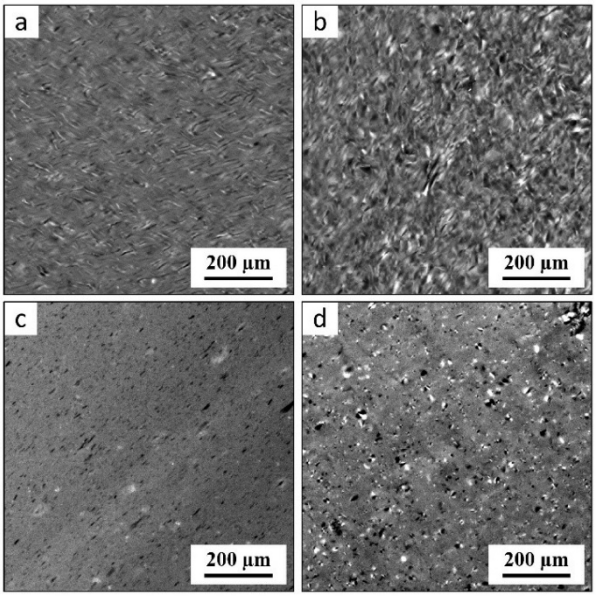


*Figure 1: PLM micrographs showing thin sections of a selection of the prepared acid-treated TPSs after SC and SC+MM: (a) 0minutes_SC, (b) 60minutes_SC, (c), 0minutes_SC+MM and (d) 60minutes_SC+MM.*

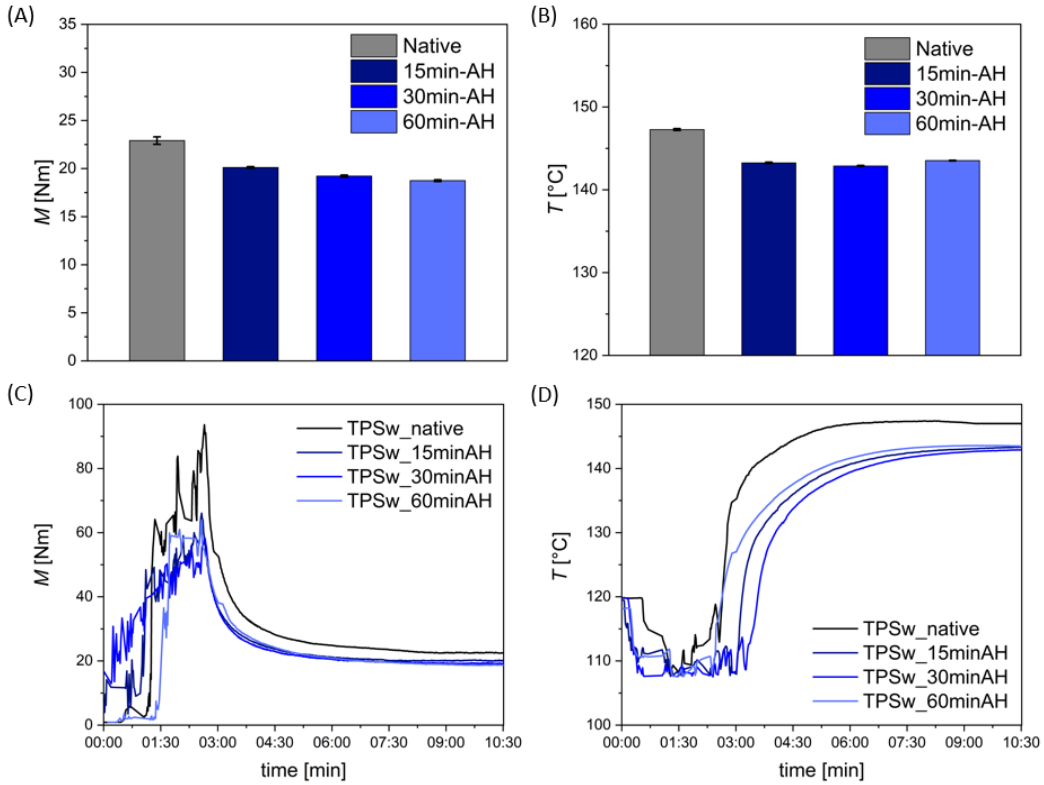


*Figure 2: In-situ measurements of torque moments (a, c) and processing temperatures (b, d) of TPS's with different times of acid hydrolysis.*

# Graphene applications in electron microscopy

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Due to its exceptional physical properties—such as flexibility, high carrier mobility, and transparency—graphene has found widespread applications in electronics, energy, and optics. Its mechanical strength, flexibility, and chemical stability make it particularly well-suited for use in solar cells, transistors, and other electronic components [1]. More recently, its applications have expanded into electron microscopy, offering significant advantages.

In transmission electron microscopy (TEM), graphene is primarily used as a support material on TEM grids. Single-layer graphene (SLG) support films are especially beneficial for high-resolution imaging of nanoparticles and low-contrast materials. However, a key challenge remains: producing free-standing graphene that remains intact during transfer while covering large areas of TEM grids [2]. Typically, graphene layers are applied to grids with hole sizes up to 10 microns or to TEM grids with lacey carbon.

Here, we demonstrate that transferred graphene on a TEM grid can also serve as a substrate for creating free-standing thin metal layers (Fig. 1), achieving 99% grid coverage. These samples are valuable for further analysis, such as electron energy loss spectroscopy (EELS), where transmitted electrons enable the study of material properties, electron behavior in different materials, and the inelastic mean free path (IMFP) of electrons.

Graphene's unique properties also make it an effective protective layer for sensitive samples, shielding them from thermal corrosion (Fig. 2). It prevents degradation of organic and biological materials under vacuum conditions and exposure to high-energy electron beams. Additionally, its excellent electrical conductivity mitigates charging effects in non-conductive samples, which is particularly crucial for imaging insulators in scanning electron microscopy (SEM).

In this work, we further explore graphene's potential in electron microscopy by demonstrating its use as a conductive coating for insulating samples, enabling their analysis in SEM.

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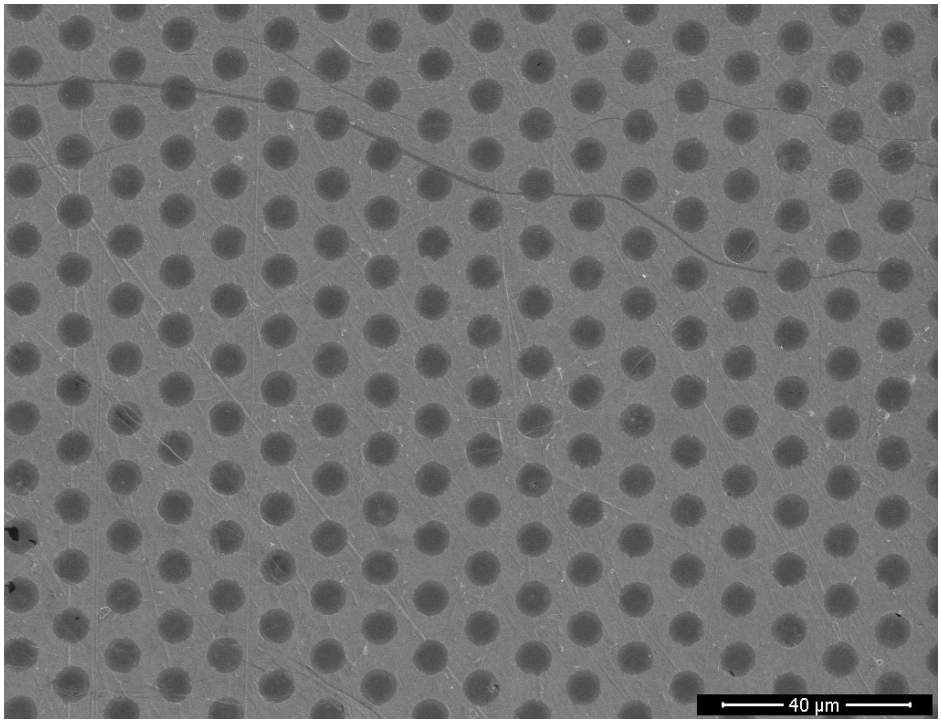


*Fig. 1: Free-standing graphene with free-standing 3 nm of ITO layer over TEM grid with holes ~ 8 µm.*

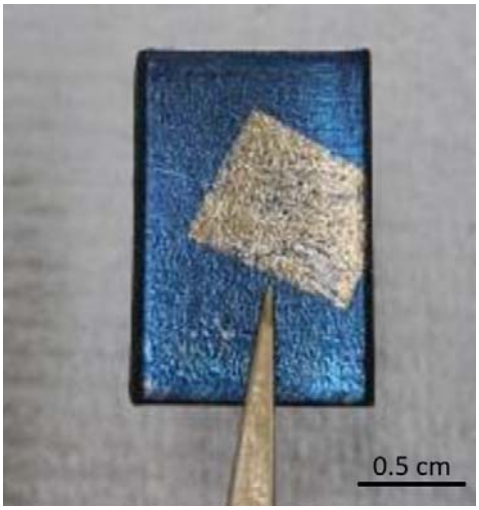


*Fig. 2: Graphene used as a thermal corrosion protection layer on the Fe sample.*

# Simulating images in a transmission electron microscope: a comparison of inelastic scattering models

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**Introduction:**

In transmission electron microscope imaging, the electrons undergo the effects of inelastic scattering primarily due to phonon excitations. Effects of inelastic scattering cause a gradual decrease in the intensity of the elastic scattering component (elastic channel) of the electron beam. The inelastic scattering on lattice vibrations, also known as thermal diffuse scattering (TDS), plays a crucial role in high-resolution transmission electron microscopy (HRTEM) and electron diffraction. The electrons that are inelastically scattered to high scattering angles, and do not backscatter towards the central beam, make up a significant portion of the intensity signal in the dark-field. This fact is utilized in high-angle annular dark-field (HAADF) imaging. [1,2]

To account for the effects of inelastic scattering, absorptive effects must be incorporated into the simulation of electron propagation through the specimen. Thus a good model for inelastic scattering is needed, not only to simulate intensity distribution correctly, but the electron imaging and HRTEM imaging in general. TDS shows up as a diffuse background in diffraction patterns and in simulations TDS background may differ in shape, intensity, and characteristic features (such as Kikuchi lines) depending on the model used. Various models, which take into account the absorption effects, exist. We investigate and compare the widely used complex absorptive potentials model to the more elaborate frozen phonon model coming from correlated atomic motion and Einstein model of atomic motion. [3,4,5,6]

**Materials and methods:**

The complex absorptive potentials model views inelastic scattering as the excitation of the crystal and uses many-body quantum mechanics to describe absorption [1]. The frozen phonon model is a semi-classical approximative model that represents electron propagation through a static lattice. In frozen phonon model the static lattice configurations, so-called snapshots, are obtained via molecular dynamics simulation (in LAMMPS software) with addition of interatomic potential or Debye-Waller factors in case of Einstein model. As specimens we use crystals of diamond and strontium titanate (STO), respectively.

For all models we performed extensive multislice calculations (in Dr. Probe software) at 300kV, which trace the evolution of traversing electron wavefunction through the diamond and STO crystals, yielding diffraction patterns. The corresponding diffraction patterns are further investigated and compared accordingly, as they contain the information about scattering and absorption.

**Conclusion:**

We will summarize theory of the inelastic electron scattering as desribed by the different models. We will present the simulation results and discuss non-negligible discrepancies between the models of frozen phonon and complex absorptive model for both diamond and STO. In conclusion, we will recommend an optimal simulation procedure when describing different types of experiments.

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**Acknowledgement:**

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# Imaging methods in 3D X-ray reciprocal space utilized for GeSi microcrystals and GeTe crystalline layers

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In the past, we have investigated the crystalline quality of individual epitaxial microcrystals of different composition (Ge, GeSi and graded ones) and different geometry using scanning X-ray diffraction in order to detect distribution images of sub-micron size lattice strain inhomogeneities induced by defects. In this work, we investigate the structural quality of GeSi microcrystals grown on regular pillar patterns on a Si substrate [1] and GeTe thin layers on $BaF_2$ with local domain structure as well. Using high resolution X-ray diffraction with reciprocal space mapping (RSM) and applying imaging methods such as Radon transform (RT), we were able to obtain 3D maps of X-ray scattering [2].

This non-typical way of 3D reciprocal space mapping of diffuse scattering around symmetric and asymmetric diffraction points realized by azimuthal sample rotation and RT reconstruction was compared to standard RSM measured with collimated beam as well. We study the crystalline quality and defect distribution using X-ray scattering in the form of variously distributed maxima in 3D reciprocal space images in GeSi locally strained microcrystals and in epitaxially grown GeTe ferroelectric layers and we compare the structural parameters with optical and electron microscopy images.

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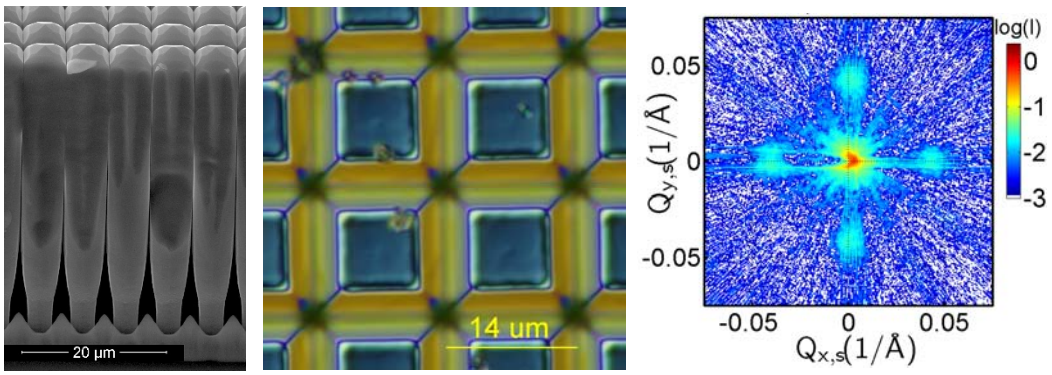


*Fig. 1: (left) SEM perspective view image of 40 µm tall SiGe epitaxial microcrystals grown on Si. (middle) DIC optical micrograph as a top view of 14×14 µm² SiGe microcrystals. (right) Reconstruction of the $Q_xQ_y$ map in reciprocal space using the inverse Radon transform applied on the sinograms can be understood as a given slice for fixed $Q_z$ through the 3D RSM. The four-fold structure of the four maxima is originating from symmetry of the microcrystal shape.*

# Sulfidic inclusions in the tektites from Laos

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Tektites (from the Greek *tēktos* = molten) are natural glasses found in geographically defined areas called strewn fields. Currently, five such fields are documented in the literature [1], with ages ranging from 35 million to 780,000 years. It is generally agreed that these glasses result from hyper-velocity impacts of extraterrestrial bodies on Earth's sedimentary or weathered surface sequences [2,3]. Based on appearance and internal structure, tektites are classified into splashform, ablated/aerodynamically shaped, and Muong Nong types (sometimes called layered, abbreviated MNT). The last type is named after Muong Nong in southern Laos, where they were first described [4]. Tektites up to 2 mm in diameter are called microtektites and are usually splashform. Other key characteristics include high $SiO_2$ content (over 70% except for some microtektites), a lack of crystalline phases, low meteorite content, water content up to a few hundred ppm, and the presence of lechatelierite (quartz glass) particles. Tektites can contain crystalline inclusions which can help us to deduce their origin,

Small, up to 20 microns in size, inclusions were studied by SEM and several TEM techniques, such as 4D STEM (ACOM), EDS, 3D ED to identify the phases present and their mutual relationship.

Two types of sulfidic inclusions were observed (Fig. 1): (A) with Fe- and (Fe,Ni)-sulfide domains and irregular edges bordered by Mg and Fe silicates, with stoichiometry resembling pyroxene-group minerals, and (B) with small worm-like Fe and Ni sulfides in a matrix of FeS. Type (A) inclusions have complex mineralogy, with pentlandite and pyrrhotite identified as main constituents. These inclusions typically feature a pyroxene-group mineral rim 100-150 nm thick, chemically identified as ferrosilite. The structural phases include both monoclinic ($P2_1/c$) and rhombohedral (*Pbca*) forms. The monoclinic phase also contains low concentrations of Ca, Mn, and Al. A layer of chalcopyrite, up to 100 nm thick, is often present between the rim and the sulfide phase, with rare hercynite domains (Fig. 2). Another spinel mineral, magnetite, is found as domains up to 500 nm within pentlandite. Hercynite additionally contains low levels of Ti, Cr, and Zn, while magnetite contains chromium. Type (B) inclusions mainly consist of pyrrhotite, with pentlandite and chalcopyrite also present. Unlike type (A) inclusions, magnetite in type (B) inclusions is pure, without other elements. Native copper is also found in these inclusions.

The presence of these sulfidic inclusions seems more consistent with the inclusion of (ultra)mafic magmatic rocks or their weathered products into tektite melt, rather than extraterrestrial material, supporting their similarity to sulfide inclusions found in terrestrial magmatic rocks [5, 6].

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Acknowledgement:

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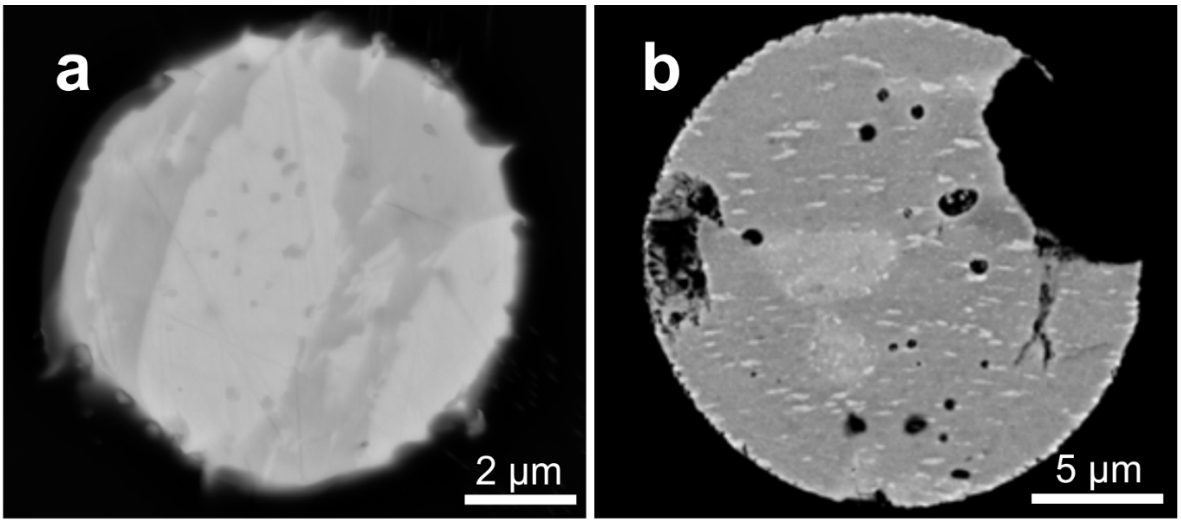


*Fig. 1: SEM micrographs of the sulfidic inclusions in the Muong Nong tektites.*

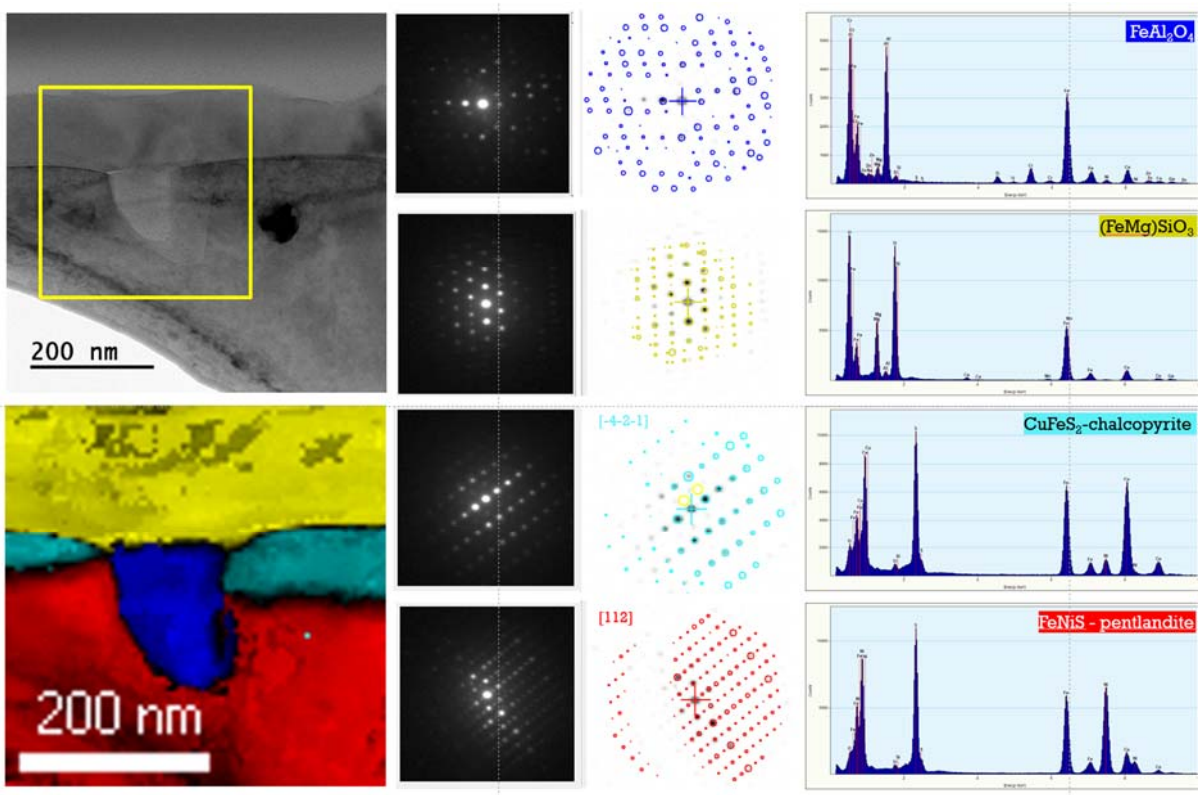


*Fig. 2: Phases present in the A-type inclusion determined by ACOM and EDX mapping.*

# Scalable workflow for analysis of dislocations in GaN and their current leakage behaviour

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## Introduction

Gallium Nitride (GaN) is a leading wide-bandgap semiconductor with high potential for power electronics. Due to the challenges and high costs associated with wafer-scale pure crystalline GaN, industry-scale fabrication relies on epitaxial growth on silicon-based substrates [1]. Despite advancements in buffer layers to mitigate lattice-mismatch-induced stress, strain-related defects remain prevalent in the functional GaN layer.

Among these, threading dislocations (TDs) are particularly critical, as they propagate parallel to the growth direction, affecting the material properties along the substrate-GaN axis. Notably, some TDs exhibit conductive behavior, leading to leakage currents that degrade the reliability and efficiency of GaN-based devices such as high-electron-mobility transistors (HEMTs) [2].

Current state-of-the-art characterization of TDs employs various techniques, including TEM [3], SEM-based methods (ECCI, EBIC) [4], scanning probe microscopy (AFM, C-AFM) [5], and cathodoluminescence (CL). However, these techniques are often used separately, limiting the broader understanding of defect behavior. Noteworthy studies, such as those by Hamachi et al. [2], have explored correlative SEM/C-AFM/TEM/multi-photon PL approaches, while Besendörfer et al. [6] investigated TDs using C-AFM and CL on AlGaN/GaN samples. However, no correlative studies have been conducted on industry-grade GaN epilayers, leaving key efficiency-limiting mechanisms unexplored.

## Project description and results

This work aims to develop a scalable method for statistically characterizing TDs in GaN, focusing on both crystallographic classification and defect conductivity. To achieve this, we employ a correlative single-defect-level analysis using Conductive AFM and Controlled Electron Channeling Contrast Imaging (cECCI).

Our experiments on industry-grade AlGaN/GaN heterostructures grown on silicon reveal edge, screw, and mixed-type TDs, with certain dislocations exhibiting significant leakage currents even at moderate voltage biases. A comprehensive statistical analysis correlating defect type with conductive behavior will be presented.

Building on these results, we introduce a cECCI image-processing engine designed for automated crystallographic classification of individual dislocations. This tool provides a statistical overview of defect density and typology as well as probable leakage behavior, offering a scalable approach for GaN quality assessment. Thanks to modular implementation approach, the engine can be used with different SEM instruments allowing cECCI micrograph acquisition. Additionally, we will discuss the mathematical frameworks and implementation challenges of this engine, paving the way for its potential adaptation to other material systems.

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# Cathodoluminescence Study of Radiation-Resistant Nanoparticles for Biomedical Applications

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Luminescent nanoparticles (LNPs) are promising for biomedical applications such as bioimaging, biosensing, and photodynamic therapy due to their unique optical properties, including photoluminescence and upconversion [1]. Specifically, garnet-based nanoparticles like LuAG:$Pr^{3+}$ have demonstrated potential in X-ray induced photodynamic therapy (PDTX), offering advantages in cancer treatment modalities [2]. In scanning electron microscopy (SEM), fast luminescence decay is beneficial, as it reduces the risk of cathodoluminescence (CL) image blurring and enables higher-resolution imaging [3]. Additionally, a faster scanning rate, even while maintaining the same radiation dose, can lower sample degradation by minimizing localized heating and charge accumulation. Optimizing these properties enhances the suitability of LNPs for advanced biomedical imaging techniques.

In this study, garnet and silicate nanoparticles were synthesized using a photochemical method within an 80-liter photochemical reactor equipped with 28 UV lamps, yielding over 250 grams of nanoparticles per synthesis. Dopants such as $Ce^{3+}$ and $Pr^{3+}$ were selected based on their intended applications in bioimaging and PDTX, respectively. The resulting nanoparticles exhibited an average size of approximately 30 nm. Characterization was performed using a DualBeam FIB-SEM Helios G4 HP microscope equipped with a Delmic SPARC CL detection system.

CL analysis revealed variations in intensity among individual nanoparticles, which may be attributed to factors such as charging effects and the spatial relationship between nanoparticles and the focal point of the CL detector's parabolic mirror. Notably, the nanoparticles demonstrated resilience under electron beam exposure at 10 keV and a current density of 0.26 A/m² over several minutes, with no observable degradation. However, a tendency for nanoparticles to form large clusters was observed. Importantly, the CL spectra remained consistent between single nanoparticles and larger clusters, indicating uniform luminescent properties.

The synthesized garnet and silicate nanoparticles exhibit promising characteristics for biomedical applications, particularly in bioimaging and PDTX. To fully harness their potential, further optimization of sample preparation techniques is necessary to mitigate nanoparticle clustering, charging effects, and variations in working distance during electron microscopy analyses.

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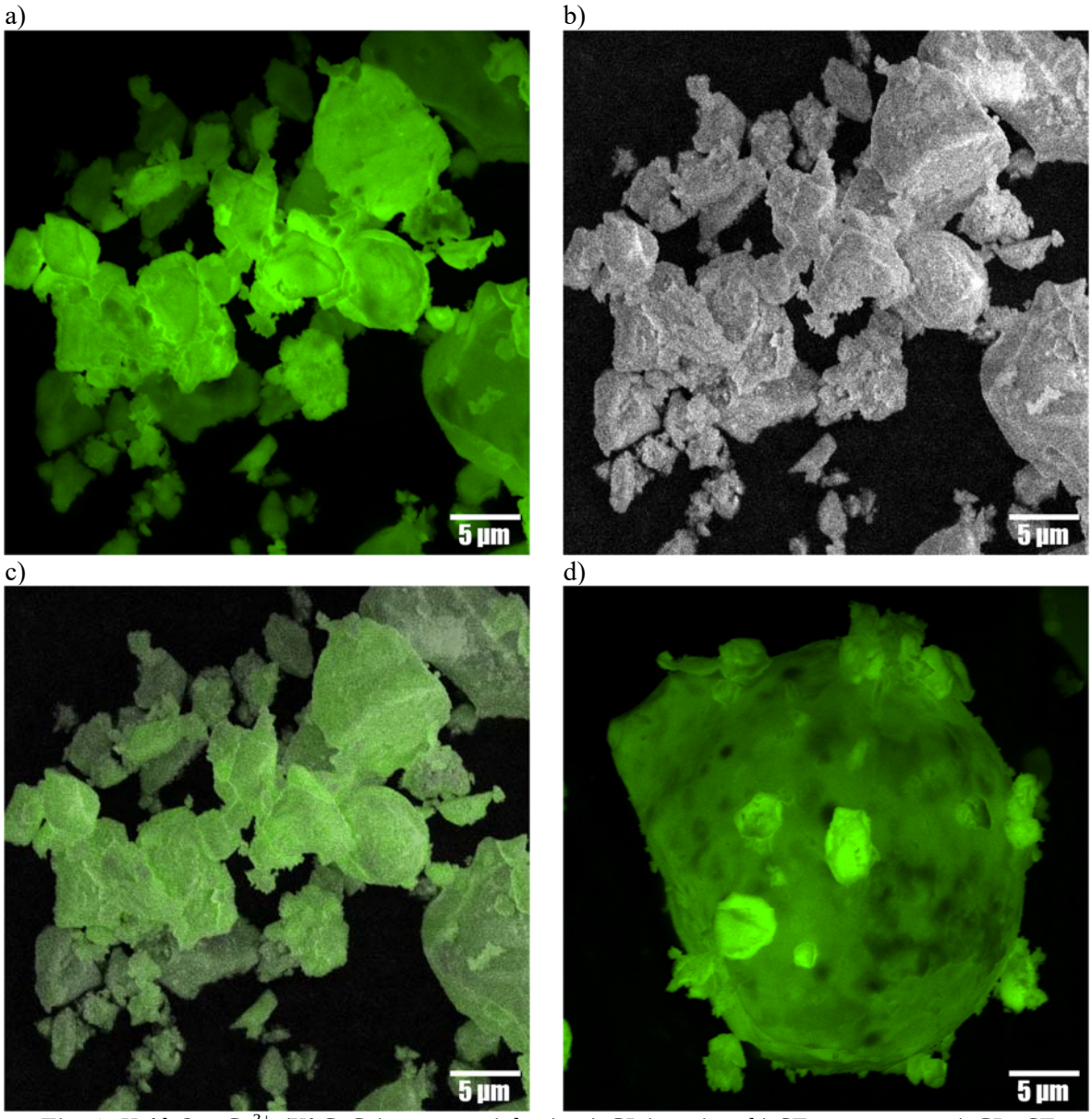


*Fig. 1: $Y_3Al_5O_{12}:Ce^{3+}$ (YAG:Ce) nanoparticles in a) CL imaging, b) SE concurrent, c) CL+SE, d) CL imaging of a big cluster*

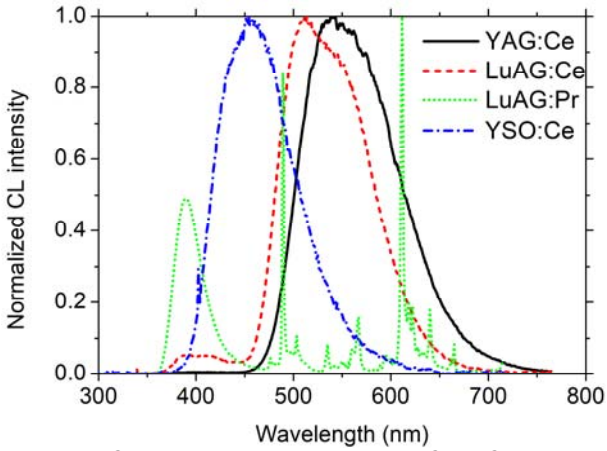


*Fig. 2: CL spectra of $Y_3Al_5O_{12}:Ce^{3+}$ (YAG:Ce), $Lu_3Al_5O_{12}:Ce^{3+}/Pr^{3+}$ (LuAG:Ce/Pr) and $Y_2SiO_5:Ce^{3+}$ (YSO:Ce) nanoparticles. Spectra were normalized to maximum CL intensity.*

# Laser Machining and Modification of SiC with Defect Analysis by Electron Microscopy

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Silicon carbide (SiC) is a popular material in the microchip industry because of its excellent mechanical, electrical, and thermal properties. Its high hardness, corrosion resistance, thermal conductivity, and chemical stability make it especially well-suited for power electronics, as MOSFETs and Schottky diodes in electric vehicles, and high-voltage and high-frequency technologies, including 5G and aerospace electronics. These characteristics make SiC one of the key materials in microchip manufacturing, especially where durability and efficiency are critical.

However, these properties that make SiC advantageous pose significant challenges during machining. Its extreme hardness and brittleness can lead to tool wear or the formation of microcracks when using traditional mechanical processing methods. Because of that, non-contact techniques like laser machining have gained traction, offering greater precision and reduced mechanical stress [1]. In our work, SiC samples from a Taiwanese university have been laser-machined with different parameters and characterized using a confocal microscope to examine surface modifications. While this method provides valuable topographical information, it lacks the resolution to reveal nano-defects and other changes in the material. We plan to use non-charging scanning electron microscopy (SEM) to study these defects, offering high-resolution imaging without the charging artifacts typical in observing insulating materials like SiC by electron microscopy.

Our research focuses on understanding how laser machining alters SiC at the micro- and nanoscale, both intentionally and unintentionally. Identifying and analyzing the defects introduced during processing is essential for optimizing machining parameters and ensuring material performance in final applications. Electron microscopy, particularly non-charging SEM, plays a critical role in this effort by providing detailed structural and compositional analysis of these modifications [2].

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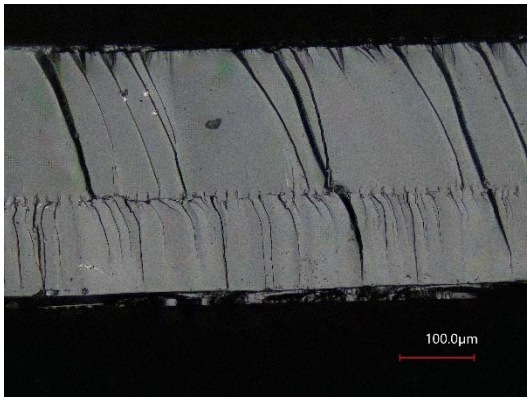


*Fig. 1: SiC wafer modifed with a femtosecond laser observed with a confocal microscope*

# Low-dose electron ptychography limitations and their link to camera length

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Electron ptychography is known for its exceptional spatial resolution [1], simultaneous sensitivity to both light and heavy elements [2], single sign contrast transfer function [3], ability to correct for aberrations through illumination recovery [4], all supported by high dose efficiency. Experimental design becomes increasingly complicated as we move from focused to highly defocused electron probes, which were introduced to reduce the applied electron dose by increasing the scan step size - reducing the real space sampling as the reconstructed pixel size is decoupled from the scan step.

The sweet spot of data acquisition parameters can be found by clever selection with prior rough knowledge of a sample. The choice of parameters can be based on empirical experience, special understanding of the physics imposed limitations, simulations with subsequent optimisation process [5] or a specific tool [6] which was co-developed.

For proper design, the most critical parameters are the probe semi-angle - its contrast transfer function must match the spatial frequencies present in a sample, the real space sampling density - the scan step size plays a crucial role in electron dose reduction, and the reciprocal space sampling which leads to the maximum probe diameter that can still be properly characterised within the probe window. Unlike real space sampling, which has a high degree of freedom, reciprocal space sampling is strictly limited by the installed detector and its geometry (physical pixel size and detection array size). The only way it can be modified is by changing the effective camera length, as it has a direct influence on these parameters. Finally, if a fast acquisition rate is required (also a way to reduce the electron dose), in some cases detector binning has to be performed, but this increases the effective pixel size with all the corresponding consequences.

Here we present the influence of varying the camera length on the reconstruction quality of a defocused probe ptychography data set and the minimum electron dose that can be achieved with such a setting for several 2D-STEM detectors.

In summary, a high-quality low-dose data acquisition design for electron ptychography is a complex task but when done properly it results in optimal contrast transfer, planned electron dose, and the ability to reconstruct high-quality images without difficult and time-consuming post-acquisition sampling modifications [7]. Initial parameter selection should be so important part of every experiment as it may save lot of time and effort lost with improperly taken data during their processing.

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# Modification of a conventional scanning electron microscope for ultrafast electron microscopy and beam shaping

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**Introduction:**

To date, there is no product on the scanning electron microscope (SEM) market that has the ability to form and modify electron pulses using light emitted by a laser. This approach is suitable for the purpose of time-resolved "ultrafast" electron microscopy [1] and especially for the development of new methods for highly sensitive imaging, which is difficult to achieve using conventional approaches. Thanks to the possibility to manipulate the spatial phase profile of an electron beam with light [2], we are also able to obtain convex and concave electron lenses with focal lengths of several millimetres and also to create aberration correctors [3]. The basic idea of our project is to modify a conventionally available scanning electron microscope into a setup that would allow the creation of electron pulses, correction of electron beam aberrations, and external stimulation of the sample, all using a high-energy pulsed synchronous laser.

**Setup diagram:**

Our approach involves a high-power pulsed femtosecond laser, which will be introduced into the SEM at three distinct locations (1, 2, and 3), as illustrated in the simplified schematic in Figure 1. The purpose of optical branch 1 is to generate electron pulses, which then propagate through the microscope. Optical branch number 2 modulates the optical pulse, which then interacts with the electron pulse and changes it into the desired form. Finally, optical branch 3 is used to stimulate the sample, enabling the investigation of dynamic processes occurring within it.

**Conclusions and results:**

In our conference contribution, we will present details on the final target setup (sketched in Figure 1), with a particular focus on the optical branch 1 and the design of a source for ultra-short electron pulses generated by a pulsed laser, which is currently under experimental testing. We will describe the construction changes made to a commercial SEM allowing for the laser injection at the Schottky electron source. Next, we will explain the optical path used for precise laser focusing on the cathode tip. Finally, we will present the results of successful electron photoemission using a pulsed laser and the first experiments.

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**Acknowledgement:**

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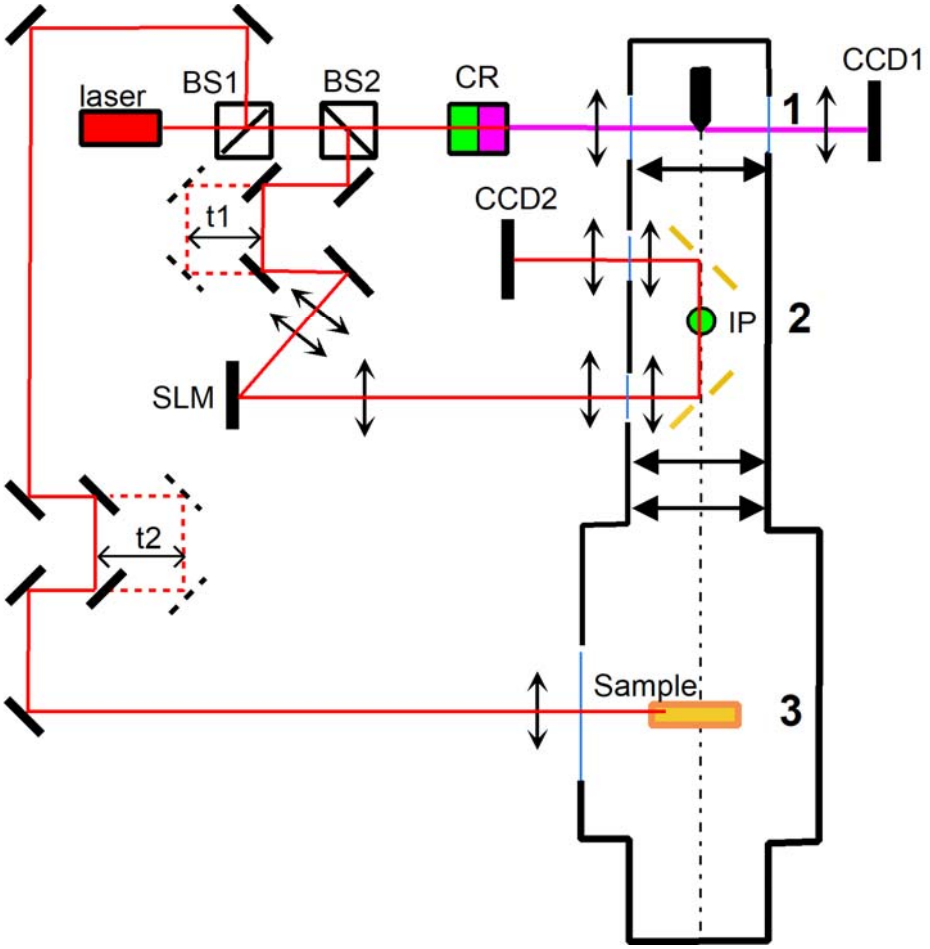


*Fig. 1:* ***Schematic of the setup for time-resolved ultrafast electron microscopy, electron beam modulation by laser and external sample stimulation.*** *Pulses emitted by the laser are incident on the first beam splitter (BS1), which transmits 90% and reflects 10% of light intensity. The beam with higher intensity is then incident on the second beam splitter (BS2), which transmits 10% and reflects 90%. The transmitted beam of lower intensity then passes through a pair of nonlinear crystals (CR), where it changes its wavelength. The beam is then focused by a lens on a Schottky cathode, and the transmitted light is imaged onto a CCD camera (CCD1). Laser pulses reflected at the beam splitter (BS2) pass through the delay line (t1) and illuminate the spatial light modulator (SLM). The modified light pulse is focused to the interaction plane (IP), where it modulates the electron pulse. The unabsorbed light is imaged onto a CCD camera (CCD2). The laser pulse reflected at the beam splitter (BS1) passes through the delay line (t2) and is focused onto the sample.*

# Introducing the Laboratory of Microscopy and Histology, Biology Centre CAS, the Czech-BioImaging research infrastructure observer

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The Laboratory of Microscopy and Histology, Biology Centre CAS is a shared service workplace of the Biology Centre and the Faculty of Science of the University of South Bohemia in České Budějovice, as part of a joint project of the School of Doctoral Studies. The laboratory provides research groups of both institutions with access to the state-of-the-art light microscopy equipment. In addition to advanced confocal and widefield microscopy, we offer unique bioluminescence and polarization microscopy. The laboratory also cooperates on several research projects focused on histological, immunohistochemical and microscopic techniques and advanced image data analysis. As part of the cooperation, we design appropriate procedures and perform experiments. We master techniques covering all levels of cell and tissue study, from macrostructure to microstructure and ultrastructure. We also offer photo documentation of the results obtained, data analysis and final preparation of results for publication purposes.

We provide several practical laboratory courses in histology and microscopy for students of the Faculty of Science of the University of South Bohemia. More than 80 students attend courses in our laboratory annually. The combination of histology and microscopy seems to be very successful, as our work attracts great interest from scientific groups. We participate in about 25 research projects annually and are involved in about 8 scientific publications. Our microscopic equipment is used by 100-120 users a year and they spend approximately 3500 user hours.

References:

For more information, visit our website https://www.entu.cas.cz/en/departments/department-of-molecular-biology-and-genetics/microscopy-centre/about-us/.

Acknowledgement:

We would like to thank the Czech-BioImaging Research Infrastructure for the opportunity to apply for membership in this organization. This involvement would benefit the development of light microscopy not only within our Biology Center and the Faculty of Science of the University of South Bohemia, but throughout South Bohemia.

**Microscopy room 1**

**Microscopy room 2**

**Microscopy room 3**

*The microscopy section of our facility*.

*Histology laboratories equipped with microtomes, cryostat and ultramicrotome for the preparation of paraplast sections, cryosections and semithin sections, respectivelly.*

# Visualisation and characterization of microstructures using optical microscopy and imaging

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In this study, we characterized quasi-periodical surface structures created by direct-writing laser lithography (DWLL) or laser interference lithography (LIL) using two complementary methods combining optical imaging and microscopy, developed and implemented as prototype laboratory setups.

With the aim of characterization of large areas covered by surface structures with highly detailed, regular patterns, an imaging system with large field of view based on optical diffractometry approach was developed. The setup is able to visualize structures with a period close to light diffraction limit (500 nm). Large-scale inhomogeneities in the very-high resolution grids could be identified with optimal lighting angles, as well as dirt and scratches notably visible with dark-field lighting. An image-processing algorithm for separation of periodic structures and defects has been implemented and tested (Fig.1), utilizing fast Fourier filtering of selected spatial frequencies to isolate the map of defects from the original structure.

As a second approach, an imaging system based on Fourier Transform and laser light source was developed for fast characterization of complex quasi-periodical structures. The diffraction images show good correlation between the structure and its Fourier-transformed representation, showing most pronounced spatial frequencies in XY dimensions. The range of frequencies that can be imaged in one shot was estimated to 2.5 um – 150 um.

Overall, our experiments demonstrates that the systems have proven ability to measure of non-periodical surface characteristics with a lateral resolution better than 2 µm, and ability to display and measure quasi-periodical surface characteristics with period of 500 nm with 20 µm lateral resolution. Both imagers can be used for tiled scanning of the large samples.

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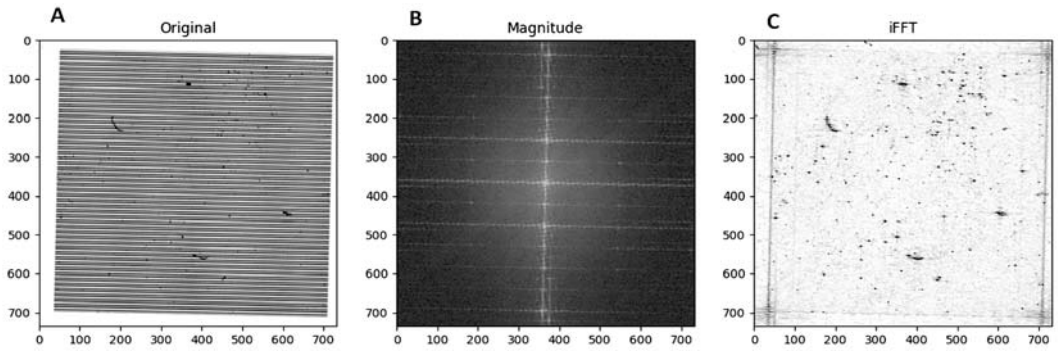


*Figure 1: A) Sample with periodic structure and some defects. B) FFT of the original image. C) Isolated defects after application of frequency filter to "remove" the original periodic structure from the picture.*

# Automation of Processing of Powder Electron Diffraction Patterns

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The recently introduced 4D-STEM-in-SEM techniques enable modern SEMs to function as user-friendly powder electron diffractometers [1]. We developed STEMDIFF (https://pypi.org/project/stemdiff), an open-source Python package that converts 4D-STEM datasets into 2D powder electron diffraction patterns [1]. These can be compared with theoretical X-ray diffraction (XRD) data to identify crystal structures, making analysis accessible even to non-experts. However, full automation requires precise pattern centering—this work presents and compares algorithms for efficient (semi) automated center determination in electron diffractograms.

We developed automated center determination within our open-source Python package, EDIFF (under development [2]). The center determination is implemented in the *ediff.center* module, featuring a manual method (user selects three points to define a circle, with its center aligning with the diffractogram center) and a list of automated methods: *intensity* (determines the center via intensity/mass distribution), *hough* (uses Hough transform to detect circular features, ideal for concentric patterns), *phase* (finds the center of symmetry though weighted intensity averaging and phase cross-correlation [3]). Refinement of the detected center coordinates is also available, either by manual shifting using the keyboard or automatically based on image properties (intensity sum maximization or intensity variance)—both contributing to precise center location determination. Once the center is satisfactorily identified, the radial distribution is computed and visualized. Background subtraction can improve compatibility with theoretical XRD profiles, aiding crystallographic analysis.

The EDIFF package, developed by all co-authors, is an intuitive, user-friendly Jupyter Notebook [4]. It serves as a template, allowing users to process 2D diffractograms via predefined workflow—successfully tested on both TEM and 4D-STEM-in-SEM data. This contribution focuses on the *ediff.center* module, which determines the key parameter for further processing—the diffractogram center.

The interactive detection method *manual* works for any diffractogram but requires user input. The *intensity* method is fully automatic but suitable only for 4D-STEM-in-SEM, as it assumes no beam stopper. *Hough* detects even incomplete circular patterns automatically but is limited to circular diffraction patterns. The *phase* method is robust across polycrystalline and single-crystal data, making it ideal for diverse crystallographic analyses. Example outputs are in Figure 1.

Additionally, some misdetections are corrected through refinement methods—users can choose between manual adjustments, which allow for precise control but introduce the risk of human error, or automatic refinement, which enhances accuracy based on pre-defined criteria, but may fail to converge to the true center if the initial detection is significantly off.

Originally developed for 4D-STEM-in-SEM, EDIFF also processes diffraction patterns from other modalities, making it a versatile tool for crystallographic analysis across various microscopy platforms. The future development will focus on image enhancement, including beam stopper removal, artifact correction, and noise/background suppression. These improvements will extend its applicability to complex crystal structures and challenging imaging conditions, further establishing EDIFF as a robust tool for crystallographic analysis.

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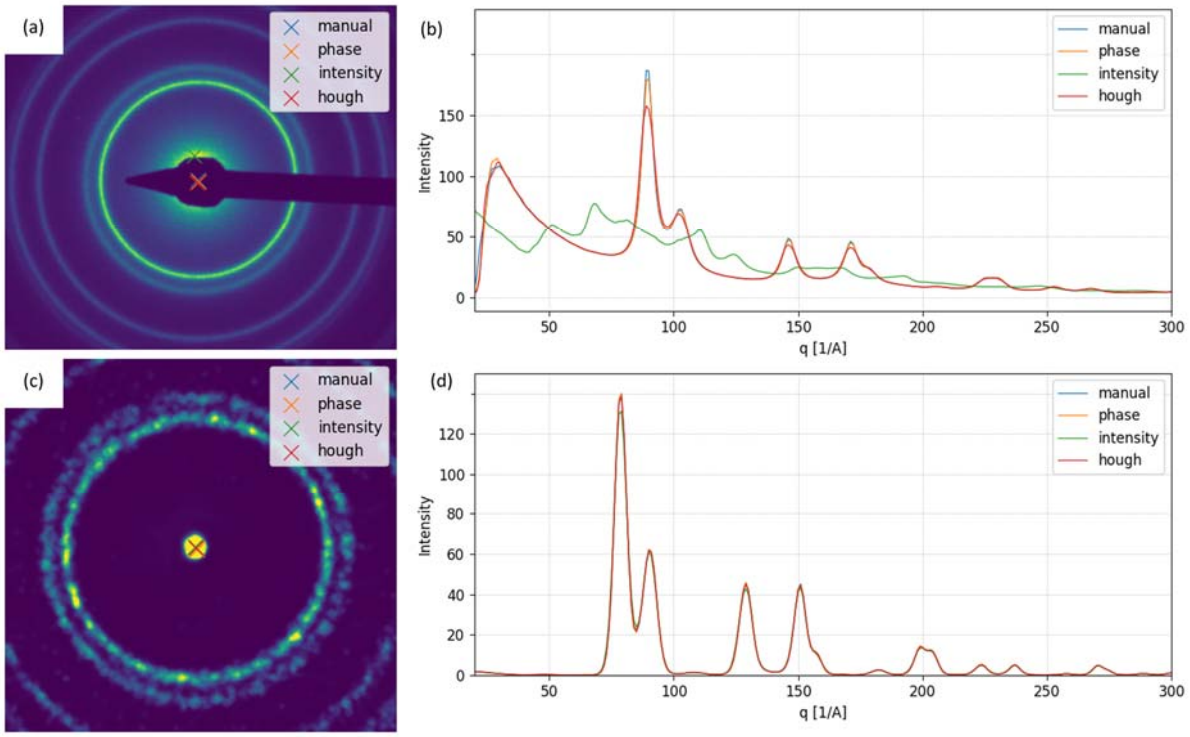


*Figure 1: Comparison of center detection methods applied to Au nanoislands using TEM/SAED (a, b) and 4D-STEM-in-SEM (c, d), shown in both 2D images (a, c) and their corresponding 1D profiles (b, d). Due to the presence of the beam stopper in (a), the intensity method fails, while the other methods perform comparably. For the 4D-STEM data, all methods perform well, with their 1D profiles being nearly identical, aside from slight differences in peak intensities*

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# Correlative AFM in SEM Microscopy in Material Science

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Correlative microscopy, in general, combines different imaging systems and benefits in understanding the principles of materials. It has become an essential tool helping us understand the complexity of the sample properties. When we imagine the setup of two complementary techniques, atomic force microscopy (AFM) and scanning electron microscopy (SEM), it has several advantages, such as multimodal measurement, under in-situ conditions, and precise localization to the area of interest.

Nowadays, a lot of effort is spent on a fundamental understanding of the surface properties of metals and alloys, thin materials, batteries, but also in the pharmaceutical industry. Mesoporous silica particles are a promising nanomaterial for advanced drug delivery. According to IUPAC, a mesoporous material has pores of 2-50 nanometers in diameter. The confinement of an Active Pharmaceutical Ingredient in such tiny pores prevents recrystallization. This can, for example, increase the solubility of an otherwise stubborn compound. Using the AFM in SEM, it was relatively easy to localize a spot of the active ingredient on the surface of a powder particle. Its topography was then measured without breaking the tip or moving the particle away. The measured roughness was lower on the active ingredient rather than on the particle itself, which confirms the idea of pores filled with the drug.

To be able to combine these techniques, a unique Atomic Force Microscope (AFM), LiteScope™, was developed by the NenoVision company for easy „plug & play“ integration into the SEMs. The connection of AFM and SEM enables to merge the strengths of both techniques, resulting in effective workflow and possibilities of complex sample analysis that was almost impossible by conventional, separate AFM and SEM instrumentation.

Thus, the AFM in SEM solution allows us analyses in in-situ conditions of several AFM modes – such as 3D topography, electrical, magnetic, or mechanical properties – and SEM capabilities like fast imaging, chemical analysis, or surface modification. This way, the Correlative Microscopy is essential not only in Material sciences but also in Nanotechnology, semiconductors, Life sciences, and other areas in both research and industry.

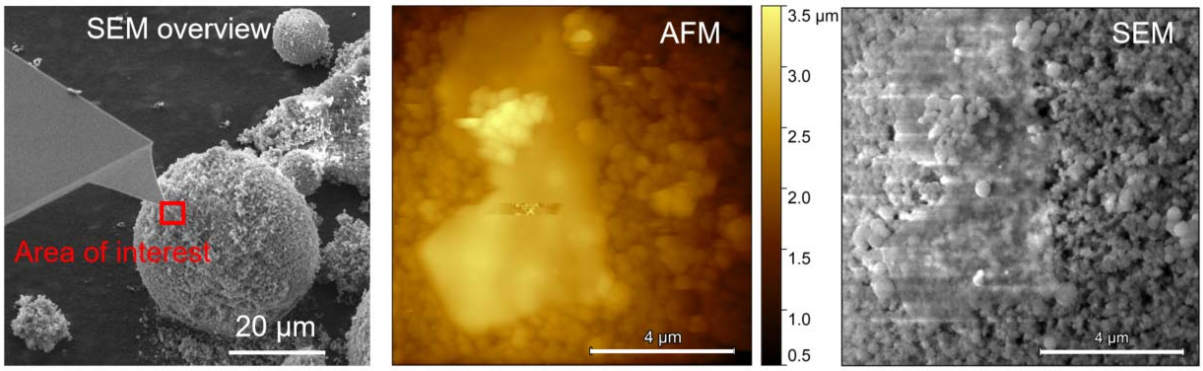


*Fig. 1: AFM tip navigation using SEM overview and simultaneous acquisition of AFM and SEM data of the treatment on powder particle.*

# TEM TITAN Themis at CEITEC Nano: Major Upgrades by a Cs-Probe Corrector and an Advanced EELS Spectrometer with Direct Electron Detectors

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An image spherical aberration (Cs) corrected high-resolution transmission electron microscope (HR-TEM), TITAN Themis 60-300 (Thermo Fisher Scientific), was installed in clean rooms (ISO 8) at CEITEC Nano Research Infrastructure at Brno University of Technology in 2016. Since then, this TEM has served the academic and industrial community in Czechia and abroad to provide state-of-the-art results in numerous investigations dealing with an R&D of progressive nanomaterials, alloys, ceramics, and polymers, or as an atomic-scale tool for a "quality control" of new nano-fabrication processes. This has been assured via an open-access system of the CEITEC Nano core laboratories, when the microscope has been operated for ~10k measurement hours and has been involved in more than 200 articles in impacted journals.

This TITAN Themis was built in a configuration to achieve cutting-edge results by using various TEM techniques. Its accelerator includes X-FEG, an ultra-stable high-brightness Schottky field emission electron source, and a Wien-type electrostatic monochromator, which narrows the energy spread of the electron source below 100 meV and reduces the effect of chromatic aberration in a low-voltage HR-TEM. The highest acceleration voltage of 300 kV offers penetration power for thicker samples and an ultimate HR-TEM resolution below 70 pm. On the contrary, the low voltage of 60 kV provides increased cross-sections for electron scattering, increased contrast of light atoms, improved spectroscopy efficiency, and reduced knock-on damage of beam-sensitive samples. An additional alignment at a mid-range voltage of 120 kV allows a compromise in illumination conditions and higher freedom in TITAN's applications, including soft materials and biological tissues. Its 3-lens condenser system is optimized for a large range of parallel illumination in TEM mode and allows a broad range of beam convergent angles in STEM. A Super-X EDX spectrometer with four 30 $mm^2$ windowless SDD detectors in a superior geometrical arrangement above a sample offers a solid angle of 0.7 srad, 100,000 spectra/sec data acquisition, and, together with the X-FEG, it is a powerful system for fast and also sensitive chemical analysis. The TITAN's image spherical aberration corrector CETCOR (CEOS) compensates all aberrations up to 3rd order (A1, B2, A2, C3, S3, A3) and boosts the resolution in the HR-TEM mode to the sub-Angström level at all the acceleration voltages. Compensated aberrations minimize the effect of delocalization in atomic imaging and thus enable the determination of artifact-free atomic coordinates, such as at interfaces. A Lorentz lens, located within the lower objective pole piece, enables L-TEM imaging of magnetic structures in field-free conditions (the objective can be completely switched off). Focusing the Lorentz lens allows imaging of magnetic domains with different properties. A four-segment differential phase contrast (DPC) detector enables STEM imaging techniques of in-plane magnetic or electric fields in a sample via measurements of beam shifts in the center of gravity of the diffraction pattern (Ronchigram). The DPC-STEM can be achieved in the field-free conditions or with the excited objective lens for atomically resolved DPC. Currently, TITAN is equipped with a post-column energy imaging filter GIF Quantum ERS/966 (GATAN) with a US1000 CCD camera for electron energy-loss spectroscopy (EELS) and energy-filtered TEM (EFTEM) imaging. Together with the monochromated electron beam, the GIF enables high-resolution EELS of both core-level and valence state transitions and thus complex information about the chemical, structural, and physical states of a sample on the atomic scale.

Although the above configuration of the TITAN microscope allows for a wealth of possibilities in the field of TEM in materials science, the microscope is undergoing two major upgrades this year to provide the scientific community with an instrument that matches the present leading TEM laboratories in the world:

The first upgrade is an installation of the latest generation of Cs-probe corrector for STEM, S-CORR (TFS). It is a unique project due to the corrector installation in the field, as aberration correctors are now always assembled and aligned in TEM factories. The S-CORR is a Rose corrector variant with two hexapoles with an additional weak hexapole placed in their crossover. This invention allows compensation for additional higher-order aberrations for all accelerating voltages, namely 5th order spherical aberration (C5) and six-fold astigmatism (A5). Correcting them allows for improved spatial resolution at the low-voltage HR-STEM. Compared to the non-corrected system, the probe corrector creates a smaller electron beam with a higher electron current. This significantly boosts TITAN's resolution in STEM (<60 pm @ 300 kV, <100 pm @ 60 kV) and its analytical performance in HR-STEM imaging, atomically resolved EELS and EDX spectroscopy mapping, DPC imaging of atomic electric or magnetic fields, etc. The new possibility of atomically resolved low-voltage HR-STEM at 60 kV allows unprecedented analyses of beam-sensitive materials, including monochromated EELS. Additionally, improvements in the S-CORR design have resulted in an order of magnitude increase in the stability of the 1st order aberrations. This translates to more time focused on imaging specimens with optimum optical conditions. The corrector is also supplied with auto alignment software to make its use easier for TEM users: Auto SCORR, which automatically corrects 1st to 4th order aberrations on a standard cross-grating sample, and OptiSTEM+, which automatically corrects 1st and 2nd order aberrations on any type of sample. Importantly, this upgrade included a complex Windows upgrade of the TEM-PC, which enables the use of the newest acquisition software.

The second upgrade is an installation of the latest generation of an EELS spectrometer, GIF Continuum HR K3+Stela (GATAN), a cutting-edge system for post-column EELS, EFTEM, and 4D-STEM diffraction measurements. Its high-end configuration selected for the TITAN has been installed only in a few laboratories around the globe up to now. The K3 (GATAN) and Stela (DECTRIS) are state-of-the-art cameras based on CMOS and hybrid-pixel detector technology, respectively, both with direct electron detection and single-electron counting capability, and superior characteristics in point spread function (PSF) and detective quantum efficiency (DQE). Thus, together with a dose attenuation function of the GIF's 100 ns electrostatic shutter, the system allows low-noise, low-dose, and ultra-fast acquisitions with a high dynamic range at all acceleration voltages of the TEM. This is, in comparison with the previous spectrometer generations equipped with CCD-based cameras, a true game changer in beam-sensitive materials analyses, low-signal acquisitions, and dynamic in-situ experiments. The spectrometer enables EELS with a high-energy range of 3000 eV, which can be combined with a DualEELS mode with a 2 keV offset to acquire low-loss spectra together with core-loss L- and M-edges of elements difficult or impossible to detect with other systems. This opens the possibility of correlations between atomically resolved monochromated EELS and "bulk" X-ray absorption spectroscopy (XAS) near-edge fine structure measurements. The GIF is equipped with a q-slit for momentum-resolved q-ω-EELS techniques as magnetic linear and circular dichroism (MLD and MCD) EELS, and the GIF's camera instrumentation can pave the way for advanced applications and development of these techniques, since they are typically associated with the low-signal acquisitions. The capability of 4D-STEM diffraction measurement with a pixelated detector has not been available on the TITAN yet, however, the new GIF comes with a system for hardware-synchronized diffraction pattern recording for each STEM probe position. The generated data cubes enable a variety of applications as strain mapping, orientation mapping and indexing, imaging with virtual apertures imitating STEM detectors, DPC-related techniques, or ptychography. Additionally, multimodal 4D-STEM and STEM-EELS spatially and temporally correlated acquisitions can be combined, synchronized, and linked in real-time during dynamic in-situ experiments, which is assured by a 10 Gb fiber-optic data transfer to a server work-station. Thus, 5D-STEM time-resolved datasets can be obtained and further synchronized with data from in-situ holders, particularly from our Fusion Select heating and biasing holder system (Protochips). The GIF system fully supports Python scripting, which allows for the highest quality data acquisition and processing, or implementation of advanced programming tools as machine learning (ML) and artificial intelligence (AI) based analyses of large and/or noisy datasets.

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# Liquid nitrogen cryostat for UV-Vis optical spectroscopy and visualisation

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Ultraviolet-visible (UV-Vis) spectroscopy (200–800 nm) is a valuable tool for studying chemical compounds in ice and aqueous solutions. This technique can provide important insights into various processes, particularly those involving freezing and lyophilisation. These processes are commonly used in biochemical laboratories and the pharmaceutical industry, where understanding the behavior of compounds under low-temperature conditions is crucial. UV-Vis spectroscopy can help answer unresolved questions regarding molecular interactions and structural changes that occur during these processes, thus offering crucial information for their improvement, such as by controlling the stability of compounds during freezing and lyophilisation [1].

We present a development version of an optical liquid nitrogen ($LN_2$) cryostat that enables UV-Vis spectroscopy studies on water ice and aqueous solution samples at low temperatures ranging from 80 K to 273 K (Fig. 1). The cryostat was dimensionally designed to fit the limited sample space (width of 125 mm, height of 180 mm, and depth of 70 mm) of a commercial Agilent Cary 5000 spectrophotometer. The cryostat allows for the installation of various samples, frozen in 12 x 12 x 45 mm cuvettes or cylindrical tubes with a diameter of 11 mm and a height of 45 mm. The cuvettes are installed into a copper sample holder and inserted into a cooled sample shaft (Fig. 1), filled with heat exchange gas ($GN_2$). The cylindrical sample shaft, made of copper, is equipped with four quartz windows with a diameter of 10 mm. The opposite quartz windows in the outer rectangular vacuum shell, made of stainless steel, have a diameter of 17 mm.

To monitor the freezing processes, we equipped the cryostat with a system for fast temperature measurement inside the samples. The temperature measurement system is equipped with two chromel-constantan thermocouples. The voltage across the thermocouples is measured with an Agilent 34410A 6½ digital multimeter. The multimeter is complemented by an OWON AG1022 function generator, which allows setting a synchronous sampling frequency of up to 1000 Hz. Recording control, data acquisition, and temperature evaluation are provided by a homemade application programmed in the LabVIEW environment.

Basic parameters of the development version of the cryostat:

- Adjustable sample temperature in the range of 83 K to 283 K
- Sample temperature stability ±0.5 °C
- Temperature measurement accuracy ±0.5 °C
- Possibility of continuously variable temperature in both directions (up and down)
- Optical finger dimensions: 40 x 40 mm, quartz windows 2 mm thick

We verified the functionality of the development version of the optical nitrogen cryostat for its planned use in UV-Vis spectroscopy by measuring the absorption spectra of a NaCl aqueous solution sample in the temperature range of 83 K to 273 K.

The final version of the cryostat should allow adjusting the sample temperature over a wider range of 65 K to 350 K. The optical finger should be exchangeable, allowing the material of the windows to be changed. Last but not least, the cryostat will be equipped with a high-speed camera for optical observation of samples during freezing processes. The final version of the cryostat will serve as an alternative with several of advantages over commercial cryostats produced by Oxford Instruments and Lakeshore Cryotronics.

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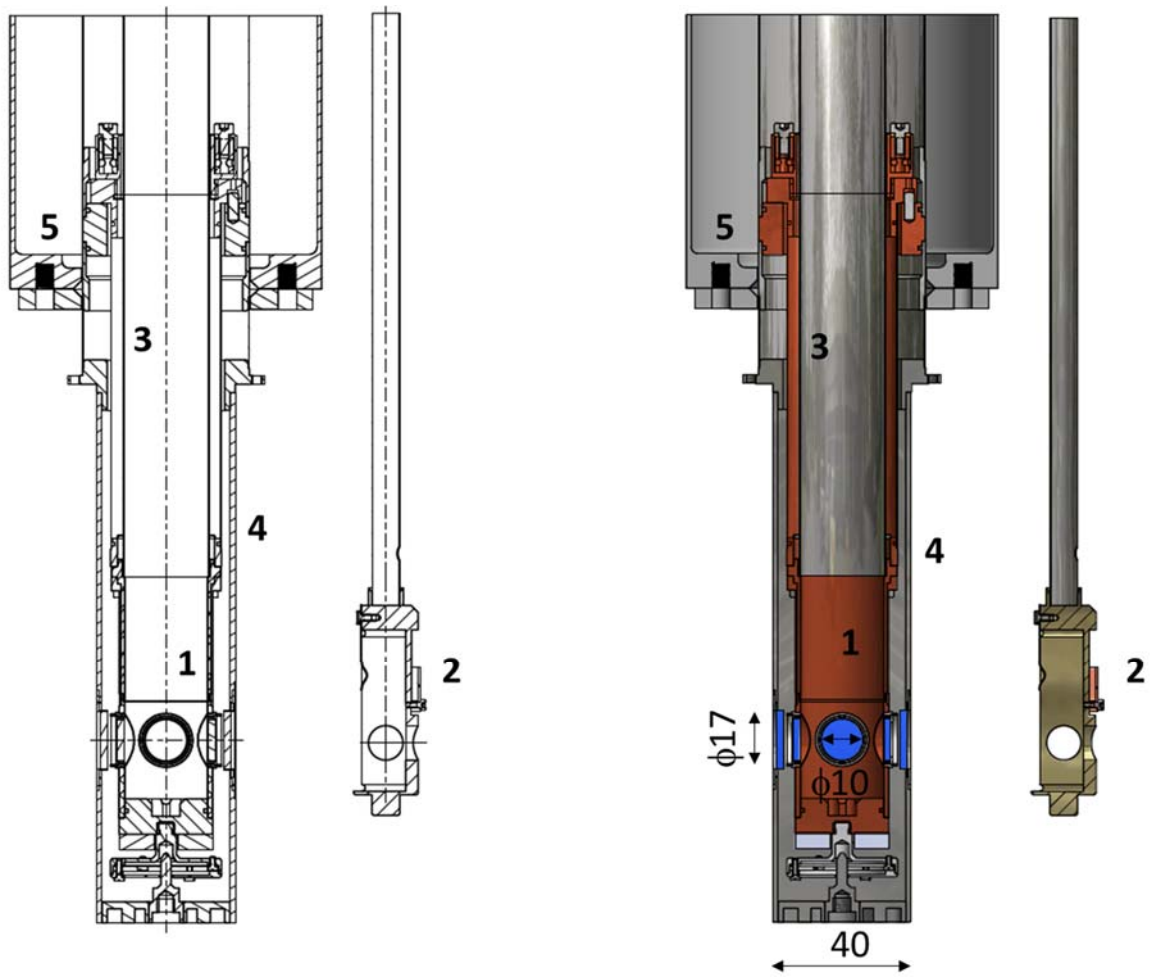



*Fig. 1: Cross-sectional view of a model of the development version of the cryostat. A removable sample cuvette holder (2) is inserted into the sample shaft (1), which is cooled via a copper thermal anchor (3) attached to the outer vacuum shell (4), which is cooled by a vessel containing $LN_2$ (5).*

# Side events

# 19.5.2025 08:00 - 13:00 TRIPS

**1. Excursion to Glassworks & Tavern AJETO in Lindava** (approx. 90-minute excursion)

- Departure by pre-booked bus from the congress venue at 9:00
- Return around 12:30
- participants only pay the **excursion fee of 150 CZK**, **max. 25 participants**, **the pre-booked bus for max. 18 participants**

**2. Hike to Ještěd** (organized group)

Public transport + walk along the blue trail to Ještěd

- Walk from the hotel to the Fügnerova tram stop – 10 minutes
- Take tram no. 3 in the direction of Horní Hanychov, get off at Horní Hanychov – 15 minutes (departure times: 8:01 / **8:17** / 8:37 / 8:57 / 9:17)
- Hike a circular route along the blue trail + Padáky educational trail
- Map: https://mapy.cz/s/hohugecepa
- Total time hotel–Ještěd–hotel: 4 hours 20 minutes
- Return around 13:00

# 21.5.2025 09:00 - 10:00 WORKSHOPS

## 1. SVEN + Hana Polášek-Sedláčková: ScanR workshop: Basics of high-content imaging

ScanR high-content screening station delivers advanced imaging capabilities to obtain high-quality and quantity data in a short amount of time. The scanR system offers fully automated image acquisition and data analysis of biological samples through deep-learning technology. During the workshop, we will showcase the key features of the system and demonstrate examples of single-cell and molecule acquisition and analysis.

## 2. yCSMS session: LiftOff: Launching Early Careers in Microscopy and Science

This section offers a supportive platform for early-career microscopists from all fields of science to present their work. The session also features an inspiring talk by an experienced scientist, followed by a discussion that encourages the audience to ask not only about science but also about the challenges of pursuing a scientific career. Both early-career and experienced scientists are welcome to join the session, share perspectives, and engage in an open dialogue!
**LiftOff** is designed to motivate, connect, and empower the future of science.

# 21.5.2025 14:30 - 16:00 EXCURSIONS and hands-on WORKSHOP

**Only 1 option is available**. Transportation for excursions within Liberec is to be arranged individually. For the excursion **to Turnov, a pre-booked bus for 18 participants will be available**.
**Departure from the hotel is 14:00**.

## 1. CXI TUL: Institute for Nanomaterials, Advanced Technologies and Innovation

- **3D Technologies Department**

The department focuses on R&D in additive manufacturing and 3D printing using state-of-the-art technologies for processing both metal and polymer materials. We specialize in the preparation of 3D CAD data, optimizing geometries for additive manufacturing technologies, including considerations for load-bearing performance. Special attention is also given to post-processing operations in order to enhance the functional properties of 3D-printed products.

- **Microscopy Laboratory**

The lab focuses on microscopic, chemical, and crystallographic analysis aimed at structural characterization, phase identification, fractography, and defectoscopy. We also support student theses and collaborate on research with academia and industry. We offer the analysis of micro and nanomaterials, composites, thin films, intermetallics, iron and non-ferrous metal alloys, polymer materials, geopolymers and natural materials. Our activities include particle size measurements, determining composition, identifying material defects and the causes of failure in machine components, distribution, and statistical processing of results for material research purposes.

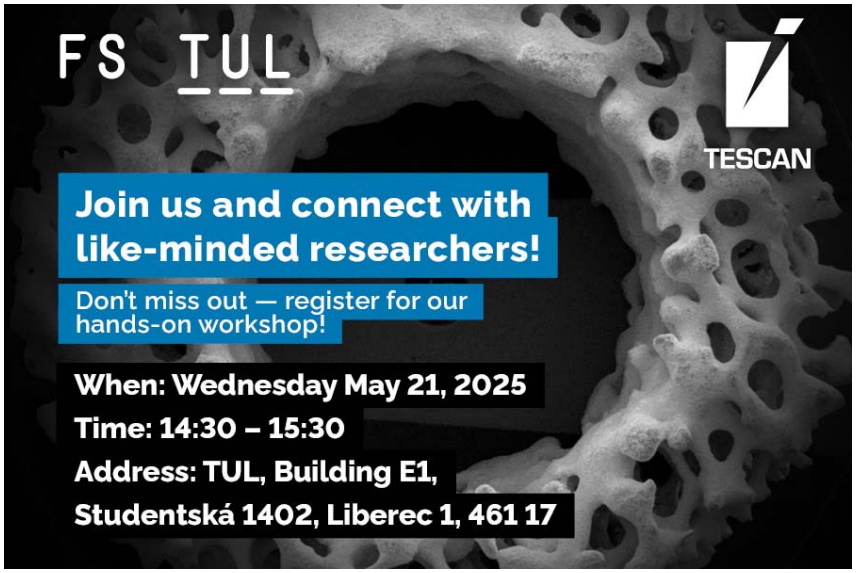
- **Nanofiber Preparation Laboratory**

The lab specializes in optimizing the production of flat nanofiber membranes tailored to the specific requirements of final applications using the electrospinning method. The facility is equipped with a Nanospider™ NS1WS500U electrospinning device, which enables production of nanofiber layers up to 0.5 meters in width. We can achieve high quality and uniformity of the nanofiber layers. The laboratory is also equipped with a system for producing composite nanofiber yarns. The prepared nanofiber layers

and composites are further utilized by specialized departments within CXI or by commercial partners, primarily for applications in filtration, acoustics, and medicine.

## 2. TESCAN workshop at the Technical University of Liberec — hands-on workshop where innovation meets real-world application

- **High-resolution analytical SEM - TESCAN MIRA - EBSD weld showcase**
- **Analytical SEM - TESCAN VEGA - waste from carbon composite processing**




## 3. CRYTUR, spol. s r.o. (Turnov)

Crytur is the provider of integrated solutions with application in electron microscopy, lasers, detection and high-power lighting for hi-tech industry and research. The Lab Tour will take you through the process of growing of synthetic garnet crystals, precision material processing, clean-room assembly of integrated opto-electronic devices and high-precision CNC workshop in the brand-new manufacturing premises in Turnov. **More info**

## 4. Institute of Plasma Physics of the CAS: toptec (Turnov)

Explore Innovative Optics at TOPTEC! Interested in precision optics and real-world applications of optical research? Visit TOPTEC, a specialized center of the Czech Academy of Sciences, located in Turnov – a town with a long tradition in optics. At TOPTEC, we develop high-precision optical systems for space, industry, and scientific research. Take a closer look at how optics shape modern technologies, from astronomy to advanced manufacturing. Our experts will introduce you to exciting projects and the unique challenges of designing and producing specialized optical components. Don't miss this opportunity to explore a research facility where science meets practical innovation. Join us at TOPTEC and see how optics make a difference! **More info**

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