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Imaging Methods Core Facility at BIOCEV

FACULTY OF SCIENCE
Charles University

CZECH BIOIMAGING
Imaging principles of life

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„Imaging Methods“ Core Facilities at Faculty of Sciences

	Optical microscopy	Electron microscopy	Flow cytometry
BIOCEV	**Imaging Methods Core Facility** Aleš Benda		
Viničná 7	**KONFMI** Ondřej Šebesta	**LEM** Miroslav Hyliš	**LC** Jozef Janda

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„Imaging Methods“ Core Facilities at Faculty of Sciences

Optical microscopy

Electron microscopy

Flow cytometry

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Viničná 7

Imaging Methods Core Facility
Aleš Benda

KONFMI
Ondřej Šebesta

LEM
Miroslav Hyliš

LC
Jozef Janda

Increasing collaboration

staff sharing, expertise sharing, best practice,
booking system, courses,
teaching, etc…

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„Imaging Methods“ Core Facilities at Faculty of Sciences

Optical microscopy	Electron microscopy	Flow cytometry

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Imaging Methods Core Facility
Aleš Benda

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Jozef Janda

Biocentrum Albertov

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	Optical microscopy	Electron microscopy	Flow cytometry
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Biocentrum Albertov	**Joined Core Facility** ????????		

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Core Facility function

To facilitate **YOUR** research

We are here to **help YOU** with your imaging and flow cytometry needs

To do so we:

1. Have equipment that you might need (microscopes, cytometers, etc.)
2. Try to keep the equipment in a good shape and up-to-date
3. Train and support users
4. Offer “measurement as a service”
5. Educate (workshops, courses, lectures) – both you and us!
6. Offer support for your microscopy project design
7. Have a booking system
8. Have formal and informal rules of operation

Core Facility means more than Service Facility

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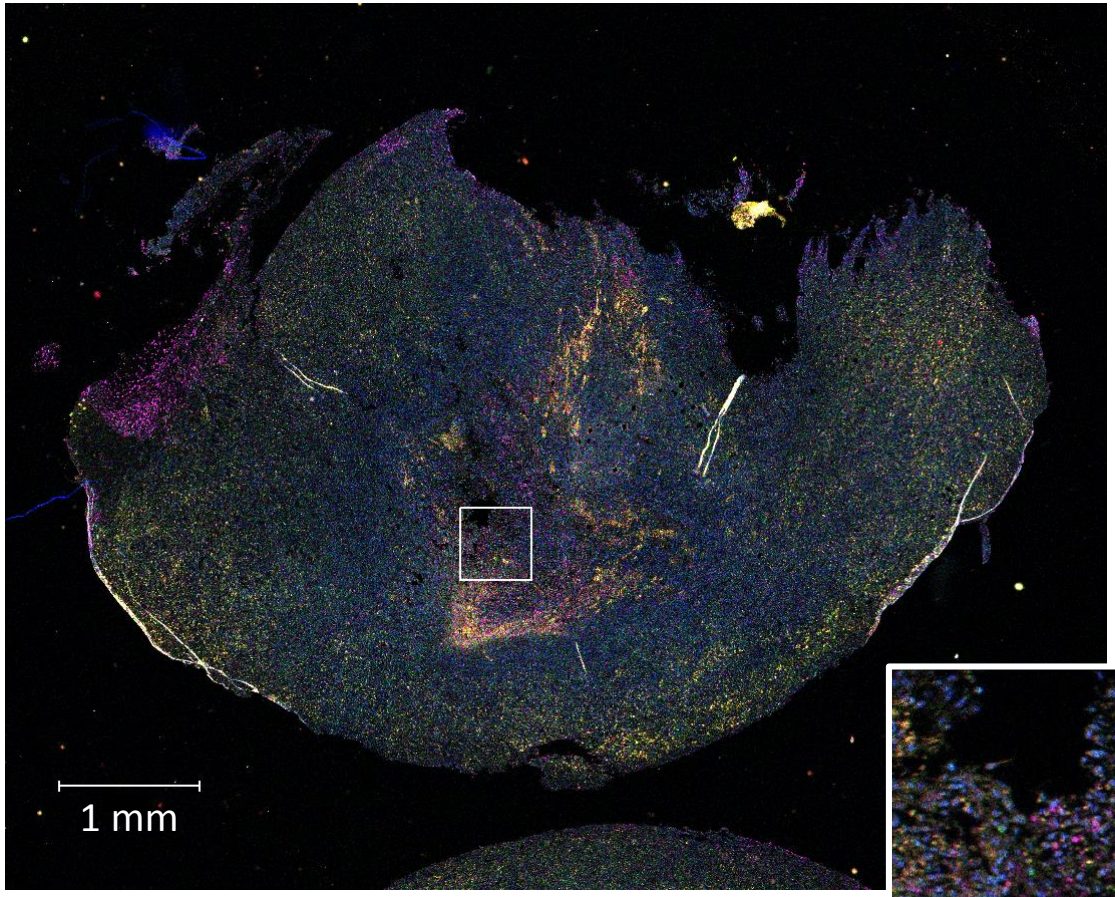
Multi-parametric Imaging
Hyperspectral Imaging



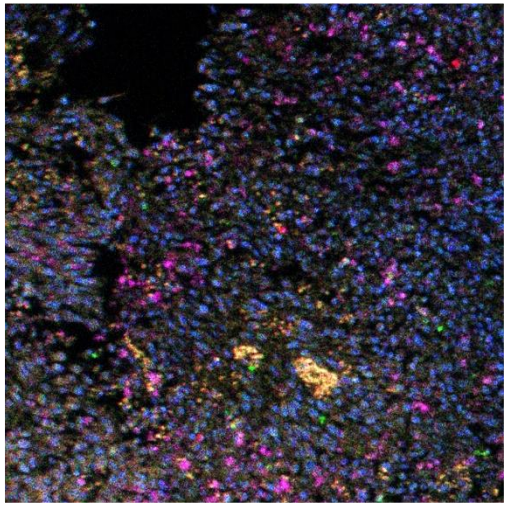
Carl Zeiss LSM 880 NLO

Objective: 5x, 0.16 N.A.

Excitation: 405 nm, 488 nm, 561 nm, 633 nm

Detection: 32 spectral channels (410 – 695 nm), additional channel 695 – 758 nm

Linear spectral unmixing with 5 spectral patterns.



Mouse tumour section stained for immune cells. Image courtesy of B. Pokrývková and R. Tachezy (Faculty of Science, Charles University).

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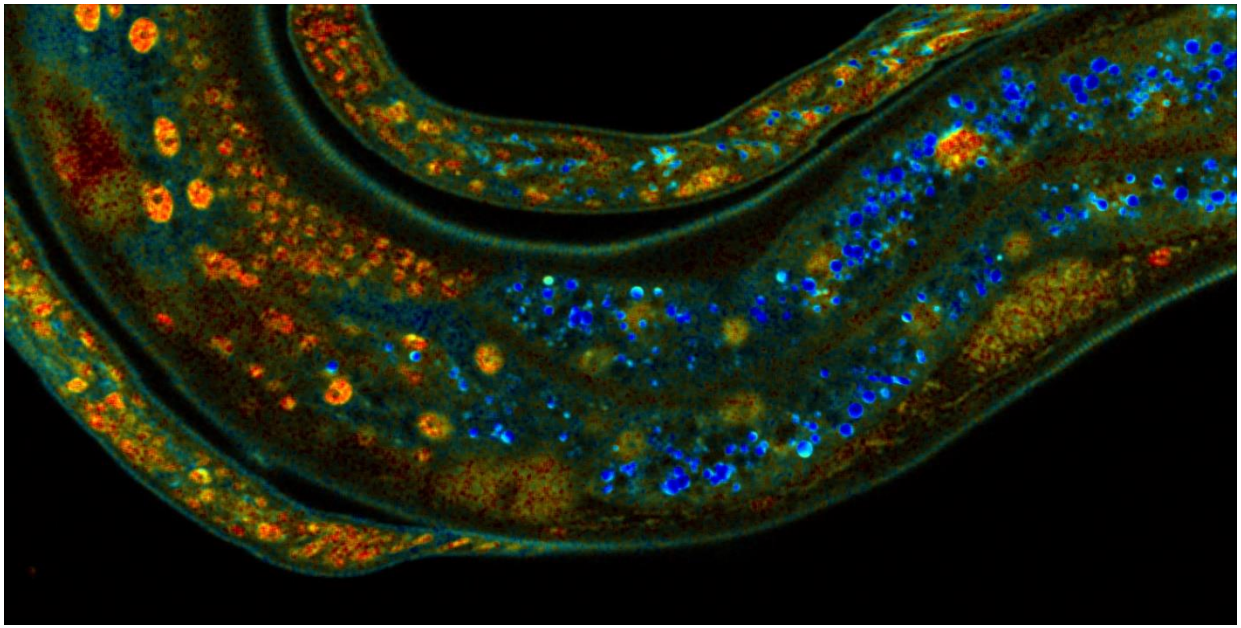
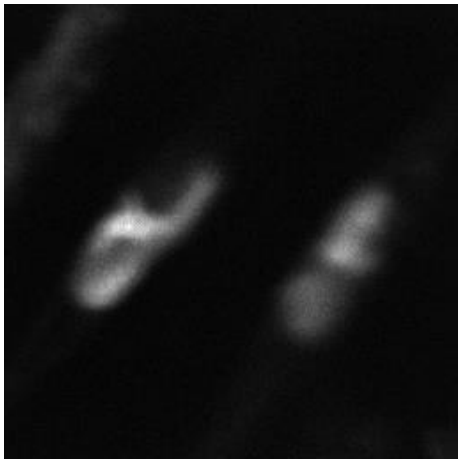
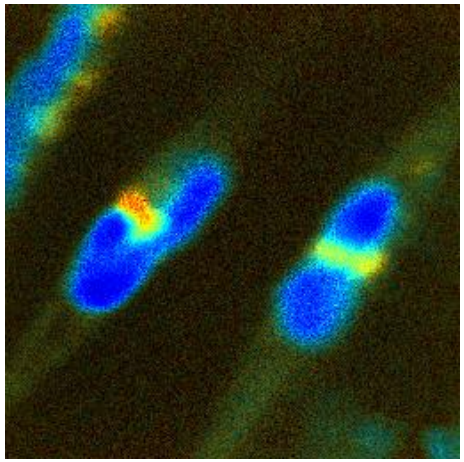
Multi-parametric Imaging
Fluorescence Lifetime Imaging



Localizing specific GFP fluorescence signal (long excited state lifetime – yellow to red) at high autofluorescence background (short excited state lifetime – blue to green) in *C. elegans*.
Data acquired in collaboration with Z. Kostrouch (1st Medical Faculty, CU).





Distinguishing between specific fluorescence signal (long excited state lifetime – yellow to red) and autofluorescence (short excited state lifetime – blue to green) in algae *Phaeodactylum tricornutum*.
Image courtesy of J. Mach (Faculty of Science, Charles University).

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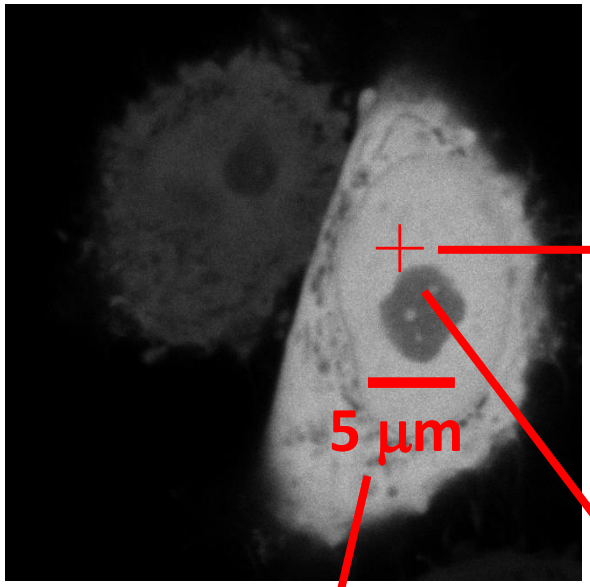
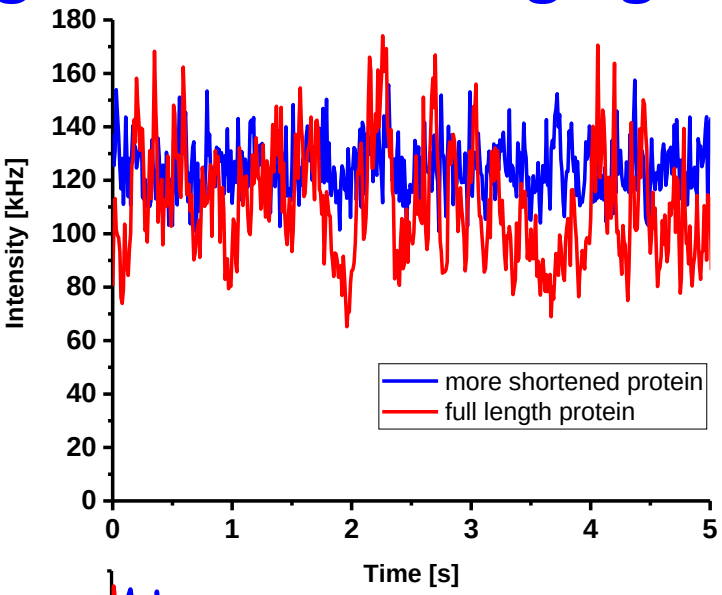
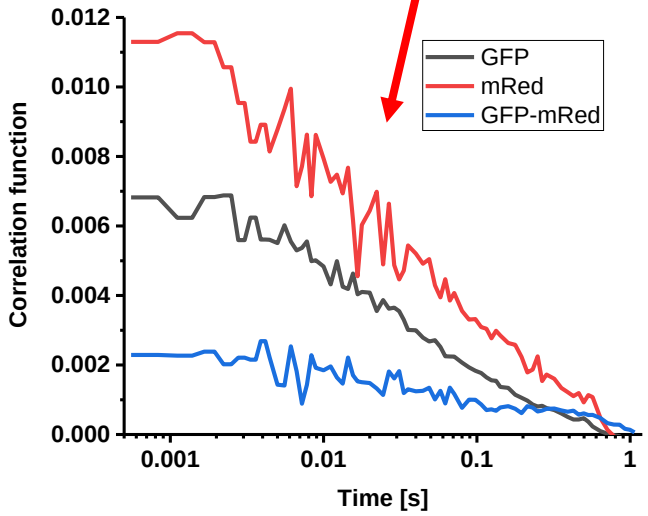
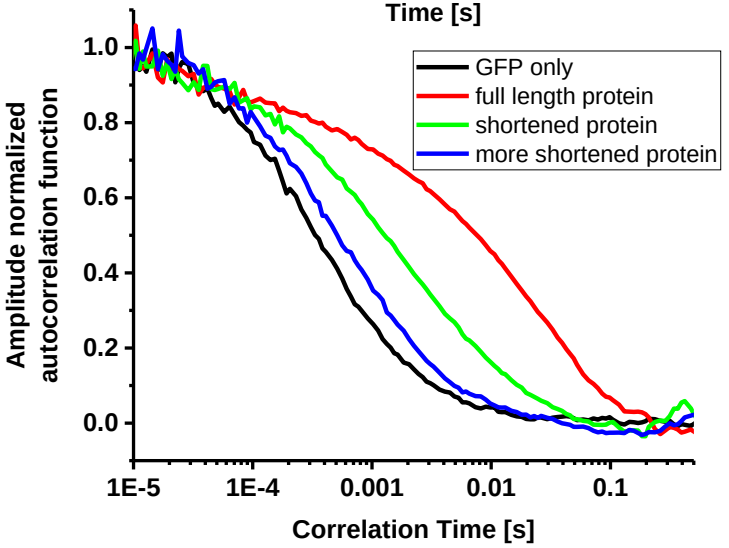
Multi-parametric Imaging
Fluorescence Correlation Spectroscopy
Single Molecule Imaging



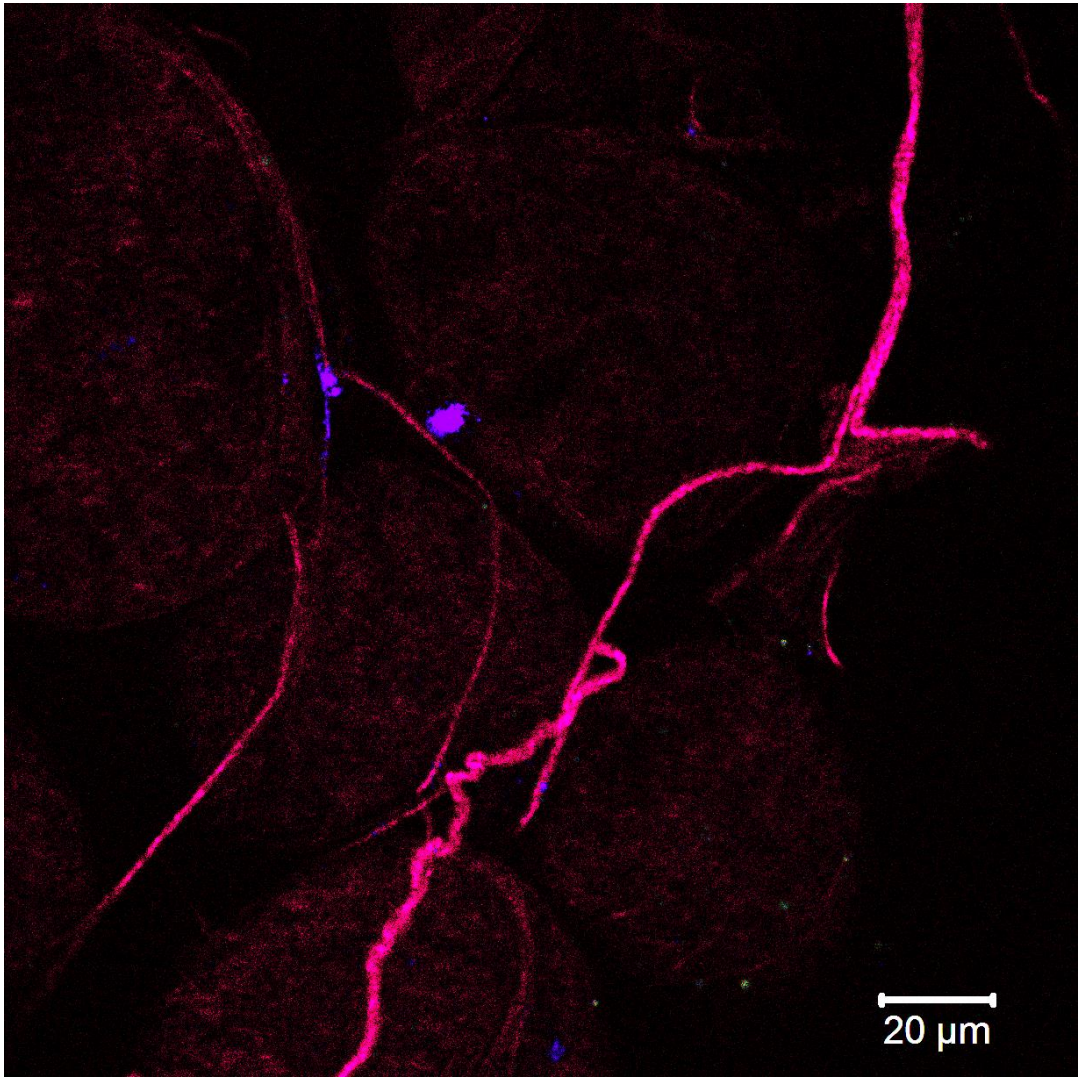


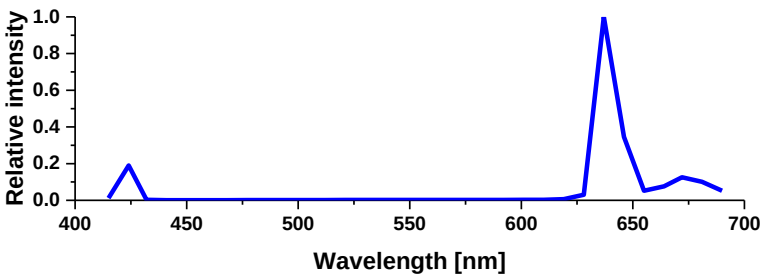




Data acquired in collaboration with David Staněk and Davide Basello (IMG AS CR).

Label-free Imaging
Second and Third Harmonic Generation
(CARS coming soon….)





Carl Zeiss LSM 880 NLO, Objective: 63x, 1.4 N.A. oil immersion Excitation: 1278 nm; internal spectral detector

Demonstration of SHG and THG signal in fatty tissue (salami Křemešník, Kostelecké uzeniny).

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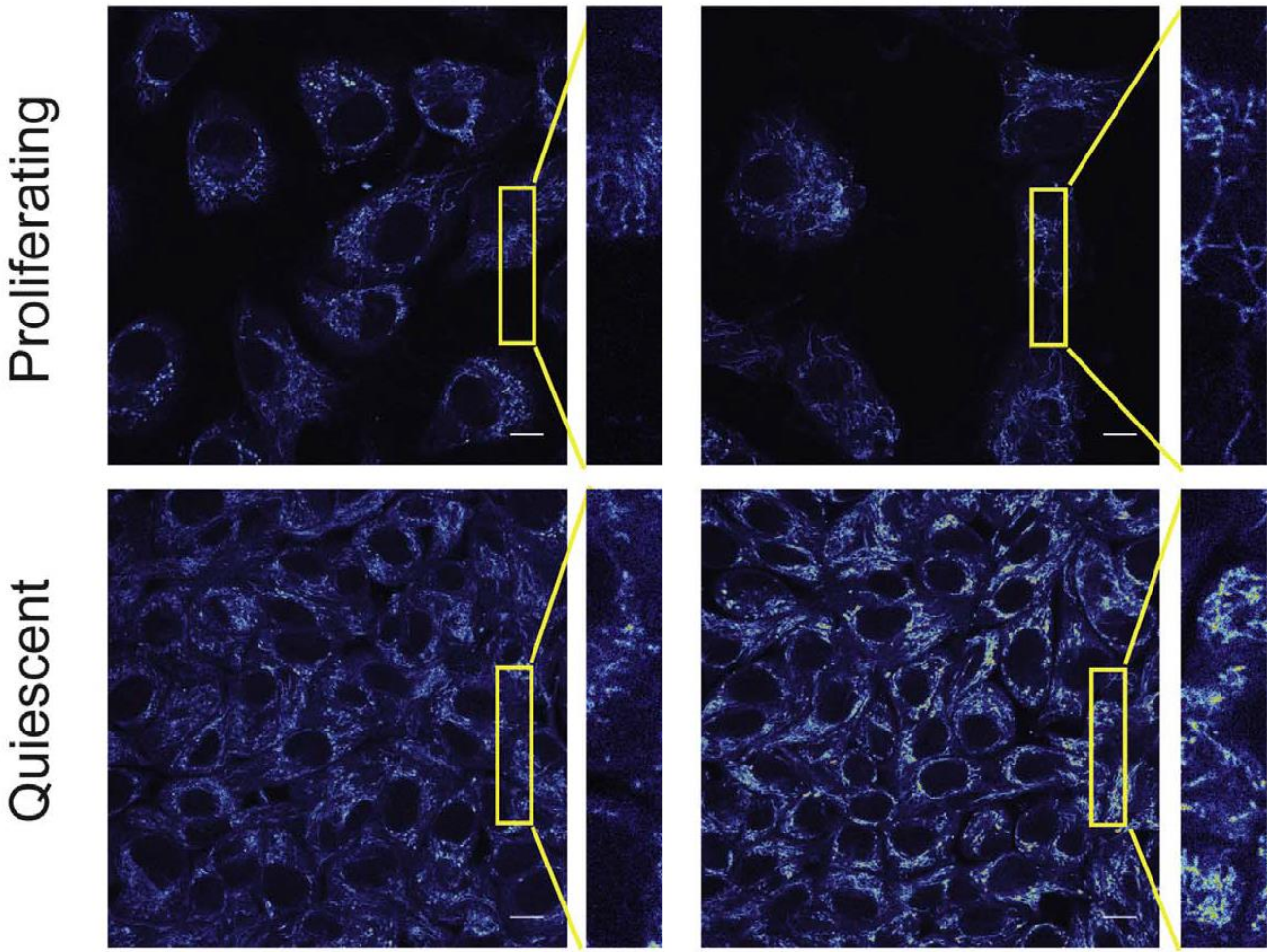
Label-free Imaging
2-photon Microscopy of NAD(P)H Autofluorescence



Carl Zeiss LSM 880
NLO, Objective: 63x,
1.4 N.A. oil immersion

Excitation: 740 nm,
Non-descanned
detection 390 – 480
nm

Quantifying mitochondrial metabolic activity.
J. Blecha et al. (2017), Free Radic Biol Med, 112: 253-256

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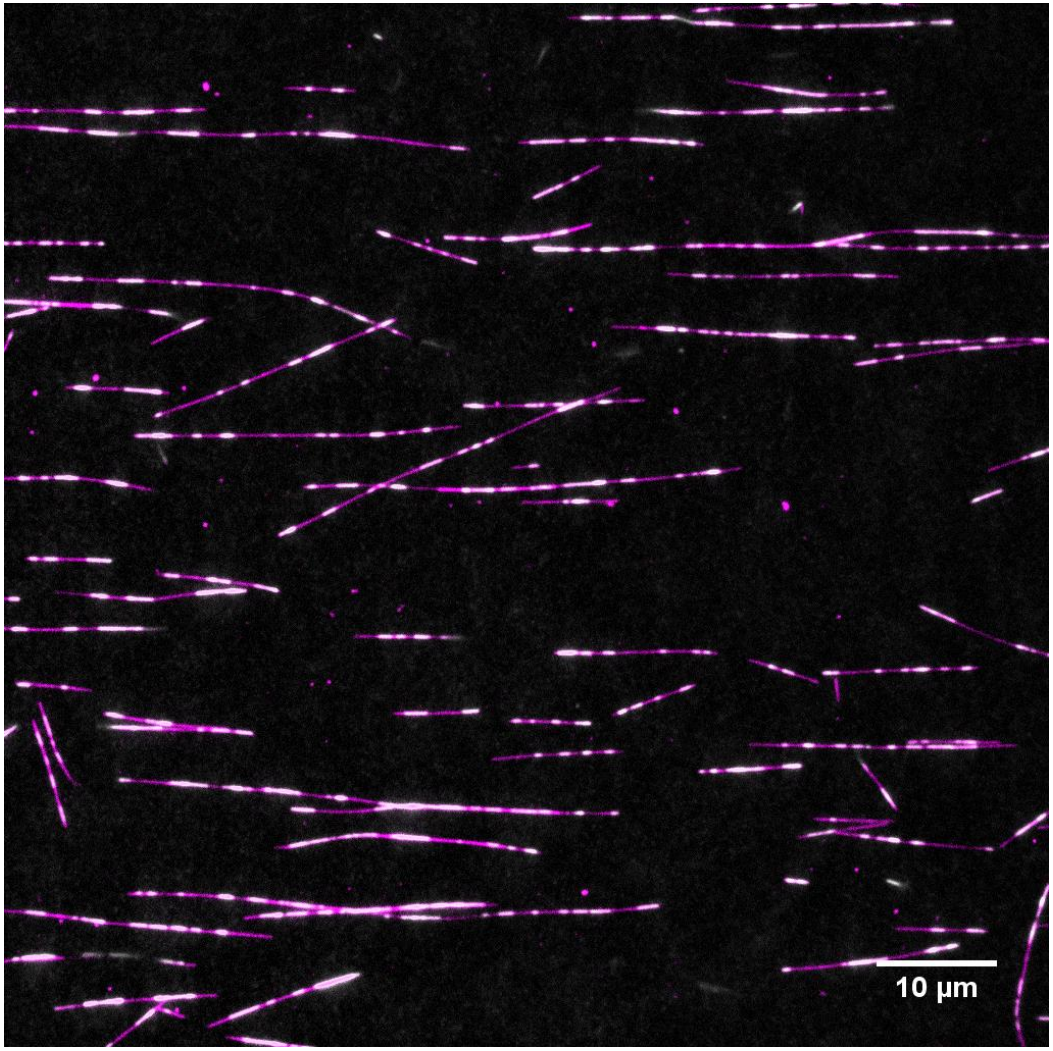
Single Molecule TIRF



Nikon Ti-E - TIRF illumination, TimeLapse
Objective: 63x, NA 1.49
Excitation: 561 and 640 nm
Detection: EM CCD camera Andor iXon Ultra DU888

Crosslinked microtubules (magenta) and actin fibers (white) imaged under TIRF illumination. Image courtesy of O. Kučera (Institute of Biotechnology AS CR).

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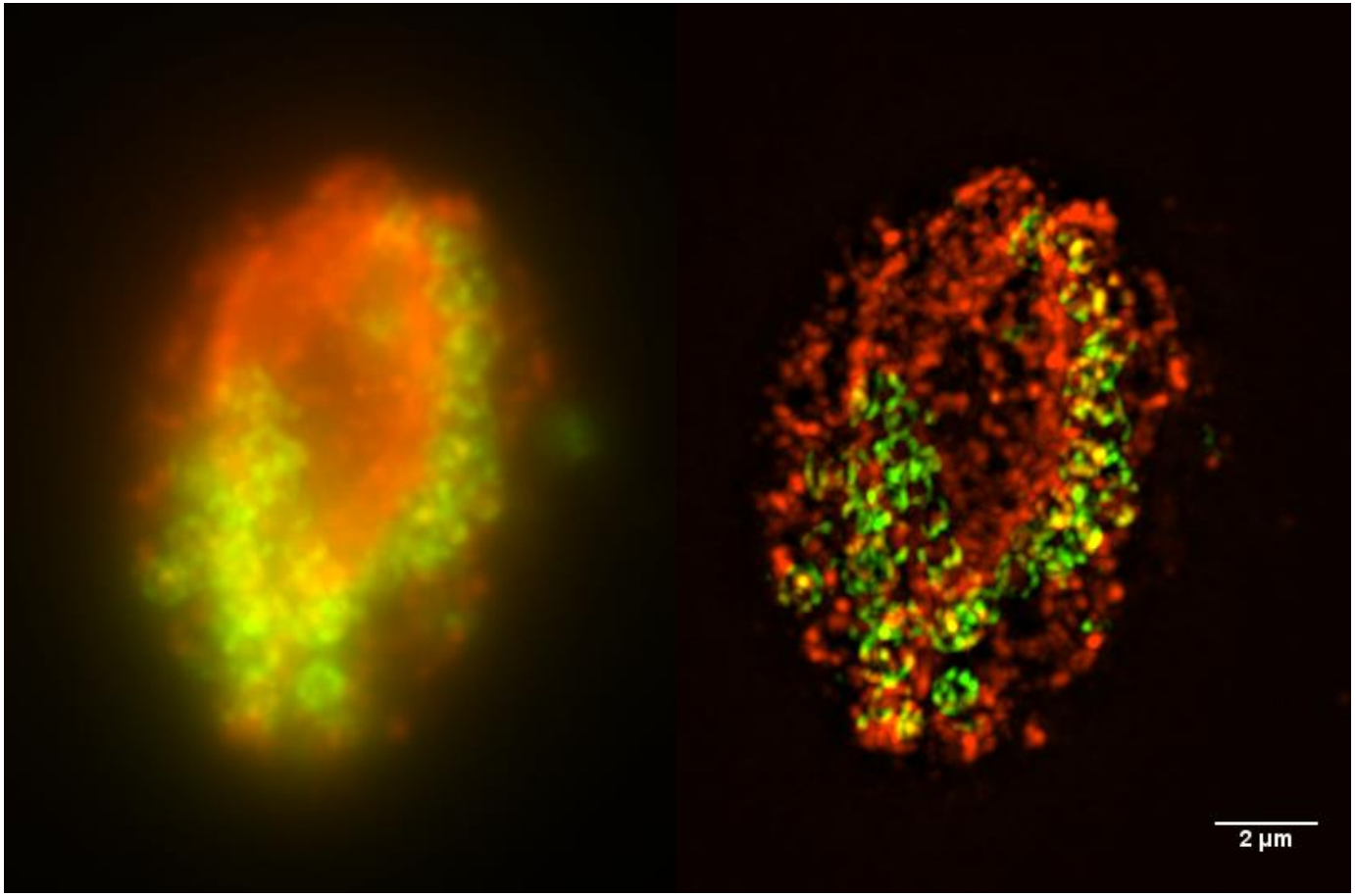
Super-Resolution Imaging
Structured Illumination Microscopy



Nikon N-SIM
Widefield and 3D SIM image (3 rotations, 5 phases)
Maximum Intensity Projection

Objective: 100x, NA 1.49
Excitation: 488 and 561 nm
Detection: EM CCD Andor iXon Ultra DU897

Widefield and SIM image of hydrogenosomes (labelled with Alexa 488) and endoplasmatic reticulum (labelled with Alexa 594) in Trichomonas Vaginalis. Image courtesy of P. Rada (Faculty of Science, Charles University).

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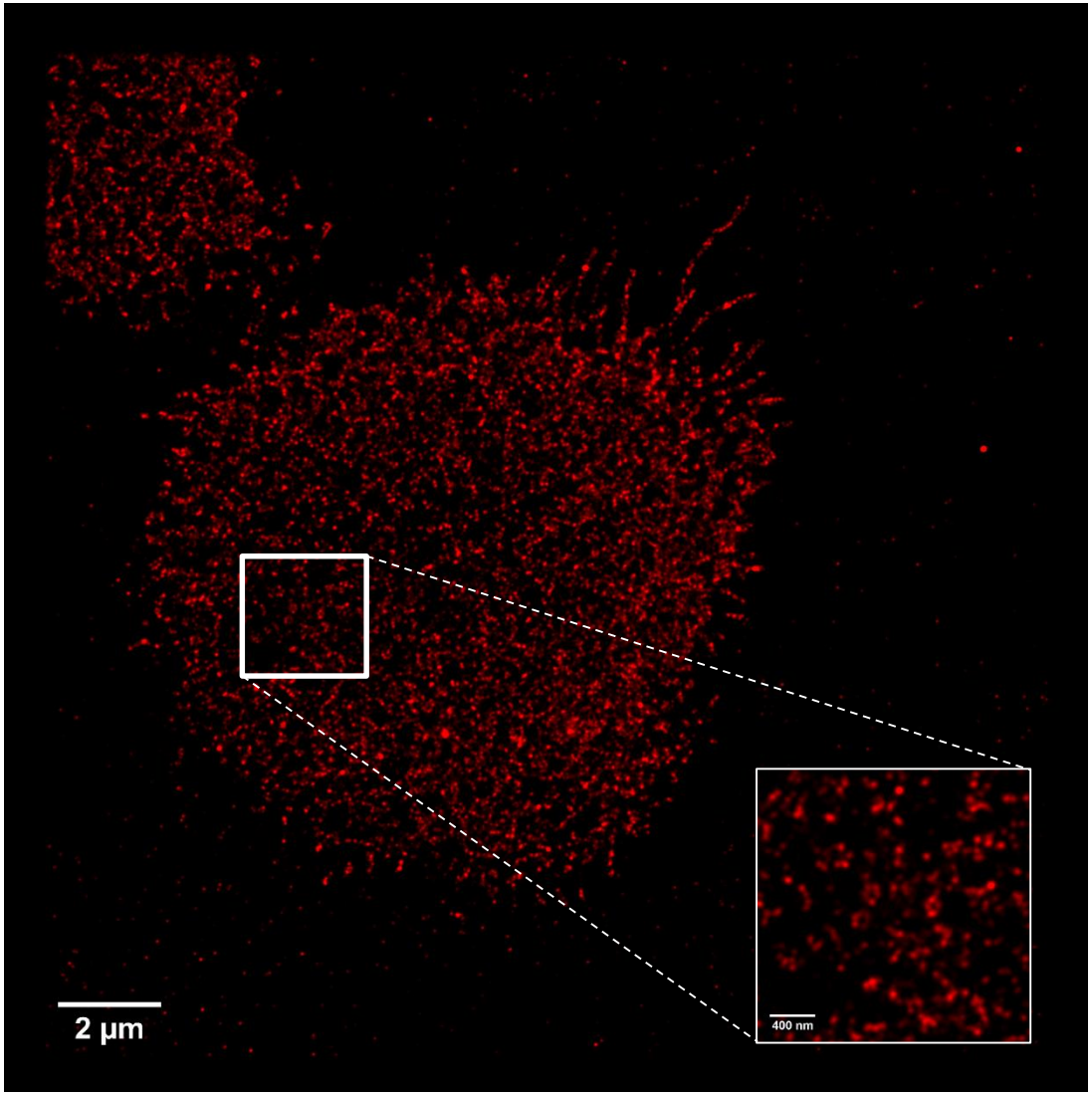
Super-Resolution Imaging Localization Microscopy



Nikon N-STORM
2D STORM, 30000 frames, Gaussian rendering, TIRF illumination
Objective: 100x, NA 1.49
Excitation: 647 nm, Activation: 405 nm

Distribution of CD4 receptor (labelled with Alexa 647) in plasma membrane of primary cells. Image courtesy of D. Glatzová (J.Heyrovsky Institute of Physical Chemistry AS CR).

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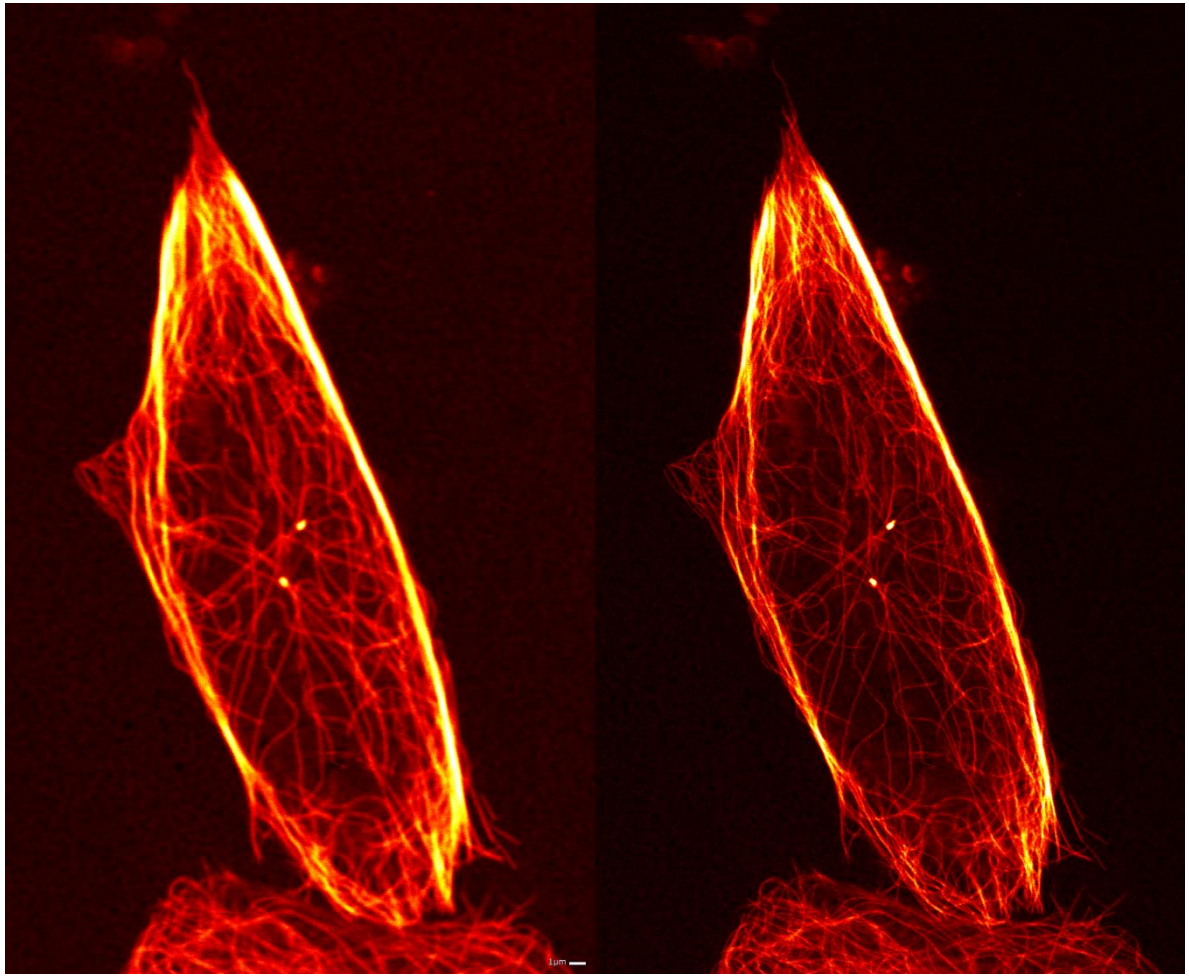
Super-Resolution Imaging STED



Abberior Instruments STED
Confocal and 2D STED image, raw data
Objective: 63xW, NA 1.2
Excitation: 640 nm, Depletion: 775 nm
Live cell imaging conditions: 37°C, 5% CO_2
Dual color 2D or 3D STED also available with
63x Oil, NA 1.4 objective.

Live cell imaging

Confocal and STED image of microtubules labelled with Silicon Rhodamine dye. Image courtesy of J. Kovářová (Institute of Biotechnology AS CR).

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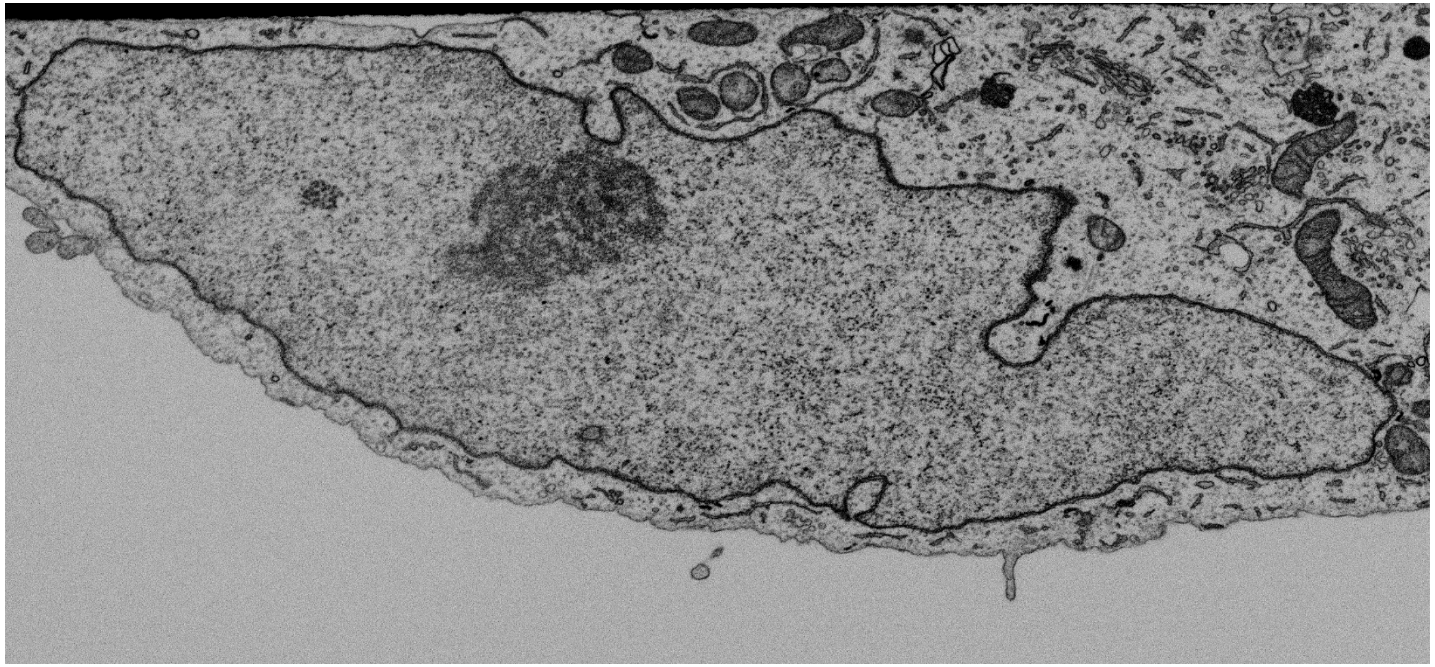
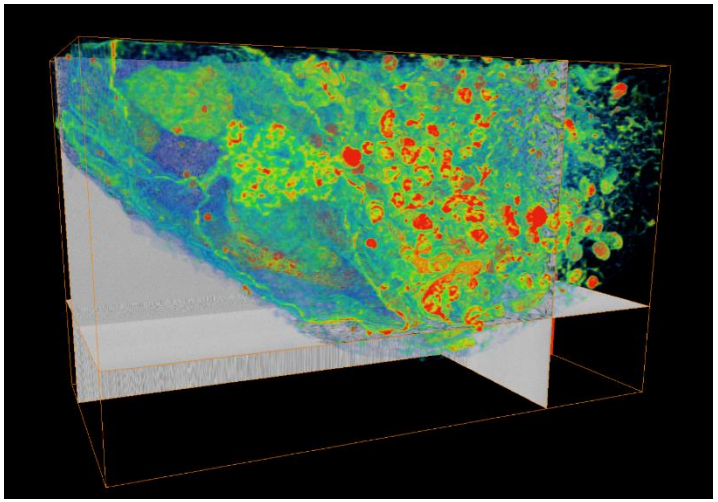
FIB-SEM



A single slice image and a 3D visualization of the HT1080 cell. The data were acquired by **Dual-beam FEI Helios Nanolab 660 G3 UC** with in-lens backscatter electron detector (ICD) at 5 nm slice thickness and 3 nm pixel size. The dataset was post-processed with **Amira Software 6.2.** The sample was provided by **Dušan Cmarko (FFM CU)**.



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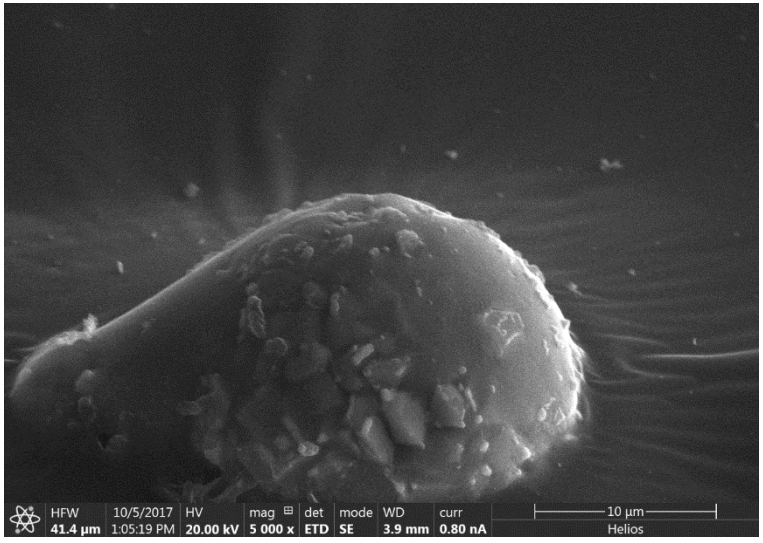
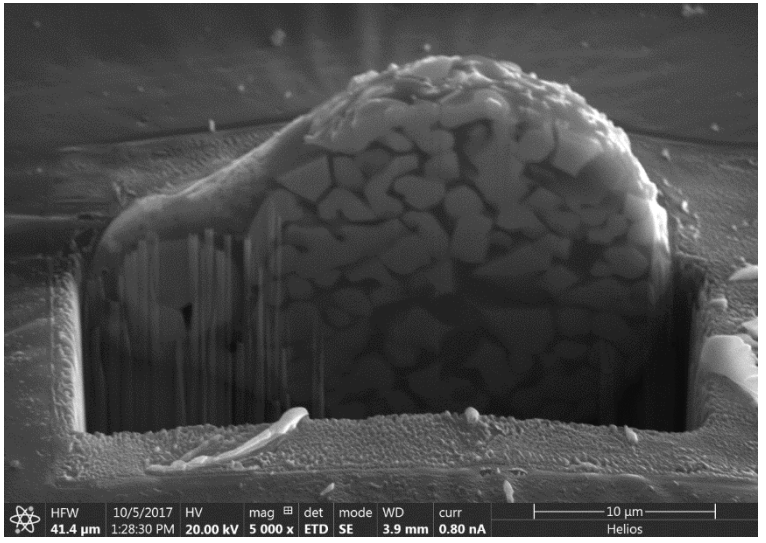
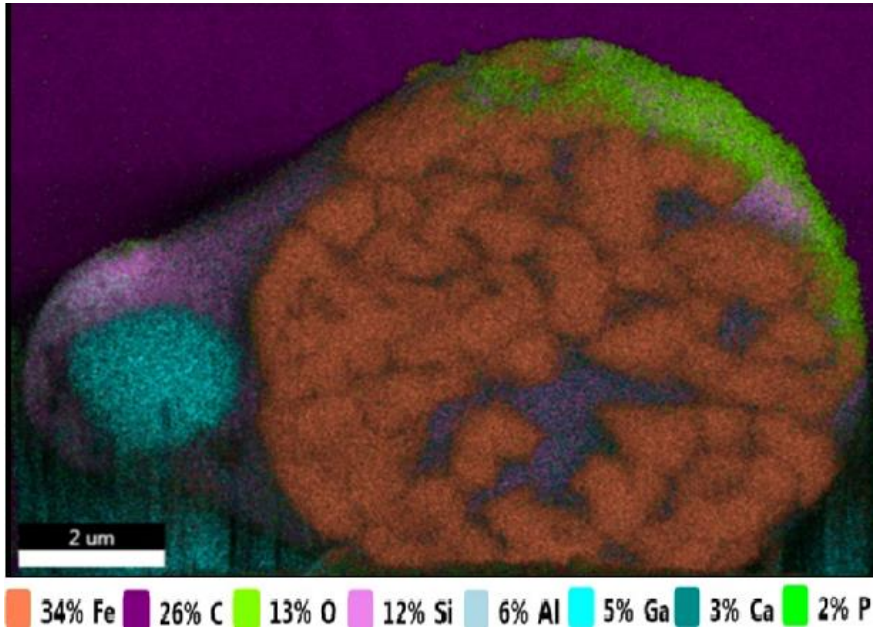
FIB-SEM with element EDS system







SEM images of a microparticle from lake sediments before and after FIB milling. **Element EDS System** provided the maps of element distributions both on the surface and inside of the microparticle. The resolution of element maps depends on the electrons penetration depth and volume from which X-rays are generated.

The sample was provided by **Gunther Kletetschka (FS CU)**.

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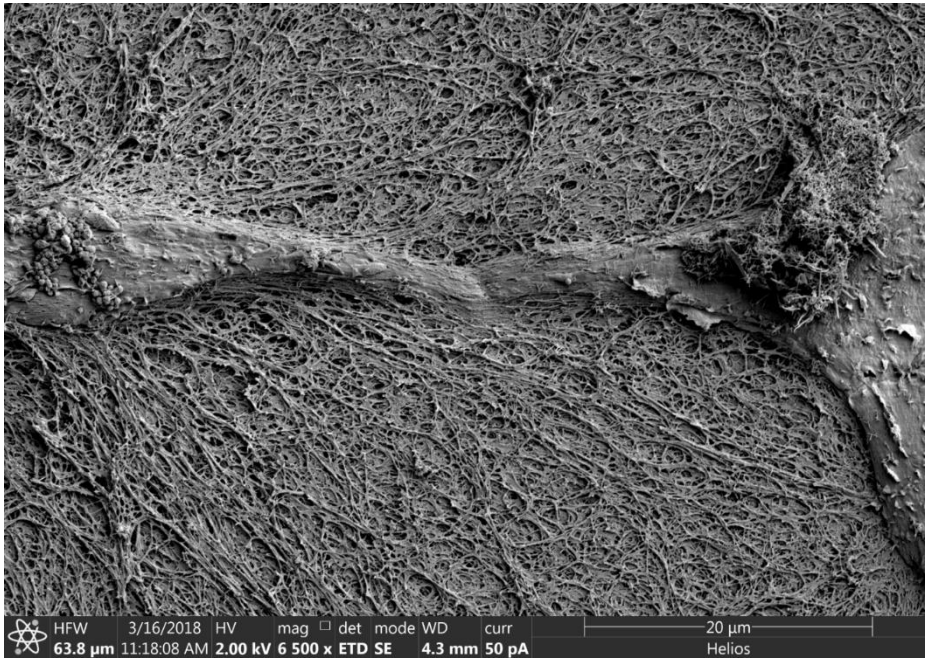
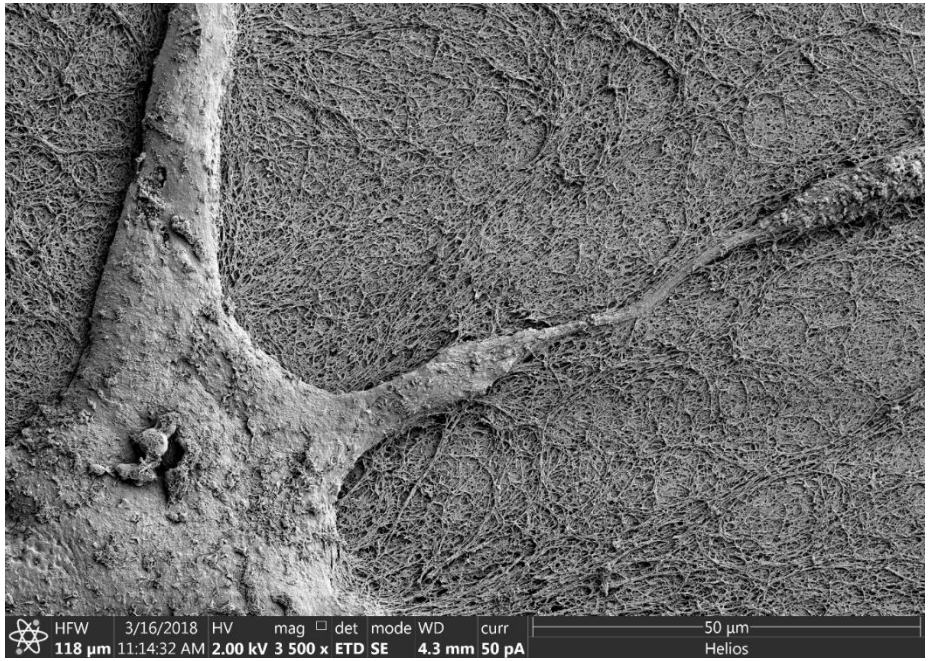
Critical point drying





Endothelial cells were grown on a hydrogel. The sample was processed by the Critical Point Drying method by **Leica EM CPD300** after chemical fixation with 2% OsO_4 .

The sample was provided by **Jana Zárubová (IPHYS CAS)**.

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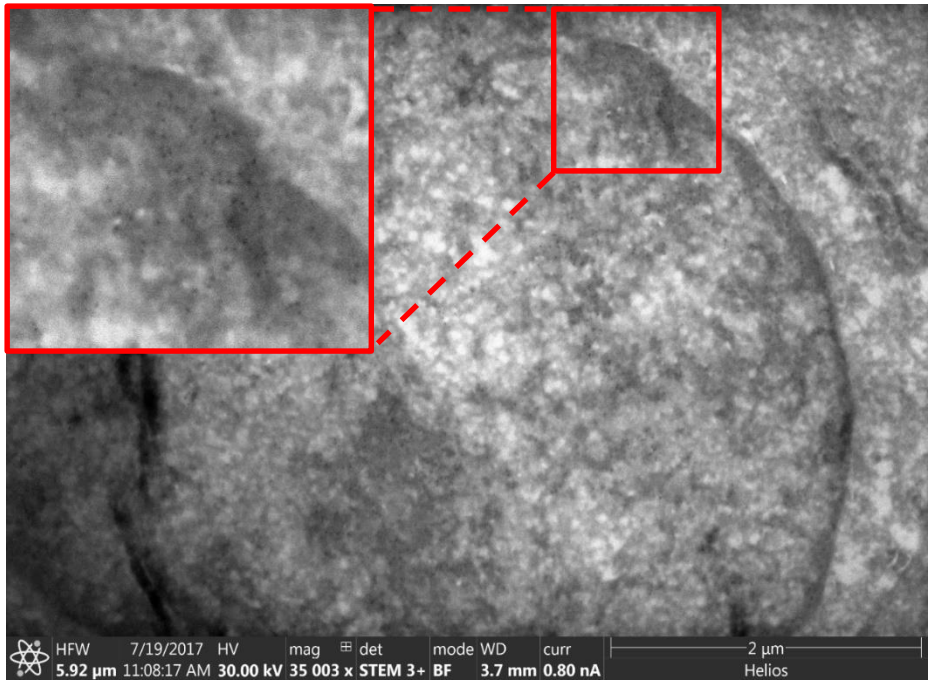
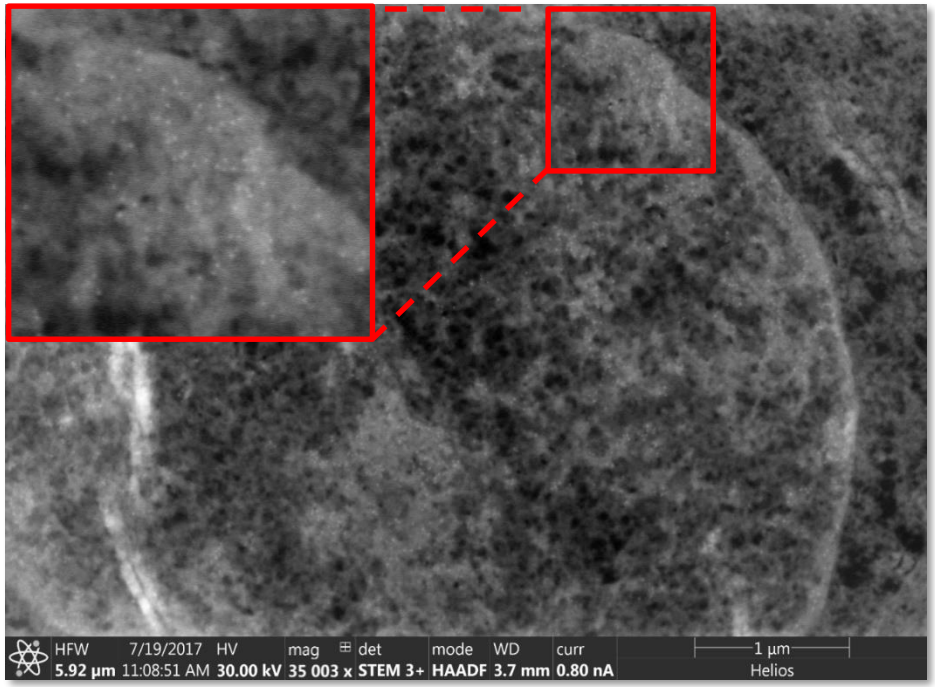
Tokuyasu technique





High-resolution STEM bright (left) and dark field (right) images of primary pancreatic beta cells, which were prepared by **Tokuyasu technique** followed by immunolabeling with 12 nm golden nanoparticles (Histon1), post-contrasted with 4% uranyl acetate and 2% lead citrate.

The sample was provided by **Andrea Dlasková (IPHYS CAS)**.

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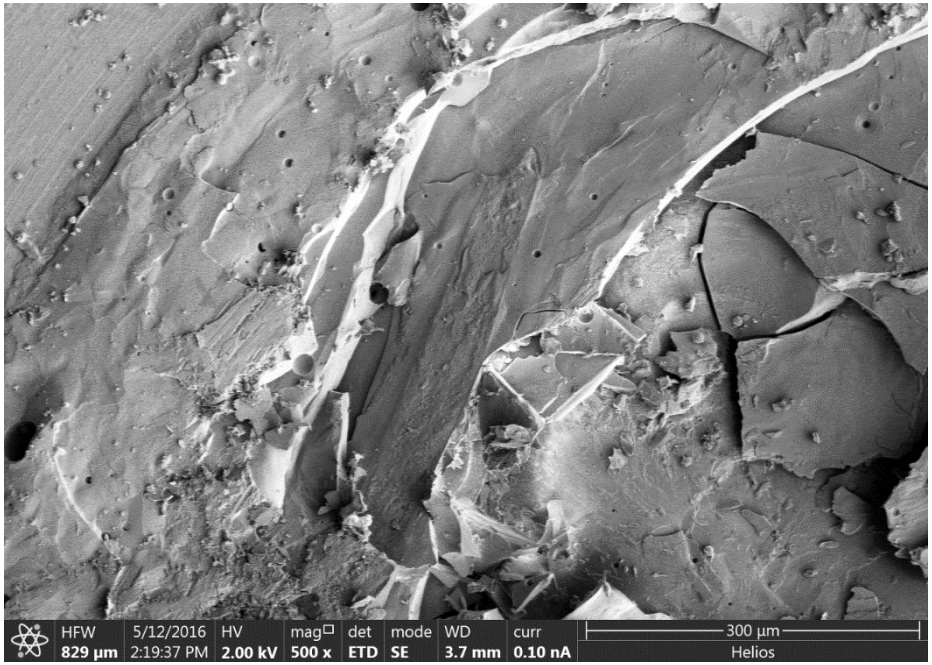
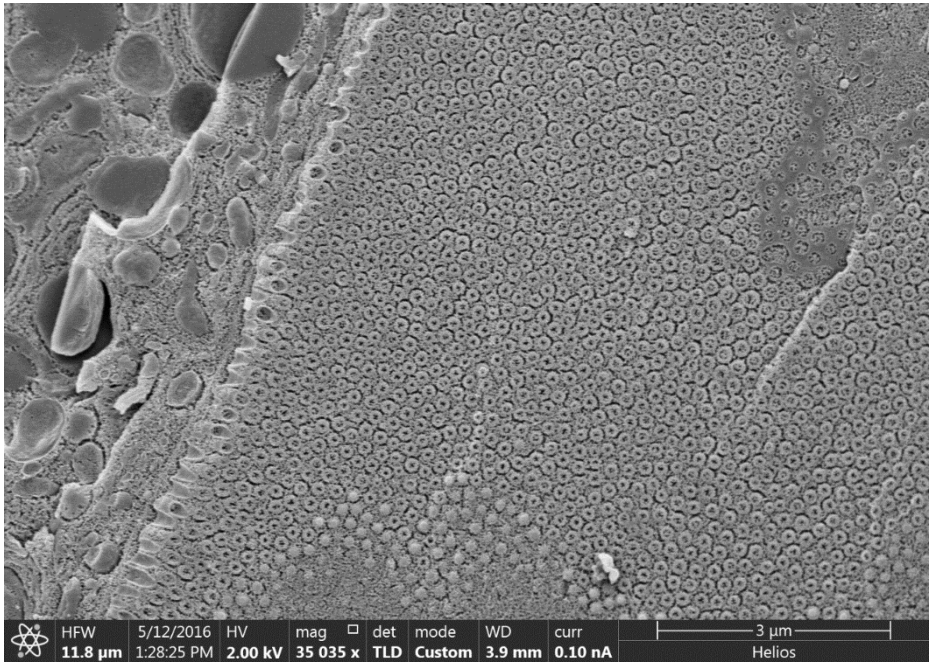
HPF and freeze fracture

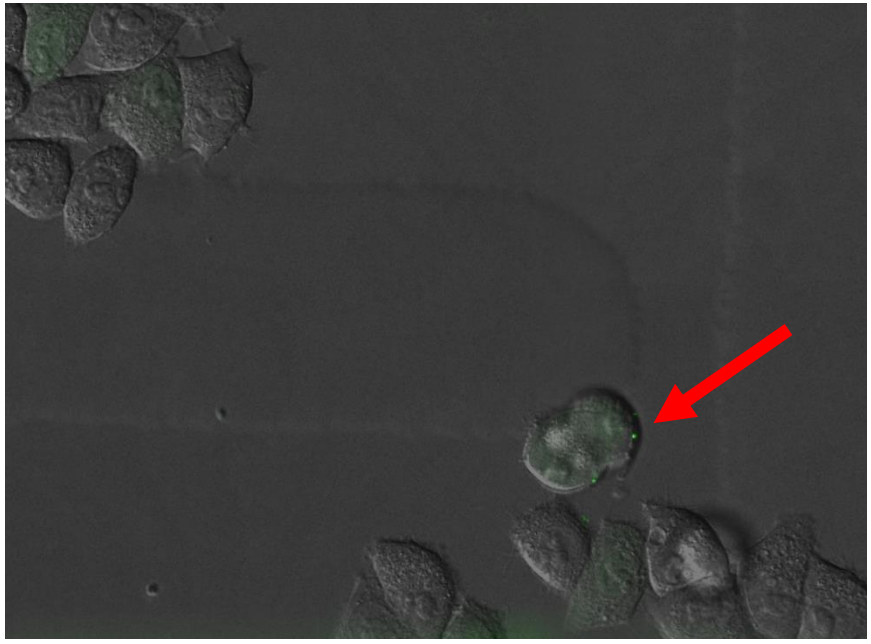


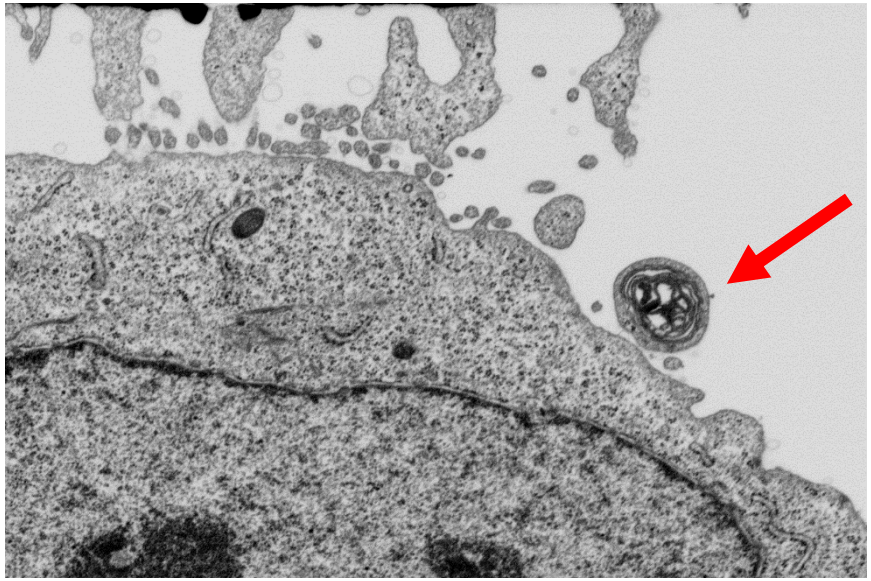


An overview and a detail SEM images of *Caenorhabditis elegans* after freeze fracture. The sample was frozen by **Leica HPM100** and then broken in the high vacuum coater **Leica ACE600**. The sample was transferred into a microscope with the shuttle **Leica VCT500** and images were recorded under cryo condition.

The sample was provided by **Christian Lanctot (FS CU)**.

BIOCEV Correlated Light and Electron Microsopy





Combined DIC and fluorescence image of HeLa cells (left, acquired on **TCS Leica SP8**) cultivated on a dish with a grid shows the accumulation regions of Oregon Green labeled peptide. After chemical fixation and sample processing for FIB-SEM, the imprinted grid (middle SEM image) was used to identify the particular cell and localize the target site (red arrow). Subsequent 3D FIB-SEM acquisition reveals the targeted volume ultrastructure (single slice image on the right).

The sample was provided by **Pavel Jungwirth (IOCB CAS) and Marek Cebecauer (JH Inst CAS)**.

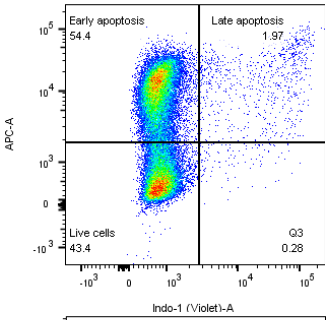
Flow cytometry analysis applications

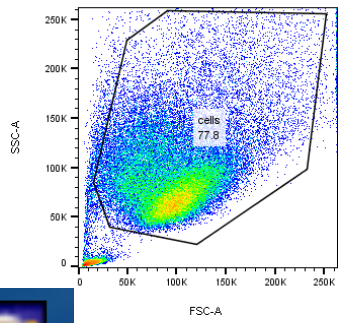
- ***Simple (1-3 colours)***

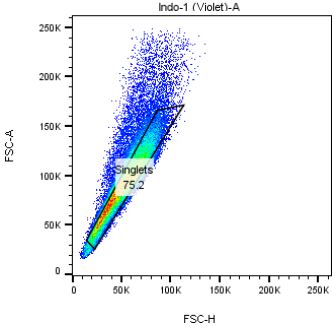
Apoptosis study in H28 mesothelioma cells transduced with lentiviral Empty vector with puromycin resistence

Drug application:
Step 1.
Homoharringtonine (HHT), 100nM for 1 hour
Step 2.
ABT737, 15uM for 2 hours
Homoharringtonine (HHT), 100nM for 2 hours

2-colour panel:
Annexin V - Dy647
Hoechst 33258

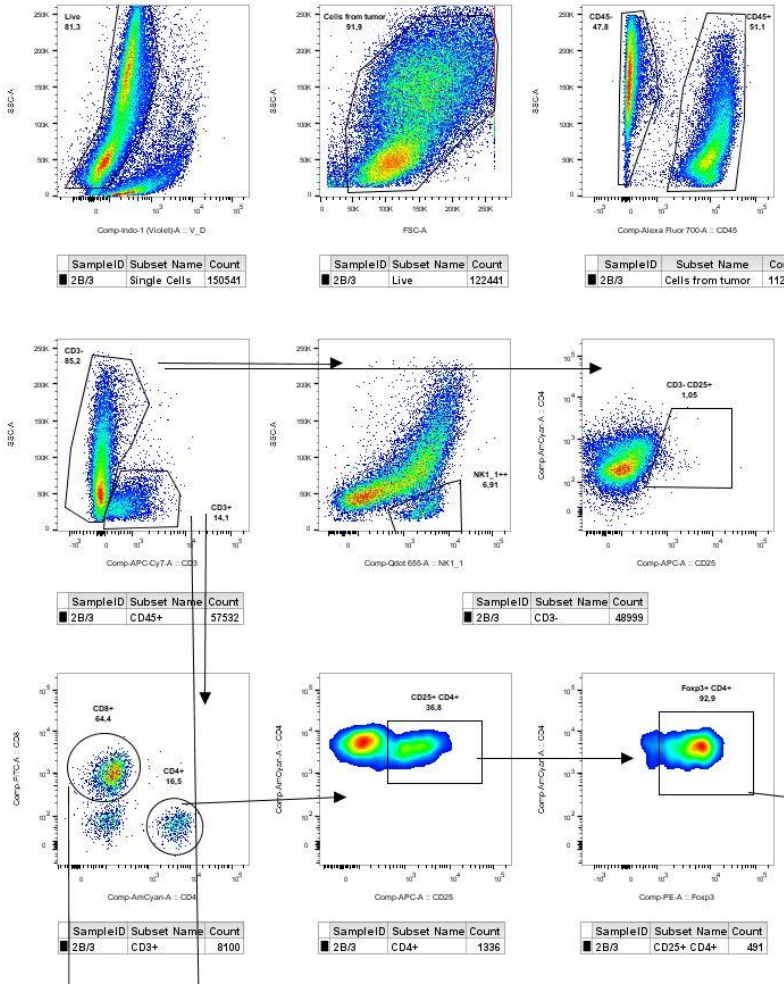






- ***Advanced (4-18 colours)***

Identification of mice tumor infiltrated cells with lymphoid origin



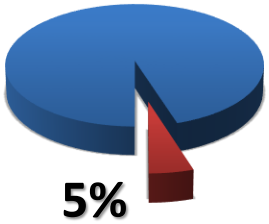
9-colour panel:
CD8-FITC
NK 1.1-BV650
CD4-BV510
Helios-BV421
Viability dye-eFluor455UV
FoxP3-PE
CD3-APC-Cy7
CD45-AF700
CD25-APC

Data courtesy of Peter Holíček, IBT

Data courtesy of RNDr. Ingrid Poláková, PhD

BD FACSDiva Software

Fluorescent Activated Cell Sorting applications

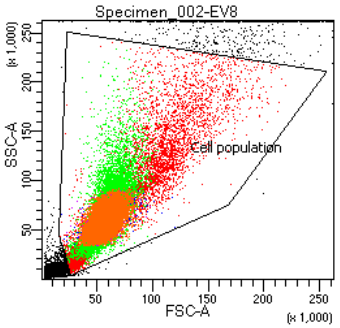


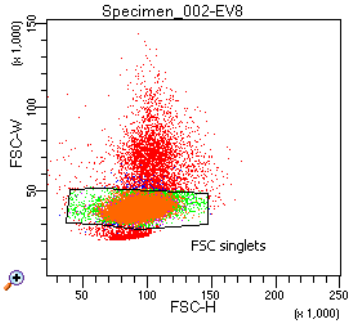
95% <u>of trivial applications:</u> - transfected cell culture (GFP-, mCherry-positive)
- singlet events

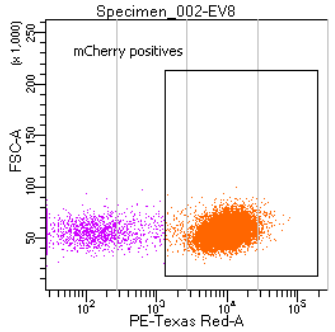
5%

<u>of non-trivial applications:</u>

- Murine fetal oocytes
- Murine thymocytes
- Sturgeon germ cells
- Algae …

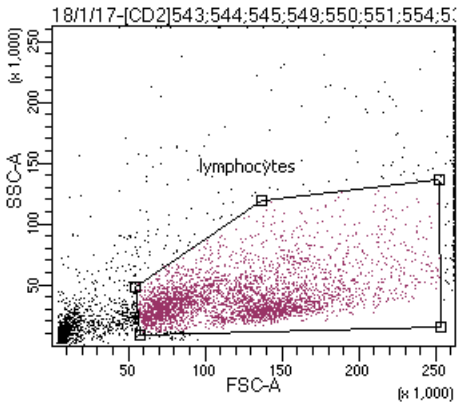


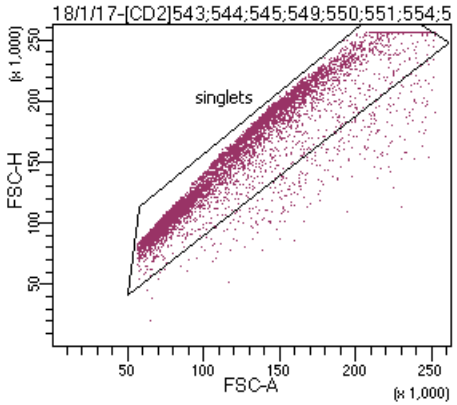


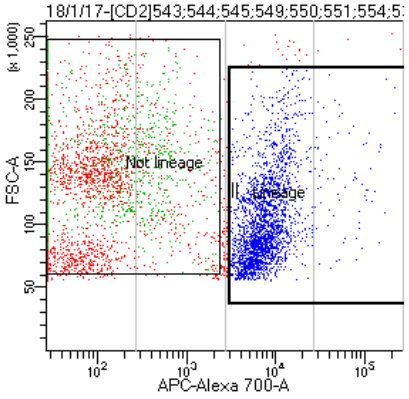


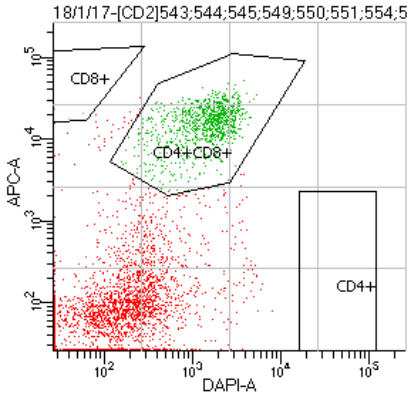
FACS of murine thymocytes from 5 weeks old mice

<u>4-colour panel</u> CD8-APC, CD4-BV421, CD71-PE Gr-1-biotin, Mac-1-biotin, B220-biotin, Nk1.1-biotin, CD11b-biotin, streptavidin-AF700









<u>Data courtesy of Tomáš Zikmund, Charles University</u>

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IMCF instruments

Flow cytometry

1. **BD FACS Aria Fusion** - Fluorescence activated cell sorter (FACS) – 5 lasers, 18 fluorescent detectors, quartz cuvette flow cell, 4-way sorter, 70 000 events per second
2. **BD LSR Fortessa SORP** - Flow cytometry analyzer - 5 non-collinear lasers, 18 fluorescent detectors, 40 000 events per second

Electron microscopy

1. **FEI Helios NanoLab G3 UC** – FIB-SEM - scanning electron microscope with focused ion beam milling
2. **120 kV TEM – 2nd half 2018** – *till then TEM at Viničná or IMG*
3. Equipment for EM sample preparation including cryo-EM
   - I. **Leica EM HPM100** - High-pressure freezing cryo-fixation
   - II. **Leica EM ACE600** – High-vacuum coater
   - III. **Leica ULTRACUT EM UC7 with Leica EM FC7 Cryo Chamber with VCT** - Ultramicrotome with cryo-chamber
   - IV. **Leica EM AFS2** - Freeze Substitution and Low Temperature Embedding System
   - V. **Leica EM CPD300 A** - Critical point drier
   - VI. **Leica EM KMR3** - Glass knifemaker

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IMCF instruments

Middle-range optical microscopes – so far **LOW USAGE**

1. **Carl Zeiss AxioZoom.V16** - Upright fluorescence macroscope with optical zoom for large specimen observation, **motorized stage**.
2. **Leica DMi8** - Inverted wide-field microscope with **motorized stage**, Cool-LED illumination and fast and sensitive EM-CCD (or sCMOS) camera
3. **MD ImageXpress Micro XLS (IBT) -** A combination of Flow Cytometry and Fluorescence Microscopy. It allows high throughput scanning of fixed samples with subsequent cytometry-like statistical analysis of cell populations.
4. **Andor DSD2 modul on Carl Zeiss Axio Observer Z1 (Palková group) -** A combination of structured illumination and spinning disk technologies with high sensitivity high dynamic range Andor Zyla 5.5 sCMOS camera allows fast confocal like 3D imaging for DAPI, GFP and RFP channels.
5. **Leica TCS SP8 DM6 CFS** - Upright confocal and wide-field microscope with manual fixed stage, suitable also for electro-physiological measurements, excitation wavelengths 405 nm, 488 nm, 552 nm and 638 nm, freely spectrally selectable detection with one high sensitivity HyD detector and three standard PMT detectors. **63x oil NA1.4 objective**

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IMCF instruments

High-range optical microscopes – wide-field systems - **HIGH USAGE**

1. **Nikon Ti-E + Laser H-TIRF System** - Wide-field inverted fluorescence microscope with TIR excitation, high sensitivity EM-CCD camera, **fast sCMOS camera and dual color emission image splitting optics** for fast multi-color single molecule or long term live cell experiments, excitation wavelengths 405 nm, 488 nm, 561 nm and 640 nm.

   Move (long term) live cell experiments to other systems

2. **Nikon Ti-E with N-SIM and N-STORM** - Fluorescence wide-field super-resolution microscope with 3D single molecule localization (SMLM) module and 3D structured illumination microscopy (SIM) module, available wavelengths 405 nm, **440 nm**, 488 nm, 561 nm and 640 nm, TIRF module and sCMOS and EM-CCD cameras, including **dual color emission image splitting optics** for fast multi-color single molecule or long term live cell experiments

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High-range optical microscopes – confocal systems - **HIGH USAGE** (STED medium)

1. **Carl Zeiss LSM 880 NLO** - Intravital inverted two-photon and confocal microscope with 32+2 channel spectrally resolved detection and with four reflected and two transmitted NDD detectors (including two highly sensitive GaAsP detectors suitable for **FLIM**), equipped with MP and OPO lasers for two colour MP excitation (690 nm – 1300 nm) and with the full set of visible cw lasers (405 nm, 458 nm, 488 nm, 514 nm, 561 nm, 633 nm) for one photon excitation. Apart from fluorescence allows for SHG imaging of collagen and other fibres, THG and **CARS**.

2. **Leica TCS SP8 WLL SMD-FLIM** - Inverted confocal microscope with fluorescence lifetime imaging module (FLIM), including pulsed white light laser (470 nm - 670 nm), pulsed 405 and **440 nm** lasers and freely spectrally selectable detection with **three** high sensitivity **SMD-HyD** detectors and two standard PMT detectors.

3. **Nikon Ti-E with STED from Abberior Instruments** - Fluorescence confocal super-resolution microscope with 3D stimulated emission depletion (STED) at 775 nm and excitations 561 nm and 640 nm and 2D STED at 592 nm with 485 nm excitation. Includes **ResCUE** option for minimizing laser exposure. In addition there is 405 nm cw laser, **4 SPAD** detectors and **FLIM module**. Long term live cell experiments.

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IMCF instruments

Data analysis software (commercial)

1. **Huygens professional** – image restoration (deconvolution), analysis and visualization software, contains deconvolution modules for confocal, wide-field, two-photon and STED data
2. **Amira** – a software platform for 3D and 4D data visualization, processing, and analysis (focus on SEM electron microscopy data)
3. **Matlab** with image processing toolbox
4. **FlowJo** – software for flow cytometry data analysis
5. **Offline licences** for Carl Zeiss ZEN, Nikon NIS-elements, Leica LAS X, Abberior ImSpector and BD FACSDiva (USB dongle server)
6. **Kaluza** – software for flow cytometry data analysis

Data analysis software (free)

1. FIJI (ImageJ)
2. Icy
3. TTTR Data Analysis (home-made FLIM software)

IMCF team at BIOCEV (7.1 FTE)

1) Coordination
   - **Aleš Benda** (0.6 FTE)
   - Administrative support – Iva Hůleová, Monika Cviková, Kateřina Jánská, BIOCEV IT
2) Flow cytometry – 0.9 FTE
   - **Galina Kislik** (0.6 FTE + 0.4 FTE at V7)
   - **Markéta Dalecká** (0.2 FTE)
   - **Petra Prokšová** (0.1 FTE)
3) Optical microscopy – 2.6 FTE
   - **Aleš Benda** (0.4 FTE) – F-methods (FLIM, FCS, etc…)
   - **Radek Macháň** (0.8 FTE + 0.2 FTE at V7) – advanced imaging and data analysis
   - **Marie Olšinová** (0.8 FTE + 0.2 FTE at V7) – super-resolution imaging
   - **Petra Prokšová** (0.1 FTE) – live cell imaging
   - **Ján Sabó** (0.5 FTE) – quality control
4) Electron microscopy – 2.5 FTE
   - **Markéta Dalecká** (0.8 FTE) – FIB-SEM operator
   - **Lenka Koptašíková** (1 FTE) – EM sample preparation also for users from V7
   - **Lucia Motlová** (0.5 FTE) – High-pressure freezing and sample preparation
   - **Adam Schröfel** (0.2 FTE) – external advisor from MPI Dresden – to return in 8.2019
5) Data analysis – 0.5 FTE
   - **Ondřej Ťupa + Martin Schätz** (0.5 FTE + 0.5 FTE at V7) – 3D data analysis

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IMCF events 2018

Past:

28.-30. May – Advanced live cell imaging practical course – **IMG**

Forthcoming:

20.-22. June – Training Workshop on Time-Resolved Techniques (TReT)

22.-26. Oct - Single molecule microscopy and manipulation - practical course - **IBT**

12.-14. Nov - Super-resolution in Light Microscopy practical course - **IMG**

26.-28. November – Fluorescence lifetime and other multi-parametric imaging and its applications - practical course **J.Hyerovský Institute of Physical Chem.**

Spring 2019 – FIB-SEM practical course

Irregular lectures:

Principles of fluorescence microscopy - Radek Macháň

Flow cytometry lecture series – Galina Kislik, Jozef Janda

EM user meetings

Instrument demonstrations

BIOCEV

IMCF teaching

Winter:

Mikroskopická technika – 5.block – excursion to BIOCEV (>200 students)

Single molecule microscopy and manipulation - 5 days practical course

Fluorescence lifetime and other multi-parametric imaging and its applications – 3 days practical course

Summer:

Seeing is believing - 2h lectures and 2 days of practicals – togather with Marek Cebecauer

??FIB-SEM practical course?? - 5 days practical course

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Booking system

- Unified for BIOCEV and Viničná 7 (Flow cytometry, Microscopy to come soon)
- **217 registered users, 38 items, 3860 reservation events per 2017**
- Paid instruments, analysis computers, software licenses, small equipment

News:

- Export to Google calendar – exists, but problematic
- **Group manager** – able to see reservations of the whole group
- Announcements – targeted for the desired user group
- Select the proper **type of service!**
- Mobile access – problematic, asking for improvements

Pricing

Imaging Methods Core Facility (IMCF) at BIOCEV, Faculty of Science, Charles University

Internal Charles University payment price list 2018

(external payment incures extra overhead/administration fee 17.65% and VAT)

Measurement type/instrument **Optical microscopy**	*Price per hour without VAT in CZK*
Brightfield (no lamp, no lasers)	100
Fluorescence widefield (lamp, no lasers)	150
Fluorescence widefield + TIRF (lasers)	200
Standard confocal	200
High-end confocal	300
Advanced and special methods (MP, CARS, super-resolution, FLIM, FCS, etc...)	350

CZ Axio-Zoom V16	Leica elphys DMi8	MD Image Xpress (IBT)	CZ + Andor DSD2	Nikon H-TIRF	Leica elphys SP8 upright	Leica SP8 FLIM + FCS	CZ LSM880 NLO	Abberior + Nikon STED	Nikon SIM STORM
X	X	X	X	X	X	X			X
X	X	X	X	X	X	X			X
				X					X
					X				
						X	X	X	
						X	X	X	X

Electron microscopy

SEM	800
SEM with FIB	950
EM sample preparation has its own price list	

- EM includes full assistance

- long automated after-hours experiments may have 50% discount

Flow cytometry

Flow cytometry - analysis	350
Flow cytometry - sort including assistence	550*

LSRFortessa	FACSAria
X	X
	X

Others

Huygens software	50
Microarray Reader	100
Microscopy expert assistance/data analysis	300#

Acknowledgment for MICROSCOPY

We acknowledge the Imaging Methods Core Facility at BIOCEV, institution supported by the Czech-BioImaging large RI projects (LM2015062 and CZ.02.1.01/0.0/0.0/16_013/0001775, funded by MEYS CR) for their support with obtaining imaging data presented in this paper.

Acknowledgment for FLOW CYTOMETRY

We acknowledge the Imaging Methods Core Facility at BIOCEV for their support with obtaining flow cytometry data presented in this paper.

**sorting outside of official sorting days incurs extra instrument switch on/off charge 2250 CZK to cover the extra costs*

 # the assistence requested above the standard training and user support (measurement and/or data analysis as a service)

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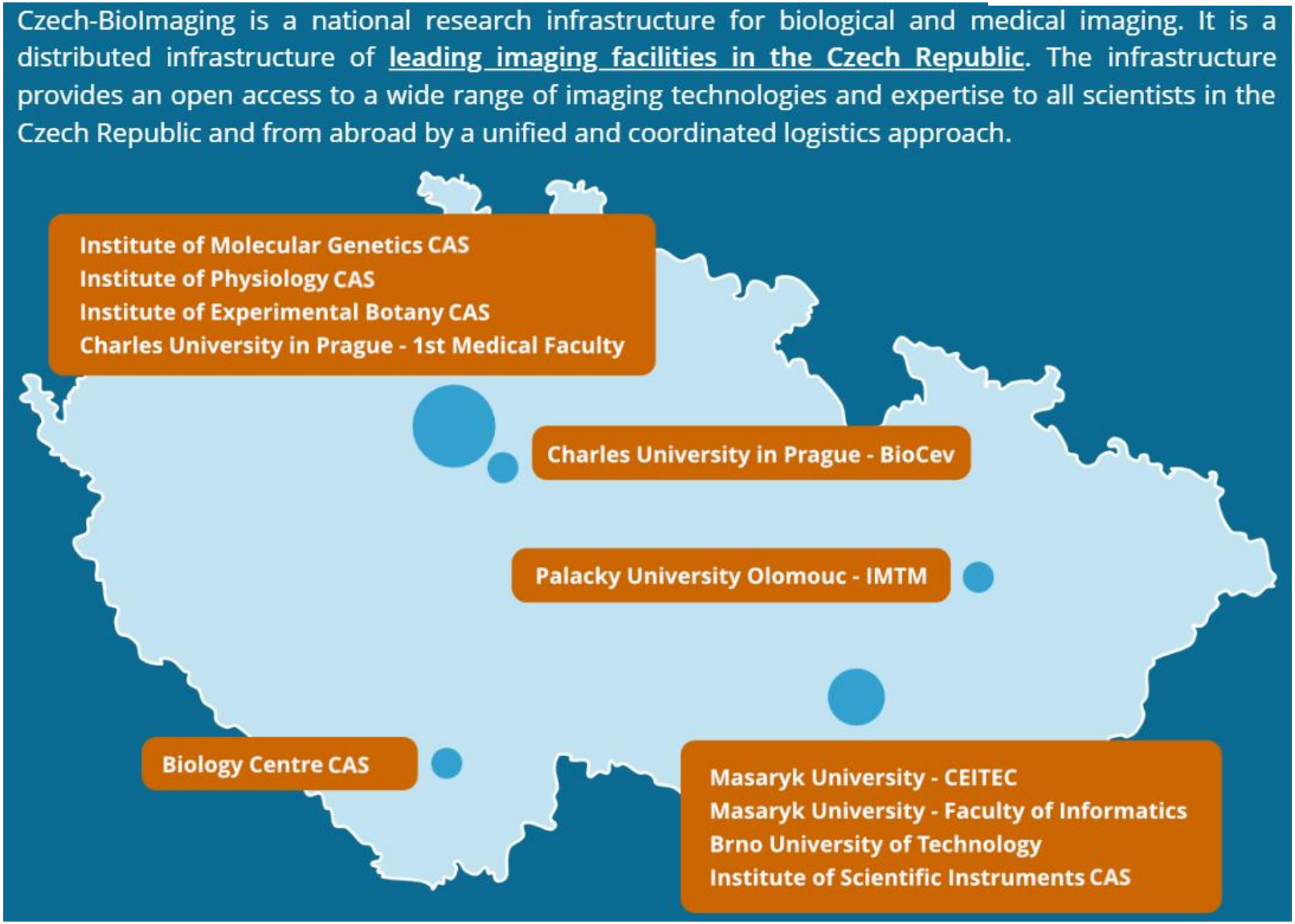
CZECH BIOIMAGING

Imaging principles of life

FACULTY OF SCIENCE
Charles University

Large Infrastructures

Czech-BioImaging is a national research infrastructure for biological and medical imaging. It is a distributed infrastructure of leading imaging facilities in the Czech Republic. The infrastructure provides an open access to a wide range of imaging technologies and expertise to all scientists in the Czech Republic and from abroad by a unified and coordinated logistics approach.



Large Infrastructures

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EURO-BIOIMAGING

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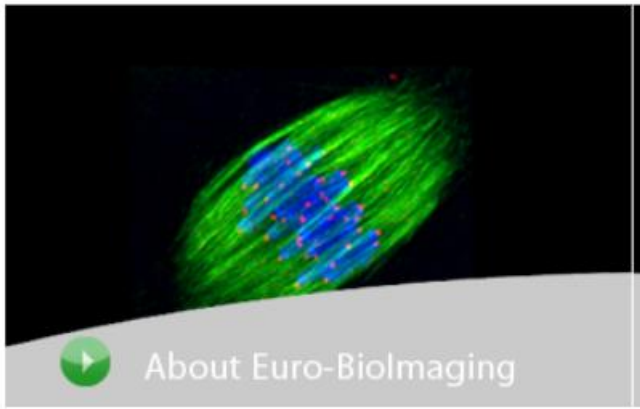
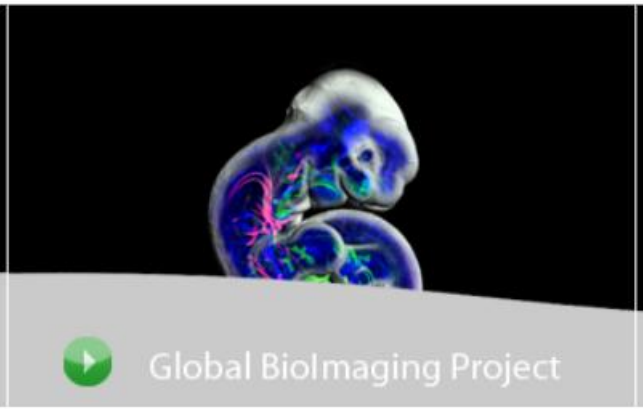
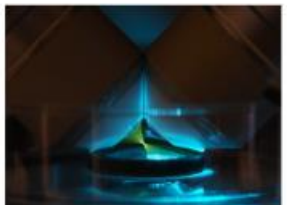




For information about 29 Euro-BioImaging Node Candidates and open access to 36 imaging technologies for biological and biomedical imaging visit:
www.eurobioimaging-interim.eu

Apply for ACCESS here

News & Media



13.06.2018

Showcasing of New Technologies for EuBI

EuBI proudly announces the first showcasing of new imaging technologies during its Interim Operation. The technological innovation of imaging technologies in the life sciences is continuous and exciting. To support the imaging community and its associated high-quality research, EuBI must remain at the technological forefront.

Mission

- To create a coordinated and harmonized plan for biomedical imaging INFRASTRUCTURE deployment in Europe
- To provide ACCESS, SERVICE and TRAINING to state-of-the-art imaging technologies
- To foster the liason and COOPERATION of all stake-holders (scientists, industry, regional and European authorities)

BIOCEV

Are we cheap or expensive?

Purchase cost of our equipment – 186 mil CZK including VAT

Running costs 2017 – 7.8 mil CZK

- Personal costs – 4.5 mil CZK (5.7 mil CZK in 2018)
- Consumables – 0.7 mil CZK
- Instrument service – 0.6 mil CZK (estimate up to 3.6 mil CZK for 2018 – 2% of purchase price per year)
- Rooms – 2 mil CZK (4 mil CZK, 50% usage by us)

Income 2017:

- User fees – 1.5 mil CZK = **19.2%**
- Czech BioImaging – 3.4 mil CZK
- OP VVV CzBI – 1.2 mil CZK
- Faculty of Sciences, Charles University – 1.7 mil CZK (+2 mil CZK)

BIOCEV

Future plans

What do you need? ☺

How much are you ready to pay for it?

- Quality control and quality assurance
- Optimize administration, daily routines, interaction with users

Investments (2020 earliest):

- (Lattice) Light-sheet (currently at IMG plus soon at Viničná 7)
- Spinning-disc confocal
- Plunge-freezer
- ?Holographic microscope
- ?Cryo-microscope for cryo-CLEM
- ?Spectral flow cytometer
- ??????