NEWSLETTER of the Core Facility for Electron Microscopy – May 2021

CORE FACILITY UPDATE

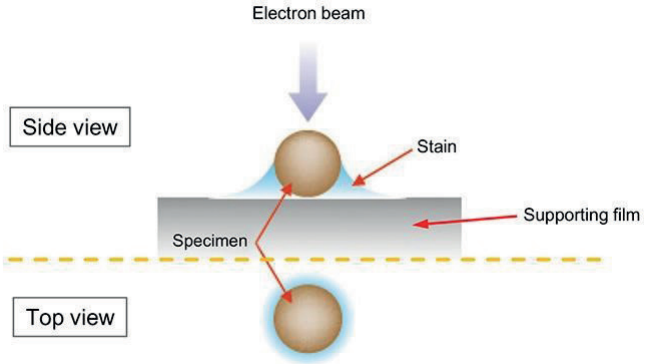
During these troublesome time of SARS-CoV-2 pandemic, we would like to inform you that our EM facility is fully operational, while implementing all necessary safety measures. As most of the projects are running in the full service mode, the workflow is not affected. Remote discussions with the users prove very efficient, and handing over the samples can be done with minimal contact. Feel free to contact us to ask for more details or arrange a meeting at any time. Since the last update, our small team has grown and gained a new pair of helping hands – our friendly colleague Helena Raabová, PhD., with years of experience (not only) in TEM of nanomaterials. Her scientific knowledge and manual skills will notably speed up the whole workflow of current and future projects.

As a major news of this issue, we are happy to announce that a fruitful collaboration between IMG, TESCAN and Leica microsystems has been established in the field of development of advanced cryo-methods for biological sample preparation and analysis with high resolution and excellent structural preservation. You can find more information on the collaboration in the recent press release, and we will devote the project a separate article in our next newsletter. To find out about new opportunities for your research provided on the equipment installed at IMG EM CF by the industrial partners, you are most warmly welcomed to attend the IMG-TESCAN-LEICA online opening seminar held on Wednesday, May 26th.

In this issue of the newsletter, you will further find a brief technical note concerning negative staining technique and its applications, a practical user guide to the facility access, and a list of provided technologies.

TECHNOLOGY HIGHLIGHT: NEGATIVE STAINING

Negative staining is a whole mount sample preparation technique that allows for electron microscopic observation of relatively small objects, such as macromolecules, subcellular components, membrane vesicles, viruses, and even bacteria, to name a few. This is a relatively fast and straightforward method, yielding the resolution down to 18-20 Å. Particles of a suspension are adsorbed onto the surface of a specimen support – a TEM grid covered with a thin carbon film. A drop of stain containing heavy metals is applied onto the specimen, incubated for a short time (seconds to minutes), removed with a filter paper, and the grid is dried in the air. The negative contrast is provided by a thin layer of stain surrounding the particles, which is electron dense, while the particles remain permeable for electrons and therefore have light appearance on the micrographs (see the scheme).



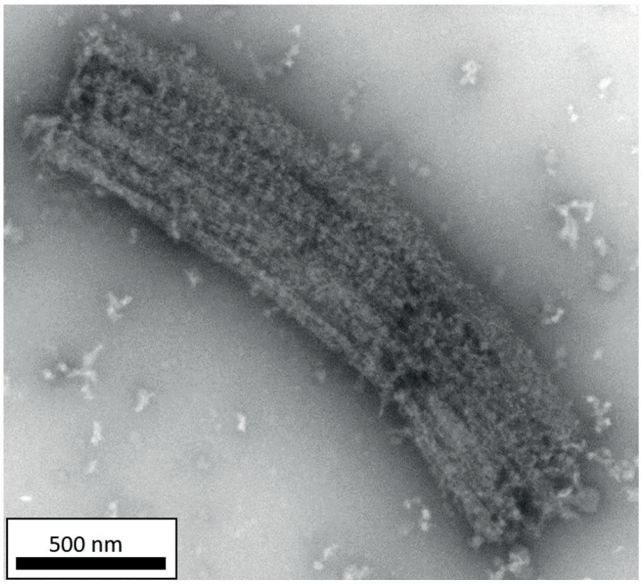
By this method, we can quickly evaluate the sample morphology and concentration, as well as study details of surface nanostructure. It is relatively sensitive to sample purity as it visualizes all the particles present in the sample, be it the object of interest or contaminants either from the original specimen or working solutions. The negative staining evaluation can be also used as a preliminary quality control preceding structural analysis of the sample by cryo-TEM.

While the method as such is quite straightforward, individual samples can behave in different ways under varying staining conditions. At the core facility, we have several well-established protocols for various kinds of samples. Usual amount of sample suspension required is 10 microliters.

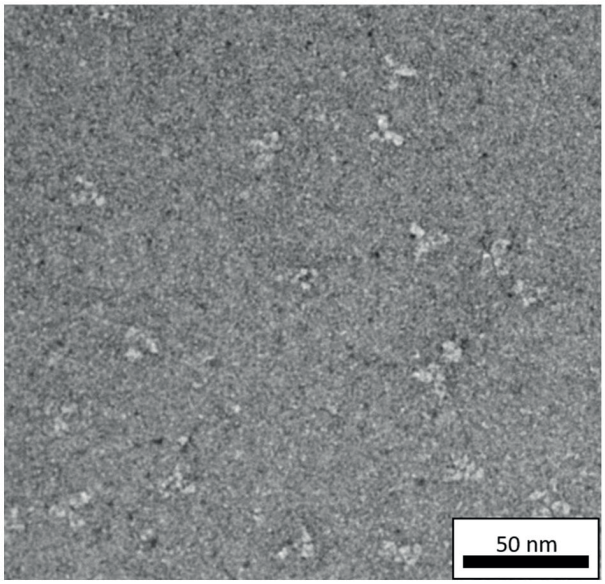
The sample is ready for observation the same or next day, examination of one sample takes half an hour up to a few hours depending on frequency of occurrence of structures of interest and absence or presence of sample contamination with other material.

You can see some examples of samples prepared and observed in our CF in the following images.

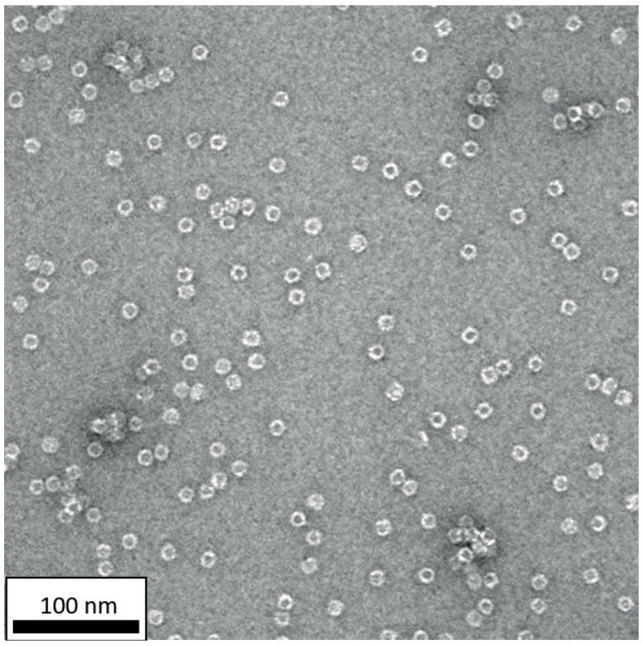
Isolated flagella.
Sample courtesy: L. Stepanek,
IMG; imaged at IMG EM CF



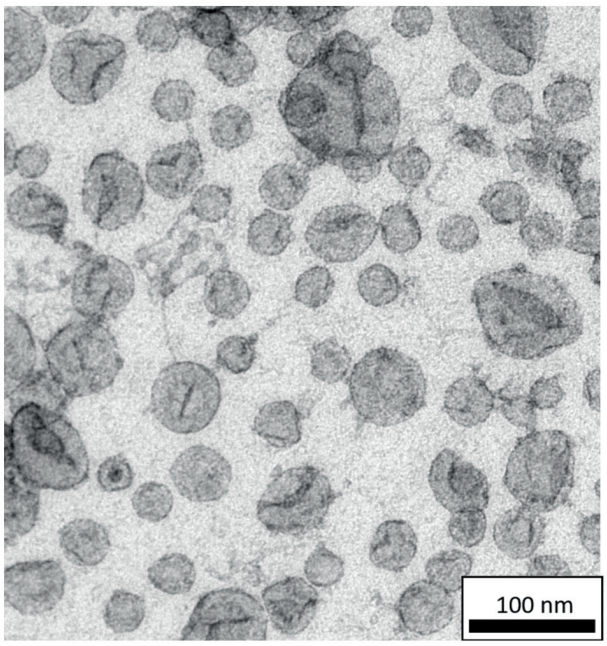
IgG molecules.
Sample courtesy: M. Sztacho,
IMG; imaged at IMG EM CF



Ferritin complexes
Sample courtesy: O. Benada,
IMIC; imaged at IMG EM CF

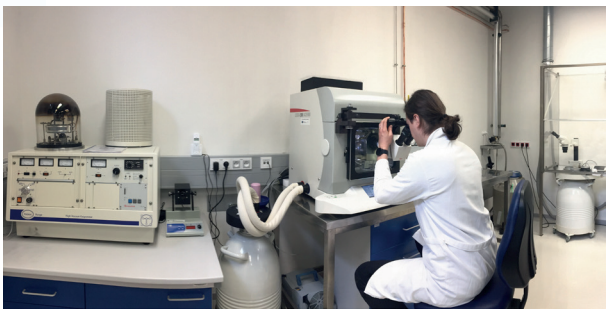


Exosomes.
Sample courtesy: M. Kolar,
IMG; imaged at IMG EM CF



USER GUIDE TO THE CORE FACILITY

We provide an open non-discriminatory access to our technologies and expertise via Czech BioImaging and Euro BioImaging infrastructures. To gain access to the core facility services, new users can use Czech-Bioimaging open access request form, EuroBioimaging technology finder, or contact us directly.



Workflow

All projects start with an initial meeting, where we discuss the aim of the project, specific features of the samples evaluate technical feasibility, and select the most appropriate technology. Currently, the prevailing mode of operation of our CF is performing the whole sample preparation and data collection procedures for the users by facility staff. We design the protocol of sample preparation, preform sample preparation, control the quality and collect first set of data for discussion. After discussion of the preliminary result with the user, we collect more data with the focus on the scientific task. We encourage the users to participate actively in data acquisition, public health situation permitting – come to the microscope session to choose the interesting objects together with the CF staff member, or operate the microscope themselves upon getting a training. We provide help with data interpretation and evaluation, preparation of figure plates, etc.

Training

Individual training is provided on request. Please contact the CF head V. Filimonenko. We also organize a yearly practical training course "Transmission Electron Microscopy in Life Sciences". Expected date of the next course is in September 2021, depending on the pandemic situation.

Instruments and technologies

The list of equipment with a brief description and key technical parameters is available here. The examples of available technologies are presented on the last page of this newsletter. **We are looking forward to seeing you at our Core facility!**

YOUR ELECTRON MICROSCOPY CF TEAM

Vlada Filimonenko, Ph.D. (Head of the facility)

Dominik Pinkas, M.Sc. (TEM specialist)

Erik Vlčák, M.Sc. (TEM specialist)

Helena Raabová, PhD (TEM specialist)

Ivana Nováková (Technician)

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COMING SOON

Technology highlight: elemental mapping by EDS
Collaborative project IMG-TESCAN-Leica
New instruments for sample preparation installed at the CF

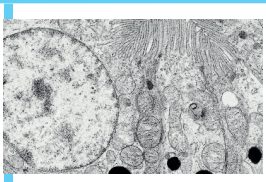
Webpage

The Electron Microscopy Core Facility is part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node.
Czech BioImaging open access form
Euro BioImaging open access form

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)".

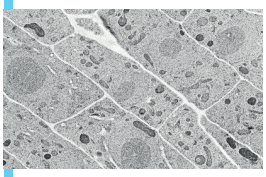
OUR SPECIALISTS ARE HAPPY TO HELP YOU WITH (NOT ONLY):



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.

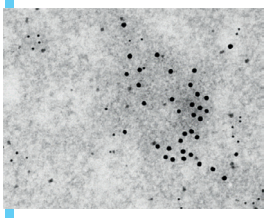
Sample: mouse kidney; courtesy: S. Novais, IBT, BIOCEV; imaged at IMG CF EM



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.

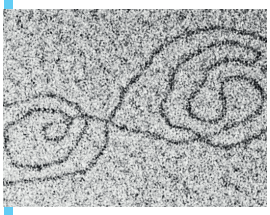
Sample: Arabidopsis root; courtesy: P. Binarová, IMIC; imaged at IMG CF EM



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.

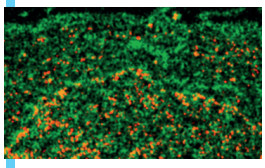
Sample: HeLa cell nucleolus, double immunogold labeling; courtesy: V. Filimonenko, IMG; imaged at IMG CF EM



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.

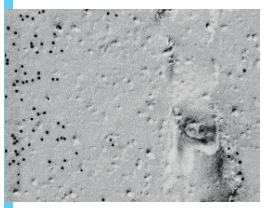
Sample: plasmid DNA; courtesy: I. Kalasová, IMG, O. Benada, IMIC; imaged at IMG CF EM



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.

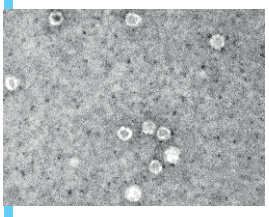
Sample: HeLa cell, mapping of proteins and nucleic acids; courtesy: V. Filimonenko, IMG; imaged at IMG CF EM



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.

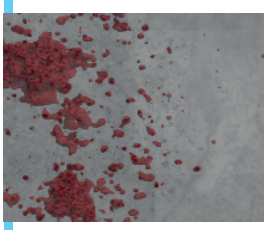
Sample: HeLa cell nucleus, immunolabeling with ani-PIP2 antibody; courtesy: Z. Lubovská, IMG; imaged at IMG CF EM EM CF



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 seriesof 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