WORKSHOP

on Cryogenic Microscopy & Spectroscopy

March 23—26, 2026

INSTITUTE OF SCIENTIFIC INSTRUMENTS
OF THE CZECH ACADEMY OF SCIENCES
KRÁLOVOPOLSKÁ 147
BRNO 61200
CZECH REPUBLIC

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Welcome Letter

Dear Colleagues and Friends,

on behalf of the organising committee, it is our pleasure to welcome you to Brno for the Workshop on Cryogenic Microscopy & Spectroscopy. This event is organized by the **Institute of Scientific Instruments of the Czech Academy of Sciences (ISI CAS)** in collaboration with the **Czechoslovak Microscopy Society (CSMS)**, under the auspices of the **European Microscopy Society (EMS)** and the **International Institute of Refrigeration (IIR).**

This workshop provides a platform for the exchange of knowledge at the intersection of electron microscopy, spectroscopy, cryogenic techniques, and cryobiology. Its aim is to bring together communities that do not always naturally overlap, fostering dialogue between microscopists, cryogenic engineers, low-temperature physicists, and cryobiologists. By connecting these perspectives, we hope to identify new research directions, address challenges associated with low-temperature microscopy, and explore innovative technical solutions.

Cryogenic approaches have become essential for studying beam-sensitive and hydrated materials, as well as biological specimens and tissues. They enable observation of samples in a near-native state under high-vacuum conditions and are increasingly important in materials science. These capabilities are driving progress across life sciences, soft matter research, materials engineering, and emerging biomedical applications, including cryopreservation.

The scientific programme reflects these developments, covering advances in cryo-imaging, spectroscopy, instrumentation, and sample preparation, with an emphasis on interdisciplinary collaboration and new experimental approaches.

The workshop will also include the inauguration of the new **Nion UltraSTEM** laboratory at ISI CAS, representing an important step forward for atomic-scale research infrastructure in Brno. Designed to operate at both room and cryogenic temperatures, the facility opens up new possibilities for the community.

Brno, with its long-standing tradition in electron microscopy and a unique ecosystem of research and industry represented by the **brnoregion microscopy platform**, serves as the perfect backdrop for our discussions. We hope you will enjoy not only the scientific sessions but also the opportunity to reconnect with friends and colleagues in this vibrant city.

We would like to thank all the speakers, contributors, participants, and sponsors for their support, and wish you an inspiring and productive stay in Brno.

Organizing Committee

- Kamila Hrubanová *(ISI CAS; President of CSMS)*
- Vladislav Krzyžánek *(ISI CAS; President of EMS)*
- Pavel Urban *(ISI CAS; Organising Committee, IIR Cryogenics Conference)*
- Jiří Gregor *(University Hospital Hradec Králové; President of IIR, Commission C1)*

In cooperation with

- Steffen Grohmann *(KIT; President, IIR Commission A1)*
- Ralf Herzog *(IIR Honorary Member; Past Head, Section A)*

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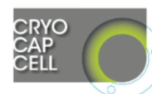

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Program

	Monday 23 March 2026
18:00	**Registration**
18:00	**Welcome reception**
21:00	**Expected end of the welcome reception**

	Tuesday 24 March 2026
8:00	**Registration**
9:00	**Welcome** **Kamila Hrubanová** (president of the Czechoslovak Microscopy Society) **Jiří Gregor** (president of International Institute of Refrigeration (IIR), Commission C1) **Pavel Urban** (Cryogenics Conference Organising Committee)
9:10	**Workshop Introduction and Objectives** **Vladislav Krzyžánek** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
9:25	**Session I: Cryogenic Electron Microscopy In Life Sciences** Chairs: Vladislav Krzyžánek & Jana Nebesářová
9:30	**EM and CryoEM in structural biology** **Richard Henderson** (MRC Laboratory of Molecular Biology, Cambridge)
10:00	**What is the best temperature for electron cryomicroscopy of biological specimens?** **Christopher J. Russo** (MRC Laboratory of Molecular Biology, Cambridge)
10:30	**High-resolution Cryo-FIB-SEM volume EM enables sub-volume averaging of cellular structures with CLEM targeting** **Pavel Křepelka** (CEITEC, Masaryk University, Brno)
10:45	**Coffee break**
11:15	**From Atoms to Cells: Cryo-EM and Cryo-FIB Enabling In Situ Structural Biology** **Radovan Spurný** (Thermo Fisher Scientific, Brno)
11:30	**Correlative CryoSEM for Structural and Chemical Identification in Hydrated Frozen Samples** **Marie Vancová** (Biology Centre, Czech Academy of Sciences, České Budějovice)
11:45	**Cryogenic SEM at the Institute of Scientific Instruments of the CAS: Bridging Microbiology and Polymer Science** **Kamila Hrubanová** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
11:55	**Panel Discussion: Current Challenges and Opportunities in Cryo-EM for Life Sciences**
12:25	**Lunch**
13:30	**Session II: Low-Temperature Physics & Cryogenic Instrumentation** Chairs: Ladislav Skrbek & Rafal E. Dunin-Borkowski
13:30	**Helium: an introduction to extraordinary properties of its cryogenic phases** **Ladislav Skrbek** (Faculty of Mathematics and Physics, Charles University, Prague)
14:00	**Imaging and spectroscopy with a liquid He-cooled sample stage** **Ondrej L. Krivanek** (Bruker AXS (formerly Nion R&D), Kirkland & Department of Physics, Arizona State University, Tempe)
14:30	**Cryogenic sample cooling in TOMO and BIO microscopes in Forschungszentrum Jülich** **Rafal E. Dunin-Borkowski** (Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons, Forschungszentrum Jülich)
14:45	**Liquid Helium TEM Sample Holder: Swift Cool-Down and Long Holding Time** **Denys Sutter** (condenZero, Zürich)
15:00	**Coffee break**
15:30	**Cryogenic conversion of room-temperature EM systems using custom LHe/LN_2 cooling technologies** **Pavel Urban** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)

15:50	**The Design of a Reverse Brayton Liquefaction and Refrigeration System Equipped with a Turbomachine for Small-Scale Applications** **Vojtech Kohut** (European Cryogenics, Prague)
16:00	**Cryo stage designs for 3-coincidental system** **Sander den Hoedt** (DELMIC, Delft)
16:15	**Managing Cryogenic Sample Preparation Workflows for Cryo FIB applications** **Andreas Nowak** (Leica microsystems, Vienna)
16:30	**Influence of condensed water and other contaminants on thermal absorptivity at cryogenic temperatures** **Tomáš Králík** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
16:40	**Panel Discussion: Current Trends and Challenges in Cryogenic Instrumentation**
17:10	**Coffee break**
17:25	**Session III: Cryogenic Fixation & Spectroscopy I.** Chairs: Thomas Burg & Dominik Heger
17:25	**Low-temperature fluidic microsystems for cryo-CLEM** **Thomas P. Burg** (Integrated Micro- and Nanosystems, Technical University, Darmstadt)
17:45	**Ultra-rapid cryo-arrest directly during fluorescence live cell microscopy for high resolution fluorescence cryo-nanoscopy and microspectroscopy** **Jan Hübinger** (Max Planck Institute of Molecular Physiology, Dortmund)
18:00	**Microfluidics and Cryo-EM: A Powerful Partnership for New Experimental Approaches at the Nanoscale** **Thomas Braun** (Biozentrum, University of Basel / cryoWrite, Basel)
18:15	**Bridging Cryo EM and Volume EM: A Unified High Pressure Freezing Pipeline for Correlative Workflows** **Xavier Heiligenstein** (CryoCapCell, Paris)
18:30	**Dinner**
19:30	**Poster Flash Presentations**
	short talks of selected posters
20:00	**Poster Session & Informal Networking (Beer & Wine)**
	Preparation of mammalian cells specimen for cryo-electron microscopy using the HPF "Waffle" method **Jana Moravcová** (CEITEC, Masaryk University, Brno)
	High-Quality Graphene for Advanced Electron Microscopy Applications **Eliška Materna Mikmeková** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
	Cryogenic fluorescence microscopy – not only CLEM. Example of Cryo-FLIM sensing **Piotr Jurkiewicz** (Imaging Methods Core Facility BIOCEV, Charles University, Prague)
	Sample chamber for LHe-Insitu-TEM investigations **Alexander Horst** (IFW Dresden)
	An LN_2 optical cryostat for UV–Vis spectroscopy of water ice and aqueous solutions **Vojtěch Krutil** (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
	BarSerNew simulation software for designing thermal links in cryogenics **Miroslav Gašpar** (Institute of Scientific Instruments, Czech Academy of Sciences & Masaryk University, Brno)
	TITAN upgrades by a probe corrector and a spectrometer with DED cameras: New horizons for TEM in the Brno region **Jan Michalička** (CEITEC, Brno University of Technology, Brno)
	Exploring the physics of plunge freezing through high-speed videography **Alok Bharadwaj** (Delft University of Technology, Delft)
	The Physics of Plunge Freezing: Revisiting Vitrification in Cryo-EM Sample Preparation **Dariush Ashtiani** (Delft University of Technology, Delft)

Time	Programme
	Microfluidic chip for high-pressure freezing of biological samples: Progress towards cryo-FIB-SEM and Raman micro-spectroscopy
	Tomáš Láznička (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
	Quantifying liquid film propagating from a solid frozen sample
	Radim Štůsek (Masaryk University, Brno)
	Design and Performance Characterization of a Cryoliquid Target System for kHz Laser–Plasma Applications
	Nina Gamaiunova (ELI Beamlines, Dolní Břežany)
	Metastable hydrates of CsCl discovered
	Ľubica Vetráková (Institute of Scientific Instruments, Czech Academy of Sciences, Brno)
	Spectroscopic and Microscopic Study of Freeze-Concentrated Solutions
	Lukáš Veselý (Masaryk University, Brno)
22:30	**Expected end of the session**

Wednesday 25 March 2026

Time	Programme
8:30	**Registration**
9:00	**Session IV: Cryogenic Fixation & Spectroscopy II.** Chairs: Thomas Burg & Dominik Heger
9:00	**Water in No Man's Land & Microsecond Time-Resolved Cryo-EM**
	Ulrich J. Lorenz (Laboratory of Molecular Nanodynamics, EPFL, Lausanne)
9:20	**Spectroscopy and Microscopy of Frozen Aqueous Solutions and their Sublimates**
	Dominik Heger (Faculty of Science, Masaryk University, Brno)
9:40	**Cryogenic Systems in Laser-Driven Particle Acceleration**
	Timofej V. Chagovets (ELI ERIC, Dolní Břežany)
9:50	**Femtosecond Structural Dynamic of Molecules and Biological Photoreceptors**
	Miroslav Kloz (ELI ERIC, Dolní Břežany)
10:05	**Panel Discussion: Current Trends and Challenges in Cryogenic Fixation & Spectroscopy**
10:25	**Coffee break**
10:40	**Session V: Cryogenic Biology** Chairs: Miroslav Kloz & Pavel Urban
10:40	**Role of the IIR C1 Commission and Cryobiology Activities at the Tissue Establishment of the University Hospital Hradec Králové**
	Jiří Gregor (Tissue Bank, University Hospital Hradec Králové)
10:50	**Potential Role of Electron Cryo-microscopy in Detection of Freezing and Thawing Damage in Cryopreservation of Cells, Tissues, and Organs: A Review**
	Pavel Měřička (Tissue Bank, University Hospital Hradec Králové)
11:05	**Physical events in cryopreservation and methods for their characterization**
	Ivan Klbik (Institute of Physics, Slovak Academy of Sciences, Bratislava)
11:20	**Principles of cell cryopreservation and working examples of STEM cell cryopreservation for clinical use**
	Yuriy Petrenko (Institute of Experimental Medicine, Czech Academy of Sciences, Prague)
11:30	**Concluding Panel Discussion: Grand Challenges in Cryogenic Microscopy**
12:00	**Workshop Conclusions and Outlook**
12:10	**Lunch**
13:00	**Official Opening Ceremony of the New UltraSTEM Laboratory**
13:00	**Inaugural Addresses & Introductory Talks**
13:50	**Live UltraSTEM Demonstration**
14:25	**Lab Excursions & Guided Tours**
	UltraSTEM inaugural demo: rotating cycles
	Parallel ISI Brno Tours: Cryogenic Technologies & Electron Microscopy
16:25	**Expected end of the inauguration**

	Thursday 26 March 2026
	Registered participants for industrial excursions to Brno companies:
8:20	Meeting point for those interested in an excursion to Brno companies at ISI Brno (workshop venue)
8:30	Shuttle Transfer: ISI Brno – Thermo Fisher Scientific
9:00	**Excursion at Thermo Fisher Scientific**
10:30	Shuttle Transfer: Thermo Fisher Scientific – TESCAN
11:00	**Excursion at TESCAN**
12:00	Bus TESCAN – ISI Brno (or hotel)

	Registered participants for excursions to ISI Brno labs (Thursday morning):
9:00	Meeting point for those interested in an excursion to ISI Brno labs (Thursday morning)
9:10	**Excursion at ISI Brno labs**
11:10	Expected end of excursions

	Registered participants for excursions to ISI Brno labs (Thursday afternoon):
13:20	Meeting point for those interested in an excursion to ISI Brno labs (Thursday afternoon)
13:30	**Excursion at ISI Brno labs**
15:30	Expected end of excursions

Abstracts

Cryogenic Electron Microscopy in Life Sciences

EM and CryoEM in structural biology

Richard Henderson[1]

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There are two reasons for cryogenic specimen preparation for structure determination by cryoEM. The first is to preserve the biological structure in its near-native state, rather than using heavy metal stain and plastic embedding to observe the structure indirectly. Dubochet's plunge-freeze procedure of specimen preparation is still the dominant method though the use of high-pressure freezing followed by FIB-milling is increasingly popular. The second reason is to minimize the consequences of radiation damage so that better quality images can be obtained. For specimens at liquid nitrogen temperature (80 K) compared with room temperature (300 K), there is a roughly 5-fold reduction in the rate of loss of structural features due to radiation damage, with a further smaller gain at even lower temperature. Specimens are typically frozen and kept cold using liquid ethane, liquid nitrogen or liquid helium, but there is increasing interest in using a Gifford-McMahon cryostat.

What is the best temperature for electron cryomicroscopy of biological specimens?

Christopher J. Russo[1]

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A decade on from the "resolution revolution", electron cryomicroscopy (cryoEM) has become the dominant technique for determining the structure of biological macromolecular complexes. Electron diffraction measurements of protein crystals at liquid helium temperatures show reduced damage when compared to liquid nitrogen. In cryoEM, there have been numerous attempts to reduce the effects of radiation damage by cooling the specimen beyond liquid-nitrogen temperatures, yet all failed to realize the potential improvement for single-particle structure determination. We recently found that this was due to the complex interaction of the electron beam with the specimen, including the reduced movement of radiolytic molecular fragments at low temperatures. Using this knowledge, we were able to eliminate the previous problems with imaging at liquid-helium temperatures and obtain structures where every micrograph was better than the equivalent using liquid-nitrogen cooling. With this in mind, we have now set out to understand what the optimal temperature for structure determination might be, and in effect, how low is low enough. This will then enable a new generation of electron cryomicroscopes to be constructed that minimize the effects of radiation damage and allow smaller and more difficult structures to be determined.

High-resolution Cryo-FIB-SEM volume EM enables sub-volume averaging of cellular structures with CLEM targeting

Pavel Krepelka[1], Jana Moravcova[1], Zuzana Trebichalska[2], Elena Buglakova[3], Maria Rosario Fernandez-Fernandez[4], Jiri Novacek[1]

[1] CEITEC, Masaryk University, Brno, Czech Republic
[2] Masaryk University, Brno, Czech Republic
[3] EMBL Heidelberg, Heidelberg, Germany
[4] IBBTEC-CSIC. Santander, Cantabria, Spain

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Conventional volume electron microscopy (volume EM) enables three-dimensional imaging of cellular architecture but typically relies on chemical fixation, dehydration, and heavy-metal staining, which can distort biological structures and limit achievable resolution. Cryo-volume electron microscopy (CVEM) overcomes these limitations by imaging vitrified specimens in a near-native state, preserving cellular ultrastructure without staining.

We present an experimental and computational workflow for high-resolution cryo-FIB-SEM volume imaging of vitrified cells and tissues. The approach combines optimized imaging geometry, ion-beam selection, and a dedicated data-processing pipeline to improve signal quality and reconstruction fidelity. To enable robust targeting of regions of interest within complex specimens, the workflow integrates cryogenic correlative light and electron microscopy (CLEM). In addition, we employ the waffle method to prepare thicker cellular samples, allowing improved milling stability and expanded sampling of cellular volumes.

Using this workflow, we achieve ~15–20 nm isotropic resolution in vitrified cells and tissues with sufficient contrast to enable sub-volume averaging directly from unstained cryo-volume EM datasets.

From Atoms to Cells: Cryo-EM and Cryo-FIB Enabling In Situ Structural Biology

Radovan Spurný[1]

[1] Thermo Fisher Scientific, Brno, Czech Republic

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This talk provides an overview of modern cryo-electron microscopy (cryo-EM), spanning single-particle analysis through cellular cryo-electron tomography (cryo-ET). It highlights how cryo-focused ion beam (cryo-FIB), including plasma-FIB, enables preparation of thin cryogenic lamellae required for in situ structural biology and high-quality tomographic data. Key steps of end-to-end workflows will be discussed—from vitrification and targeting (including correlative approaches) to FIB milling, data acquisition, and visualization/analysis—along with practical considerations for automation, throughput, and typical life-science and drug-discovery applications.

Correlative CryoSEM for Structural and Chemical Identification in Hydrated Frozen Samples

Marie Vancová[1], Jana Nebesářová[1], M. Fluchs[1]

[1] Biology Centre CAS, České Budějovice, Czech Republic

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Electron microscopy provides high-resolution imaging of biological structures in a near-native state, but morphology alone often does not allow unambiguous identification. This is especially true for targets for which specific labeling probes are unavailable. To address this, we correlate ultrastructural information with complementary chemical and/or optical readouts acquired from the same hydrated frozen sample. At the Laboratory of Electron Microscopy, this correlation is enabled by a cryoSEM system that integrates fluorescence imaging, cathodoluminescence, EDS, and Raman spectroscopy. We will illustrate the approach with applications such as the in situ identification of plastics, lipids, and carotenoids.

Cryogenic SEM at the Institute of Scientific Instruments of the CAS: Bridging Microbiology and Polymer Science

Kamila Hrubanová[1], Anna Havlíčková[1], Kateřina Mrázová[1], Martin Súkeník[2], Petr Sedláček[2], Vladislav Krzyžánek[1]

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[2] Faculty of Chemistry BUT, Brno, Czech Republic

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The transition toward sustainable agriculture requires innovative strategies to reduce dependence on inorganic fertilizers. Plant growth-promoting rhizobacteria such as Azotobacter vinelandii represent a promising approach due to their beneficial interactions with plant roots and their ability to produce extracellular alginate. In this study, bacterially produced alginate was cross-linked with Ca^{2+} ions to form a hydrogel encapsulating bacterial cells, creating a model system at the interface of microbiology and polymer science.

Low-temperature scanning electron microscopy was employed to investigate the ultrastructure of encapsulated cells and the surrounding polysaccharide matrix. The cryo-SEM infrastructure at the Institute of Scientific Instruments of the CAS will be presented, including instrumentation and workflow for high-pressure freezing, freeze substitution, resin embedding, and STEM imaging.

The influence of freeze-substitution solvents on hydrogel preservation will be discussed, highlighting how sample preparation parameters affect ultrastructural interpretation. These results emphasize the importance of optimized cryogenic workflows for reliable structural analysis of polysaccharide-based biomaterials.

Low-Temperature Physics & Cryogenic Instrumentation

Helium: an introduction to extraordinary properties of its cryogenic phases

Ladislav Skrbek[1]

[1] Faculty of Mathematics and Physics, Charles University, Prague, Czechia

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We introduce cryogenic gaseous, normal liquid and superfluid phases of both stable isotopes of helium – ^{4}He and ^{3}He – possessing extraordinary physical properties. They provide a variety of cooling methods reaching very low temperatures down to a milliKelvin range, serve as coolants for countless laboratory experiments and offer a playground for basic research utilizing them as an object of investigation for thermodynamics, fluid dynamics or cosmology. In particular, we discuss their potential use in cryogenic microscopy & spectroscopy.

Imaging and spectroscopy with a liquid He-cooled sample stage

Cameron W. Johnson[1], Michael T. Hotz[1], Benjamin Plotkin-Swing, Tracy C. Lovejoy[1], Ondrej L. Krivanek[1,2]

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The observation of working quantum materials and many other electron microscopy applications require the sample to be cooled to below liquid N2 temperature (77 K), and temperatures close to liquid He are beneficial for low-dose biological imaging [1].

We have therefore designed a liquid He-cooled sample stage for our UltraSTEM® and Nion HERMES® scanning transmission electron microscopes (STEMs). The design is based on a low-vibration liquid He cryostat attached to the sample chamber, with the cryostat tip cooled close to 4.2 K and coupled to the sample holder via flexible copper braids optimized for mechanical flexibility and high heat conduction [2].

Working with the stage has shown that it has excellent stability, with sample drift rates as low as 0.3 nm per minute, minimized shakes that allow 1 Å imaging, ability to reach temperatures < 9K at the sample, holding time >10 hours, and essentially unobservable ice contamination. Initial applications have focused on imaging, diffraction, and spectroscopy of materials with charge density waves, and on measuring the sample temperature by gain-loss spectroscopy.

[1] Dickerson JL et al., Proc. Natl. Acad. Sci. U.S.A. 122 (17) e2421538122.
[2] Johnson CW et al., Microscopy and Microanalysis 31 (7), 2025, 1377–1379

Cryogenic sample cooling in the TOMO and BIO microscopes in Forschungszentrum Jülich

Joachim Mayer[1], Rafal E. Dunin-Borkowski[1], Carsten Sachse[1], Hugo van Leeuwen[2], Eric Van Cappellen[3]

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The "ER-C 2.0" project for a "National research infrastructure for high-resolution electron microscopy" in Forrschungszentrum Jülich, Germany is based on five next-generation electron microscope platforms. Two of the new instruments are supplied by Thermo Fisher Scientific and allow the sample to be cooled to below 30 K with minimal sample vibration by using an innovative solid state cooler (a thermal battery), which acts as an intermediary between a compressor unit and the sample.

The first instrument (referred to as TOMO) is a double-Cs-corrected, monochromated, ultra-high-vacuum (S)TEM, which is combined in a single instrument with a fully functional co-located laser-equipped atom probe tomography capability, an exchangeable local electrode, magnetic-field-free imaging with 0.3 nm spatial resolution, pre-tilting and post-tilting of the beam for imaging in high electric fields, workflows for tomography using both (S)TEM and atom probe tomography, ultra-high-vacuum specimen transfer, additional ports and electron biprisms. It is intended to allow the evolution of the tip apex and the true electric extraction field to be observed during atom probe tomography.

The second instrument (referred to as BIO) is a double-Cs-corrected cryo TEM equipped with additional Cc correction of the objective lens, additional electron optical elements for novel phase plate designs, an electron biprism, autoloader-based sample loading for cryo transfer and a sample transfer workflow to a next-generation cryo plasma focused ion beam scanning electron microscope. It is intended to improve spatial resolution in cryo electron microscopy and enable studies of thicker samples using both Cc corrected imaging and novel tunable phase plate concepts.

Liquid Helium TEM Sample Holder: Swift Cool-Down and Long Holding Time

Denys Sutter[1], Joachim Dahl Thomsen[2], Peng-Han Lu[2], Hooman Hosseinkhannazer[3], Dominik Biscette[1], Rafal E. Dunin-Borkowski[2]

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Quantum materials host unique electronic and magnetic properties—including superconductivity, charge and spin ordering—predominantly observed at cryogenic temperatures [1,2]. While progress in cryogenic transmission electron microscopy (TEM) methodologies have led to the development of liquid nitrogen cooled side-entry sample holders and microscopes tailored to suit the demands of life sciences, the exploration of phase transitions within quantum materials typically necessitates temperatures with a base in the liquid helium (LHe) range [3].

LHe solutions for high-resolution imaging in electron microscopes have been developed with base temperatures as low as 1.5 K maintainable over a continuous five-hour timespan [4]. Until recently, commercially available LHe side-entry holders have been limited by considerable mechanical and

thermal instability, as well as short base-temperature holding times due to the limited cryogen storage capacity of the dewar attached to the holder.

We present recent innovations of an ultra-low-temperature LHe TEM sample holder, with 8 electrical contacts for biasing capabilities. From room temperature, a base temperature of 5.2 K (as measured adjacent to the sample) can be attained within one minute and sustained with a stability of +/- 2.5 mK for days. Here, we demonstrate our recent achievements in the latest LHe cryo-TEM setup and upcoming developments with MEMS devices for heating and biasing [5, 6].

1. Y. Zhu, Acc. Chem. Res. 54, 3518-3528 (2021).
2. A. M. Minor, P. Denes, D. A. Muller, MRS Bulletin, 44, 961-966 (2019)
3. R. E. A. Williams, D. W. McComb, S. Subramaniam, MRS Bulletin, 44, 929-934 (2019)
4. Y. Fujiyoshi et al. Ultramicroscopy, 38, 241 (1991)
5. Y.-H. Kim et al., Ultramicroscopy, 280, 114263 (2026)
6. J. D. Thomsen et al., preprint, arXiv:2601.13298 (2026)

Cryogenic conversion of room-temperature EM systems using custom LHe/LN_2 cooling technologies

Pavel Urban[1], Pavel Hanzelka[1], Kamila Hrubanová[1], Vojtěch Krutil[1], Vladislav Krzyžánek[1], Ivan Vlček[1]

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Cryogenic electron microscopy (cryo-EM) enables the observation of samples in a near-native state under high-vacuum conditions while significantly reducing radiation damage [1,2]. Conventional cryo-EM systems rely on liquid-nitrogen or liquid-helium cooling, but dedicated commercial instruments remain prohibitively expensive. A cost-effective alternative is to convert existing room-temperature EMs into cryo-EM systems by integrating custom cryogenic cooling technology. We present an overview of cryogenic technologies developed for both bath and flow cooling, enabling the conversion of room-temperature EMs into fully functional cryo-EM platforms [3]. These include an LN_2 Dewar with a cooled copper finger, an LHe/LN_2 flow cryostat with two heat exchangers, a low-loss vacuum-insulated transfer line with controlled flow, and a cryogenic sample holder with spring-loaded contacts and a thermally insulating InBallPad interface [4]. Using this instrumentation, we have converted several electron microscopes, including a Helios G4 FIB/SEM, a Magellan 400L SEM and the EM ACE 600 unit, achieving sample temperatures as low as 30 K and enabling research on biodegradable materials and biotechnologically relevant microorganisms.

Supported by TA ČR (SIGMA, TQ11000042).

[1] pnas.org/doi/10.1073/pnas.2421538122
[2] dx.doi.org/10.18462/iir.cryo.2025.0076
[3] dx.doi.org/10.18462/iir.cryo.2025.0064
[4] doi.org/10.1016/j.ijrefrig.2021.09.019

The Design of a Reverse Brayton Liquefaction and Refrigeration System Equipped with a Turbomachine for Small-Scale Applications

Vojtech Kohut[1], Luboš Polák[1]

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The increasing demand for refrigeration systems with small liquefaction or cooling capacities creates opportunities for the development of compact cryogenic systems based on the reverse Brayton thermodynamic cycle using turbomachinery (referred to as the Turbo-Brayton cycle). Such systems offer a promising alternative to conventional cryogenic technologies due to their simplicity, robustness, and good scalability.

Several configurations of this cycle can be applied. For small-scale applications, requirements such as low system complexity, simple design, ease of control, high reliability, and low maintenance lead to the configuration using a single turbomachine that performs both compression and expansion.

Such a design is mechanically simple and can satisfy the main operational requirements. Due to its good scalability, the system can be adapted to a very wide range of operational conditions. On the other hand, certain operational limitations of the turbomachine may affect the cycle efficiency.

This paper describes the basic principles, advantages, and disadvantages of this concept and presents the design of a system intended for the liquefaction of nitrogen boil-off vapours or for refrigeration at liquid-nitrogen temperature levels over several capacity ranges.

Cryo stage designs for 3-coincidental system

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Delmic has built a number of prototype tri-coincidental systems (fluorescence, ions and electrons), see: https://doi.org/10.7554/eLife.82891. A key challenge in making these systems was the design of the cryo stage. The design was complex – driven primarily by the required accessibility of the light microscope objective. The team also tried to develop methods that did not require traditional liquid nitrogen cooling. This presentation will give an overview of the different design considerations, the solution eventually chosen for the prototypes and the latest developments and insights.

Managing Cryogenic Sample Preparation Workflows for Cryo FIB applications

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Reliable cryo EM results critically relies on carefully controlled sample preparation workflows to preserve native structures and ensure reproducible, high quality imaging results.

Cryo FIB milling has become an essential technique for preparing electron transparent lamellae from vitrified specimens, particularly for cryo electron tomography. This presentation emphasis on vacuum cryo transfer Leica VCT 500 –based handling and the cryo sample preparation (vitrification) workflow prior to sample transfer into the Cryo- FIB.

Influence of condensed water and other contaminants on thermal absorptivity at cryogenic temperatures

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Low thermal absorptivity of metallic surfaces plays a key role in minimizing radiative heat load in cryogenic systems and cryo-electron microscopy. However, these favorable radiative properties can be significantly altered by the presence of condensed water and other common contaminants.

In this contribution, we investigate the impact of thin layers of water ice and selected technical contaminants on the thermal absorptivity of aluminium surfaces at cryogenic temperatures. Using a dedicated apparatus for measuring radiative heat transfer between parallel surfaces, contaminated samples were exposed to thermal radiation from a controlled source over a wide cryogenic temperature range. The studied contaminants included water originating from outgassing processes as well as substances typically encountered in vacuum environments.

Our results demonstrate that even small amounts of deposited material can substantially increase the absorptivity of otherwise low-emissivity metallic surfaces. These findings highlight the critical importance of contamination control in cryogenic environments and underline the relevance of surface cleanliness for accurate thermal management in cryo-electron microscopy and related cryogenic applications.

Cryogenic Fixation & Spectroscopy

Low-temperature fluidic microsystems for cryo-CLEM

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Correlative Light and Electron Microscopy (CLEM) is a powerful tool for investigating the connections between the structure and function of healthy and diseased cells. Performing CLEM on vitrified cells at cryogenic temperature (cryo-CLEM) is of particular interest, as the structure is preserved in a near-native state under these conditions. However, cryo-CLEM technology today suffers from two significant limitations. First, cryofixation by high-pressure freezing or plunge freezing interrupts live-cell imaging by additional sample preparation and transfers. Second, only low-resolution air objectives are available in cryogenic light microscopy. To overcome the first limitation, our group has introduced a microsystems-based cryofixation technology that allows cryofixation of thin samples (below ~20 µm) within fully operating microfluidic devices directly in the light microscope. Recently, we have been investigating applications of this approach to studies of cell biomechanics and cryopreservation. To address the second limitation, we designed a new type of light microscope and cryo-immersion objectives. I will describe recent advances towards integrating this system into a new immersion-based correlative light and electron microscopy workflow, which we termed cryo-iCLEM. We expect that, together, microfluidic cryofixation and cryo-iCLEM will be of particular interest for studying dynamic relationships between cell stimulation, function, and structure at the nanometer scale.

Ultra-rapid cryo-arrest directly during fluorescence live cell microscopy for high resolution fluorescence cryo-nanoscopy and microspectroscopy

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We have developed ultra-rapid cryo-arrest of cells directly during fluorescence live cell imaging. This enables to connect live cell imaging with high-resolution fluorescence micro- and nanoscopy as well as microspectroscopy of the cryo-arrested cells. The sample is cooled by a burst of liquid nitrogen pressurized by helium through a diamond heat exchanger. The superior thermal conductivity of the diamond thereby enables very high cooling rates. The sample is mounted on a standard microscopy slide, which enables high-resolution fluorescence micro- and nanoscopy. Theory and experiment have shown that this cryo-arrest circumvents fundamental resolution barriers, which are normally imposed by molecular motion and photobleaching on fluorescence microscopy. The resolution gain is general in all kinds of fluorescence microscopy. However, microspectroscopy or nanoscopy methods show an even higher gain due to I) longer acquisition time and II) advantageous photophysics and photochemistry under cryogenic conditions. A higher fluorescence quantum yield enabled better dynamic range in FRET-FLIM microspectroscopy, thereby molecular states on small structures such as intracellular vesicles could be determined with unprecedented precision. STED nanoscopy under high depletion laser power (>MW/cm2) was enabled by superior thermal dissipation over the diamond and dramatically reduced photobleaching under cryogenic conditions.

Microfluidics and Cryo-EM: A Powerful Partnership for New Experimental Approaches at the Nanoscale

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Sample preparation remains a major bottleneck in cryo-EM, particularly for scarce, heterogeneous, or sensitive specimens. Conventional blotting requires large sample volumes, exposes proteins to uncontrolled air–water interfaces, and integrates poorly with upstream biochemical workflows—limitations that become critical for low-input or fragile samples.

We present the cryoWriter platform, which couples microfluidic sample processing directly to cryo-EM grid preparation via capillary writing in nanoliter volumes. By controlling liquid layer thickness without blotting, cryoWriter enables direct transfer from biochemical manipulation to vitrification under defined conditions.

We demonstrate applications including preparation of fragile organelles, microfluidic isolation of endogenous or tagged protein complexes compatible with cell-free translation, single-cell lysis combined with high-resolution analysis of amyloid assemblies, and nanoliter-scale biochemical assays with structural readout.

This integration improves sample efficiency and establishes cryo-EM as an analytical platform linking biomedical and biochemical perturbations to structural outcomes, with implications for facility workflows and low-input structural biology.

Bridging Cryo EM and Volume EM: A Unified High Pressure Freezing Pipeline for Correlative Workflows

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Over the past decade, two major trends have reshaped the landscape of electron microscopy. Cryo electron microscopy has undergone a “resolution revolution,” recognized by the 2017 Nobel Prize; yet plunge freezing is now reaching its practical limits. Biological samples suffer from blotting induced surface tension, and cellular specimens frequently exceed the 1–2 µm thickness that can be efficiently vitrified. Consequently, an increasing number of projects now require high pressure freezing combined with large scale cryo FIB milling workflows[1–4].

In parallel, correlative light and electron microscopy (CLEM), which expanded rapidly in the 2010s, has evolved toward volume electron microscopy. Many advanced preparation protocols—initially developed for high pressure freezing and freeze substitution—must therefore be adapted to volume EM to enable high resolution biomorphological analysis of samples immobilized with state of the art cryogenic techniques[3–5].

At CryoCapCell, we have developed an integrated pipeline that begins with live imaging of samples maintained either in the CryoCapsule[6] or in an Autogrid. After live observation, samples are rapidly cryo immobilized by high pressure freezing in the HPM Live µ. Vitrified samples—already pre clipped in Autogrids—can be directly loaded into the TFS Autoloader for cryo EM analysis. Alternatively, samples

cultured in the CryoCapsule can be transferred to our newly developed benchtop, liquid nitrogen free freeze substitution system for embedding in R221 resin[7], enabling robust volume EM CLEM workflows.

Our advances on the resin based pipeline have recently been published[8], while development of the full cryogenic workflow is ongoing.

1. Kuba, J. et al. *J. Microsc.* **281**, 112–124 (2021).
2. Kelley, K. et al. *Nat. Commun.* **13**, 1857 (2022).
3. Tacke, S. et al. *J. Struct. Biol.* **213**, (2021).
4. Sibert, B. S. et al. https://doi.org/10.1101/2021.06.06.447251 (2021).
5. Hoffman, D. P. et al. *Science* (1979). **367**, (2020).
6. Heiligenstein, X. et al. *Traffic* **15**, 700–716 (2014).
7. Heiligenstein, X. & Lucas, M. S. *Front. Cell Dev. Biol.* **10**, (2022).
8. Heiligenstein, X. et al. *Scientific Reports* 2025 15:1 **15**, 43175- (2025).

Water in No Man's Land & Microsecond Time-Resolved Cryo-EM

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During vitrification, cryo-EM samples have to safely traverse so-called no man's land, where the supercooled liquid crystallizes so fast that a systematic characterization has remained elusive. Yet, this regime promises to hold the key to understanding the origin of water's many anomalies. We have recently been able to record the complete evolution of the diffraction pattern of water as it cools from room temperature to cryogenic temperatures. Our approach involves preparing water in no man's land by flash melting an amorphous ice sample and capturing a diffraction pattern with an electron pulse before crystallization occurs.

A twist on the flash melting approach allows us to observe protein dynamics. Our understanding of protein function has remained fundamentally incomplete, as observing proteins in action is not generally possible. My group has introduced microsecond time-resolved cryo-EM, a novel technique that enables such observations by flash melting and revitrifying cryo-EM samples. I will discuss the properties of our technique and present time-resolved observations that highlight its potential to advance our understanding of protein function. Finally, I will also describe a complementary approach for microsecond time-resolved cryo-EM based on time-resolved jet vitrification that we have recently introduced and that dramatically expands the range of dynamics that can be observed.

Spectroscopy and Microscopy of Frozen Aqueous Solutions and their Sublimates

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This study employs environmental scanning electron microscopy (ESEM) and UV-VIS spectroscopy (absorption and luminescence) to investigate frozen aqueous solutions, specifically focusing on the freeze-induced solute aggregation of 1.

The research addresses three primary areas:

- Physicochemical Dynamics in Pharmaceuticals: We characterize the structural and compositional changes induced by freezing, cooling, warming, and sublimation to elucidate the processes that influence protein stability during pharmaceutical manufacturing and lyophilization2-4.
- Cryospheric Environmental Science: We investigate fundamental processes occurring in the natural cryosphere, including the time-dependent morphological evolution of frozen seawater and the spatial distribution and density of saline particles following ice sublimation5-7.
- Surface Interface Phenomena: We propose a mechanistic link between the quasi-liquid layer (QLL) on ice surfaces and the precursor nanofilm—an ultrathin solution layer responsible for the lateral transport and spreading of dissolved salts.

1. Vesely, L., et al. Int. J. Pharm. 2024, 650, 123691.
2. Vesely, L., et al. Int. J. Pharm. 2026, 691, 126584.
3. Vesely, L., et al. Int. J. Pharm. 2025, 676, 125593.
4. Lay-Fortenbery, A., et al. J. Pharm. Sci. 2024.
5. Vetráková, L., et al. Atmos. Chem. Phys. 2023, 23, (7), 4463-4488.
6. Závacká, K., et al. Geophys. Res. Lett. 2022, 49, (8), e2021GL097098.
7. Vetráková, Ľ., et al. The Cryosphere 2019, 13, (9), 2385-2405.

Cryogenic Systems in Laser-Driven Particle Acceleration

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Laser-driven particle acceleration relies on the interaction of ultra-intense, ultrashort laser pulses with matter, generating dense plasmas that sustain electric fields far exceeding those of conventional accelerators. These extreme fields enable ion and electron acceleration over micrometer distances, providing a route toward compact particle and radiation sources. During the interaction, the irradiated region is rapidly ionized, forming an expanding plasma plume accompanied by particle emission and local destruction of the target material.

High-repetition-rate operation therefore requires target systems capable of rapid and reliable renewal. Cryogenic liquid and supercritical jets offer a continuous, debris-free interaction medium with high chemical purity and well-defined geometry. After each laser shot, the damaged volume is naturally replaced by fresh material due to the continuous flow, eliminating mechanical target exchange.

The cryogenic platform developed at ELI Beamlines employs a closed-cycle cryocooler and a temperature-controlled cell with micron-scale nozzles, enabling stable extrusion of nitrogen, argon, and krypton jets under vacuum conditions compatible with kHz laser systems. Precise thermodynamic control allows tuning of jet diameter and density, improving reproducibility and acceleration stability.

Femtosecond Structural Dynamic of Molecules and Biological Photo-receptors

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We specialize in femtosecond Raman spectroscopy to study ultrafast structural dynamics of cofactors in biological photoreceptors directly in solution. By exploiting resonance effects, we can detect changes in specific chemical bonds across entire proteins. Our work bridges ambient and cryogenic conditions, providing a critical complement to cryo-EM and crystallographic data, which are typically collected at cryogenic temperatures.

One of our flagship achievements is probing photoactivation of human blue and green cone opsins, based on extremely sensitive and scarce samples. While our collaborators focus on cryo-EM structures, we provide detailed dynamical insight into activation pathways, intermediates, and rates. We can track processes continuously from femtoseconds to seconds—spanning roughly twelve orders of magnitude—capturing both ultrafast events such as retinal isomerization (~200 fs) and slower processes like retinal dissociation that triggers G-protein signaling. This unified temporal approach reduces ambiguity compared to combining separate experiments and delivers a more consistent picture of biologically relevant dynamics.

Cryogenic Biology

Role of the IIR C1 Commission and Cryobiology Activities at the Tissue Establishment of the University Hospital Hradec Králové

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The International Institute of Refrigeration (IIR) is an independent intergovernmental organization and the only global body dedicated to gathering scientific and technical knowledge across all fields of refrigeration. Founded in 1908, it has built a worldwide network of leading experts.

The IIR is committed to disseminating refrigeration knowledge to improve quality of life while respecting the environment and considering economic needs.

The C1 Commission deals with cryobiology, cryomedicine, and health products. Electron cryomicroscopy may be a promising tool for generating evidence on the structural changes that occur during the freezing of cells and tissues.

The Tissue Establishment of the University Hospital Hradec Králové, founded in 1952 as one of the first facilities of its kind, produces and cryopreserves hematopoietic stem cells, donor lymphocytes, and starting material for CAR-T therapies. It also cryopreserves arterial and venous grafts, along with a broad range of connective tissues and bone fragments.

Potential Role of Electron Cryo-microscopy in Detection of Freezing and Thawing Damage in Cryopreservation of Cells, Tissues, and Organs: A Review

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Two ways assure survival of cells, tissues and organs after freezing:1. Equilibrium (slow) freezing in the presence of cryoprotectants; 2. Vitrification in the presence of cryoprotectants. In the first case, crystallization occurs in the extracellular space, while intracellular crystal formation is avoided. In the second case, crystallization is avoided during freezing; nevertheless, attention must be paid to preventing devitrification during thawing. Electron cryo-microscopy may contribute to more accurate detection of morphological changes encountered in these events. Experimental design should include both detection of damage immediately after thawing and possible damage repair after thawing. Equilibrium freezing in the presence of DMSO or DMSO-free media is still the most frequently used method of cryopreservation of cells and tissues. The cells shrink during freezing as water is pushed out by the osmotic pressure gradient. In freezing cell suspensions extracellular ice does not cause cell damage, while in freezing tissues extracellular ice causes detachment of cells from basement membranes and disconnection of intercellular junctions, which leads to separation of cell layers or of individual cells. In cryopreservation of organs vitrification approach prevents volume changes leading to ruptures of organs during freezing. The difficult step is warming of cryopreserved organs and prevention of devitrification, which may lead to fatal bleeding after transplantation.

Physical events in cryopreservation and methods for their characterization

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The freezing of biological samples is governed by complex physical processes that determine the success of cryopreservation. Central to these processes are phase diagrams of aqueous and biomolecular systems, defining ice crystallization, eutectic transitions, freeze-concentration, and the balance between crystalline and amorphous water. During slow freezing, extracellular freeze-concentration drives osmotic dehydration, limiting intracellular ice but exposing cells to eutectic crystallization and high-salt stress. In contrast, vitrification uses rapid cooling to suppress ice formation by trapping the system in a glassy state, typically requiring high concentrations of cryoprotective agents with potential toxicity. Both cooling and heating rates are critical to prevent recrystallization during thawing. Cryoprotective agents depress the freezing point, reduce ice formation, inhibit salt crystallization, influence glass transition, and limit salt freeze-concentration. These phenomena are studied using differential scanning calorimetry, cryomicroscopy, Raman and infrared spectroscopy, dielectric spectroscopy, and cryo-electron microscopy, which together reveal thermal, structural, and dynamical aspects of freezing. These principles are essential for cryopreservation, freeze-drying, food science, and structural biology.

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Principles of cell cryopreservation and working examples of STEM cell cryopreservation for clinical use

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Cryopreservation offers unique opportunities in biomedicine by enabling long-term storage and off-the-shelf availability of living cells, tissues, and tissue-engineered constructs for both research and therapeutic applications. The lecture will provide a comprehensive overview of cell cryopreservation, starting from fundamental principles and extending to practical implementation in clinically compliant settings. Core topics will include general cryopreservation protocols, the mechanisms of action, benefits, and toxicity of cryoprotective agents, as well as the critical importance of optimized cooling and thawing rates and appropriate post-thaw testing strategies. In addition to fundamental aspects, the lecture will address the development of clinical-grade cryopreservation protocols, showed by working examples involving multipotent mesenchymal stromal cells. Special attention is given to cryoprotectant-associated toxicity, strategies for dimethyl sulfoxide reduction, cell processing workflows, and assessment of post-thaw cell quality and functional potency. Together, these topics will provide an integrated view of how basic cryobiological principles are translated into safe cryopreservation approaches for clinical stem cell applications.

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Posters

Preparation of mammalian cells specimen for cryo-electron microscopy using the HPF "Waffle" method

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Cryo-electron microscopy (cryo-EM) enables a high-resolution insight into cellular ultrastructure. Requirement for acquisition and collection of high quality data is properly vitrified cellular specimen. However, sample preparation still presents challenges in thicker specimens as bigger cells or cellular clusters typically frozen using conventional plunge freezing method and may suffer with improper vitrification. Here we focus on the "Waffle" method [1], which combines application of sample onto a electron microscopy (EM) grid with a technique of high pressure freezing (HPF), that provides an advantage of proper vitrification of specimens thicker than 15um.

Rat pancreatic INS-1E cell suspension was fluorescently labelled and high-pressure frozen on EM grid, producing a 20–30 µm vitrified layer that was thinned to ~200 nm lamellae using cryo-focused ion beam milling (cryo-FIB) prior to cryo-EM imaging. A key step was the selection of a suitable cryoprotectant; 20% polyvinylpyrrolidone provided optimal osmotic protection and preserved cellular integrity, enabling proper vitrification.

We demonstrate the feasibility of preparing mammalian cell specimens on EM grids using the HPF "Waffle" method. Proper vitrification of cells was confirmed by successful preparation of cryo-FIB lamellae. Ice quality and structural preservation were verified by visualization of mitochondria and insulin granules, demonstrating the suitability of this approach for high-resolution cryo-EM analysis.

High-Quality Graphene for Advanced Electron Microscopy Applications

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High-quality graphene grids represent a promising platform for transmission and scanning transmission electron microscopy (TEM/STEM). Owing to their atomic thickness, high electrical conductivity, mechanical strength, and exceptional transparency to the electron beam, graphene significantly reduces background scattering, improves signal-to-noise ratio, and enhances image contrast compared to conventional amorphous carbon supports.

Graphene grids enable high-resolution imaging of low-contrast and beam-sensitive specimens, including nanomaterials and biological structures. In addition, graphene can serve as a protective layer, limiting beam-induced damage, contamination, and oxidation of sensitive materials. Well-defined graphene membranes may also function as reference samples for instrument calibration and resolution benchmarking.

A critical parameter for practical implementation is surface wettability. Intrinsically hydrophobic graphene can complicate sample preparation, particularly in cryo-EM. Controlled surface modification

(e.g., plasma treatment, UV/ozone exposure, or chemical functionalization) enhances hydrophilicity, enabling uniform sample spreading and reproducible vitrification. Optimizing wettability is therefore essential for minimizing artifacts and achieving reliable, high-quality data.

Cryogenic fluorescence microscopy – not only CLEM. Example of Cryo-FLIM sensing

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Optical microscopy at cryogenic temperatures is often used to support electron microscopy: providing overview, checking sample quality, or locating fluorescent labels for correlative light–electron microscopy (CLEM). We show that cryogenic fluorescence microscopy can also report properties of the sample just before freezing.

Using a confocal microscope upgraded for fluorescence lifetime imaging (FLIM), we demonstrate membrane sensing with Flipper-TR both at 310 K and below 100 K. The probe conformation is preserved during vitrification and reflects membrane lipid packing.

FLIM benefits from cryo conditions that allow long acquisitions with improved photon statistics and minimal photobleaching. Cryo-FLIM remains a rare technique with largely unexplored potential. It can complement fluorescence specificity used in CLEM with information on molecular-level organization beyond the reach of electron microscopy.

This approach enables detailed study of cryo-arrested cells and provides a sensitive measure of how well samples were preserved during vitrification. We present current results and invite discussion and collaboration.

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Sample chamber for LHe-Insitu-TEM investigations

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Cryogenic Transmission Electron Microscopy (TEM) and Electron Energy Loss Spectroscopy (EELS) explores structural and electronic properties of low temperature states of matter such as superconductors, chiral magnetic textures, and charge density waves [1,2]. Here, we present the extension of a probe-corrected Hitachi HF3300S 60-300 kV TEM with an in-situ sample stage module that facilitates sample motion in six degrees of freedom, cooling below 10K, four-contact electrical biasing as well as magnetic field-free environment (Fig. 1). The incorporated SmarAct tripod stage enables sample movement over several millimetres and rotation around three independent axes over ±10° with a nominal precision of appr. 1nm and 1µrad, respectively [3]. A continuous-flow LHe cryostat (ColdSpot Tmin=1.7 K) in conjunction with counter-heating near the sample realizes a temperature range from room-temperature down to less than 10K. The system ensures full high-vacuum (HV) compatibility as well as X-ray safety shielding and is equipped with a fast entry lock with a sample magazine and a vacuum transfer unit for

small turnaround times. The integration into the micro column provides cryogenic (S)TEM imaging with a spatial resolution of a few nanometres and EELS measurements with sub-eV energy resolution.

[1] https://doi.org/10.1021/acs.accounts.1c00131
[2] https://doi.org/10.1021/acs.nanolett.1c02146
[3] SmarAct: https://www.smaract.com

An LN_2 optical cryostat for UV–Vis spectroscopy of water ice and aqueous solutions

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Ultraviolet–visible (UV–Vis) spectroscopy is a valuable tool for studying chemical compounds in ice and aqueous solutions, particularly in processes involving freezing and lyophilisation, where molecular stability under low-temperature conditions is critical. We present a development version of an optical liquid-nitrogen (LN_2) cryostat designed for UV–Vis spectroscopy of water ice and aqueous samples in the temperature range 80–273 K. The cryostat is dimensionally compatible with the sample compartment of an Agilent Cary 5000 spectrophotometer and accommodates samples frozen in standard cuvettes or cylindrical tubes mounted in a copper sample holder. The cooled sample shaft is equipped with quartz optical windows and filled with nitrogen heat-exchange gas to ensure uniform thermal coupling. Fast temperature monitoring inside the samples is enabled by two thermocouples connected to a high-speed acquisition system operating at up to 1000 Hz. Initial tests, including UV–Vis absorption measurements of NaCl aqueous solutions between 83 and 273 K, confirm the functionality of the prototype. The final version of the cryostat will extend the temperature range to approximately 65–350 K and offer interchangeable optical fingers, providing a flexible alternative to commercially available cryostats.

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BarSerNew simulation software for designing thermal links in cryogenics

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Cryogenic instrumentation, such as thermal links or cold fingers need a fast and reliable simulation tool. BarSerNew software enables rapid prototyping in the early design stages with a simple GUI without the need for CAD geometry.

BarSerNew solves the steady-state one-dimensional heat-flow boundary problem by modelling the system as a chain of one-dimensional bar elements connected in series. User defined boundary conditions are temperatures at the ends, inward/outward heat fluxes along the system, and the ambient temperature (thermal radiation calculations).

BarSerNew is based on the BarOne1 and BarSer1 simulation programs [1] developed for MS-DOS. The original software lacked a GUI, access to detailed data export, data plotting and a built-in geometry calculator. BarSerNew includes all of these with reduced computation time.

The software was used to design a copper cold finger for an LN_2 Dewar vessel intended for the conversion of a SEM into a Cryo-SEM [2], followed by subsequent optimization. The temperatures at the tip of the cold finger predicted by the simulation for both the original and optimized designs are presented together with experimental verification. The simulation results show good agreement with measurements obtained on a functional prototype. These results confirm that BarSerNew is a useful tool for cryogenic system design. Supported by TA ČR (SIGMA, TQ11000042).

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[2] dx.doi.org/10.18462/iir.cryo.2025.0064

TITAN upgrades by a probe corrector and a spectrometer with DED cameras: New horizons for TEM in the Brno region

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Two major upgrades have been performed on the CEITEC's monochromated and image corrected TITAN Themis to provide the community with a cutting-edge instrument:

1) Installation of an S-CORR, the latest TFS's Rose variant probe corrector, enabling compensation of the 5th-order spherical aberration (C5) and six-fold astigmatism (A5) across all accelerating voltages. Compared to the non-corrected system, STEM resolution (<50pm@300kV) and analytical performance in atomically resolved EELS/EDXS or DPC/COM imaging were significantly enhanced.

The S-CORR ability of C5 correction below 1 mm at 60 kV opened low-voltage HR-STEM with large convergence angles forming beam ~100 pm sharp. Together with monochromated beam, this opened HR-EELS (~100meV) of beam-sensitive materials at the atomic scale.

2) Installation of a GIF Continuum HR K3&STELA, the latest GATAN's filter with a unique configuration including direct single-electron detection and counting cameras, 100 ns electrostatic shutter, q-slit, In-Situ option, or SW precession. Compared to the previous GIF Quantum, this system opened unprecedented capabilities in EELS, EFTEM, 4D-STEM and EMD applications, especially for very beam sensitive materials. Thanks to the cameras' characteristics, fast shutter, and beam smart scanning, the system enables low-dose, low-beam damage, drift-controlled, and ultra-fast acquisitions at all acceleration voltages of the TEM.

The poster will present first achieved results.

Exploring the physics of plunge freezing through high speed videography

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Plunge freezing has been highly successful for producing vitreous ice specimens for cryogenic electron microscopy (cryo-EM). Despite this success, a detailed physical description of the fluid-structure interactions governing vitrification is still lacking. Here, we investigate these processes using subsurface high-speed videography to directly visualize the interaction between cryo-EM substrates and liquid ethane during cryogen entry and immersion. For conventional TEM grids, we observe the formation of a shallow, quasi-static air cavity pinned near the liquid surface, while the grid below the pinned contact line rapidly establishes and maintains direct contact with the cryogen. Combined with heat-transfer simulations, these observations show that conductive cooling through edge-only contact is insufficient to vitrify supported thin films at relevant rates, and that effective vitrification requires direct liquid contact and high interfacial heat flux. In contrast, microfabricated substrates and pre-clipped assemblies exhibit deeper, persistent air cavities with delayed closure, substantially reducing the extent and duration of liquid contact and thereby inhibiting heat transfer. By quantifying cavity collapse dynamics across substrate geometries, we identify a regime in which impact-induced inertia competes with curvature-driven capillary forces and wettability to control cavity persistence.

The Physics of Plunge Freezing: Revisiting Vitrification in Cryo-EM Sample Preparation

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Plunge freezing physics has remained underexplored in cryogenic electron microscopy (cryo-EM), largely because vitrification has appeared almost “magically” reliable since its introduction by Jacques Dubochet in the 1980s. Since then, developments have focused on technological refinement to improve grid preparation reproducibility, from early blotting-based systems such as the Thermo Fisher Scientific Vitrobot to more recent jet vitrification approaches such as VitroJet, emphasizing practical usability over fundamental physical understanding. Nevertheless, imperfect vitrification, devitrification, and grid variability remain persistent challenges, often addressed through empirical adjustments rather than first-principles insight.

We argue that a deeper physical understanding of plunge freezing can improve reproducibility. We investigate grid entry dynamics, including air-to-cryogen transition, fluid–grid interaction, transient boiling regimes, and heat transfer mechanisms governing cooling rates. We quantify cooling rates under different conditions, identify critical parameters affecting vitrification, and develop a physical framework that provides guidance for diagnosing unsuccessful vitrification due to suboptimal cooling.

By dissecting the coupled hydrodynamic and thermal processes, we aim to establish a coherent physical basis for cryo-EM vitrification and offer practical guidance to improve grid preparation reliability.

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

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This study introduces a novel microfluidic chip developed for the high-pressure freezing (HPF) of biological samples using liquid nitrogen. The device is constructed from Polydimethylsiloxane (PDMS) layers and features tailored microchannels for precise fluid manipulation. To ensure highly efficient heat transfer during vitrification, the chip incorporates a sapphire or copper base alongside dedicated ports for seamless sample embedding.

By utilizing HPF, this method allows for the rapid vitrification of samples up to 600 µm in thickness without detrimental ice crystallization [1]. To evaluate freezing efficiency and ultrastructural preservation, we process vitrified samples via freeze substitution. Samples are then embedded in resin, cut into ultrathin sections, and observed via electron microscopy.

These results demonstrate structural preservation, paving the way for the project's primary goal: enabling direct, in-situ multi-modal analysis using cryogenic focused ion beam scanning electron microscopy (cryo-FIB-SEM) and Raman micro-spectroscopy. A key application includes investigating microorganisms producing polyhydroxyalkanoates (PHAs), a sustainable alternative to conventional petrochemical plastics [2].

[1] Mejia, Y. X., et al.: Lab on a chip, 14 (2014), 3281-3284.
[2] Anjum, A., et al.: Int. J. Biol. Macromol., 89 (2016), 161-174.

Quantifying liquid film propagating from a solid frozen sample

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One of the key properties of liquids is their ability to spread. Prior to macroscopic spreading, a thin precursor film is emitted from the liquid droplet. The existence of this nm-thin, effectively “invisible”, film was already postulated in the 18th century by Benjamin Franklin in his famous oil drop experiment. Early studies primarily focused on film spreading on liquid substrates, where the process was easier to observe.

Later, attention shifted to spreading on solid surfaces, where direct observations of the nanometric film using techniques such as TEM or ellipsometry confirmed the earlier assumptions. However, these direct methods are often experimentally demanding, prone to significant uncertainty and unusable for frozen samples. As a result, indirect approaches remain widely used. Although they do not capture the dynamics of spreading, they provide reliable information about the final state of the system.

In this work, we employ an ex-post indirect method to detect the presence of a precursor film through its remnants. A CsCl solution droplet was frozen outside of the microscope, transported in the cryo-chamber and left there, allowing the film to spread from the droplet‘s perimeter. Subsequent sublimation of ice revealed salt crystals deposited several milimeters beyond the original droplet boundary, providing clear evidence of precursor film formation. Moreover, by counting the particles, we assessed the „concentration“ and distribution of salt in the film.

Design and Performance Characterization of a Cryoliquid Target System for kHz Laser–Plasma Applications

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Laser-driven particle acceleration uses ultra-intense ultrashort laser pulses interacting with matter and requires renewable high-purity targets for high-repetition-rate operation. Liquid targets are well-suited due to fast renewability and shot-to-shot reproducibility [1]. Among them, cryoliquid targets offer continuous, high-purity, debris-free source of accelerated particles and radiation, which is crucial for secondary beam quality and compatibility with sensitive optics [2].

The ELI BL cryotarget system enables stable extrusion of high-purity, reproducible cryoliquid flow for kHz laser operation. The setup is based on closed-cycle helium-free cryocooler and custom condensation cell operating down to 4 K. The cold head inside a helium exchange gas volume ensures low vibration and efficient thermal coupling to cold plate supporting brass or copper cell. The cell exchangeable nozzle with apertures from 5 to 25 µm defines the liquid jet diameter.

Cryojets of nitrogen, argon, and krypton are already obtained. Current efforts focus on optimizing the extrusion performance. For instance, stable 10 µm nitrogen column jet with adjustable speed of 5–20 m/s has been achieved at 70 K and 0.5–7 bar, while chamber pressure of 10^{-4} mbar is suitable for laser-driven ion acceleration. Ongoing work focuses on expanding the range of liquefied gases [3] and minimizing jet spatial fluctuations.

[1] doi.org/10.1117/12.2665607
[2] doi.org/10.3389/fphy.2021.754423
[3] doi.org/10.1063/10.0012652

Metastable hydrates of CsCl discovered

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Ice plays a crucial role in our environment. Not only it serves as a vital habitat for polar wildlife and helps regulate the climate, it also acts as a reservoir for anthropogenic pollutants and other chemicals. These chemicals can accumulate, be stored, undergo (photo)transformation into other species. Understanding these processes requires detailed knowledge of the ice morphology and how it varies based on the freezing method used.

We studied the low-temperature behaviour of concentrated aqueous solutions of cesium chloride. Although the literature data show that cesium chloride does not form any hydrates,[1] we discovered the existence of two CsCl hydrates that were formed upon cold-crystallization of fully vitrified hyperquenched solution.[2] We determine the composition of these hydrates to be $CsCl \cdot 5H_2O$ and $CsCl \cdot 6H_2O$ and find evidence for their existence in calorimetry, X-ray diffraction, and ESEM. Our findings contradict the current knowledge that alkali chlorides only have hydrates for the smaller cations Li^+ and Na^+, and pave the way for future determination of CsCl-hydrate crystal structures. The principles outlined here might be more generally applicable and found in nature, e.g., in comets or on interstellar dust grains, when glassy aqueous solutions crystallize upon heating.

1 Chen, L. et al. J. Phys. Chem. Lett. 11, (2020). DOI:10.1021/acs.jpclett.0c02046
2 Závacká, K. et al. ACS Phys. Chem. Au 5, (2025). DOI:10.1021/acsphyschemau.4c00093

Spectroscopic and Microscopic Study of Freeze-Concentrated Solutions

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The freezing of aqueous solutions causes water segregation in ice crystals and freeze- concentrated solutions (FCSs). The formation of FCS was shown to be coupled with freezing-induced acidity, polarity and aggregation changes. While all these processes contributed to an altered reactivity during freezing, the understanding of the underlaying mechanisms is still far from being complete. Aggregation is a fundamental process in chemistry, with molecules associating to form larger structures, commonly referred to as aggregates. We report on the freezing-enhanced aggregation of methylene blue with respect to the concentration of inorganic salts (NaCl, CaCl2), and sugars (glucose, sucrose, and trehalose). We detail the impact of the cooling rates and salts/sugars concentration, proposing that a low concentration or slow cooling rates lead to a several orders of magnitude increased aggregation than a high concentration or high cooling rates. Furthermore, we have observed a "sweet spot" of salt concentration, where the MB aggregates least throughout all studied salts. These results are rationalized by differences in the specific surface areas revealed via microscopic images. Our samples were analyzed via UV-VIS spectroscopy to examine the enhanced aggregation on molecular level and by means of environmental scanning microscopy to inspect the inner ice structures and contaminants.

How to get to the venue

Address: Královopolská 147, Brno 612 00

To access the workshop venue, please enter the parking area in front of the institute and walk through gate (A) to the backyard. You will pass a yellow house on your right and enter another parking area. Diagonally across this area, there is a large grey gate (B), which will be open.

Enter the building there and take the elevator to the second floor, where the workshop lecture hall is located. The elevator opens directly in front of the lecture hall, which will be on your right.