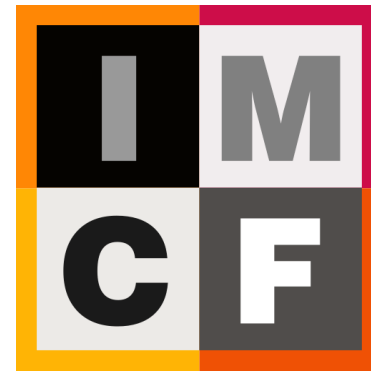


BIOCEV



# FIB SEM in Biology Research

Markéta Dalecká



FACULTY OF SCIENCE  
Charles University





# Imaging Methods Core Facility at

operated by Faculty of Science, Charles University



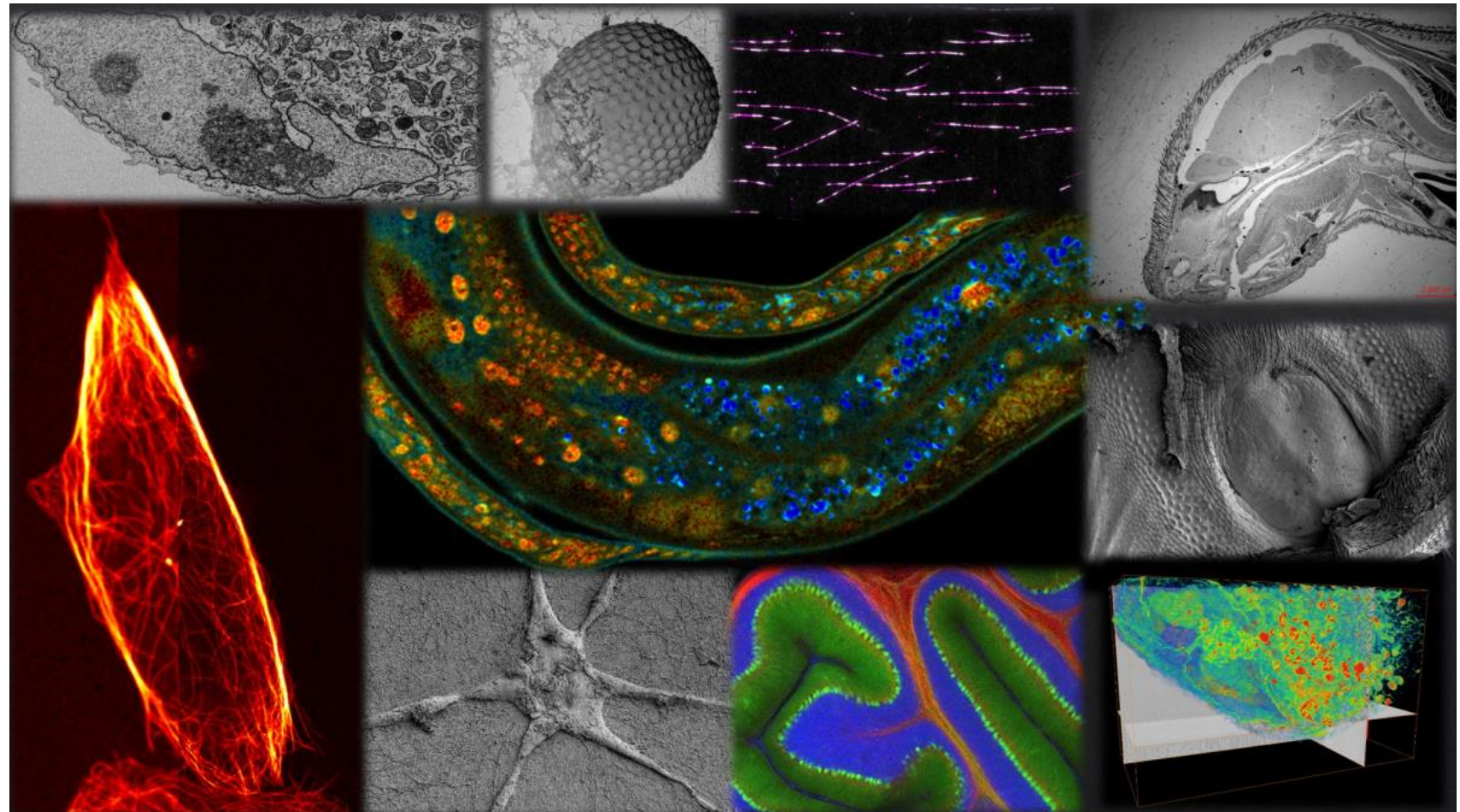
BIOCEV



**OPEN ACCESS**



**Advanced microscopy for everyone!**



## 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

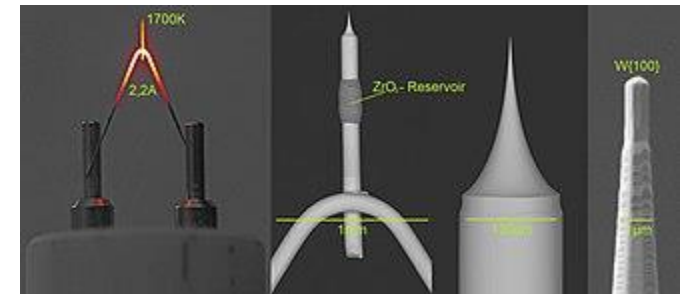
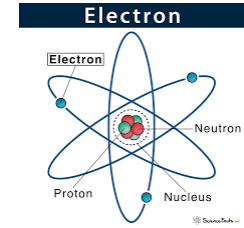
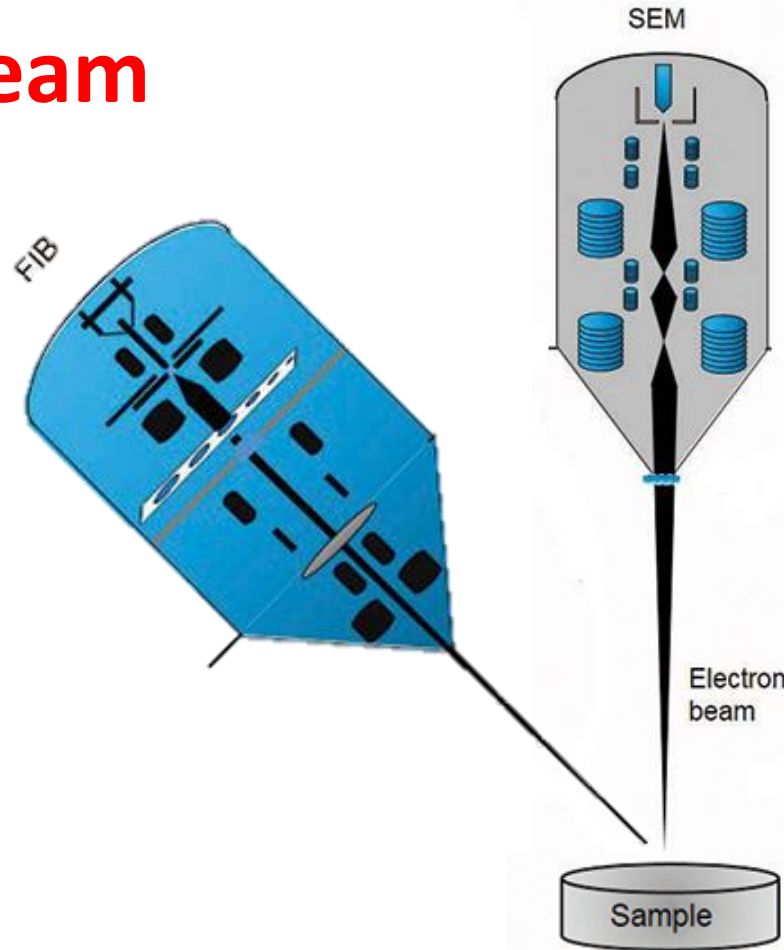
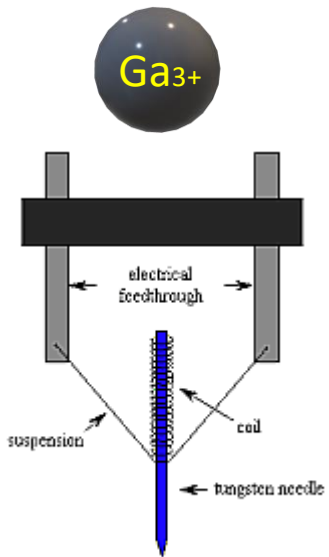
## 4. Data Analysis

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

# FIB-SEM

## Scanning Electron Microscope

### Focused Ion Beam

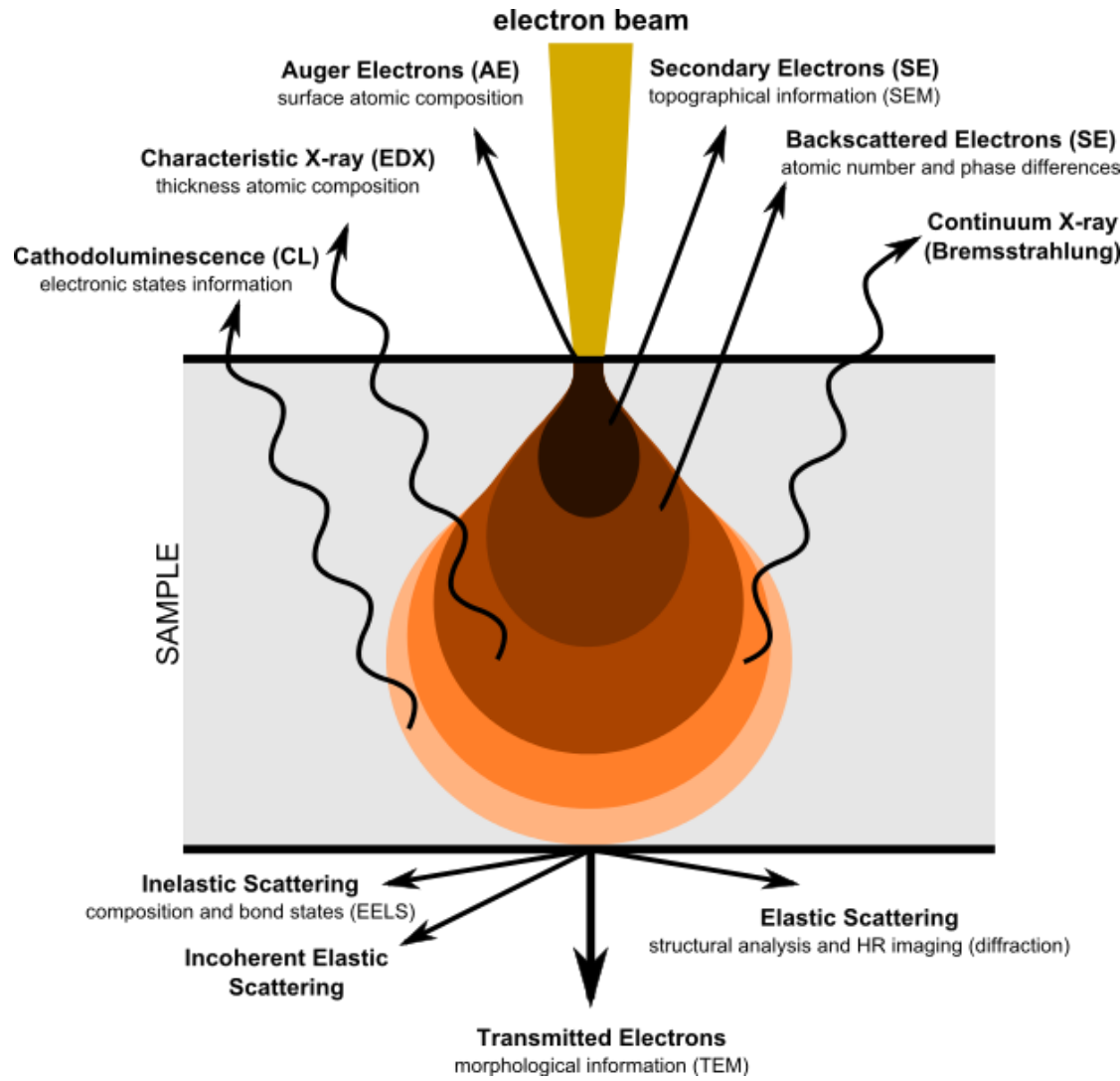


Take home messages 😊

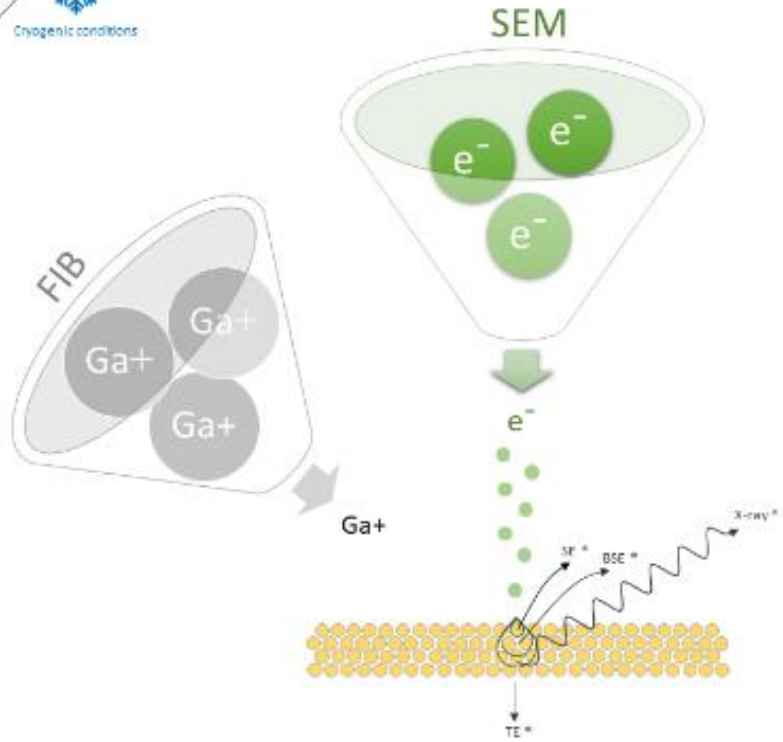


# FIB-SEM

Interaction volume



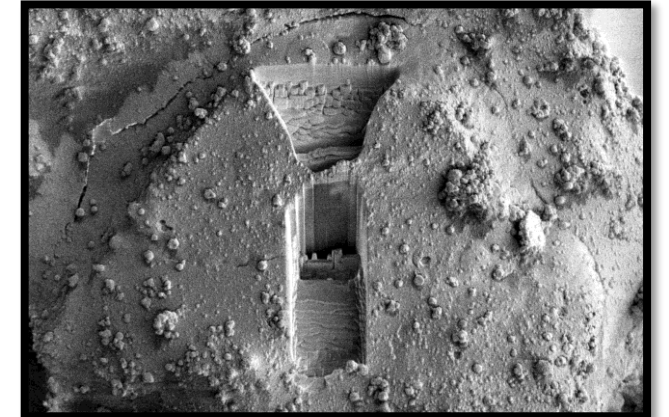
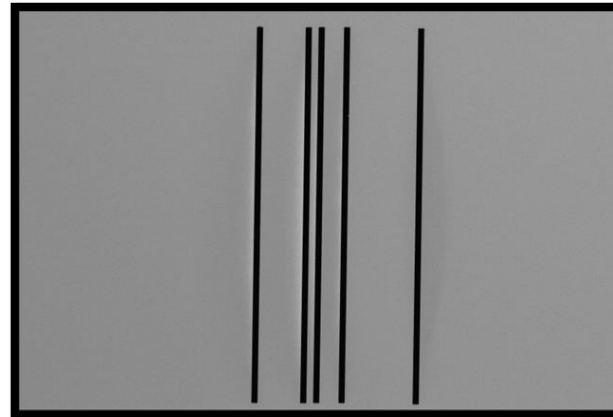
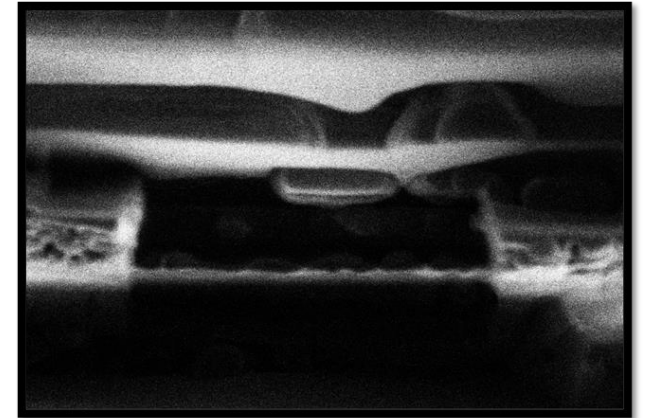
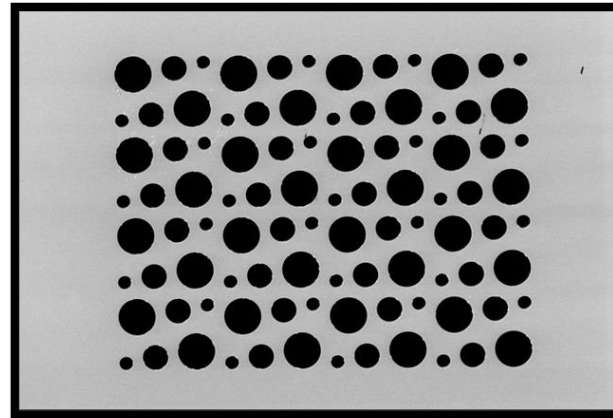
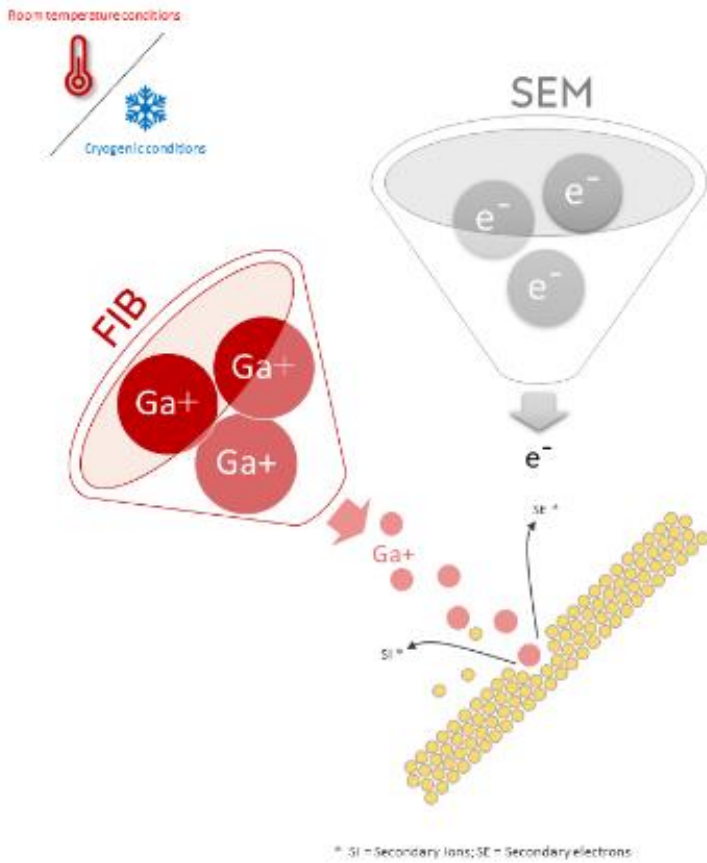
# Scanning Electron Microscope



<sup>+</sup> SE = Secondary electrons; BSE = Backscattered electrons; X-ray = Characteristic X-rays; TE = Transmitted electrons

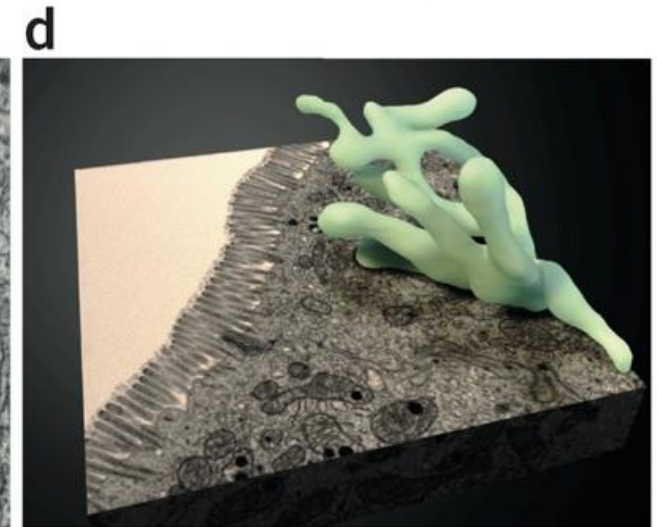
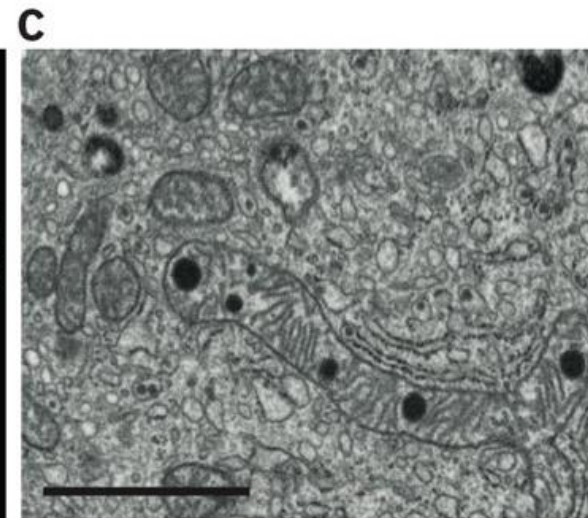
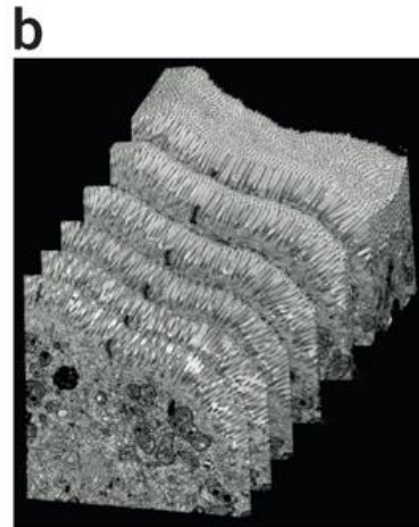
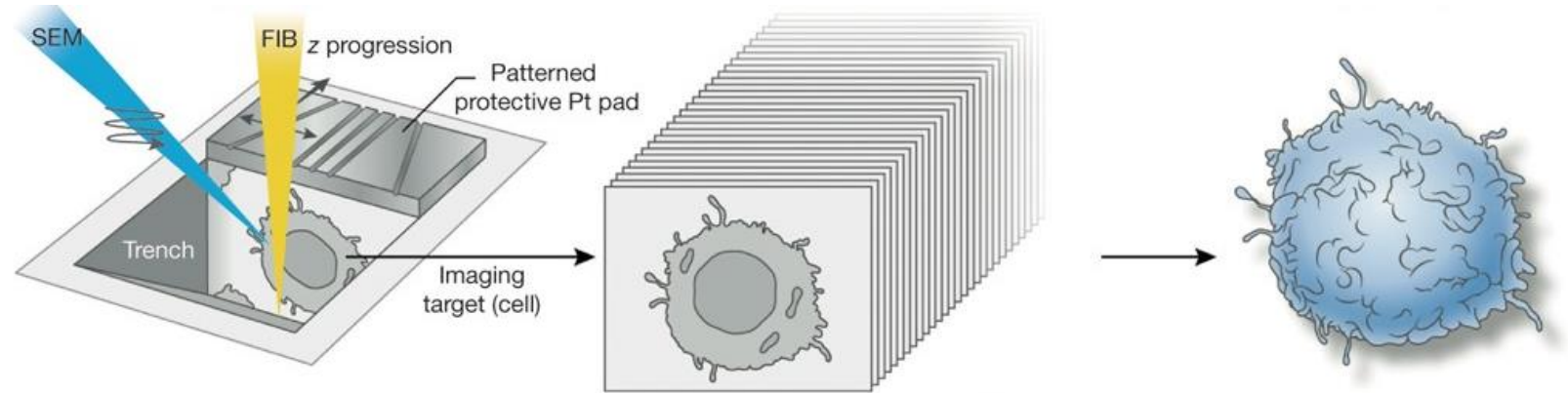
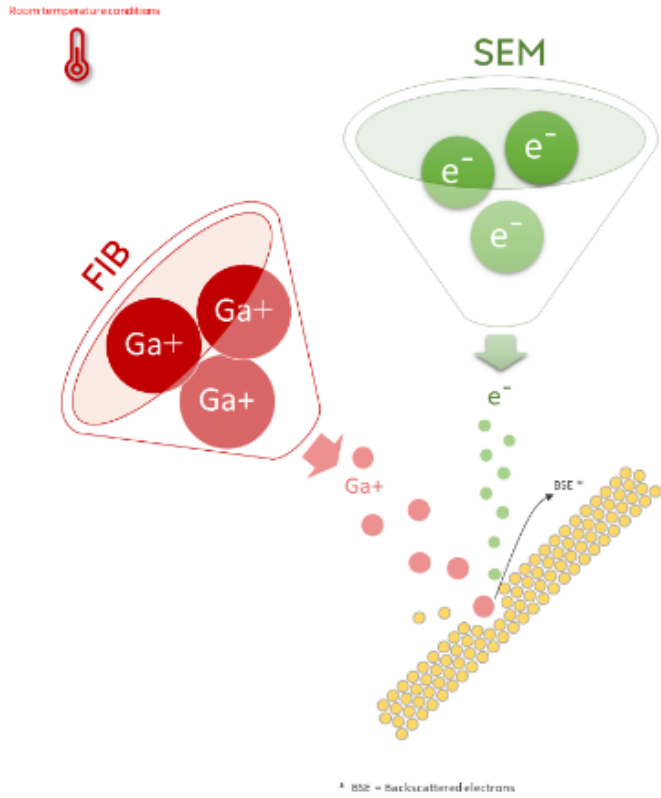


## Focused Ion Beam

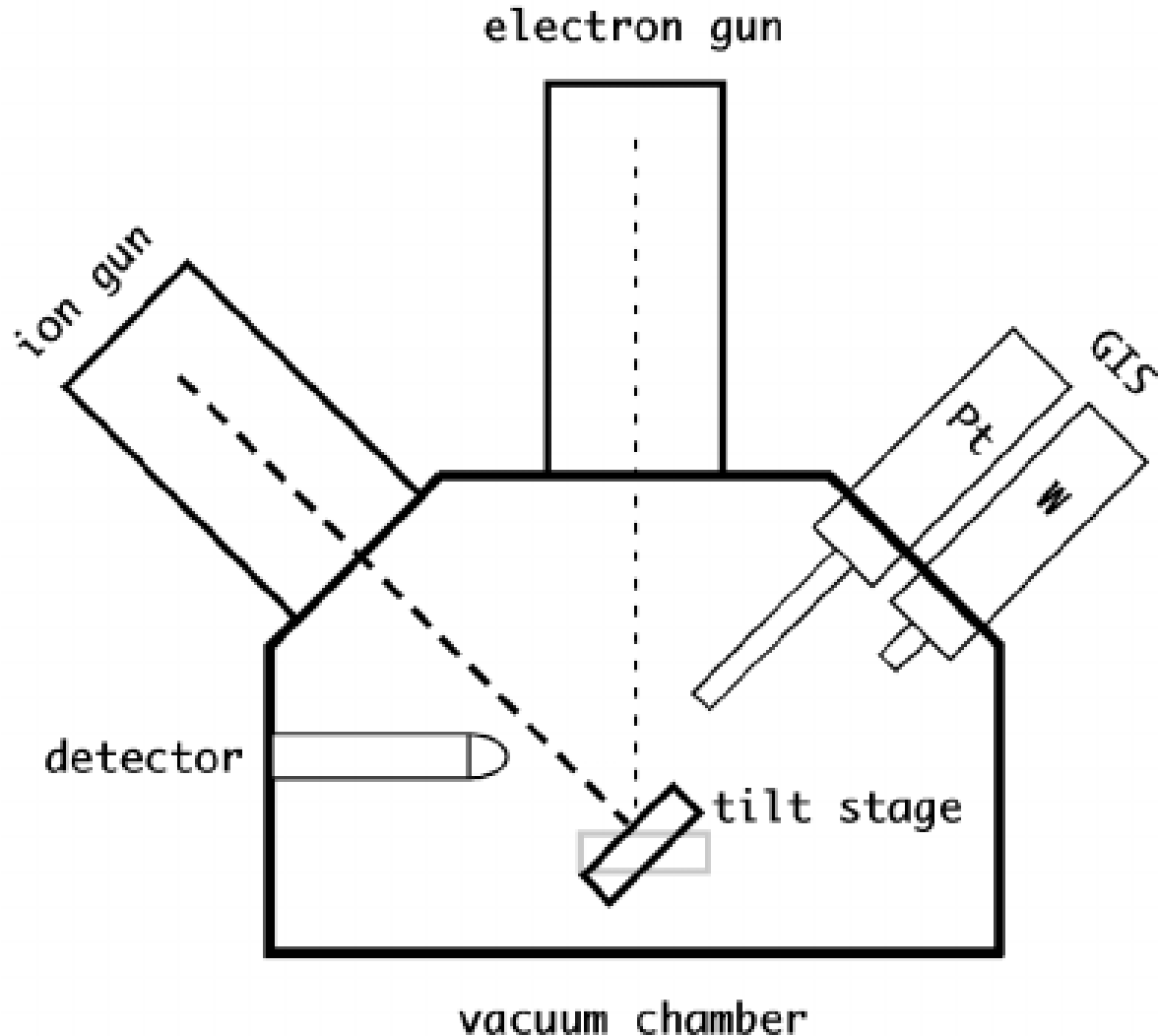


# FIB-SEM

Volume EM technique



# MICROSCOPE



Microscope at IMCF

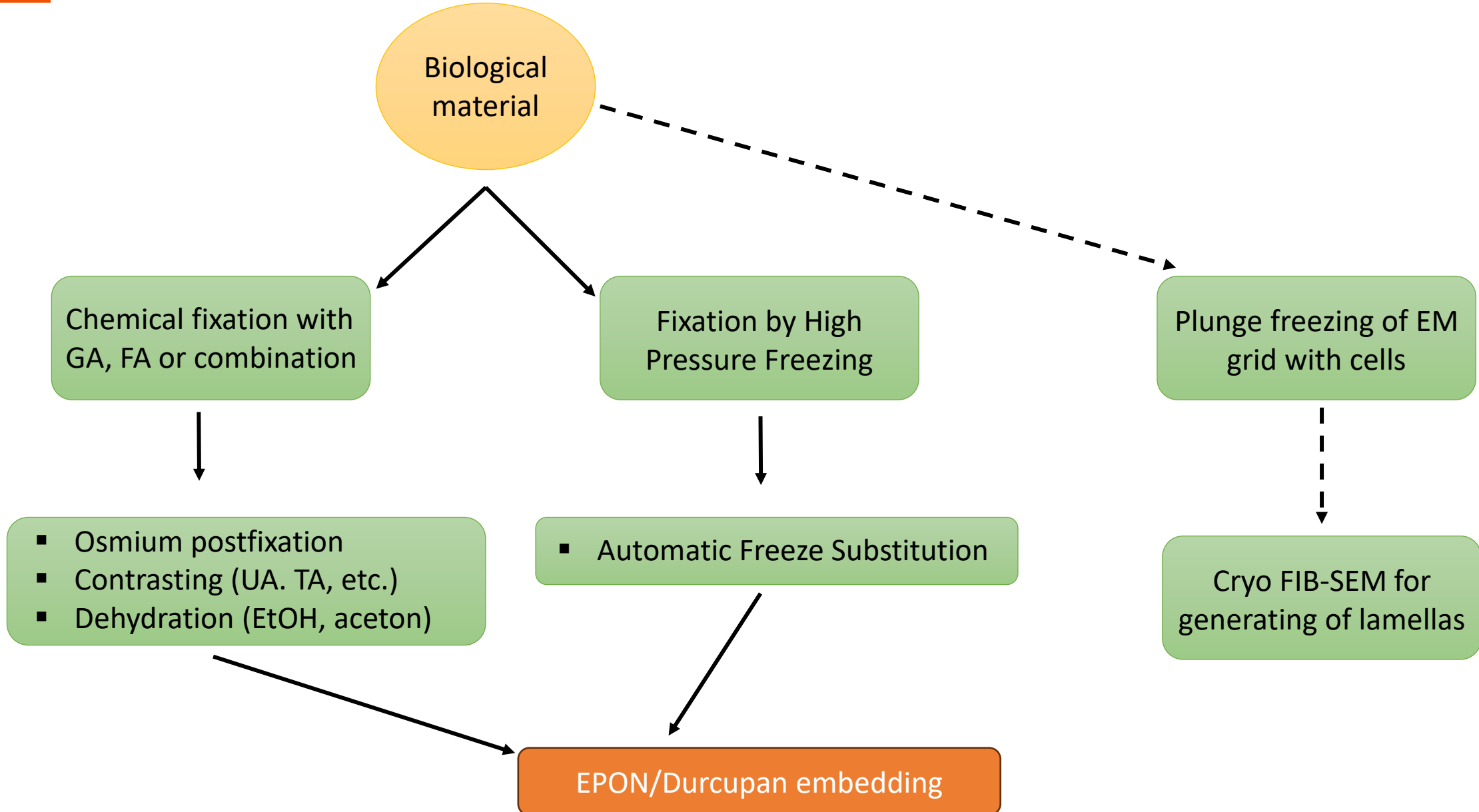


## FEI Helios NanoLab G3 UC

- 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



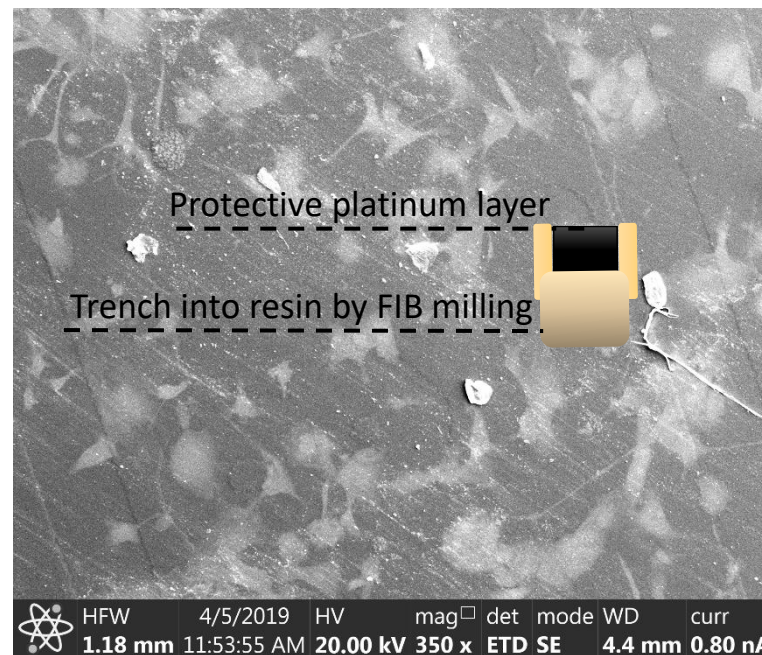
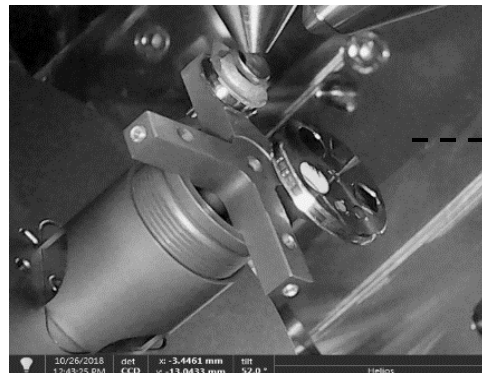
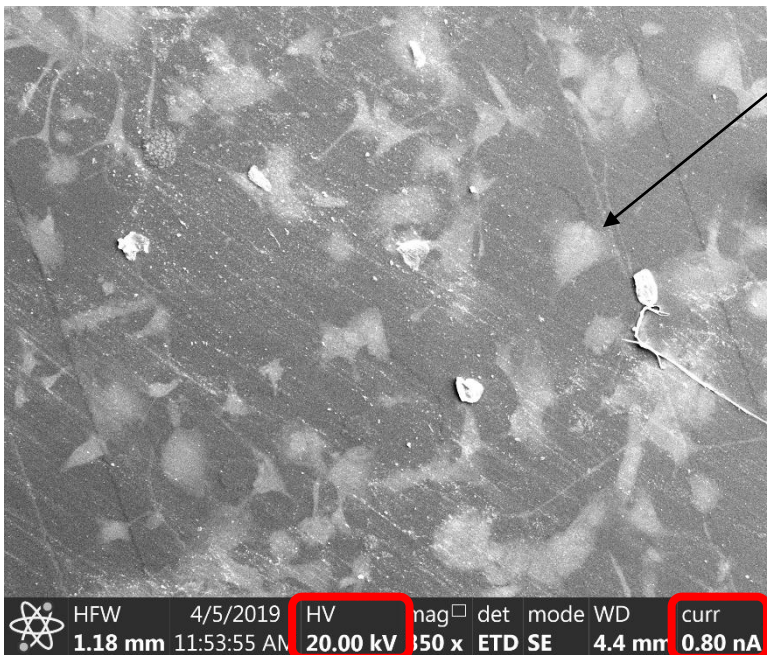
# Sample preparation for FIB-SEM







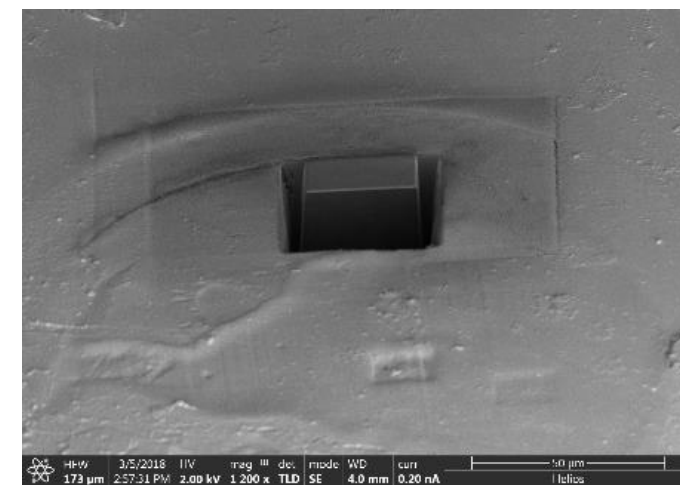
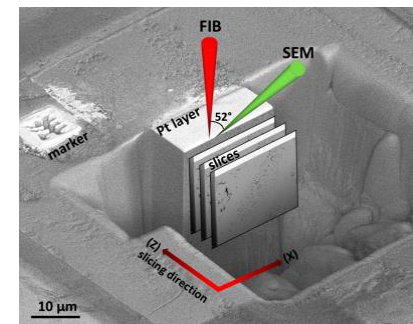
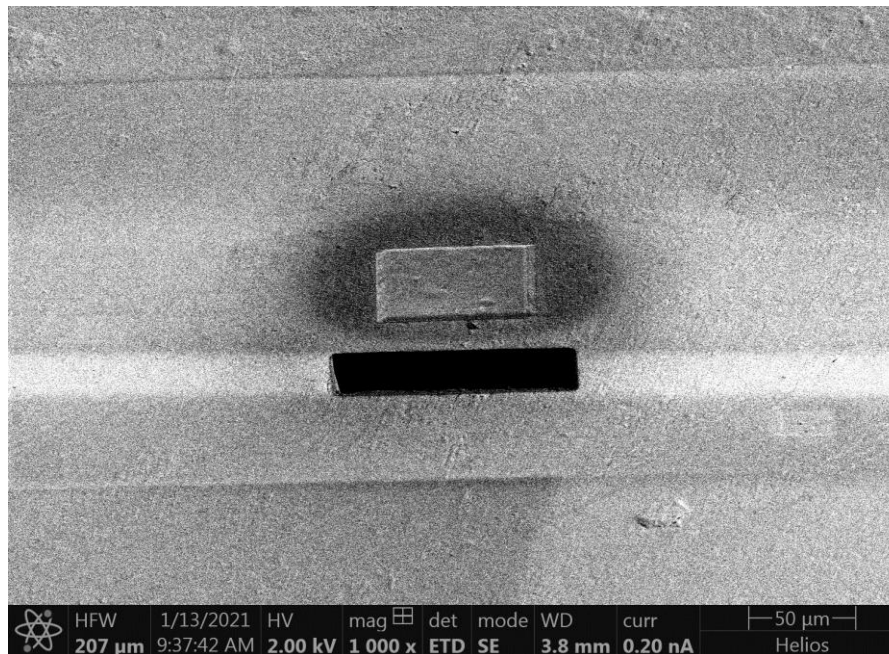
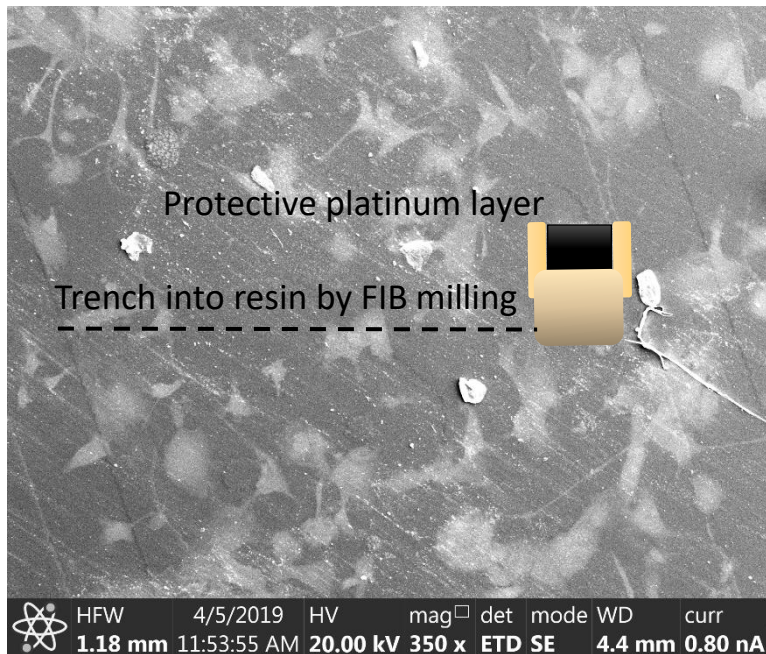
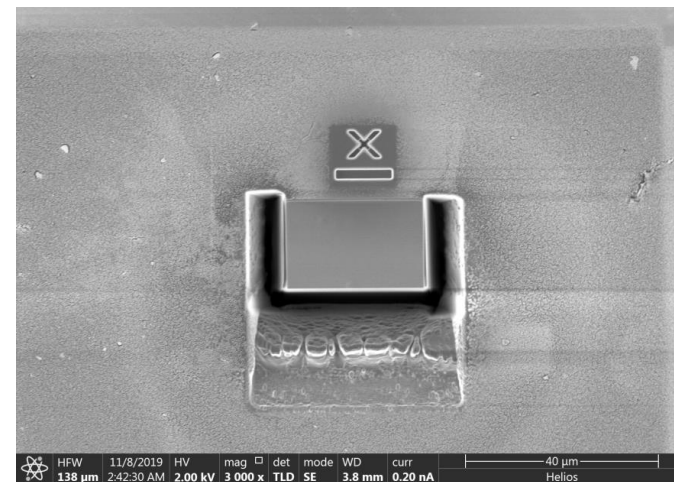
# FIB-SEM imaging of samples in the resin



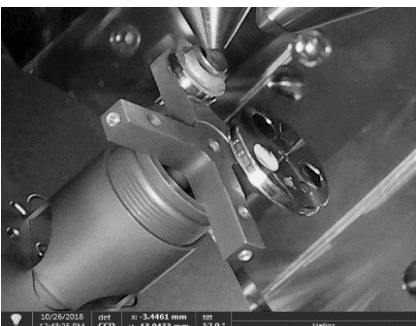


Why tilt the stage?

### VIEW FROM SECONDARY IONS

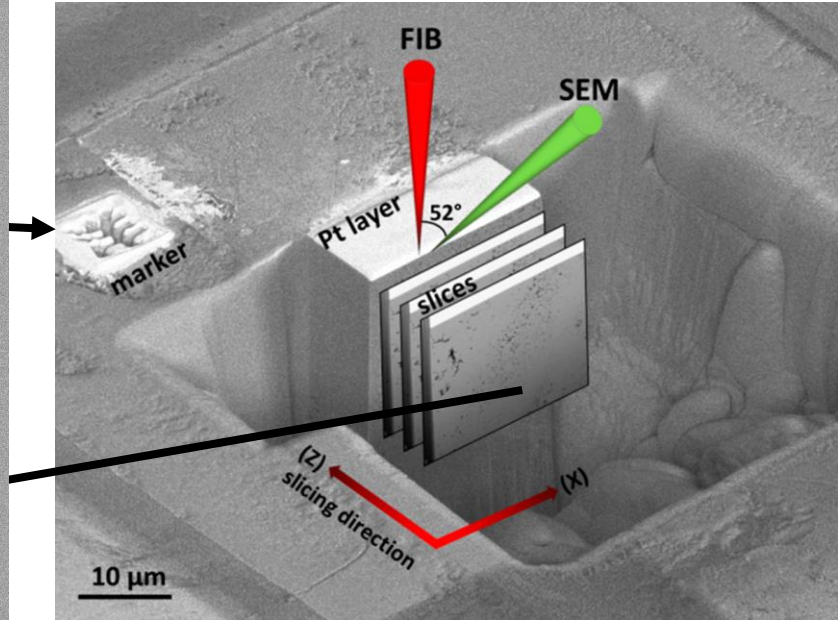
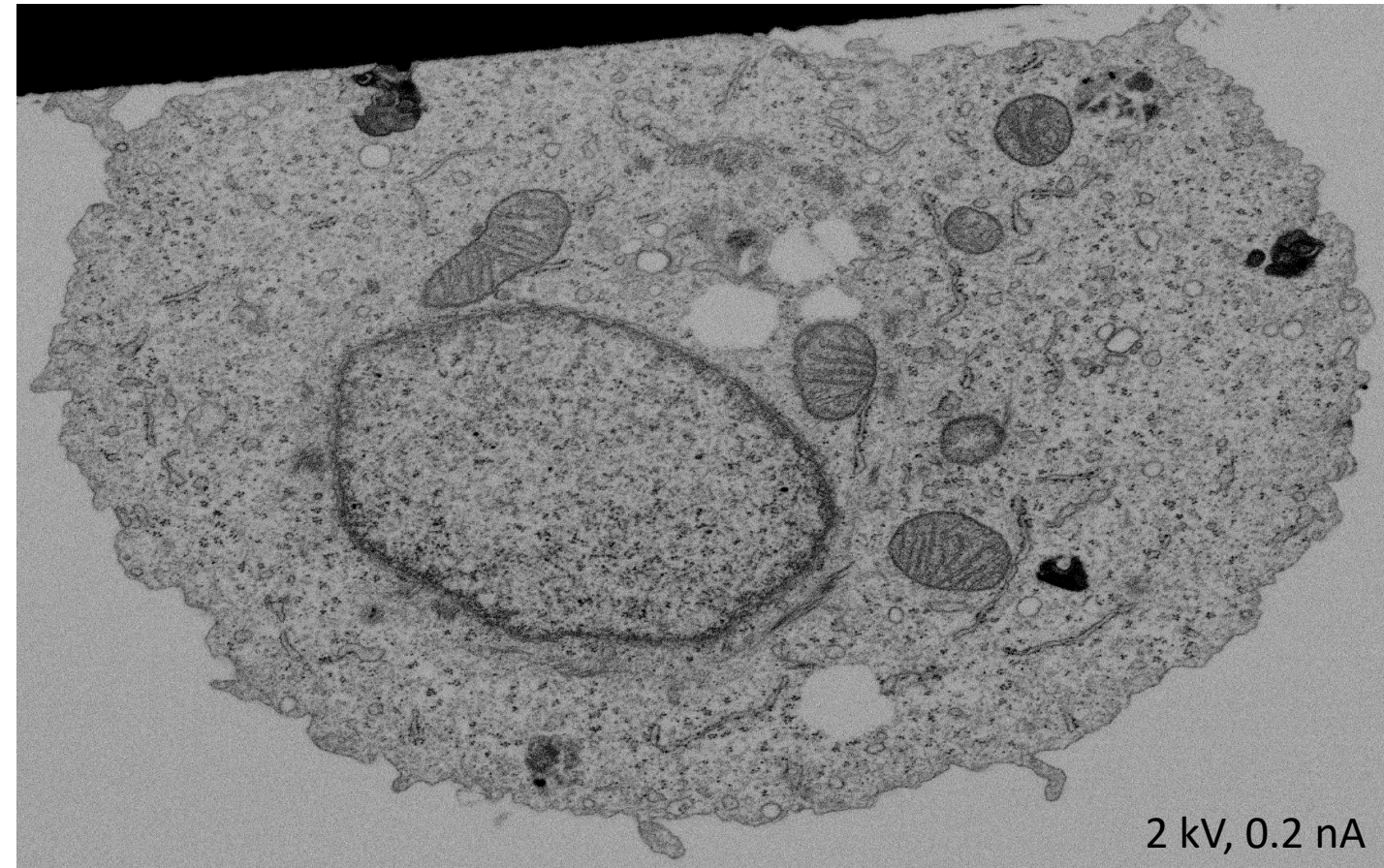


### VIEW FROM SECONDARY ELECTRONS



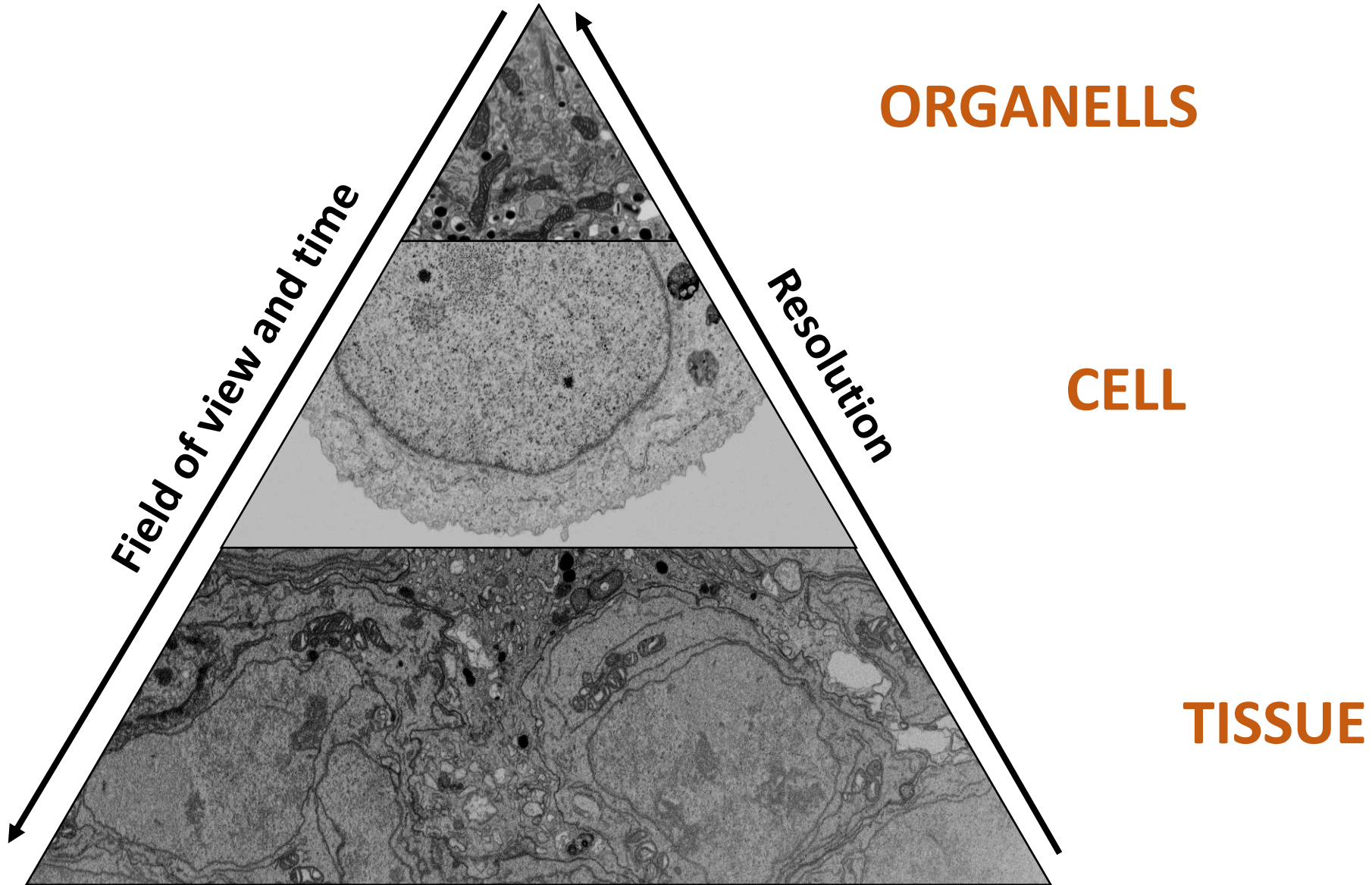


# FIB-SEM imaging





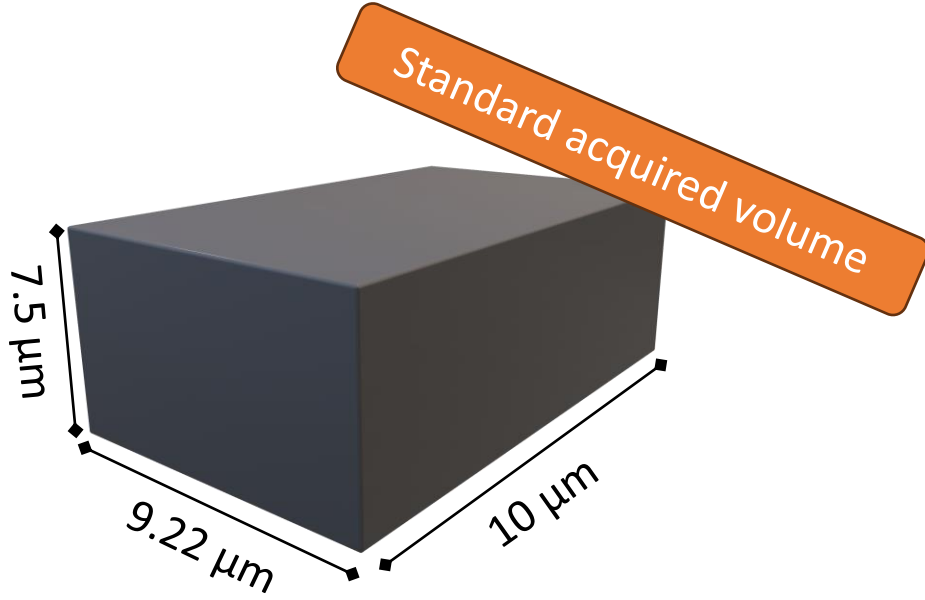
# FIB-SEM in biology



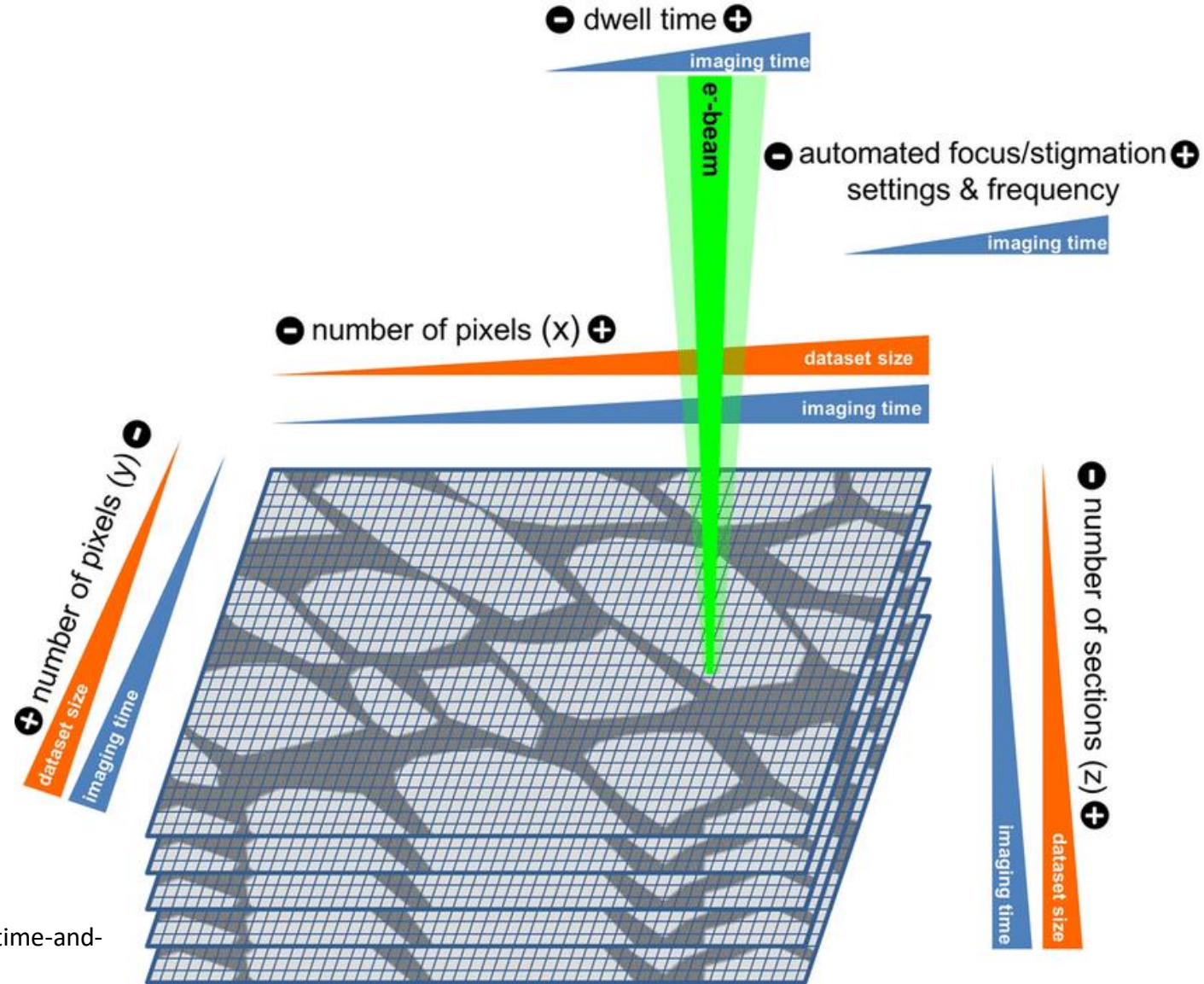




# Volume vs Time vs Sampling



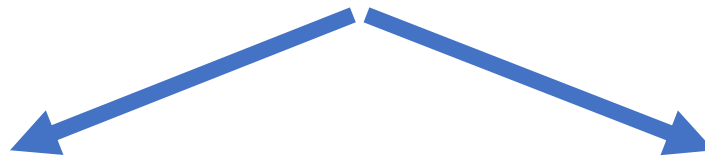
X,Y,Z = 3x3.8x5 nm  
Acquisition time = 67 hours





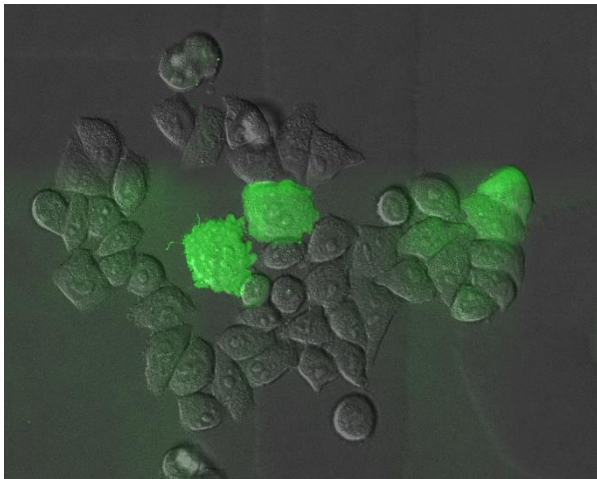
# CLEM – Correlative Light and Electron Microscopy

combination of fluorescence microscopy with high-resolution electron microscopy



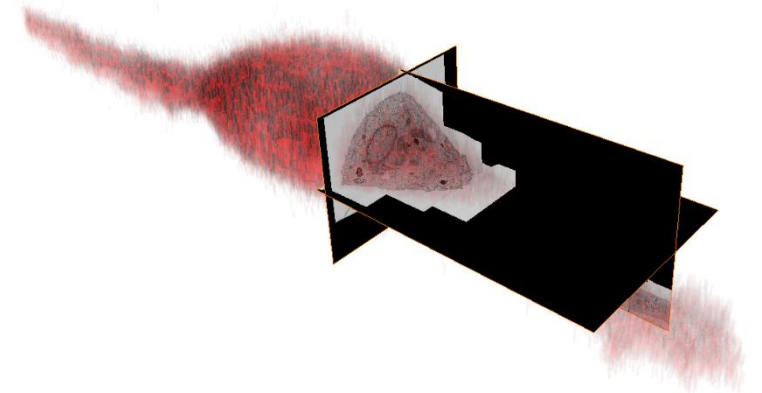
## CLEM for selection of the cell

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



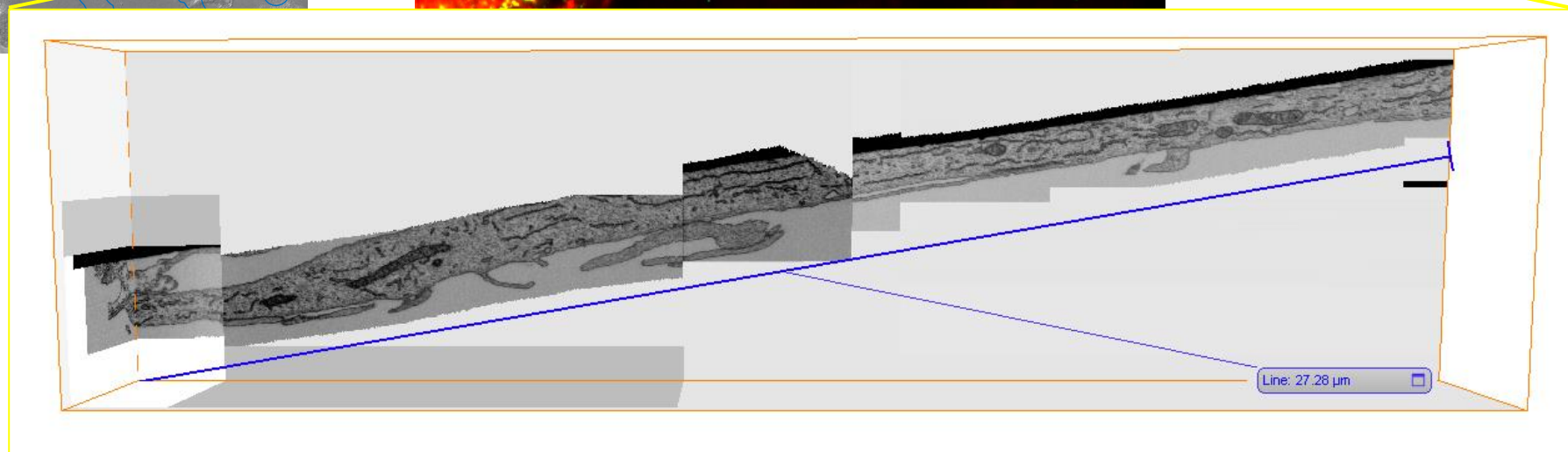
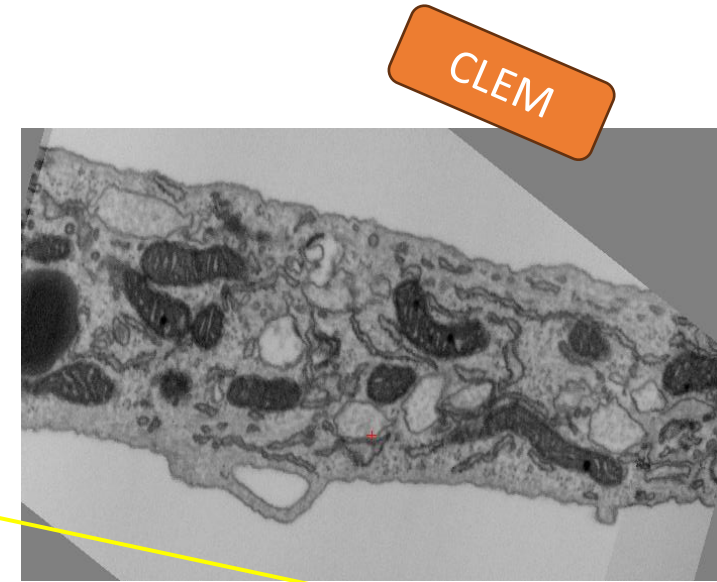
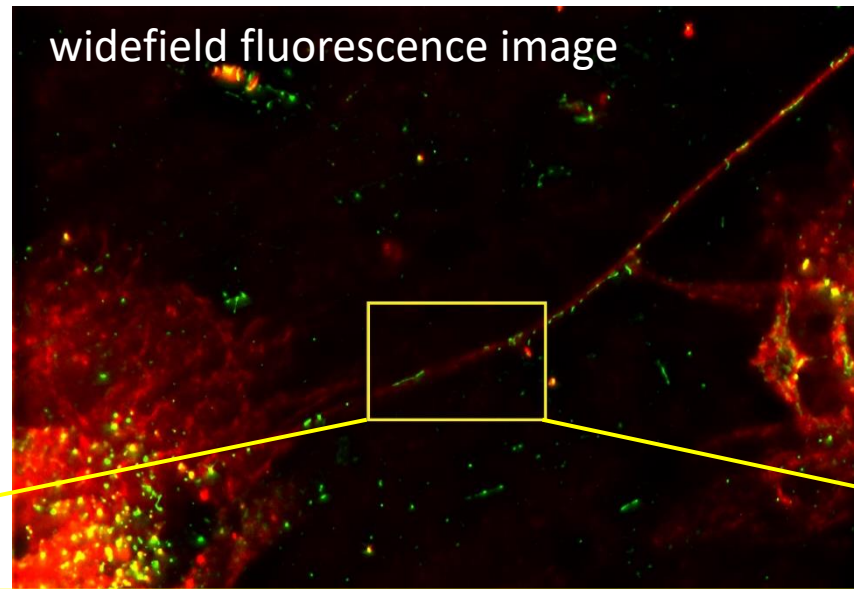
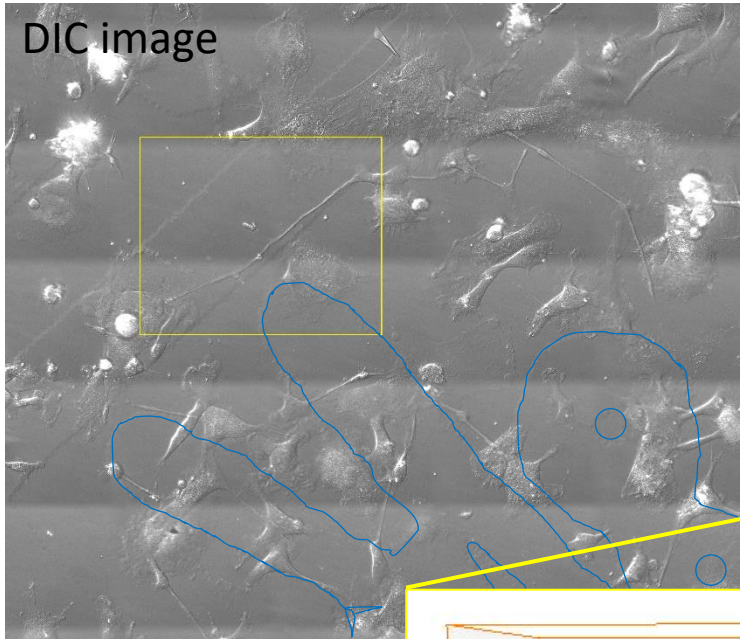
## CLEM for registration of datasets

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



# Examples of CLEM application

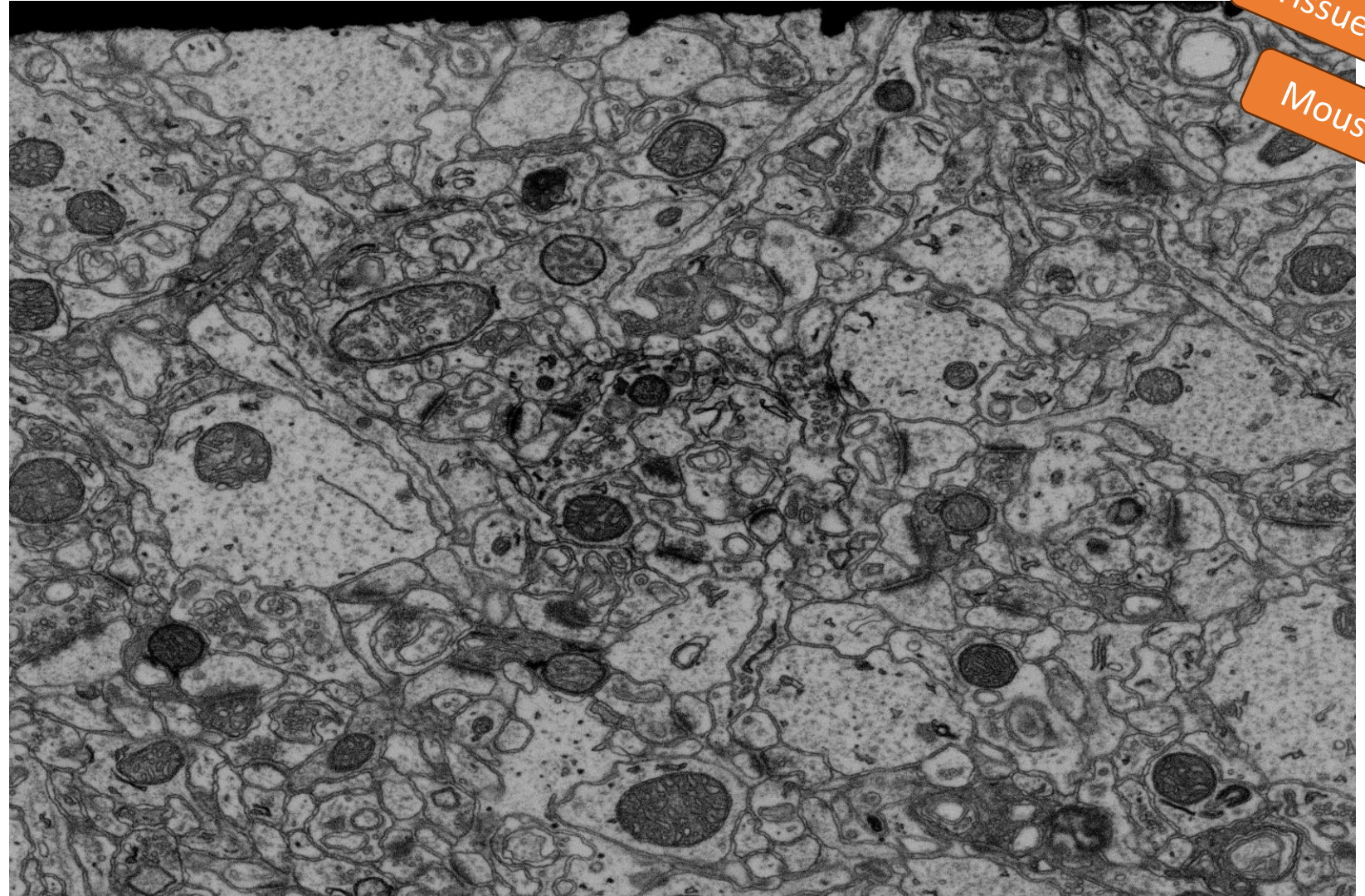
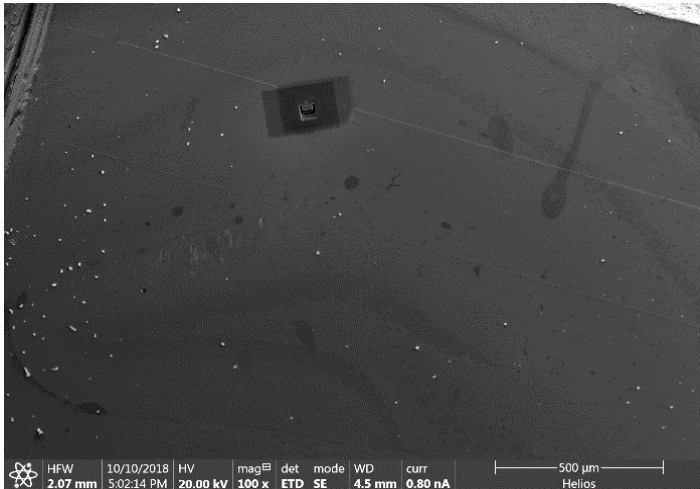
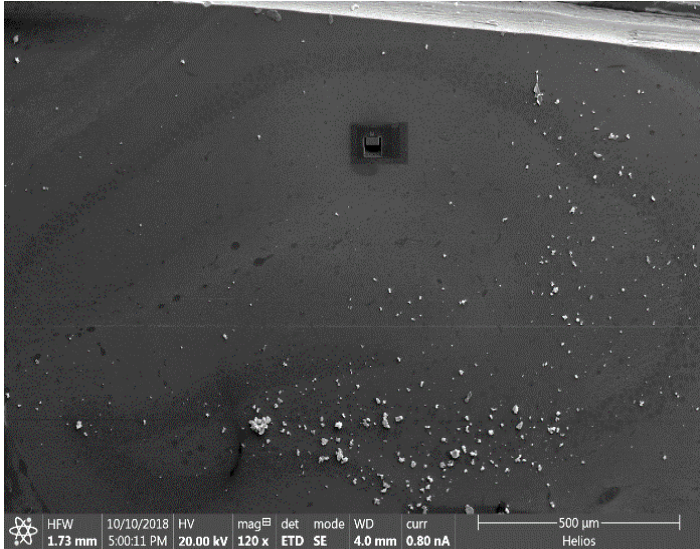
*Mitochondria transiting through tunneling nanotubes*





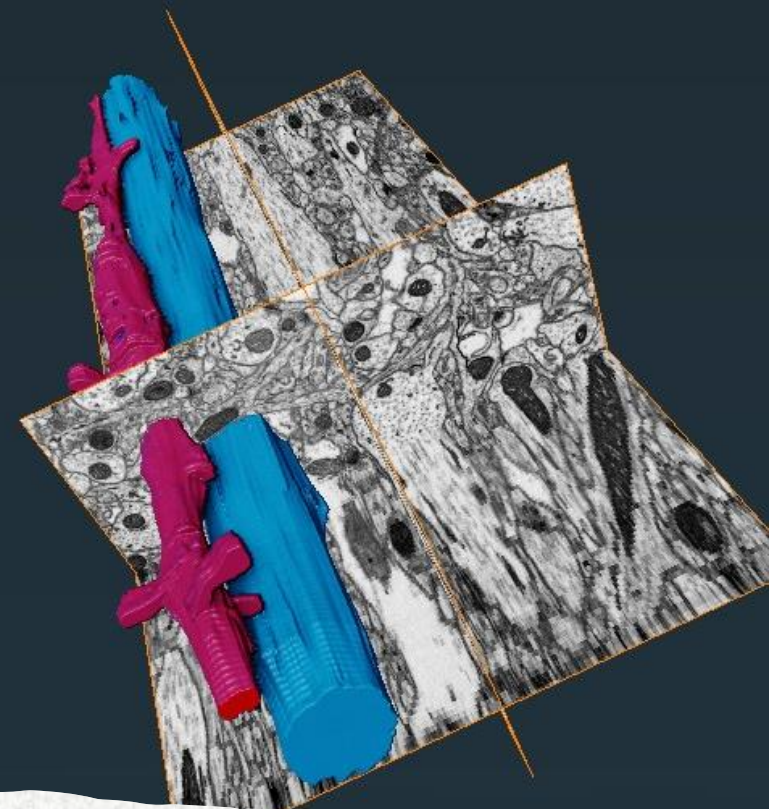
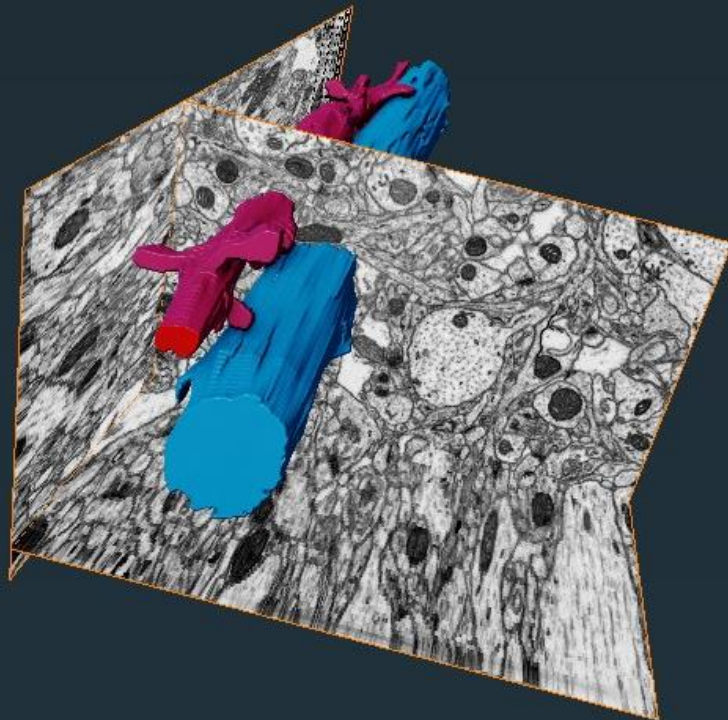
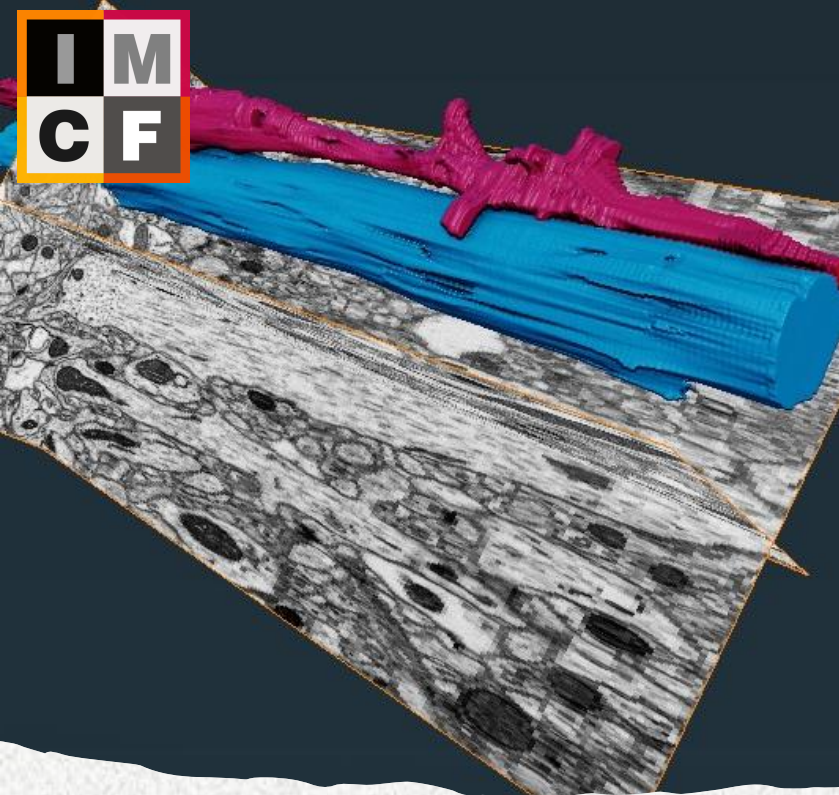
# Examples of FIB-SEM application in IMCF

## Counting neuron synapses in mouse brain



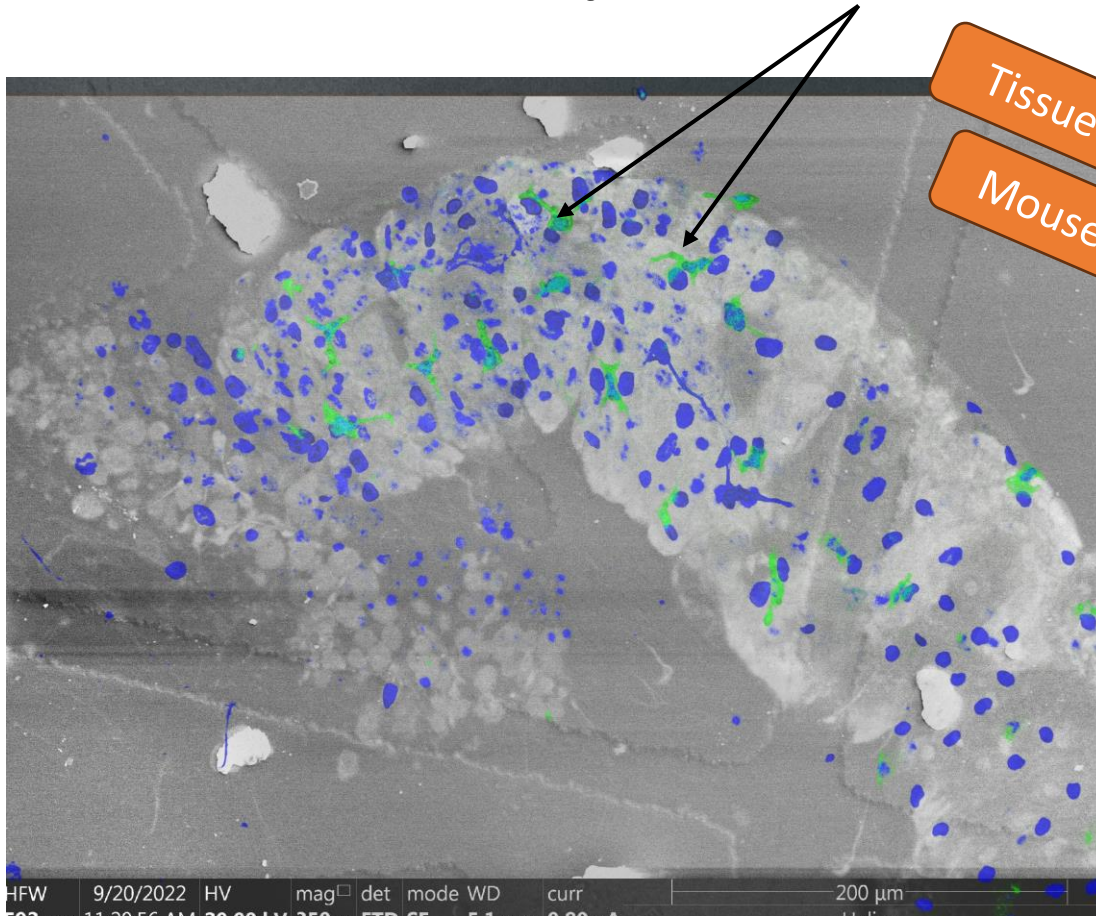
Tissue  
Mouse





# Examples of FIB-SEM application in IMCF

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



Tissue  
Mouse

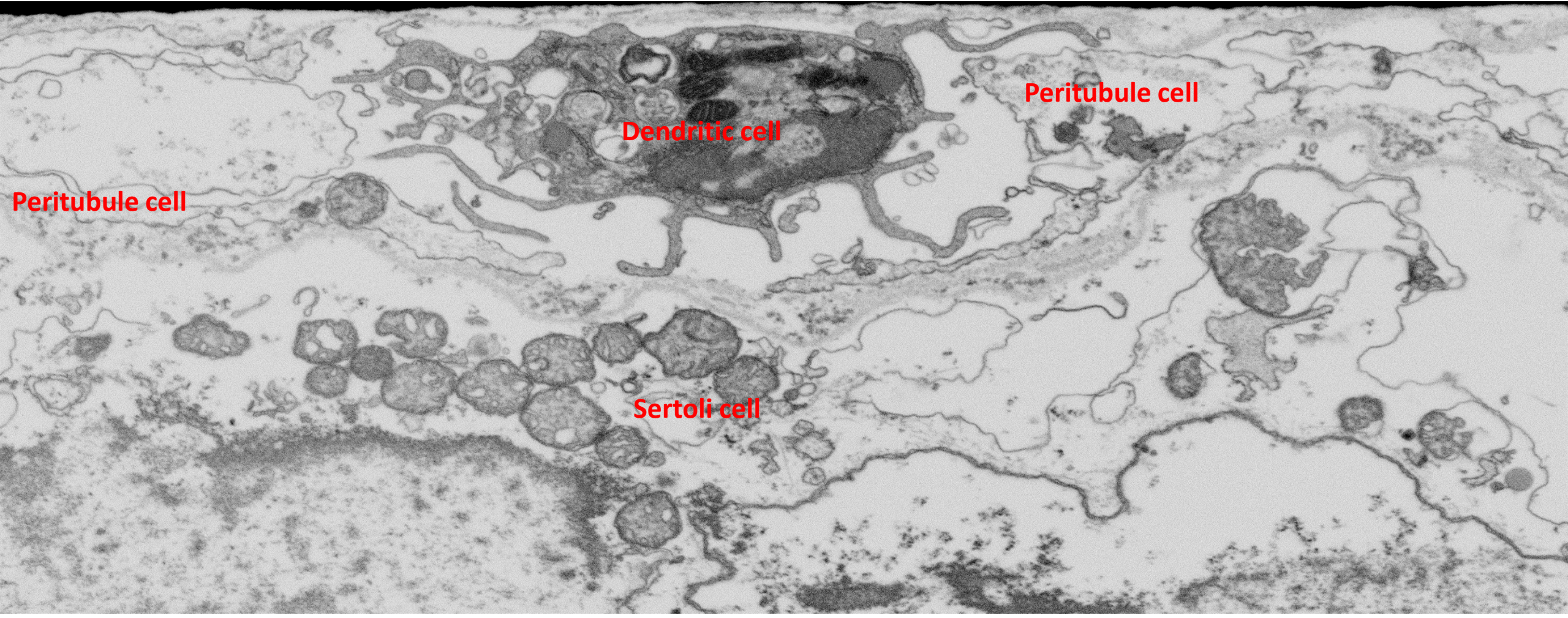
## CLEM application for targeting specific position on FIB-SEM

- 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



# Examples of FIB-SEM application in IMCF

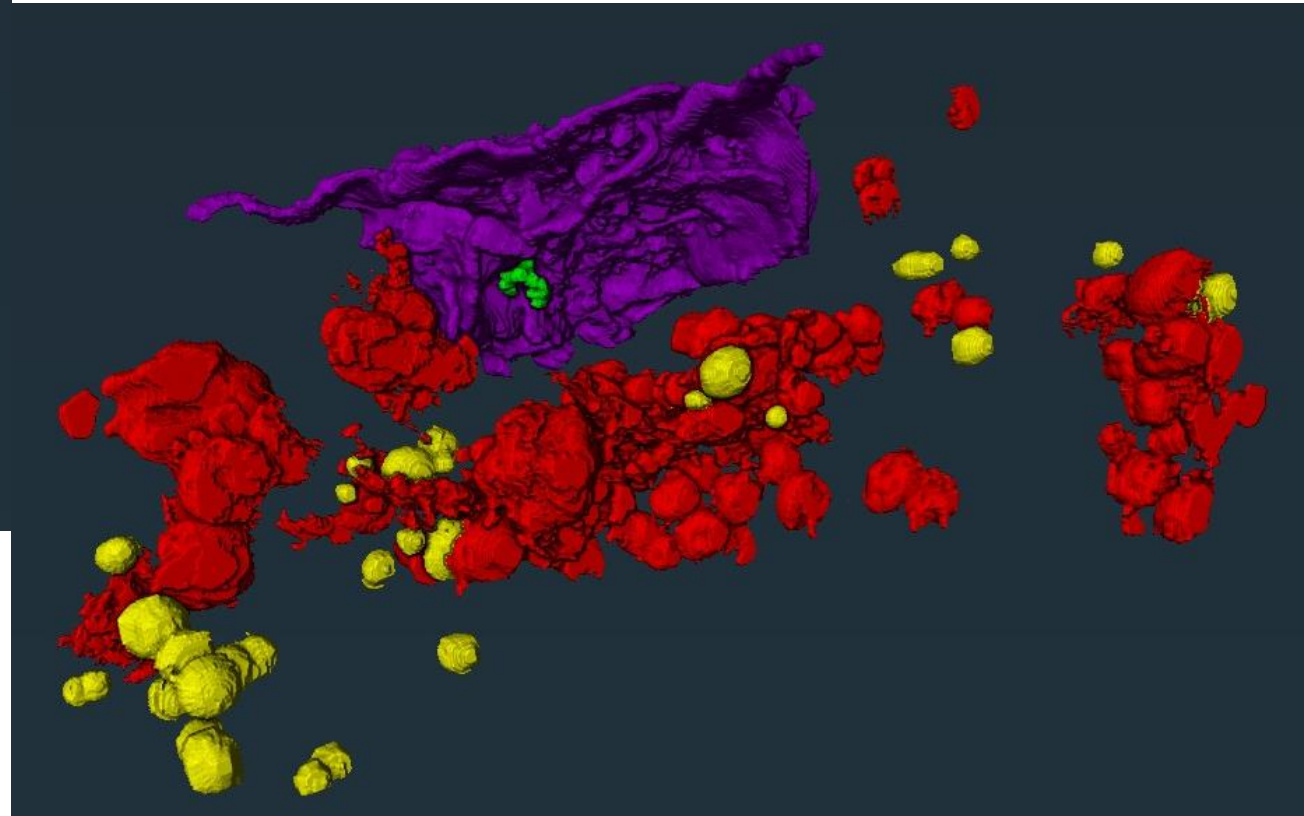
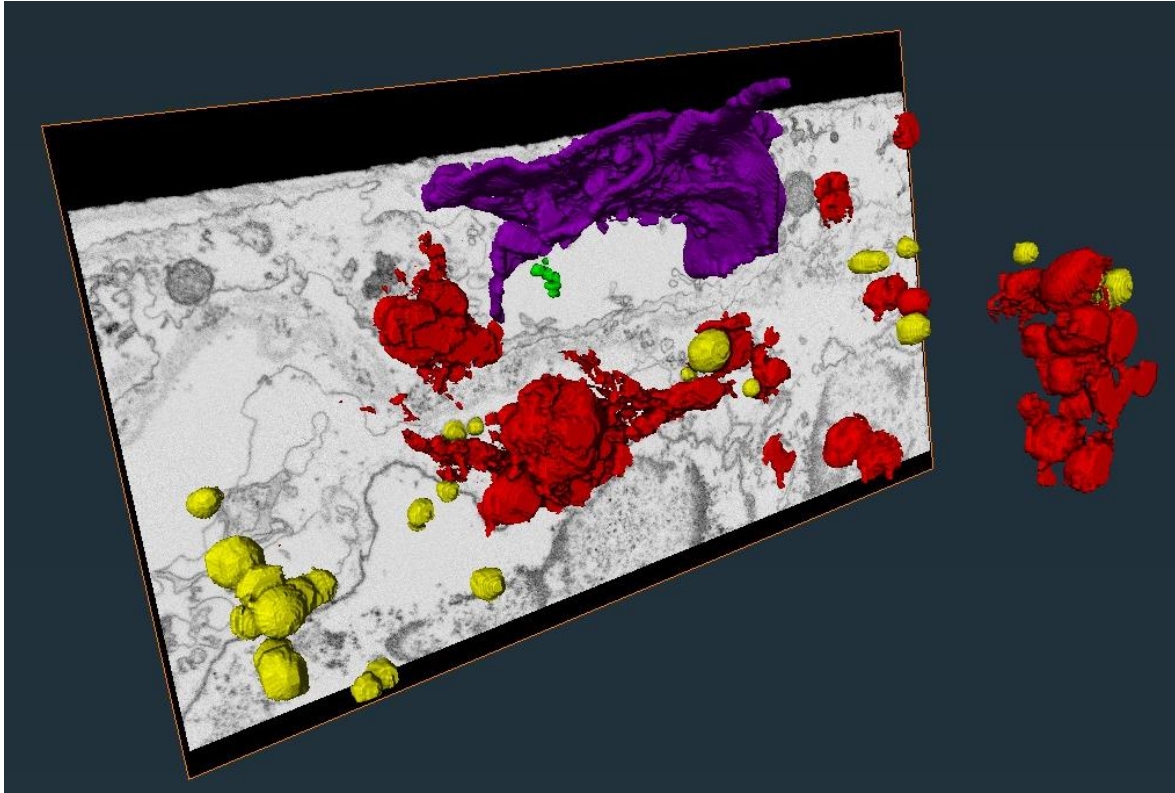
*3D reconstruction of the dendritic cell and the surrounding vesicles in seminiferous tubule*





# Examples of FIB-SEM application in IMCF

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



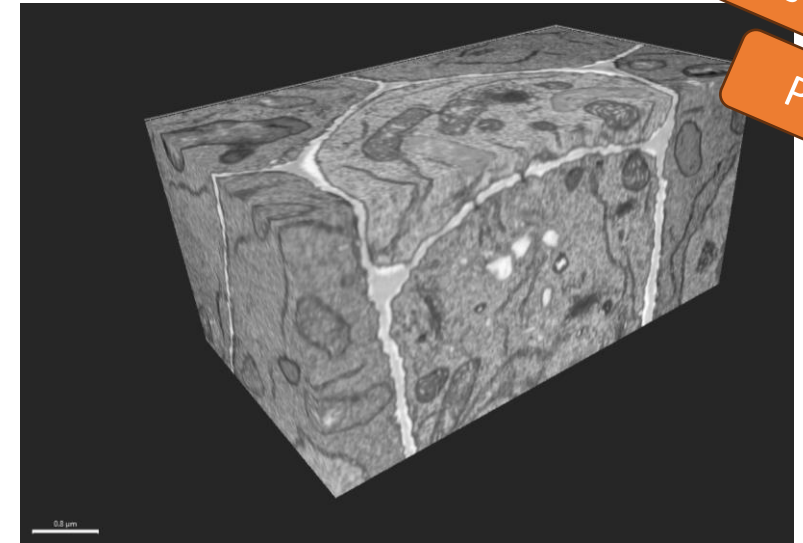
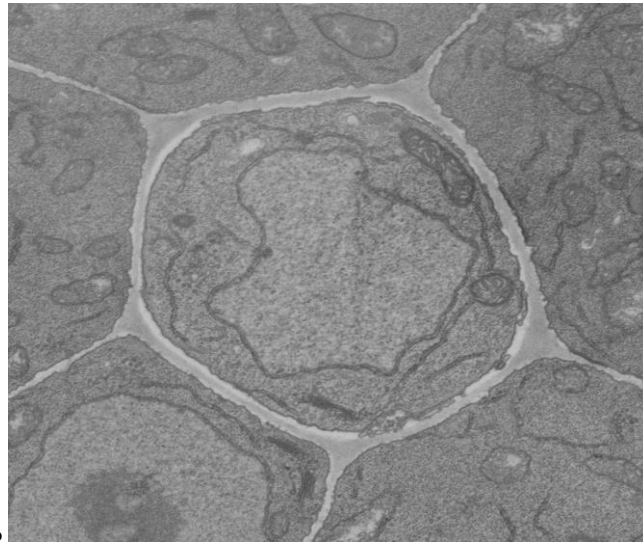


# Examples of FIB-SEM application in IMCF

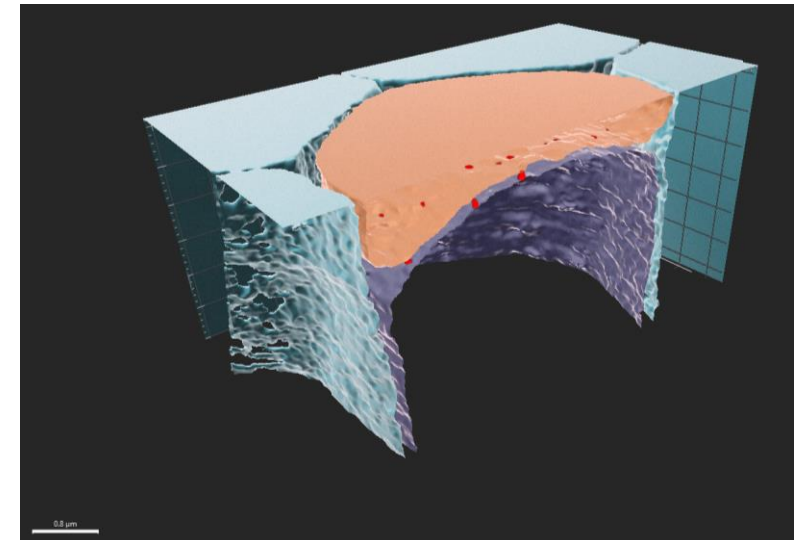
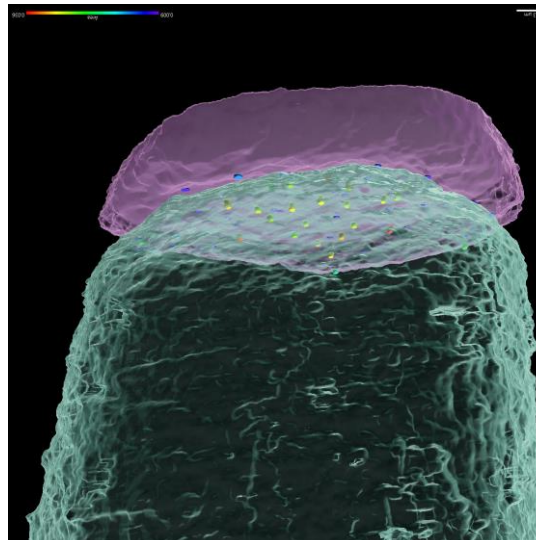
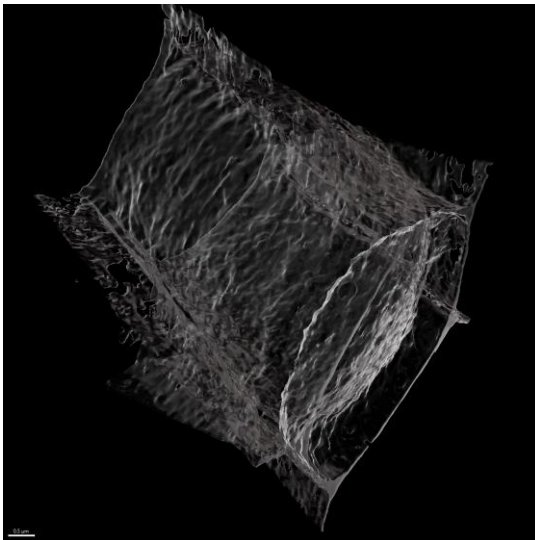
## *3D ultrastructure of dividing protophloem cells in Arabidopsis thaliana roots*

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

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

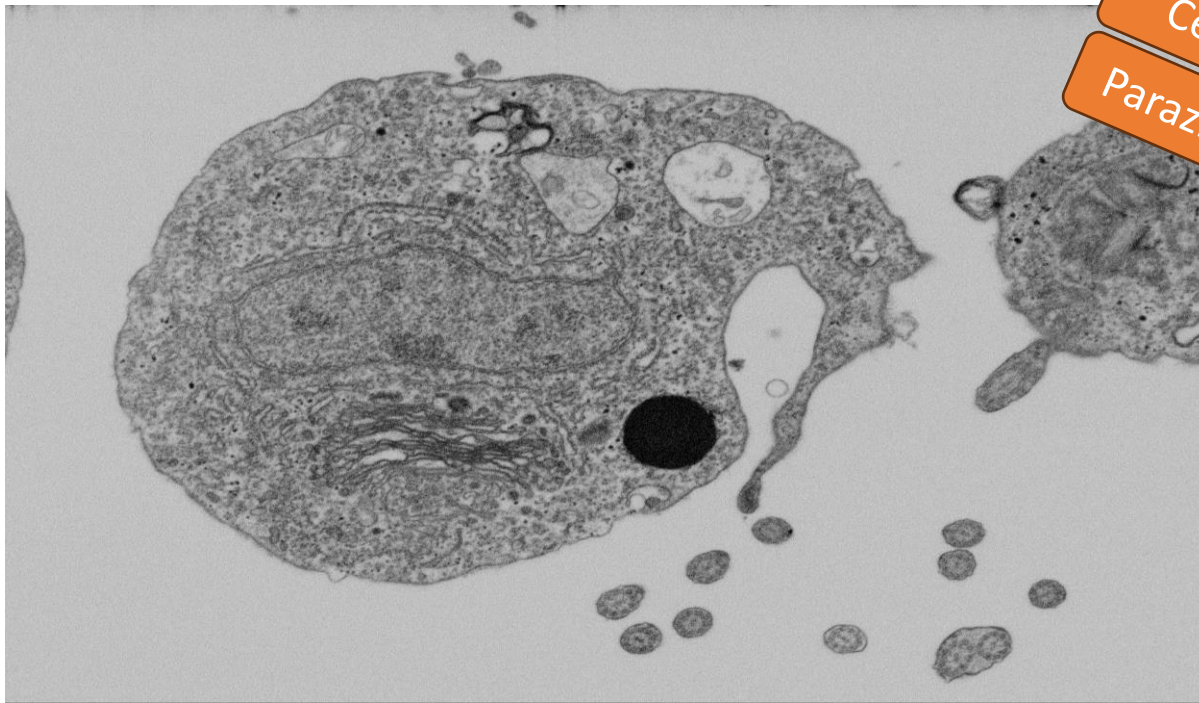


Tissue  
Plant

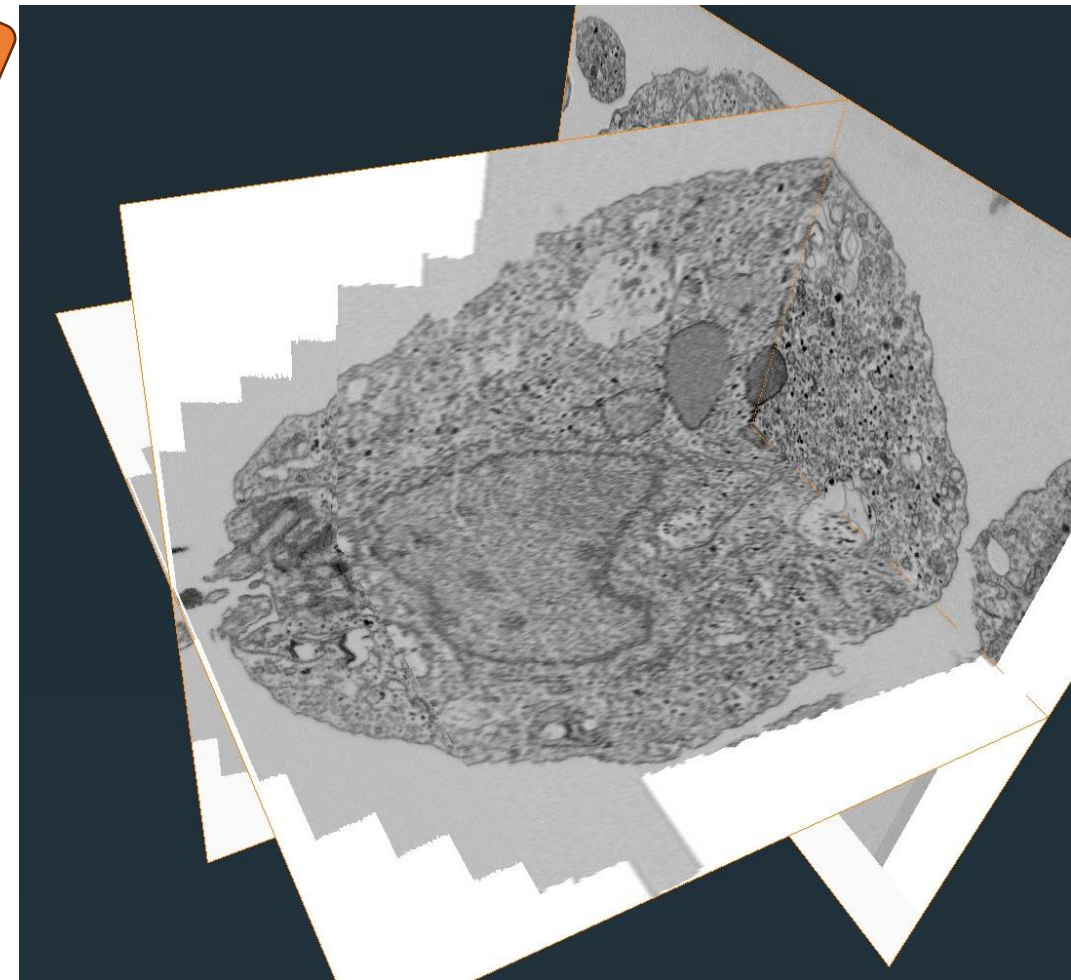


# Examples of FIB-SEM application in IMCF

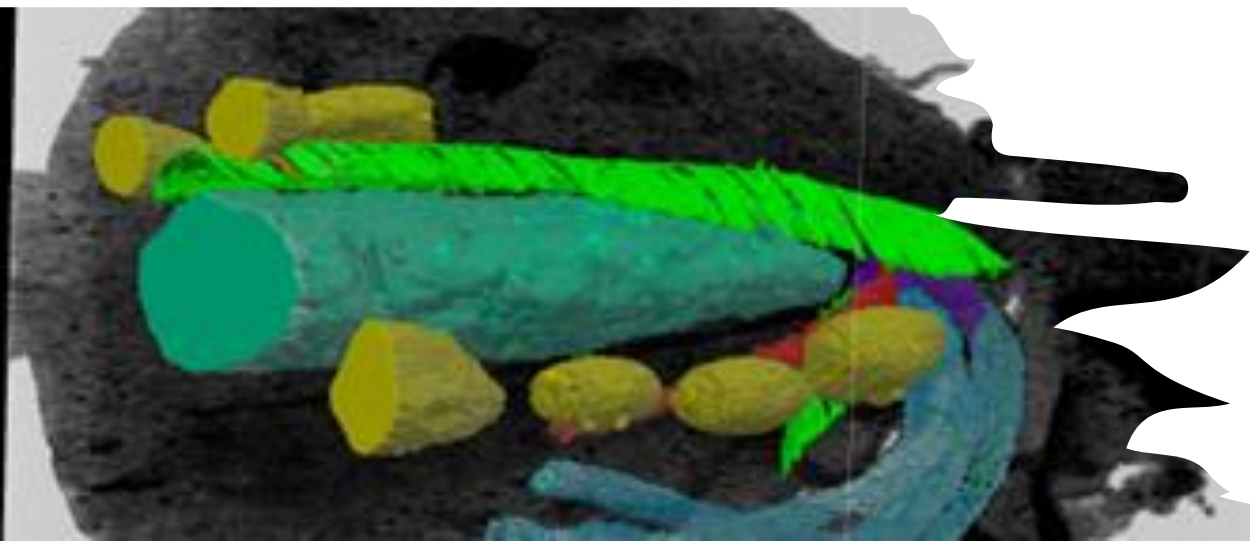
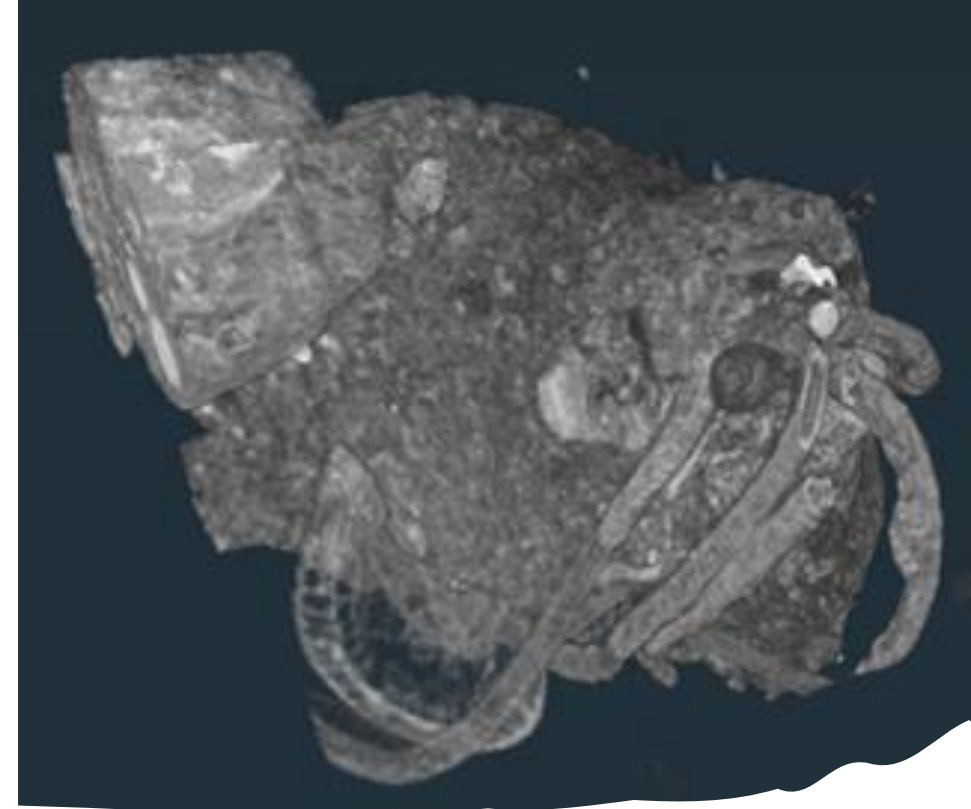
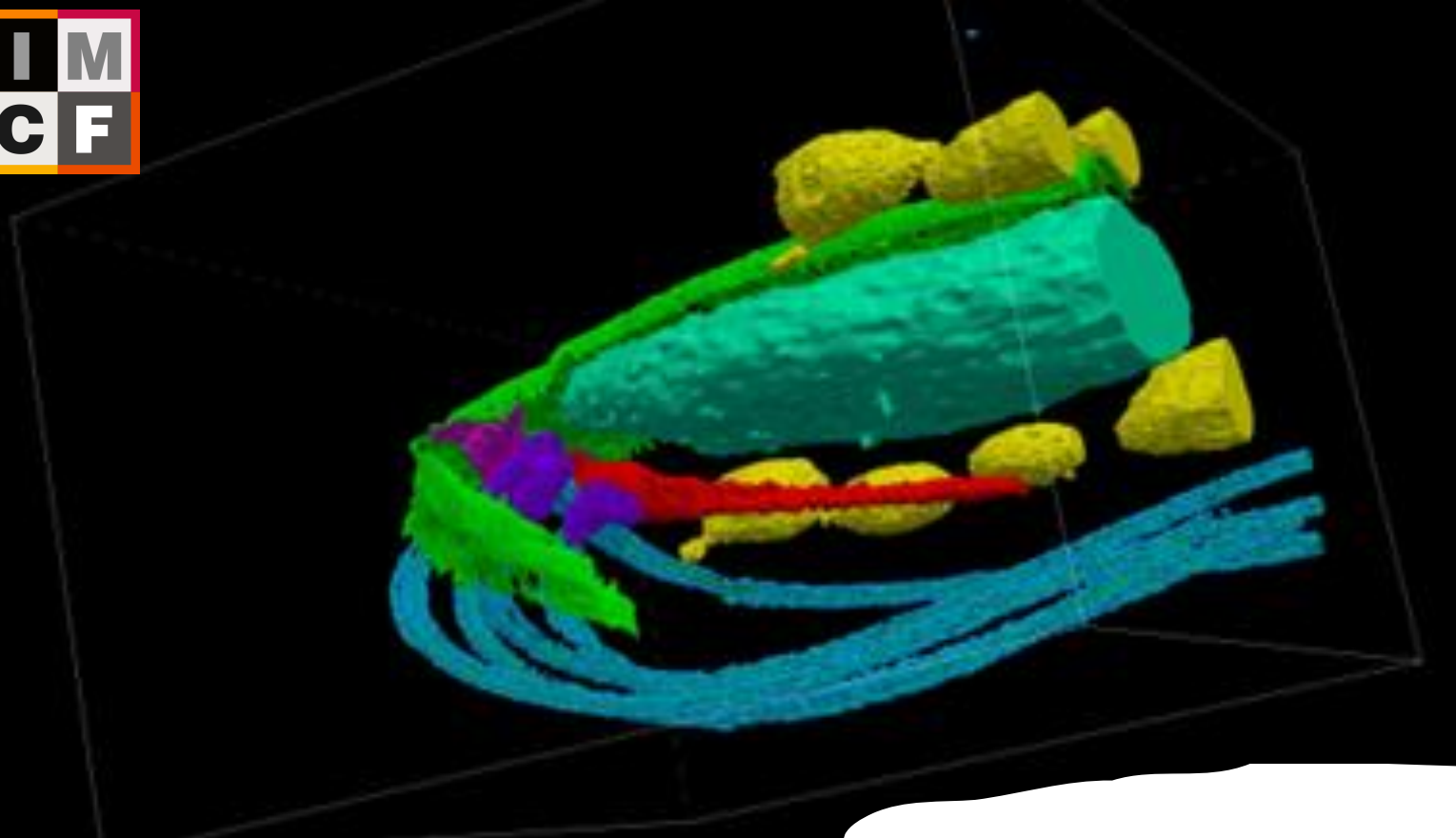
## *3D ultrastructure of Trichomonas vaginalis*



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



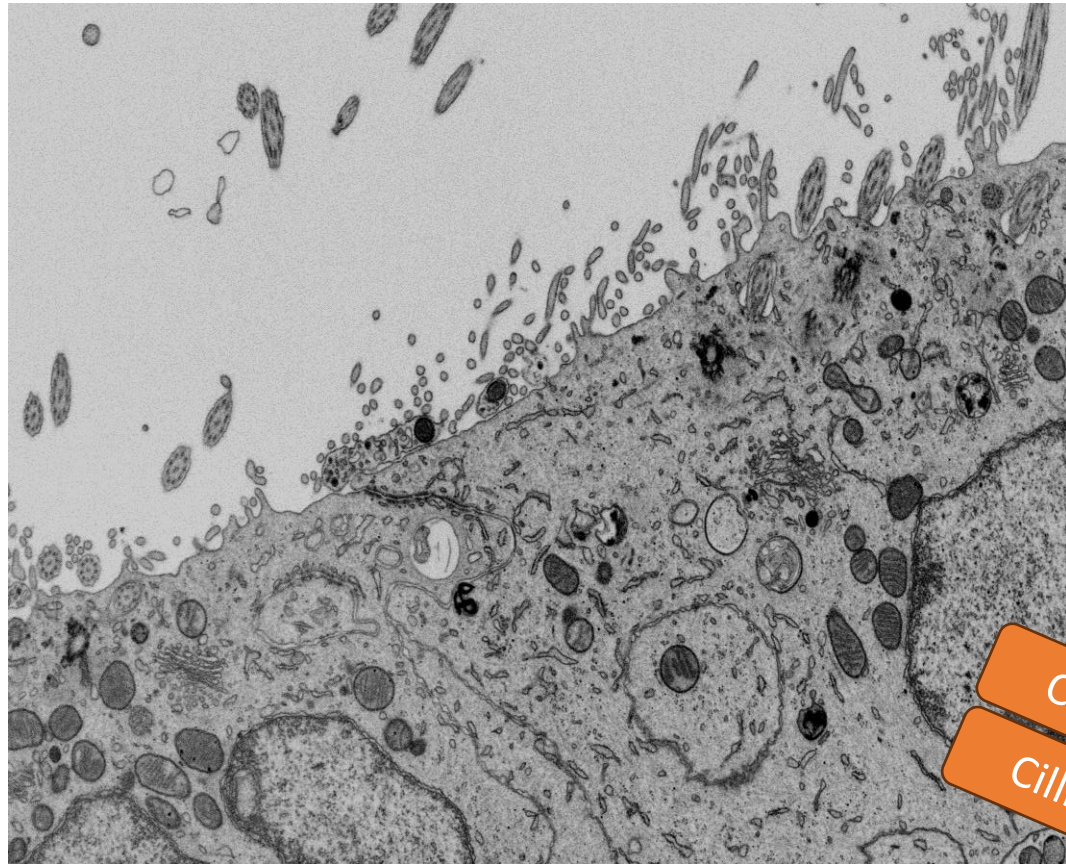




*3D ultrastructure of  
Trichomonas vaginalis*

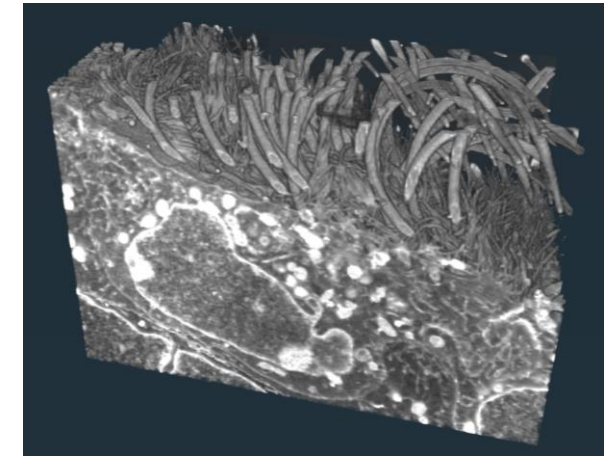
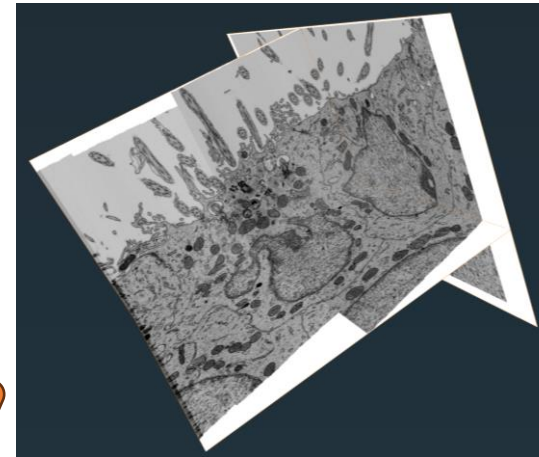
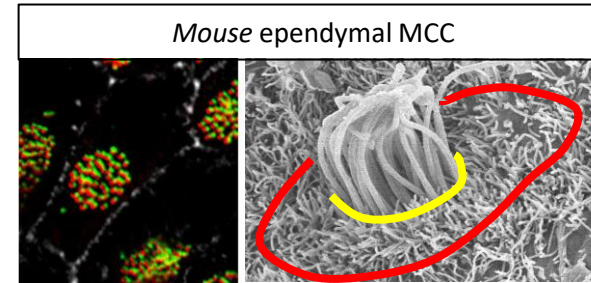
# Examples of FIB-SEM application in IMCF

## *Organization of cilia anchoring in the ependymal cells*



Images courtesy by Camille Boutin  
Aix-Marseille Université, France

Cells  
Cilia

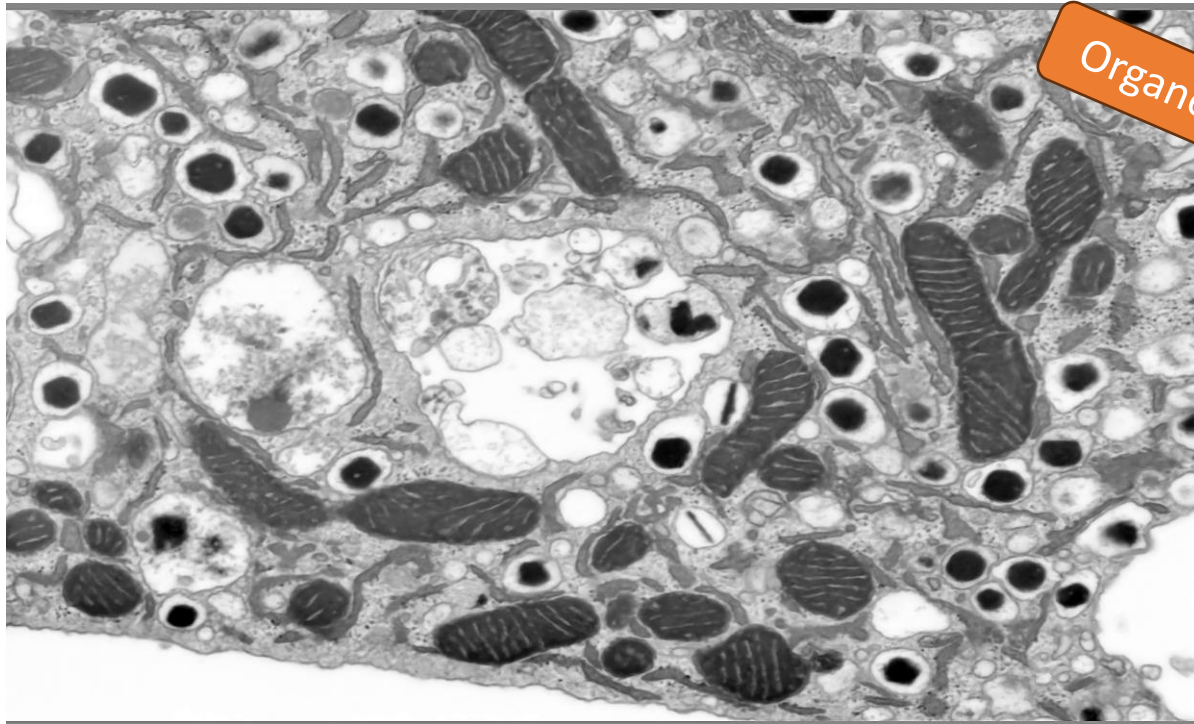


FIB-SEM acquisition to reconstruct the organization of ependymal multiciliated cells to access the organization of their apical surface and distribution of main components inside the cells.

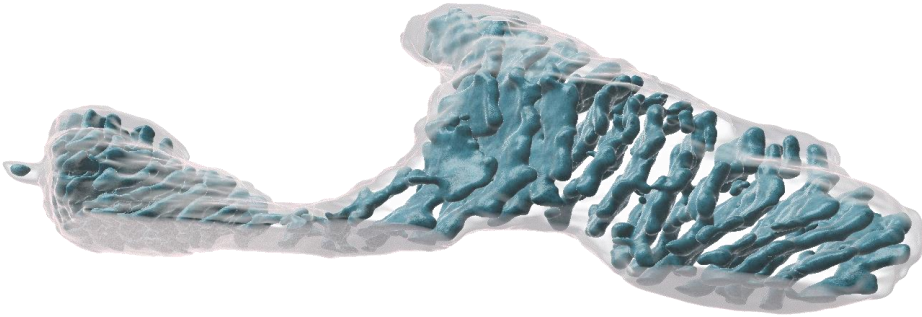
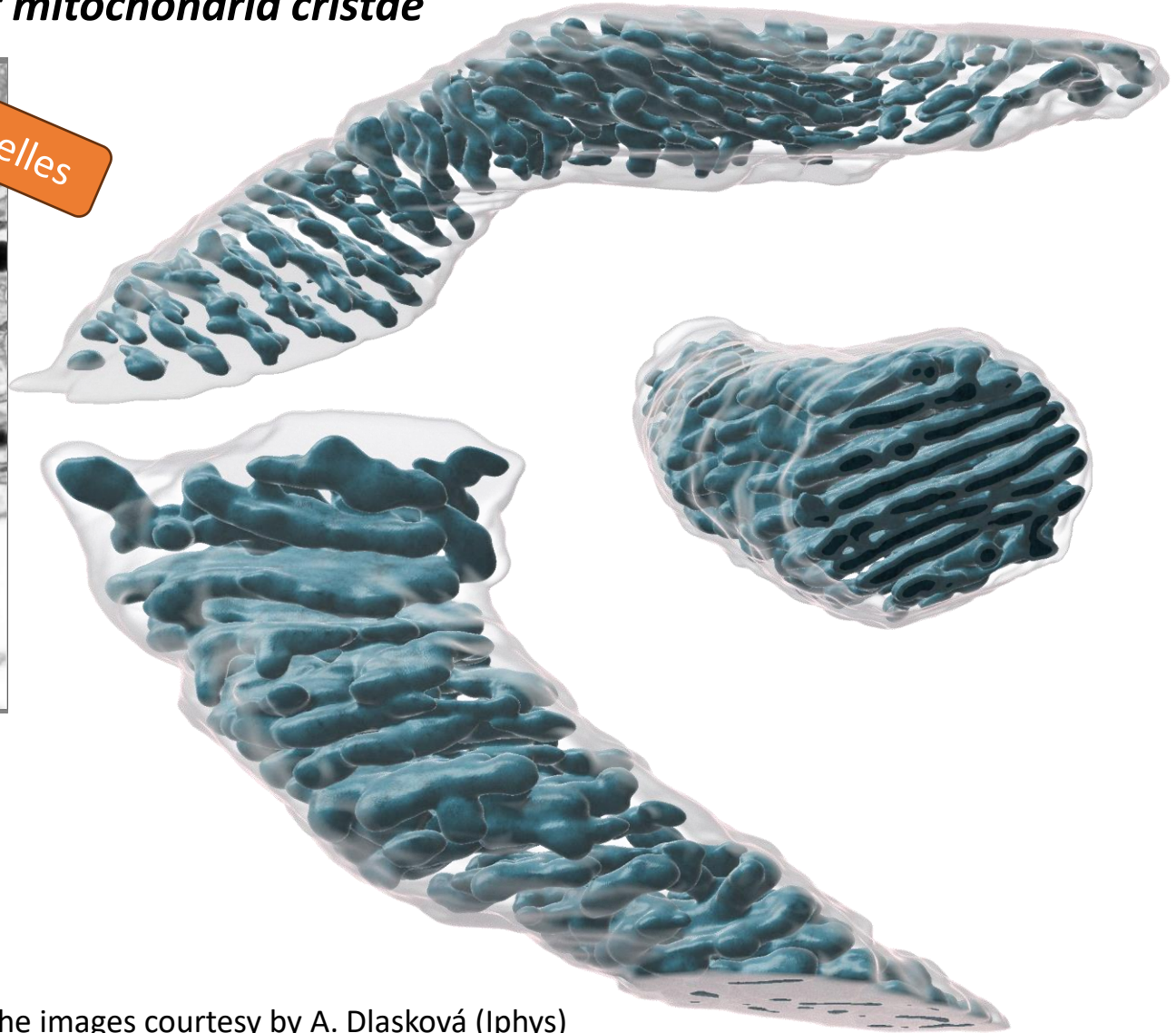


# Examples of FIB-SEM application in IMCF

*Quantification of mitochondria cristae*



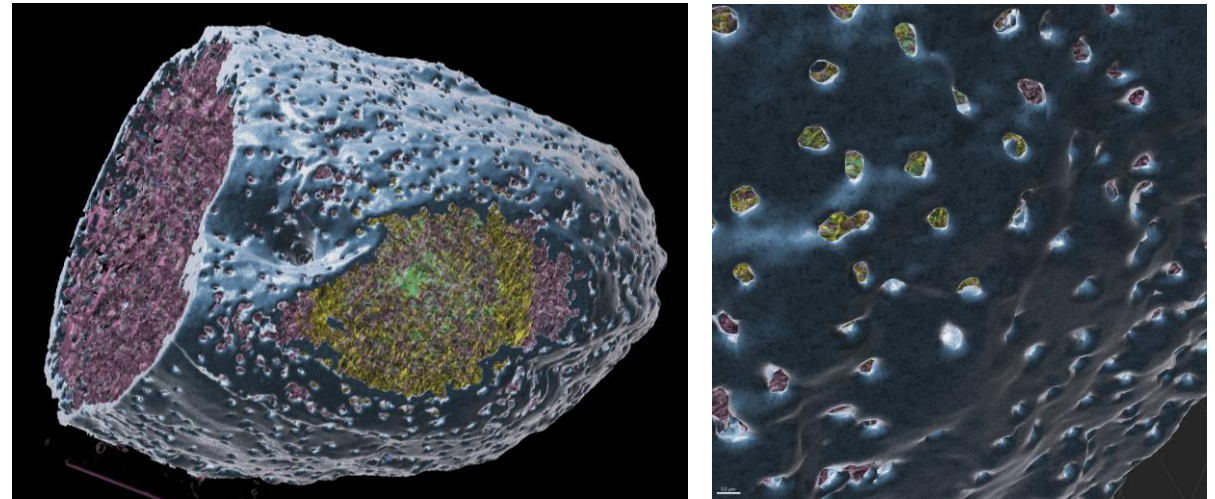
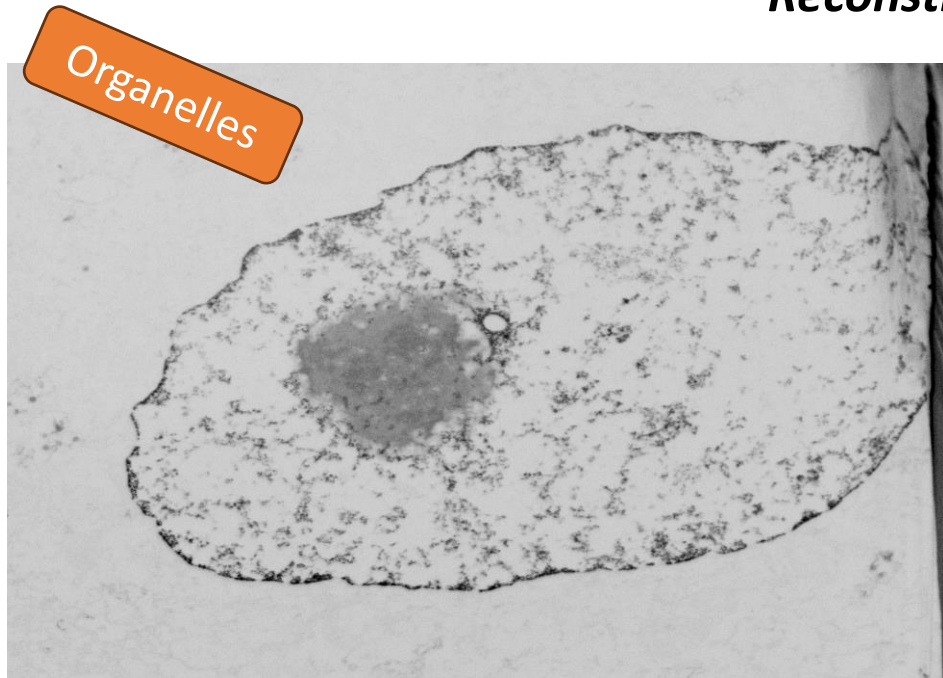
Organelles



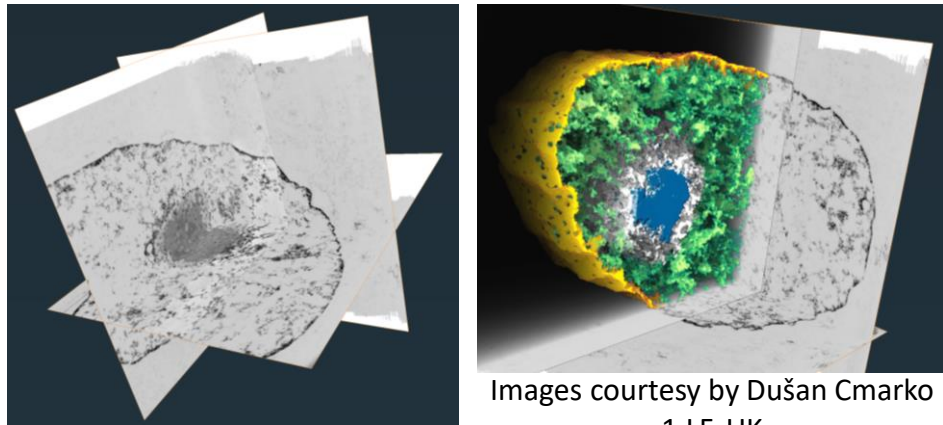


# Examples of FIB-SEM application in IMCF

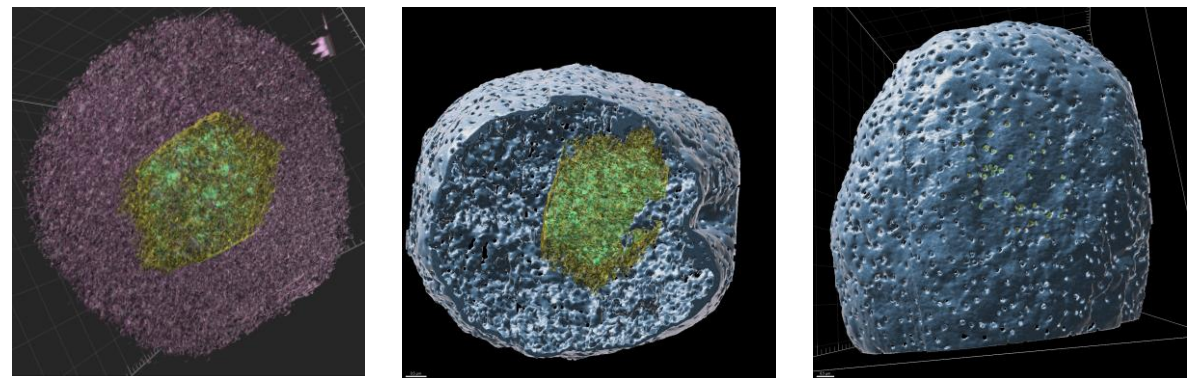
## *Reconstruction of chromatin subpopulations*



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



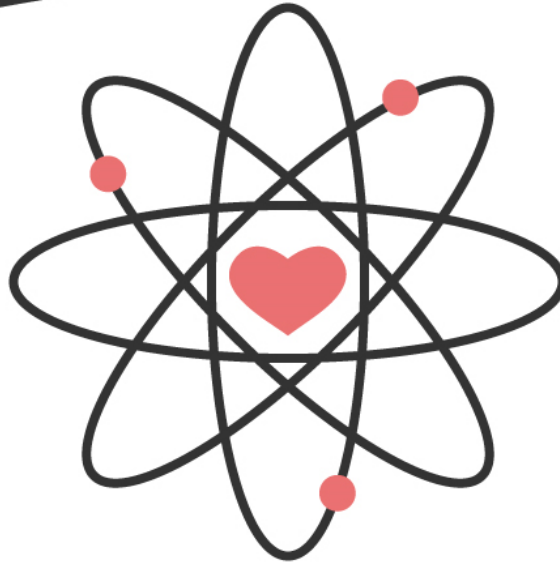
Images courtesy by Dušan Cmarko  
1.LF, UK





Thank you for your attention!

**ELECTRONS ARE NEGATIVE**



*but they're positive in my heart*