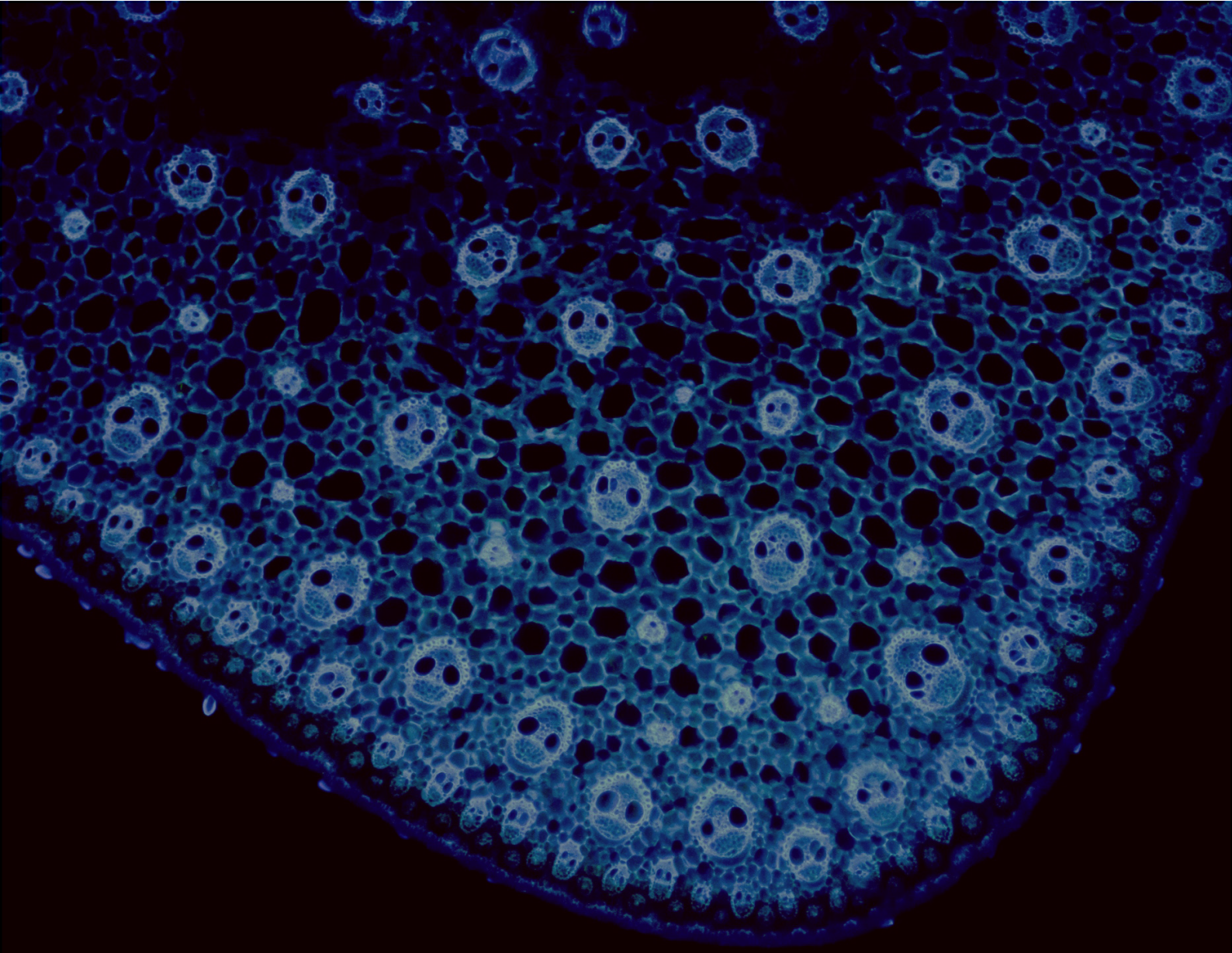

CZECH-BIOIMAGING NEWSLETTER

Issue 10 / Summer 2024

CZECH BIOIMAGING

Imaging principles of life

www.czech-bioimaging.cz

USER PROJECT HIGHLIGHTS

INVESTIGATION OF siRNA DELIVERY INTO CELLS BY CATIONIC POLYMER-COATED NANODIAMONDS

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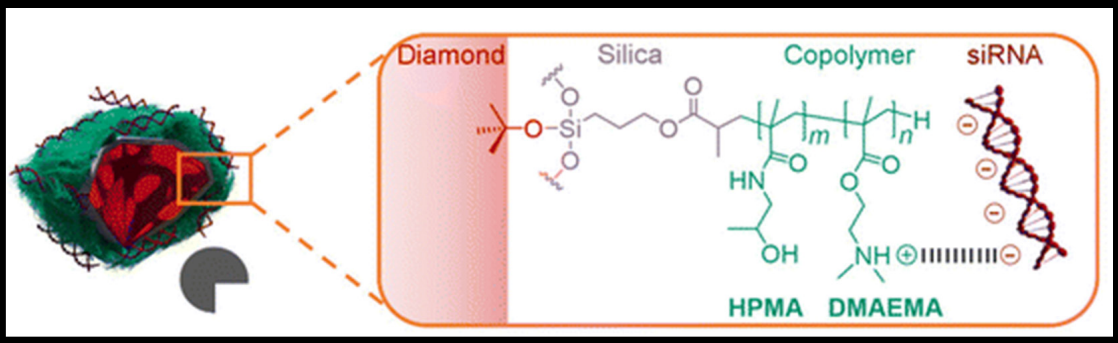
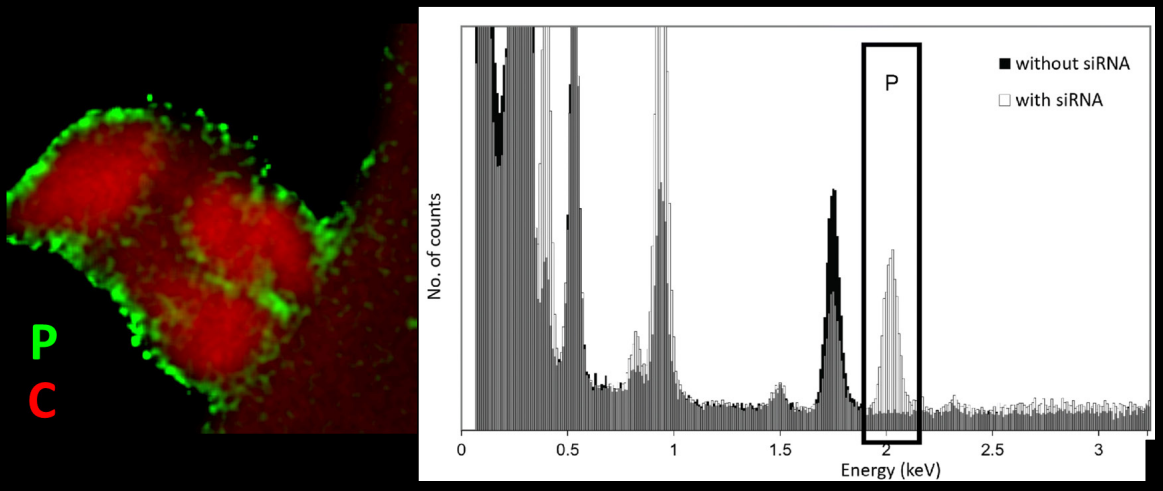
Gene silencing using small interfering RNAs (siRNAs) is a selective and promising approach for treatment of numerous diseases. However, broad applications of siRNAs are compromised by their low stability in a biological environment and limited ability to penetrate cells. Nanodiamonds coated with cationic polymers can enable cellular delivery of siRNAs. Recently, **Petr Cigler's group** at the Institute of Organic Chemistry and Biochemistry developed a new type of nanodiamond coating based on a random copolymer consisting of (2-dimethylaminoethyl) methacrylate (DMAEMA) and N-(2-hydroxypropyl) methacrylamide (HPMA) monomers. These hybrid nanodiamond-polymer particles overcome many of the previous limitations by exhibiting very low toxicity, forming stable complexes with siRNA in serum, and effectively delivering siRNA to cells in vitro and in vivo.

The objective of this study was to investigate the mechanism of cellular uptake and intracellular fate of nanodiamond-polymer complexes with siRNA. To this end, the complexes were prepared under standardized robust conditions and thoroughly characterized. To confirm the binding of siRNA to the copolymer layer, the elemental composition of the layer was analyzed using high-angle annular dark-field scanning transmission electron microscopy with energy dispersive X-ray spectroscopy (EDS). The data were obtained at the **Electron Microscopy Core Facility at IMG Prague** on a 200 kV transmission electron microscope Jeol JEM-F200 equipped with an EDS detector. The focus of the analysis was on the phosphorus content, as this element is present solely in siRNA, but not in the original polymer layer. The X-ray signal of phosphorus was successfully used to detect even a single layer of nucleic acid on a carbon-based support. The advantage of the electron microscopic analysis was the ability to visualize individual nanoparticles and details of their structure.





siRNA carrier complexes. *Top: schematic structure of the nanodiamond particle (red) with copolymer coating (green) and attached siRNA. Bottom: EDS elemental map of carbon in nanodiamond core and phosphorus in attached RNA; the graph shows comparison of spectra between particles with and without RNA.*

X-ray spectra were collected from each pixel of a STEM image and elemental maps were calculated based on the collected data. The core diamond particles are carbon-based and are surrounded by a layer of polymer containing a significant percentage of nitrogen. Correspondingly, no P signal was observed in control particles without siRNA. These results allowed us to confirm at nanoscale resolution that siRNA binds to the polymer layer and remains bound in Cop+-FND : siGAPDH under the experimental conditions used. Using these particles as a delivery platform, effective transport of siRNA into U-2 OS human bone osteosarcoma cells, was achieved, leading to a remarkable inhibition of targeted GAPDH mRNA.

Cellular uptake and fate of cationic polymer-coated nanodiamonds delivering siRNA: a mechanistic study
Jan Majer, Marek Kindermann, Dominik Pinkas, David Chvatil, Petr Cigler, and Lenka Libusova
Nanoscale, 2024,16, 2490-2503
DOI: 10.1039/D3NR05738K

WELCOMING NEW INTERNATIONAL ADVISORY BOARD MEMBERS

We are delighted to announce and welcome to our latest additions to the Czech-BioImaging International Advisory Board. They will accompany the current members in expert panels:

Light microscopy (LM)
Electron microscopy (EM)
Medical imaging (MI)
Data management (DATA)

Please join us in welcoming them. We believe that their experience and expertise will be instrumental in shaping the future direction of Czech-BioImaging and we are excited to have them on board.

- **Prof. Uwe Himmelreich**,
  Head of Biomedical MRI Research Group, Katholieke Universiteit Leuven, Belgium

- **Dr. Eija Jokitalo**,
  Electron Microscopy Unit (EMBI) Institute of Biotechnology, University of Helsinki, Finland

- **Prof. Karel Pacák**,
  National Institutes of Health (NIH), USA - ***joining in August 2024***

- **Prof. Klaus Qvortrup**,
  Core Facility for Integrated Microscopy, University of Copenhagen, Denmark

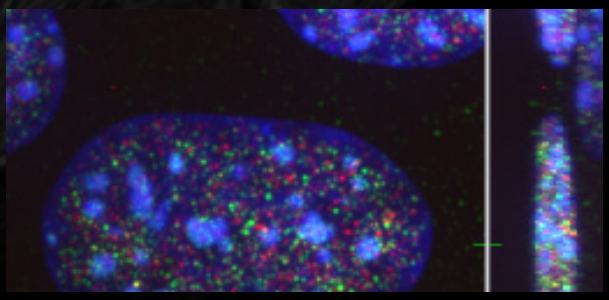
- **Dr. Paula Sampaio,**
  Advanced Light Microscopy, i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal

- **Dr. Sébastien Tosi**,
  Formerly:Head of the Danish BioImaging Infrastructure Image Analysis Core Facility, Denmark

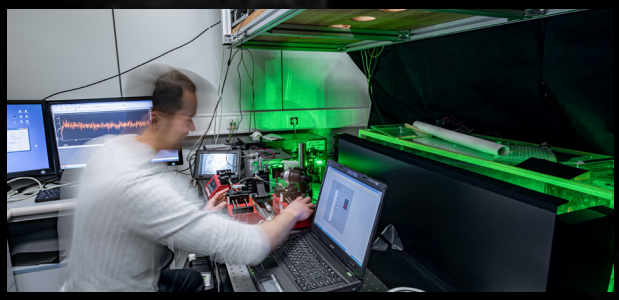
EURO-BIOIMAGING NODES UPGRADE

We would like to announce a successful Node upgrade, which was officially approved on May 22 by the Euro-BioImaging Board during their 10th meeting in Heidelberg.

BRNO NODE IS NOW UPGRADED BY:

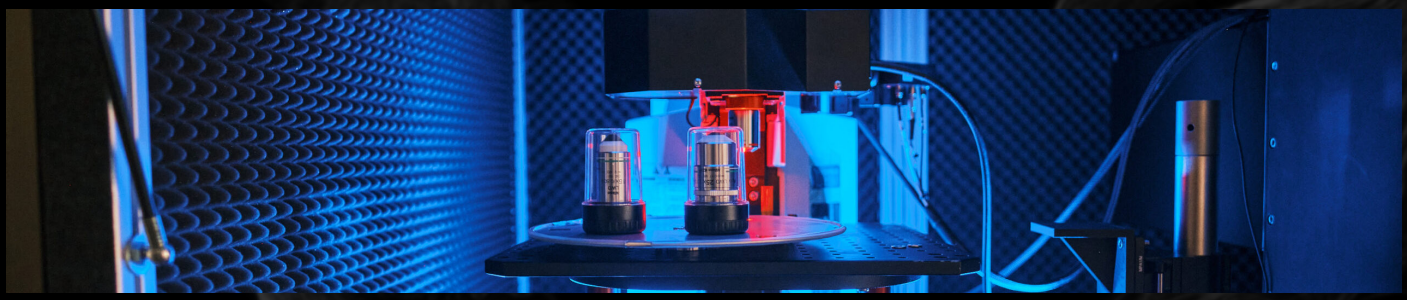


Centre for Biomedical Image Analysis (CBIA), MUNI



Core Facility Electron Microscopy and Raman Spectroscopy, Institute of Scientific Instruments, Czech Acad. Sci.

PRAGUE NODE IS NOW UPGRADED BY:



Microscopy Service Centre, Institute of Experimental Medicine, Czech Acad. Sci.

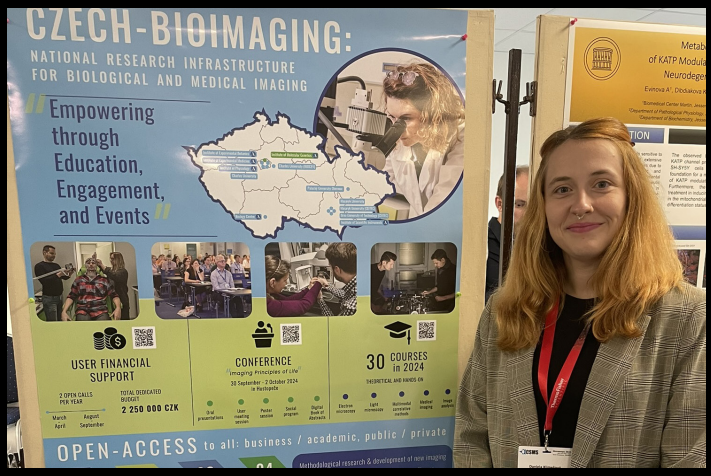
The upgraded Nodes are now available under the Euro-BioImaging open access and have been included in the Euro-BioImaging portfolio.

See all Czech Nodes at www.euro-bioimaging.com.

CZECH-BIOIMAGING AT THE EUROBIOIMAGING ALL HANDS MEETING AND THE CSMS MICROSCOPY CONFERENCE

Czech-BioImaging proudly participated in the annual Euro-BioImaging All Hands Nodes Meeting held in Torino, Italy, from May 22-24, 2024. This annual event gathers representatives from imaging facilities across Europe to share knowledge, discuss advancements, and strengthen collaborations within the imaging community. CzBI's presence was marked by insightful presentations from Ales Benda, the Scientific Representative of the Prague Node in the Panel of Nodes and Dominik Pinkas representing the IMG EM CF.

In addition to our participation in the EuroBioImaging meeting, CzBI was actively involved in the Microscopy Conference organized by the Czechoslovak Microscopy Society (CSMS) in Novy Smokovec, Slovakia, from May 26-30, 2024. This conference serves as a vital platform for microscopy professionals and researchers to exchange ideas, present their latest findings, and explore innovative microscopy techniques. Our team presented a variety of services including open access opportunities, educational courses, plans for acquiring progressive technologies, and available financial support options. We emphasized that our infrastructure is dedicated to supporting users. During the poster session, we presented a poster titled "Empowering Through Education, Engagement, and Events." The conference provided an excellent opportunity for networking with users and industrial representatives.



UPCOMING COURSES

AUG - NOV 2024

CHECK HTTPS://WWW.CZECH-BIOIMAGING.CZ/COURSES FOR UPDATES.

ADVANCED 3D IMAGE VISUALIZATION AND QUANTIFICATION
June 18-19, 2024 | Institute of Experimental Medicine of the Czech Academy of Sciences, Prague

FUNDAMENTALS OF LIGHT MICROSCOPY
June 18-20, 2024 | CELLIM CEITEC MU, Brno

IMAGE ANALYSIS AND DATA PROCESSING IN SUPERRESOLUTION MICROSCOPY
August 5-9 2024 | VMCF CUNI, Prague

ADVANCED MICROSCOPY METHODS IN BIOLOGY
September 10-12, 2024 | CELLIM CEITEC, Brno

ADVANCED COURSE ON PRECLINICAL IMAGING
September 16-20, 2024 | Joint course at the Institute of Experimental Medicine, Institute of Physiology and Institute of Scientific Instruments of the Czech Academy of Sciences

EXPERIMENTAL BIOPHOTONICS
September 26-27, 2024 | Brno University of Technology, Brno

MULTI-MODAL LIGHT MICROSCOPY IMAGING IN PLANT RESEARCH
October 16-17, 2024 | Institute of Experimental Botany of the Czech Academy of Sciences, Prague

NEUROIMAGING: MAPPING THE FUNCTION AND STRUCTURE OF BRAIN
November 11-13, 2024 | MAFIL CEITEC, MU, Brno

INTRODUCTION TO PROGRAMMING FOR IMAGE PROCESSING
November 13-15, 2024 | Institute of Experimental Medicine of the Czech Academy of Sciences, Prague

FLIM NOT ONLY FOR BIOLOGISTS, PRACTICAL ASPECTS WITH HANDS-ON EXPERIENCE
November 18-20, 2024 | BIOCEV, CUNI, Vestec

MECHANICAL CHARACTERIZATION OF BIOLOGICAL SAMPLES USING CORRELATIVE METHODS
November 19-21, 2024 | Institute of Physiology of the Czech Academy of Sciences, Prague

ANALYSIS OF MR SPECTRA WITH AMARES
November 22, 2024 | Institute of Scientific Instruments of the Czech Academy of Sciences, Brno

TRANSMISSION ELECTRON MICROSCOPY IN LIFE SCIENCES
November 25-29, 2024| Institute of Molecular Genetics of the Czech Academy of Sciences, Prague

UPCOMING USER MEETINGS AND CONFERENCE

We are excited to announce the upcoming Czech-BioImaging Conference Imaging Principles of Life (IPL 2024) scheduled for September 30 - October 2, 2024, in Hustopeče. This annual event is a cornerstone for scientists, researchers, and professionals in the field of bioimaging, offering a unique opportunity to share knowledge, explore cutting-edge technologies, and foster collaborations.

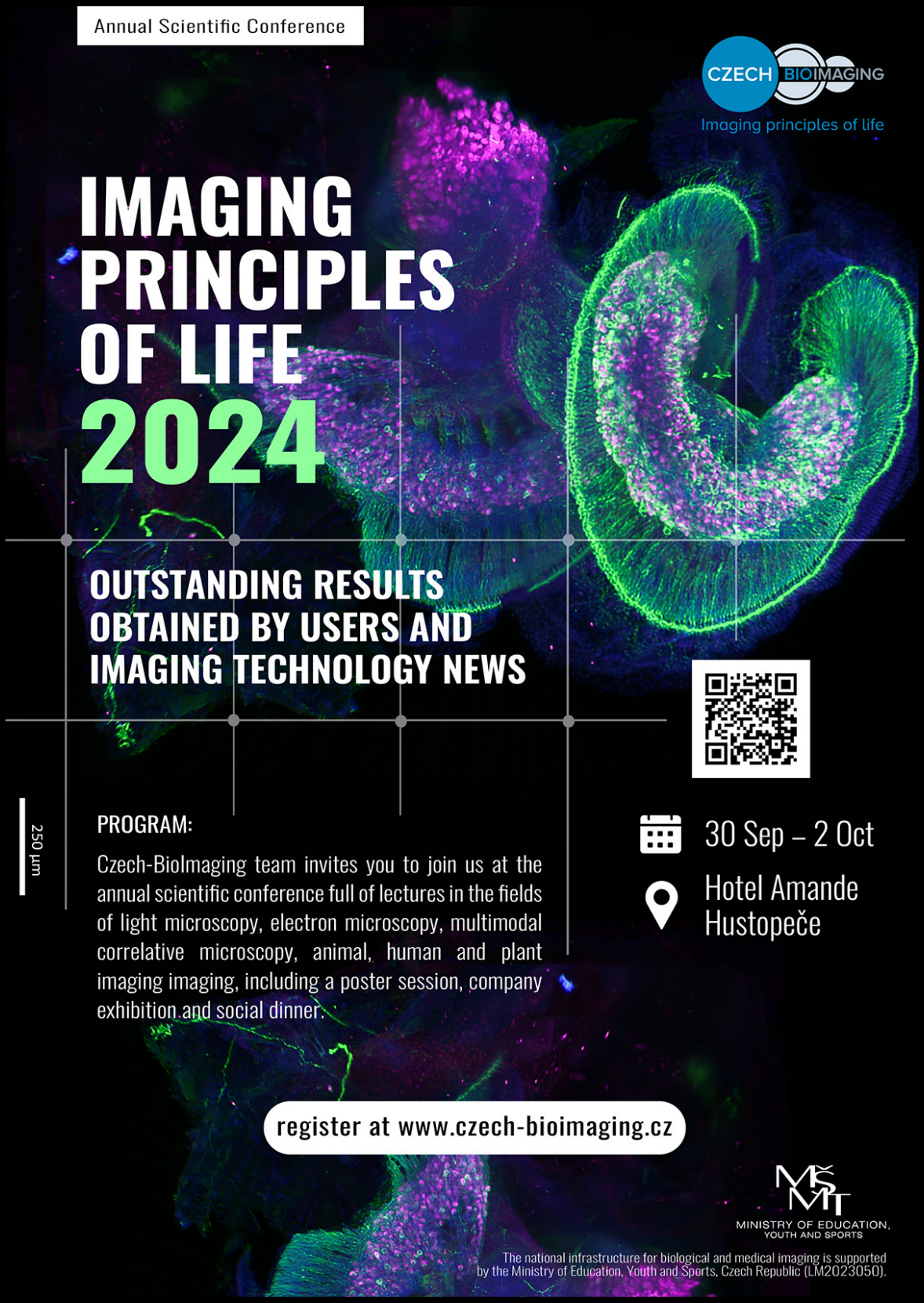
Leading up to the conference, join us for the "Photons, Electrons & Sausages 2024: CzBI & EuBI User Meeting & BBQ," (PES 2024 for short) a special user meeting for local users of Prague core facilities. Organized by Czech-BioImaging and the Prague Node, this event will take place on June 18, 2024. It's a perfect occasion for local users to engage with our experts in an informal setting, discuss their imaging needs, and learn about the latest advancements and services offered by our facilities. Plus, enjoy a BBQ and network in a relaxed atmosphere.

This year, the Czech-BioImaging Conference will feature a new section titled "Czech-BioImaging Meets Users." This special segment aims to enhance engagement between our core facilities and the user community. Attendees will have the chance to discuss their imaging needs directly with our experts, learn about the latest services and technologies available, and explore opportunities for collaboration and support.

Join us for both the user meeting and the conference!

REGISTER FOR PES 2024

REGISTER FOR IPL 2024



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

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Co-funded by the European Union