Newsletter of the Core Facility for Electron Microscopy – June 2020

Who we are

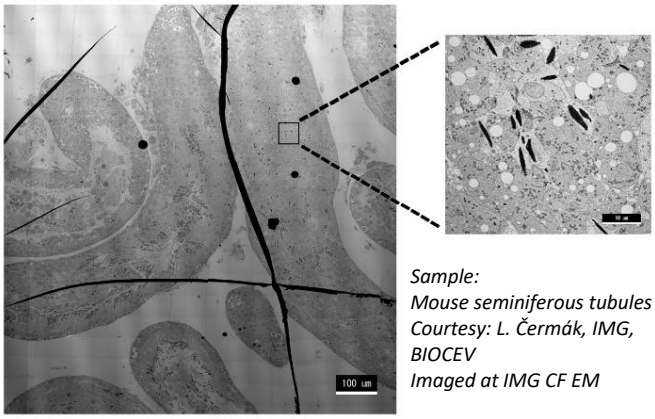
Our Core Facility is a part of the Czech-BioImaging and Euro-BioImaging research infrastructures. We offer a wide range of state-of-the-art electron microscopy instruments and techniques for analysis of biological samples to any user under the „open access“ policy. Our staff provides professional consultation services, sample preparation, data acquisition, image processing and analysis, as well as help with interpretation of the obtained results. We organize individual trainings for users, as well as annual one-week practical course of transmission electron microscopy for beginners.

What's new

Our facility underwent a full-scale reconstruction in 2019 aimed at modernization to meet the strict technical requirements for installation of two new transmission electron microscopes. The 120 kV instrument Jeol JEM-1400Flash is intended for fast and user-friendly routine imaging of sample ultrastructure and immunolabeling, while the advanced 200 kV instrument Jeol JEM-F200 "F2" allows for high resolution TEM, STEM, 3D electron tomography, cryo-electron microscopy, and EDS elemental analysis. To obtain high-quality data, the sample must be properly prepared, ideally in a hydrated close-to-the native state, and our facility is equipped for the whole range of current methods of biological sample preparation. The techniques include chemical fixation and cryo-fixation with Leica EM ICE high pressure freezing machine, plunge-freezing with Leica EM GP2, freeze fracturing and making of surface replica with Leica EM ACE 900, immunolabeling, cryo-substitution with two Leica AFS2 instruments and subsequent ultramicrotomy with Leica UC 6 at room temperature or in cryo conditions.

Technology highlight

A very useful new feature of our new instruments is a Limitless Panorama (LLP). It enables the user to capture a large-scale picture of the whole sample or its big part, consisting of thousands of high-magnification images seamlessly stitched together. This process if fully automated, easy to setup and provides an interesting opportunity to study small cellular components in the context of tissue or cell culture. It also greatly enhances the chance to spot rare cellular events. Individual areas of interest can be easily selected and exported from LLP at high resolution. Moreover, the LLP dataset allows us to track back the interesting features in the microscope and obtain additional images at different magnification, when needed.



Sample:
Mouse seminiferous tubules
Courtesy: L. Čermák, IMG, BIOCEV
Imaged at IMG CF EM

Selection of recent papers with contribution of our core facility

Bajzíková M. et al. (2019) Reactivation of dihydroorotate dehydrogenase-driven pyrimidine biosynthesis restores tumor growth of respiration-deficient cancer cells. *Cell Metabolism* 29: 1 – 18 (IF: 20.565)

Ganesh S. et al. (2020) The most abundant maternal lncRNA Sirena1 acts post-transcriptionally and impacts mitochondrial distribution. *Nucleic Acids Research* 48(6): 3211 – 3227 (IF: 11,147)

Fáberová V. et al. (2020) Super-resolution localisation of nuclear PI(4)P and identification of its interacting proteome. *Cells* 9(5): 1191. (IF: 5.656)

Kállai B. M. et al. (2019) γ-tubulin interacts with E2FA, E2FB and E2FC transcription factors, regulates proliferation and endocycle in Arabidopsis. *Journal of Experimental Botany* pii: erz498. doi: 10.1093/jxb/erz498. (IF: 5.360)

Krchlíková V. et al. (2020) Antiviral activity and adaptive evolution of avian tetherins. *Journal of Virology* 94(12): 416 – 420. (IF: 4.324)

Brzicová T. et al. (2019) Molecular responses in THP-1 macrophage-like cells exposed to diverse nanoparticles. *Nanomaterials (Basel)* 9(5): 687. (IF: 4.034)

Angelisová P. et al. (2019) The use of styrene-maleic acid copolymer (SMA) for studies on T-cell membrane rafts. *Biochimica et Biophysica Acta - Biomembranes* 1861: 130 – 141. (IF: 3.790)

Open Access

The Electron Microscopy Core Facility associated to the IMG Microscopy Centre is part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node. You can apply for open access on Czech-BioImaging or Euro-BioImaging webpages, or contact us directly.

Czech BioImaging open access form
Euro BioImaging open access form

Contact us

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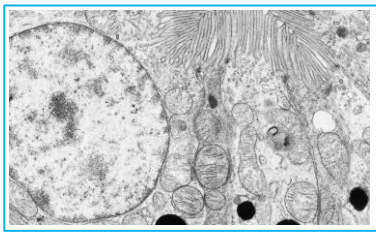
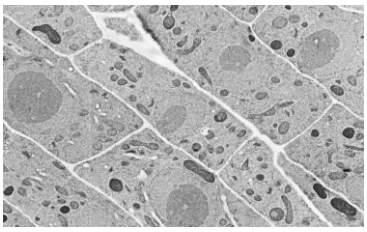
Our specialists are happy to help you with (not only ☺):



Ultrastructural morphology

Study of ultrastructural morphology is one of the most common classical procedures. For this purpose, samples are chemically fixed, usually with a mixture of glutaraldehyde and formaldehyde, followed by contrasting, dehydration, infiltration by epoxy or acrylic resin and subsequent polymerization in oven or under UV light. Ultra-thin sections of 70-90 nm are then observed after heavy metal contrasting.

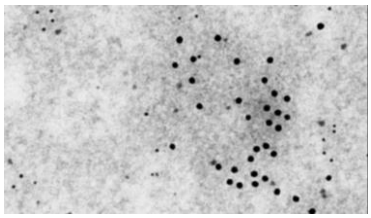
Sample: mouse kidney; courtesy: S. Novais, IBT, BIOCEV; imaged at IMG CF EM



Close-to-native sample preparation

With sensitive samples where chemical fixation may disturb the structure and antigenicity, we can take advantage of immediate cryofixation using the high pressure freezing machine Leica EM ICE. Under a very high pressure applied to the sample at the moment of freezing with liquid nitrogen, water inside the sample vitrifies without crystallization and fixes the sample without the need for any chemical. Subsequently, vitrified water within the sample is replaced gradually at cryo conditions by organic solvents and resin. This process, named cryo- or freeze-substitution, is carried out in automated Leica AFS2 machines.

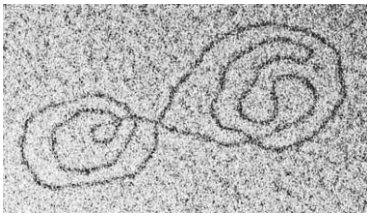
Sample: Arabidopsis root; courtesy: P. Binarová, IMIC; imaged at IMG CF EM



Ultrastructural immunodetection of macromolecules

Localization of specific biomolecules in the sample can be assessed by specific antibodies and visualized in TEM using metal nanoparticles coupled to primary or secondary antibodies. The use of nanoparticles with different sizes for different antigens allows us to visualize and clearly distinguish several types of biomolecules simultaneously and thus evaluate their mutual localization, clustering or compartmentalization. These data can be statistically analyzed with in house developed tools.

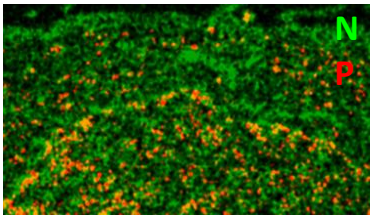
Sample: HeLa cell nucleolus, double immunogold labeling; courtesy: V. Filimonenko, IMG; imaged at IMG CF EM



Nucleic acid visualization

As the only laboratory in Czech Republic to date, we are equipped with state-of-the-art sputter coater Leica EM ACE 900. This machine allows to coat the surface of the sample with a very thin layer of platinum under vacuum while the sample itself is rotating around its axis in a set angle to platinum beam. This process is called rotary shadowing and greatly enhances the contrast of extremely small molecules, such as strands of DNA.

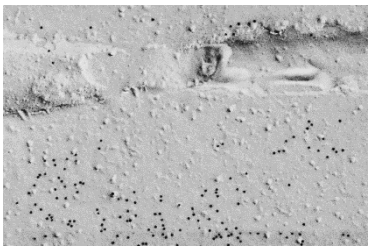
Sample: plasmid DNA; courtesy: I. Kalasová, IMG, O. Benada, IMIC; imaged at IMG CF EM



Mapping of elements in biological samples

Upon interaction of electron beam with the sample, multiple signals are produced. Some of these signals provide element-specific information. The 200 kV TEM Jeol JEM F200 is equipped with a highly sensitive SDD windowless detector of specific X-rays to map the elemental composition of the sample at any given point or area. The technique is useful e.g. for mapping protein or nucleic acid-rich regions in the cell (Figure), detection of nanoparticles, assessing the levels of specific elements in cells and organisms under various conditions.

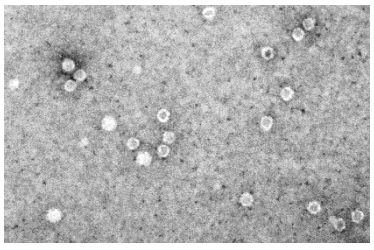
Sample: HeLa cell, mapping of proteins and nucleic acids; courtesy: V. Filimonenko, IMG; imaged at IMG CF EM



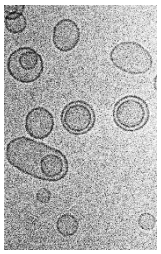
Imaging membranes and their composition

For studies of dynamic lipid-containing cellular structures, freeze-fracture replica immunolabeling technique is especially suitable. The sample is fast frozen in 20 ms in the Leica EM ICE between two carriers and split still in frozen state by a blade in the Leica ACE 900 in two parts. This effectively tears apart the sample along weak portions, typically membranes. The exposed surface is then coated with a layer of carbon and platinum, creating a replica. The replica will retain the attached biomolecules from the fractured surface which can be immunolabeled.

Sample: HeLa cell nucleus, immunolabeling with ani-PIP2 antibody; courtesy: Z. Lubovská, IMG; imaged at IMG CF EM

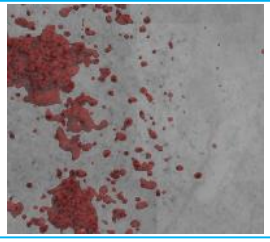


Characterization of macromolecular complexes



Isolated macromolecular complexes or other small objects, such as viruses or membrane vesicles, can be visualized directly without necessity of prior thin sectioning. A negative staining is a quick and simple method for evaluation of sample morphology and concentration. A more advanced technique, observation of frozen-hydrated samples, ideally preserves the native structure of biological objects. Samples are plunge-frozen in thin layer using Leica EM GP2 and directly observed in the electron microscopes equipped for operation in cryo-mode.

Samples: left: horse heart ferritin (Koch-Light); courtesy: O. Benada, IMIC; imaged at IMG CF EM
right: frozen liposomes; courtesy: M. Hof, UFCH JH; imaged at IMG CF EM



Volume information

Images obtained in transmission electron microscope represent two-dimensional projections of a three-dimensional object. To retrieve volume information, electron tomography can be used. Both of our transmission electron microscopes are equipped with tilting stage, special sample holders and software allowing for collection of a series of images of a sample at many different angles. From this series, high-resolution 3D reconstruction of the object is calculated. You can then slice through this model along X, Y or Z axis and see specific structure in its whole volume.

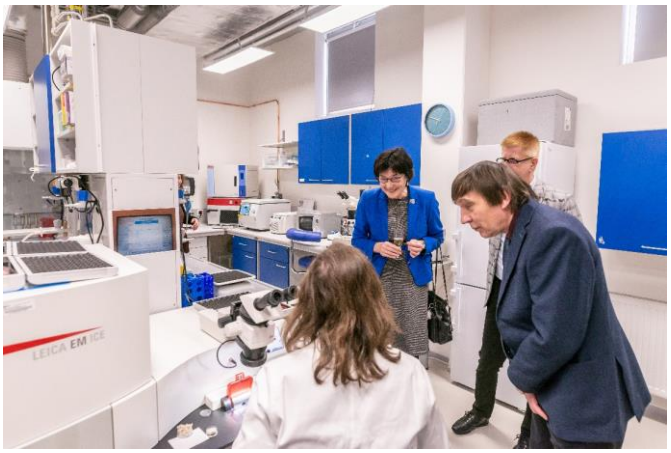
Sample: HeLa cell nucleus, pre-embedding immunolabeling of PIP2; courtesy: M. Sobol, IMG; imaged at IMG CF EM

Official opening after reconstruction

The official opening of the reconstructed Core Facility for Electron Microscopy at the Institute of Molecular Genetics of the Czech Academy of Sciences took place on the 27th of February 2020. The event began with short greetings from Director of the IMG Dr. Petr Dráber, Chair of the Czech Academy of Sciences prof. Eva Zažímalová, Director of the Czech BioImaging research infrastructure prof. Pavel Hozák, and President and General Director of the Jeol company Dr. Bruno Achard. After cutting the ribbon, the guests had an opportunity to see the facility premises and equipment with demonstrations from Core Facility and Jeol specialists. In the afternoon, a methodological seminar with introduction of new available technologies at the Core Facility was followed by facility tour for all interested.

Cutting the ribbon (left to right): Dr. Petr Dráber, prof. Eva Zažímalová, prof. Pavel Hozák, and Dr. Vlada Filimonenko.

Dr. Bruno Achard introducing the Jeol company vision in development of powerful instrumentation for life sciences during his opening speech.



Facility specialist Dominik Pinkas discussing workflow of sample preparation by high pressure freezing with prof. Eva Zažímalová, prof. Pavel Hozák and Dr. Petr Dráber.

Jeol application specialist Dr. Régis Ravelle Chapuis discussing advanced imaging possibilities of the new electron microscope Jeol JEM F200 with prof. Eva Zažímalová.

Our team (left to right):

Erik Vlčák, M.Sc. (TEM specialist)
Dominik Pinkas, M.Sc. (TEM specialist)
Vlada Filimonenko, Ph.D. (Head of the facility)
Ivana Nováková (Technician)
Lenka Pišlová (Secretary)

Funding: The extensive modernization of equipment was supported by OP RDE (CZ.02.1.01/0.0/0.0/16_013/0001775 "Modernization and support of research activities of the national infrastructure for biological and medical imaging Czech-BioImaging") with participation of IMG, and the reconstruction was fully financed by IMG. The activities of the IMG Core Facility for Electron Microscopy are supported from the program for large research infrastructures of the Ministry of Education, Youth and Sports within the project "National Infrastructure for Biological and Medical Imaging (Czech-BioImaging – LM2018129)".