

Central European Institute of Technology BRNO | CZECH REPUBLIC







Mgr. Milan Esner, Ph.D.

Cellular Imaging Core Facility

CELLIM

Ceitec MU, Brno, Czechia

September 6, 2023 Bio AFM summer workshop

cellim@ceitec.muni.cz https://cellim.ceitec.cz





### **CELLIM** overview









https://www.czech-bioimaging.cz/

### **CzBI Open Access**

Czech Biolmaging offers an open non-discriminatory access to the top technologies (instrumentation and expertise) in the field of biomedical imaging.

#### CzBI open access mode

It is an excellence-driven access mode, which is exclusively dependent on the scientific excellence, originality, quality and technical feasibility of an application as evaluated by the respective Czech-Biolmaging core facility expert (complex projects can be additionally evaluated externally).

All users are welcome to apply for the CzBI open access. The Czech-BioImaging Access Policy is formulated with accordance to the **European Charter for Access to Research Infrastructure**. CzBI open access is efficient, supportive, transparent, and open at the point of service for the user. The CzBI user will be granted access to required resources (e.g. access to instrumentation, expertise, training, data software and analysis tools) at all stages of the research project.

CzBI facilities operating in open access are located in Prague, Brno, Olomouc, and Ceske Budejovice.

#### **CzBI Grant Scheme**

#### For a special support to conduct a project within the Czech-Biolmaging

The aim is to promote open access to the core facilities participating in the Czech-Biolmaging research infrastructure. Czech-Biolmaging provides financial support in performing projects within the Czech-Biolmaging open access to users from all over the world.





### **CELLIM** overview





### **CZECH REPUBLIC**

# Advanced Light Microscopy and Medical Imaging Node Brno CZ

Cellular Imaging Core Facility - Ceitec MU
Experimental Biophotonics Facility – Ceitec BUT
Multimodal and Functional Imaging Laboratory – Ceitec MU
Magnetic Resonance and Cryogenics - Institute of Scientific Instrumentation CAS

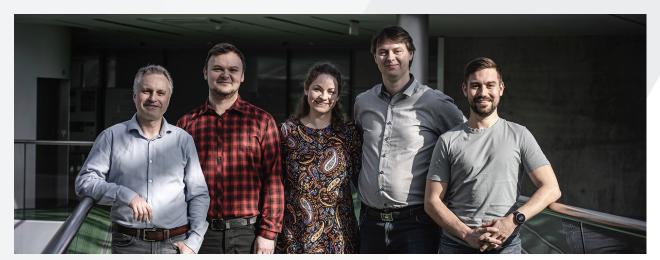
https://www.eurobioimaging.eu/





### **CELLIM** overview





	2019	2020	2021	2022
Number of users	117	133	151	166
User publications	17	16	16	32

#### PLANT SCIENCE

#### Meiotic exit in *Arabidopsis* is driven by P-body-mediated inhibition of translation

Albert Cairo<sup>1</sup>, Anna Vargova<sup>1</sup>, Neha Shukla<sup>1</sup>, Claudio Capitao<sup>2</sup>, Pavlina Mikulkova<sup>1</sup>, Sona Valuchova<sup>1</sup>, Jana Pecinkova<sup>1</sup>, Petra Bulankova<sup>2</sup>+, Karel Riha<sup>1</sup>\*

Meiosis, at the transition between diploid and haploid life cycle phases, is accompanied by reprograming of cell division machinery and followed by a transition back to mitosis. We show that, in Arabidopsis, this transition is driven by inhibition of translation, achieved by a mechanism that involves processing bodies (P-During the second meiotic division, the meiosis-specific protein THREE-DIVISION MUTANT 1 (TDM: incorporated into P-bodies through interaction with SUPPRESSOR WITH MORPHOGENETIC EFFEC GENITALIA 7 (SMG7). TDM1 attracts eIF4F, the main translation initiation complex, temporarily seq in P-bodies and inhibiting translation. The failure of tdm1 mutants to terminate meiosis can be over chemical inhibition of translation. We propose that TDM1-containing P-bodies down-regulate expre

meiotic aranscripts to facilitate transition of cell fates to postmeiotic gametophyte differentiation. Continuous double-strand break induction and their differential processing sustain chiasma formation during Caenorhabditis elegans meiosis

> Tara Hicks, 1 Shalini Trivedi, 2 Mikayla Eppert, 1 Richard Bowman, 1 Hui Tian, 1 Amna Dafalla, 1 Caroline Crahan, Sarit Smolikove,1,\* and Nicola Silva2,3,\*

<sup>1</sup>Department of Biology, The University of Iowa, Iowa City, IA 52242, USA

<sup>2</sup>Department of Biology, Faculty of Medicine, Masaryk University, Brno 625 00, Czech Republic

\*Correspondence: sarit-smolikove@uiowa.edu (S.S.), silva@med.muni.cz (N.S.) https://doi.org/10.1016/j.celrep.2022.111403

CelPress

#### **Structure**

Molecular mechanisms underlying the role of the centriolar CEP164-TTBK2 complex in ciliopathies

Ivan Rosa e Silva, 1,2,\* Lucia Binó,3 Christopher M. Johnson,2 Trevor J. Rutherford,2 David Neuhaus,2 Antonina Andreeva,2 Lukáš Čajánek,3 and Mark van Breugel1.2.4.\*

Queen Mary University of London, School of Biological and Chemical Sciences, 2 Navark Street, London Ed 201 Lik <sup>2</sup>Medicial Research Council – Laboratory of Molecular Biology, Franc Published Online: 19 April, 2022 | Supp Info: http://doi.org/10.26508/sa.202201421 <sup>3</sup>Department of Histology and Embryology, Faculty of Medicine, Mas Downloaded from life-science-alliance.org on 25 April, 2022

\*Correspondence: m.vanbreugel@qmul.ac.uk (M.v.B.), ivan.silva@alu Resource https://doi.org/10.1016/j.str.2021.08.007

**Cell**ress





LuminoCell: a versatile and affordable platform for real-time monitoring of luciferase-based reporters

Kamila Weissová<sup>1</sup>, Bohumil Fafilek<sup>2,3,4</sup>, Tomasz Radaszkiewicz<sup>5</sup>, Canan Celiker<sup>1</sup>, Petra Macháčková<sup>6</sup>, Tamara Čechová<sup>3,4</sup>, Jana Šebestíková<sup>1,5</sup>, Aleš Hampl<sup>1,4</sup>, Vítězslav Bryja<sup>5</sup>, Pavel Krejčí<sup>2,3,4</sup>, Tomáš Bárta<sup>1,5</sup>





# Cellular Imaging Core Facility - CELLIM



Light microscopy facility of Ceitec Masaryk University.

### What we are doing:

Providing access to a wide spectrum of light microscopes, including training and assistance services

Providing access to image processing and analysis tools (hardware and software), including training

Maintenance (QC, cleaning, calibration) of all our microscopes

Providing project design assistance and help with planning experiments, sample preparation

Developing and applying new methods and protocols for and in collaboration with our users

Full service – sample preparation, image acquisition, image analysis – dependent on staff availability

Educational activities – courses, workshops, demos of new instruments ...

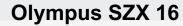




# Large specimen imaging

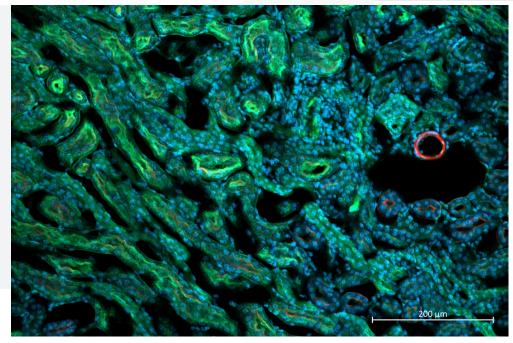


### Zeiss AxioZoom – Apotome 2









Mouse kidney section



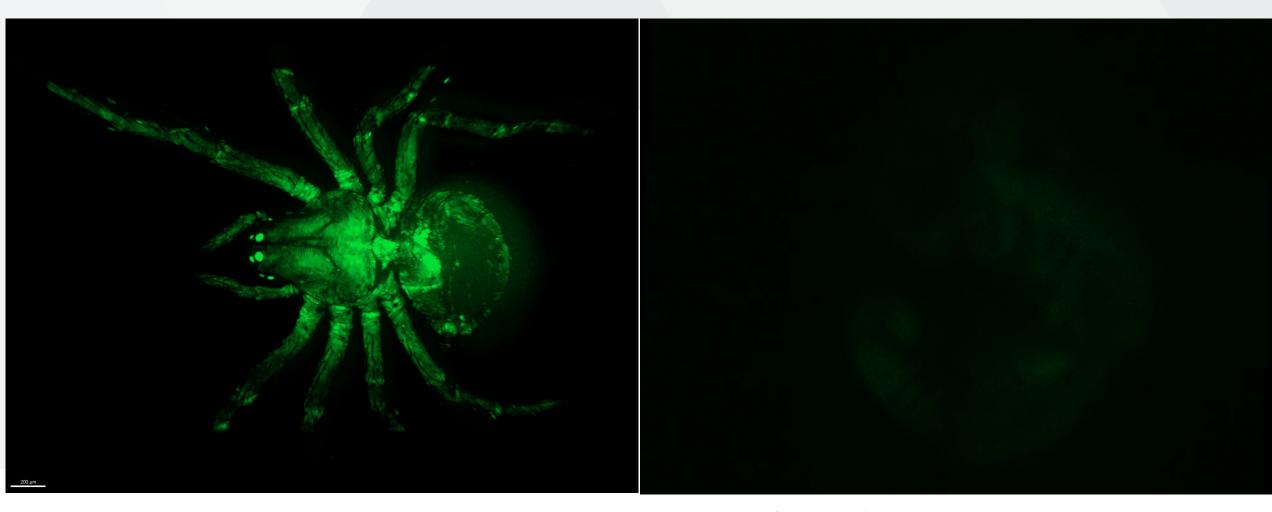
Head louse - Pediculus capitis





# Optical sections with Apotome





Courtesy of Nela Jandova and Marcela Buchtova Institute of Animal Physiology and Genetics, CAS CZ



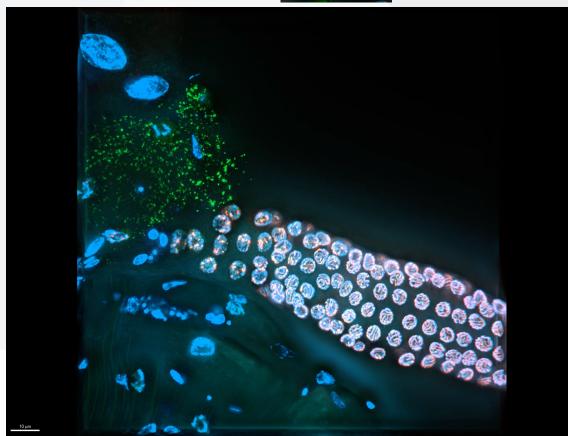


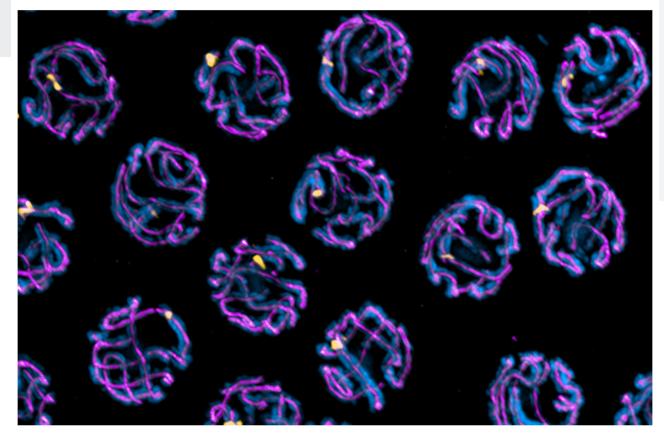
# Widefield microscopy with deconvolution



Zeiss Axiolmager – Apotome 3











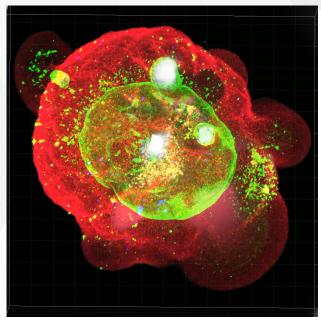
## Laser scanning confocal microscopy



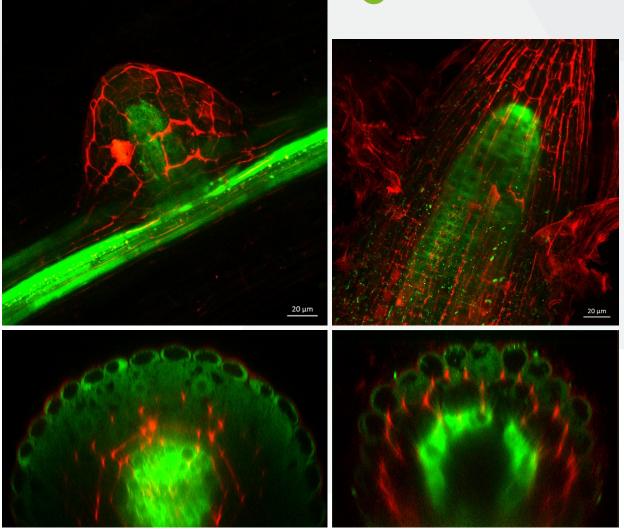








Organoid from stem cells
Courtesy of Tomas Barta and Jan Krivanek
Faculty of Medicine, Masaryk University



Arabidospis thaliana root apical meristem Courtesy of Marketa Samalova and Jan Hejatko Ceitec Masaryk University





# Airyscan – entry level to superresolution

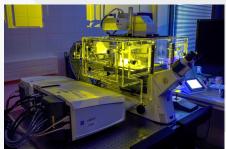


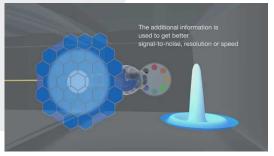
Higher resolution, higher SNR compare to standard confocal microscope

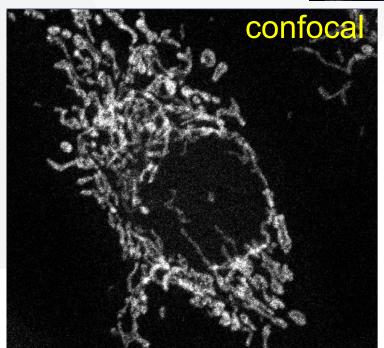
**Zeiss LSM 780-Airy** 

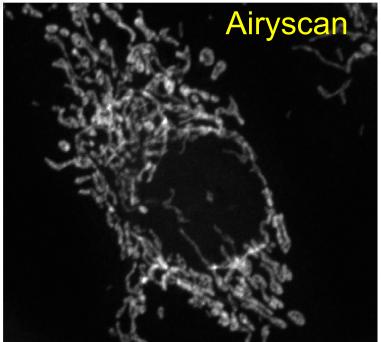
Zeiss LSM 880-Airy





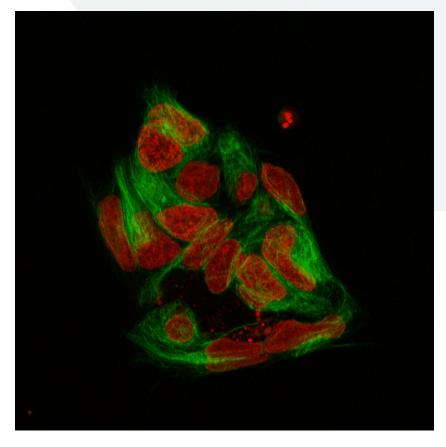






Temperature control CO2 control DefinitFocus2

Piezo Z drive Water immersion objectives

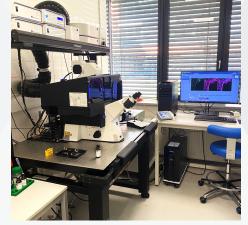


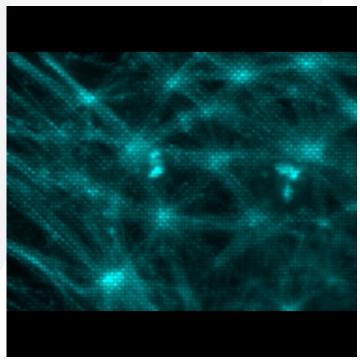


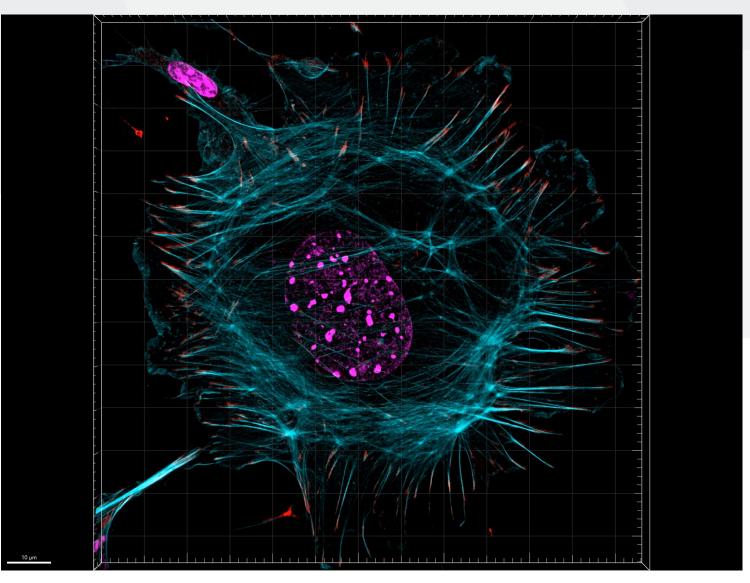


# Carl Zeiss Elyra 7- Lattice SIM, SMLM













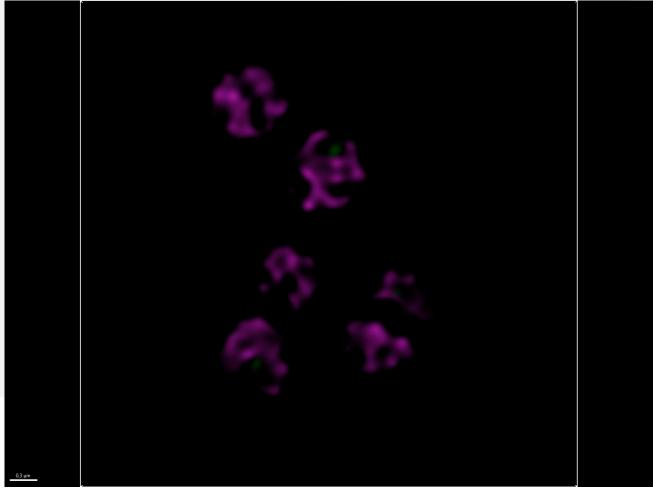
# Lattice structured illumination microscopy



Staphyloccocus cells

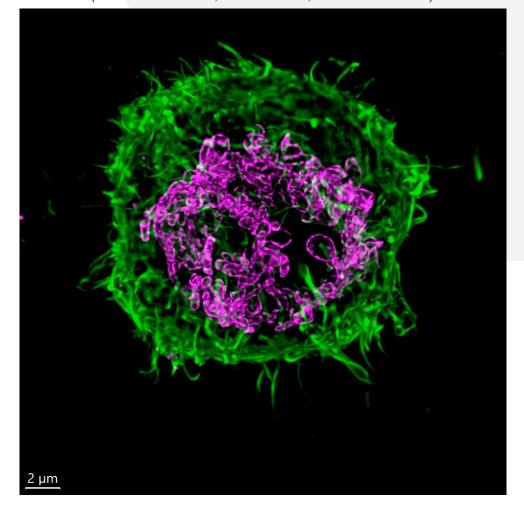
Cell mebrane (green), Nuclei (purple)

(Michaela Procházková, Pavel Plevka, Ceitec MU Brno)



3D reconstruction of Lymphocyte cell

Actin filament (green), mitochondria (magenta). (Pedro Faria Zeni, Marek Mráz, Ceitec MU Brno)

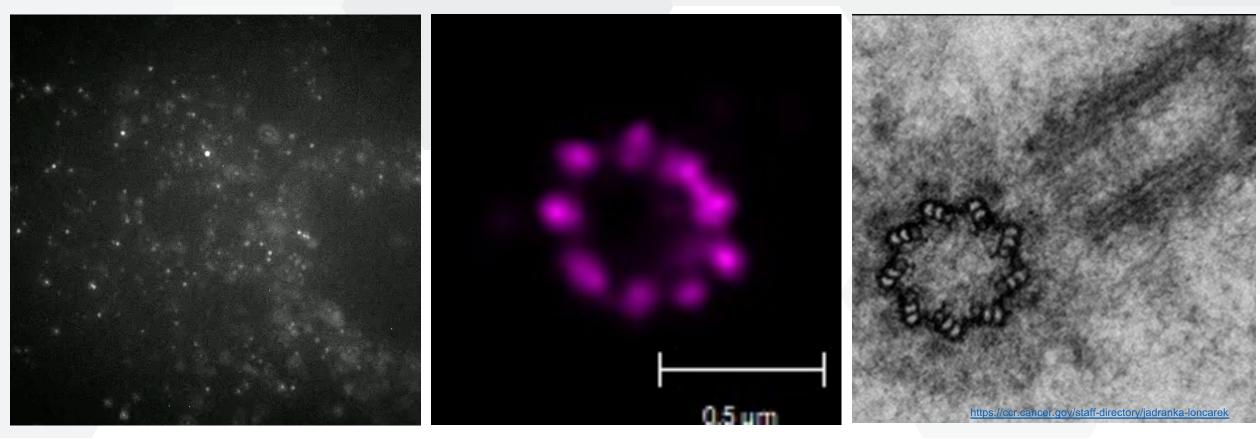






# Single molecule localization microscopy





Centriole dSTORM microscopy centriole (magenta) (Ondřej Bernatík, Lukáš Čajánek group, LF MU)

**Centriole TEM microscopy** 





# Lightsheet microscopy



### **Zeiss Lightsheet 7**











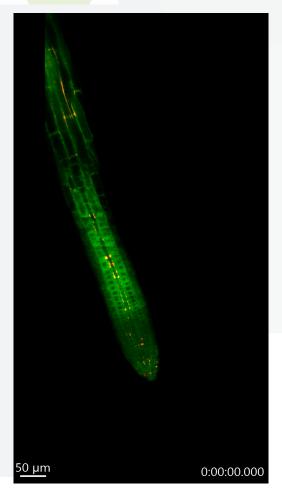


Organic solvent- based clearing protocols	Ethyl cinnamate (ECi) RI ≈ 1.5 – 1.6	Klingberg et al. 2017	
Aqueous-based clearing protocols	60% Glycerol RI ≈ 1.47	Vargas et al. (1999)	
	learing protocols CUBIC 2 RI ≈ 1.45-1.48		

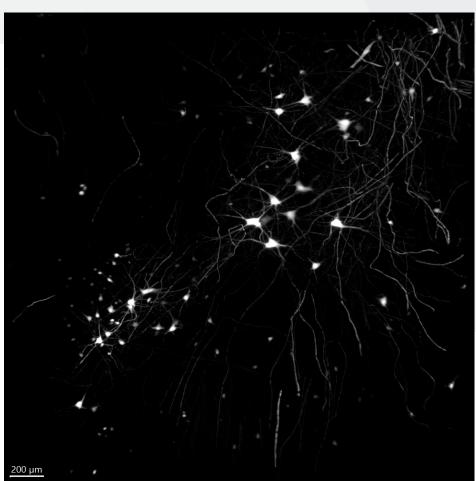


# Lightsheet microscopy









Arabidospis thaliana root growth Water (Ri= 1.33), Plan-Apochromat 20x/1.0 Courtesy of M. Samalova and P. Macháčková

**Detailed view of mouse brain**BABB/Eci (Ri=1.50), EC "Plan-Neofluar" 5x/0.16 M27
Courtesy of M. Goudarzi, P. Macháčková, W. Jesionek





## Temporal data storage



# Storage/analysis server Acquifer ODEON



1 TB RAM, Nvidia RTX6000, 2 x 24 Intel XEON CPU, 300TB storage space 10 GB data link to all microscopes – direct data saving from microscopes account on demand



1 licence, full pack Airyscan, SIM processing Deconvolution Lightsheet processing



Open source
Objects segmentations,
measurements
Lightsheet processing



1 licence Objects segmentations, measurements, tracking 3D rendering

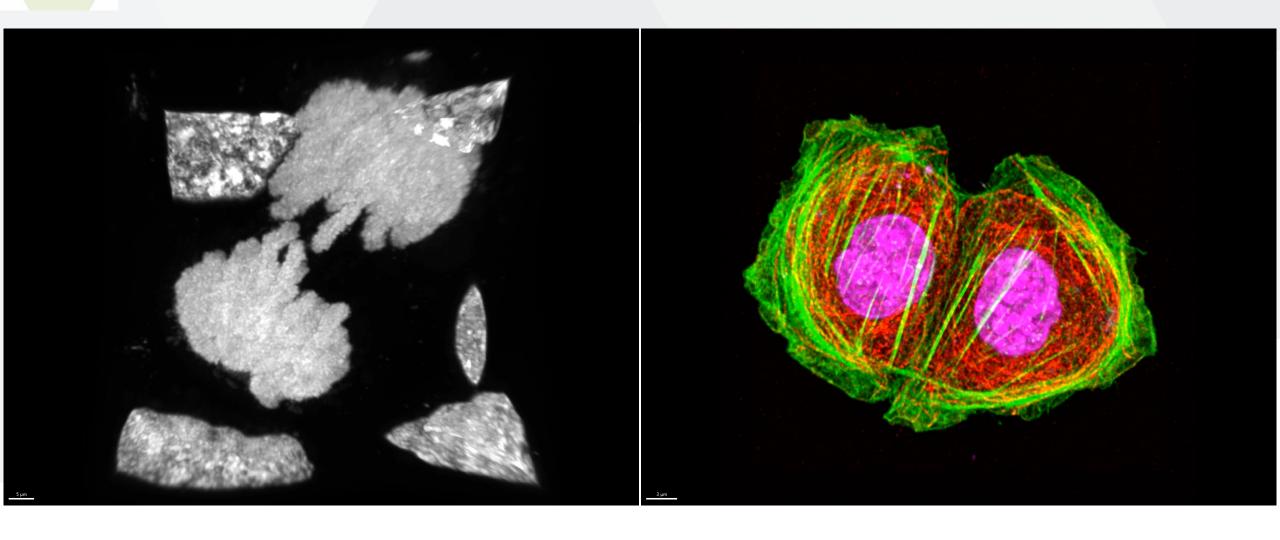


Open source
Objects segmentations,
measurements
High throughput



# Image processing and analysis







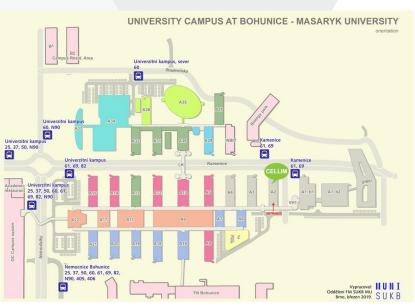


## Cellular Imaging Core Facility - CELLIM



#### http://cellim.ceitec.cz







### **Cellular Imaging Core Facility - CEITEC**

@Ceitec\_CellimCF

Light microscopy facility of @CEITEC\_Brno @muni\_cz. Part of #CzechBioimaging and @EuroBioimaging research infrastructures.

https://twitter.com/Ceitec\_CellimCF





### Upcoming events





### FAST AND EASY LIVE CELL SUPER RESOLUTION BASED ON SPINNING DISK CONFOCAL

Spinning disk confocal is the most suitable microscopy technique when it comes to 3D imaging of fast processes in living cells and/or 3D imaging over long periods of time. Nikon's leading platform, the Eclipse Ti2 inverted microscope, already offers high throughput and high speed automation for advanced imaging. The X Light V3 is the next generation of spinning disk. It relies on the cutting edge technology, advanced optical design and engineering solutions developed by CrestOptics to meet the very high end specifications required by modern fluorescence microscopy applications. Super resolution, down to 100 nm, is now achievable by simply using the DeepSIM Super resolution module based on a multi spot structured illumination system.

### PROGRAM: 12.09.2023

# ROOM E35/145 10:00 – 12:00 INTRODUCTION OF THE CREST X LIGHT V3 SPINNING DISK CONFOCAL SYSTEM WITH DEEPSIM SUPER RESOLUTION MODULE ROOM C02/113 13:00 – 17:00 HANDS ON SESSION 13. & 14.09.2023 ROOM C02/113

HANDS ON REGISTRATION:

ondrej.sedlak@nikon.com







CrestOptic







**Cellular Imaging Core Facility** and **OptiXs s.r.o.** would like to invite you to workshop

### ANDOR FLAGSHIP HIGH-SPEED CONFOCAL SYSTEM: DRAGONFLY 600 DEEPER IMAGING. DEEPER INSIGHTS. DEEPER UNDERSTANDING.

The new **Dragonfly 600 multimodal spinning disk confocal system** with SMLM delivers outstanding multi-dimensional images from subcellular (nm) to whole organism (cm), while significantly boosting productivity. It integrates super-resolution solutions compatible with confocal, widefield or TIRF. Furthermore, it incorporates the newly developed High Power Laser Engine (HLE) and new B-TIRF imaging modality.

The new B-TIRF and super-resolution modules reveal smaller details, such as the dynamics of viral infection and the ultrastructure of chromatin or organelles.

#### PROGRAM:

#### 10.10.2023

#### **Room C03/117**

09:00 – 09:15 WELCOME & INTRODUCTION (MILAN EŠNER, CELLIM, BLANKA SCHUSTEROVÁ, OPTIXS) 09:15 – 10:00 INTRODUCTION TO DRAGONFLY 600 SYSTEM (BRUNG COMBETTES, ANDOR)

#### **ROOM C02/113**

10:15 – 11:00 DRAGONFLY 600 OVERVIEW DEMO GROUP 1
11:00 – 11:45 DRAGONFLY 600 OVERVIEW DEMO GROUP 2

13:00 - 17:00 HANDS-ON SESSION

#### 11. & 12.10.2023

#### **Room C02/113**

09:00 - 17:00 HANDS-ON SESSION

#### **WORKSHOP MAIN TOPICS:**

- SINGLE-MOLECULE STUDIES
   DSTORM, PALM & DNA-PAINT
- LIVE-CELL SUPER-RESOLUTION
   PHOTOSTIMULATION









HANDS-ON REGISTRATION: 回题模型图

CAPACITY IS LIMITED.

IF YOU WANT TO TRY YOUR OWN SAMPLES,

QXEQER ANDOR

PLEASE CONTACT SCHUSTEROVA@OPTIX
FOR MORE DETAILS OR REGISTER HERE





**WORKSHOP** 

17-19.10.2023

### ADVANCED LIGHT MICROSCOPY METHODS IN BIOLOGY

We would like to invite you to a practical course on advanced methods in light microscopy, where you will learn about the main methods of super-resolution imaging and light-sheet microscopy used in biology. The theoretical part will be followed by a hands-on session where you will have the opportunity to try out different techniques on real samples, including data analysis and visualisation.

The course is primarily aimed at students who already have experience with standard microscopy

techniques.

Places are limited. Registration is required - see the link below.

Registration fee: 1000 CZK + VAT academic users, 4000 CZK + VAT commercial users. Coffee and refreshment will be provided during the course.

#### PROGRAM:

#### 17.10.2023

#### ROOM E35/1S102 09:00 - 17:00

- MILAN ESNER: DIFFRACTION LIMITS IN LIGHT MICROSCOPY
- JAKUB POSPISIL: DIFFERENT APPROACHES TO BREAK THE DIFFRACTION BARRIER
- PETRA KUCEROVA: LIGHT-SHEET MICROSCOPY
- · JOSEF LAVICKY: LIGHTSHEET MICROSCOPY FOR STUDYING EMBRYONIC DEVELOPMENT
- SURENDRA SADDALA: EXPLORING BIOLOGICAL CONDENSATES IN PLANTS THROUGH

SUPER-RESOLUTION MICROSCOPY

#### 18.10.2023

ROOM C02/CELLIM 09:00 - 17:00

PRACTICAL SESSION

#### 19.10.2023

ROOM E35/1S102 09:00 - 17:00

WOJCIECH JESIONEK: IMAGE PROCESSING AND ANALYSIS

REGISTRATION:

https://gr.page/g/3VoyggdVF9E















09:00 - 17:00 HANDS ON SESSION



