



### **FIB SEM in Biology Research**

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### Imaging Methods Core Facility at



operated by Faculty of Science, Charles University





### 1. Light microscopy

11 high-end systems user operated

#### 2. Electron microscopy

FIB-SEM + sample preparation, TEM + sample preparation staff and superuser operated

#### **3. Flow cytometry**

1 sorter + 2 analyzers user and staff operated

#### 4. Data Analysis

http://imcf.natur.cuni.cz/IMCF/



# **FIB-SEM**

### **Scanning Electron Microscope**



Electron Proton Nucleus





https://analyticalscience.wiley.com/do/10.1002/was.00070009/



#### **FIB-SEM** Interaction volume electron beam **Scanning Electron Microscope** Secondary Electrons (SE) Auger Electrons (AE) topographical information (SEM) surface atomic composition Backscattered Electrons (SE) Characteristic X-ray (EDX) atomic number and phase differences thickness atomic composition Continuum X-ray (Bremsstrahlung) Cathodoluminescence (CL) SEM electronic states information Cryogenic conditions SAMPLE Same Ga+ Inelastic Scattering composition and bond states (EELS) Elastic Scattering structural analysis and HR imaging (diffraction) Incoherent Elastic Scattering TE\* Transmitted Electrons \* SE = Secondary electrons; BSE = Backsraftered electrons; X-ray = Characteristic X-rays; TE = Transmitted electrons

morphological information (TEM)





### **Focused Ion Beam**









https://www.nature.com/articles/nmeth.3623





https://www.researchgate.net/figure/Basic-components-of-SEM-FIB-systems-vacuum-chamberion-optical-column-electron-optical\_fig1\_254740167





#### FEI Helios NanoLab G3 UC

MICROSCOPE

- Field Emission Gun (0,5-30 kV) with resolution 0.9 nm at 15 kV (SEM)
- Liquid Metal Ions Source (Ga+) with resolution 3 nm at 30 kV (FIB)
- Full cryo-compatibility



### Sample preparation for FIB-SEM





### FIB-SEM imaging of samples in the resin



Cell localization



Stage tilt 52° Protective platinum layer Trench into resin by FIB milling MEW 4/5/2019 HV mag det mode WD curr 1.18 mm 11:53:55 AM 20.00 kV 350 x ETD SE 4.4 mm 0.80 nA



#### **VIEW FROM SECONDARY IONS**



HFW 4/5/2019 HV mag□ det mode WD curr **1.18 mm** 11:53:55 AM **20.00 kV 350 x ETD SE 4.4 mm 0.80 nA** 





#### **VIEW FROM SECONDARY ELECTRONS**









### **FIB-SEM** imaging





## **FIB-SEM in biology**





# Volume vs Time vs Sampling



# **CF** CLEM – Correlative Light and Electron Microscopy

combination of fluorescence microscopy with high-resolution electron microscopy

#### **CLEM for selection of the cell**

- images from the optical microscope are used only for targeting one specific cell or spot of interest in the electron microscope



#### **CLEM for registration of datasets**

- datasets from optical and electron microscope are overlaid for studying the complex relation between form and function in biology



# **Examples of CLEM application**

C

Mitochondria transiting through tunneling nanotubes



Counting neuron synapses in mouse brain



CE





Ruzicka, J. et al. Perineuronal nets affect memory and learning after synapse withdrawal. Molecular Psychiatry, In Review

Movie and images courtesy by Jiří Růžička Institute of Experimental Medicine, AS CR



3D reconstruction of the dendritic cell and the surrounding vesicles in seminiferous tubule



Images courtesy by Jana Petrusová Institute of Molecular Genetics, AS CR

# CLEM application for targeting specific position on FIB-SEM

-isolated seminiferous tubules from MHCII-gfp expressing male at 14 and 30 PND were mounted on MatTek glass bottom dish with gridding and stained with DAPI

-identifying of the area with MHCII positive cell body and dendrite using confocal microscopy



3D reconstruction of the dendritic cell and the surrounding vesicles in seminiferous tubule



# C F

# **Examples of FIB-SEM application in IMCF**

3D reconstruction of the dendritic cell and the surrounding vesicles in seminiferous tubule



3D ultrastructure of dividing protophloem cells in Arabidopsis thaliana roots

Dividing protophloem cells line targeted in the whole root. Ultrastrucrure details were acquired from cells connection parts.

> Images courtesy by Jan Petrášek Institute of Experimental Botany, AS CR











3D ultrastructure of Trichomonas vaginalis



Comparison of ultrastructure changes in TAX1 mutant of *Trichomonas vaginalis* and WT. Focused on the organelles of cytoskelet (basal bodies, axostyle, costa atc.)



Images courtesy by Jan Tachezy Faculty of Science, CU





# *3D ultrastructure of Trichomonas vaginalis*

# **EXAMPLES OF FIB-SEM application in IMCF**

Organization of cillia anchoring in the ependymal cells



Images courtesy by Camille Boutin Aix-Marseille Université, France



FIB-SEM acquisition to reconstruct the organization of ependymal multiciliated cells to access the organization of their apical surface and distribution of main components inside the cells.

Quantification of mitochondria cristae



# **EXAMPLES OF FIB-SEM application in IMCF**







Images courtesy by Dušan Cmarko 1.LF, UK

Reconstruction of chromatin subpopulations



Sample prepared by NAMA-Ur. Protocol which selectively stains chromatin, data segmented using AI in NIS-elements software







### Thank you for your attention!

