BIOCEV

IMCF

Microscopy at Faculty of Science, Charles University

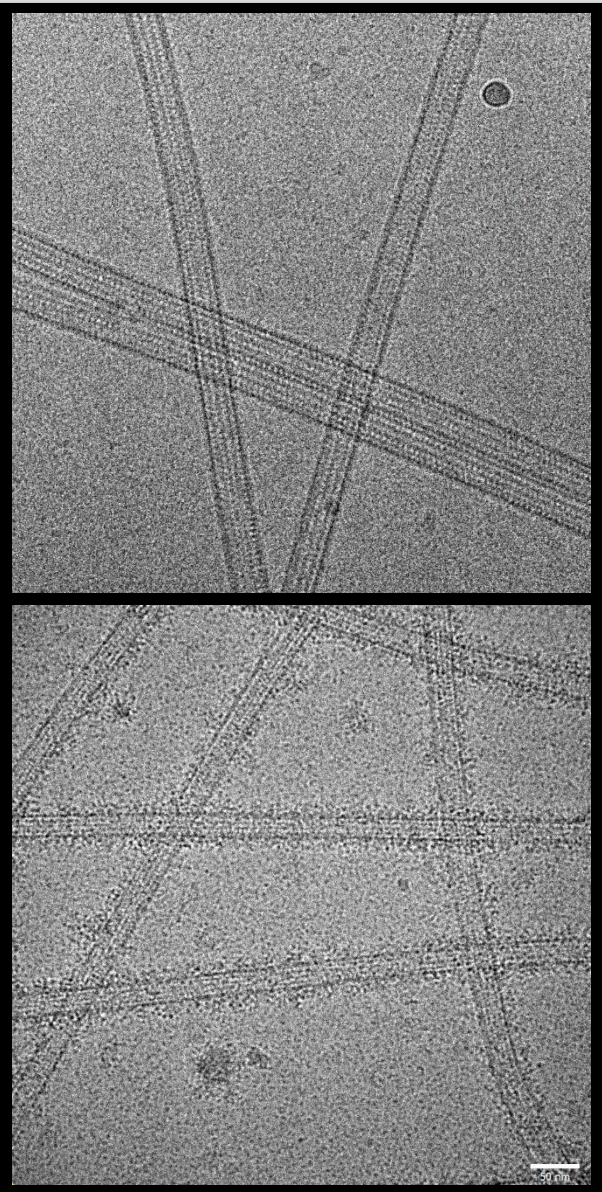
Aleš Benda

FACULTY OF SCIENCE
Charles University

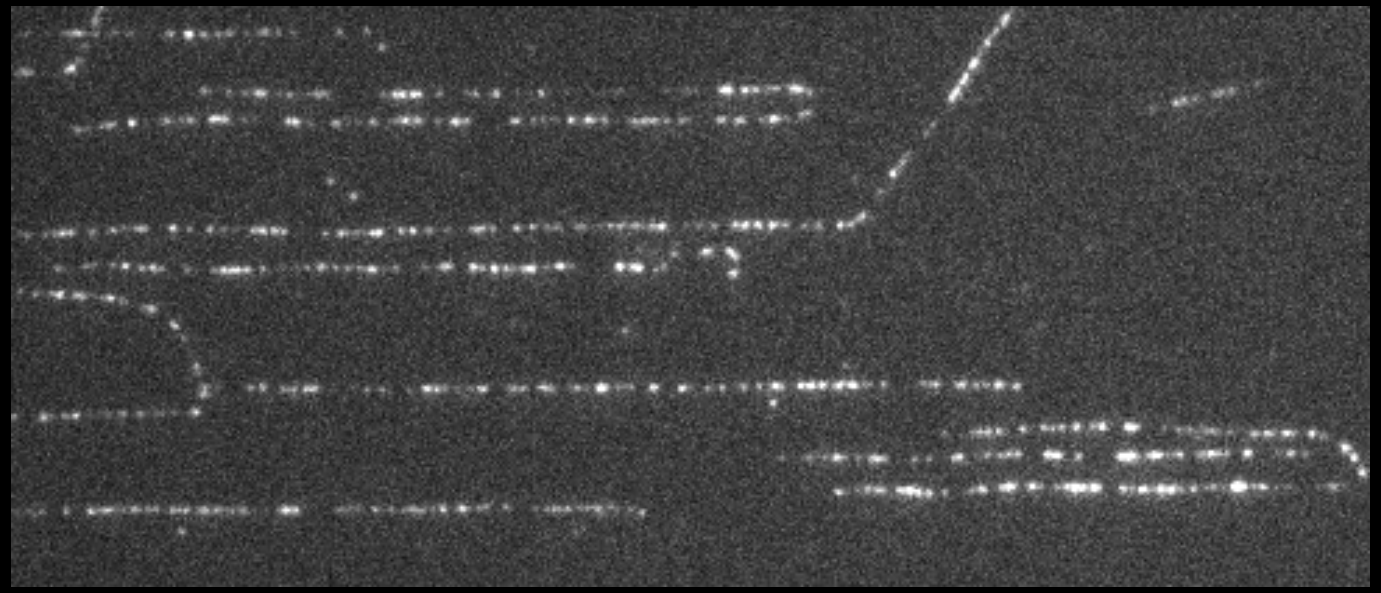
CZECH BIOIMAGING
Imaging principles of life

EURO-BIOIMAGING

Molecular principles of life

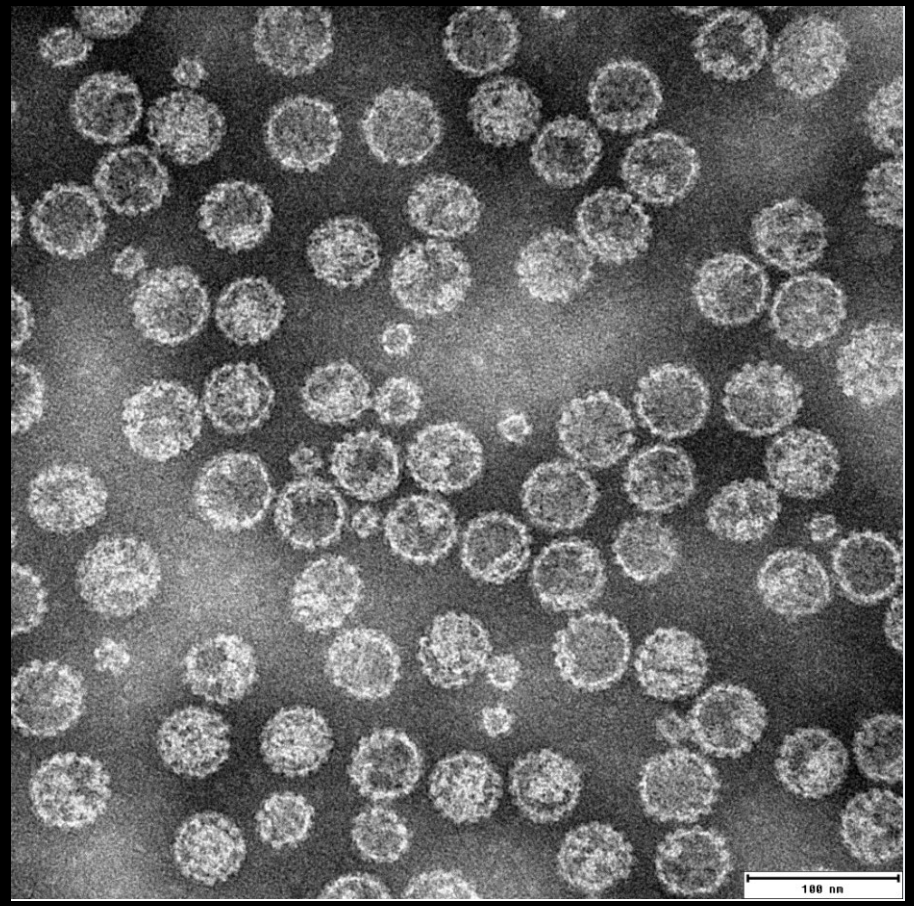


Microtubules with
and without proteins

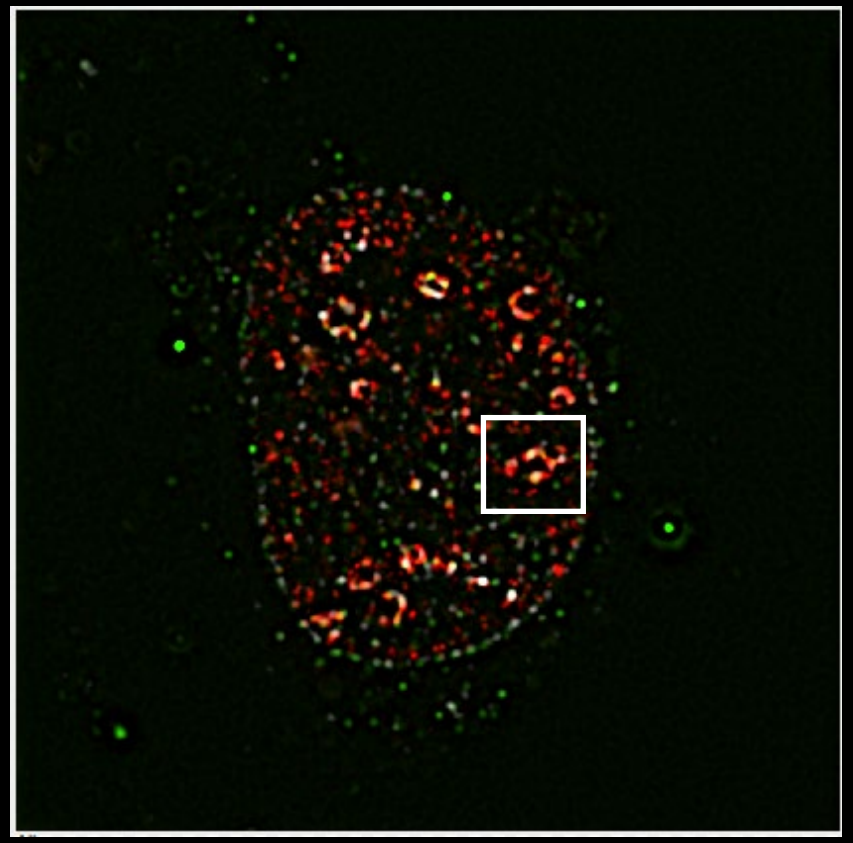


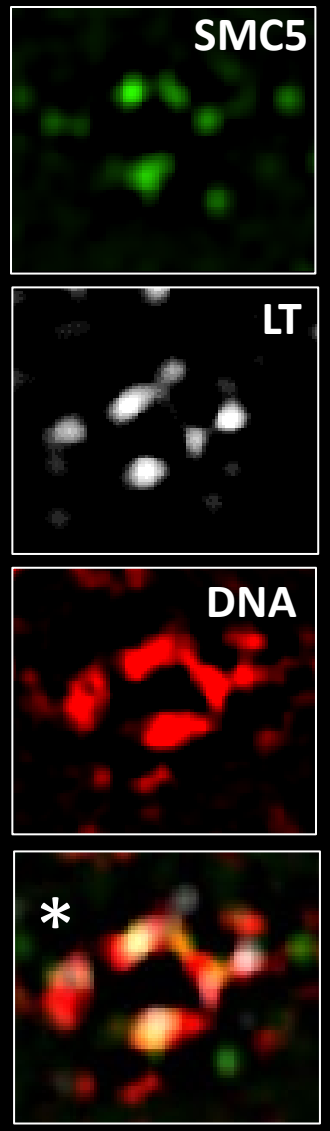
Molecular motors walking along microtubules

Viruses and their interactions with host cells



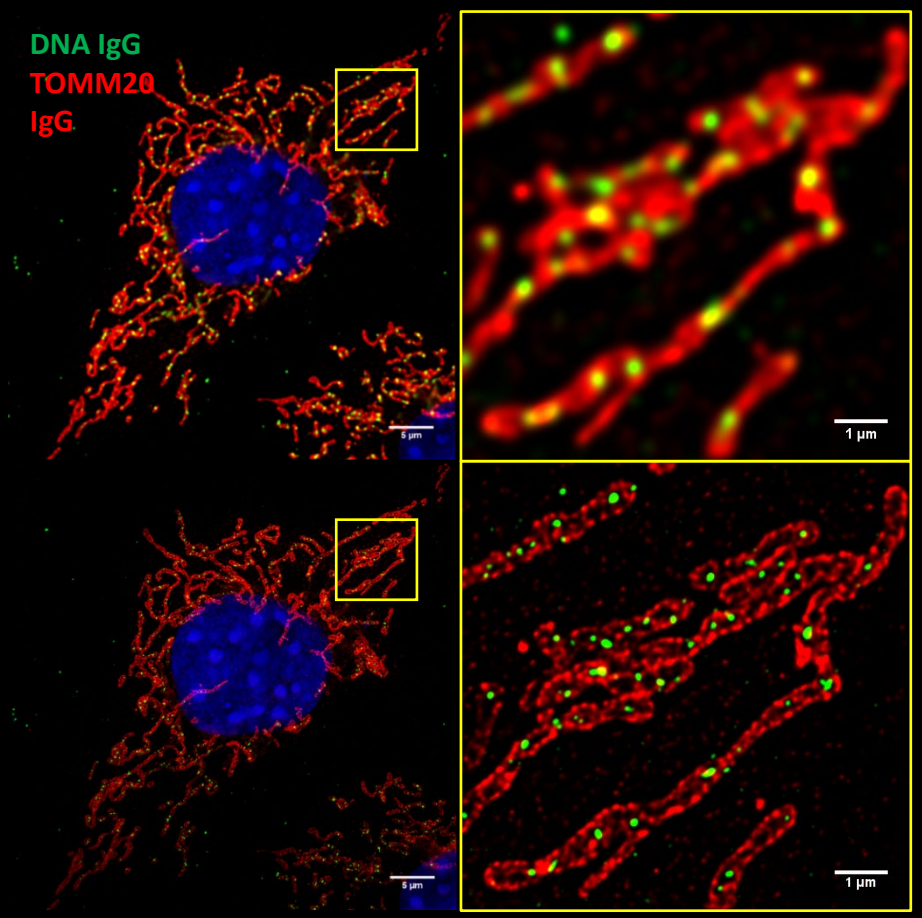
Murine polyomavirus (viral capsides)

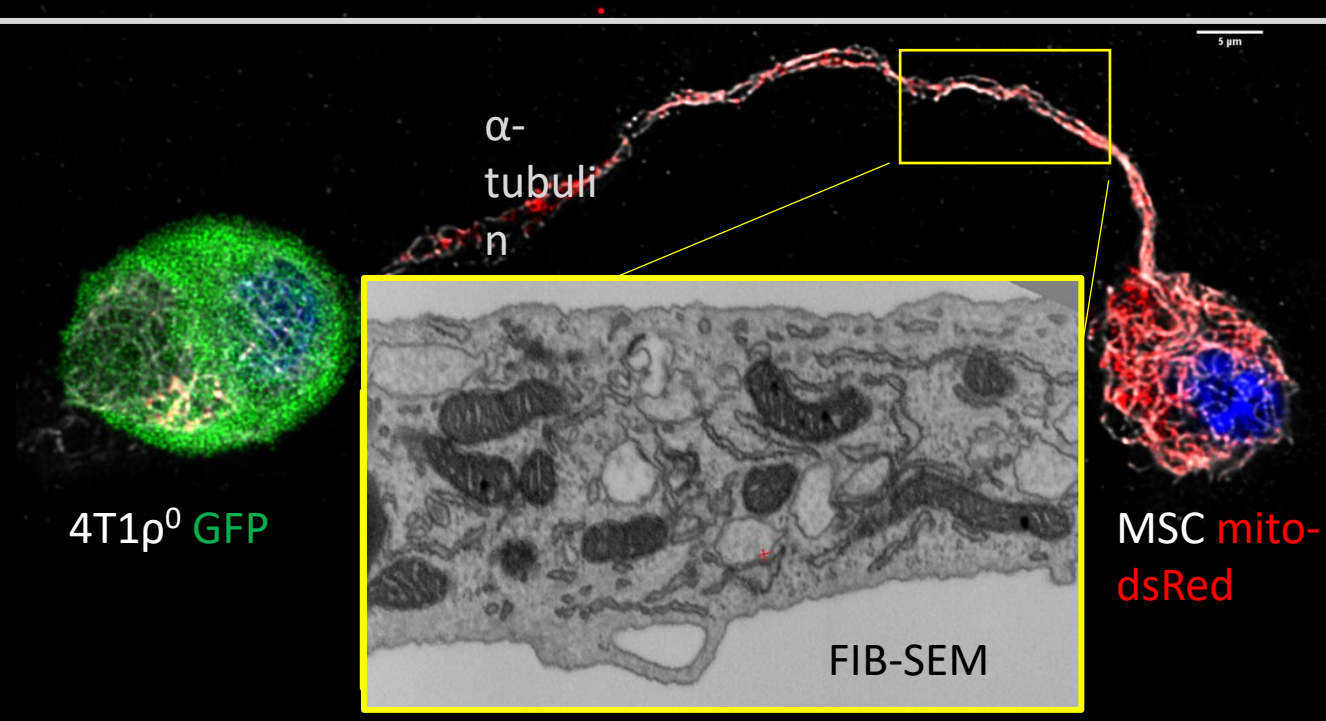


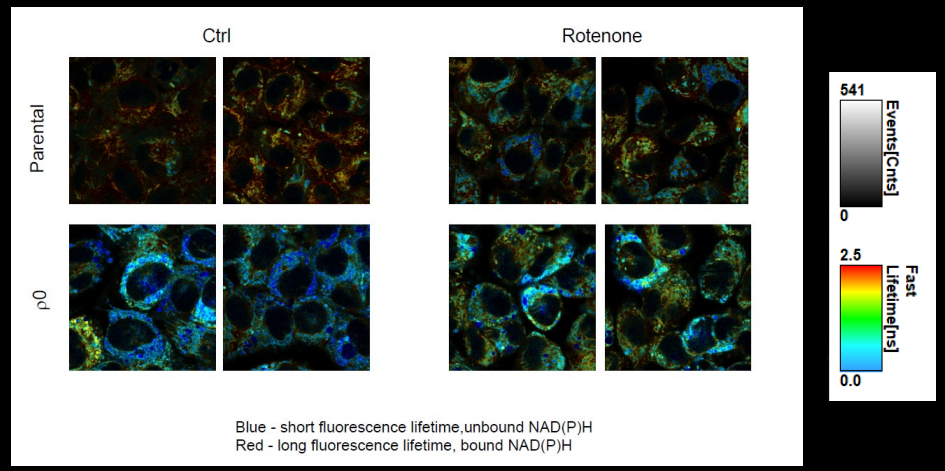


viral DNA escaping replication center

Mitochondria in the life and death of a cancer cell – Jiří Neužil group, IBT CAS

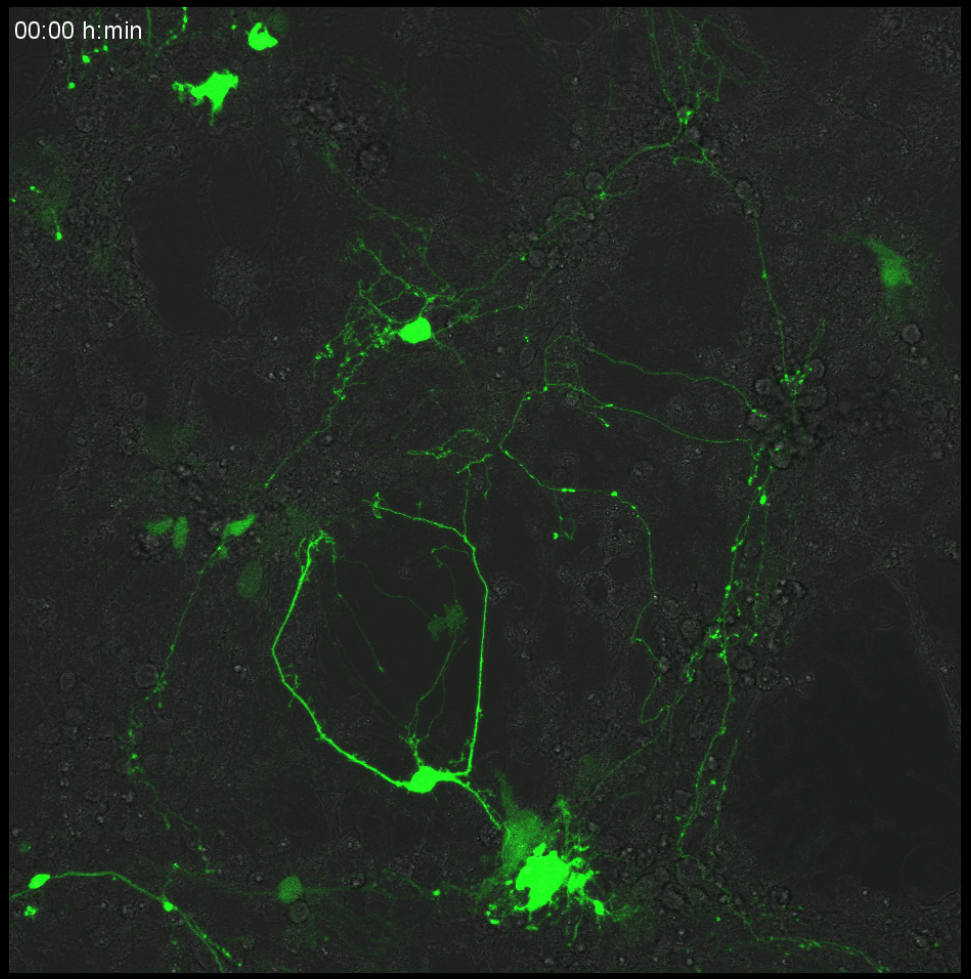






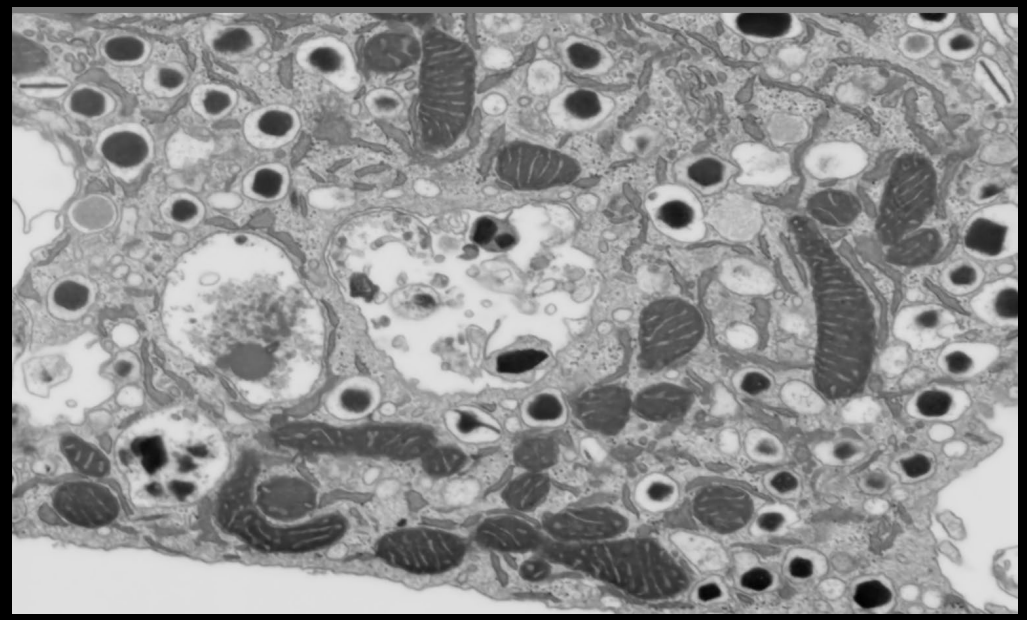
Neurology

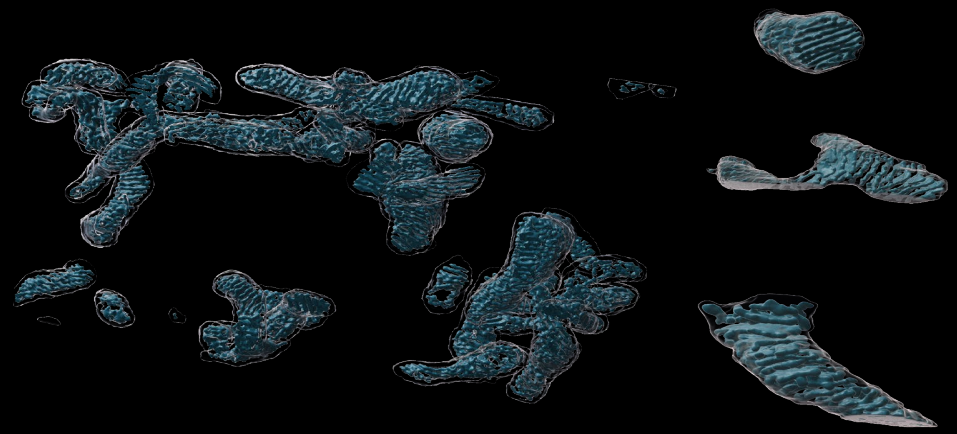
Mouse brain – counting neuronal synapses



Neuron regeneration after axotomy

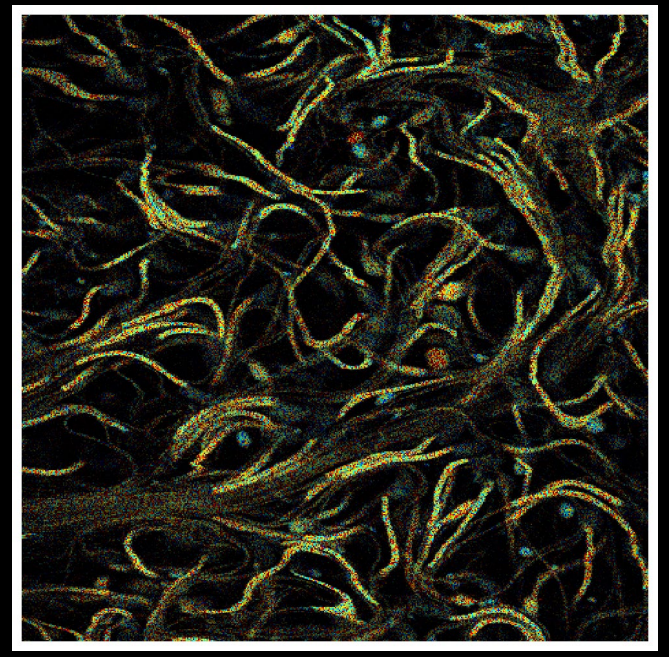
Diabetes

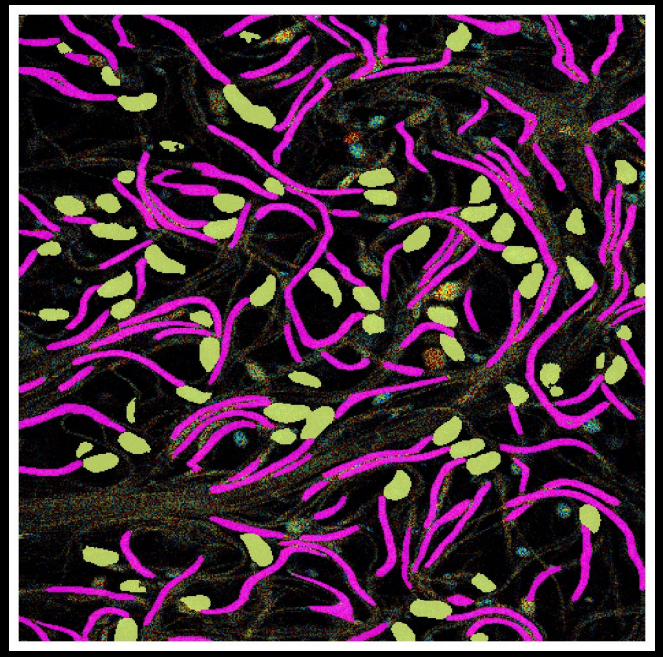




Morphological changes in pancreatic tissues

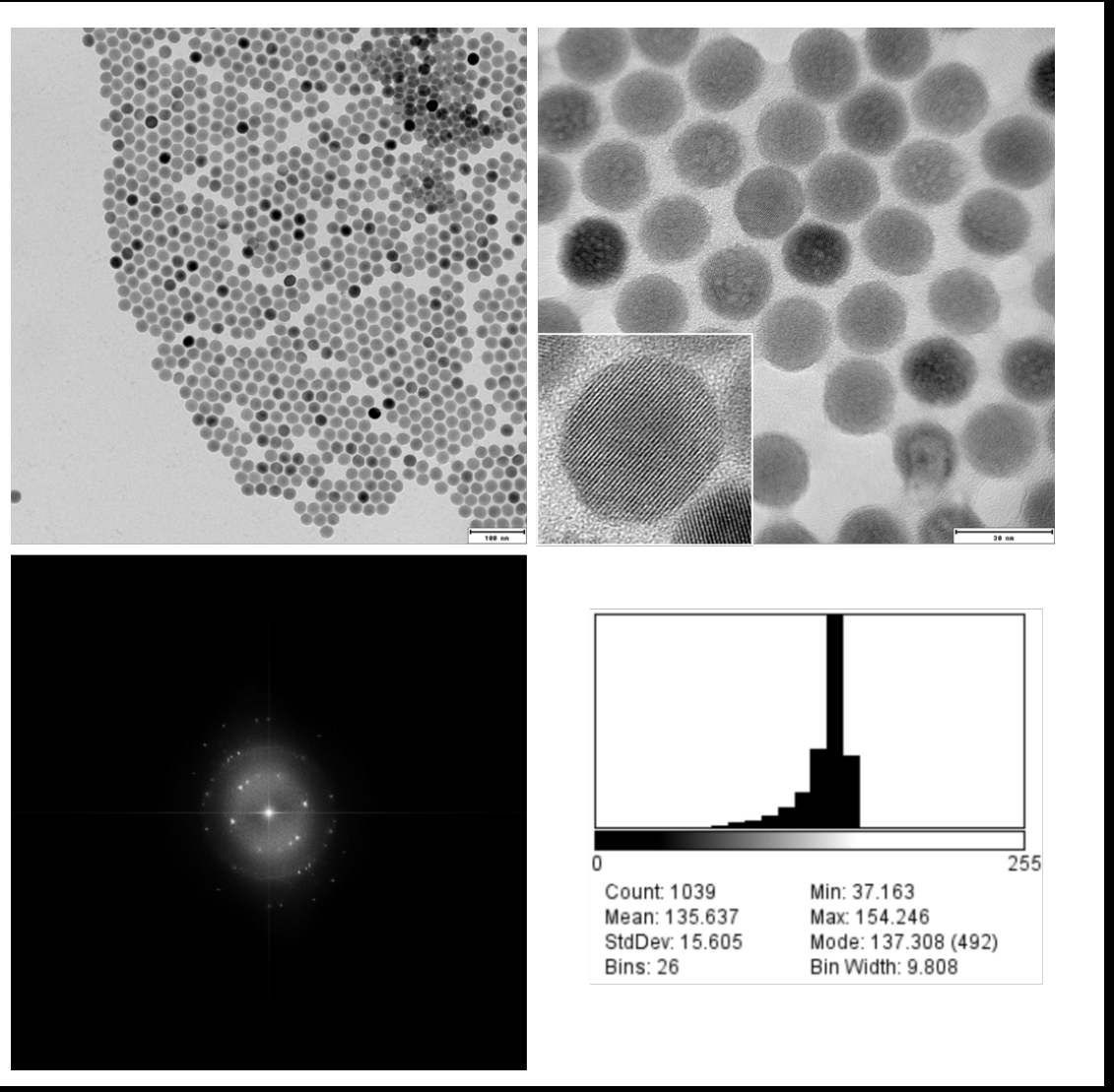
Fertility



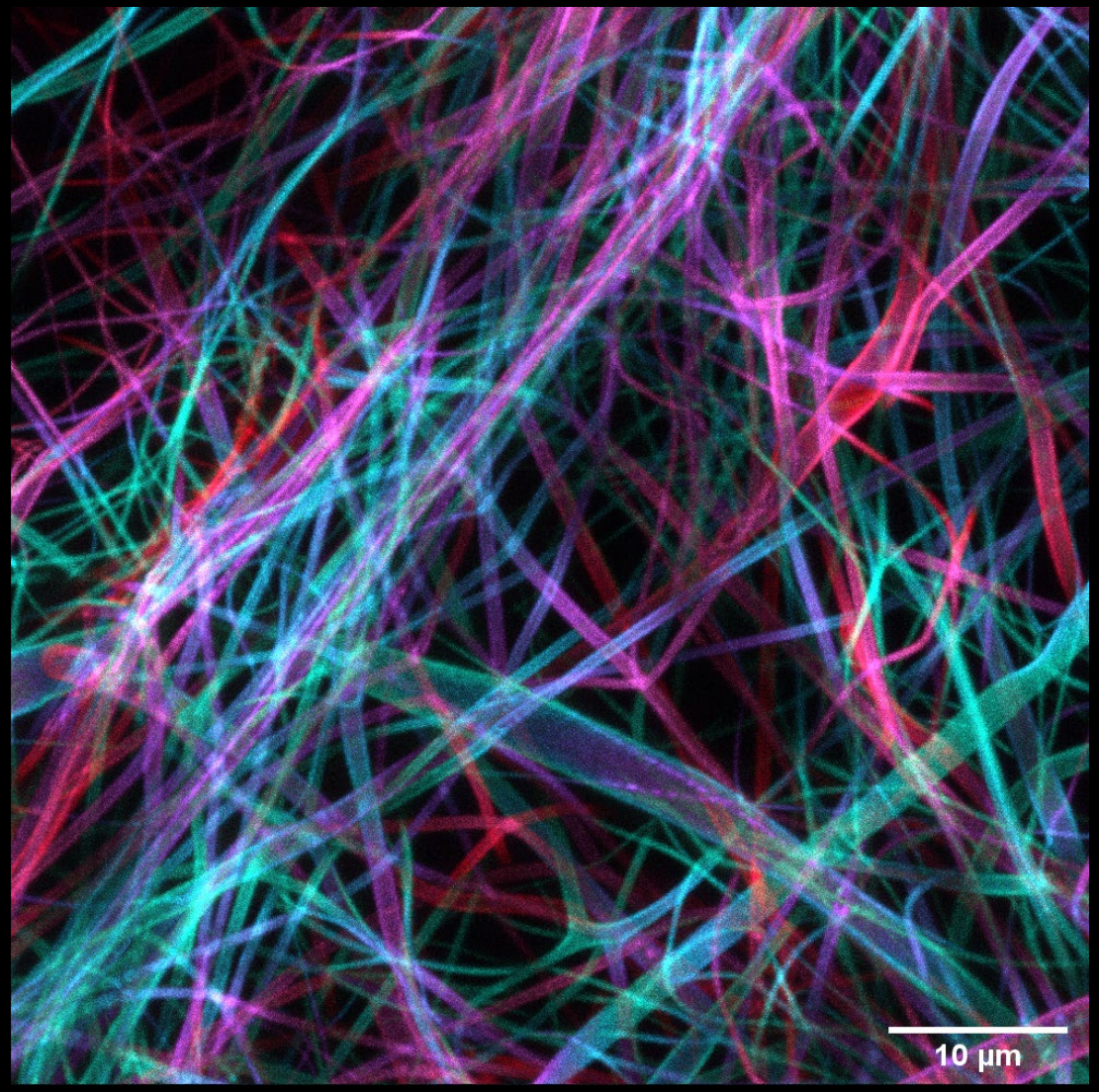


Metabolic changes in normal and defective sperms

Material science



Ultrastructural characterization of upconverting nanoparticles



Confocal image of 3D fabric fibers

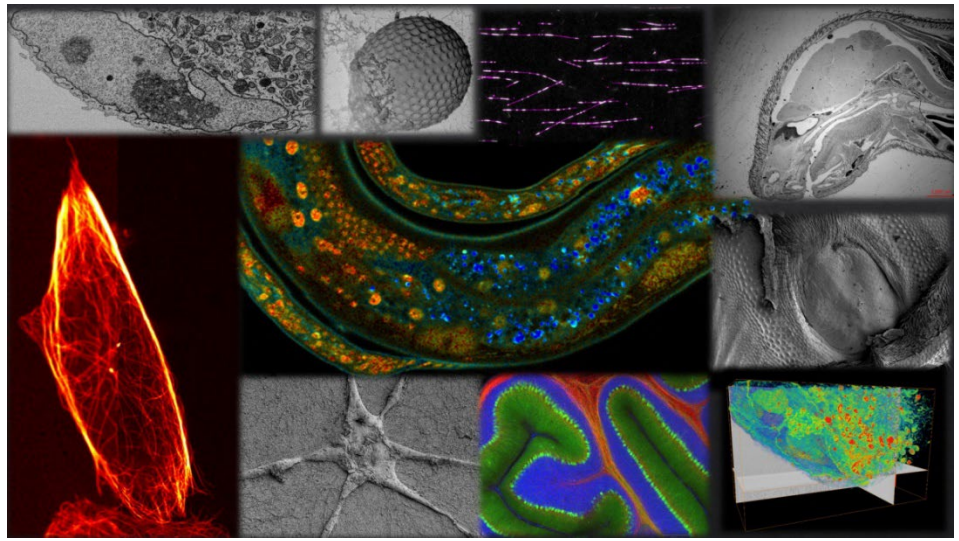
Imaging Methods Core Facility at BIOCEV

operated by Faculty of Science, Charles University

OPEN ACCESS = advanced microscopy for everyone

ONLY users' projects and user inspired methodologial projects

1. **Light microscopy**
   13 high-end systems
   (mostly) user operated
2. **Electron microscopy**
   FIB-SEM, TEM
   + sample preparation
   (mostly) staff operated
3. **Flow cytometry**
   2 analyzers + 1 sorter
   user and staff operated
4. **Image Data Analysis**
   Commercial software + hardware
   assistance



- **250 mil. CZK (10 mil EUR) in equipment**
- **around 250 academic users per year**
- **~11 000 imaging instrument hours per year**
- **14 staff (12 FTE)**
- **Companies – BeneMeat, LIM, Sotio, Diana, …**

http://imcf.natur.cuni.cz https://x.com/IMCF_BIOCEV

CHARLES UNIVERSITY

VMCF

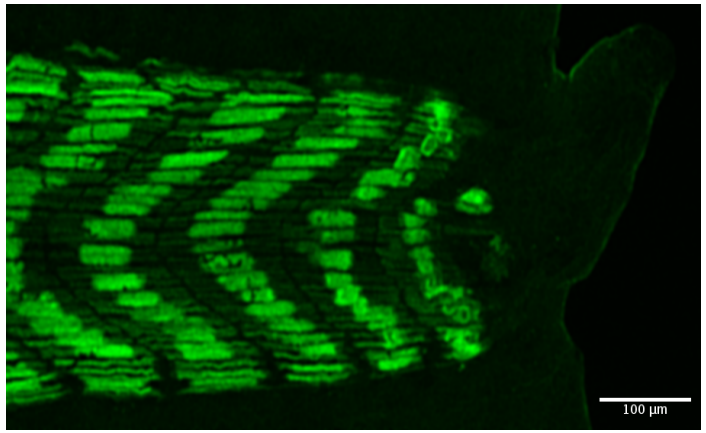
Viničná Microscopy Core Facility
Faculty of Science Charles University, Prague

Staff (2024):

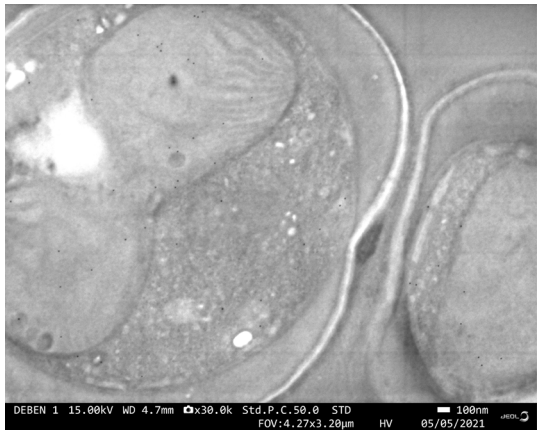
Electron Microscopy Section: RNDr. Miroslav Hyliš,Ph.D.,Ing. Jana Nebesářová,CSc., Mgr.František Kitzberger, Eva Kirchmannová

Light Microscopy & Bioimage analysis Section: Mgr. Ondřej Šebesta, Ing. Martin Schätz,Ph.D., Mgr. Jan Pačes, Mgr. Zuzana Burdíková Ph.D., Mgr. Paula Alejandra Build Maldonado

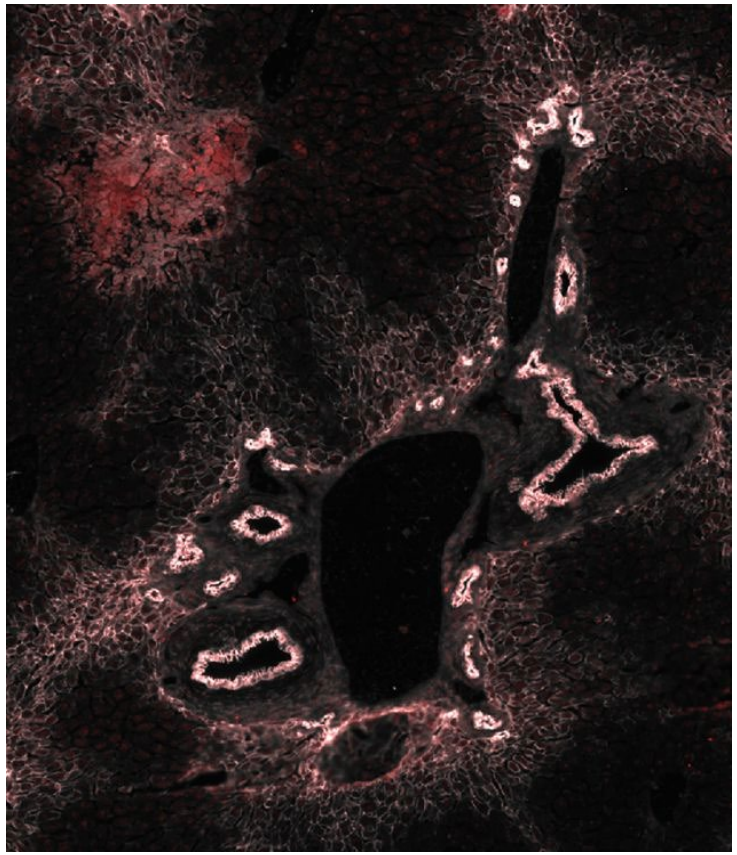
Cytometry Section: MVDr. Josef Janda Ph.D., Mgr. Zuzana Brůhová



Confocal image maximum projection of tail muscles in Xenopus embryo. Courtesy of Qing Zhao
Zhao Q, Mertová I, Wróblová A, Žabková S, Tlapáková T, Krylov V. Immunomodulatory role of Xenopus tropicalis immature Sertoli cells in tadpole muscle regeneration via macrophage response modulation. Stem Cell Res Ther. 2024 Nov 13;15(1):421. doi: 10.1186/s13287-024-04050-2. PMID: 39533333.



High-resolution SEM operating at low accelerating voltage was used to evaluate the immunolabelling of Chromera velia using rabbit anti-histone H2A primary antibodies and protein A as secondary antibodies conjugated to 10 nm AuNPs.
Kitzberger, F. et al.: An advanced fast method for the evaluation of multiple immunolabelling using gold nanoparticles based on low-energy STEM. Sci Rep 14, 10150, 2024.



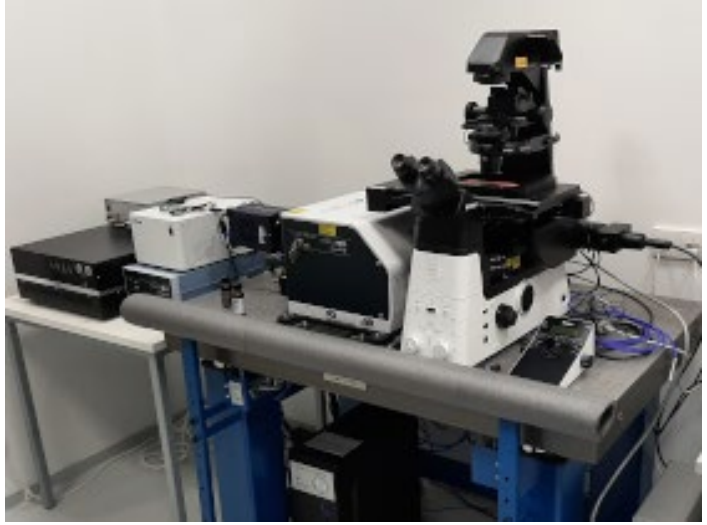
Fluorescence image of liver tissue section. Courtesy of Anna Maria Frontino and Daniel V. Oliveira
Mašek J, Filipovic I, Van Hul N, Belicová L, Jiroušková M, Oliveira DV, Frontino AM, Hankeova S,
He J, Turetti F, Iqbal A, Červenka I, Sarnová L, Verboven E, Brabec T, Björkström NK, Gregor M,
Dobeš J, Andersson ER. Jag1 insufficiency alters liver fibrosis via T cell and hepatocyte differentiation defects.
EMBO Mol Med. 2024 Nov;16(11):2946-2975. doi: 10.1038/s44321-024-00145-8. Epub

Optical microscopes - selection

Carl Zeiss LSM 880 NLO

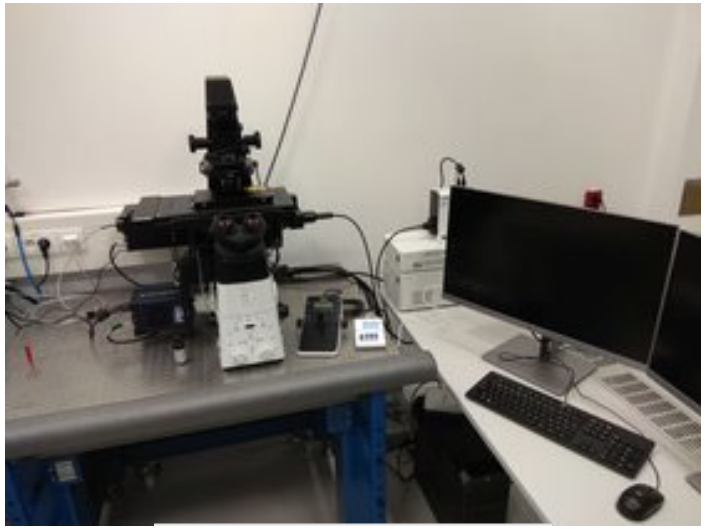
Leica TCS SP8 WLL SMD-FLIM

Abberior Instruments STED



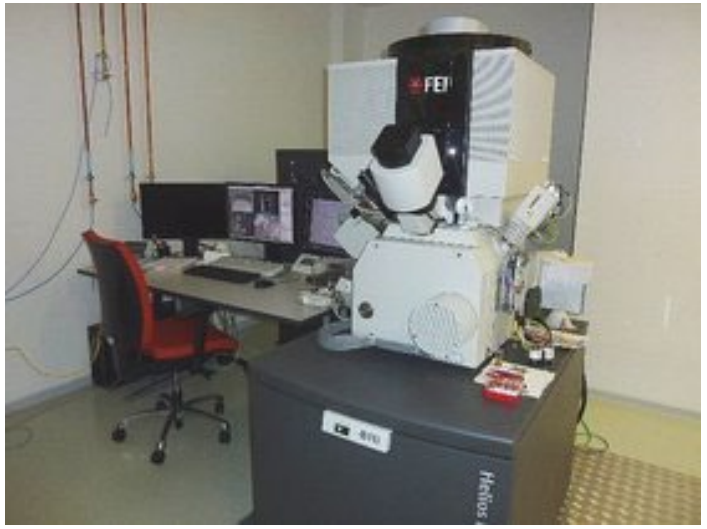
Nikon CSU-W1

Nikon N-SIM & N-STORM



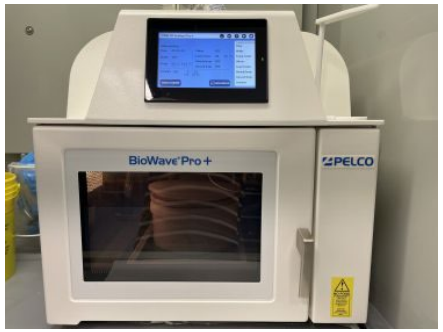
Nikon H-TIRF 2

Electron microscopes



FEI Helios NanoLab 660 G3 UC

JEOL JEM 2100-Plus 200kV



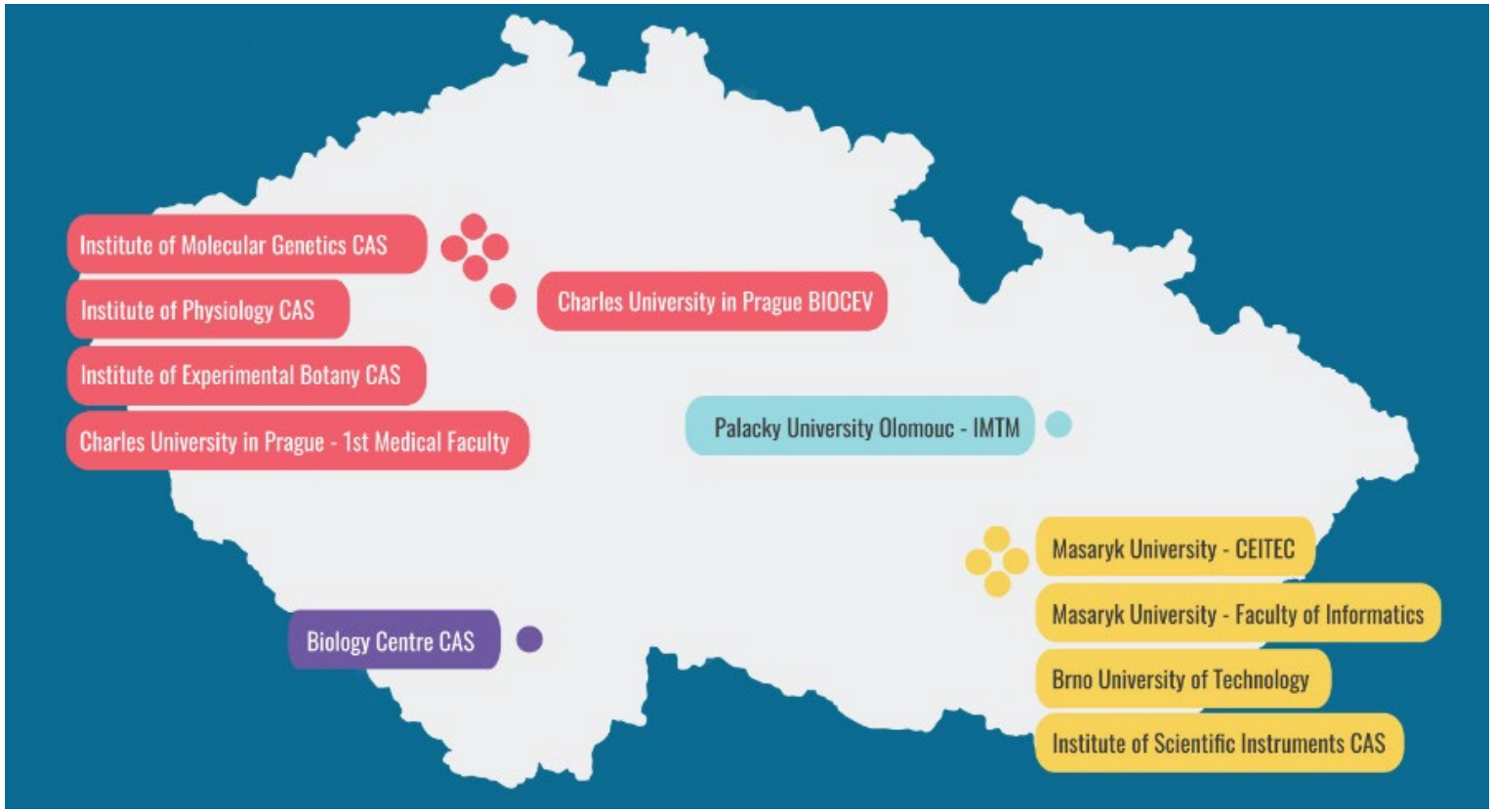
Complete toolbox for EM sample preparation

Aims and focus of the CFs

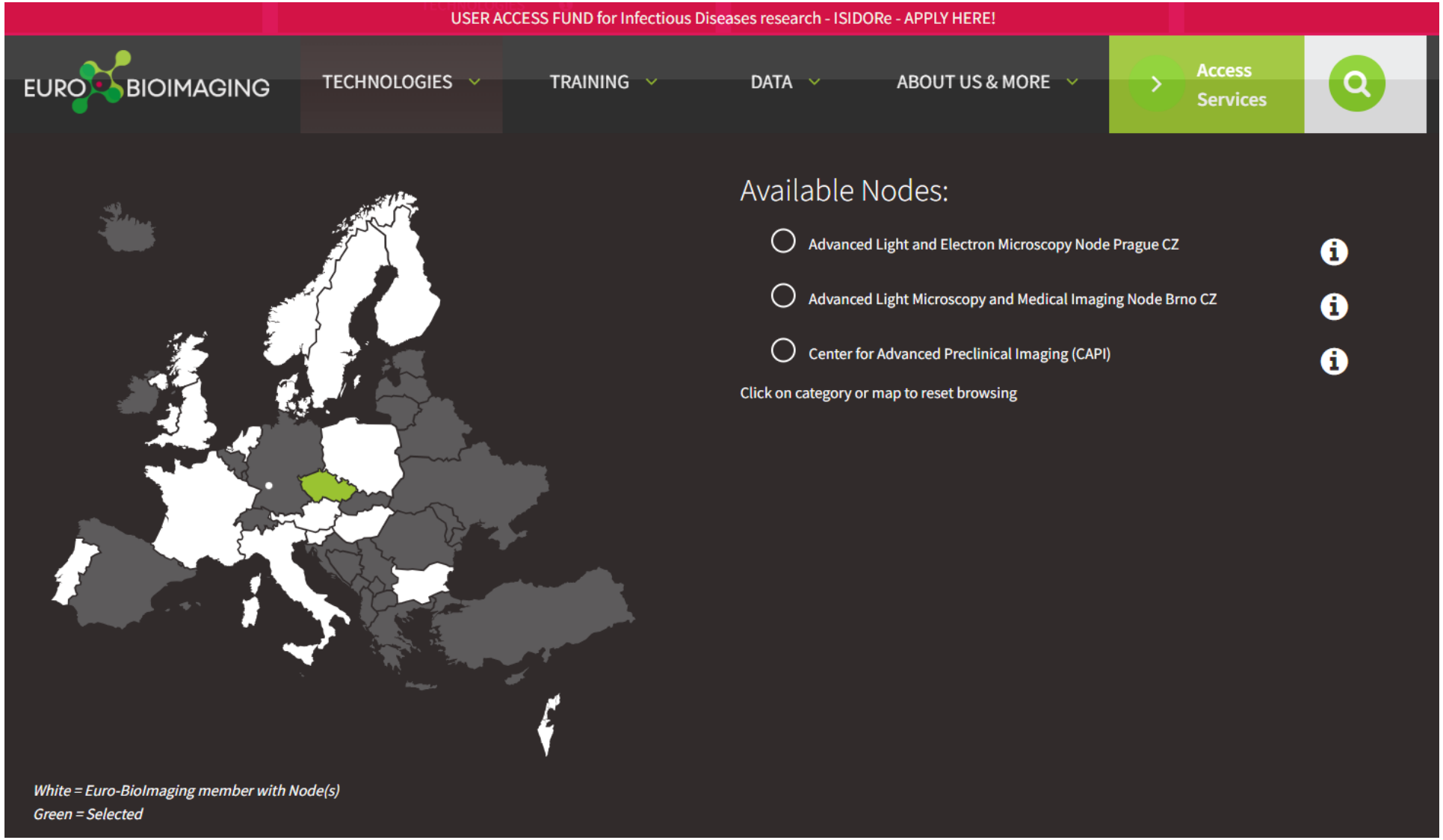
- Our motto is: Advanced microscopy for everyone
- IMCF consists of four closely collaborating units – **Light microscopy**, **Electron microscopy**, **Data analysis** and **Flow cytometry**
- Fully focused on **Open Access – serving all users regardless their affiliation**
- Operational mode – from **independent trained users,** via **assisted users** to **full service**
- **Provision of complex fee-for services** comprising sample processing, microscopy analysis and beyond
- **Expert consulting** on experiment design, sampling, sample preparation for analysis, and data evaluation
- **Training** of and **assistance** to users of the facility (both internal and external)
- Practical training **courses** on advanced microscopy methods
- Maintenance and upgrades of tools, instrumentation, and facility infrastructure
- **Development**, **implementation,** and **application** of advanced methodical approaches and analytical workflows
- **Collaboration** on user's projects, grant proposals, presentations and manuscripts for publishing

Czech-BioImaging

- Distributed national research infrastructure for biological and medical imaging
- 10 institutions – 16 facilities - 3 EuroBioImaging Nodes (11 facilities)
- Funded by "National Infrastructure for Biological and Medical Imaging - LM 2023050„ MEYS CR



EuroBioImaging



Acknowledgment

- **All our users**
- All Prague Node staff
- To MEYS CR (LM2018129, LM2023050) and ERDF (CZ.02.1.01/0.0/0.0/18_046/0016045)

Thank you for your attention

Courses

Upcoming and past courses on advanced techniques in microscopy and image analysis organized by IMCF:

FLIM not only for biologists, November 18-21, 2024

Cryo-Imaging of Biological Samples, April 7-9, 2025

Molecular Dynamics in Living Cells, Spring 2025

Python for Analysis and Processing of Images, October 20-21 & October 27-28, 2025

3D-CLEM: Imaging function and ultrastructure, November 2025

CryoEM Data Processing Seminar, September 24, 2024

Napari for beginners – image analysis course, May 27-28, 2024

EM-Sample Preparation Series, April 22-25, 2024

Neural Networks in Bioimage Analysis, May 9-11, 2023

Single Particle Analysis and Cryo-Electron Tomography, May 15-19, 2023

Single Molecule Microscopy and Manipulation, October 9-13, 2023

Search

QUICK NAVIGATION

- Acknowledgment
- Booking system
- New users
- MyScope microscopy training
- Temperatures in the rooms
- Sample preparation for cytometry analysis [PDF]
- Price lists [PDFs]
- Price list for sample preparation for EM [PDF]
- OMERO
- IMCF Feedback Form
- GDPR [PDF]

RECENT POSTS

- Invitation to the course FLIM not only for biologists, November 18-21
- Czech-BioImaging call for research projects
- Course: FLIM not only for biologists

IMCF Teams

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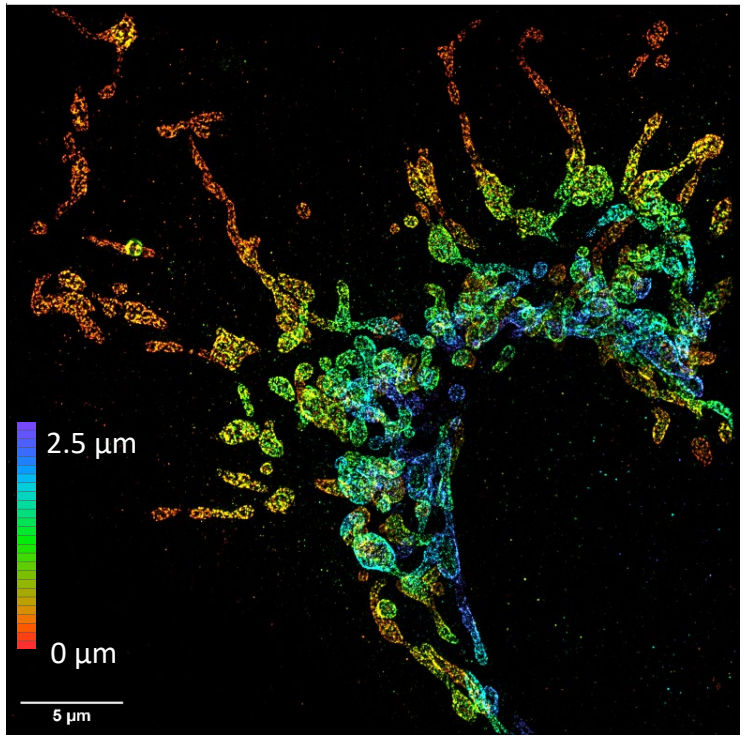
https://imcf.natur.cuni.cz/IMCF/team/

Fluorescence super-resolution by all means

Single Molecule Localization Microscopy (SMLM)



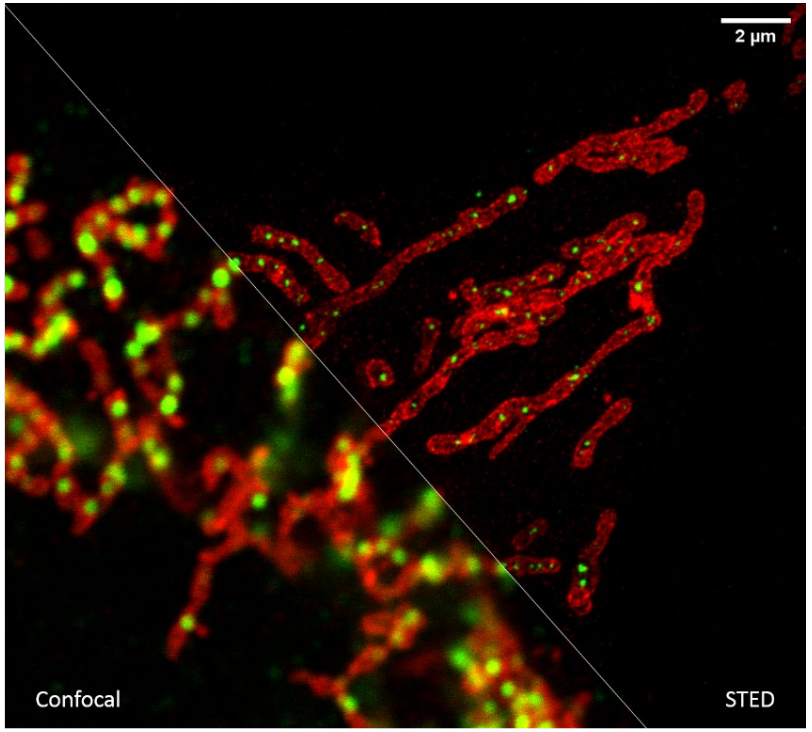
3D DNA-PAINT of mitochondrial receptor TOM20 located at outer mitochondrial membrane stained with MASSIVE-AB 1-PLEX (Atto655) kit

2D or 3D (biplane, helix, astigmatism)

Multi-colour (488 nm, 561 m, 640 nm)

Spectral demixing option

Stimulated Emission Depletion Microscopy (STED)



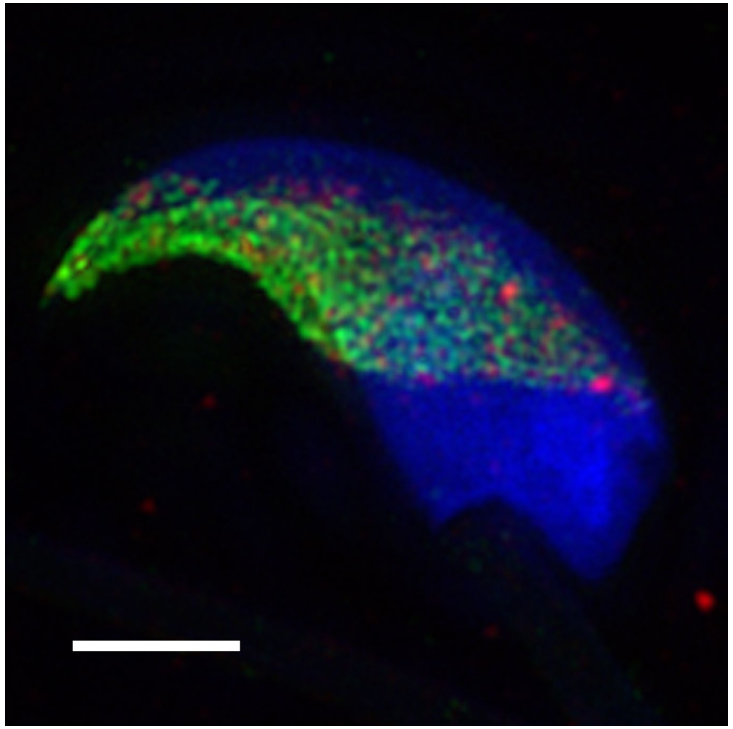
Comparison of raw confocal and STED images acquired on the Abberior Instruments STED microscope at IMCF BIOCEV. Red: TOM20 - Abberior STAR635P; green: nucleoids – Abberior STAR 580

2D or 3D depletion, oil, water or silicon immersion

Multi-colour excitation, depletion 775 nm

DyMIN and FLIM modules, adaptive optics, MATRIX array detectors

Structured Illumination Microscopy (SIM)



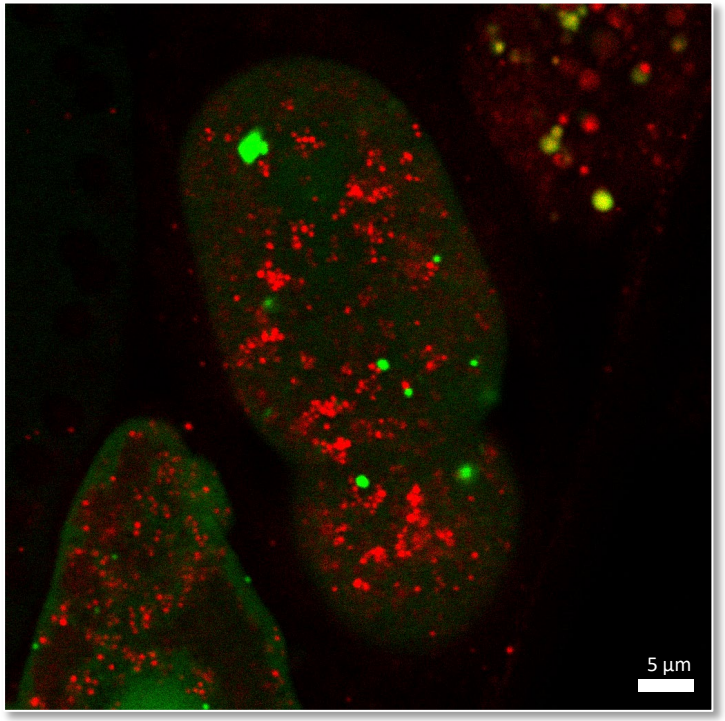
Visualization of mutual position of CD151 (green) and alpha6 integrin (red) on mouse acrosome intact sperm by SIM, DAPI counterstaining (blue). Scale bar: 2 µm.

2D, 3D or TIRF modes

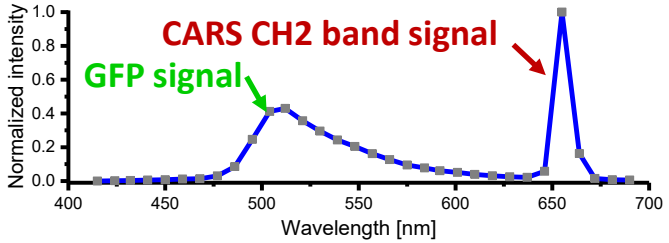
Multi-colour (405, 488, 561 or 640 nm)

Multiple flavors of label-free imaging

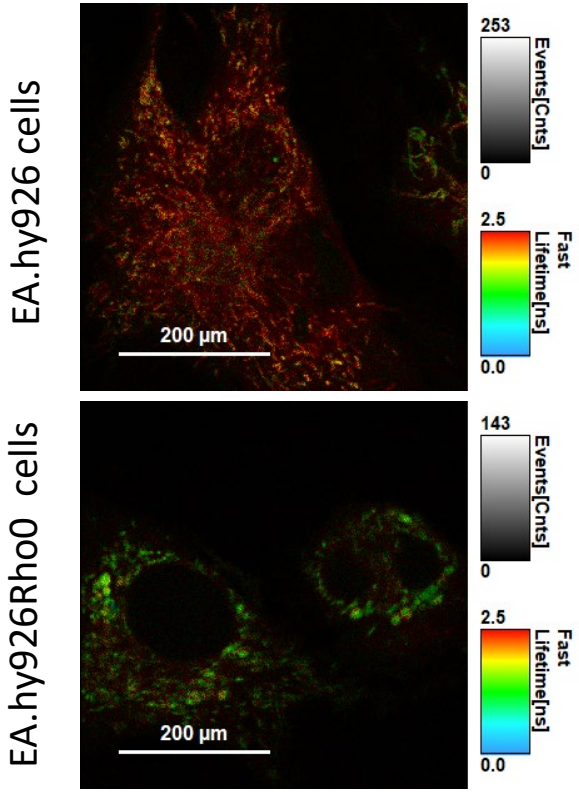
Coherent Anti-Raman Stokes (CARS) Imaging



Simultaneous GFP and lipid imaging in *C.Elegans*

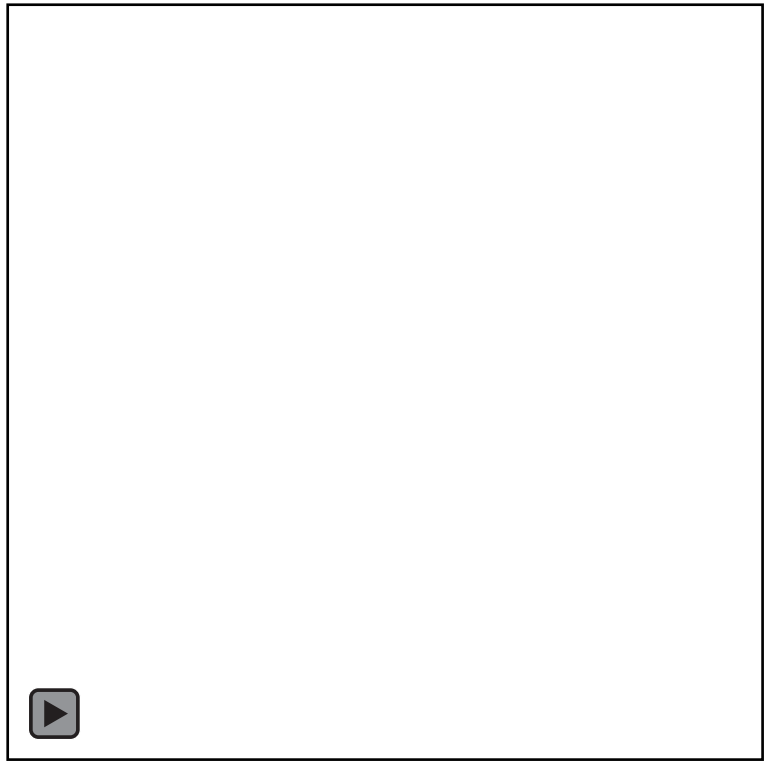


Metabolic imaging by 2-photon FLIM Microscopy of NAD(P)H



Defect in respiration increases the fraction of free NAD(P)H (shorter lifetime) compared to bound version (longer lifetime). 740 nm excitation, autofluorescence signal 420-480 nm.

Quantitative Phase Imaging (QPI)



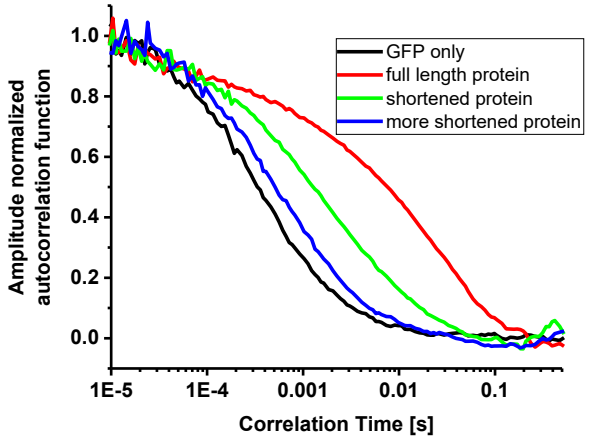
Long-term live-cell dynamics of fibroblasts. MIP of RI, 240x240um, 5h@1min per frame

Other options: Second (SHG) and Third (THG) Harmonic Generation

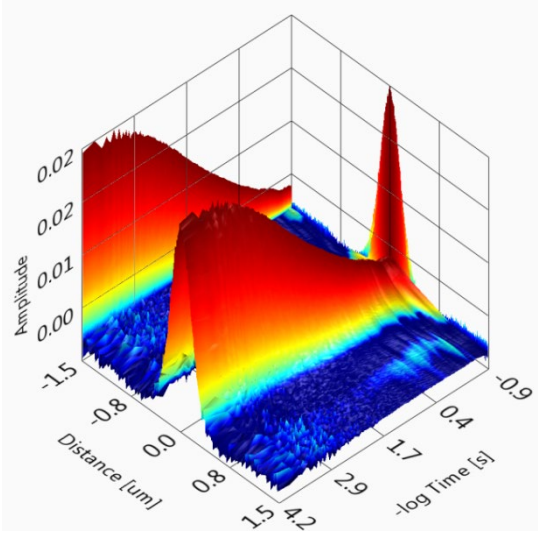
Nanolive CX-A - 3D holotomography

Unique toolset for functional microscopy

Fluorescence Correlation Spectroscopy (FCS) and its variants



Effect of protein modifications on its interaction within living cells followed by FCS



Example of spatio-temporal correlation obtained by line-scan FCS analysis

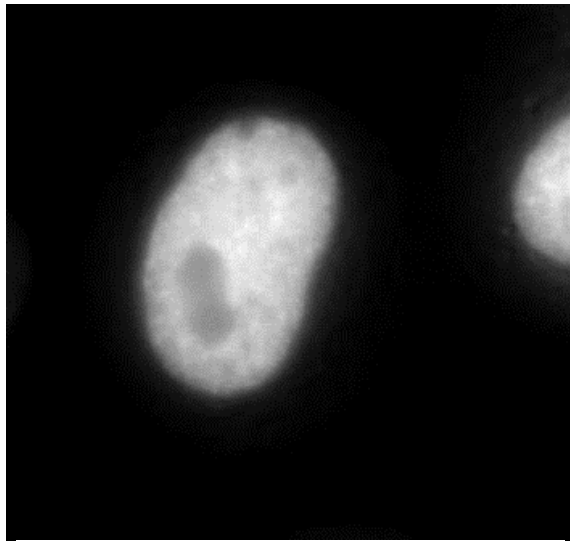
Point multicolor (PIE) cross-correlation analysis

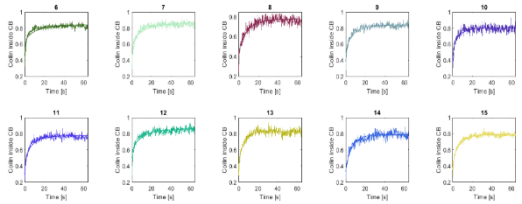
Fluorescence Lifetime or Spectral correlation analysis

STED – FCS Line-scan FCS Image-FCS

Custom software for semi-automated analysis

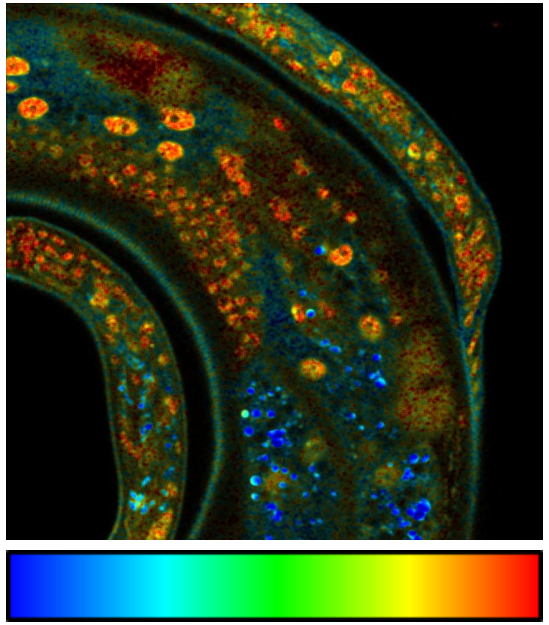
Fluorescence Recovery After Photobleaching (FRAP)





FRAP (Photoactivation) module combined with fast Spinning Disk or Ring-TIRF excitation

Fluorescence Lifetime Imaging (FLIM)



lifetime

One- or Two- photon excitation

WLL or laser diodes with PIE mode

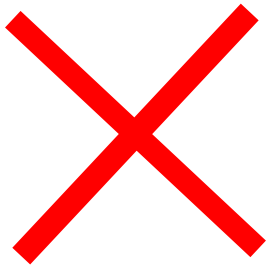
Up to 4 HyD or SPAD detectors

Ultrafast TCSPC card with short deadtime

Custom TTTR Data Analysis software

Open-access research infrastructure
Shared research infrastructure

Instruments “owned” by research groups



Open-access instruments and expertise

Core facilities

Benefits:

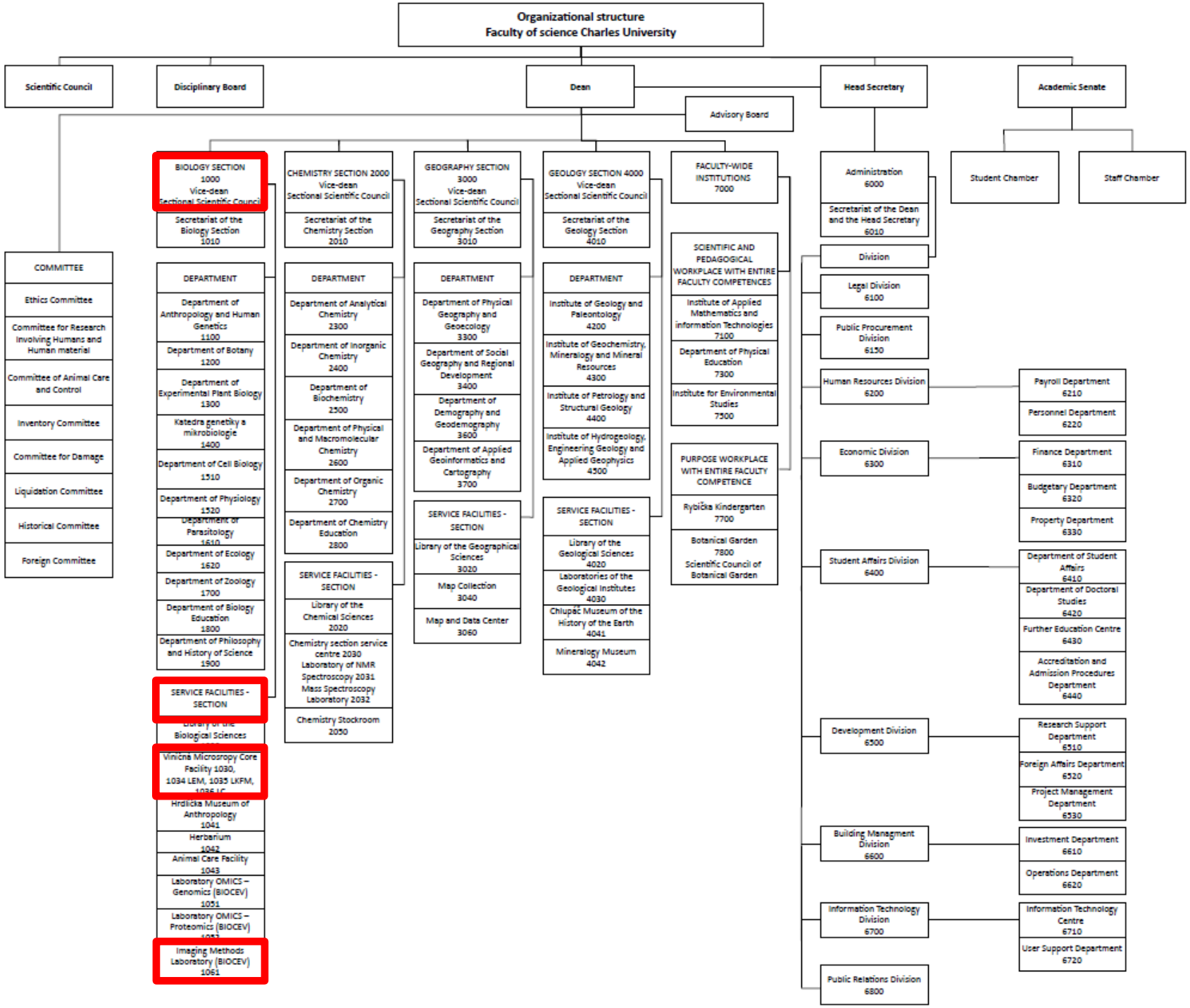
- Scientists have an instant access to a wide range of high-end technologies without the need to „own them“ and becoming a technology expert themselves
- Continuous build-up of methodological know-how and its long-term preservation
- Economicaly efficient – sharing means less instruments with higher usage
- Economicaly efficient – getting results in shorter time - „time is money“

Faculty of Science

- Chemistry
- Geography
- Geology
- Biology

Service facilities

- ➢ Viničná Microscopy Core Facility
- ➢ Imaging Methods Core Facility at BIOCEV



VMCF aims and focus

- CF provides microscopic service and overall support in the fields of electron and advanced light microscopy, image analysis, histology and cytometry for research and educational purposes (for both internal and external users; CF is directly connected to major educational institution - Faculty of Science Charles University)
- Confocal microscopy applications: FRAP, FRET, FLIM, RICS, Live Cell Imaging, Time Lapse
- Superresolution techniques: SIM, PALM\STORM, TIRF
- Electron microscopy: routine TEM, SEM; HR SEM, STEM, Microarray tomography
- Consultation on widefield, fluorescent, confocal, multiphoton, light-sheet, electron microscopy and cytometry projects
- Special consultations: image analysis, training for AI image analysis, cryo & immuno EM, immunocytochemistry
- Hosting special seminars, symposia, and open-house events, organizing many courses, workshops and webinars (localization of the facility in the center of the capital city offers favorable conditions for events, users' visits and cooperation with local and foreign institutions)

https://natur.cuni.cz/en/biology/departments-and-work-places/service-facilities/vinicna-microscopy-core-facility