CSMS

CONFERENCE

MICROSCOPY2026

1 - 3 JUNE 2026

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Microscopy 2026

Book of abstracts

Vladislav Krzyžánek, Kamila Hrubanová
(eds.)

Czechoslovak Microscopy Society

Brno, 2026

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Content

Welcome Letter

Microscopy 2026 – Czechoslovak Microscopy Society (CSMS) Annual Conference

June 1–3, 2026, Rožnov pod Radhoštěm, Czech Republic

Dear Colleagues and Friends,

On behalf of the organizing committee and the Czechoslovak Microscopy Society (CSMS), I am delighted to welcome you to Microscopy 2026, held this year in the vibrant town of Rožnov pod Radhoštěm.

Our meeting continues a proud tradition of bringing together researchers, students, and professionals from the life sciences, materials science, and instrumentation fields, all united by a shared passion for microscopy and imaging technologies. The conference features a rich scientific program, including invited lectures, oral and poster sessions and company presentations. We are also pleased to support the next generation of microscopists through student sessions - the yCSMS LiftOff event. Beyond the scientific program, we hope you will enjoy the social events, excursions, and the beautiful surroundings of Rožnov pod Radhoštěm. These activities offer excellent opportunities to reconnect with colleagues, establish new collaborations, and engage in informal discussions that often inspire future research.

I would like to express my sincere thanks to all presenters, session chairs, sponsors, and members of the organizing committee for their dedication in preparing this year's conference.

Thank you for being part of Microscopy 2026. I wish you an inspiring, productive, and enjoyable stay in Rožnov pod Radhoštěm!

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

Partners

Program: Microscopy 2026

	Monday 1st June 2026
8:00	**Trip to the mountains with yCSMS / Onsemi Factory Tour / Wallachian Open Air Museum**
14:00	**Registration** (until 19:00)
15:00	**Members' meeting of CSMS** **Kamila Hrubanová** (president of CSMS)
16:00	**Coffee break**
16:30	**Conference opening**
16:35	**Announcement of the winner of the Thermo Fisher / CSMS scholarship competition:** **Eliška Šiška Virágová** *(CEITEC BUT, Thermo Fisher Scientific, Brno)*
16:38	**Preliminary in-situ SEM heating observations of non-conductive HA–Al_2O_3 chunks on MEMS chips** **Eliška Šiška Virágová** *(CEITEC BUT, Thermo Fisher Scientific, Brno)*
16:53	**Announcement of the winner of CSMS scholarship competition:** **Ivana Michálková** *(CEITEC BUT, Brno) – Lecture Jakub Bělín June 3, 12:35*
16:54	**Announcement of the Best PhD thesis award – ZEISS:** **Klára Steklíková** *(Charles University, Prague)*
16:57	**New factors in the tooth development and its molecular control** **Klára Steklíková** *(Charles University, Prague)*
17:12	**Announcement of the Best yCSMS paper:** **Michael Foltýn** *(Brno University of Technology)*
17:15	**Announcement of the CSMS Award:** **Dušan Chorvát** *(The International Laser Centre, SCSTI, Bratislava)*
17:18	**Lasers, waves and life** **Dušan Chorvát** *(The International Laser Centre, SCSTI, Bratislava)*
18:00	**Short break**
18:15	**News in microscopy** Chair: Jana Nebesářová, Vladislav Krzyžánek
18:15	**EMS - Supporting the Community and Looking Ahead** **Vladislav Krzyžánek** *(president of EMS)*
18:22	**Building the Czech optical ecosystem** **Petr Přikryl** *(Český optický klastr, Olomouc)*
18:29	**News from Brnoregion Microscopy** **Radka Novák** *(Brnoregion Microscopy, JIC)*
18:36	**Connect, Learn, Share: The Young Microscopists of CSMS** **Eva Ďurinová** *(Charles University, BIOCEV, Prague)*
18:43	**The study programs "Mikroskopie" and "Microscopy" at the Masaryk University** **Petr Mikulík** *(Masaryk University, Brno)*
18:50	**Czech-BioImaging: Infrastructure Dedicated to Users** **Pavel Hozák** *(Institute of Molecular Genetics of the CAS, Prague)*
19:00	**Biological Imaging LM/EM Data in the Czech National Repository Platform (NRP)** **Michal Kozubek** *(Masaryk University, Brno)*
19:10	**Welcome reception**

Tuesday 2nd June 2026	
9:00	**Registration**
9:00	**Momentum-resolved STEM in Materials and Life Sciences** Plenary lecture by **Knut Müller-Caspary** *(Ludwig-Maximilians-University, Munich)*
9:50	**Session I: Material sciences** Chairs: Eliška Materna Mikmeková, Lukáš Průcha
9:50	**Pushing SEM to the eV Limit: Super-Slow Electrons for Advanced Steels Characterization** Invited lecture by **Šárka Mikmeková** *(Institute of Scientific Instruments of the CAS, Brno)*
10:20	**Diffract, reveal, retract: High speed 4D-STEM imaging with a retractable hybrid pixel detector for diffraction in SEM** **Pavel Stejskal** *(AdvaScope, Brno)*
10:35	**Coffee break & Poster session**
11:20	**Work-Function-Resolved Imaging of Relaxation Oscillations and Chemical Spillover in CO Oxidation over Platinum Surfaces** **Miroslav Kolíbal** *(Brno University of Technology, Brno)*
11:35	**Elucidation of internal damage using FIB tomography** **Stanislava Fintová** *(Institute of Physics of Materials of the CAS, Brno)*
11:50	**New capabilities for 3D imaging and surface texture measurement in optical microscopy** **Karel Jiřikovský** *(SVEN BioLabs s.r.o., Prague)*
12:05	**4D-STEM-PNBD with Frame- and Event-based Detectors: Walking through Dimensions** **Miroslav Šlouf** *(Institute of Macromolecular Chemistry of the CAS, Prague)*
12:20	**Lunch**
13:20	**Session II: Instrumentation and Optics** Chairs: Dušan Chorvát, Kateřina Mrázová
13:20	**Light enters electron microscopy** Invited lecture by **Martin Kozák** *(Charles University, Prague)*
13:40	**Advent of new capabilities of the TITAN Themis enabled by upgrades with the latest Cs-probe corrector, post-column energy filter, and direct electron detection cameras** **Jan Michalička** *(CEITEC BUT, Brno)*
13:55	**TruePix: an optimized integrated EBSD workflow** **Jakub Holzer** *(Thermo Fisher Scientific, Brno)*
14:10	**Phase contrast in 4D-STEM and aberration measurement** **Zvonimír Jílek** *(Institute of Scientific Instruments of the CAS, Masaryk University, Brno)*
14:25	**Near-Field Scanning Optical Microscopy: A Powerful Tool for Next-Generation Photonic Applications** Invited lecture by **Dusan Pudis** *(University of Žilina, Žilina)*
14:45	**New approaches for investigating beam-sensitive materials by TEM/STEM** **Guillaume Brunetti** *(JEOL, Paris)*
15:15	**Coffee break & Poster session**
16:05	**Session III: Life sciences** Chairs: Josef Lazar, Veronika Huntošová
16:05	**Extending Lateral-Shearing Digital Holographic Microscopy Beyond Conventional Coherence Limits** Invited lecture by **Jaromír Běhal** *(Palacký University, Olomouc)*
16:35	**In situ protein crystallography – a light microscopy–guided cryo-FIB pipeline for direct protein structure determination from a single intracellular crystal** **Dominik Pinkas** *(Institute of Molecular Genetics of the CAS, Prague)*
16:50	**Recent advances in 200kV Cryo Electron Microscopy** **Jana Skarupova** *(Thermo Fisher Scientific, Brno)*
17:05	**Coffee break**

17:20	**From Cells to Molecules: How Cryo-Electron Microscopy Technologies Are Transforming Structural Biology** **Jana Šmídová** *(Tescan Group, Brno)*
17:35	**Fighting Malaria with Volume EM** **Jiří Týč** *(Biology Centre of the CAS, České Budějovice)*
17:50	**FIB SEM volume imaging in life sciences: Femtosecond laser as versatile sample preparation tool** **Ivana Burianová** *(ZEISS Group, Prague)*
18:05	**Automated High-Resolution Cryo-FIB-SEM Volume EM Enables Sub-Volume Averaging of Cellular Structures** Pitch talk by **Pavel Křepelka** *(CEITEC MUNI, Brno)*
18:08	**Break**
19:00	**Conference dinner**
23:59	**Expected end of the dinner**

Wednesday 3rd June 2026

9:00	**Session IV: yCSMS** Chairs: Eva Ďurinová
9:05	**The Road to Scientific Excellence - Talk & Discussion with Knut Müller-Caspary** Invited talk by **Knut Müller-Caspary** *(Ludwig-Maximilians-University, Munich)*
9:45	**Bismuth Plasmonic Antennas** The best publication award yCSMS **Michal Foltýn/Michal Horák** *(CEITEC BUT, Brno)*
10:00	**Advanced Characterisation of 2D Materials Using SLEEM/ToF** **Veronika Pizúrová** *(Institute of Scientific Instruments of the CAS, Brno)*
10:10	**Short break**
10:20	**Session V: Interdisciplinary** Chairs: Miroslav Šlouf, Pavlína Sikorová
10:20	**4D-STEM Meets Biology: Promises, Constraints and Practical Limitations** Invited lecture by **Radim Skoupy** *(Laboratory of Biological Electron Microscopy, EPFL, Lausanne)*
10:40	**BRUKER EMA: Unique Range of Analytical Tools for Electron Microscopes** **Cristian Vailati** *(Měřicí technika Morava, Zastávka u Brna)*
10:55	**The ideal microscope (not only) for a microscopy facility** **Pavel Krist** *(ZEISS Group, Prague)*
11:10	**Design and Development of Calibration Standards for Electron Microscopy** Pitch talk by **Daniel Burda** *(Institute of Scientific Instruments of the CAS, Brno)*
11:13	**Coffee break & Poster session**
11:45	**AI Tools for Spectroscopy – Current Progress at ISI CAS** Invited lecture by **Ondřej Vaculík** *(Institute of Scientific Instruments of the CAS, Brno)*
12:05	**Future trends in Electron microscopy, sample preparation and imaging** **Martin Bačík** *(Specion, Brno)*
12:20	**SEM-EDX as a Tool in Biological Anthropology: From Skeletal Remains to Dental Calculus** **Dana Buriánková Fialová** *(Masaryk University, Brno)*
12:35	**Holographic Incoherent-light-source Optical Diffraction tomography: algorithms and their performance analysis** CSMS scholarship **Jakub Bělín** *(CEITEC BUT, Brno)*
12:50	**Conference closing**
12:55	**Lunch**
13:55	**Transfer to excursions**
14:25	**Excursion in ON Semiconductor Czech Republic, s.r.o. (14:30-15:30)**

Plenary session

Chairs: Kamila Hrubanová and Dušan Chorvát

Momentum-resolved STEM in Materials and Life Sciences

Müller-Caspary K.[1], Lorenzen T.[1], Leidl M.[1], Xia Y.[1], Jehle A.[1], Dushimineza F.[1], Sturm S.[1], Zell R.[1], Amaseder M.[1], Wenzel P.[1], Mann D.[2], Sachse C.[2], Stahlberg H.[3]

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Since the introduction of ultrafast diffraction cameras more than a decade ago, the methodological portfolio of scanning transmission electron microscopy (STEM) has been extended and diversified dramatically. Recording a full 2D diffraction pattern at each position of the 2D scan grid yields a 4D data set which comprises detailed information on local strain, chemical composition, specimen thickness, electric fields, magnetic fields, and the atomic structure. Using ptychography algorithms, specimen and probe can be deconvoluted to yield spatial resolutions far below the conventional limits set by the electron optics [1].

This contribution motivates 4D-STEM using nano-beam electron diffraction-based strain mapping applied to cross-sections of multi-quantum well core-shell nanowires [2] as an example. Particular focus is laid on the development of ultrafast cameras [3] capable of recording in the order of 10^6 diffraction patterns in a few seconds, and the recent performance of event-based detection with CERN's Timepix4 chip is reported. One of the key applications of 4D-STEM is centre-of-mass (COM) imaging, yielding electric fields in thin specimens quantitatively down to the subatomic scale [4], providing excellent contrast for both light and heavy atoms, and being dose-efficient. The COM method is introduced briefly using basic quantum mechanics and Ehrenfest's theorem, and applications to the atomically-resolved mapping of electric fields, charge densities and electrostatic potentials in 2D transition metal dichalcogenides [5] are presented. The tuneable, high sensitivity to weak meso-scale built-in electric fields is furthermore demonstrated by presenting the electric fields across a pn-junction in GaAs which is invisible in conventional TEM and STEM imaging [6]. In addition, the measurement of piezoelectric and spontaneous polarisation fields in wurtzite GaN/AlN nanowires for optoelectronic applications is shown [7], for which atomically-resolved 4D-STEM data is averaged across crystal unit cells and compared to simulations so as to estimate systematic errors.

Attention is then drawn to advanced processing of 4D-STEM data using ptychographic algorithms. To this end, the basics and the workflows of prevalent direct and iterative algorithms are briefly introduced and exemplified via applications to low-dose imaging of beam-sensitive specimens, such as covalent and metal-organic frameworks (C/MOFs), and perovskite nanostructures for optoelectronics. Inverse multislice including both partial coherence of the optical setup as well as frozen phonon averages over different thermal snapshots of the specimen to include temperature accurately is outlined in terms of neural networks [8], and the computational efficiency of imposing atomicity of the specimen is demonstrated. As an application, we show the measurement of ferroelectric displacements in a PZT ferroelectric with a precision below 5pm [9].

Finally, progress of interdisciplinary activities is reported, namely the mutual implementation of materials science-related 4D-STEM methodologies on the one hand, and biological sciences-governed automation and data processing pipelines in cryogenic 4D-STEM on the other hand [9,10]. In this respect, the current status of cryo-ptychography of protein such as tobacco mosaic virus and apoferritin is presented in terms of developed workflows and obtained 3D densities, whereas potential strategies to further improve spatial resolution and dose efficiency are discussed.

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[4] Müller K. et al.: Nature Communications 5(2014), 5653.
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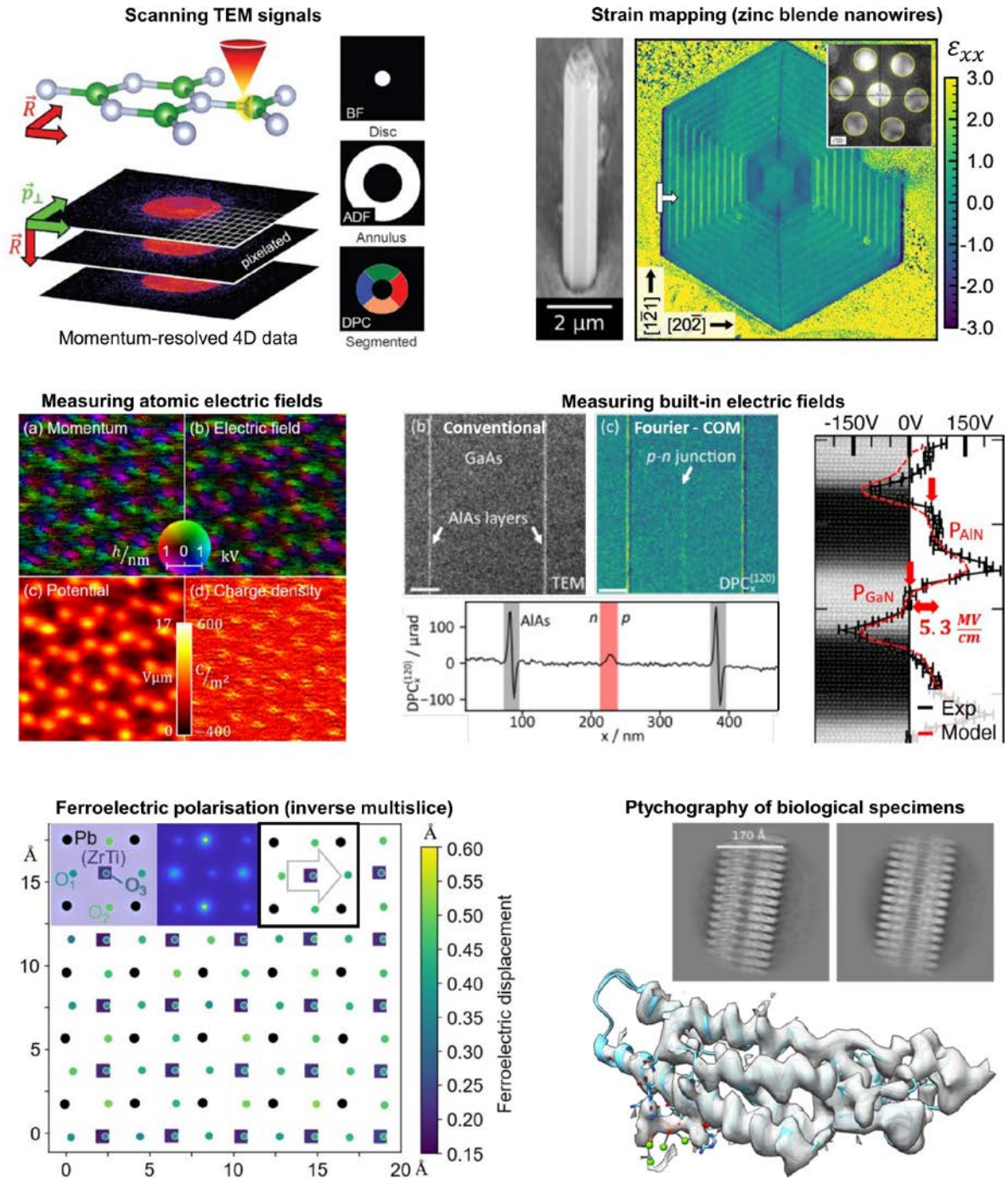

Fig. 1: Overview of momentum-resolved STEM methodologies and applications. *<u>First row:</u> Recording of full diffraction patterns at each probe position in 4D-STEM (left); Mapping of strain in a hexagonal multi-quantum well cross-section of a nanowire (right). <u>Second row:</u> Centre-of-mass imaging of a 2D material (left); Electric field across a pn-junction and in w-GaN/AlN nanowire heterostructures. <u>Third row:</u> Inverse multislice ptychography including frozen phonons yields ionic displacements of O and (ZrTi) in Pb(ZrTi)O_3 (colour-coded, left); Selected 2D classes and 3D density of tobacco mosaic virus from cryo-ptychography (right).*

Exhibitor talks and posters

Talks are included within the corresponding scientific sessions

Diffract, reveal, retract: high speed 4D-STEM imaging with a retractable hybrid pixel detector for diffraction in SEM

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Four-dimensional scanning transmission electron microscopy (4D-STEM) is emerging as a powerful diffraction-based technique within scanning electron microscopes (SEMs). It involves the acquisition of a two-dimensional diffraction pattern at every probe position during a scan, producing a rich four-dimensional dataset. While widely established in transmission electron microscopy, its implementation in SEMs has historically been constrained by limited detector sensitivity at low primary beam energies and the lack of sufficiently compact geometries compatible with SEM chambers.

Recent advances in hybrid-pixel detector technology—specifically in low-energy sensitivity, readout speed, and noise suppression—now enable efficient 4D-STEM acquisition directly inside SEMs. In this work, we introduce a retractable hybrid-pixel detector optimized for low-energy electrons and designed specifically for diffraction experiments in SEM. The retractable geometry allows the detector to be inserted below the sample for 4D-STEM operation and withdrawn when required, ensuring seamless integration with existing SEM workflows.

A key aspect of this development is the detector's ability to operate in true real-time, providing rapid feedback for live sample navigation, dynamic focusing, and stigmation. This capability removes a major historical barrier to the adoption of pixelated detectors in SEMs: the lack of responsive, immediate visualization during scanning. The high acquisition speed enables the collection of large diffraction datasets with dwell times on the order of hundreds of nanoseconds, facilitating efficient mapping, virtual imaging, and quantitative analyses without compromising routine SEM usability.

Our results demonstrate that the detector can fully replace a conventional STEM detector while dramatically expanding its functionality. In addition to standard bright-field and dark-field imaging, the pixelated acquisition allows for flexible virtual imaging through arbitrary mask definitions and comprehensive post-processing, including center-of-mass and phase-contrast analysis, strain mapping, and other advanced 4D-STEM workflows. The combination of retractability, high speed, and low-energy performance thus establishes a practical and versatile pathway for integrating full 4D-STEM capability into SEM platforms.

New capabilities for 3D imaging and surface texture measurement in optical microscopy

EVIDENT/Olympus Lext OLS5500 3D Profilometer – All-in-one device
(Laser Confocal Scanning Microscope, White Light Interferometer and Opto-Digital 3D Microscope)

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Currently, there is a growing demand for non-standard methods of measuring very fine components, coatings, and joints, as well as for monitoring of structures of various materials and inspecting surface textures or roughness with high measurement accuracy. Optical devices—specifically confocal laser scanning microscopes and 3D profilometers—are ideally suited for these purposes, enabling submicron-level imaging of material surfaces with exceptional resolution and the capability for precise three-dimensional measurement.

The new Evident LEXT OLS5500 3D profilometer combines a confocal laser microscope, a white-light interferometer (WLI), and a color 3D optical digital microscope (VZ) into a single device, designed for more than just materials science applications.

It represents a new generation of optical systems capable of high-precision 3D imaging and measurement, offering new possibilities for the development and inspection of various materials and components. It is particularly well-suited for emerging applications in the micro- and nanotechnology sectors, which place ever-increasing demands on non-standard methods of non-contact measurement and inspection of materials, miniature components, very fine joints, lithographic holograms and wafers, as well as the inspection of surface texture (roughness) with submicron precision.

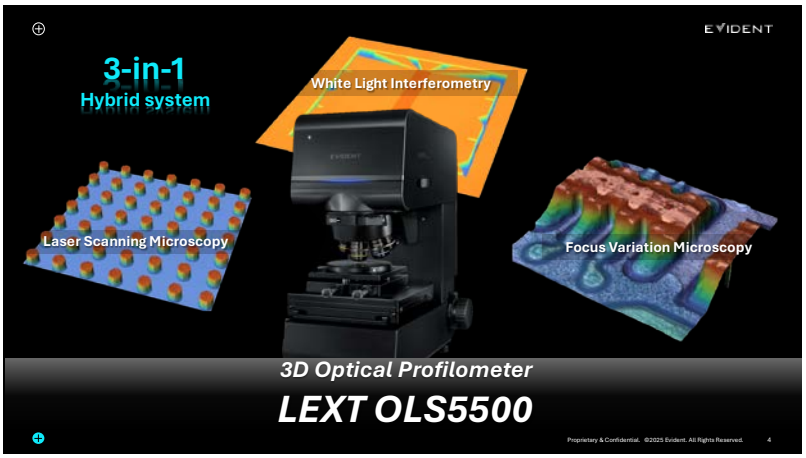

Fig. 1: The new Evident LEXT OLS5500 3D Profilometer (Universal configuration)

Currently, contactless 3D profilometer technology for material applications (Figure 1) enables imaging ranging from an overview image, composed of multiple fields of view combined, to submicron imaging of material surfaces and components with non-standard 4K resolution in lateral imaging of up to 120 nm and the capability of guaranteed, highly accurate 3D measurement in the z-axis of up to 1 nm.

The typical optical magnification range of 50x to 17,500x meets the needs not only of research and development professionals who work at the intersection of optical light microscopes and scanning electron microscopes (SEM). Samples can be placed directly on the microscope stage in the 3D profilometer without the need for a vacuum chamber.

Scanning takes place in real time, and no surface preparation of the sample is required.

Measuring the surface texture of materials using a 3D non-contact profilometer

Fig. 2. Endoprosthesis – evaluation of the surface texture of functional (metal) surfaces and the passivated portion (hydroxyapatite)

Highly suitable application of the new 3D profilometer is the ability to evaluate the texture of various material surfaces in accordance with international standards. It is suitable for both linear roughness measurement according to ISO 4287 (ISO 16 610) and the increasingly common area roughness measurement according to ISO 25 178. The system performs surface evaluation non-contact, making it highly suitable for evaluating of very soft and delicate surfaces (e.g., plastics, Cu, Al alloys...), PVD and CVD coatings, surfaces after laser machining, as well as highly complex surfaces and shapes (Fig. 2).

References:

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TruePix: an optimized integrated EBSD workflow

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Electron backscatter diffraction (EBSD) is a powerful technique for linking microstructure to performance in advanced materials, particularly in metals and battery systems where crystallographic orientation, phase distribution, and grain-scale heterogeneity strongly influence functional behavior. In this presentation, we highlight the Thermo Scientific TruePix EBSD detector integrated into the Thermo Scientific Apreo electron microscope as a single, coordinated platform for high-quality crystallographic characterization across these application areas.

A major advantage of this solution is the integration of the EBSD detector directly into the microscope platform, creating one streamlined system and workflow for crystallographic analysis. This reduces setup complexity, improves ease of use, and enables faster, more reliable data collection. For researchers studying engineering alloys, this supports efficient investigation of grain structure, deformation behavior, recrystallization, phase transformations, and texture development. In battery materials, the same integrated workflow helps address increasingly complex questions related to cathode particle structure, degradation mechanisms, phase evolution, and microstructural variation across heterogeneous electrodes.

The TruePix detector is based on direct electron detection, avoiding the losses associated with conventional electron-to-photon conversion approaches. This improves sensitivity and pattern quality and enables strong EBSD performance at lower accelerating voltages. Low-kV operation is especially important in both metals and battery materials: in metals, it can improve spatial resolution for fine-grained or multiphase microstructures; in battery materials, it can reduce interaction volume and help preserve localized information from small features, surface layers, and beam-sensitive regions. The ability to acquire high-quality Kikuchi patterns under these conditions expands the practical range of EBSD for challenging samples.

By combining deep system integration with the advantages of direct electron detection, the TruePix-on-Apreo platform enables more accessible, efficient, and application-relevant EBSD analysis for materials scientists working on metals, batteries, and other complex material systems.

New approaches for investigating beam-sensitive materials by TEM/STEM

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Since 1949, JEOL's legacy has been one of the most remarkable innovations in the development of instruments used to advance scientific research and technology. JEOL has more than 75 years of expertise in the field of electron.

Beam-sensitive materials—including 2D crystals, metal–organic frameworks, zeolites, battery components, soft matter, and biological specimens—often undergo rapid structural and chemical alteration under electron irradiation, making conventional TEM/STEM characterization dose-limited. This work presents a practical framework for preserving both structural and chemical information under ultra-low-dose conditions by shifting from global beam reduction to precise dose delivery and information-efficient acquisition. After reviewing key damage mechanisms and the limits of standard parameter tuning (accelerating voltage and probe current), we outline a roadmap built around four complementary strategies: (i) dose efficiency, extracting more information per electron through high-efficiency STEM detection modes (e.g., OBF/SAAF), optimized EDS geometry and solid angle, and frame-integration approaches that maintain resolution at reduced dose; (ii) dose control in time, using electrostatic dose modulation and pulsed operation to deliver electrons only when useful, while preserving alignment and resolution; (iii) dose control in space, minimizing unnecessary exposure via true area scanning, ROI-only acquisition, and spatial "dose painting" masks; and (iv) event-based acquisition, replacing fixed dwell time with event-driven imaging (TEMPO) to stop acquisition once sufficient information is collected (Fig. 1). We also highlight the emerging synergy between dose control and AI-based denoising to push imaging toward extreme low-dose regimes. Together, these approaches reposition dose from a limiting factor to a controllable parameter for robust TEM/STEM analysis of fragile materials.

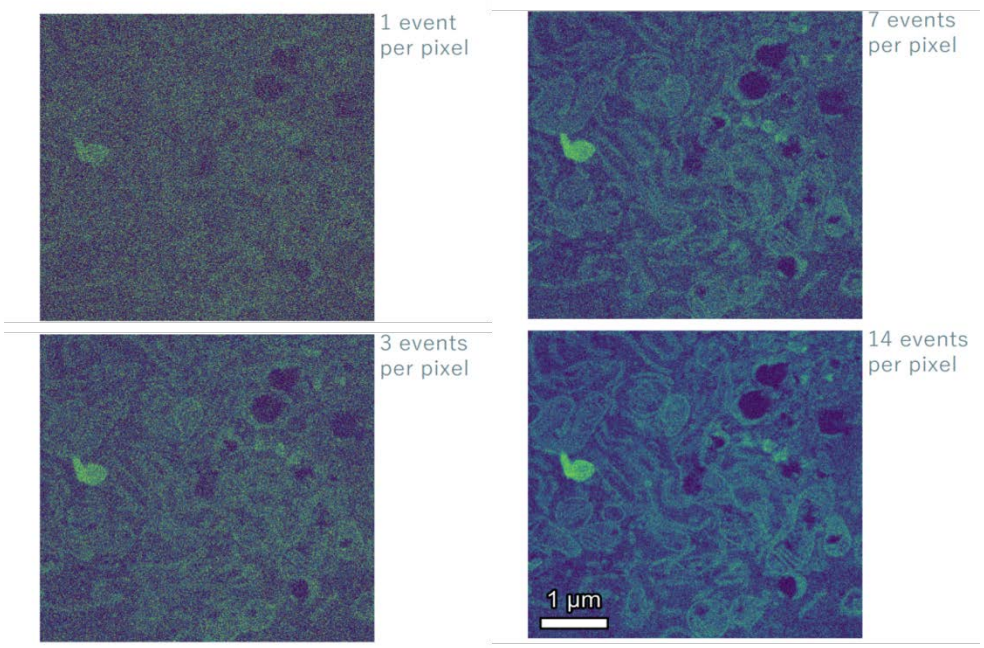

Fig. 1: TEMPO images of human macrophage cells recorded with the beam blanker triggering after various event numbers. Contrast represents scattering-rate values. Data credit: turboTEM.

Recent advances in 200kV Cryo Electron Microscopy

Yu L.[1], Raschdorf O.[1], Kocian O.[2], Kara P.[2], Knap Z.[2], Bayer J.[2], Barinka D.[2], Kachtik L.[2], Fadaeinia M.[1], Walek P.[2], Skarupova J.[2], Lengyel J.[2]

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Cryo-electron microscopy (cryo-EM) has rapidly advanced in recent years. Researchers can now achieve near-atomic detail of proteins and macromolecular structures in vitreous ice with single particle analysis (SPA) and explore cellular structures in their native context using cryo-electron tomography (cryo-ET).

To convert these technical advances into greater productivity and broader adoption, Thermo Fisher Scientific is introducing integrated hardware and software innovations designed to enhance performance while lowering infrastructure barriers.

Among cryo-EM platforms, 200 kV transmission electron microscopes (cryo-TEMs) offer an optimal balance between high-performance imaging and accessibility. We present several new features for the 200 kV Glacios 3 Cryo-TEM platform, including enhanced system stability, an essential factor for cryo-EM applications that require long data acquisition times. In addition, new optical improvements increase productivity in single particle workflows while improving overall usability.

With these advancements, the Glacios 3 Cryo-TEM delivers high resolution, increased throughput, and improved ease of use in 200 kV imaging. This makes it a powerful and versatile solution for high-resolution single particle analysis, cryo-ET, and MicroED applications across biological research, drug discovery, and beyond.

From Cells to Molecules: How Cryo-Electron Microscopy Technologies Are Transforming Structural Biology

Jana Šmídová[1]

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How can we explore the inner organization of cells while preserving structures in their native state? Cryogenic methods are reshaping the way scientists study biological systems across scales.

CryoFIB-SEM is unlocking new possibilities in in situ structural biology—from targeted lamella preparation to three-dimensional analysis of vitrified samples. However, widespread adoption remains limited by practical challenges, including long preparation times, user-dependent variability, and difficulties in accurately targeting regions of interest.

Addressing these challenges requires both technological innovation and workflow optimization. Advances in plasma FIB milling and integrated cryo-correlative microscopy are enabling faster, more reproducible sample preparation and more precise targeting within complex biological specimens. Together, these developments support more efficient workflows, improved data quality, and more reliable access to structures in their native cellular context.

FIB SEM volume imaging in life sciences: femtosecond laser as versatile sample preparation tool

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FIB-SEM volume imaging has become a key technology for nanoscale characterization in life sciences, enabling detailed three-dimensional analysis of biological ultrastructure. However, one of the major bottlenecks remains efficient, precise, and scalable sample preparation, particularly when targeting rare or spatially confined regions of interest.

In this contribution, we present an advanced workflow integrating femtosecond (fs) laser technology directly into FIB-SEM platforms to significantly enhance sample preparation capabilities. The fs laser enables rapid, high-precision material removal with minimal thermal damage, allowing users to access deeply buried regions of interest and to define sample geometry in a highly flexible manner. This approach overcomes limitations of conventional trimming techniques and dramatically reduces preparation time.

We highlight how integrated fs laser processing enables the extraction of multiple targeted volumes from a single resin-embedded biological specimen, supporting higher throughput and improved experimental efficiency. Furthermore, the ability to generate application-specific sample geometries—such as cylindrical pillars optimized for X-ray tomography or volumes tailored for FIB-SEM acquisition—opens new opportunities for correlative and multimodal imaging workflows.

By bridging large-volume access with nanoscale resolution, this integrated solution empowers researchers to streamline their workflows from targeting to high-resolution imaging. The presented approach demonstrates how next-generation FIB-SEM systems can expand the boundaries of life science research by combining speed, precision, and workflow flexibility in a single platform.

BRUKER EMA: Unique Range of Analytical Tools for Electron Microscopes

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Bruker's electron microscopy analyzers, including Energy Dispersive X-ray Spectroscopy (EDS), Electron Backscatter Diffraction (EBSD), and Micro-X-ray Fluorescence (Micro-XRF) for scanning electron microscopy (SEM), provide a comprehensive platform for compositional, structural, and crystallographic characterization of materials. Full integration of these complementary analytical techniques within the ESPRIT software environment enables efficient correlative analysis and advanced data interpretation across a wide range of materials science applications.

The latest generation of QUANTAX EDS systems incorporates the XFlash® 7 detector series, offering exceptionally large X-ray collection solid angles, high count-rate capability, and excellent performance for high-throughput elemental analysis, rapid mapping, and low-kV applications.

In the field of crystallographic analysis, Bruker's eWARP represents a new generation of Electron Backscatter Diffraction detectors based on pixelated sensor technology specifically developed for EBSD. The combination of wide area pixels, CMOS architecture, and optimized signal acquisition enables high-speed orientation mapping with excellent pattern quality, even under challenging acquisition conditions.

Micro-X-ray Fluorescence (Micro-XRF) integrated into SEM significantly expands the analytical capabilities of conventional EDS by enabling non-destructive trace element detection, analysis of layered structures, and improved sensitivity for low-concentration elements. The combination of SEM imaging, EDS, EBSD, and Micro-XRF provides a powerful multimodal approach for advanced materials characterization.

The contribution will also include a brief overview of newly available instrumentation and characterization capabilities within the portfolio of Měřicí technika Morava (MTM), highlighting recently expanded opportunities in multimodal materials characterization.

Fig. 1. Bruker multimodal detector ecosystem for EM analysis

The ideal microscope (not only) for a microscopy facility

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The ideal microscope should be able to digitize specimens in an easy, reliable, reproducible way and should create high-quality virtual microscope slides. It should be able to acquire fluorescence, brightfield, polarization in a fully automated way and must be easy to operate. The challenging research tasks as well as routine scanning applications must be supported by powerful hardware and perfectly featured software. The acquisition must be performed quickly with high-speed scanning, while retaining consistently high quality. The acquisition profiles must be easy to create and applied, even in complex fluorescence experiments. And of course, there must be subsequent image analysis tools available which can process data accurately and in an unbiased way. The acquired virtual slides must be available anytime, no matter where or what operating system is used. Sharing images online with colleagues and organizing entire projects, even on the go is beneficial too.

Classical and most modern techniques like multiplexed spatial profiling, multiplex immunofluorescence (mIF) staining with multiple biomarkers etc. must be available. Broad application range like Life Science Research, Pharma&Biotech, Clinical Application, Geology&Materials are needed.

Does such microscope exist? YES – find more information at:

https://www.zeiss.com/microscopy/en/products/imaging-systems/axioscan-7.html

Future trends in Electron microscopy, sample preparation and imaging

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This lecture provides a general introduction to sample preparation and imaging techniques used in electron microscopy. It outlines the fundamental principles of preparing specimens for microscopic analysis and discusses how preparation methods can influence image quality and analytical performance.

The lecture also presents an overview of the main electron microscopy techniques and their typical applications in research, development, and industrial practice. Emphasis is placed on understanding the relationship between sample preparation, imaging conditions, and instrument capabilities in order to support effective workflow selection for a wide range of analytical tasks.

DynaMic 1K: an ultrafast confocal microscope for 3D imaging of biological dynamics

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Laser-scanning confocal fluorescence microscopy allows 3D imaging of biological samples. However, confocal microscopy is usually rather slow, requiring several seconds for each image, and therefore several minutes for each 3D image stack. In collaboration with laboratories at KU Leuven and Charles University, we have now developed a new design of a confocal microscope [1] that allows acquiring up to one thousand images per second, allowing real-time 3D imaging of complex biological samples. Furthermore, thanks to combining our confocal design with the technique of linear dichroism microscopy, the microscope (DynaMic 1K) allows not only traditional applications, but also observations of a large class of novel molecular biosensors, termed FLIPs [2,3,4]. Because FLIPs allow observing many molecular processes of cell signaling, the DynaMic 1K opens many new possibilities in microscopy imaging, including in pharmaceutical drug discovery and development.

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Acknowledgement:

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CSMS awards

Chairs: Kamila Hrubanová and Dušan Chorvát

Preliminary in-situ SEM observations of sintering process in non-conductive ceramics chunks on MEMS chips

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Hydroxyapatite (HA) based composites reinforced with alumina (Al_2O_3) platelets are promising materials for biomedical applications; however, their sintering behaviour is accompanied by complex microstructural evolution at the HA-Al_2O_3 interface. In-situ scanning electron microscopy offers a direct way to observe thermally induced changes in such systems, but the preparation and observation of non-conductive ceramic chunks on MEMS heating chips remain challenging. This contribution presents preliminary results towards the development of an in-situ SEM methodology for HA based composites containing 1 vol.% Al_2O_3 platelets, in line with the broader research focus on interfacial microporosity formation during sintering.

As a proof of concept, ceramic chunks (5x5x5 μm) were prepared from HA based material containing 1 vol.% Al_2O_3 platelets after annealing in a nitrogen atmosphere, retaining residual carbon that enabled chunks preparation of this otherwise non-conductive system. Chunks were heated in situ in SEM up to 1300 °C at a heating rate of 1 °C s^{-1} with a dwell time of 20 min under both low-vacuum and high-vacuum conditions. During heating, the samples remained stable on the MEMS chip without visible drift or cracking, while shape changes and shrinkage were clearly observed.

To determine the maximum chunk size that can be effectively heated on a MEMS chip, ceramic chunks were evaluated after in-situ heating. The observations made it possible to define suitable sample dimensions and their optimal placement on the MEMS chip. The results indicate that micro-samples with dimensions commonly used for conductive materials may also be applicable in the present non-conductive HA–Al_2O_3 system. Although halo-like microporosity was not observed in the present experiments, larger micro-samples will be prepared in the next stage to enable more representative investigation of interfacial phenomena around alumina platelets.

The current results demonstrate the feasibility of in-situ SEM heating of non-conductive HA-Al_2O_3 chunks on MEMS chips and indicate that sample geometry and material volume are critical parameters for future experiments. The next step will involve the preparation of larger chunks and detailed microstructural analysis of the heated material, including lamella preparation after in-situ heating and comparison with the same material conventionally sintered in a furnace. This comparison will be used to evaluate porosity development, neck formation, grain growth, interface evolution around alumina platelets, and the overall densification pathway.

Acknowledgement:

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New factors in the tooth development and its molecular control

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The development of teeth and the tooth row is studied as a representative model of the development of ectodermal derivatives. It is based on interactions between the epithelium and underlying mesenchyme. The tooth row development is controlled by epithelial signalling centres (EK, enamel knot), which periodically appear antero-posteriorly in the growing jaw . Their appearance and signalling function then determine the future dental pattern. This periodicity is based on interactions between activator and inhibitor molecules. Based on this, we modelled their appearance mathematically and we have shown that even an established pattern can be rewritten during normal embryonic development. The communication between the established and arising centres is dynamic and their fates may differ (disappearance, fusion with other centres, or initiation of tooth germ development itself (1). However, in all those processes a strict spatiotemporal control is very important. Its disruption leads to defects in the tooth row development. Activation and inhibition are related not only to the pattern establishment and tooth row formation but also to each single tooth development (1,2). To identify molecules associated with the establishment and maturation of EKs and the differentiation of the tooth germs, we used RNA sequencing and consequent RNA scope validation. We identified new players in this process, as Cdkn2b, Sema3b and others (2). Results that are part of this work further showed the importance of the epithelium not only in the early stages of tooth development, but also during the production and maturation of dental hard tissues. We showed that the epithelium determines not only the shape of developing teeth, but it also affects the maturation of adjacent dentin and its mineralisation (3).

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Acknowledgement:

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Bismuth Plasmonic Antennas

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This contribution presents the findings published in the paper entitled *"Bismuth Plasmonic Antennas"* published in *ACS Nano* [1], which has been awarded by the **yCSMS Best Paper Award**. The paper addresses the limitations of gold as a plasmonic metal by introducing bismuth as a suitable substitute offering comparable plasmonic performance.

Gold-based plasmonic applications show great potential; however, their widespread use is limited by high fabrication costs and the limited plasmon energy range imposed by band transitions. These challenges could be addressed by the use of alternative non-noble metals. Among them, bismuth is a particularly promising alternative plasmonic metal because of its theoretically predicted wide spectral bandwidth [2,3]. The current studies of bismuth-based plasmonic nanostructures are largely limited to optical spectroscopy of large ensembles of nanoparticles. As a result, a direct correlation between plasmon energy and nanostructure size and shape, crucial for evaluating their application potential, remains unexplored. In the awarded paper, we experimentally demonstrate the correlation between the shape and size of individual bismuth plasmonic antennas and their optical properties, using STEM-EELS.

Our experimental findings demonstrate that these antennas support localized surface plasmon resonances, and their dipole modes can be tuned through their size from the near-infrared to the entire visible spectral region. Furthermore, our findings demonstrate that bismuth exhibits a plasmon dispersion relation nearly identical to that of gold (Fig. 1), while maintaining its plasmonic performance even at higher plasmon energies. The validated plasmonic performance of bismuth antennas highlights their potential as a viable substitute for gold. In addition, the lower cost of bismuth, together with its biocompatibility and resistance to oxidation, make it a suitable candidate for use, especially in industrial and large-scale plasmonic applications.

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Acknowledgement:

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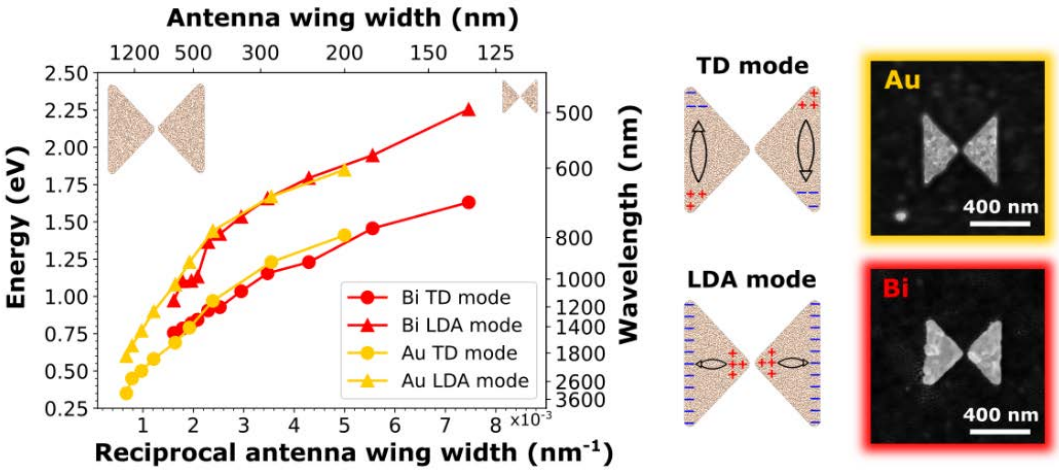

Fig. 1: Dependence of the plasmon resonant modes energy on the reciprocal antenna width for bismuth and gold antennas [1].

Lasers, waves and life

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In this contribution, I summarize results of my work in the area of biophotonics and optical imaging over more than 30 years of my career. Biophotonics is the interdisciplinary field devoted to development and applications of light-based technologies to study, monitor, and manipulate biological systems ranging from molecules and cells to tissues and organisms. It combines life sciences (ecology, biology, medicine) with state-of-the-art imaging, spectroscopy and time-resolved detection, as well as light-control techniques.

In my case the path started with observations of natural ecosystems with more and more sophisticated methods and instruments, and peaked in applications of advanced time-resolved spectroscopy methods combined with laser microscopy for assessment of cell metabolic state. My interests ranged from cardiac cell models through patient biopsies, through bacterial systems used in biotechnology to plants and algae with ecologic importance. Second area of my activity was focused to research of hydrogels and polymer structures, including fundamental research of cell/polymer interactions up to development of methods for controlled laser-based 3D printing of microstructures. Last but not the least, in my presentation I will introduce my activities in the field of scientific popularization, such as science shows, festivals and development of hands-on science museum exhibitions.

Nowadays, a strong focus is placed on the breakthrough performance of automatic systems for data processing and analysis. In spite of the widely discussed potential of these methods, the viewpoint acquired during my career suggests that radically new knowledge will not be gained without a proper understanding of the functional principles that control the complex behavior of cells and organisms. In our previous work [1] we indicated that a deep interconnection may exist between biology and generalized 'wave mechanics,' paving the way for a conceptually new approach to understanding and describing biological and social systems. Such an approach should emphasize the observation of living beings and their dynamics—allied with the construction of a new language and tools to describe these observations—rather than fitting pre-defined concepts to experimental data.

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Acknowledgement: I deeply acknowledge my family for support of my scientific endeavours.

Fig. 1: Spiral calcium wave patterns in rat ventricular myocytes.

Holographic Incoherent-light-source Optical Diffraction tomography: algorithms and their performance analysis

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Optical Diffraction Tomography (ODT) is a branch of Quantitative phase imaging (QPI) that enables volumetric reconstruction of refractive index distribution (RID) allowing for three-dimensional microscopic imaging without the need for external labeling. This label-free capability makes ODT highly suitable for medical and biological applications, particularly those requiring live-cell imaging [1–3].

Conventional ODT systems are typically based on a transmission interferometric setup using a coherent light source and sequential illumination of the sample with plane waves at varying angles. However, this approach faces several challenges, including coherence noise and the need for complex beam-steering mechanisms.

Recently, and alternative ODT strategy based on the Holographic Incoherent-light-source QPI (hiQPI) system [4] in combination with axial scanning, has been introduced [5]. This approach, named the, Holographic Incoherent-light-source ODT (hiODT) offers several advantages, including a coherence-gating effect that reduces coherence noise artifacts and provides inherent optical sectioning. Nevertheless, appropriate reconstruction algorithms are still required to address the missing cone problem.

Here, we present two such reconstruction approaches: a physics-driven, iterative optimization-based method, and a neural network (NN)-based reconstruction framework. Both methods are validated using simulated and experimental data. We further compare their respective advantages and limitations and discuss future directions for improving the overall hiODT approach, with a focus on reconstruction accuracy and computational efficiency, as well as ongoing efforts toward these goals.

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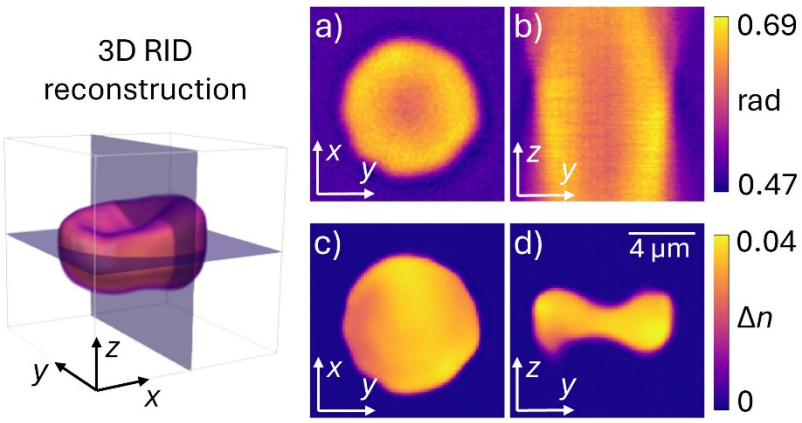

Fig. 1: Example of 3D refractive index distribution of a red blood cell using a neural network-based reconstruction algorithm. a)-b) cross sections of quantitative phase z-stack, which served as an input to the reconstruction. c)-d) cross sections of the reconstruction

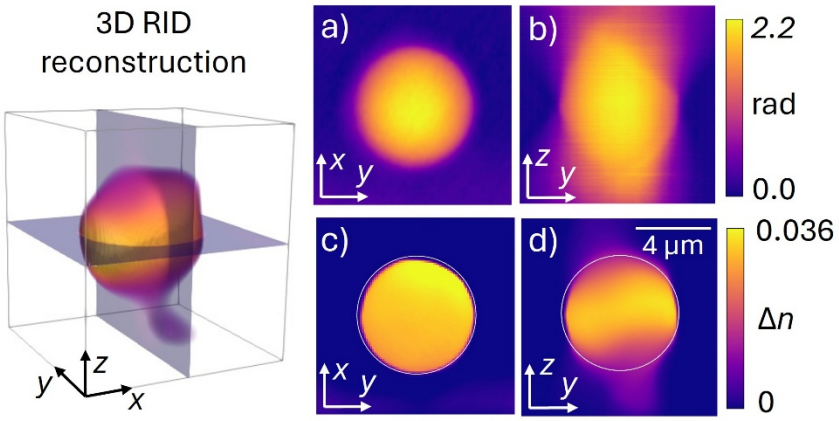

Fig. 2: Example of 3D refractive index distribution of bead embedded in oil using the physics-driven, iterative optimization-based reconstruction algorithm. a)-b) cross sections of quantitative phase z-stack. c)-d) cross sections of the reconstruction

Material sciences

Chairs: *Eliška Materna Mikmeková and Lukáš Průcha*

Pushing SEM to the eV Limit: Super-Slow Electrons for Advanced Steels Characterization

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Recent advances in scanning electron microscopy (SEM) enable operation at near-zero landing energies in the electron-volt range, referred to as super-slow electrons, providing a powerful approach for highly surface-sensitive materials characterization. In contrast to conventional SEM operating at keV energies, where the electron interaction volume extends deep into the material, ultra-low landing energies drastically reduce penetration depth and confine signal generation to the topmost nanometers. As demonstrated in recent studies [1–4], such conditions can be reliably achieved in commercial high-vacuum SEM instruments using beam deceleration techniques, allowing routine access to the eV regime without the need for dedicated ultra-high vacuum systems.

At these energies, electron–matter interaction is dominated by surface-sensitive processes, including variations in secondary electron yield, electron reflectivity, and local work function. This results in contrast mechanisms that are strongly influenced by crystallographic orientation, phase composition, and surface electronic structure. Experimental studies [2–4] have shown that very low energy SEM enables visualization of microstructural features that remain indistinguishable under conventional imaging conditions, including enhanced phase contrast in multiphase steels and sensitivity to surface oxidation and segregation effects.

In particular, operation at landing energies approaching units of eV has been shown to enable clear differentiation of multiple steel phases, such as ferrite, martensite, and austenite, even in cases where compositional contrast is minimal [4]. The reduced interaction volume further improves spatial localization of the signal while minimizing beam-induced damage and charging effects, making this approach suitable for both metallic and more beam-sensitive materials.

Recent work has also highlighted the importance of detection strategies and signal selection in the low-energy regime [1,3]. The use of in-lens detectors, energy filtering, and optimized electrostatic fields enables selective enhancement of specific signal components, significantly increasing the information content of SEM images. These approaches provide access not only to morphological contrast but also to information related to electronic structure and surface states.

Although operation at near-zero landing energies requires careful control of surface cleanliness and experimental conditions, the ability to perform such measurements in widely available commercial SEM instruments makes super-slow electron imaging an accessible and versatile tool. Beyond steels, this methodology is applicable to a wide range of material systems, including thin films, coatings, nanostructured materials, and functional surfaces, where surface and near-surface properties are critical.

Overall, SEM imaging with super-slow electrons represents a significant advancement in materials characterization, offering a unique combination of high surface sensitivity, reduced interaction volume, and novel contrast mechanisms. These capabilities open new possibilities for the investigation of complex materials and highlight the growing importance of low-energy electron techniques in modern microscopy.

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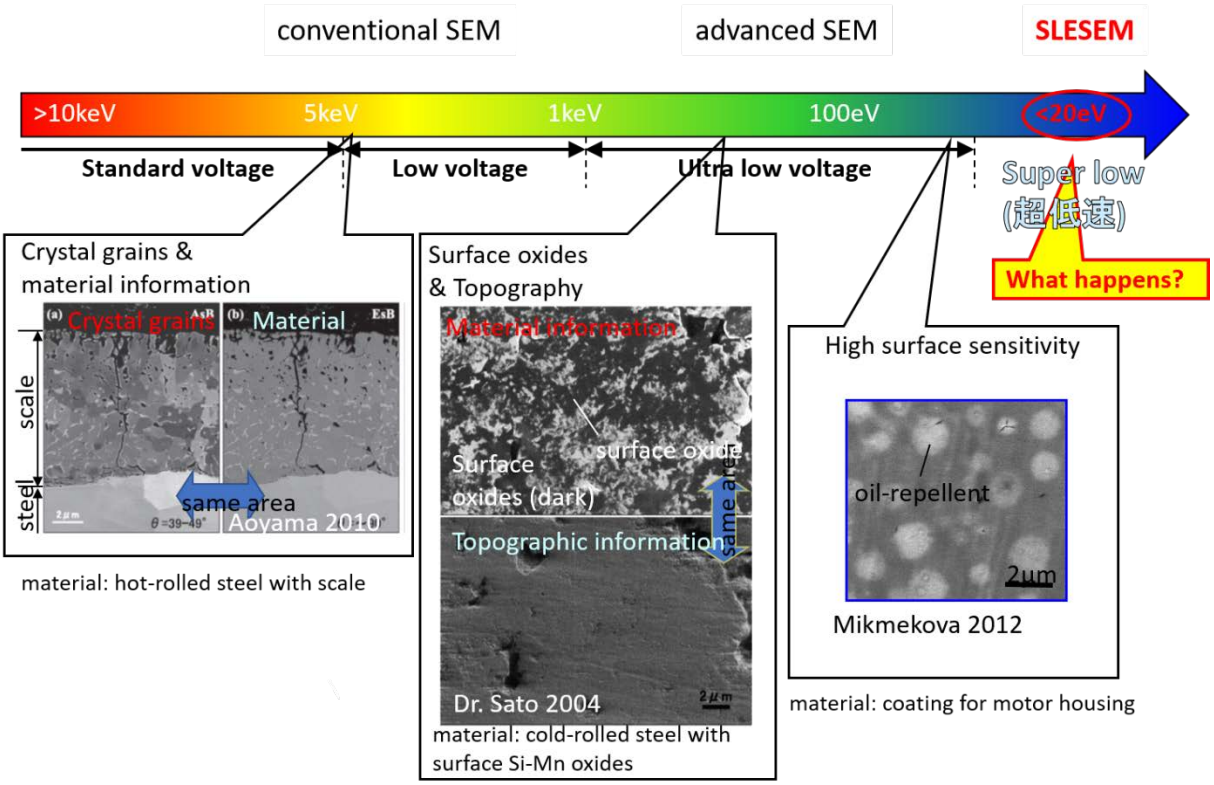

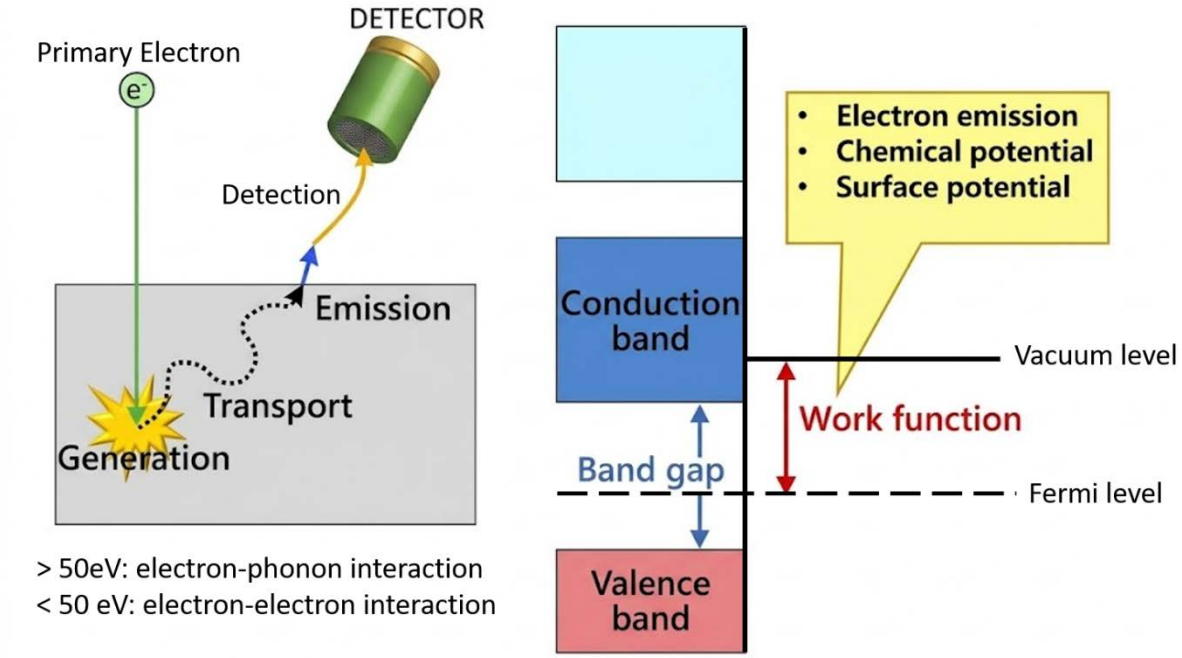

Work-Function-Resolved Imaging of Relaxation Oscillations and Chemical Spillover in CO Oxidation over Platinum Surfaces

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Introduction.

Spatio-temporal oscillations during catalytic CO oxidation on Pt surfaces represent a prototypical example of self-organization in heterogeneous catalysis [1]. While techniques such as photoemission electron microscopy (PEEM) and scanning electron microscopy (SEM) [2] have revealed complex wave patterns, the physical origin of the observed contrast and the local reaction dynamics remain only partially understood. In particular, intensity-based imaging does not provide direct information about local surface chemistry or work-function variations.

Materials and Methods.

In this work, we combine operando SEM with frequency-modulated Kelvin probe force microscopy (FM-KPFM) to simultaneously image reaction fronts and measure local contact potential difference (V_{CPD}) on a Pt(110) single crystal during CO oxidation. SEM provides high-resolution visualization of propagating reaction waves, while FM-KPFM enables spatially and temporally resolved mapping of surface work function. Experimental observations are complemented by spatially resolved reaction–diffusion simulations incorporating adsorption, desorption, and diffusion kinetics of CO and O species.

Results.

The combined measurements allow a direct correlation between SEM contrast and local work-function variations, enabling unambiguous assignment of bright and dark regions to CO- and O-covered Pt, respectively. The measured V_{CPD} values (up to 0.46 eV) exclude the presence of extended surface or subsurface oxide phases, indicating that the reaction proceeds via adsorbed species. Time-resolved KPFM signals reveal pronounced asymmetry of the reaction fronts, which is not discernible in SEM alone. Phase portraits constructed from the KPFM data exhibit a triangular shape, characteristic of relaxation oscillations with strong time-scale separation. Furthermore, the transition to the oxygen-covered state occurs at locally varying work-function values, indicating spatially heterogeneous threshold behavior. Simulations reproduce the observed front morphology, temporal asymmetry, and internal gradients of adsorbate coverage within the propagating wave.

Conclusion.

The combination of SEM and FM-KPFM provides a powerful operando approach for resolving the local dynamics of catalytic reactions. The results demonstrate that CO oxidation on Pt(110) proceeds in a relaxation-type regime governed by local kinetics and adsorbate interactions, refining the classical mean-field description and highlighting the importance of spatially resolved work-function measurements.

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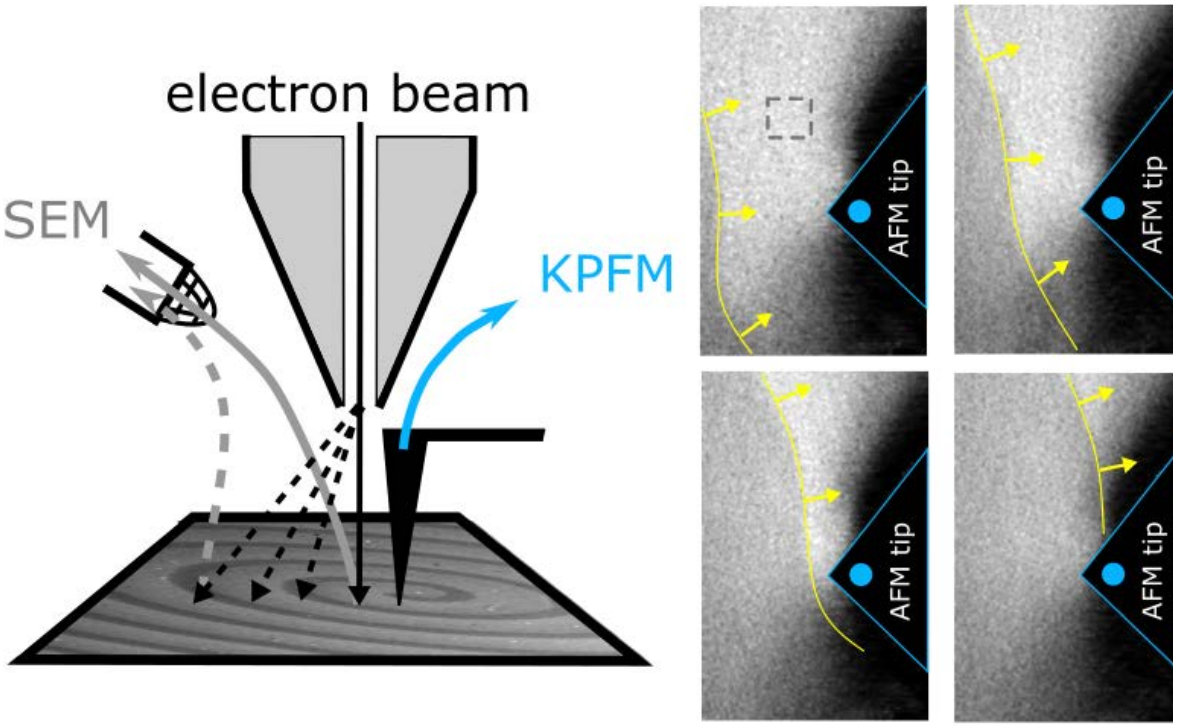

Fig. 1: Correlative SEM and FM-KPFM imaging of spatio-temporal reaction patterns during CO oxidation on Pt surfaces. Left: Schematic of the experimental configuration combining SEM and frequency-modulated Kelvin probe force microscopy (FM-KPFM). Right: Sequence of SE images showing the evolution of a reaction front within a single grain on a polycrystalline Pt sample in the vicinity of the AFM tip (blue, see further) in FEI Versa 3D SEM. Experimental conditions: p(CO) = 3×10^{-3} Pa and p(O_2) = 1.6×10^{-2} Pa, T = 216 °C. The yellow line marks the position and the direction of the reaction front; yellow arrows show the propagation direction.

Elucidation of internal damage using FIB tomography

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Fatigue loading is a complex process that results in irreversible changes in the material, which are subsequently responsible for failure. As a response of the material to the cyclic loading, the localization of the cyclic plastic deformation occurs, resulting in the evolution of damage in the interior of the material. Only grains with a crystallographic orientation preferred for slip exhibit damage. Since most of the engineering materials exhibit a structure free of texture, only several grains are exposed to the plastic deformation. Also, the cyclic loading of the material is not homogeneous throughout the sample. Mostly, the middle part of the sample (depending on the loading type) is exposed to the highest stress / plastic formation. As a consequence, surface grains of the tested sample in the middle region are the most loaded and usually responsible for the frack initiation.

Many theories of the fatigue crack initiation mechanism have been proposed. However, most of them were proposed based on the low-frequency data and are fully applicable for the low-cycle (up to 10^5 cycles) and mostly also for the high-cycle fatigue regions (up to 10^7-10^8 cycles). Nowadays, the ultrasonic testing systems is being used as well, to reach a number of cycles up to and even above 10^{10} cycles, speeding up the test and prolonging the range of the lifetime for the component usage. However, most of the time, frequency-dependent results are measured. With increased test frequency, the lifetime also increased. This increase is, however, artificial and can not be considered when predicting the real working life of the material.

Results of several fatigue tests performed at different frequencies and the fatigue crack initiation mechanism studied by SEM are presented in this study. Since the slip markings were observed to be responsible for fatigue crack initiation at all test frequencies, their evolution was analysed in greater detail. The focused ion beam (FIB) cutting and slicing were adopted to reveal the slip marking structure beneath the surface. FIB cutting through slip markings (Fig. 1) revealed their evolution during cycling, followed by slicing through the whole slip marking.

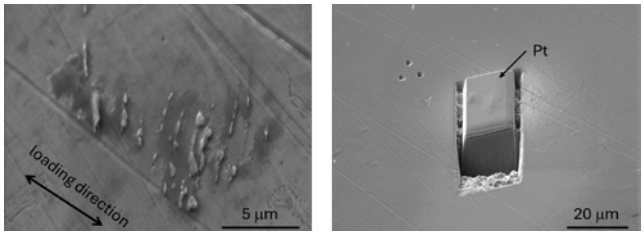

Fig. 1: Slip markings on the surface and coverage with Pt for FIB slicing. Slicing was performed perpendicular to the slip markings. Fatigue sample from the ferritic-pearlitic steel tested at 20 kHz.

Individual FIB cuts through the slip markings revealed different damage extension in different locations (Fig. 2). Thus the FIB slicing was proposed as a method allowing the inspection of the volume instead of the site specific inspection. For the FIB slicing, the slip markings on the surface were fully covered with Pt layer to protect the surface and eventually to preserve the evidence of the early stages of the fatigue crack initiation. Afterwards, the trenches around the covered areas were milled by FIB. The final step was to process the FIB slicing (slice thickness of 100 nm) through the whole volume of interest. As

the last step, the image processing by Image J software and 3D reconstruction using Tescan 3D Analysis suite was performed (Fig. 3).

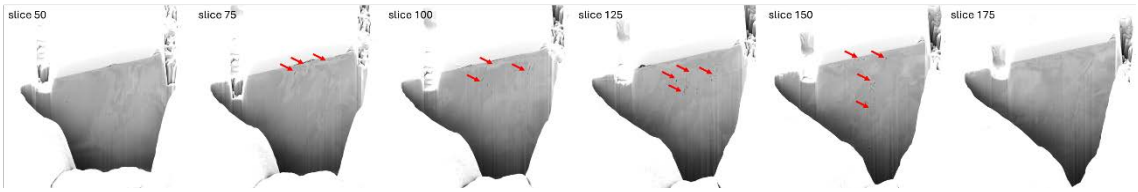

Fig. 2: Individual cuts of the slip markings on the surface showing the damage evolution under the material surface (cavities are marked by arrows).

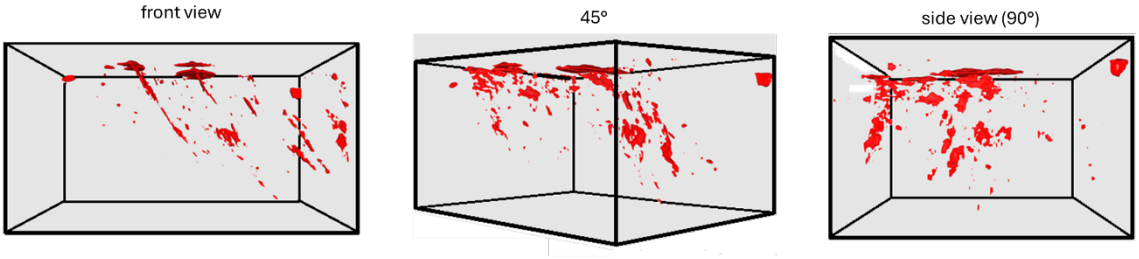

Fig. 3: 3D reconstruction of the damage under the surface due to the cyclic loading. See the cavity location along the slip plane.

The detailed study showed that there is no difference between the damage mechanisms acting at different frequencies and stress amplitudes. The only change was the extent of the damage. With the increasing frequency, the deceleration of the damage mechanism was revealed. This phenomenon is connected to the formation of cavities and their diffusion, further responsible for the formation of slip marking on the surface and crack initiation. Detailed analysis of the data revealed the evolution of cavities and their localization along the slip planes.

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4D-STEM-PNBD with Frame- and Event-based Detectors: Walking through Dimensions

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The advent of 2D array detectors of transmitted electrons (pixelated detectors) have revolutionized the field of electron microscopy. A microscope equipped with a pixelated detector scans an ultrathin specimen, recording the position of the electron beam on the specimen (*X, Y*) *together with positions of all scattered electrons* on the detector (*x, y*). If the scanning beam is quasi-parallel, the individual images (x, y) are nanobeam diffraction patterns (NBD). The resulting scanning transmission electron microscopy (STEM) dataset is four dimensional (*X, Y, x, y*) and the method is called 4D-STEM [1].

Traditional pixelated detectors are frame-based, collecting data in the straightforward form described above as a 2D array of 2D diffraction patterns. Modern pixelated detectors can operate in an event-based mode, recording a continuous stream of individual electron events characterized by their detector position (*x, y*), time of arrival (*t*; ToA), and energy-related signal measured as time over threshold (*E*; ToT). The frame-based detectors (Fig. 1) provide classical, structured, intensity-calibrated diffraction data that are well suited for established 4D-STEM techniques such as phase, orientation, and strain mapping, as well as ptychography or differential phase contrast. The event-based detectors (Fig. 1) excel in high-speed, low-dose, and/or time-resolved experiments; however, the resulting streams of individual events require more elaborate processing. The 4D-STEM techniques are well established in high-energy, high-resolution transmission electron microscopy (4D-STEM-in-TEM; ref. [1]), but remain much less explored in scanning electron microscopy, where the lower electron energies lead to stronger electron-matter interactions, increased inelastic scattering and reduced penetration depth, requiring special data treatment (4D-STEM-in-SEM; refs. [2,3]).

In our project, we develop accessible, open-source software packages for 4D-STEM-in-SEM. The packages (Reader4D [2], STEMDIFF [3], and EDIFF [4]) are based on popular Python programing language and simple templates in Jupyter environment. The templates are designed and documented so that they could be easily adjusted by common SEM users, i.e. by non-programmers and non-experts on electron diffraction. The first of our packages (Reader4D) reads/visualizes/checks 4D-STEM data, the second (STEMDIFF) converts them to 2D powder diffraction patterns and the third (EDIFF) converts the *experimental* 2D-diffractograms to radially averaged 1D-profiles (ELD) and compares them with the *theoretical* powder X-ray diffraction patterns of the expected crystal structure (XRD). The calculation of theoretical XRD diffractograms is fully automated, the only input is a CIF file downloadable from literature or open crystallographic databases [5]. If ELD an XRD patterns overlap, the nanocrystal structure in the sample is identified (Fig. 2). This is the principle of our newly introduced method named 4D-STEM-PNBD (powder nanobeam diffraction in four-dimensional scanning electron microscopy [3,4].

Recently, we have been extending and improving our packages in several ways: (i) Reader4D now reads both frame-based data (traditional detectors) and event-based data (newly installed event-based detector in the laboratory of the last author), (ii) the STEMDIFF package enabled us to analyze the first event-based datasets (proof-of-concept stage), (iii) the EDIFF package was completely re-written towards full automation, simplicity and user-friendliness, including accurate auto-detection of diffractogram center, auto-removal of diffraction background, and auto-comparison of ELD and XRD patterns, which reduces the processing to a small template that is fully automated for standard diffraction patterns and easily adjustable for more difficult cases.

In this contribution, we will explain how we reduce 4D datasets obtained in 3D space to 2D patterns and final 1D profiles. Moreover, we will discuss the specific processing of datasets from frame-based detectors (4D-STEM as 2D array of 2D-NBDs) and event-based detector (4D-STEM as 1D stream of events). Finally, we will outline the future development of our software tools. We plan numerous extensions, enhancements and improvements, the most important of which are AI-based algorithms for background removal (to facilitate processing of both monocrystalline and polycrystalline diffraction patterns) and novel algorithms for peak detection and indexing (to enable structure identification from single, monocrystal diffraction patterns from selected locations).

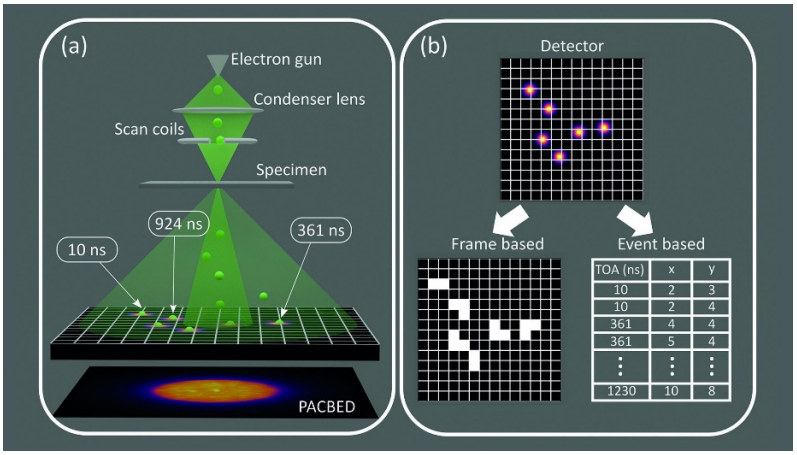

Fig. 1. *Frame-based and event-based detectors for 4D-STEM. The frame-based readout consists in the collection of 2D-array of 2D-NBD patterns. The event-based readout produces a 1D stream of data packets, where each packet corresponds to a single event – when electron strikes the detector chip. Image from internet, ref. [6].*

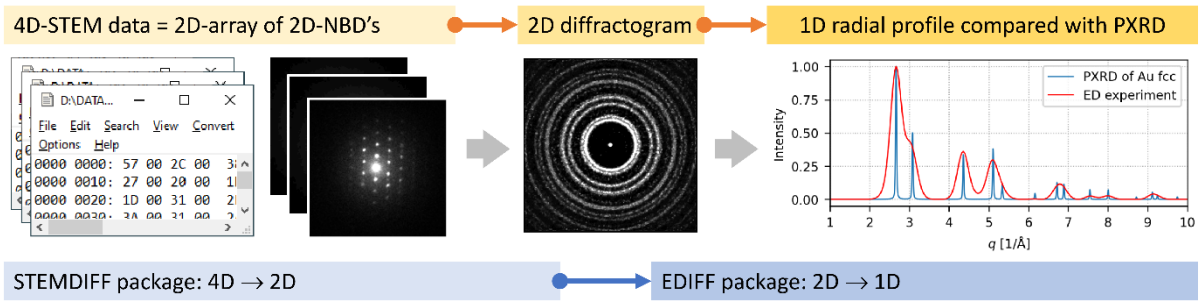

Fig. 2. *Principle of our 4D-STEM-PNBD method: the conversion of a 4D-STEM dataset to a powder electron diffraction pattern and its comparison with theoretically calculated X-ray diffractogram.*

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[6] https://analyticalscience.wiley.com/content/article-do/4d-stem-event-based-electron-detection

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Instrumentation and optics

Chairs: Dušan Chorvát and Kateřina Mrázová

Light enters electron microscopy

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Electron microscopy has enabled observation of the fundamental building blocks of matter, individual atoms and molecules in solid state structures. Besides static imaging and spectroscopy of materials, there is a large demand for techniques allowing to capture dynamical evolution of electronic, vibrational and other excitations in a sample brough out of equilibrium by external stimulus. This talk will review recent developments in the field of ultrafast electron microscopy and diffraction, which utilize femtosecond laser pulses to reach both atomic spatial and sub-picosecond time resolutions. I will discuss the recent theoretical proposals and experimental applications of the interaction between coherent light and freely propagating electrons, focusing on advanced spatio-temporal shaping of electron wave function [1-7], aberration correction of electron optics using light [8-10] and time-resolved imaging of optical near-fields of nanostructures using ultrafast 4D scanning transmission electron microscopy [11].

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Advent of new capabilities of the TITAN Themis enabled by upgrades with the latest Cs-probe corrector, post-column energy filter, and direct electron detection cameras

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Major and unique upgrades were successfully implemented on a monochromated and Cs-image-corrected TITAN Themis 60–300 installed in CEITEC Nano infrastructure, creating a TEM instrument that meets current requirements for cutting-edge materials analyses at the atomic scale, at low dose conditions, and utilizing machine-learning and artificial-intelligence.

The first upgrade was a world-unique field installation of an S-CORR, the latest Rose-variant Cs-probe aberration corrector from Thermo Fisher Scientific, enabling compensation of 5th-order spherical aberration (C5) and six-fold astigmatism (A5) across all accelerating voltages of the microscope. Compared to the previous non-corrected configuration, the STEM lateral resolution was significantly improved, reaching the highest standards of 40 pm at 300 kV and a sub-ångström resolution at 60 kV.

The second upgrade was globally the first installation and integration of a complex GIF Continuum HR K3&STELA system on the TITAN Themis platform. It is the latest spectrometer and image filter from GATAN with a unique configuration providing unprecedented capabilities in EELS, EFTEM and 4D-STEM applications, especially for very beam sensitive materials or for detection of extremely low signals. This is assured mainly by the K3 (GATAN) and ELA (DECTRIS) cameras with direct single electron detection and counting and very high frame rates, a 100 ns electrostatic shutter, an In-situ and STEMx packages for very fast and precise S/TEM data cube acquisitions with the real-time output, or a new DigiScan3 unit enabling beam-smart control and programable scanning strategies mitigating beam damage and image drift. Additionally, the system includes a q-slit allowing for ultimate ω-q-EELS acquisitions of magnetic chiral signals via EMCD or EMLD techniques and a new SW-controlled beam precession electron diffraction important in crystallography measurements. Above, the fully supported Python-scripting environment of the newest control software, GMS 3.62, together with the entire GIF system, provides a robust platform for development and implementation of advanced ML- and AI-based analyses of complex datasets.

Thanks to the upgrades, different advanced and new methods are being developed and implemented. Aside the new performance of the renovated TEM system, it will be presented, for example:

- A new robust approach to 4D-STEM data analysis, which allows to detect non-structural asymmetries in the odd component of individual ronchigrams, taking another step toward reliable atomically resolved spin detection in antiferromagnets.
- Very-low dose rate EELS mapping at atomic scale with a dose-fractionated spectrum imaging using the fast and single electron counting cameras.
- Low-voltage HR-STEM imaging with application of the monochromator.

Acknowledgment:

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Phase contrast in 4D-STEM and aberration measurement

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Phase contrast techniques are essential for imaging radiation-sensitive samples that interact weakly with the electron beam. A major challenge in conventional electron microscopy arises when imaging biological or light-element specimens (composed primarily of carbon, oxygen, and hydrogen). These low-Z elements scatter electrons more weakly than heavier atoms, making standard high-angle annular dark-field (HAADF) imaging highly inefficient or even impossible. Phase contrast, therefore, offers a powerful alternative to visualize these weak interactions.

While phase contrast has traditionally been the domain of the transmission electron microscope (TEM), modern scanning transmission electron microscopy (STEM) offers advanced alternatives. Beyond conventional bright-field (BF) STEM and integrated differential phase contrast (iDPC), the advent of pixelated detectors enables 4D-STEM. By processing complete diffraction patterns using techniques such as ptychography, high-quality phase contrast can be achieved.

In this work, we focus on Single Side Band (SSB) ptychography, a direct phase-retrieval method that reconstructs the sample phase by analyzing interference in the double overlapping regions of diffracted disks. Furthermore, we introduce a novel algorithm for aberration measurement. Unlike previously demonstrated methods [1, 2] that fit the phase of the G-set (obtained via a 2D Fourier transform of the 4D dataset with respect to the real-space scanning coordinates), our algorithm fits the difference in the phase of the G-set. This approach completely eliminates the need for complex phase unwrapping procedures.

Unwrapping is necessary when evaluating the phase of the G-set, as any phase shifts larger than 2π are mathematically wrapped. Traditional algorithms must mathematically unwrap these discontinuities to reconstruct the continuous phase before fitting. By fitting the phase difference instead, we bypass this unwrapping step and reduce the total number of fitting parameters. As a trade-off, calculating phase differences amplifies noise, and the fitting procedure requires a non-linear optimization approach rather than a linear one.

To validate the algorithm, we applied it to a simulated 60 keV (34 mrad aperture semi-angle, $5 \cdot 10^4$ e/Å^2 dose) 4D-STEM dataset of a carbon nanotube on graphene (Fig. 1). Aberrations (e.g., defocus, astigmatism, and spherical aberration) were deliberately introduced to make the probe non-ideal, as shown in Fig. 2a. By applying our algorithm, we successfully recovered the aberrations, yielding a probe profile (Fig. 2b) that closely matches the original non-ideal simulation.

The knowledge of probe aberrations is crucial for robust image recovery. While SSB ptychography typically requires an optimal probe, it can account for non-ideal conditions if the aberrations are known. Reconstructions without and with accounting for the measured aberrations are compared in Fig. 3a and Fig. 3b, respectively. Incorporating the measured aberrations was successful, as individual atoms became clearly visible. The resulting image (Fig. 3b) achieves a fidelity comparable to that of the SSB reconstruction using true aberrations known a priori from the simulation (Fig. 3c).

In conclusion, the proposed aberration measurement algorithm is not only useful for precise microscope alignment but also highly valuable for post-processing. The ability to use these measured aberrations to reconstruct accurate images from non-ideal 4D datasets offers a significant advantage for practical high-resolution phase-contrast imaging.

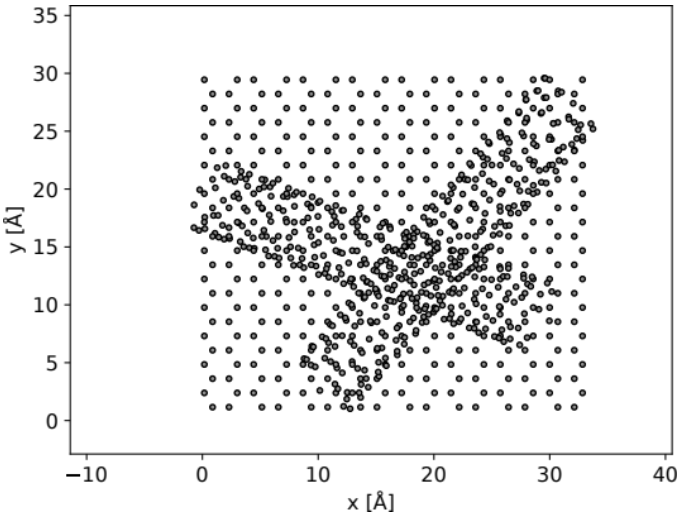

Fig. 1: Atomic model of the sample

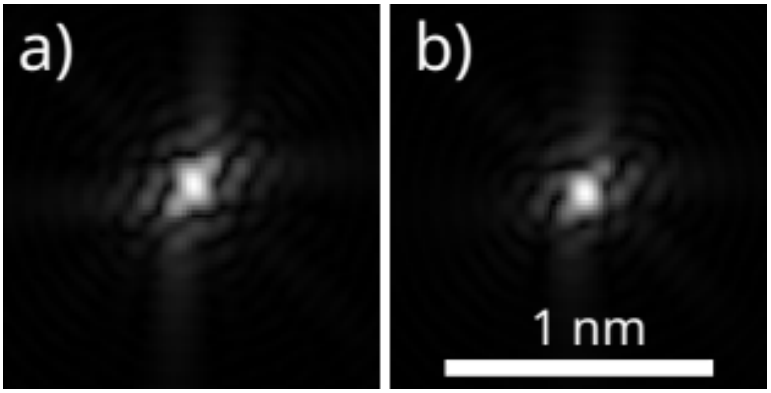

Fig. 2: Intensity of the a) true and the b) recovered probe

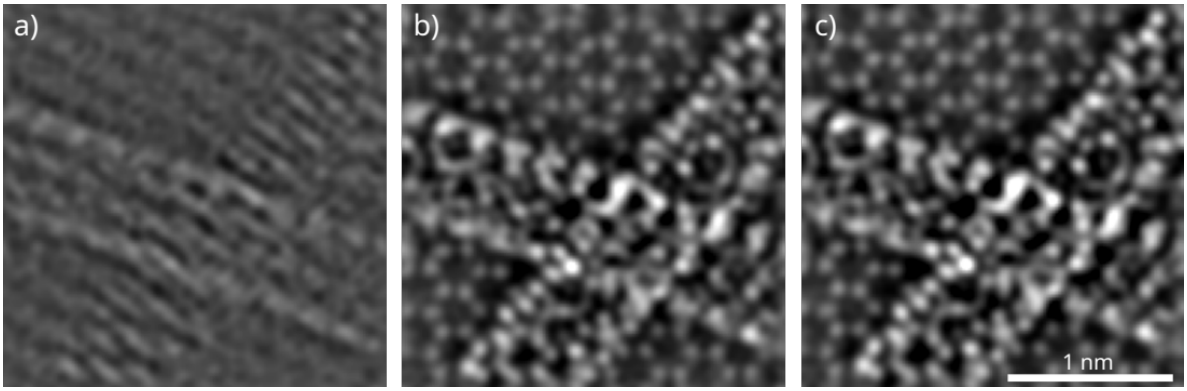

Fig. 3: Reconstructed phase of the sample using SSB a) without aberration correction, b) with aberration correction using measured aberrations, and c) with aberration correction using true aberrations.

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Acknowledgement:

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Near-Field Scanning Optical Microscopy: A Powerful Tool for Next-Generation Photonic Applications

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The diffraction limit of conventional far-field optics remains a significant barrier to the direct observation of light-matter interactions at the nanoscale. Near-field Scanning Optical Microscopy (NSOM) effectively overcomes this limitation by capturing evanescent fields, enabling optical resolution far below the Rayleigh criterion.

This contribution presents a comprehensive NSOM system designed for high-resolution imaging of photonic devices and structures. A part of the contribution is focused on the development of next-generation optical probes fabricated using 3D laser lithography via two-photon polymerization. Unlike traditional fiber-pulling or chemical etching methods, 3D lithography allows for highly tailored 3D geometries.

Furthermore, we demonstrate the efficacy of this NSOM system in analyzing various photonic devices and nanostructures. By mapping local electromagnetic field distributions in real space, the system provides critical insights into mode propagation and field confinement. Our results show that the integration of custom-tailored 3D probes with NSOM results in a powerful tool for the characterization of next-generation nanophotonic devices.

Life sciences

Chairs: Josef Lazar and Veronika Huntošová

Extending Lateral-Shearing Digital Holographic Microscopy Beyond Conventional Coherence Limits

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Digital holographic microscopy (DHM) has evolved into a versatile platform for quantitative phase imaging, enabling label-free investigation of biological samples with applications ranging from high-resolution cell imaging to holographic flow cytometry and tomographic reconstruction. In particular, lateral-shearing configurations and related common-path interferometric schemes have demonstrated enhanced robustness to environmental perturbations, improved phase stability, and compatibility with high-throughput modalities, including in-flow measurements and machine-learning-assisted analysis.

A key operational regime in lateral-shearing digital holographic microscopy (LS-DHM) arises when the shear exceeds the sample dimensions, allowing the formation of an effective reference wave within a single field of view. Under coherent illumination, this approach enables stable, single-shot quantitative phase reconstruction and has been successfully employed in a variety of biological imaging scenarios. However, despite these advances, LS-DHM fundamentally relies on mutual coherence between laterally shifted wavefronts, which usually restricts its operation just to coherent light sources.

In contrast, partially coherent illumination can be utilized in two-arm interferometric systems, where independent optical paths and dispersive elements enable compensation of coherence mismatch. While such approaches allow operation with reduced spatio-temporal coherence, they sacrifice the inherent stability and simplicity of lateral-shearing geometries.

In this work, we overcome this long-standing limitation by demonstrating lateral-shearing interferometry in a regime where the shear exceeds not only the sample size but also the coherence area of the illumination. In this previously unexplored regime, interference is preserved in a manner that effectively generates a reference wave even under illumination with limited spatio-temporal coherence.

Based on this concept, we introduce an incoherent LS-DHM approach that combines simplicity and robustness of the common-path interferometry with the advantages of the illumination with reduced coherence. The method enables single-shot quantitative phase imaging with high accuracy, improved phase stability, and increased space–time bandwidth product. We experimentally validate the approach across multiple microscope configurations and demonstrate its scalability in terms of resolution and field of view.

Finally, we demonstrate the applicability of the method to biological imaging, including cheek cells, diatoms, and yeast, highlighting its potential for high-resolution, label-free investigation of subcellular structures. By overcoming the coherence constraint inherent to lateral-shearing interferometry, the proposed approach establishes a new paradigm for quantitative phase imaging with reduced coherence requirements.

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Acknowledgement:

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In situ protein crystallography – a light microscopy–guided cryo-FIB pipeline for direct protein structure determination from a single intracellular crystal

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Intracellular crystallisation is an emerging approach in structural biology that bypasses the need for protein purification. In 2024, the InCellCryst [1] pipeline was introduced for structural studies of intracellular crystals using serial X-ray crystallography. However, serial crystallography requires exposure of tens of thousands of cells containing intracellular crystals, precluding high-resolution studies of proteins that crystallize in only a few cells, and it requires access to synchrotron facilities, which significantly limits the accessibility of this approach.

We have developed IncelluloED [2], a method that leverages light and fluorescence microscopy–guided cryo-FIB lamella preparation to access intracellular crystals for in situ 3D electron diffraction. The approach relies on expression of a fluorescence marker in the cytoplasm of transfected cells, exclusion of this marker from the crystals to enable crystal localization and on cell surface imaging with reflected light to target the region of interest for FIB. In the ideal case, IncelluloED achieves high-resolution structures from a single crystal within a single cell. Experiments on a microcrystal of the HEX-1 protein from Magnaporthe grisea, grown inside an insect cell, yielded a structure at 1.9 Å resolution from an exposed volume of ~1.6 µm³. By comparison, serial X-ray crystallography achieved 1.8 Å resolution from more than 50,000 crystals, corresponding to a multimillion-fold gain in efficiency.

IncelluloED uses widely available cryo-EM tools and brings high-resolution structural biology into laboratories without access to large-scale infrastructure.

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Acknowledgement:

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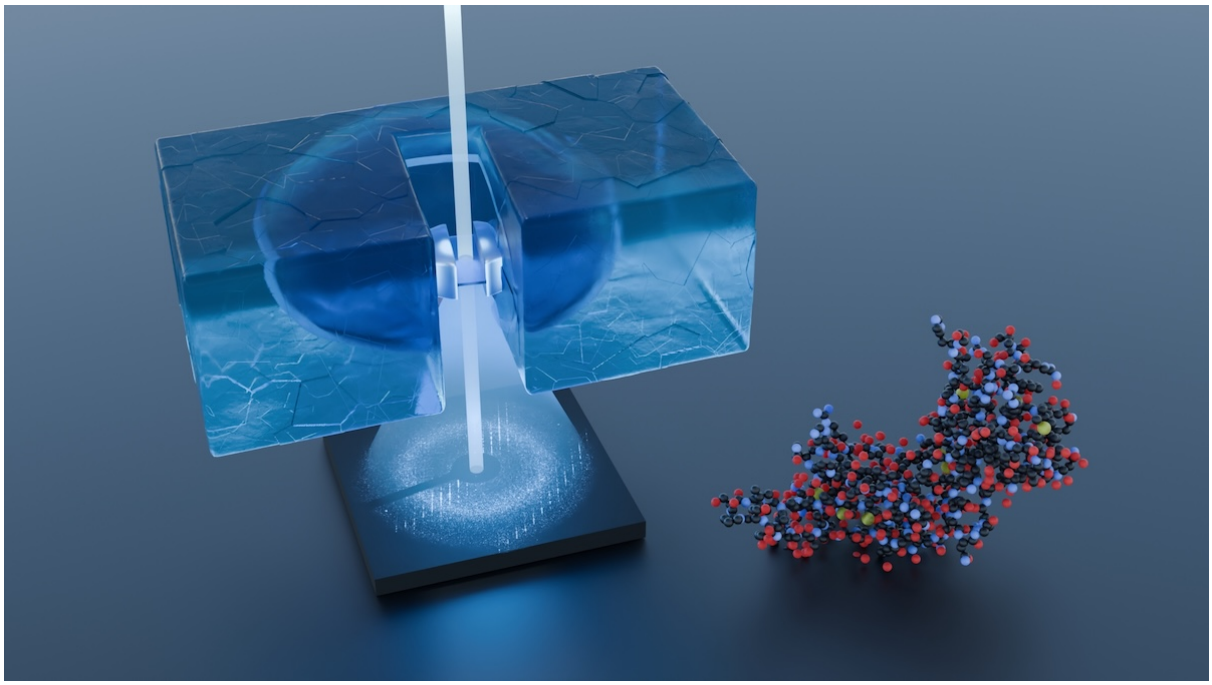

Fig. 1: Artistic illustration of IncelluloED method by Lucas J. Martin, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Fighting Malaria with Volume EM

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Malaria remains one of the world's deadliest infectious diseases, and blocking parasite transmission is a major goal in the fight against it. To do so, we need to identify vulnerable steps in the parasite life cycle - including those that are difficult to access by conventional microscopy. This is where volume electron microscopy (volume EM) becomes particularly powerful, by linking three-dimensional cellular organization to biological function across complex developmental stages.

In the human malaria parasite Plasmodium falciparum, transmission to the mosquito depends on specialized secretory organelles that enable progression through mosquito tissues and host cell invasion, yet the mechanisms controlling their function remain poorly understood. Here, we focused on GEST, a critical and understudied component of secretory organelles in multiple mosquito-stage forms of P. falciparum, including gametocytes, ookinetes, and sporozoites. Using in vitro gametocyte assays and experimental infections with a GEST-deficient parasite line (GEST-KO), we showed that loss of GEST significantly impairs host cell disruption and severely compromises parasite transmission.

To uncover the structural basis of this phenotype, we applied Array Tomography-Scanning Electron Microscopy (AT-SEM), complemented by fluorescence microscopy for parasite localization when needed. This approach provided detailed structural insight into secretory organelles across transmission stages and revealed a severe defect in organelle secretion upon parasite activation in the absence of GEST.

Together, these findings establish GEST as a key factor in secretory organelle function, provide mechanistic insight into malaria transmission, and highlight how volume EM can help uncover transmission-stage vulnerabilities that may be exploited to interfere with parasite spread.

Automated High-Resolution Cryo-FIB-SEM Volume EM Enables Sub-Volume Averaging of Cellular Structures

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Volume FIB-SEM microscopy enables three-dimensional imaging of biological ultrastructure but relies on dehydration and heavy-metal staining, which can perturb native cellular organization. Cryo-volume electron microscopy (CVEM) preserves samples in a near- native state; however, its broader application has been limited by low image contrast and charging artifacts that complicate data interpretation.

Here, we present an integrated experimental and computational workflow that suppresses charging artifacts and enhances imaging contrast in CVEM. Using this approach, we acquire three-dimensional datasets of cells and tissues at ~15–20 nm isotropic resolution. Subvolume averaging of 113 nuclear pore complexes extracted from CVEM data resolves nuclear pore architecture to 9.8 nm resolution.

These results establish CVEM as a powerful approach for the automated acquisition of high-resolution volumetric data under near-native conditions.

Interdisciplinary sciences

Chairs: Miroslav Šlouf and Pavlína Sikorová

4D-STEM Meets Biology: Promises, Constraints and Practical Limitations

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Electron ptychography has demonstrated the ultimate resolution limit imposed by atomic vibrations for materials sciences samples [1]. Unfortunately, its application to biological samples is more challenging. Specimens in structural biology are composed of light atoms with inherently low contrast, embedded in non-conductive ice of varying thickness, and are highly sensitive to electron dose, leading to rapid degradation. For such samples, electron ptychography remains far from realizing the resolution routinely achieved by conventional cryo-TEM and currently is still at the stage of proof-of-principle method development [2].

With biological cryo samples, under conditions of extremely low electron doses, limited high-angle scattering, large space-dependent defocus gradients, beam-induced specimen drift and rapid sample degradation, iterative ptychography reconstruction strategies often fail to converge. Furthermore, attempts to increase resolution through usage of higher probe angles further reduces signal contrast, making reconstruction even more challenging.

For challenging samples such as biological structures, no straightforward experimental design or processing strategy has so far shown optimal results. Instead, a more complex approach is required, which may draw on methods developed for materials science samples but must be substantially adapted to account for the fundamentally different nature of biological specimen.

Here, we present a 4D-STEM workflow that combines selected reconstruction methodologies to optimize performance for cryogenic biological imaging. The workflow enables dose-fractionation through multi-layer electron beam scanning, and combines parallax bright-field defocus estimation with shadow montage-based beam position and sample drift correction and applies this to a hierarchical, recursive ptychographic reconstruction.

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[1] Zhen Ch. et al.: Science 372 (2021), 826-831.
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Design and Development of Calibration Standards for Electron Microscopy

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We introduce two calibration standards fabricated using advanced e-beam lithography for use in electron microscopy techniques. The first calibration standard is intended for the evaluation of material contrast in SEM detectors, such as EDS, and for the optimization of detector imaging parameters. It features unique continuous and lateral planar metal layers deposited on a silicon substrate, enabling direct comparison of detector response and contrast performance. A detail of the calibration standard for different detector filter settings featuring a periodically repeating pattern with sharply defined metal–metal interfaces (Cr and Ag in this case) is shown in Fig. 1.

The second calibration standard is designed for geometric and imaging calibration in SEM and is shown in Fig. 2. It consists of high-orthogonality calibration grids that provide high contrast in secondary electron imaging due to sharply defined edges. The substrates are silicon-based, non-charging and compatible with high voltage operation in an electron microscope. Typical use cases include evaluation of image contrast in secondary and backscattered electron modes, calibration of distance measurements, magnification verification, orthogonality calibration, and distortion analysis. Both calibration standards can be adapted for different experimental setups and measurement tasks.

Acknowledgement:

The research was supported by the Infrastructure of the Czech Academy of Sciences (RVO:68081731) and by the TQ11000042 Support for ‘Proof-of-Concept’ activities at the Institute of Scientific Instruments of the Czech Academy of Sciences, which is co-financed with support from the Technology Agency of the Czech Republic under the SIGMA Program.

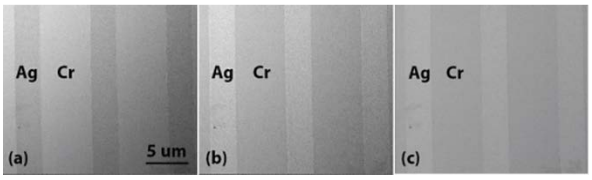

Fig. 1: Detail of the material contrast of the Cr-Ag planar standard, a), b), and c), corresponds to the changing of a detector filter.

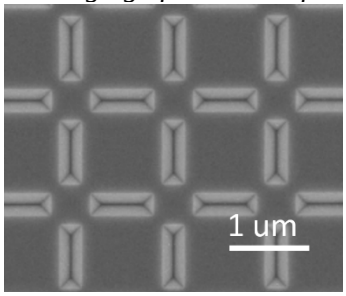

Fig. 2: Detail of the orthogonal calibration grid of the silicon-based calibration SEM test sample.

AI Tools for Spectroscopy – Current Progress at ISI CAS

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The growing use of imaging spectroscopy techniques produces high-dimensional, storage-demanding datasets that are beyond the scope of manual analysis [1]. Integrating artificial intelligence (AI) and machine learning algorithms into Raman spectroscopy and hyperspectral imaging (HSI) offers a solution to the significant data volume challenge. Machine learning takes advantage of such large data volumes using advanced algorithm architectures, such as 3D Convolutional Neural Networks (CNNs) for synergistic spatial-spectral feature extraction and Graph Neural Networks (GNNs) for direct spectrum prediction. These algorithms can to automate complex tasks including denoising, segmentation, and molecular identification [2].

However, the inherent black-box nature of these nonlinear models creates the need for Explainable Artificial Intelligence (XAI) frameworks. These frameworks can decompose black-box decisions into human-interpretable visualizations, helping us to understand the decision mechanisms inside the models. Examples of these frameworks include 3D Gradient-weighted Class Activation Mapping (Grad-CAM), SHapley Additive exPlanations (SHAP), and LIME-inspired masking [3]. These XAI methodologies prioritize transparency through volumetric attribution and ranking the contribution of specific wavenumbers. This ensures that AI-driven insights remain chemically grounded and compatible with FAIR (findable, accessible, interoperable, and reusable) principles and encourages scientific trust [4].

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Acknowledgement:

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SEM-EDX as a Tool in Biological Anthropology: From Skeletal Remains to Dental Calculus

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Introduction

This study explores the analytical potential of Scanning Electron Microscopy coupled with Energy-Dispersive X-ray Spectroscopy (SEM-EDX) for the investigation of diverse bioanthropological materials. The integration of high-resolution topographic imaging and elemental microanalysis provides a non-destructive pathway to understanding past human lifeways, medical history, and funerary practices.

Materials and methods

The methodology involved analysing a diverse sample set, including historical dental prostheses, dental calculus, human teeth, and an unidentified specimen recovered from the mummy's orbital cavities. Samples were analysed using variable-pressure SEM without modifications (or coating) to accommodate fragile archaeological tissues, with EDX mapping used to identify inorganic inclusions and elemental distributions.

Brief results

SEM analysis of ancient dental calculus offers insights into the dietary patterns of past populations. The EDX detector also revealed microparticles of specific elements, reflecting unique socio-cultural habits [1]. Furthermore, the characterisation of material compositions in historical dental prostheses and fillings proved to be a powerful tool for potential dating and chronological placement based on known technological milestones in restorative dentistry. In unidentified samples, such as those recovered from the orbital cavities, SEM-EDX provides a critical diagnostic pathway to determine the specimen's origin.

Conclusion

Given the non-destructive nature of this analytical approach, we have established a formal recommendation for archaeologists to prioritise SEM-EDX as a preliminary diagnostic step before proceeding with more invasive or destructive methods in ancient dental calculus [2]. However, this can be applied to a wider range of bioanthropological samples. These findings demonstrate that SEM-EDX is an essential, preservation-friendly tool in biological anthropology, offering high precision for identifying unknown substances and interpreting the socio-cultural context of skeletal remains.

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News in microscopy community

Chairs: Jana Nebesářová and Vladislav Krzyžánek

Building the Czech optical ecosystem

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Since its establishment, the Czech Optical Cluster has focused on strengthening cooperation between industry, research organisations, universities and public institutions across the entire value chain in optics, photonics, optomechatronics, optoelectronics and related advanced technologies.

In recent years, the cluster has significantly expanded its international activities and strategic partnerships, contributing to the growing visibility of the Czech photonics ecosystem in Europe and Asia. During 2025 and 2026, the Czech Optical Cluster actively participated in major international initiatives and events, including OPIE Japan, EXPO 2025 Osaka, Laser World of Photonics, and several European technology and industrial platforms.

A strong emphasis has been placed on international collaboration projects such as PhotonQBoost, supporting the acceleration of quantum and photonic technologies in Europe, and Laser-Pro, focused on strengthening European laser ecosystems, industrial cooperation, technology transfer and advanced manufacturing capabilities. These activities have enabled stronger connections between Czech stakeholders and leading European and global innovation ecosystems.

Photonics is recognized at the EU level as a Key Enabling Technology and a strategic technology for Europe’s industrial competitiveness, resilience and technological sovereignty. Its applications are transversal across sectors such as semiconductors, mobility, healthcare, defense, manufacturing, agrifood and quantum technologies.

The presentation will highlight how the Czech Optical Cluster contributes to building a resilient and internationally connected optical and photonics ecosystem in the Czech Republic through strategic networking, cluster collaboration, support for SMEs, international partnerships and ecosystem development activities. Special attention will be dedicated to the role of photonics in strengthening Europe’s economic security, industrial resilience and future technological autonomy.

Acknowledgement:

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News from Brnoregion Microscopy

Novák 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 that whenever the world talks about (electron) microscopy, the first association is #brnoregion.
Within the strategic framework for 2026–2027, we are focusing on four key areas:

1. COMMUNITY DEVELOPMENT – Supporting networking, knowledge sharing, active community engagement and talent development across the Brnoregion Microscopy ecosystem.
2. FORMAL EDUCATION AND EXCELLENT SCIENCE – Strengthening cooperation with secondary schools and universities, supporting international training activities, workshops and scientific conferences.
3. COMMERCIALISATION SUPPORT – Transferring research results into practice, supporting proof-of-concept activities, spin-offs and commercialization culture within research institutions.
4. PR AND MARKETING – Coordinated promotion of Brno as an internationally recognized microscopy region and increasing awareness of microscopy among students, researchers and global audiences.

For the MICROSCOPY 2026 conference, we have prepared an update on the latest developments within Brnoregion Microscopy, including our current activities, strategic goals and plans for the upcoming two-year period focused on strengthening the regional microscopy ecosystem and its international visibility. We warmly invite all attendees to join us, 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://www.brnoregion-microscopy.com/

[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.

- ✓ October 15, 2026, from 15:00 to 18:30 in the atrium of the CEITEC BUT, Brno.
- ✓ 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 contact us at novak@jic.cz or visit our website.

Connect, Learn, Share: The Young Microscopists of CSMS

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The young microscopists of the Czechoslovak Microscopy Society (yCSMS) connect young people during their studies and at the beginning of their careers across all scientific fields related to microscopy. We create a friendly space for sharing experiences, broaden career opportunities by facilitating connections to academia and industry, and offer opportunities to gain knowledge. We also share information on funding possibilities and support community growth — all while having fun!

In this talk, we will present some of our past activities and plans for the future, together with an invitation to our MICROSCOPY 2026 program.

Everyone is invited to join our activities during the conference. We begin with a hike on Monday morning — a great opportunity to network before the official program starts. We also encourage you to join our next networking activity during the conference dinner on Tuesday evening, where participants will search for their scientific soulmates through an easy and fun connecting game.

Our program becomes more serious on Wednesday morning during the LiftOff session. The spotlight will be on several of our members, who will introduce their projects in short talks, followed by a not-so-typical presentation from the conference plenary speaker on the ups and downs of pursuing an academic career. The session will include time for discussion, and we warmly invite everyone to take part.

In addition to the program, you can visit our photobooth throughout the conference. We also invite everyone to approach us with questions, suggestions, or ideas related to our program and activities.

More information, updates, and contact details are available at our webpage [1]. We invite everyone to follow us on social media (see fig. 1) to stay connected and up-to-date with our latest activities.

References:

[1] https://ycsms.org/

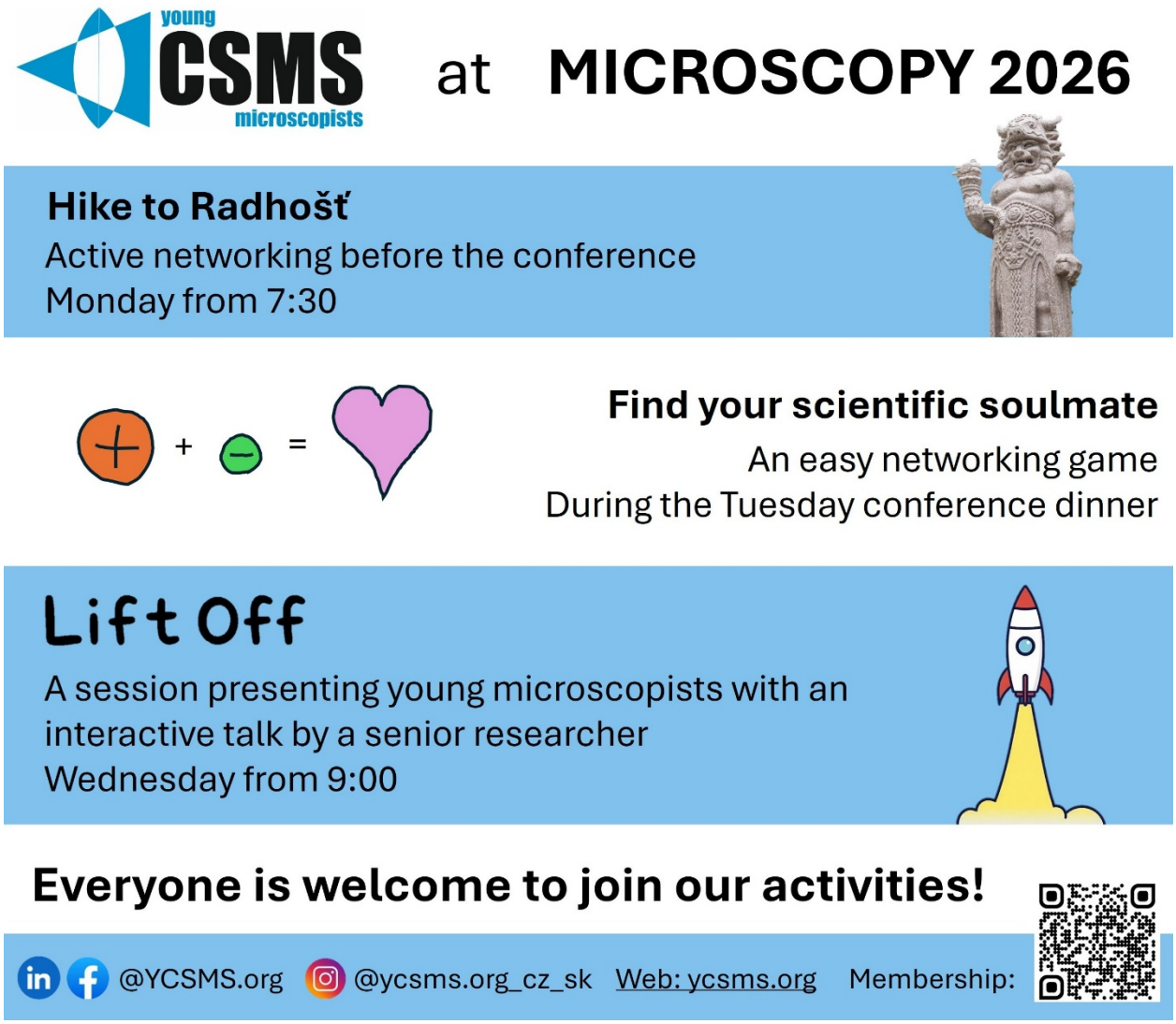

Figure 1: Invitation to yCSMS activities and social media.

The study programs “Mikroskopie” and “Microscopy” at the Masaryk University

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The new microscopy-related study programme “Mikroskopie” in Czech has started in the Autumn semester 2024; the first students will graduate this summer. Recently, the English version of this programme named “Microscopy” has been accredited and it will open in the near future expecting foreign students enthusiastic about the microscopy field in the Brno region. Both are two-year follow-up master's degree studies 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, as well as companies, participate in courses. These institutions are also discussing their broad activities as members of Brnoregion Microscopy platform [2].

The aim of the microscopy-related study programme in either language is to provide students with the professional qualification to become experts in microscopic methods. It focuses on electron and light microscopy as well as other imaging methods useful for research, development and applications. The study aims to both consolidate and deepen the education students received in their previous bachelor's programme with a physical, mathematical-physical or biophysical focus. The programme covers areas of light and particle optics, the properties of materials, the structure of biological samples, and physics related to the properties and construction of imaging devices. The students acquire specialised knowledge and skills in physics, materials science, 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 on practical training at standard and development workplaces of manufacturing companies and at the academy.

References:

[1] https://www.sci.muni.cz/pro-uchazece/navazujici-magisterske-studium/26987-mikroskopie
[2] https://brnotechregion.eu/brnoregion-microscopy

Acknowledgement:

We thank all the people, institutions and companies supporting the microscopy study programmes.

Fig. 1: Flyer of the Microscopy programme.

Czech-BioImaging: Infrastructure Dedicated to Users

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Czech-BioImaging is the national research infrastructure advancing biological and medical imaging in the Czech Republic. Since its launch in 2015, it has provided open access to cutting-edge imaging technologies and expert support for researchers across the scientific community. In 2025, Czech-BioImaging celebrated 10 years of providing open access to imaging services for the scientific community.

The infrastructure connects 16 core facilities in five major Czech cities, offering a broad portfolio of imaging techniques to users from academic, medical, and private sectors. Technologies include light and fluorescence microscopy, super-resolution and electron microscopy, correlative light and electron microscopy (CLEM), and preclinical and clinical medical imaging—supported by comprehensive image processing and data analysis tools. 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.

As host of three Euro-BioImaging Nodes, Czech-BioImaging is part of the European research infrastructure landscape, fostering international collaboration and scientific excellence. Its distributed model ensures regional accessibility and interdisciplinary cooperation nationwide.

Beyond technology access, Czech-BioImaging supports the community through training courses, workshops, user project support and its annual conference, and collaboration with instrumentation manufacturers, contributing to both education and innovation in 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 "Modernisation of the VVI Czech-BioImaging" CZ.02.01.01/00/23_015/0008205).

Biological Imaging LM/EM Data in the Czech National Repository Platform (NRP)

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In the Czech Republic, access to cutting-edge biological imaging technologies (LM as well as EM) is primarily provided by the large-scale research infrastructure Czech-BioImaging (CzBI). At the suggestion of the International Advisory Board, which conducts regular infrastructure evaluations, CzBI decided to build its own licensed repository for biological imaging data. This repository is being built as one of the four pilot repositories of the Czech National Repository Platform (NRP) [1], which is in turn part of the National Data Infrastructure (see Fig. 1). The work started in January 2025 and should finish by December 2028. Currently, the first trial version was released.

There is also an international archive of biological imaging data called BioImage Archive [2], which focuses on data related to significant publications. These are submitted to the BioImage Archive manually via a web interface. The CzBI repository further elaborates the standards and principles used in the BioImage Archive and will support not only manual but also automated collection of data and metadata and comprehensive work with a wide range of outputs from the microscopes (including so-called negative results) and their sharing. It will support large multidimensional and multimodal data and adhere to the FAIR principles. Visualization, annotation and analysis tools are also planned.

References:

[1] https://www.eosc.cz/en/projects/national-repository-platform-for-research-data-nrp/
[2] https://www.ebi.ac.uk/bioimage-archive/

Acknowledgement:

The authors acknowledge funding from the Ministry of Education, Youth and Sports (Projects CZ.02.01.01/00/23_014/0008787 National Repository Platform and LM2023050 Czech-BioImaging).

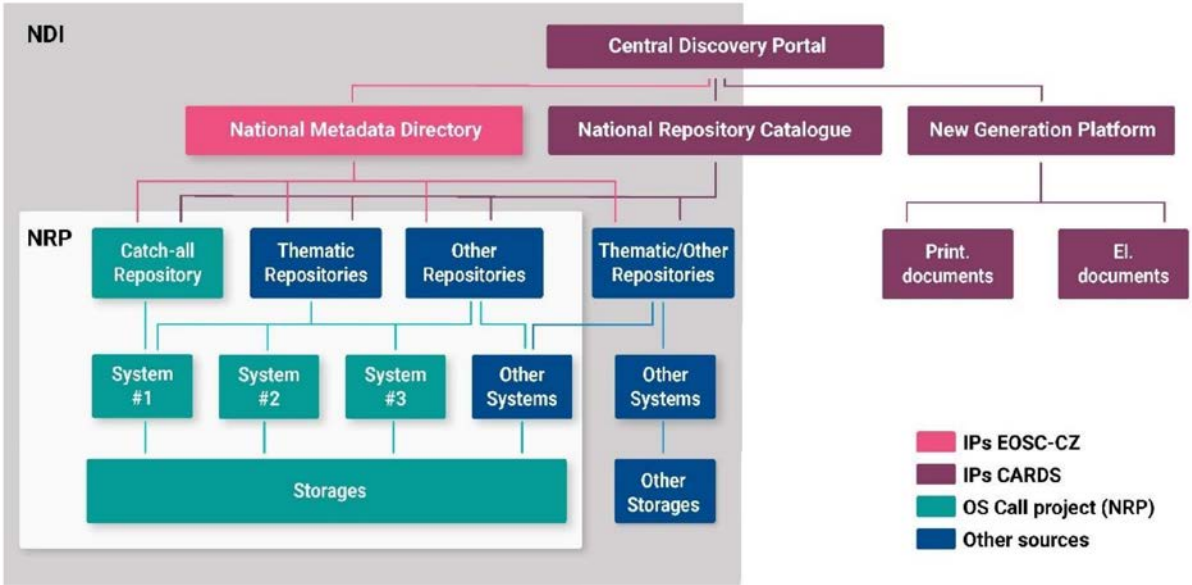

Fig. 1: Concept of National Data Infrastructure (NDI) and National Repository Platform (NRP)

yCSMS session

LiftOff: Launching Early Careers in Microscopy and Science

Chairs: Eva Ďurinová

Advanced characterisation of 2D materials using SLEEM/ToF

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Many interesting properties emerge when at least one dimension of a material is reduced to a sub-nanometre size, forming a two-dimensional (2D) material. In such systems, the motion of particles, such as electrons, becomes confined, giving rise to physical behaviour and material properties that are absent in their bulk counterparts [1]. The electronic structure and collective excitations of 2D materials are highly sensitive to surface chemistry and thickness, which necessitates the use of surface-sensitive characterisation techniques operating at low electron energies.

In this work, graphene, hexagonal boron nitride (h-BN), molybdenum disulfide (MoS_2), and titanium carbide (Ti_3C_2) MXene were investigated using complementary techniques, including scanning low-energy electron microscopy (SLEEM), scanning transmission electron microscopy (STEM), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and scanning low-energy electron microscopy combined with time-of-flight spectroscopy (SLEEM/ToF). An advanced characterisation approach based on SLEEM/ToF demonstrates the ability to obtain electron energy loss spectra (EELS) that provide information on the electronic structure and collective excitations of 2D materials.

Low-energy EELS were obtained, enabling direct probing of π and $\pi+\sigma$ plasmon excitations with enhanced surface sensitivity and reduced beam-induced damage compared with conventional high-energy electron techniques. For graphene, Ti_3C_2, and h-BN, the EELS spectra exhibited two plasmon peaks, whereas the spectrum of MoS_2 showed a more complex structure with more than two distinct plasmon features. For graphene, quantitative determination of the inelastic mean free path (IMFP) in the 10–50 eV energy range was achieved.

These results demonstrate SLEEM/ToF as a powerful tool for surface-sensitive analysis of 2D materials. While EELS simulations and low-energy measurements are well established for graphene [2], h-BN [3], and MoS_2 [4], comparable studies for Ti_3C_2 MXene remain limited. The present findings provide valuable experimental data and highlight the need for further theoretical and experimental investigations of plasmon dispersion in MXenes.

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[4] Hong J. et al.: (2021).

Acknowledgement:

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Posters

Quantitative 3D Analysis of Au Nanoparticle Embedding in TiO_2 Inverse Opals via Double-Tilt Electron Tomography

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This work aims to precisely characterise the embedding and strong metal–support interactions (SMSI) of Au nanoparticles (NPs) in TiO_2 raspberry-colloid templated inverse opals (RC-IOs) using double-tilt electron tomography (ET) and high-resolution electron microscopy. Nowadays, decarbonization is one of the most studied fields in science because of the push for global carbon neutrality. An elegant approach to achieving a carbon-neutral industry is the utilisation and valorisation of CO_2 by water-gas shift (RWGS) reaction. A promising photocatalyst for RWGS reaction is TiO_2 with the deposition of noble metals onto the surface, such as Au. [1,6] An approach for synthesising active and stable Au/TiO_2 photocatalysts with controlled and tunable structural properties is the raspberry colloid-templating strategy.[2] This method represents a new approach to prepare supported metal catalysts within a highly ordered three-dimensional photonic structure.[3,4] This structure prevents particle growth under high illumination intensities and elevated temperatures (up to 800 °C)[5] by the partial embedding of the NPs into the support structure. The embedding and resulting catalytic activity can also be tuned through high-temperature reductive pre-treatment, creating a SMSI and overgrowth of the Au NPs with a thin TiO_x layer. SMSI have ussually positively affected the photocatalytic activity.[6]

Transmission electron microscopy offers a unique opportunity to visualise the structure of RC-IOs at the nanoscale. However, it provides only 2D images, which are projections of a 3D structure. As a result, we cannot accurately determine the extent to which Au NPs are embedded. To precisely quantify embedding, the missing spatial dimension must be reconstructed.[7,8,10] Images of RC-IOs catalysts with and without reductive pre-treatment conditions were acquired for evaluation of embedding and SMSI by Talos 200X and Spectra 300 from Thermo Fisher Scientific. Double-tilt electron tomography[14] was acquired in a TEM, where the sample was imaged at a range of tilt angles ±70°, 2° step and by an HAADF detector by Velox.[11] For alignment of tilt-series IMOD software was used. The reconstruction is carried out using i-ART [15] and segmentation in Avizo 3D[7]. The segmented RC-IO models were quantitatively analysed in Avizo 3D and MATLAB, providing information on particle size distribution, morphology, and embedding depth.[5]

A methodology for double-tilt electron tomography was successfully developed, from acquisition to segmentation across different software packages, to achieve maximum reconstruction quality. This methodology enabled the calculation of Au NP embedding into the carrier, which would not have been possible using any other method. The samples showed identical embedding of 84% before and after pre-treatment, but the average diameter was slightly reduced from 18.83 to 17.21 nm. HR-TEM images

enabled the observation of SMSI and the correlation of structural changes in the catalyst with catalytic tests.

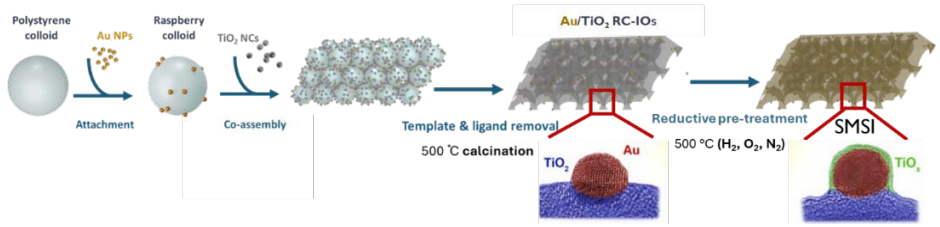

Figure 1: Sample preparation scheme. In the two-step co-assembling of TiO_2 nanocrystals with Au nanoparticles and polystyrene colloid. The third step is polystyrene removal, then reductive pre-treatment, which creates SMSI.[6]

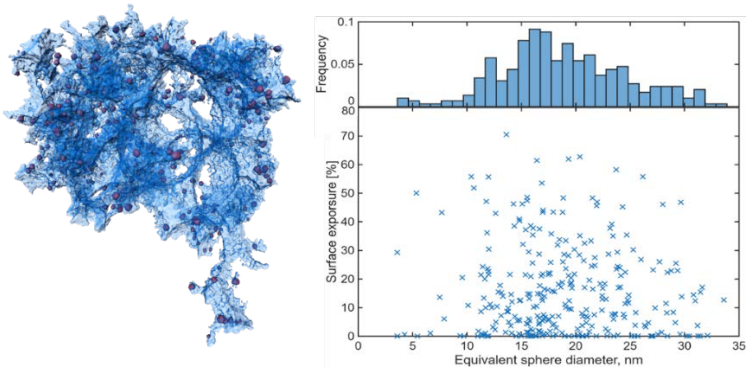

Figure 2: 3D model of RC-IO before pre-treatment. Particle size distribution and embedding of each Au nanoparticle.

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Optimization of embedding resins with improved contrast and higher resistance to electron-beam damage in (S)TEM and 3D-SEM applications

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Electron microscopy (EM) is a fundamental technique for characterization of soft biological materials, including tissues, cells, and organelles, derived from both plants and animals. A critical step in EM analysis is the preparation of samples in a manner that preserves their structural integrity. Embedding specimens in synthetic polymer resins is a widely used approach, providing effective protection and fixation, and is therefore one of the preferred methods for preparing samples for EM. Commercial embedding resins are typically optimized for transmission electron microscopy (TEM), which relies on high-energy transmitted electrons. However, modern volume EM techniques, such as 3D SEM, employ lower electron energies, backscattered electrons, and higher electron doses. Conventional embedding resins are often unsuitable for these methods, due to these resins sensitivity to charging and to damage caused by the electron beam.

To overcome these limitations, we modified commercially available epoxy resins using various chemical stabilizers that mix smoothly and rapidly with the resins. Additionally, we also synthesized siloxane-based resins which are inherently more resistant to electron beam exposure. The siloxane resins were gradually optimized in our laboratory through minor chemical modifications, to enhance their adhesion to biological samples and improve their cuttability in ultramicrotomes, facilitating their routine use in biological laboratories. Although the laboratory-prepared siloxane resins showed promising results, their synthesis remains time-consuming and may yield slight variations of properties between batches. Therefore, we explored commercially available siloxane-based alternatives, that maintain comparable EM performance while being easier to handle. We further improved the mechanical properties of the commercial siloxane-based systems by incorporating additional comonomers.

The present study focuses on the modification and testing of both siloxane and epoxy resins with high resistance to electron beam damage, intended for the embedding of biological samples. These materials were evaluated for volume EM, as well as for other advanced microscopic techniques, including STEM and FIB-SEM. Our results indicate that both types of newly developed resins represent promising embedding media for volume EM and related microscopic applications.

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Structural and morphological changes of TES-ADT thin films after thermal annealing and during aging

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We have studied effects of thermal annealing and ageing on the crystal phase and morphology of organic semiconductor 5,11-bis(triethyl silylethynyl) anthradithiophene (TES-ADT) thin films. The thin films are prepared by spin-coating and subsequently annealed at 135 ˚C to achieve transformation from as-deposited α_2 to thermally induced β crystal phase. We use polarisation optical microscopy to spatially resolve grains of a particular phase. X-ray diffraction is employed to identify dominating crystal phases in the samples. From a relatively smooth as-deposited film, during annealing the film undergoes partial dewetting and the β phase forms needle-like structures with widths of tens of micrometres and heights of units of micrometres. After the annealing step and sample quenching to room temperature, we observe the disappearance of the beta phase on a time scale of tens of days.

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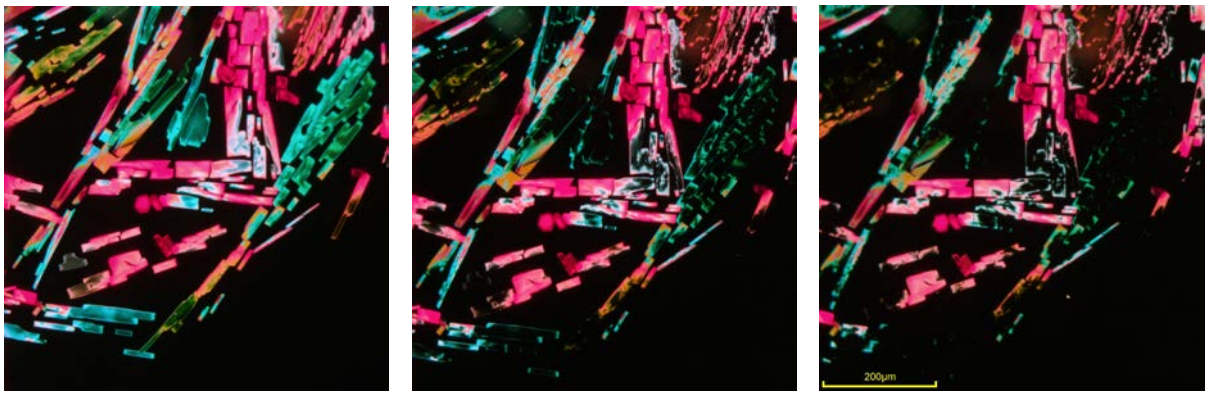

Fig. 1: Cross-polarization microscopy images of the TES-ADT film in the β phase, recorded on day 1, 5 and 15 after the annealing. The scale bar is 200 µm.

The Influence of Solution-Annealing Parameters on the Microstructure of Additively Manufactured IN939

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Introduction

Nickel-based superalloys are the primary material for components required to withstand high-temperature exposure and loading, owing to their exceptional resistance to oxidation, creep, and their remarkable mechanical strength. These alloys are widely used in turbine hot sections, nuclear reactors, and aerospace applications [1,2]. A typical degradation mechanism in such components is low cycle or thermomechanical fatigue (TMF), driven by large plastic deformation resulting from high stresses and thermal gradients. Traditional manufacturing routes such as casting or forging are increasingly being replaced by additive manufacturing (AM) techniques [3–5]. Although AM still faces several challenges, optimizing laser powder bed fusion (L-PBF) parameters has enabled the production of defect-free IN939 bulk material with a fine specific microstructure, promising improved strength and fatigue performance. Nevertheless, the influence of L-PBF processing on damage mechanisms during low-cycle fatigue and TMF has not been adequately addressed. The IN939 alloy typically undergoes a three-step heat treatment comprising solution annealing and two precipitation-hardening steps. This contribution examines the influence of solution-annealing parameters on the tensile properties of additively manufactured IN939.

Experiment

The nickel-based superalloy IN939 prepared by laser powder bed fusion was studied. The as-built microstructure consisted of elongated grains approximately 14 µm in size perpendicular to the build direction and 50–100 µm along the build direction, exhibiting a pronounced crystallographic texture along the build direction, as shown in Fig. 1 (left). Dog-bone tensile specimens were machined from the as-built material according to the drawing shown in Fig. 1 (right). Subsequently, the specimens were subjected to solution annealing heat treatments at 1175 °C, 1200 °C, and 1225 °C for 2 hours. The microstructure of the as-built condition and all heat-treated specimens was characterized by scanning electron microscopy (SEM). Additionally, electron backscatter diffraction (EBSD) analyses were performed, and the kernel average misorientation (KAM) and geometrically necessary dislocation (GND) density parameters were evaluated. Tensile tests were performed at 800 °C at a constant strain rate until fracture. From the tensile tests, the ultimate tensile strength, total elongation at fracture, and yield strength were determined.

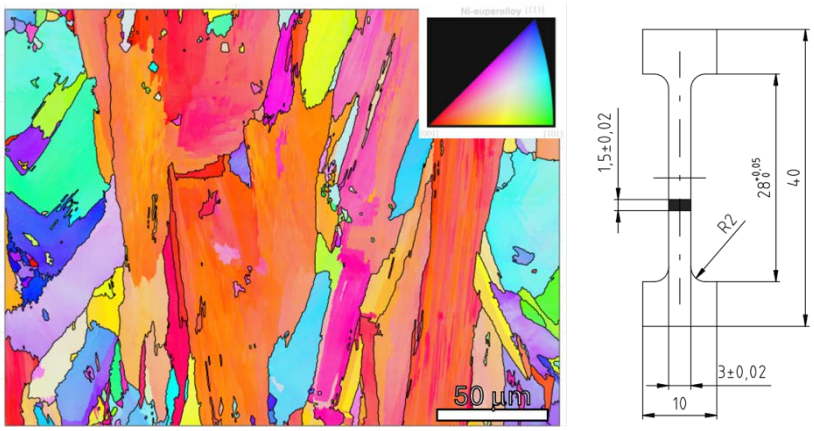

Fig. 1: EBSD map along the build direction (left); drawing of the dog-bone tensile specimen (right).

Results

The upper part of Fig. 2 shows the tensile stress–strain curves of the specimens after each solution-annealing treatment. It is evident that both the ultimate tensile strength and yield strength decrease with

increasing annealing temperature. The elongation at fracture for the highest annealing temperature (1225 °C) is significantly lower compared to the other conditions. These changes may be attributed to the evolution of the specific as-built microstructure during solution annealing. To address these changes, a detailed EBSD analysis was performed for all treated states. KAM and GND maps were constructed for each condition; an example for the highest annealing temperature is shown in the lower part of Fig. 2. Both the mean values and the scatter of KAM and GND were evaluated from these maps. The GND values were in the order of 10^{14} m^{-2}, which is typical for the specific AM microstructure. Compared to the as-built condition, the mean KAM and GND values decreased after the two lower annealing temperatures, whereas for the highest temperature (1225 °C), these values remained nearly unchanged.

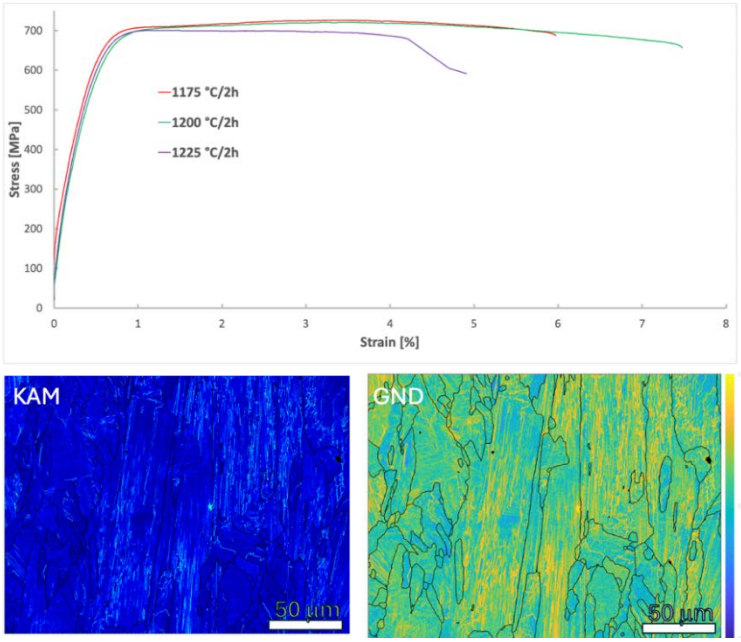

Fig. 2: Tensile test results for all heat treatment conditions (top); KAM and GND maps of the specimen after solution annealing at 1225 °C for 2 hours (bottom).

Conclusion

Based on the experiments, the following conclusions can be drawn:

- The as-built material exhibited a typical AM microstructure, with grains approximately 14 µm in size perpendicular to the build direction and elongated grains along the build direction.
- The tensile test results revealed a clear dependence of the mechanical properties on the solution-annealing temperature.
- KAM and GND analysis revealed that the dislocation density decreased after annealing at 1175 °C and 1200 °C, while it remained comparable to the as-built state after annealing at 1225 °C, suggesting that the retained dislocation substructure contributes to the observed reduction in ductility.

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Ultra-clean Graphene for Advanced Electron Microscopy

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Our research is focused on the preparation of high-quality and ultra-clean graphene for advanced applications in electron microscopy. The developed material is intended particularly for TEM grids, free-standing membranes, protective layers, and detector systems, where the combination of atomic thickness, high electrical conductivity, mechanical stability, and near-ideal electron transparency is essential. The main objective is the development and optimization of an ultra-clean exfoliation process enabling the production of graphene with minimal contamination and high structural homogeneity, which is crucial for demanding applications in both transmission and scanning electron microscopy.

The prepared graphene layers are systematically characterized using advanced microscopic and spectroscopic techniques, namely SLEEM, Raman spectroscopy, scanning transmission electron microscopy (STEM), and time-of-flight (ToF) spectroscopy.[1,2,3] Together, these methods provide comprehensive information on structural quality, defect density, purity, electronic properties, and the interaction of low-energy electrons with the material. This multimodal approach enables detailed correlation between the fabrication process and the resulting graphene quality at the atomic scale.

The experimental results demonstrate that the developed process significantly reduces contamination and improves both electron transparency and mechanical stability of graphene layers. These improvements substantially broaden the potential of graphene for advanced electron microscopy applications. As a result, graphene represents a versatile platform material for imaging of beam-sensitive specimens, contrast enhancement, protection against radiation damage, and improved performance of imaging and detection systems.

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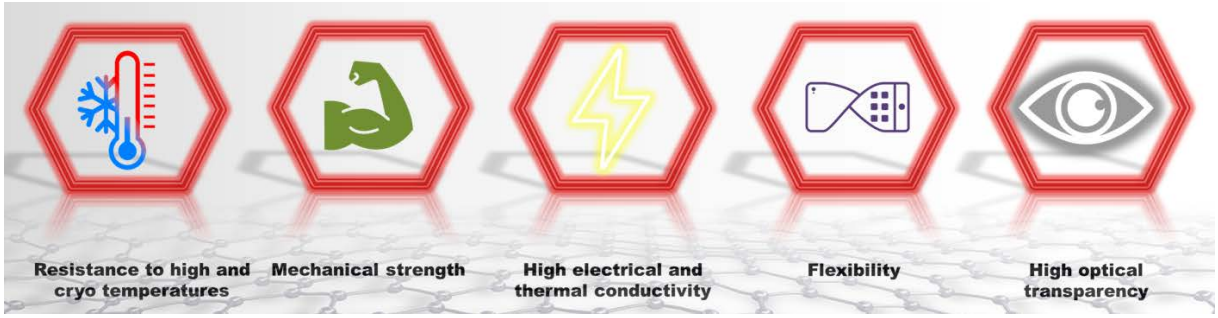

Fig. 1: Key properties of graphene: Atomic thickness, high mechanical strength, excellent electrical and thermal conductivity, chemical stability, flexibility, and high electron transparency

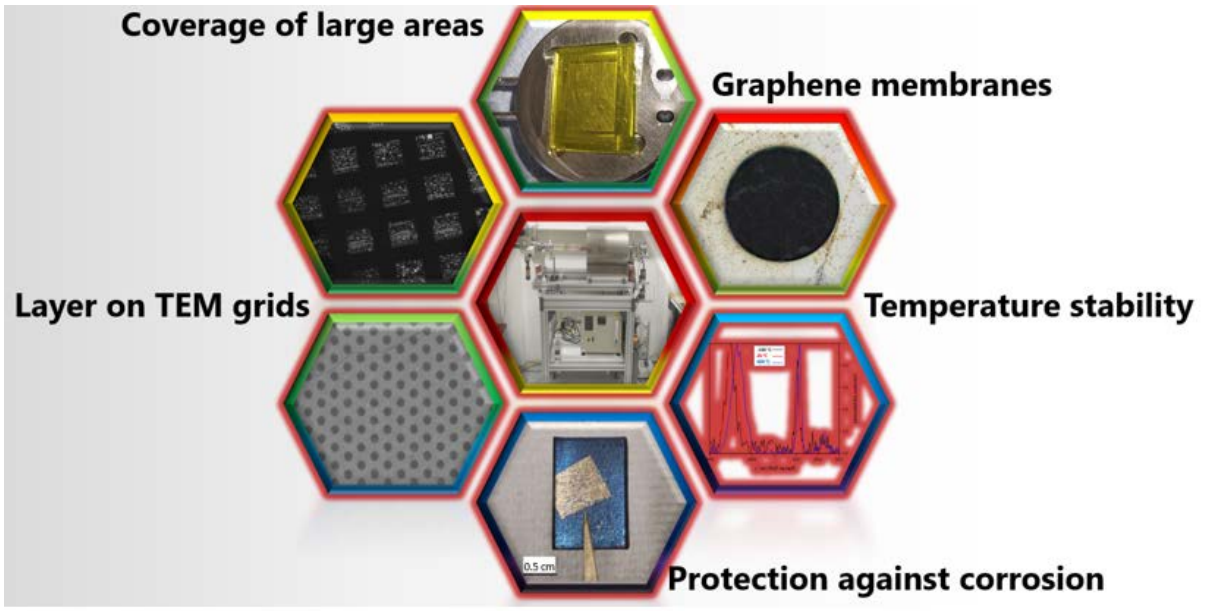

Fig. 2: Graphene applications in advanced electron microscopy

Creep–fatigue interaction under thermomechanical loading in additively manufactured Inconel 939

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Nickel-based superalloys produced by laser powder bed fusion (PBF-LB/M) are increasingly considered for high-temperature turbine applications, where components are subjected to complex thermomechanical loading histories involving simultaneous temperature and mechanical strain cycling. Among the governing damage mechanisms, the interaction between fatigue and creep — particularly under dwell-period loading — represents a critical life-limiting factor that remains insufficiently understood for additively manufactured microstructures. The anisotropic grain morphology and texture inherent to PBF-LB/M processing introduce additional complexity, as building direction is known to influence mechanical response. Our recent work established the thermomechanical fatigue (TMF) behaviour of PBF-LB/M IN939 under in-phase (IP) and out-of-phase (OP) loading for both vertically and horizontally built specimens [1], and characterised the isothermal low-cycle fatigue (ILCF) and TMF response of vertically built IN939 in terms of deformation mechanisms and damage evolution [2]. The present work extends these investigations to include the effect of dwell periods on TMF cycling, with the aim of quantifying creep–fatigue interaction and its damage mechanisms across both building orientations.

Cylindrical specimens of IN939 were fabricated by PBF-LB/M in two building orientations — vertical (V, parallel to the build direction) and horizontal (H, perpendicular to the build direction) — using the same processing parameters and three-step heat treatment (solution annealing at 1175°C/45 min, followed by two-step precipitation hardening at 1000°C/6 h and 800°C/4 h) as described in [1,2]. This ensured the precipitation of the γ′ strengthening phase, which ultimately acts as an obstruction in dislocation movement. Specimens were subjected to strain-controlled TMF cycling in the temperature range of 400–800°C under both IP and OP phase configurations, with an introduction of a dwell period of 600 s at either maximum or minimum applied strain. Reference datasets without dwell (LCF, TMF-IP, TMF-OP) from [1,2] provide the baseline for direct comparison. Post-mortem characterisation was built around a correlated, multi-scale electron microscopy strategy. Scanning electron microscopy (SEM) was employed to assess fracture surface morphology, crack initiation sites, and macroscopic propagation paths. Electron backscatter diffraction (EBSD), performed on longitudinal sections of the gauge length, provided crystallographic mapping of grain-level fracture behaviour, local misorientation accumulation, and texture-dependent crack deflection mechanisms in the vicinity of fatigue cracks. Transmission electron microscopy (TEM), operating in bright-field and high-angle annular dark-field scanning transmission (HAADF-STEM) modes, enabled direct nanoscale observation of dislocation substructures — including stacking fault formation, persistent slip band development, zig-zag dislocation networks characteristic of creep, and precipitate–dislocation interactions within the γ/γ′ microstructure — resolving the elementary deformation mechanisms activated under dwell-period thermomechanical loading.

Lifetime curves across all loading conditions reveal a consistent anisotropy, confirming the life advantage of vertically built specimens over horizontally built counterparts established in [1]. The introduction of 600 s dwell periods resulted in a pronounced reduction in fatigue lifetime relative to standard TMF cycling at equivalent strain amplitudes, with the lifetime reduction evident across both IP and OP configurations. The lifetime data (Fig. 1a) demonstrate that dwell-induced creep damage and oxidation substantially accelerate failure, particularly at intermediate strain amplitudes where time-dependent mechanisms compete most strongly with cyclic fatigue damage. SEM-EBSD mapping revealed that dwell periods intensified intergranular (Fig. 1b) damage in IP loading, with grain boundary

crack networks more extensive than those observed under equivalent no-dwell conditions, while SEM fractography captured the transition in surface crack morphology between dwell and no-dwell conditions. TEM analysis directly linked the lifetime reduction to specific changes in rich dislocation substructure: dwell periods promoted denser dislocation networks (Fig. 1c) and stacking faults within γ′ precipitates and persistent slip bands (Fig. 1d).

Building direction and dwell period duration are both critical parameters governing the thermomechanical fatigue performance of PBF-LB/M IN939. The correlated SEM–TEM microstructural characterisation proved essential in bridging the gap between macroscopic lifetime data and the elementary damage mechanisms responsible for dwell-induced damage accumulation, revealing grain-boundary-driven fracture and creep-specific dislocation configurations as the key life-limiting features.

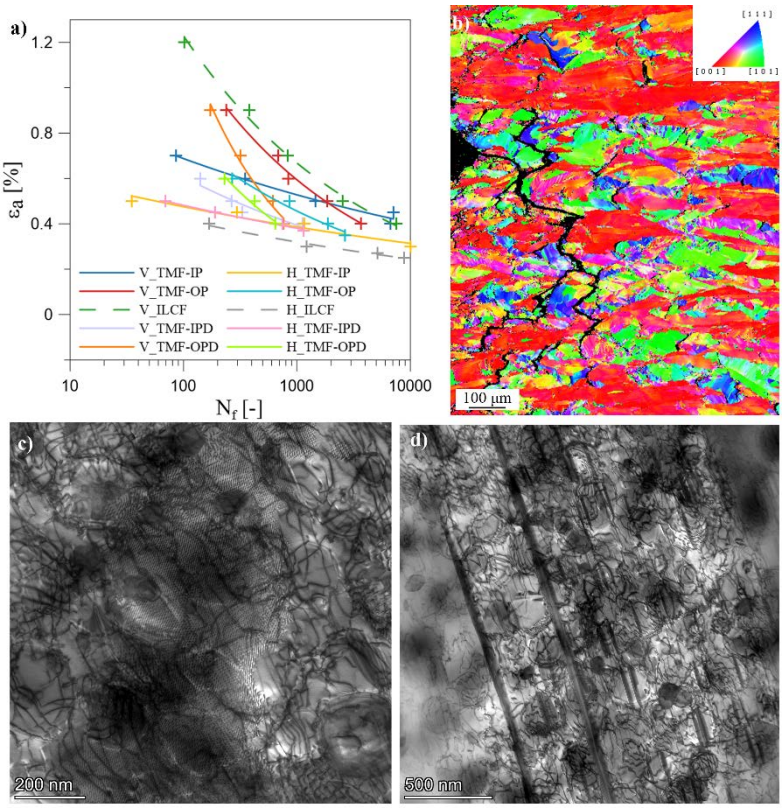

Fig. 1: a) lifetime courves of PBF-LB/M IN939 subjected to TMF, TMF-dwell and ILCF fatigue, b) representative SEM-EBSD micrograph of intergranilar crack propagation in horizontally build sample after TMF-dwell fatigue, c) representative STEM–BF micrograph of rich dislocation microstructure, d) representative STEM–BF micrograph of persistent slip bands and stacking faults within γ′ precipitates

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Influence of Surface Preparation on Secondary Electron Dopant Contrast in SiC Investigated by SEM

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Silicon Carbide (SiC) is a crucial material for high-power-density applications, where precise doping is a fundamental factor influencing device functionality. Consequently, the demand for high-resolution, non-destructive dopant characterization has increased significantly. Secondary Electron Dopant Contrast (SEDC) has emerged as a promising technique for two-dimensional profiling; however, its efficiency is often compromised by surface artifacts and mechanically induced damage.

In this work, we examine the impact of specimen surface preparation on the visualization of dopant concentrations in SiC using Scanning Electron Microscopy (SEM). We provide a systematic comparison between conventional mechanical cleaving/polishing and Focused Ion Beam (FIB) milling of SiC epitaxial layers. While traditional mechanical preparation introduces subsurface strain, defects, and partial amorphization, which degrade the SEDC signal, FIB milling also induces ion-related damage. We demonstrate that an optimized protocol involving low-energy ion cleaning effectively reduces the damaged layer.

When combined with Scanning Low-Energy Electron Microscopy (SLEEM) and high-pass energy filtering, this approach substantially enhances contrast sensitivity and the signal-to-noise ratio at the sample interface. Our analysis reveals a direct correlation between the thickness of the amorphous surface layer and the accuracy of dopant signal detection. By mitigating surface-state effects, the integration of optimized FIB cross-sectioning with low-voltage electron detection enables SEM to transition toward semi-quantitative dopant profiling. This refined workflow establishes a robust framework for failure analysis and the advancement of next-generation wide-bandgap power electronic devices.

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Influence of Glycerol Content on the Morphology and Properties of Thermoplastic Starch

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The globally increasing pollution due to highly durable and hardly degradable plastics has led to growing interest in the biodegradable biopolymers. Starch is one of the most widely studied biopolymers, mainly due to its availability, low cost, perfect biocompatibility, and complete biodegradability. It is employed in the food industry as a packaging material, in agriculture for mulch films and seed coatings, in pharmaceutics, cosmetics industries, and many other fields [1]. Native starch cannot be processed by standard protocols due to its high molecular weight combined with strong hydrogen bonds. However, it is possible to convert the native starch to thermoplasticized starch (TPS) using low molecular weight plasticizers such as glycerol, citric acid or modern eutectic liquids. TPS is usually prepared by two preparation procedures – solution casting (SC) or melt-mixing (MM). Glycerol has long been used as a plasticizer and remains one of the most commonly employed plasticizers today. The amount of glycerol most commonly used for plasticization appears to be in the range of 30–40 wt.%.

However, there is no standardized preparation procedure and there are any comprehensive studies examining the effect of glycerol quantity on the resulting morphology and properties of TPS.

TPS samples were prepared by three preparation procedures: (i) solution casting (SC), (ii) melt-mixing (MM) and (iii) their combination (SC+MM). During the SC preparation, the starch is mixed at an elevated temperature with a plasticizer (water + glycerol) and, once a gelation is complete, TPS is casted onto the foil and left to dry at ambient conditions. During the MM preparation, the starch is mixed directly with glycerol in the chamber of a twin-screw laboratory mixer (Brabender Plasti-Corder; Germany) and compression molded. Our preparation procedure combines the SC and MM to achieve highly homogenous samples [2]. The TPS samples were prepared by means each of the three preparation protocols (SC, MM, and SC+MM), with the glycerol concentration ranged from 20 to 50 wt.% with step 5 wt.%, which makes as much as 21 samples (3 preparations × 7 glycerol concentrations = 21). The homogeneity of all obtained TPS's was characterized by polarized light microscopy (PLM), scanning electron microscopy (SEM), and wide-angle X-ray scattering (WAXS). The influence of starch homogeneity on the macro- and micromechanical properties was studied by dynamic mechanical analysis (DMA) and instrumented microindentation hardness testing (MHI).

The amount of glycerol affected both the TPS morphology and the resulting mechanical properties. Figure 1 shows the SEM-SE fracture surfaces of all samples: upper row – SC, middle row – MM and bottom row SC+MM samples.

The low-glycerol-content TPS's (SC, MM and SC+MM samples with 20 and 25 wt. % glycerol) were homogeneous, with a very small amount of non-plasticized granules. A low glycerol content as a lubricant caused significant friction, which results in the effective homogenization of the starch granules. The SC preparation procedure was a slightly less efficient method of plasticization than the other two methods. However, regardless of the preparation method chosen, the low-glycerol content samples were hard and brittle, which would significantly limit their practical applications.

The high-glycerol-content TPS's (SC, MM, and SC+MM samples with 40, 45, and 50 wt.% of glycerol) were very soft, with a rubbery-like behavior. However, samples exhibited a high degree of inhomogeneities (non-plasticized granules). This could be attributed to the fact the high glycerol content resulted in rather thin molten mixture during the MM step. As a result, the friction forces acting on the starch granules were too low to cause their complete destruction and homogenization.

The medium-glycerol-content TPS's (SC, MM, and SC+MM samples with 30 and 35 wt.% of glycerol) showed the best results for all three preparation methods. The amount of non-plasticized granules was minimized in comparison with both the low- and high-glycerol TPS. The mechanical properties were balanced: the samples were relatively hard, but not brittle. SC+MM protocol yielded the most homogenous samples. Melt-mixing provided the samples with the similar micromechanical properties, but the amount non-fully plasticized granules was somewhat higher as demonstrated Fig. 1.

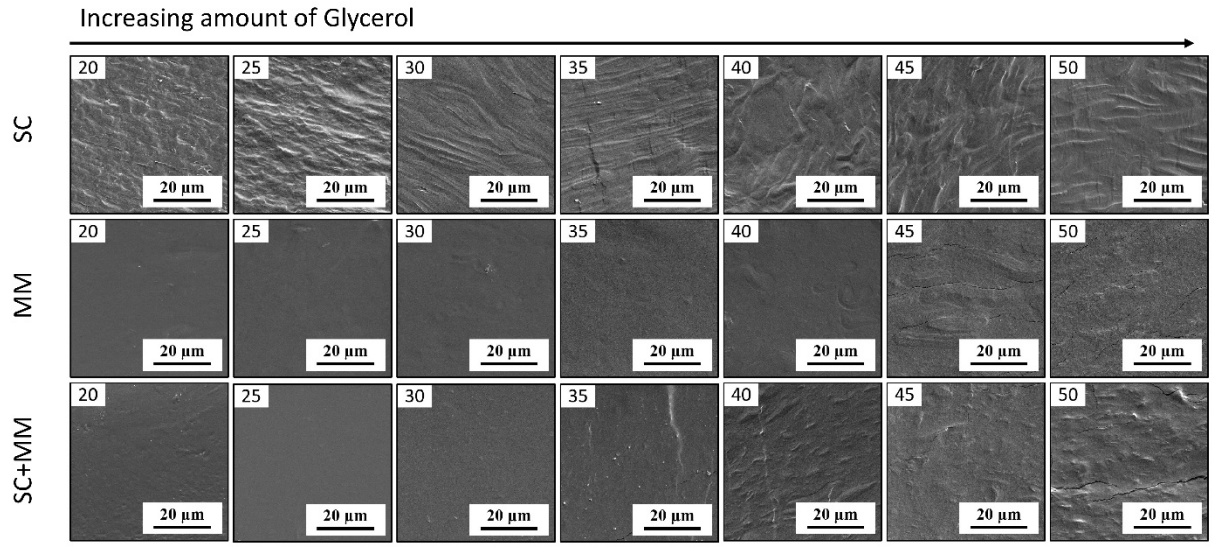

Figure 1: *SEM-SE fracture surfaces of TPS/Gly samples prepared by different preparation procedures: solution casting (SC) – upper row, melt-mixing (MM) – middle row and solution casting combined by melt-mixing (SC+MM) – bottom row. The amount of glycerol increases from left to right from 20% to 50%.*

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High Sensitivity Automated Spectroscopy Station

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We present a newly developed automated station for Raman spectroscopy designed for integration into measurement and analytical workflows. The system is equipped with two excitation lasers (785 nm and 532 nm), a high-sensitivity spectrometer coupled with a low-noise detection camera, and a preview imaging camera with integrated illumination for precise sample positioning.

The optical configuration includes an automated filter cube exchanger with dedicated excitation and emission filters for each laser, as well as a motorized objective turret enabling flexible measurement conditions. The entire system is fully automated and can operate either in manual mode via side access doors or in a fully automated mode using a robotic manipulator with a gripper. The robot is capable of loading samples through a motorized drawer system.

The station supports measurements of samples prepared on specialized dishes or standard microscope slides. It also features an integrated decapping mechanism for sample containers, further enhancing automation capabilities. While primarily designed for biological samples, the system is also suitable for general solid and liquid sample analysis.

To reduce fluorescence background, photobleaching techniques are implemented prior to spectral acquisition. All components, including electrical infrastructure, are integrated into a compact and mobile unit. The station also incorporates multiple safety features to ensure safe operation and user protection.

Control of the system is provided by an embedded computer for manual operation, while in automated mode the station is connected to a central control system.

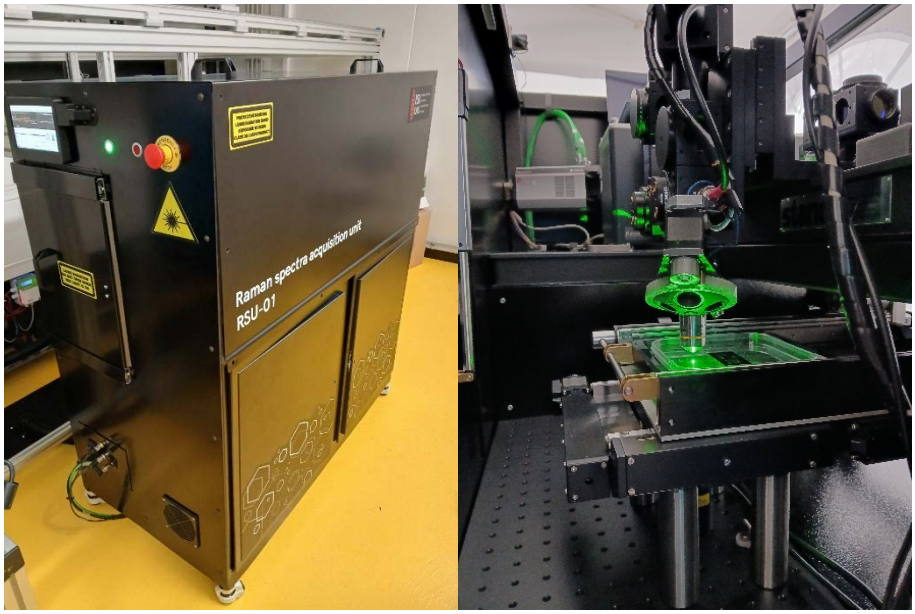

Fig. 1: High Sensitivity Automated Spectroscopy Station

Acknowledgement:

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Ultrafast SEM with electron beam shaping

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To the best of the current knowledge, no scanning electron microscope (SEM) has been developed based on ultrafast electron pulses that can be further modified using structured light. This approach is particularly well-suited for the domain of time-resolved (ultrafast) electron microscopy [1] and has proven to be instrumental in the development of novel methods for highly sensitive imaging, a feat that has historically been challenging to achieve using conventional approaches. The capacity to manipulate the spatial phase profile of an electron beam with light [2] has enabled the fabrication of convex and concave electron lenses with focal lengths ranging from several millimeters, facilitating the correction of aberrations [3,4].

The objective of this project is to modify a commercial scanning electron microscope into a system that will facilitate the generation of electron pulses, the rectification of electron beam aberrations, and the external stimulation of the specimen. The proposed methodology entails the utilization of 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 depicted in Fig. 1. The purpose of optical branch 1 is to generate electron pulses based on laser injection at the Schottky electron source, which then propagate through the microscope. The optical branch number 2 modulates the optical pulse, which subsequently interacts with the electron pulse and transforms it into the desired form. Finally, optical branch 3 is used to stimulate the sample, enabling the investigation of dynamic processes occurring within it. In this conference contribution, we will present the target setup and preliminary experimental results.

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Acknowledgement:

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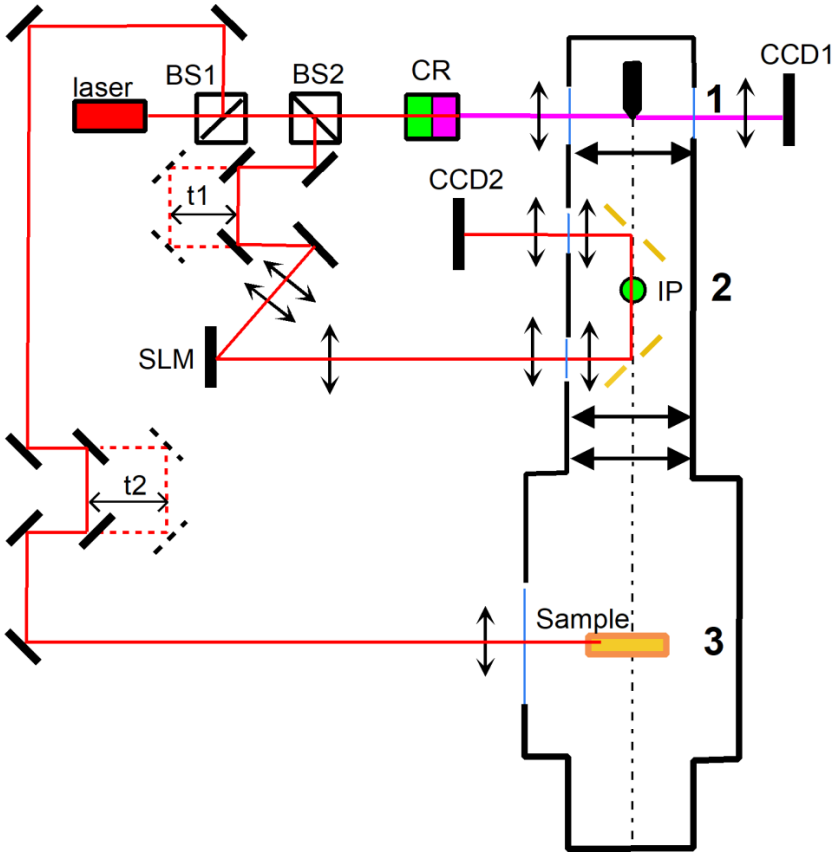

Fig. 1: Schematic of the setup for time-resolved ultrafast electron microscopy, electron beam modulation by laser and external sample stimulation.

High-power Raman microspectroscopy for the analysis of enzymatic degradation of an anthropogenic pollutant

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Raman microspectroscopy is a powerful analytical tool offering chemical analysis with submicron spatial resolution, rapid data acquisition, and minimal sample preparation. However, it is often limited by low sensitivity, particularly for certain analytes present at low concentrations. To overcome this, we present a novel approach – the use of a high-power laser (6 W) in our custom-built Raman microspectroscope to enhance the Raman signal.

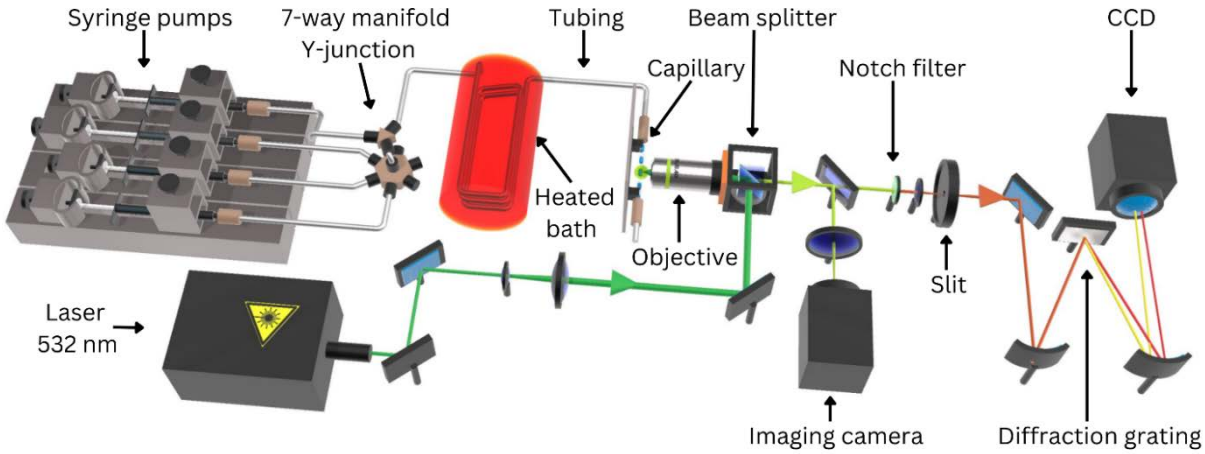

Fig. 1: scheme of the in-house built high-power Raman microspectroscope, including the microfluidic setup

This strategy is unconventional within the field, which typically relies on surface-enhanced Raman spectroscopy (SERS) to improve detection limits [1]. SERS, however, introduces variability due to the need for metallic nanoparticles or nanostructured surfaces, and does not offer consistent enhancement across different molecules, making it often unsuitable for complex samples.

Our study applies high-power Raman microspectroscopy to the analysis of enzymatic degradation of the environmental pollutant 1,2,3-trichloropropane (TCP), a persistent organic contaminant and potential carcinogen. The enzymatic degradation of TCP into the harmless product glycerol is facilitated by a cascade of three enzymes [2]. The method is implemented in a microfluidic droplet environment, enabling precise control of reaction conditions but requiring rapid and sensitive detection. With the utilization of high-power Raman, we tested different enzyme ratios to establish the efficiency of the reaction and to find the optimal enzyme ratio for maximum glycerol production. The sensitivity of the method far exceeded conventional Raman, reaching submillimolar detection in highly dynamic and complex environment.

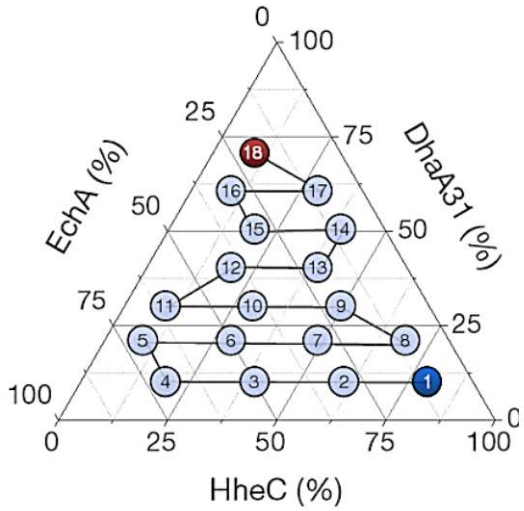

Fig. 2: sequence of the tested enzyme ratios

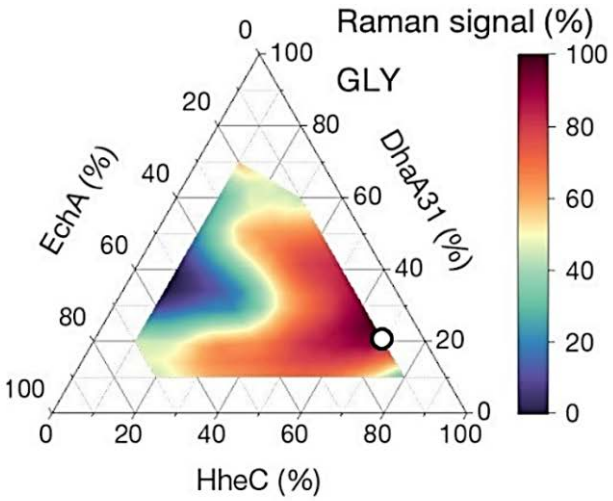

Fig. 3: relative Raman signal of the produced glycerol (GLY) depending on the enzyme ratios

The use of high-power Raman microspectroscopy significantly enhances detection sensitivity, enabling reliable monitoring of complex enzymatic reactions in microfluidic environments without the limitations of SERS. This approach provides a robust and consistent alternative for analyzing low-concentration analytes, with potential for broader applications in chemical and environmental analysis.

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Acknowledgement:

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Cryo-workflows at the IMG Electron Microscopy Core Facility

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Continual advancements in cryo-electron microscopy (cryo-EM) have established it as the method of choice for reliable analysis of biological samples in their close-to-native state. Our facility consistently updates cryo-workflows to provide professional solutions for current scientific demands.

Our 200 kV Jeol JEM-F200 transmission electron microscope, equipped with a cryo polepiece, cold field emission gun, Volta phase plate, Gatan Alpine direct electron detector and a 4k CMOS camera (TVIPS XF-416), is optimized for diverse cryoTEM applications. These include observing dehydration-sensitive small objects (e.g., small organisms, DNA origami), quality checks of purified protein samples before SPA, collecting diffraction patterns from frozen protein crystals, and cryo-electron tomography of subcellular structures.

The core facility now offers complete cryo-TEM tomography workflow for frozen hydrated lamellae, developed through a collaboration with TESCAN Company. This optimized on-grid workflow involves plunge freezing (Leica EM GP2), quality checks (Leica THUNDER cryo-CLEM), sample mounting and transfer to TESCAN Amber Cryo FIB-SEM for lamella fabrication, and final transfer to cryoTEM for tilt series acquisition. Larger samples vitrified via high-pressure freezing (Leica EM ICE HPF with light-stimulation) can also be imaged in a frozen hydrated state after cryo FIB lift-out, or processed into resin blocks following freeze-substitution (Leica EM AFS2). This workflow has successfully enabled observations and volume reconstructions of subcellular structures in *C. elegans*, *S. cerevisiae*, *Chlamydomonas*, *Chlorella* and HeLa cells.

Additionally, our facility provides other specialized vitrification-based workflows: freeze-fracture replica immunolabeling (Leica EM ACE900), cryo-CLEM imaging with reliable image correlation, and cryo-sectioning followed by immunolabeling after Tokuyasu.

As part of Czech-BioImaging and Euro-BioImaging, the EM Core Facility offers open access to all described technologies with professional support throughout user projects.

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Microfluidic chip for high-pressure freezing of biological samples: Progress towards cryo-FIB-SEM and Raman micro-spectroscopy

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Introduction

Preservation of biological samples close to their native state is essential for reliable ultrastructural analysis. High-pressure freezing (HPF) is a well-established method enabling vitrification of hydrated specimens while minimizing ice crystal formation. However, conventional workflows often lack precise control over sample positioning and handling prior to freezing.

Microfluidic technologies offer a promising approach to overcome these limitations by enabling controlled manipulation of small sample volumes and reproducible positioning of biological material. Their optical accessibility and compatibility with live-cell handling make them suitable for integration with advanced analytical techniques and cryo-fixation workflows.

In this work, we present a microfluidic chip designed as a carrier for HPF, aiming to improve sample handling and establish a reproducible workflow for subsequent electron microscopy (EM) analysis. The platform is designed to be compatible with Raman micro-spectroscopy, enabling non-destructive monitoring of cells prior to freezing. In addition, the concept allows future integration of Raman-based optical trapping (Raman tweezers), which would enable targeted selection and positioning of individual cells before vitrification [1].

Technical Realization

The platform is based on a polydimethylsiloxane (PDMS) microfluidic chip fabricated using standard soft lithography [2]. It consists of microchannels connected to a central circular chamber designed for stable sample positioning and controlled fluid handling, while remaining compatible with high-pressure freezing (HPF) requirements.

To improve thermal performance, the chip is combined with copper components. During preparation, it is enclosed between a flat copper plate and a copper mold surrounding the circular chamber, defining the final sample geometry. The assembled system is then inserted into standard HPF carriers (e.g., Leica EM ICE, HPM100).

An integrated trimming mechanism within the HPF half-cylinders removes excess microfluidic structures during loading, leaving only the compact sample chamber enclosed by copper parts. This configuration ensures suitable thickness and efficient heat transfer for rapid vitrification.

The complete workflow includes chip fabrication, sample loading, assembly into HPF carriers, freezing, freeze substitution, resin embedding, ultramicrotomy, and EM analysis.

Results and Discussion

Initial experiments using *Azotobacter vinelandii* demonstrate that the developed microfluidic platform is compatible with HPF and subsequent EM processing. Variability in preservation quality was observed across samples. In some cases, structural artifacts such as partial membrane disruption or cytoplasmic inhomogeneity were present. These effects are likely related to limitations in heat transfer efficiency and variations in effective sample thickness during the freezing process.

The results suggest that while the concept of microfluidic-assisted HPF is valid, further optimization is required. Key parameters under investigation include chip geometry, thickness of PDMS layers, and the

design and placement of cooper heat-transfer elements. Improving these aspects is expected to increase the reliability and reproducibility of vitrification.

Conclusion

We have developed a microfluidic platform designed for use in HPF workflows and subsequent EM analysis. The system enables controlled sample manipulation prior to vitrification and is compatible with standard HPF instrumentation.

The obtained results confirm the feasibility of this approach, although further optimization is necessary to achieve consistent ultrastructural preservation. Future work will focus on improving thermal performance and adapting the design for more robust and reproducible operation.

Overall, the presented approach represents a promising step toward more controlled and versatile sample preparation strategies in electron microscopy.

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IMG Electron Microscopy Core Facility

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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 a FIB-SEM instrument, which, among other applications, is being used for the development of an original cryo-lamella lift-out workflow.

Standard and advanced techinques 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.

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Development of functionalized modular nanoparticles for multimodal bioimaging and photodynamic therapy of breast cancer

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Advances in microscopy and spectroscopic imaging have accelerated the development of multifunctional nanomaterials for cancer diagnostics and therapy. In this work, we introduce modular metal–organic framework (MOF)–based nano and microparticles engineered for multimodal microscopic imaging, targeted delivery, and photodynamic therapy (PDT) in breast cancer models. MIL-101(Al) microparticles [1] and UiO-66(Zr) nanoparticles [2] were synthesized and structurally optimized to achieve high porosity, enhanced molecular loading, and improved colloidal stability. Using fluorescence microscopy, Raman spectroscopy, and optical coherence tomography, we monitored nanoparticle uptake, intracellular localization, and the resulting morpho molecular responses in 2D and 3D cancer cell systems.

Photosensitizers introduced into the MOF pores enabled fluorescence guided photodiagnostics and efficient formation of reactive oxygen species upon light activation. Surface functionalization with folic acid, histidine, proteins, and cancer targeting ligands significantly enhanced cell specific accumulation, resulting in spatially localized photodynamic effects observable with high resolution microscopy. For therapeutic enhancement, methylene blue (MB) and 5 fluorouracil were co loaded into UiO-66(Zr) nanoparticles. Microscopy based live cell analysis revealed improved intracellular MB retention after surface modification and demonstrated synergistic PDT–chemotherapy cytotoxicity.

Finally, the nanoplatform was validated in the quail chorioallantoic membrane model [1-3], enabling 3D microscopy of biodistribution, controlled drug release, and treatment induced tissue responses in a vascularized, tumor like environment. Overall, the developed MOF based constructs represent a robust multimodal system integrating targeted delivery, microscopic visualization, and dual modality treatment, demonstrating strong potential for future translational applications in breast cancer diagnostics and therapy.

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Volume Electron Microscopy Methods for Investigating Nitrogen Handling and Cellular Vision in Microscopic Algae

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Recent advancements in volume electron microscopy (vEM) have enabled biologists to visualize well-preserved organic structures with exceptional resolution, making these methods increasingly vital for studying complex biological systems. Among the various techniques—such as focused ion beam and serial block-face scanning electron microscopy (FIB-SEM and SBF-SEM), array tomography, and electron tomography—each offers distinct advantages, though some have limitations. Combining these methods based on the specimen's specific characteristics is often essential for obtaining the most informative results.

In this study, we investigated *Effrenium voratum* (Symbiodiniaceae, Dinoflagellata, Alveolata, SAR), a unicellular marine eukaryote related to symbionts of corals, anemones, and clams. We focused on a challenging biogenic crystalline material localized in two compartments: vacuoles with metabolic functions [1,2] and the eyespot, which plays a key role in cellular vision [3,4]. In conventional TEM sections, crystals often drop out due to their brittle mechanical properties, making vEM techniques crucial for preserving intact crystal structures.

To explore the structural and optical properties of the eyespot, we employed serial section electron tomography and FIB-SEM. While electron tomography offers excellent resolution in the nm to sub-nm range, its limited section depth (200–300 nm per slice) restricts the volume that can be imaged. FIB-SEM was used to overcome this constraint, enabling whole-cell scanning at a resolution of 3×4×20 nm.

Our results provide new insights into the arrangement of guanine crystals within the cell, allowing us to determine the eyespot’s size, location, and orientation, along with initial findings on its formation process. The eyespot in *E. voratum* consists of multiple crystalline layers of guanine with a high refractive index (1.83), separated by cytoplasmic layers with a lower refractive index (1.34)—each approximately 70 nm thick. Our detailed 3D reconstruction of the eyespot arrangement was used to predict its reflectance spectra via finite-difference time-domain optical simulations, establishing a functional link to the eyespot's role in microbial phototaxis—the ability to navigate marine environments.

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Ruthenium Red for Enhanced Visualization of Alginate Hydrogels Encapsulating *Azotobacter vinelandii* Cells

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Sustainable agriculture is increasingly seeking strategies that reduce dependence on inorganic fertilizers while maintaining plant productivity. Beneficial soil microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), can support plant development through mechanisms related to nutrient availability, stress tolerance, and other factors [1]. Among them, *Azotobacter vinelandii* is notable for its ability to synthesize phytohormones and extracellular alginate. This polysaccharide contributes to the stabilization of the rhizosphere environment and improves water retention around plant roots, which can help plants better tolerate environmental stress [2].

In this study, bacterially produced alginate was used to form a hydrogel matrix that encapsulated the cells. The encapsulation was achieved by the addition of $CaCl_2$, inducing cross-linking of alginate chains through Ca^{2+} ions. Due to the polysaccharide-rich nature of the resulting material, visualization of its structural properties using electron microscopy is challenging because commonly used staining agents have limited affinity for these components. To enhance the contrast of polysaccharide structures, ruthenium red was applied as a selective agent with high affinity for acidic polysaccharides and glycoconjugates, where it can also enhance osmium tetroxide contrast [4,5]. As ruthenium red is poorly soluble in common freeze substitution solvents, such as acetone, methanol, or ethanol, it was added directly to the alginate cross-linking solution prior to high-pressure freezing to ensure its presence during cryofixation.

After high-pressure freezing, samples were freeze-substituted in either acetone or methanol, embedded in epoxy resin, and ultrathin sections were prepared. Additional contrast was mostly provided by lead citrate. The samples were analysed using a scanning electron microscope (Helios G4 HP, Thermo Fisher Scientific) equipped with a STEM3+ detector. Imaging was performed in STEM bright field mode at an acceleration voltage of 20 kV and probe current of 13 pA.

Ruthenium red significantly improved visualization of polysaccharide-rich structures. Both the capsule surrounding *A. vinelandii* cysts and the surrounding alginate hydrogel network exhibited enhanced contrast while preserving distinguishable fibrillar features. On the other hand, conventional lead citrate post-staining increased overall electron density but partly obscured fine structural details of the cyst capsule, although it improved visualization of the hydrogel matrix.

The freeze-substitution solvent also influenced hydrogel morphology. Samples substituted in methanol showed a more open and less dense fibrillar network than those substituted in acetone, possibly due to partial loss of Ca^{2+} ions or dissolution of hydrogel components. However, given the biological variability of the samples, local heterogeneity must also be considered when interpreting these differences.

Overall, these results highlight the importance of optimizing sample preparation and staining strategies for reliable ultrastructural analysis of polysaccharide-based materials. Future work will focus on commercially available alginate hydrogels as chemically defined model systems to distinguish preparation-induced artifacts from intrinsic structural features. The applicability of ruthenium red will also be further evaluated in both cryogenic and conventional chemical fixation protocols to improve contrast strategies for polysaccharide-rich biomaterials in electron microscopy.

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Acknowledgement:

This research was supported by the projects GAČR 25-18143S (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).

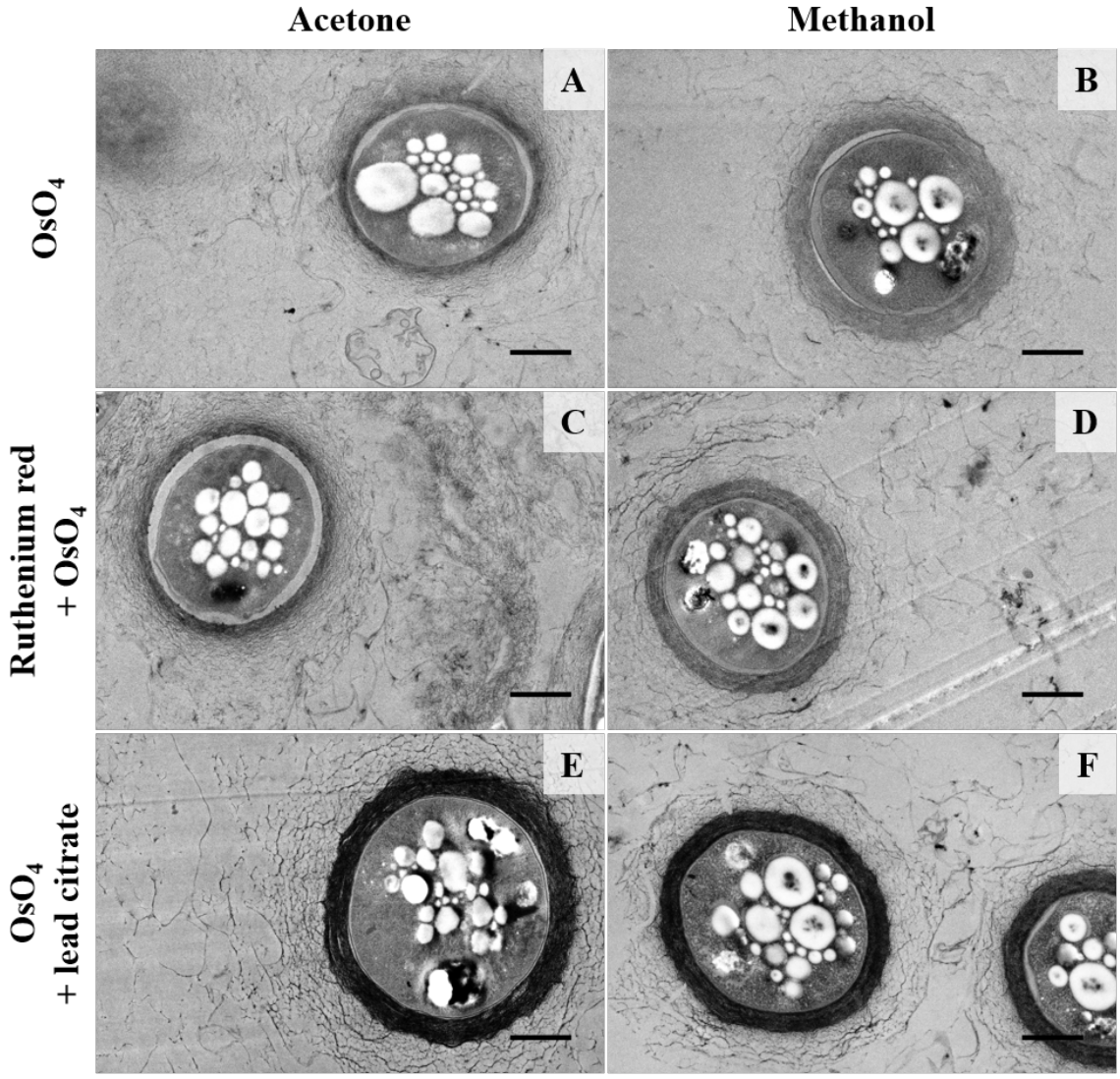

Fig. 1: STEM images of Azotobacter vinelandii DSM 87 encapsulated in alginate hydrogel. Samples A and B were stained only with OsO4 during freeze substitution. Samples C and D were stained with ruthenium red during cross-linking and OsO4 during freeze substitution. Samples E and F were stained with OsO4 during freeze substitution and post-stained with lead citrate. Acetone was used as a dehydrating agent for A, C and E, while methanol was used for B, D and F. The scale bar is 1 µm for all images.

Endoplasmic Reticulum Stress–Mediated Immune Dysregulation in Ovarian Cancer: Implications for Immunotherapy

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Introduction:

Ovarian cancer (OC) remains the most lethal gynecological malignancy, with a five-year survival rate below 30% in advanced stages. Although immunotherapy represents a promising therapeutic approach, its efficacy is often limited by immune evasion and resistance mechanisms. Interactions between cancer and immune cells within the tumor immune microenvironment (TIME) contribute to immunosuppression. Disruption of endoplasmic reticulum (ER) homeostasis induces chronic ER stress (ERS), which promotes tumor progression and impairs immune cell function. Additionally, transmissible ER stress (TERS), a process by which stressed cancer cells propagate ER stress to surrounding cells, has emerged as a novel mechanism contributing to cancer progression.

Materials and Methods:

The study investigates the effects of ERS and TERS on immune responses in OC, with a focus on TUSC3 and treatment response prediction. Peripheral blood mononuclear cells (PBMCs) were exposed to TERS conditions, and changes in viability and effector functions were assessed at gene (e.g. qPCR) and protein (e.g. fluorescent microscopy, western blot) levels. ERS conditions were modulated using tauroursodeoxycholic acid (TUDCA) in in vitro models. In parallel, the TIME of OC patients treated with conventional therapy or immunotherapy is being characterized using spatial phenotyping and MALDI imaging. The role of TUSC3 in immunoediting and therapy resistance is being examined. Additionally, MALDI-TOF MS is being implemented as a tool for treatment response prediction and patient stratification.

Results:

ERS marker expression and tumor-infiltrating lymphocytes (TILs) within the TIME correlated with treatment response in OC patients. In our previous research, we described, that loss of TUSC3 is being associated with reduced ERS and enhanced tumor cell viability, migration, and growth, while increasing sensitivity to ERS induction. TERS conditions impaired PBMC viability and effector functions, accompanied by upregulation of ERS and immunosuppressive markers. These effects were partially reversed by TUDCA, indicating the reversibility of ERS–mediated immune dysfunction. Preliminary data suggest that ERS cancer cells release soluble factors that contribute to immune suppression. Finally, MALDI-TOF MS analysis highlights the potential of mass spectrometry-based approaches for treatment response prediction and patient stratification.

Conclusion:

Targeting ERS pathways and understanding TERS and TUSC3-related mechanisms could enhance immunotherapeutic strategies and support personalized oncology. Spatial phenotyping and MALDI MS offers sensitive, high-throughput profiling of tumor proteins and their spatial distribution. These methods provide new opportunities to explore OC TIME mechanisms and identify biomarkers for improved therapy. Moreover MS approaches based on liquid biopsies can be utilized for rapid and effective identification of patient response to treatment that could lead to the streamlining of treatment and better patient quality of life.

Acknowledgement:

Supported by Ministry of Health of the Czech Republic, grant nr. NW24J-03-00038. All rights reserved. Supported by the project SALVAGE (OP JAC; reg. no. CZ.02.01.01/00/22_008/0004644) – co-funded by the European Union and by the State Budget of the Czech Republic. RB is funded by Brno city municipality (Brno Ph.D. Talent Scholarship).

What information can we extract from microscopic data – Anatomical parameters and mesophyll conductance of leaves of plants inhabiting wide range of altitudes

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Mesophyll conductance (g_m) is a critical physiological parameter that describes the ease with which CO_2 diffuses from sub-stomatal cavities to the site of carboxylation within the chloroplast stroma. Analyzing anatomical parameters and calculating anatomical g_m for plants inhabiting diverse environments, such as those across varying altitudes, provides vital information on how leaf anatomy constrains physiological function. From these data, researchers can learn how plants adapt their internal anatomy to cope with environmental stressors such as varying irradiance and temperature.

To calculate the anatomical g_m, several parameters have to be precisely extracted from the optical microscopy and TEM images of semi-thin and ultra-thin sections of the leaf samples, respectively (an example of TEM image analysis is displayed in Figure 1). The image analysis results provide insights into a plant's resource economics, revealing how leaf structural robustness – often characterized by increased cell wall thickness – mediates the trade-off between carbon fixation and survival in harsh high-altitude environments. The next step in our research is complex multivariate analysis of the collected data and image processing automation via machine and/or deep learning approaches to further accelerate analysis of future samples and reduce human-induced variability.

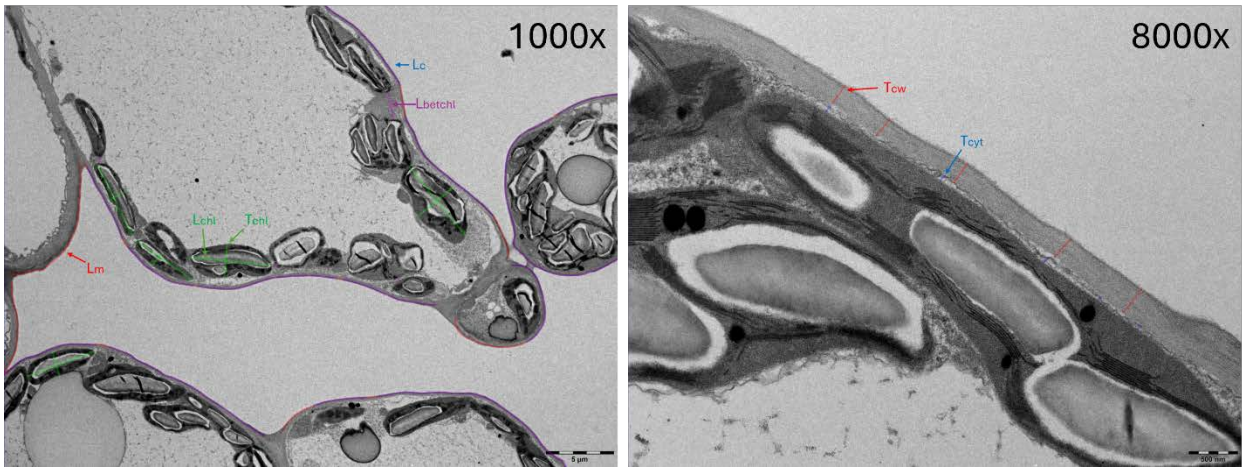

Figure 1. Example of TEM images of ultra-thin sections of leaves at 1000× (left) and 8000× (right) magnification. The analyzed parameters for each image are displayed. These are the length of cell walls (Lm), the length of cell walls with neighboring chloroplasts (Lc); the length (Lchl) and thickness (Tchl) of chloroplasts, distance between chloroplasts (Lbetchl), from the 1000× magnified images, together with thickness of the cell wall (Tcw) and thickness of chloroplast cytoplasmatic membrane (Tcyt) from the 8000× magnified images.

Acknowledgement:

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Morphological Diversity and Arrangement of Silk Glands in Lepidopteran Caterpillars

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Micro-computed tomography (microCT) is a powerful, non-invasive imaging technique increasingly applied in studies of insect morphology. The method enables visualization of delicate tissues and small organs in their native state (in situ), providing insights into their function and interaction with other anatomical features without the need for physical dissection [1, 2]. In this study, we use microCT to examine and compare the morphology and spatial arrangement of silk glands (SGs) in caterpillars of selected species from the families Saturniidae, Sphingidae, and Bombycidae.

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 10.0 (Oxford Instruments, UK) using the Surface module: Surpass - Contour Surface.

Three-dimensional reconstructions generated in Imaris software enabled precise localization, volumetric analysis, and comparative assessment of SGs across the examined taxa. Caterpillars of Saturniidae, known for producing large protective cocoons, exhibited well-developed SGs occupying approximately 18% of the larval body volume. The glands were arranged in a distinct vertical folding pattern, with relatively thinner middle silk glands (MSG) and robust posterior silk glands (PSG).

In contrast, representatives of Sphingidae – species that produce only minimal silk at the end of larval development prior to pupation – displayed narrow, irregularly arranged SGs, occupying roughly 3% of body volume. Bombycidae larvae, specialized in intensive silk production, showed the most extensive SG development, with glands filling up to 25% of the larval body. In this group, thick MSGs were aligned longitudinally, while thinner PSGs followed a zigzagging shape within the body cavity.

The observed inter-family differences in SG morphology and spatial organization reflect varying degrees of silk production and represent distinct evolutionary adaptations among Lepidoptera. Our results demonstrate the utility of microCT imaging combined with 3D modelling as an effective, non-destructive method for high-resolution analysis of soft tissue morphology in insects.

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Acknowledgement:

The work was supported by Biology Centre CAS, Institute of Entomology (RVO 60077344).

Low-Voltage Electron Microscopy Study of Cupriavidus necator in the presence of Magnetite Nanoparticles

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Introduction
Electron microscopy plays a key role in investigating bacterial ultrastructure and intracellular organization, enabling visualization at the nanometer scale. In particular, low-voltage electron microscopy (LVEM) has proved to be an effective technique for imaging biological samples with enhanced image contrast and reduced staining requirements.

Cupriavidus necator is a model microorganism widely studied for its ability to produce polyhydroxybutyrate (PHB), a biodegradable polymer accumulated as intracellular granules. The morphology and internal organization of these cells can be influenced by cultivation conditions and the presence of external particulate materials (1).

This study focuses on the application of low voltage electron microscopy, especially LVEM 25E, to analyze structural changes in *C. necator* H16 and PHB^{-4} strains cultivated in media supplemented with magnetic nanoparticles. The aim is to evaluate how these conditions affect cellular morphology and to demonstrate the capabilities of LVEM techniques, including TEM, STEM, SEM, and energy-dispersive spectroscopy (EDS), for comprehensive characterization of biological samples.

Materials and Methods
Magnetite nanoparticles were synthesized in the laboratory and characterized using dynamic light scattering (DLS). The particles (≥200 nm) were added at varying concentrations to cultivation media inoculated with *C. necator* H16 and PHB^{-4} strains.

Bacterial growth was monitored by optical density measurements using UV-VIS spectrophotometry. Cell viability was assessed by flow cytometry. PHB content was quantified using gas chromatography with flame ionization detection (GC-FID).

For ultrastructural analysis, bacterial samples were prepared for low-voltage electron microscopy (LVEM). Standard ultrathin sections were prepared using conventional fixation, dehydration, embedding, and sectioning protocols. Due to the enhanced contrast at low accelerating voltages, staining requirements were reduced compared to conventional TEM preparation.

Samples were analyzed using LVEM 25E in multiple modes:

- TEM mode (25 kV) for high-resolution internal structure visualization
- STEM mode (15 kV) for enhanced contrast and particle localization
- EDS analysis for elemental mapping and confirmation of Fe_3O_4 nanoparticle presence within or associated with bacterial cells (2)

Results
The presence of Fe_3O_4 nanoparticles influenced bacterial growth and viability in a concentration-dependent manner. Spectrophotometric and flow cytometry data indicated moderate effects at lower concentrations and inhibitory trends at higher concentrations. However, flow cytometry analysis showed that bacterial cells remained largely viable even in the presence of Fe nanoparticles, demonstrating a notable level of resistance. The wild-type strain *C. necator* H16 exhibited greater tolerance than the PHB^{-4} mutant, which can be attributed to the protective role of intracellular PHB granules. GC-FID analysis revealed changes in PHB accumulation, particularly in the wild-type strain.

Electron microscopy showed alterations in cell morphology and internal organization. LVEM imaging enabled clear visualization of intracellular structures with reduced staining artifacts. TEM, STEM and EDS analyses confirmed the presence and localization of Fe_3O_4 nanoparticles, suggesting their interaction with bacterial cells and possible internalization or surface association.

Conclusion

Magnetit nanoparticles affect both morphology and metabolic activity of *C. necator* strains. LVEM proved to be a suitable method for analyzing biological samples with improved contrast and reduced sample preparation requirements. The combination of TEM, STEM, and EDS provides comprehensive insight into nanoparticle–cell interactions. These findings contribute to understanding the role of nanoparticles in microbial biotechnology and biopolymer production.

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Acknowledgement

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Histological and immunohistochemical analysis of structural and chemical changes in the silk produced by the silk glands of the Mediterranean flour moth, *Ephestia kuehniella*

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Silk is an important natural biopolymer that has played a vital role in human history and remains a valuable raw material. It is best known as the product of the domesticated silkworm, *Bombyx mori*, which has been used for centuries as a textile material. Silk is valued for its distinctive properties, including luster and high strength. Silkworm silk has a tensile strength of up to 700 MPa (depending on fiber diameter) and an elongation of up to 35%, making it suitable for applications beyond the textile industry.

Silk production in Lepidoptera is a complex biological process involving the highly organized synthesis, secretion, and structural transformation of silk proteins along the silk gland. This study examines the histological and immunohistochemical analysis of structural and chemical changes in silk within the silk glands of the Mediterranean flour moth, *Ephestia kuehniella*. Special attention was given to previously observed changes in Masson's trichrome staining reaction of silk proteins and their possible relationship to biochemical and structural modifications of silk proteins, particularly pH changes, phosphorylation levels, and ultrastructural changes.

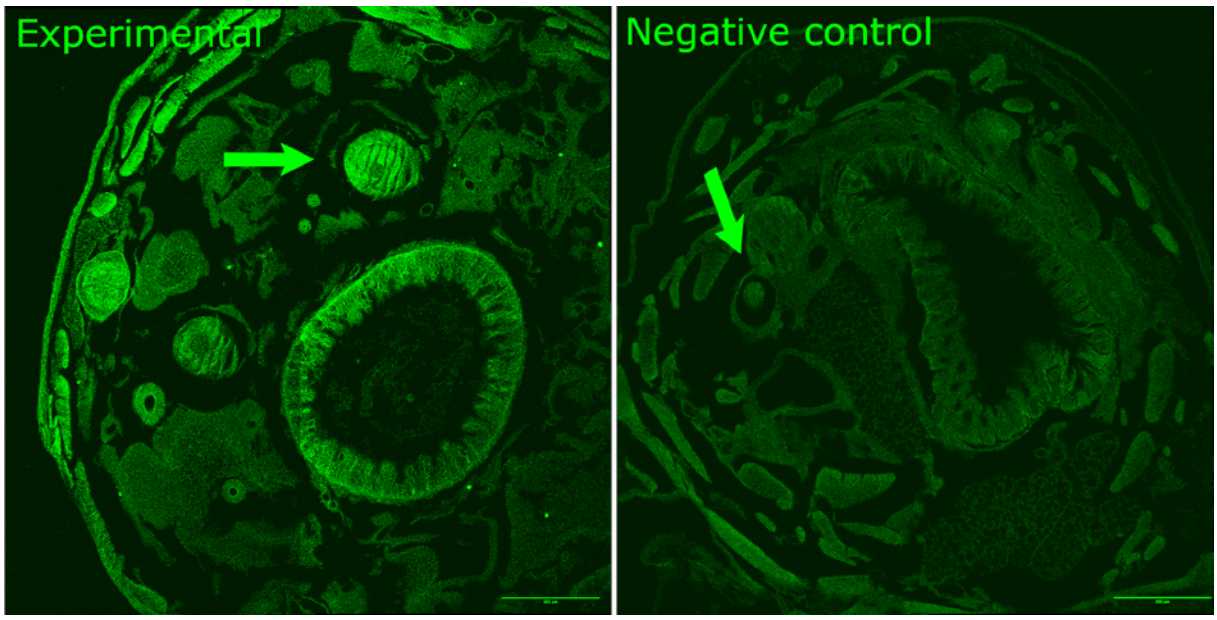

Figure 1: Detection of phosphorylated serines in the silk gland of Ephestia kuehniella using fluorescence confocal microscopy. Experimental sample stained with anti-phosphoserine antibody shows a fluorescence signal (arrow), whereas the negative control (without primary antibody) shows no comparable signal. Images were acquired under identical microscopy settings.

Acknowledgement:

The authors used services of the Czech-BioImaging research infrastructure, specifically the Light Microscopy Core Facility, Biology Centre CAS, supported by project LM2023050 funded by the Ministry of Education, Youth and Sports of the Czech Republic, with the instrumental equipment co-financed by the European Union.

RNA-FISH using RNAscope™ system – benefits and limitations

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Commercial kits for RNA-FISH have become increasingly popular in recent years, offering optimized protocols, standardized reagents, advanced signal amplification systems, and professionally designed probes. As a result, they provide improved reproducibility, specificity, and sensitivity compared to custom, lab-developed protocols.

The RNAscope™ Multiplex Fluorescent Reagent Kit v2, recently established in our lab, enables signal amplification and detection of up to three transcripts simultaneously. The system uses a set of oligonucleotides that form a detection and amplification complex, which is subsequently labeled with fluorophores using horseradish peroxidase. Although the protocol is primarily designed for histologic sections, our experience shows it is also applicable to whole-mount samples.

Using this kit, we achieved detection with very strong signals using a simple two-day protocol. However, our results also showed that certain combinations of probes may be problematic due to probable crosstalk during the labeling step. This limitation must be considered in experimental design, but otherwise, the method provided excellent results and has become a valuable tool in our team's sample preparation skill set.

Application of active and passive biomonitoring using lichens to monitor anthropogenic air pollution

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This work focuses on the use of lichens for active and passive biomonitoring of anthropogenic air pollution. Samples were placed at seven locations with different expected exposure levels for active biomonitoring; one sample was collected from each location every month (September 2025 – March 2026). For passive biomonitoring, additional samples were collected from various locations in Brno. Monitored elements were selected in accordance with legislation in the Czech Republic.

Atomic absorption spectrometry was used to analyse the total concentrations of risk elements in the collected samples. The measured elements were lead, nickel, mercury, and cadmium. Electron microscopy in combination with energy-dispersive spectroscopy was employed to analyse the spatial distribution of the measured elements in lichen tissue and to visualize dust particles from which these elements originate. The results will be compared with values measured by the Czech Hydrometeorological Institute [1] to assess the effectiveness of using lichens for biomonitoring of air pollution.

References:

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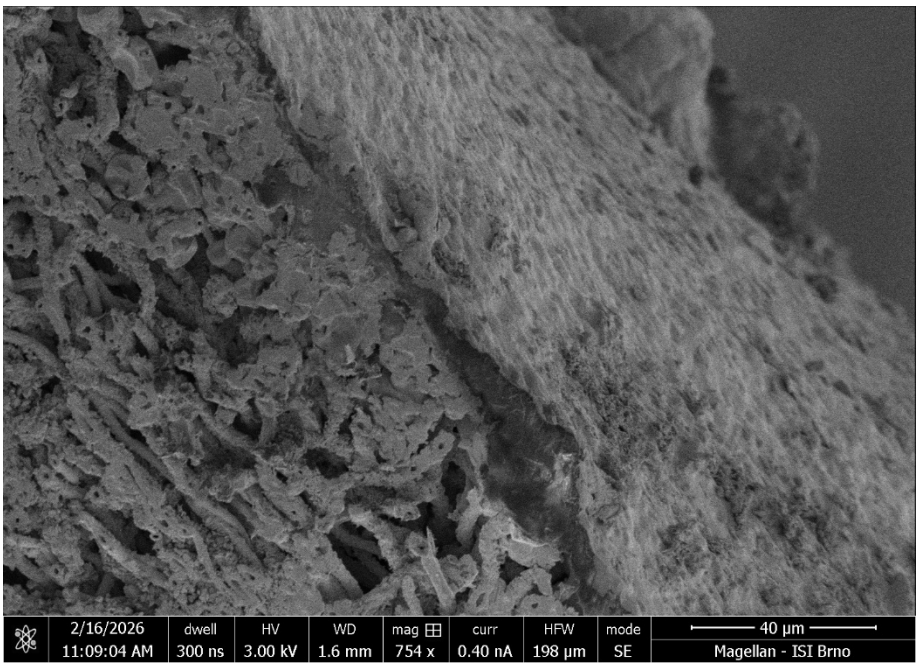

Fig. 1: Lichen and dust particles in scanning electrone microscope

Current research projects requiring the expertise and technical support of the Light Microscopy Core Facility, Biology Centre CAS

Sehadová H.[1,2], Šauman I.[1,2], Rouhová L.[1], Král M.[1,2], Podlahová Š.[1,2], Erbert D.[1,2], Konzalová J.[1,2], Barešová A.[1,2], Matyášová A.[1,2]

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The Light Microscopy Core Facility of Biology Centre CAS (BC LM) is located in the Institute of Entomology of Biology Centre CAS and consists of two workplaces: Light Microscopy Service and Light Microscopy Support.

Light Microscopy Service provides access to state-of-the-art light microscopy equipment and software for advanced image data processing. The facility offers full open access to a wide range of instruments, including two confocal microscopes (one with FLIM detection and the other featuring a unique module for polarization microscopy), five widefield microscopes (three with motorized XYZ positioning), a bioluminescence microscope, and a fluorescence macroscope with motorized XYZ positioning. In addition to technical support and professional training for microscopy device users, the team assists users with experimental design, training in various microscopy methods, data acquisition, analysis, and interpretation. Upon request, we develop new methods for microscopy sample preparation, imaging, and the use of advanced image analysis algorithms.

Light Microscopy Support offers assistance with the sample preparation for light microscopy on the basis of scientific collaboration. To illustrate the breadth of activities, several ongoing projects are presented, spanning diverse areas of biological research. These projects rely on advanced light microscopy techniques for high-resolution imaging, quantitative analysis, and visualization of cellular and subcellular processes. Access to state-of-the-art instrumentation, as well as methodological guidance provided by the facility, is essential for ensuring data quality, reproducibility, and innovation in experimental design.

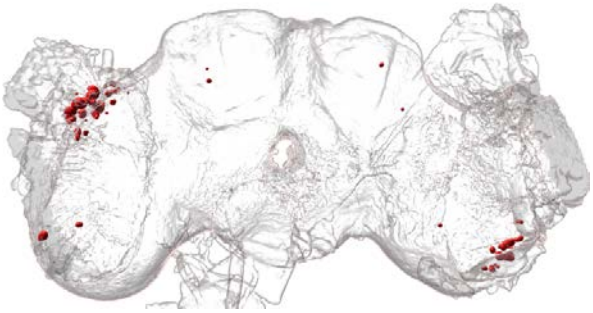
Collaboration with the Light Microscopy Core Facility enables researchers to apply cutting-edge imaging approaches, optimize sample preparation protocols, and implement sophisticated image analysis workflows, thereby significantly enhancing the scientific output and impact of their research.

References:

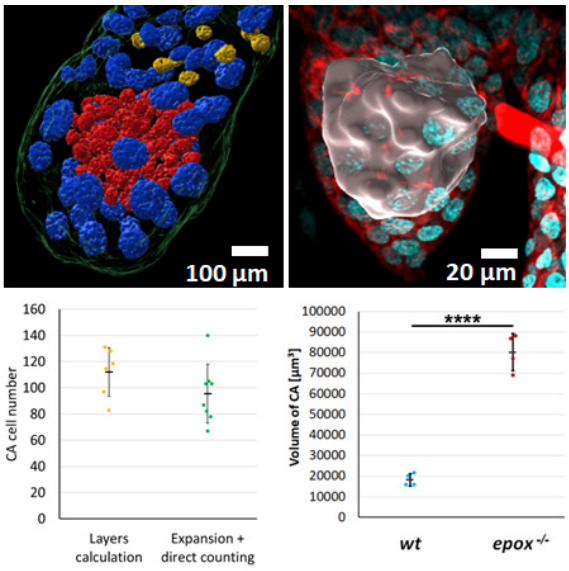
For more information, visit our website https://www.entu.cas.cz/en/departments/department-of-molecular-biology-and-genetics/microscopy-centre/about-us/.

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.

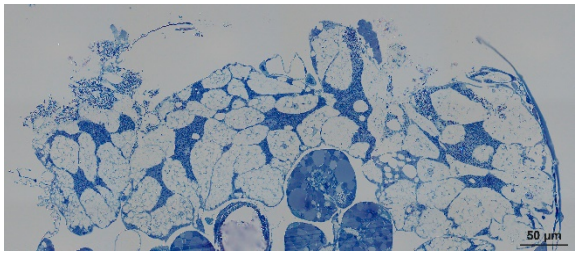


Automated 3D neurodegeneration analysis in Drosophila using deep learning

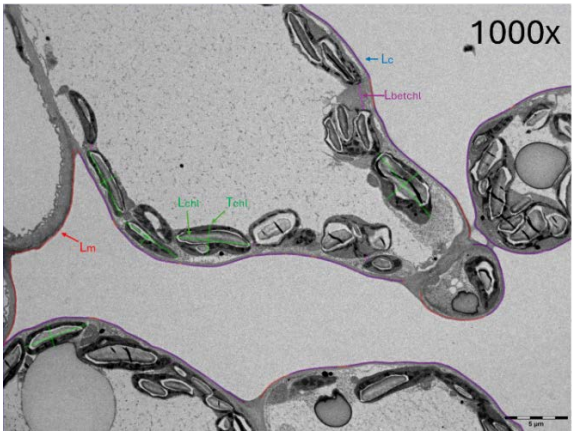


Study of neurohemal organs in mosquito larvae using histochemical techniques and advanced image data analysis

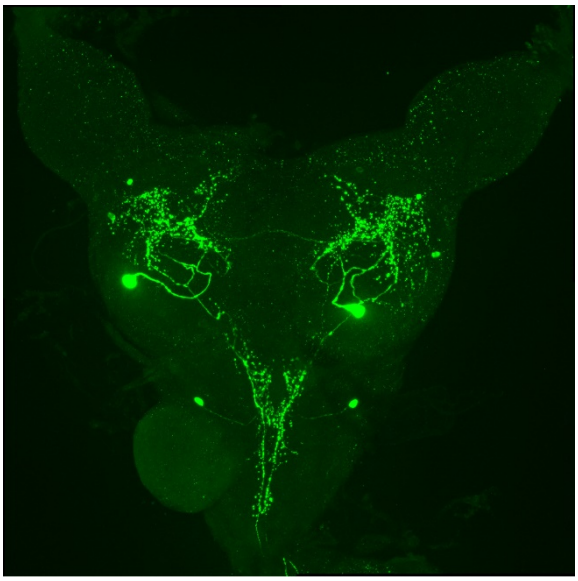
Evolutionary diversification of silk gland morphology in Bombycoidea



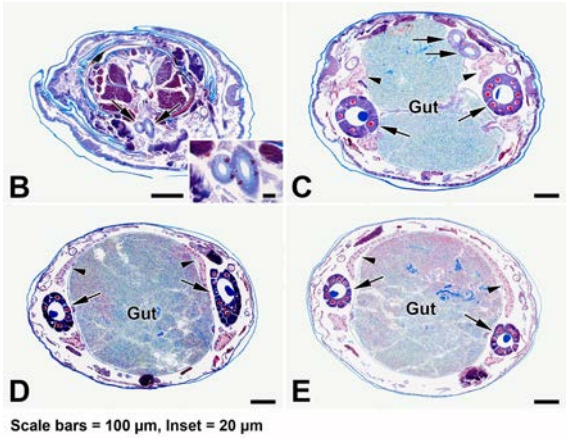
Morphology and adaptations of the defensive gland in termites



Deriving mesophyll conductance from semithin and ultrathin leaf section imaging



Mapping distribution of neuropeptides and circadian proteins in the brain of Pyrhocoris apterus



Structural and compositional properties of silk-like materials produced by the larval salivary glands of Keroplatus testaceus (Diptera)

Structure Matters: Microscopic Analysis of Nanoparticle-Based Skin Delivery Systems

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The human skin represents a complex biological barrier with essential protective, regulatory, and immunological functions. Its outermost layer, the *stratum corneum* (SC), plays a key role in preventing water loss and limiting the penetration of external factors like pathogens and toxins[1, 2]. While this barrier function is crucial for maintaining homeostasis, it also significantly restricts the transdermal delivery of active compounds[1-3].

As a result, the SC represents a major limiting factor for effective topical and transdermal drug delivery. Many therapeutically relevant compounds, including antifungal and anticancer agents, exhibit poor skin permeability and low bioavailability when applied in their conventional form[3]. To overcome this limitation, drugs are commonly formulated into nanoscale delivery systems. Poorly soluble compounds can be stabilized as nanocrystals using suitable stabilizers to prevent aggregation and enhance dissolution[4, 5]. Alternatively, active compounds can be incorporated into vesicular systems, such as liposomes or cerosomes, which facilitate interaction with skin lipids and may enhance dermal delivery[5]. Non-vesicular lipid-based carriers, such as lipid nanocarriers (LNCs), represent another approach, combining structural stability with enhanced delivery properties[2, 5, 6].

Different types of skin disorders (skin conditions) require distinct therapeutic approaches and, consequently, different drug delivery strategies. Vesicular systems such as liposomes, cerosomes, and bicelles are an alternative strategy focuses for the treatment of inflammatory skin conditions, on restoring the SC barrier by directly replenishing the deficient lipids[1, 2]. In contrast, nanocrystals are particularly suitable for poorly soluble drugs where enhanced dissolution and local availability are critical. Despite the diversity of these delivery systems, their performance is closely linked to their internal structure and morphology. Therefore, this study aimed to investigate multiple nanocarrier platforms from a microscopic perspective, focusing on structural differences between vesicular systems and nanocrystals.

A range of nanoparticle systems was prepared, including liposomes, cerosomes, bicelles, and lipid nanocarriers, and nanocrystals. Vesicular systems were prepared using the thin lipid film hydration method[1], followed by size reduction techniques such as filtration, wet-milling, and high-pressure homogenization to achieve the desired particle size and homogeneity. Nanocrystals were produced via wet-milling process, while anticancer drug-loaded systems were obtained using nanoprecipitation and phase inversion techniques[4, 7].

The presence of crystalline structures within vesicular formulations was evaluated using optical microscopy, enabling detection of phase separation or crystallization phenomena. Therefore, understanding the structural organization of these systems is essential for predicting their behaviour upon application to the skin. The overall morphology and structure of all systems were further characterized using transmission electron microscopy (TEM). TEM imaging allowed detailed visualization of particle shape, size, lamellarity, and internal organization, as well as clear differentiation between vesicular and crystalline systems.

TEM analysis demonstrated distinct morphological differences across the studied systems. Vesicular carriers formed spherical structures with varying degrees of lamellarity, while bicelles exhibited characteristic disc-like morphology and lipid nanocarriers appeared as compact spherical particles with relatively uniform structure. In contrast, nanocrystals showed well-defined angular structures with high

electron density, reflecting their crystalline nature. The particle size distributions obtained by dynamic light scattering (DLS) were consistent with the size ranges observed in TEM images, despite the expected differences between hydrodynamic and dry-state measurements. Overall, vesicular systems exhibited higher structural variability, whereas nanocrystals were more uniform.

This comparative study provides a comparative microscopic evaluation of multiple nanocarrier systems used in skin therapy, linking their structural characteristics to their intended application. The results confirm that microscopy, particularly TEM, is essential for distinguishing between nanocarrier types and identifying structural features such as lamellarity and crystallinity that are not accessible through conventional techniques. Understanding these structural differences is crucial for selecting appropriate delivery systems for specific skin conditions and for the rational design of advanced dermal formulations.

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Acknowledgement:

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Influence of Sample Preparation Parameters on the Ultrastructural Preservation of Alginate Hydrogels with Encapsulated Bacterial Cells

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Hydrogel-based systems represent highly hydrated and structurally delicate materials, posing a significant challenge for electron microscopy. [1, 2] This is particularly critical in complex bio-hybrid systems, where bacterial cells are encapsulated within a polymeric matrix, and both components must be preserved simultaneously. In this study, we investigate how key parameters of chemical sample preparation influence the ultrastructural preservation of alginate hydrogel encapsulating *Azotobacter vinelandii*, using low-voltage scanning transmission electron microscopy (LV-STEM).

Alginate hydrogels containing bacterial cells were prepared by ionic crosslinking with $CaCl_2$ and processed using a modified TEM preparation protocol. Special attention was given to the concentration and timing of $CaCl_2$ addition during fixation, post-fixation, and dehydration steps. Three preparation strategies were systematically compared, differing in the presence and concentration of the crosslinking agent. Ultrathin sections were analysed using a scanning electron microscope (Magellan 400/L, FEI) equipped with a STEM3+ detector, both with and without additional lead citrate staining, to evaluate contrast-related effects. [3]

The results demonstrate that both the concentration and timing of $CaCl_2$ addition critically affect not only the stability of the hydrogel network but also the integrity of the cellular ultrastructure. Variations in $CaCl_2$ concentration (0,5% vs. 1%) and its timing of addition during the preparation protocol influenced the preservation of the polymer network, with higher concentrations generally promoting a more clearly defined fibrillar structure extending beyond the immediate vicinity of the cells. However, premature exposure of cells to $CaCl_2$ during fixation resulted in osmotic damage. Furthermore, spatial heterogeneity within the samples revealed diffusion-limited effects, with reduced structural preservation towards the sample interior. Additional staining enhanced the visibility of fine polymer fibres but could obscure intracellular details due to over-contrasting.

These findings highlight the delicate balance between structural stabilization and artefact formation during electron microscopy sample preparation of hydrogels. The study provides practical insights into how preparation-induced changes can influence the interpretation of ultrastructural data and demonstrates that careful optimisation of crosslinking conditions is essential for reliable imaging of complex soft-matter biological systems.

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Acknowledgement:

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

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FLIPs are a versatile class of genetically encoded fluorescent biosensors that take advantage of directionality of optical properties of fluorescent proteins. The FLIP molecular design offers high sensitivity, multiplexing ability, ratiometric output and resilience to bleaching artifacts, without requiring any modifications to the proteins of interest. We have used FLIPs for real-time single-cell microscopy imaging of activation of a number of overexpressed G protein-coupled receptors (GPCRs), G proteins, arrestins, small GTPases, as well as receptor tyrosine kinases. However, protein overexpression can lead to non-physiological artifacts, such as ectopic sub-cellular localization, erroneous formation of protein complexes or aberrant organelle morphology. Motivated by a desire to understand molecular processes of cell signalling under physiological conditions, we have now demonstrated, on several examples, the ability of FLIPs to report on cell signalling by non-modified, endogenously expressed proteins.

Optical microscopy at Imaging Methods Core Facility BIOCEV

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The Imaging Methods Core Facility (IMCF) at BIOCEV, Vestec, is a part of the Czech-BioImaging and Euro-BioImaging infrastructures and offers open access to advanced microscopy technologies and expert support in experimental design, sample preparation, data acquisition, and analysis. Our facility comprises four divisions (Optical Microscopy, Electron Microscopy, Flow Cytometry, and Data Analysis), with this contribution focusing on the Optical Microscopy core.

Within the Optical Microscopy division, we provide a comprehensive toolbox ranging from widefield transmitted-light, fluorescence and holographic imaging, through laser scanning and spinning disc confocal, TIRF, super-resolution and nonlinear modalities, to functional imaging (FLIM, FCS, FRAP, etc.). Highlighted in this poster is the selection of specialized imaging platforms where the IMCF team demonstrates unique expertise and cutting-edge instrumentation:

- ZEISS Lattice Lightsheet Microscopy: High-speed volumetric imaging of live cells, embryos, organoids, and tissue models with near-isotropic resolution, minimal phototoxicity, and automated sample alignment for long-term experiments.
- STED Super-Resolution Microscopy: Sub-diffraction spatial resolution (typically below 50 nm) for precise mapping of protein localization and nanoscale cellular structures. System come with matrix detectors and adaptive optics.
- Fluorescence Correlation Spectroscopy (FCS): Quantitative analysis of molecular diffusion and concentration at the single-molecule level using point- and line-scan configurations, processed via our proprietary TTTR analysis tools. Together with FCS, we offer also FRAP, FRET and FLIM.
- Data analysis: Integrated support in data management using OMERO for FAIR-compliant data sharing, and a range of analysis tools including Fiji, Python/Napari, and in-house scripts for FCS and FLIM processing, together with commercial software NIS- Elements, Imaris and Huygens.

IMCF BIOCEV welcomes new collaborations across academic and industrial programs and provides training, consultations, and remote access to visualization and analysis platforms. We invite participants to explore our poster to discuss specific workflows and outcomes across diverse imaging modalities.

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Evaluation of Strategies for Automated Segmentation of Cryo-FIB-SEM Volume EM Data

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Cryo-FIB-SEM volume EM (cryo-FIB-SEM vEM) enables the three-dimensional reconstruction of biological structures in their near-native state at nanometer-level resolution. However, the manual segmentation of these vast datasets remains a significant bottleneck. This study evaluates different strategies for automated segmentation using deep learning architectures, specifically U-Net [1] and DeepLabv3+ [2], implemented within the DeepMIB [3] environment. The research utilized low-contrast cryo-vEM fungal data to optimize spatial and computational parameters across 2D, 2.5D, and 3D training approaches.

The findings indicate that incorporating a broader spatial context through multi-dimensional training is essential for identifying organelles in low-contrast images. Optimization of input configurations and training parameters, such as batch size, proved necessary for maintaining gradient stability and enhancing the network's ability to generalize complex biological features. Furthermore, the results suggest that models benefit from exposure to the wider cellular environment, as restricting the training focus with selective masking was found to decrease performance. In conclusion, these results demonstrate that the evaluated deep learning strategies can achieve a functionally usable level of automated detection. This approach provides a viable alternative to exhaustive manual annotation, helping to address the current limitations in volume electron microscopy data processing.

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Illuminating *Streptomyces*: Detecting Secondary Metabolites in *Streptomyces* with Raman Spectroscopy

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Streptomyces are filamentous bacteria recognized as producers of various secondary metabolites, including polyenes with significant antifungal activities [1]. Our work utilizes Raman microspectroscopy, a non-destructive vibrational technique, to analyze bacterial colonies directly on Petri dishes and identify characteristic molecular fingerprints associated with their overall molecular composition (Figure 1).

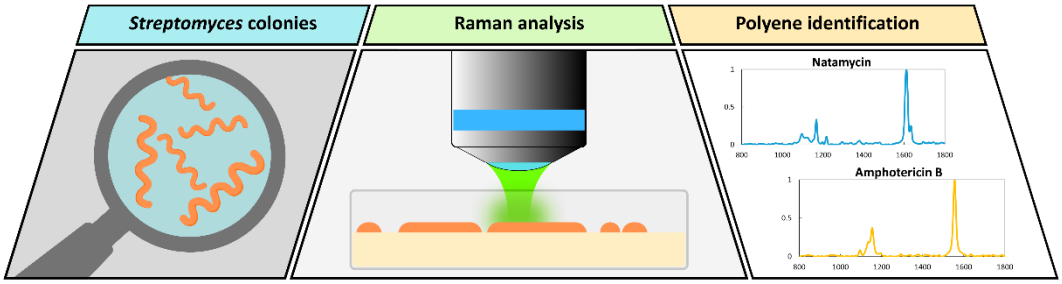

Figure 1 Demonstration of Raman spectroscopy applied to bacterial colonies

We investigate how culture conditions influence the spectral profiles of *Streptomyces* colonies, specifically focusing on the induction and modulation of secondary metabolite production. By comparing Raman spectra acquired from colonies grown on minimal and rich media, we aim to identify specific spectral features that reflect microbial adaptation to different growth conditions.

Polyene antibiotics generate intense resonant signals at approximately 1150 cm^{-1} and in the 1550-1650 cm^{-1} region, corresponding to the characteristic structural features of the polyene chain. This chain consists of alternating single and double carbon-carbon bonds, which give rise to the most prominent Raman bands observed in these compounds. The signal for double bonds in the 1550-1650 cm^{-1} region, and its position, depends on the length of the polyene chain [2].

We cultivated several strains of *Streptomyces* on different media and measured the differences among their growth on different nutrient sources. It was possible to detect differences in production on different media, and we demonstrate Raman spectroscopy as a rapid screening tool for detecting the production of polyenes and distinguishing their basic structural characteristics.

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MyImg/iLabels: Open-Source Python Workflow for Semi-Automated Classification of Nanoparticle Signals in Low-Voltage (S)TEM

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Low-voltage STEM imaging of ultrathin sections can capture nanoparticle signals originating from both surfaces of a section. Accurate interpretation requires discrimination between intensity profiles associated with top- and bottom-surface localization. This classification is typically performed manually, making the process time-consuming, subjective, and difficult to reproduce across users and datasets.

To address this challenge, we developed MyImg/iLabels [1, 2], an open-source, Python-based workflow for automated detection, annotation, and classification of nanoparticle signals in LV-STEM data. Implemented in a user-friendly Jupyter notebook environment, the workflow supports reproducible analysis of nanoparticle intensity profiles while reducing operator bias. In its current form, the software enables interactive signal annotation, structured dataset characterization, and automated classification of nanoparticles by means of machine learning (random forest classifier). This provides a practical basis for standardized analysis.

The workflow was motivated by recent imaging advances that enable ultrastructural visualization using backscattered electron signals alone, while increasing the need for robust computational discrimination between nanoparticle signals from opposite section surfaces. By shifting this task from subjective manual interpretation to computer-assisted analysis, MyImg/iLabels improves consistency, transparency, and throughput in LV-(S)TEM image evaluation.

References:

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Acknowledgement: TACR, program NCK2, project TN02000020.

Automated Analysis of Spatiotemporal Cell Behavior Across Scales: From Single Cells to Organoids

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Automated bioimage analysis is an indispensable part of the services provided by the national research infrastructure Czech-BioImaging (CzBI). The leading core facility providing such services tailored to specific user applications is the Centre for Biomedical Image Analysis (CBIA) [1] of Masaryk University. Although CBIA tackles various tasks, it specializes in analyzing cell behavior at various scales: from individual live cells to complex dynamic systems such as spheroids or organoids.

For time-lapse 2D and 3D live-cell imaging, CBIA develops algorithms for cell detection, segmentation, and tracking, along with annotation tools to create reference (gold-standard) results for training and testing automated methods. In addition, CBIA coordinates the established international benchmark for cell detection, segmentation and tracking called Cell Tracking Challenge (CTC) [2,3].

For tumor spheroids, CBIA has developed methods for quantitative assessment of anticancer drug efficacy by measuring drug penetration into the spheroid volume [4]. Recently, a method for quantifying cellular invasion into surrounding tissue was published [5].

For organoids, we initially focused on automated deep-learning-based quantitative analysis of their spatiotemporal development in 2D time-lapse sequences [6]. Later, we developed a workflow for the segmentation and tracking of individual cells within organoids in large 3D volumes with stained cell nuclei, enabling quantification of nuclei characteristics (size, shape) and their dynamics (speed, division/death, inter-nucleus distances, etc.).

CBIA offers help with image analysis to biologists working on similar tasks.

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Acknowledgement:

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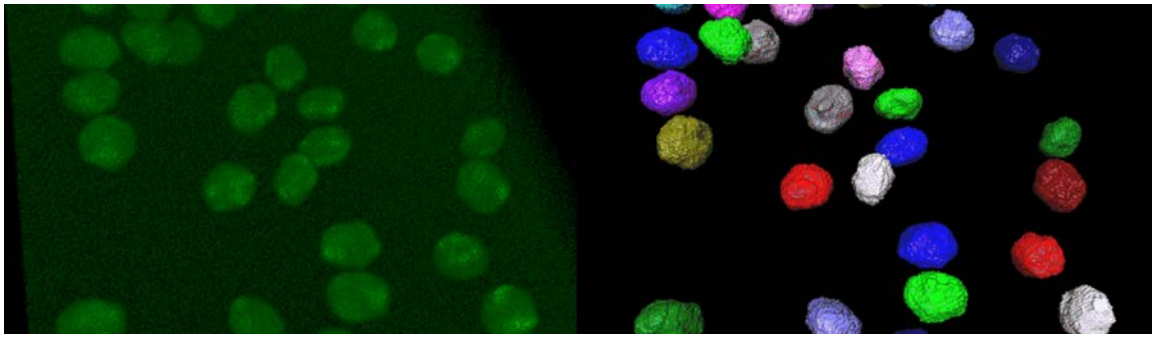

Fig. 1: CBIA develops methods for the detection, segmentation and tracking of cells across scales

Computational Methods for Automated Center Determination in Electron Diffraction Patterns

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Accurate center localization is critical for diffraction analysis but remains challenging for low-symmetry patterns and often relies on computationally intensive spot-fitting approaches. Here, we evaluate a range of fully automated algorithms, including methods adapted from outside crystallography, to improve robustness and efficiency.

The algorithms were tested on TEM/SAED and 4D-STEM-in-SEM datasets comprising both polycrystalline and monocrystalline patterns. The workflow is implemented in the *ediff.center* module of the EDIFF Python package [1] and employs a two-stage pipeline: (i) automated center detection using six algorithms with optional preprocessing and distortion correction, and (ii) center refinement using three optimization methods. All routines are integrated into a reproducible pipeline accessible via Jupyter notebooks.

Performance depends strongly on pattern type. For polycrystalline data (Debye–Scherrer rings), phase cross-correlation achieved the highest robustness and sub-pixel accuracy, showing resilience to noise and beamstoppers. For monocrystalline patterns, curve-fitting and intensity-based approaches performed best due to their sensitivity to local features. Examples are in Fig. 1.

EDIFF provides a robust, open-source, and modality-agnostic framework for automated center localization. By integrating complementary algorithms within a unified pipeline, it enables accurate, high-throughput processing of diverse diffraction data, including electron, X-ray, neutron, and optical diffraction. [2]

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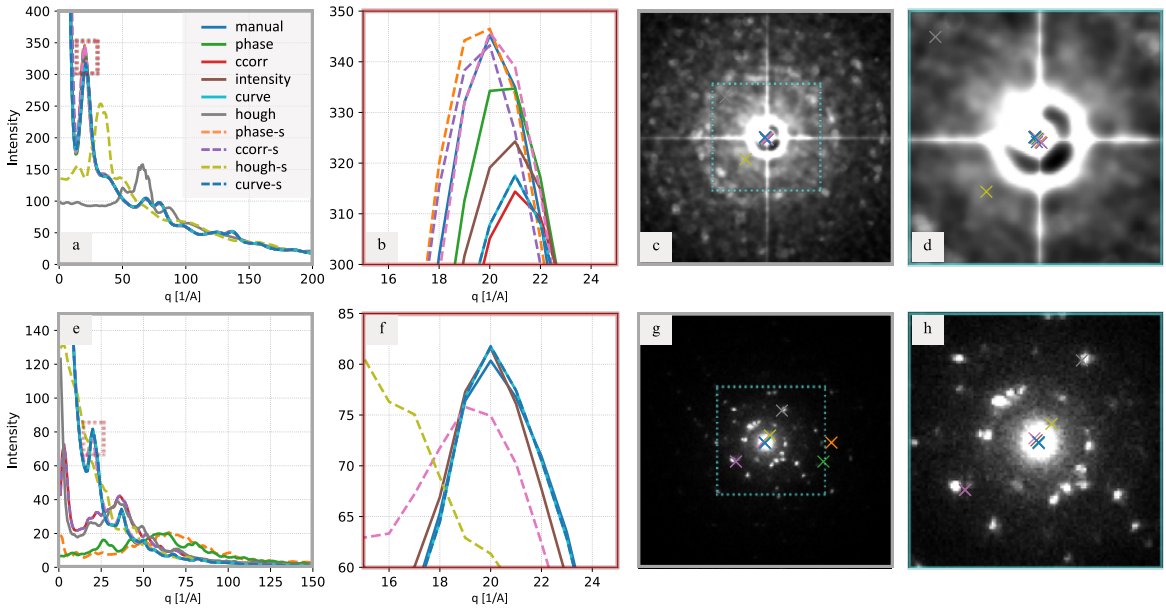

Fig. 1: Comparison of detection methods in diffractograms. (a) and (e) 1D radially averaged profiles of diffractograms calculated from center locations obtained by all detection methods. (b) and (f) Close-up views of the most intense diffraction peak [highlighted by the red rectangle in panels (a) and (e), respectively], illustrating how inaccuracies in center location affect the peak's intensity, location and shape. (c) and (g) Diffractograms with detected center locations. (d) and (h) Close-up views of the detected center positions.

An ultrafast confocal microscope for 3D imaging of organoids

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Laser scanning confocal microscopy allows 3D imaging of complex biological samples. However, practical applications of this ability are generally limited by slow imaging speed. Although fast 3D imaging can be achieved by approaches such as spinning disk microscopy and light sheet microscopy, both have their own disadvantages, including price and geometrical requirements. In order to overcome these obstacles, we have now developed a novel design of a confocal fluorescence microscope (a so-called tri-scanning microscope)[1,2]. The design achieves up to 1 kHz imaging frame rates, with only minor reduction in image quality in comparison with point scanning confocal microscopy. The tri-scanning confocal microscope is suitable for fast 3D imaging of organoids, as well as for continuous imaging of fluorescent biosensors in living cells. The design is now commercially available.

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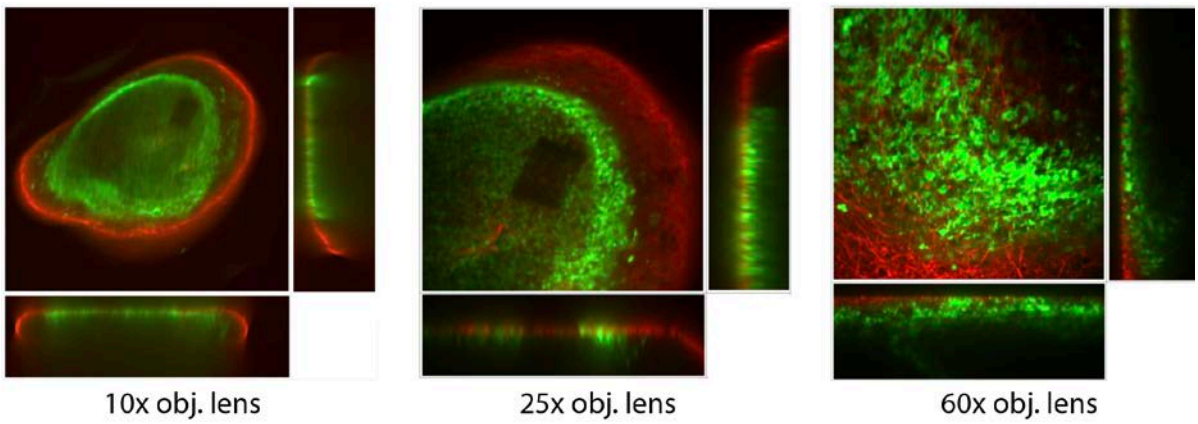

Fig. 1: An immunostained brain organoid imaged by a tri-scanning confocal microscope

Development and validation of a deep learning-based pipeline for quantification of neurodegeneration from 3D images of whole-mount *Drosophila melanogaster* brains

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Neurodegeneration is a central hallmark of major human disorders such as Alzheimer's disease, characterized by the accumulation of toxic protein aggregates including amyloid-β (Aβ) and Tau. The model organism *Drosophila melanogaster* enables targeted expression of human disease-related genes, such as *Aβ42* and *Tau*, using the GAL4/UAS system, providing a robust platform for studying neurodegenerative processes in vivo. Whole-mount brain preparations combined with confocal microscopy allow three-dimensional visualization of neuronal structures and degeneration patterns (Figure 1), however, quantitative analysis of such data remains challenging due to high complexity and user-dependent variability.

This study aims to develop and validate a standardized pipeline for quantifying neurodegeneration from 3D confocal images (Figure 2) of whole-mount adult *Drosophila* brains expressing human Aβ42 and Tau proteins. The workflow includes optimization of dissection, fixation, immunostaining and imaging parameters to ensure high-quality and reproducible datasets. Special attention is given to minimizing artefacts and improving signal-to-noise ratio in volumetric data.

A central component of this work is the implementation of deep learning-based approaches for automated detection and quantification of neurodegenerative features, such as vacuolization, neuronal loss, and structural disorganization. The developed models are integrated into an image analysis pipeline (e.g., Fiji/ImageJ, Imaris) to enable high-throughput and unbiased analysis.

The proposed approach demonstrates how the integration of advanced microscopy and artificial intelligence can significantly improve the accuracy, reproducibility, and scalability of neurodegeneration analysis. This work contributes to methodological standardization and provides a foundation for future large-scale genetic or pharmacological screening studies targeting neurodegenerative diseases.

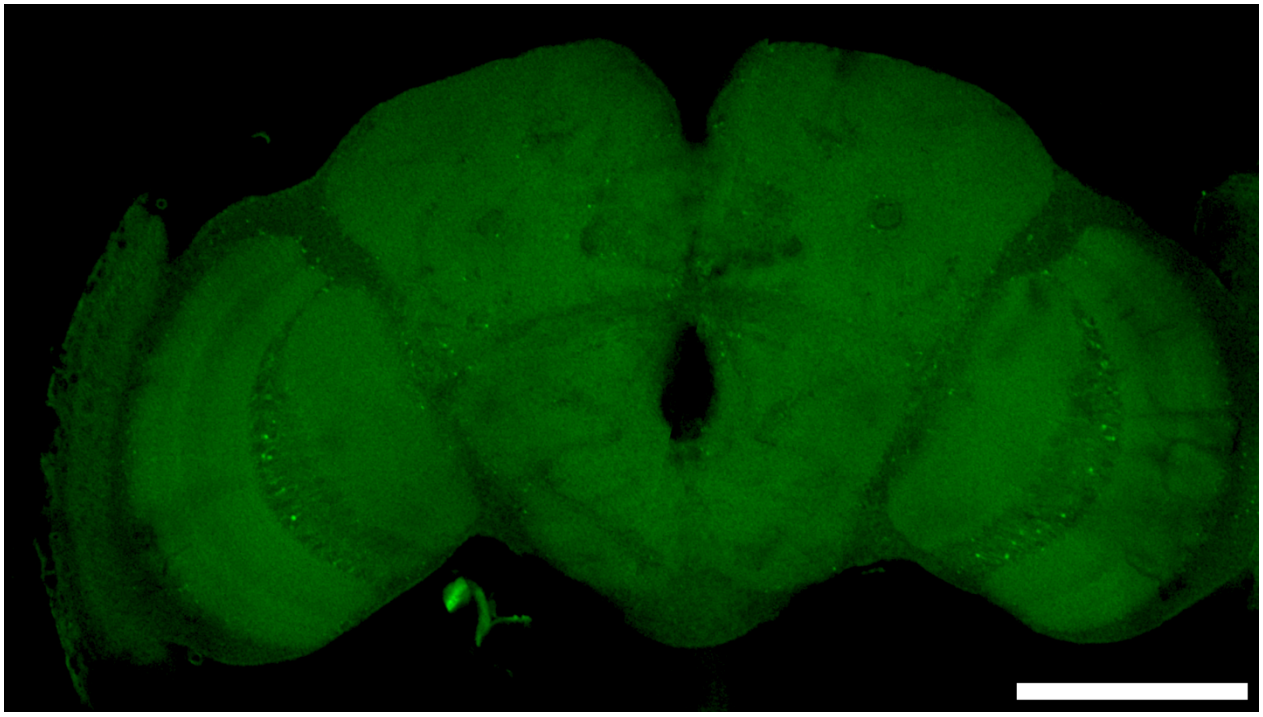

Figure 1. *Confocal microscopy image showing a single optical section of a brain from a 30-day-old Drosophila melanogaster (w^{1118} line). The image was acquired at 20× magnification; scale bar = 100 µm.*

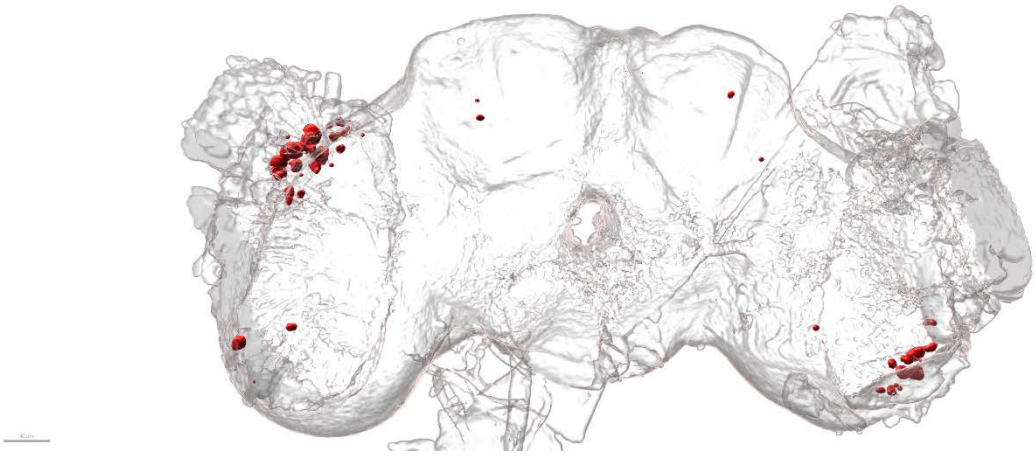

Figure 2. *Three-dimensional reconstruction of the Drosophila melanogaster brain generated from confocal z-stack images using Imaris software. Regions of neurodegeneration are highlighted in red.*

Cathodoluminescence Response of $Ce_xLa_{1-x}F_3:Tb^{3+}$ Nanoparticles for Electron-Driven Luminescence Applications

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Lanthanide-doped fluoride nanoparticles represent a promising class of materials for applications in electron microscopy, where their luminescence can be directly excited by an electron beam and utilized for high-resolution cathodoluminescence (CL) imaging [1]. In particular, $Ce_xLa_{1-x}F_3:Tb^{3+}$ nanoparticles combine efficient energy transfer between Ce^{3+} and Tb^{3+} ions with good chemical stability, making them suitable candidates for correlative CL–SEM techniques and electron-beam-driven optical contrast [2]. For such applications, a detailed understanding of their CL response, stability, and recombination dynamics is essential.

In this contribution, we present a systematic CL study of $Ce_xLa_{1-x}F_3:Tb^{3+}$ nanoparticles with varying cerium and terbium concentrations, focusing exclusively on their behavior under electron-beam excitation. CL imaging and spectral measurements were performed in a scanning electron microscope DualBeam FIB-SEM Helios G4 HP (Thermo Fisher Scientific) equipped with a SPARC CL detector (Delmic). Time-resolved CL measurements were carried out using a specially adapted CL apparatus [3], enabling study over temperature range of 100–500 K.

Continuous irradiation experiments at high current density of 23.5 $A \cdot m^{-2}$ applied for up to 200 s were used to monitor the evolution of CL spectra, revealing significant intensity changes while preserving the spectral shape, indicating stable emitting centers under electron excitation. Special attention was paid to long-term stability and surface-related effects. CL spectra recorded after prolonged storage under ambient conditions exhibited a noticeable red shift of emission bands, with a reduced shift observed for nanoparticles coated with a thin indium–tin oxide (ITO) layer. These findings highlight the importance of surface conditions for reliable CL-based characterization and imaging.

CL decays were carried out using pulsed electron excitation at low current density (four orders of magnitude lower than in spectral studies) minimizing specimen degradation. The results show that nanoparticles with higher Tb^{3+} concentration exhibit increased CL intensity but slower decay dynamics. This effect directly influences CL imaging, where prolonged emission leads to partial blurring of adjacent pixels and thus affects spatial resolution.

Overall, this work demonstrates that CL provides a powerful and application-relevant tool for evaluating luminescent nanoparticles in electron microscopy, linking their composition, surface state, and recombination dynamics to practical imaging performance.

References:

[1] Jacobsohn L. G. et al.: Opt. Mater. 33(2010), 136-140.
[2] Lytvynenko X. et al.: Radiat. Meas. 189(2025), 107536.
[3] Bok J. et al.: Rev. Sci. Instrum. 82 (2011), 113109.

Acknowledgement:

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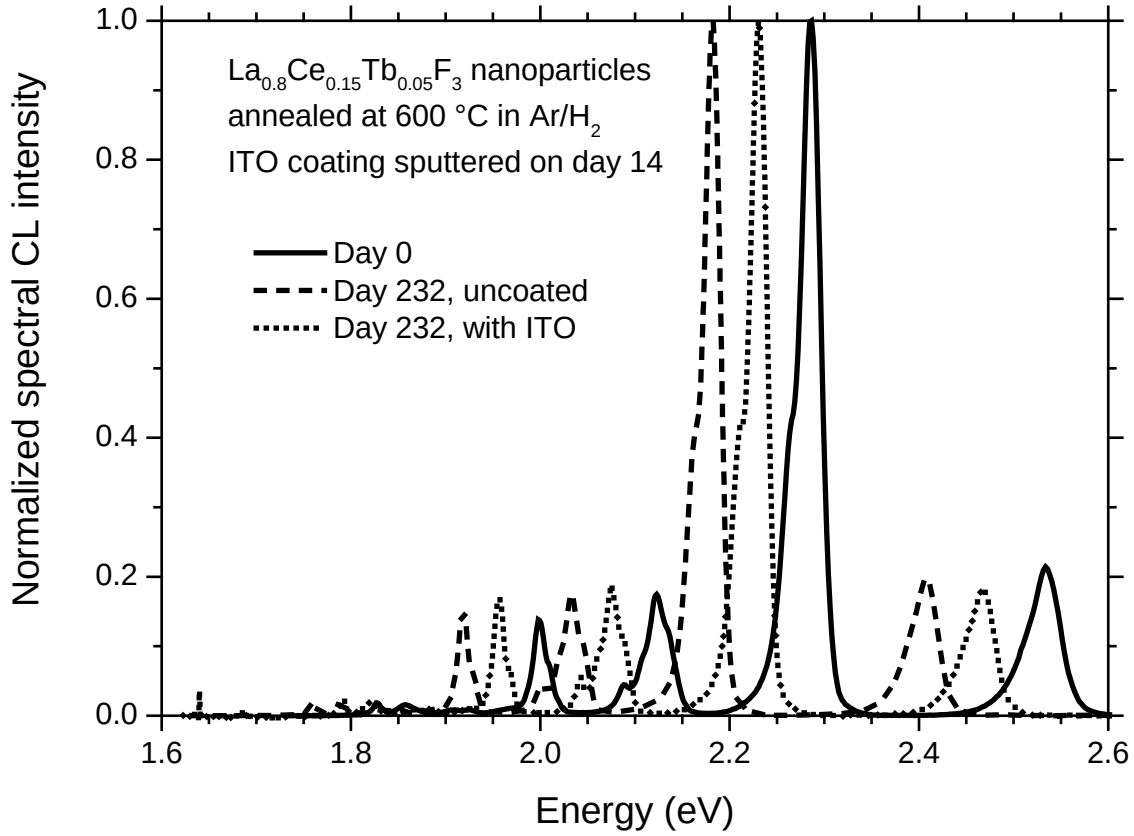

Fig. 1: Normalized CL spectra of $La_{0.8}Ce_{0.15}Tb_{0.05}F_3$ nanoparticles. Reference measurement conducted early after specimen preparation (Day 0) and part of specimen coated with 50 nm of ITO, supptered on day 14.

Fourier Transform in Image Analysis of Micrographs

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Abstract

The Fourier transform (FT) is a useful tool for extracting quantitative information from micrographs by transforming spatial data into the frequency domain. In this work, we developed an open-source Python package named MyImg/FFT [1], which automates the application of discrete fast Fourier transform (DFFT) in image analysis. MyImg/FFT package calculates Fourier transforms for selected 1D intensity profiles (Fig. 1) and 2D image regions (Fig. 2). The calculated 1D and 2D-FT results can be further analysed (to determine periodic distances in 1D; Fig. 1) or transformed into radial and azimuthal profiles (to reveal and quantify periodicities and symmetry in 2D; Fig. 2).

Principles of FT in Image Analysis

Image preprocessing was performed using ImageJ, primarily for the extraction of one-dimensional intensity profiles from micrographs [2]. In this context, ImageJ serves as a convenient tool to convert image data into numerical (x, f(x)) datasets/profiles. The profiles are input to the subsequent 1D discrete fast Fourier transform (1D-FFT) analysis made with MyImg/FFT [1]. For 1D-DFFT, the output is a one-dimensional spectrum in which spatial coordinates are converted into frequencies (f), enabling determination of periodic distances, whereas 2D-DFFT produces a two-dimensional Fourier image representing spatial frequency distributions with the zero-frequency component centered. To further quantify periodicity and symmetry, 2D-DFFT data can be numerically transformed into one-dimensional radial profiles (intensity vs. radius) and azimuthal profiles (intensity vs. angle), obtained by averaging intensity values over concentric rings or angular sectors, respectively.

Numerical analysis was carried out in Python using the MyImg package [2] alongside standard scientific Python libraries. MyImg/FFT was also developed to ensure fast, easy, and reproducible extraction of radial and azimuthal intensity distributions from 2D-DFFT outputs, enabling consistent quantitative characterization of structural features [3].

Applications

The methodology was demonstrated on micro- and nanostructured materials. FT analysis enabled identification of periodic spacings in lamellar systems (Fig. 1), detection of hidden hexagonal symmetry in polymers, and quantification of partial ordering in gold nanoparticle assemblies (Fig. 2). In all cases, results were consistent with complementary experimental techniques such as Small-angle X-ray scattering [4, 5].

Conclusion

FT is a valuable method for extracting quantitative structural information from micrographs. Although not always routinely applied, it provides clear insights into periodicity and symmetry of materials. Combined with modern computational tools such as Python, FT analysis offers a flexible and efficient approach for studying micro- and nanostructured systems.

References:

[1] https://pypi.org/project/myimg/
[2] Schindelin, J., Arganda-Carreras, I., Frise, E. et al. Nat Methods 9, (2012), 676–682.

[3] https://mirekslouf.github.io/myimg/docs/pdoc.html/myimg/apps/fft_utils.html
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[5] D. Gromadzki, J. Lokaj, M. Šlouf, P. Štěpánek, Polymer 50, (2009), 2451.

Acknowledgement: TACR, program NCK2, project TN02000020 and GA JU 04-115/2025/P.

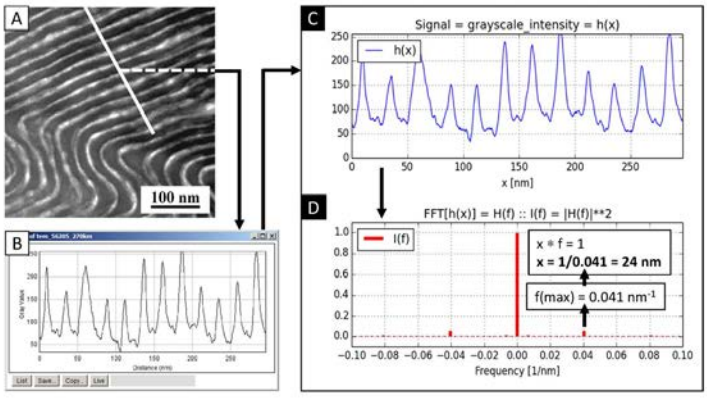

Figure 1. Determination of the periodicity of the lamellae in PS-b-SAN polymer blend: *(A) TEM micrograph, (B) intensity profile in ImageJ, and (C, D) output from a Python script—the loaded profile, its 1D-DFFT, and the calculation of the periodic spacing.*

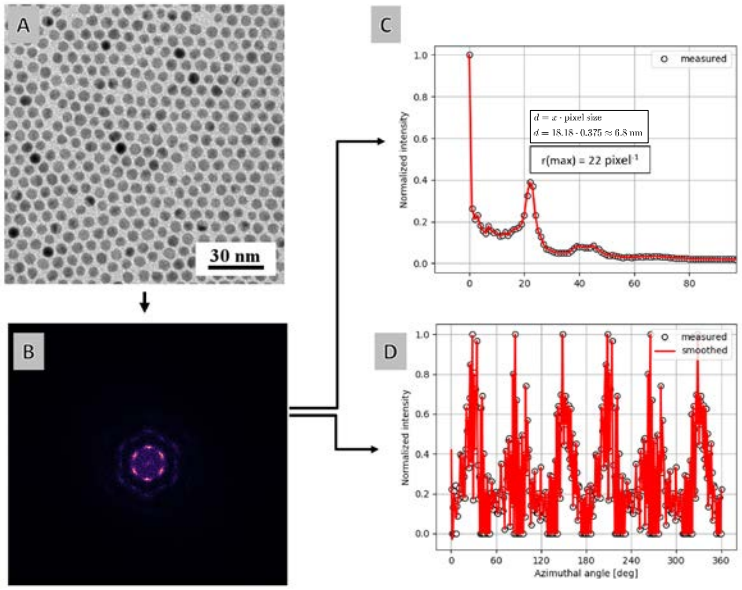

Figure 2. Characterization of the self-assembly of Au nanoparticles on a thin carbon film: *(A) TEM micrograph, (B) 2D-DFFT image of the entire micrograph, (C) radial profile, (D) azimuthal profile confirming hexagonal self-assembly.*

Side events

Excursions and Hike with yCSMS

Contact email: conference@mikrospol.cz

Excursion to Onsemi, Monday June 1 2026

Meeting Time: 9:00 (by car 8 min, by walk 40 min or by bus No. 979 at 9:08 from station „Rožnov p.Radh., rest.U eroplánu“) contact: Kamila Hrubanová, Anna Havlíčková

Meeting Point: Hotel Relax, next to the reception / gatehouse

Excursion Time: 10:00 – 11:00

Onsemi contact: David Pléha, +420 571 753 820

Please be on time, as the group will depart from the hotel precisely at 9:30 to ensure we arrive at the Onsemi facility before the official start.

Important Requirements (Safety & Dress Code): Since we are visiting a high-tech manufacturing and technology facility, please adhere to the following rules:

ID Required: Please bring a valid ID card or passport, as it may be required at the entrance security check.

Dress Code: Wear comfortable, closed-toe walking shoes (no sandals or high heels).

Excursion to Onsemi, Wednesday June 3 2026

Excursion Time: 14:30 – 15:30

More Information: Visit Registration desk

Excursion to the Wallachian Open Air Museum, Monday June 1 2026

Meeting Time: 9:30

Meeting Point: Hotel Relax, next to the reception

Departure: 9:45 (we will walk to the museum together)

Contact: Radka Novak Guided Tour: 10:30 – 11:30

Important: We are walking! Please note that we will be walking to the museum from the hotel. The walk takes approximately 20 minutes each way.

What to Wear & Bring: As the museum is an open-air facility and we will be walking: Please wear comfortable walking shoes and appropriate clothing. In case of bad or unpredictable weather, do not forget to bring a raincoat or an umbrella.

Admission Fee & Payment Price: 190 CZK per person. The admission fee will be collected at the registration desk during the conference. Please make sure to settle this payment before the excursion day

Lunch Option: On our way back to the hotel, there will be an option to stop by a nearby traditional restaurant for lunch.

Hike with yCSMS, Monday June 1 2026

Meeting Time: 7:30 (at the latest at 7:45!)

Meeting Point: Hotel Relax, in front of the hotel

Expected Return: Between 13:30 and 15:00 (depending on the chosen route)

Route Options: There are two scenic routes prepared. You can choose your preferred option on the spot.

To PUSTEVNY on foot, back by BUS

Details: 12 km walking, +790 m elevation gain. A steep hike from the hotel to Radhošť (1129 m), then to the Radegast statue, finishing at Pustevny. From there, we will take a direct bus back to Rožnov (leaves at 12:35 or 13:45, arriving 2 km from the hotel).

Refreshments: Possible stops at Hotel Radegast and Pustevny.

Estimated return: 13:30 – 15:00.

To RADHOŠŤ and back on foot

Details: 18 km walking, +740 m elevation gain. A steep hike up to Radhošť (1129 m) and Hotel Radegast, returning to the hotel via a different trail.

Refreshments: Possible stops at Hotel Radegast and Chata Mír. Estimated return: 14:00 – 15:00. PDF guide with maps and elevation profiles here:

https://mikrospol.cz/wp-content/uploads/2026/04/MICROSCOPY-2026-hike.pdf

What to Pack & Wear

Since we are heading into the mountains, please make sure you are well-prepared:

Footwear & Clothing: Proper, comfortable hiking shoes are highly recommended. Dress in layers (mountain weather changes quickly) and do not forget a raincoat or a waterproof jacket.

Cash (CZK): Please bring enough cash with you for personal expenses along the way, such as the return bus ticket (for Route 1) and refreshments/lunch at the mountain huts.

Essential items: Snacks, plenty of water, and, most importantly, a good mood!

A Small Surprise from yCSMS: To make our trip even more pleasant, the yCSMS team has prepared small gifts for all participants to take on the journey! Let's stretch our legs and start the networking a bit earlier! We look forward to hitting the trails with you

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