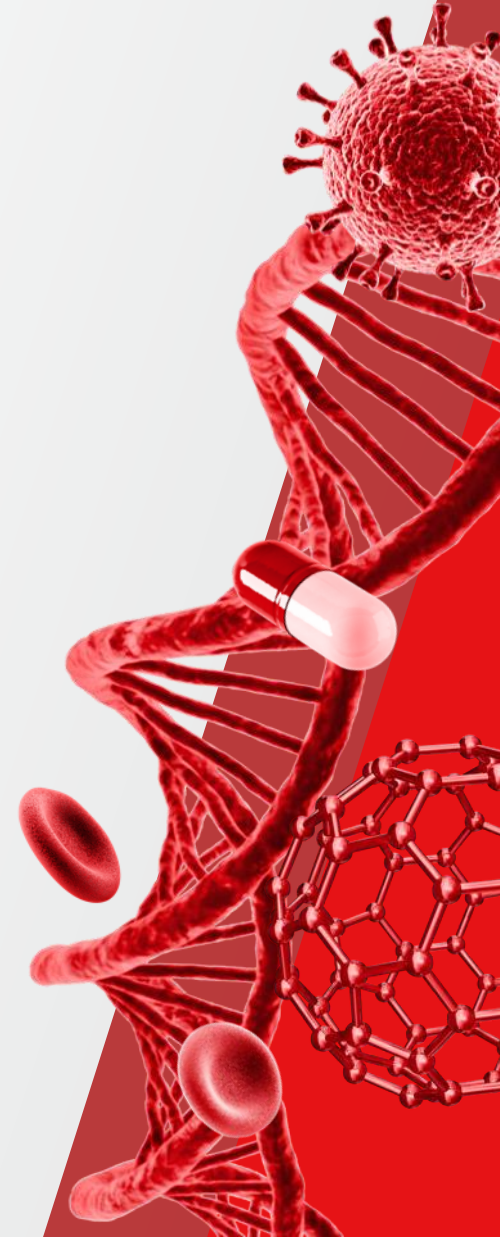


Correlative Microscopy of Vitrified Bio-Samples

Miloš Hovorka

October 7th, 2021

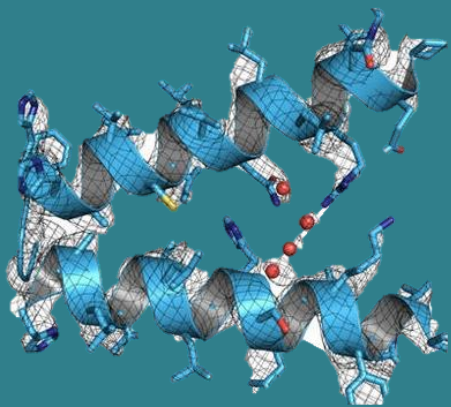
 The world leader in serving science



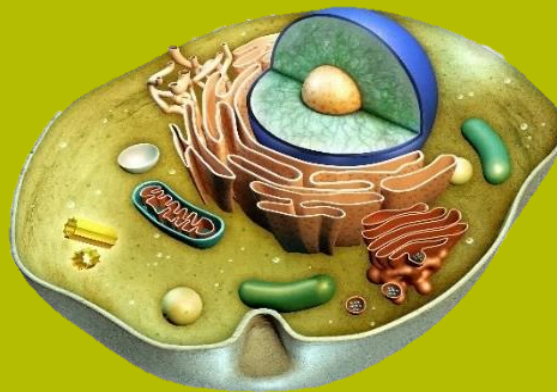
- **Cryo(SDB)-Electron Tomography Workflow.**
- **Correlative Microscopy with iFLM.**

Cryo-EM | Two main imaging methods

Structure of isolated proteins



Protein function in cells



Single particle analysis

Cryo-tomography

...information about the molecular landscape
inside the cell.



Illustration: Charis Tsevis (flickr)

Cryo(SDB) Tomography Workflow



Vitrification

Milling and Correlation

TEM Tomography

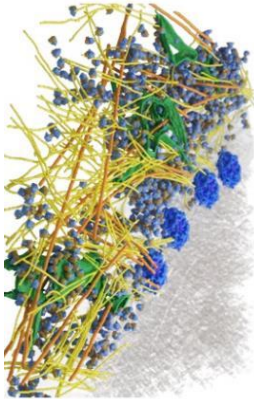
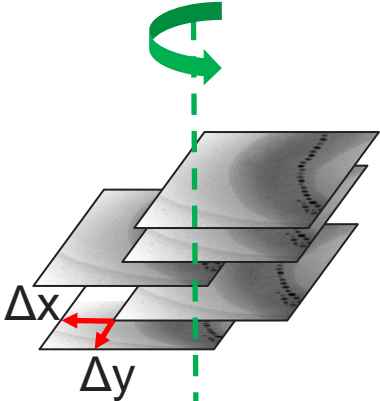
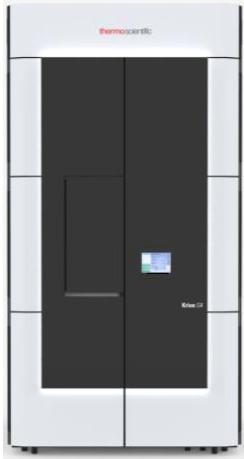
Tomo Reconstruction

Data Analysis

Sample

Lamella

Data



SAMPLE
FREEZING

LAMELLA
PREPARATION
(optical, E- and I-
imaging)

DATA ACQUISITION,
RECONSTRUCTION
(TOMOGRAMS)

VISUALIZATION and
ANALYSIS

Freezer

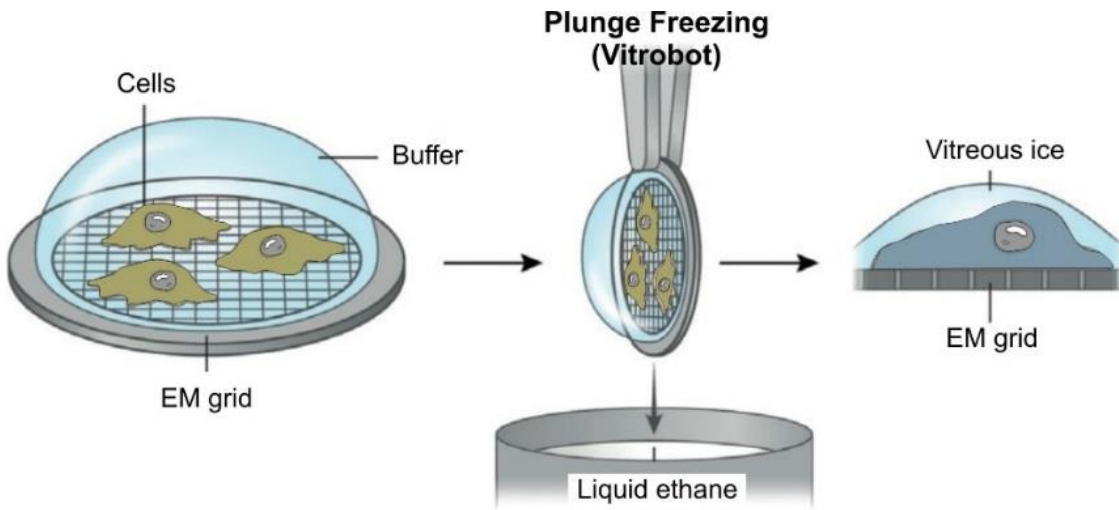
(cryo)SDB

(cryo)TEM

Reconstruction and post-processing pipeline

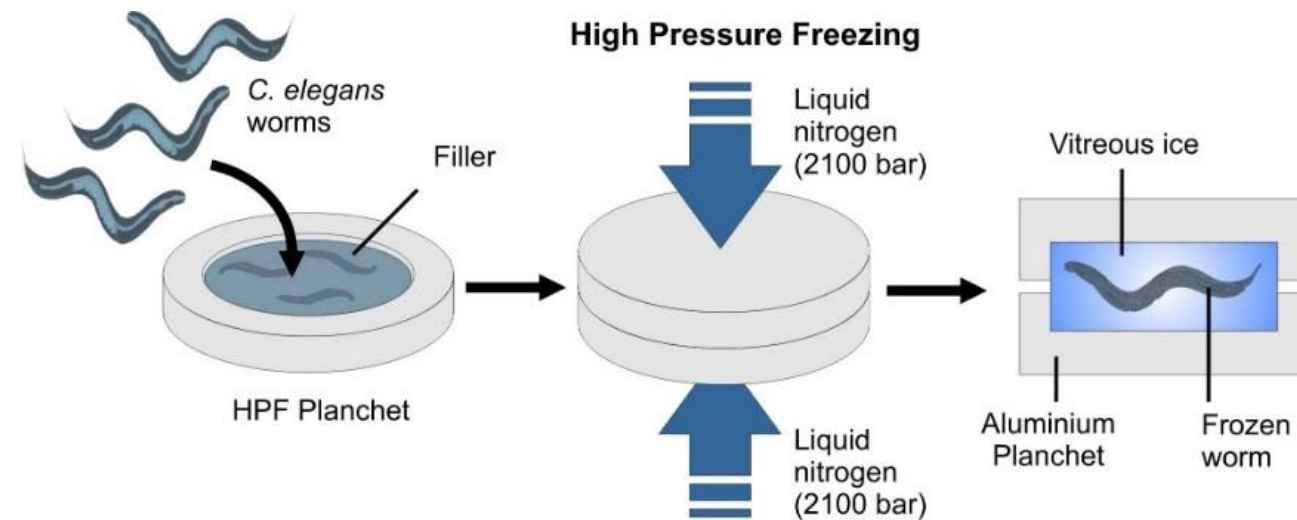
Sample vitrification methods (to arrest cells in a close-to-native state)

Proteins or Cells



From Narayan & Subramaniam, *Nature Methods* (2015)

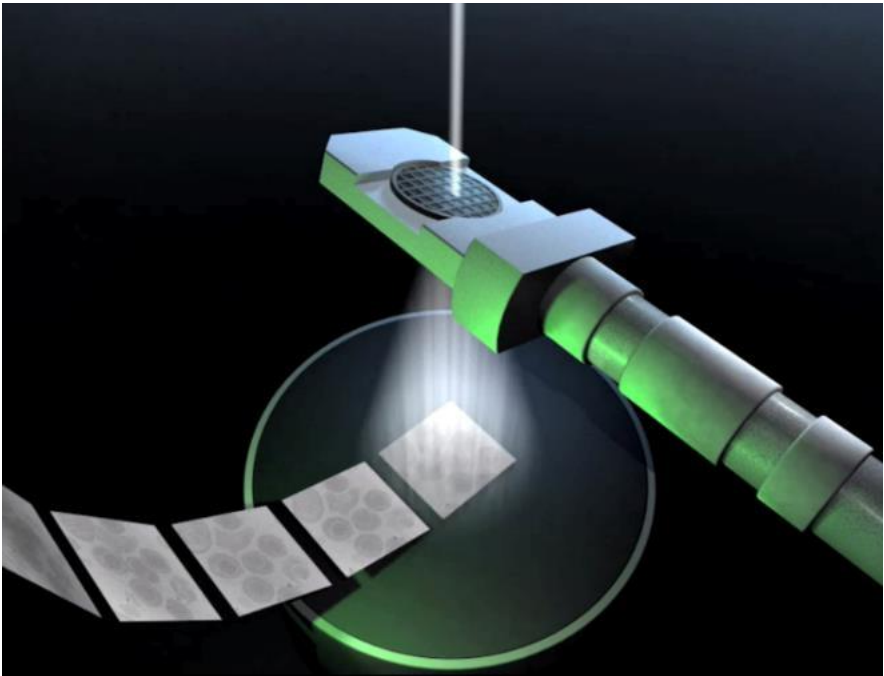
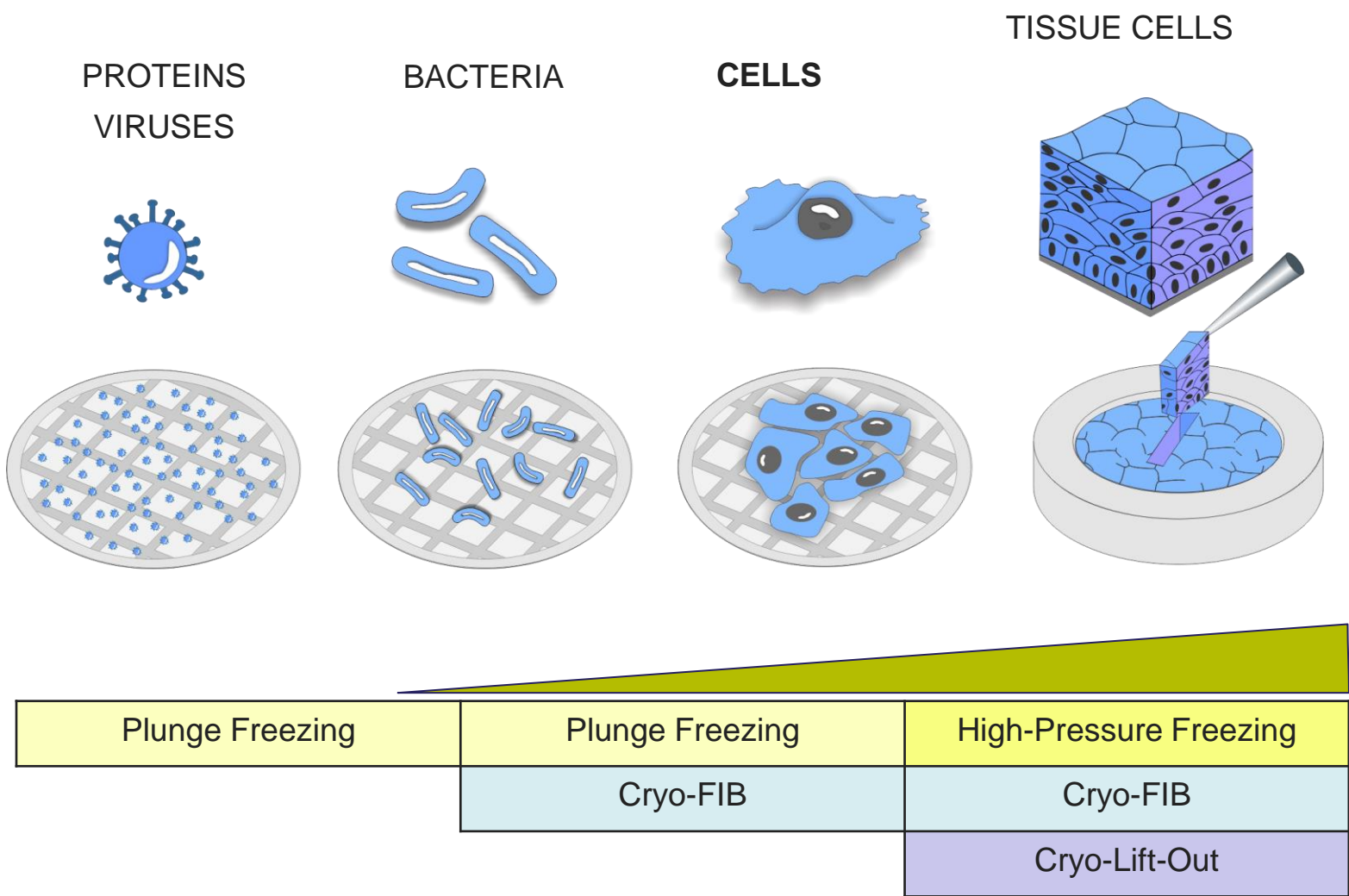
Small organisms or tissue biopsies



Plunge Freezing (Thin-Film Vitrification)
($< 5 \mu\text{m}$)

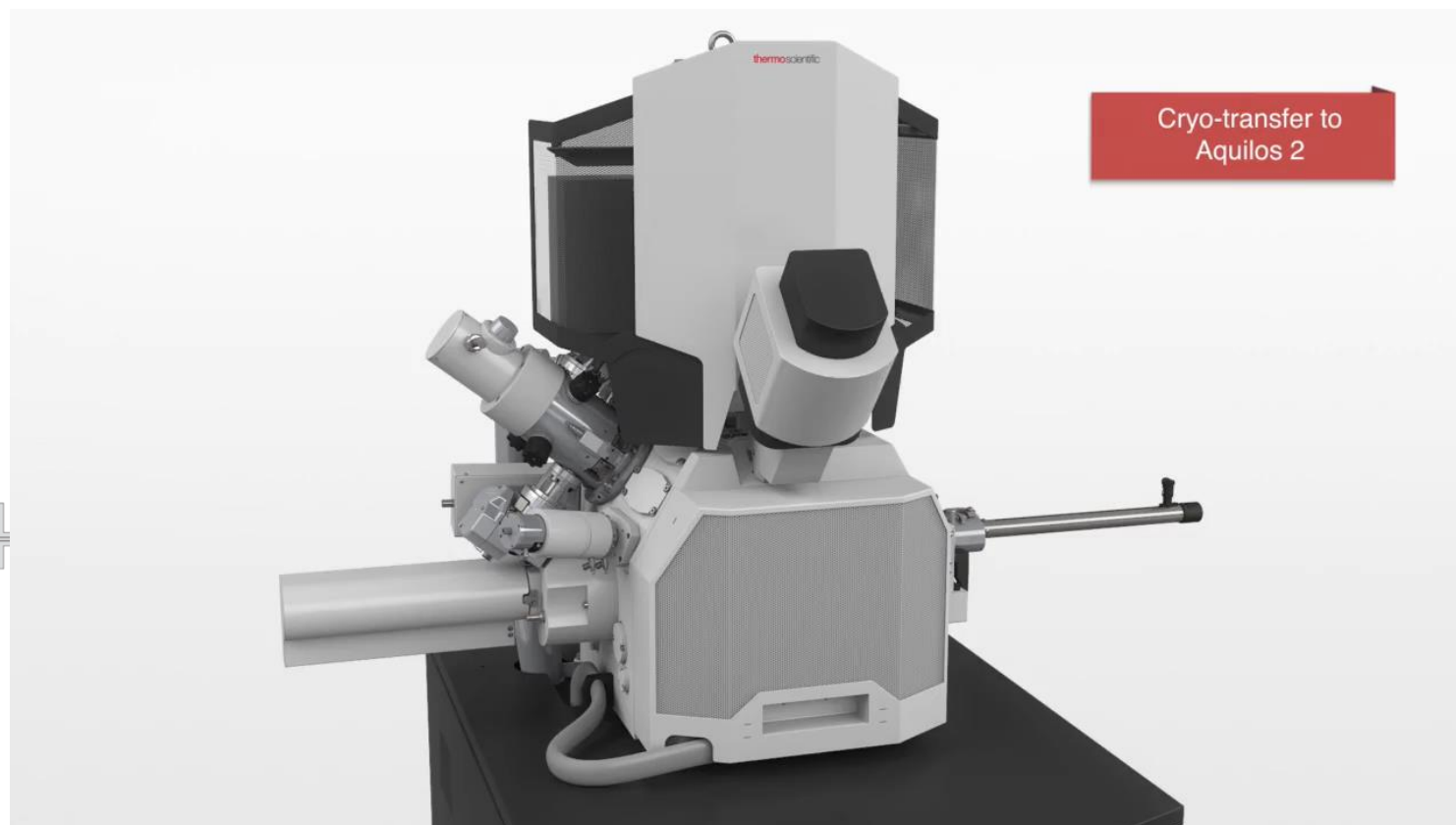
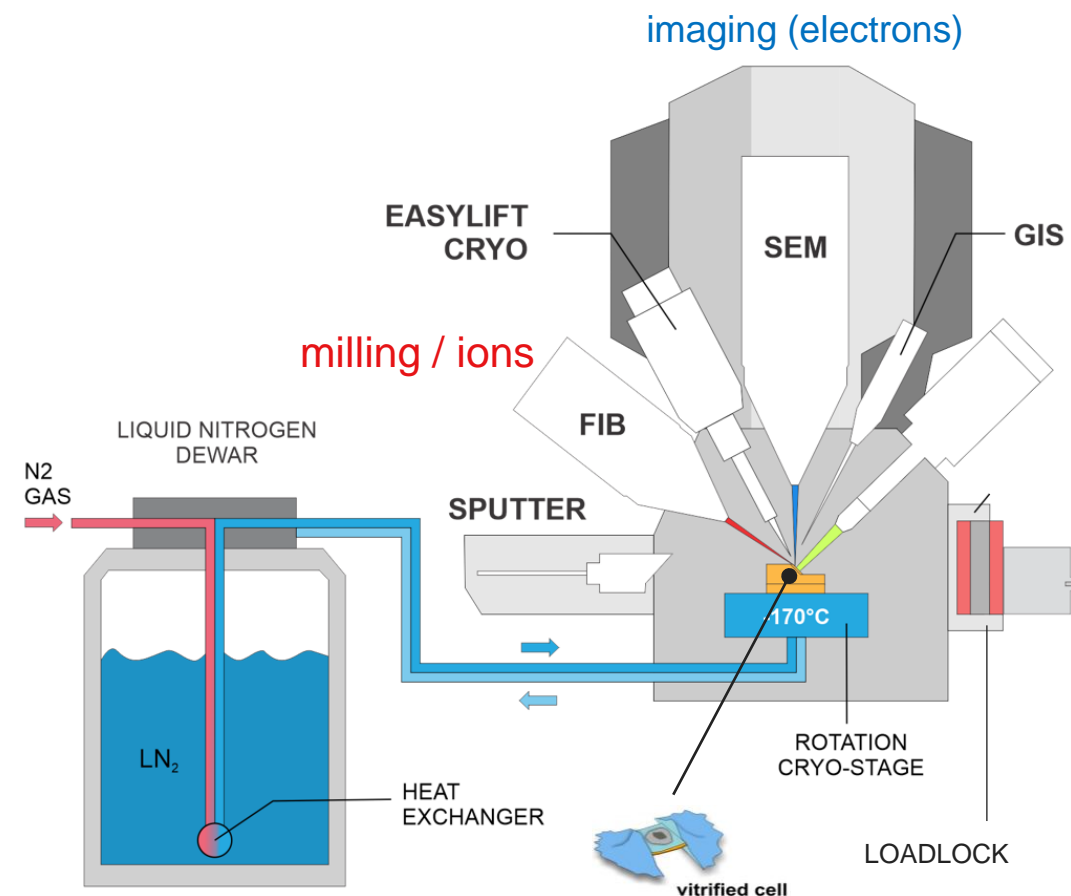
High-Pressure Freezing
($< 200 \mu\text{m}$)

Tomography can be done on different types of samples



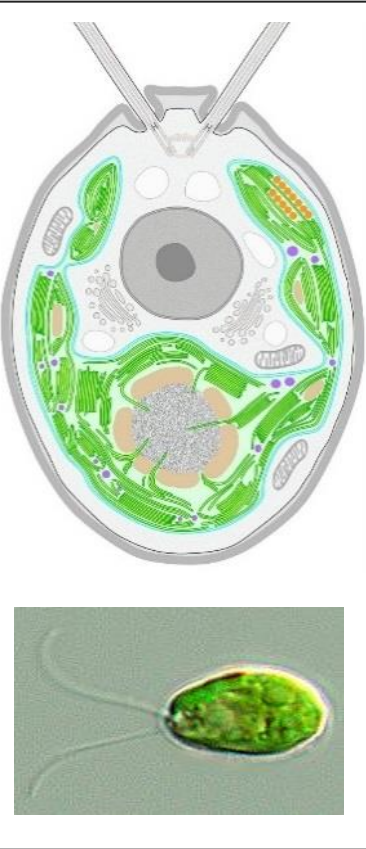
- **Difficulty in localizing the region of interest.**
- **Sample thinning.**

Thinning cells for tomography

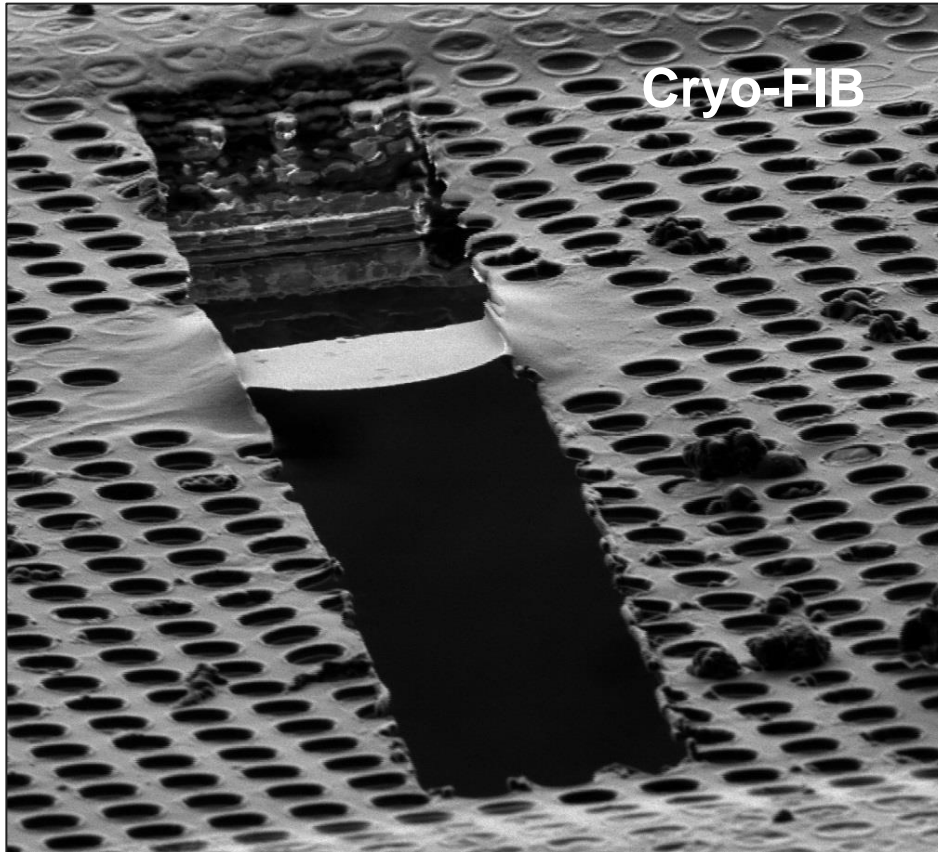


In situ lamella milling with the **Aquilos 2 Cryo-FIB**.

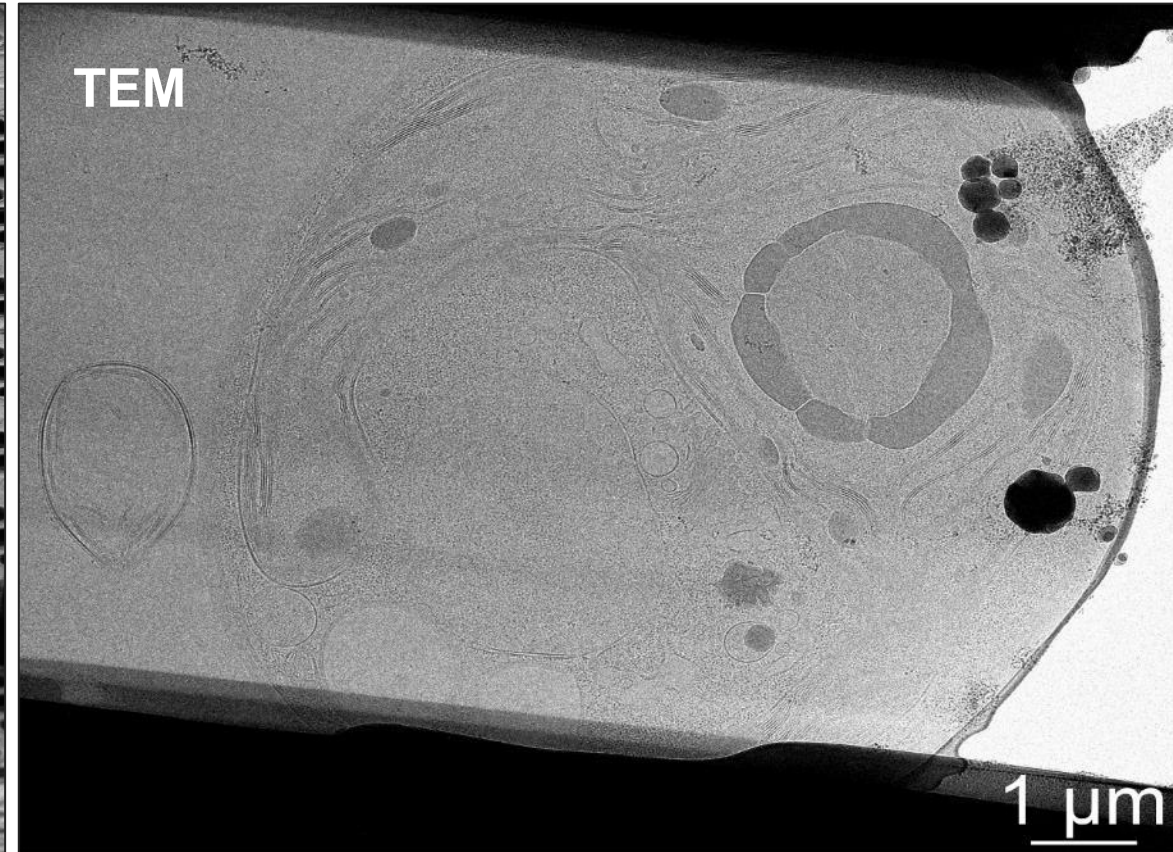
Cryo-lamella produced by *in-situ* cryo-FIB milling



model
organism

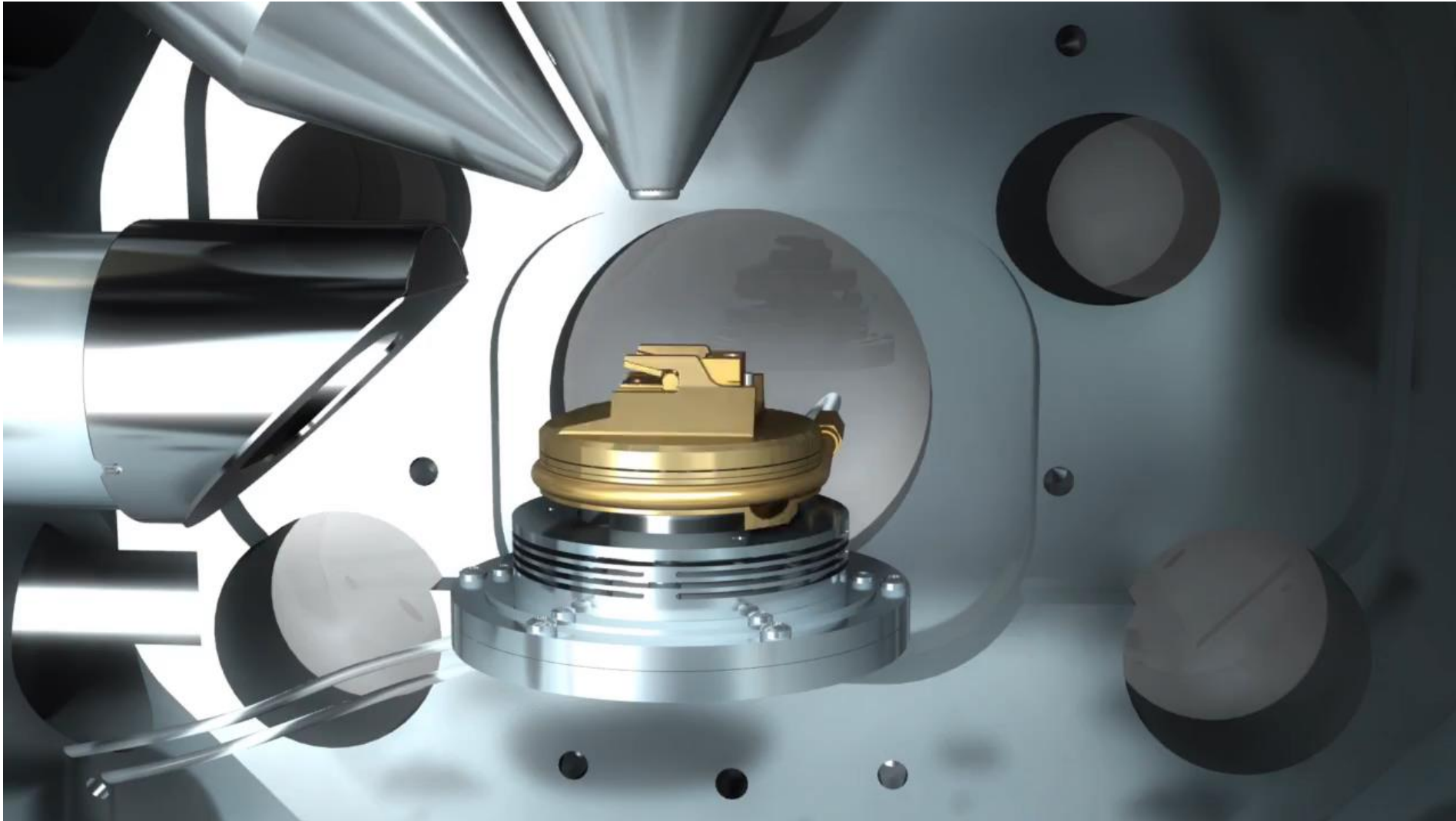


'On-the-grid' *in situ* lamella prepared
with the Aquilos Cryo-FIB.



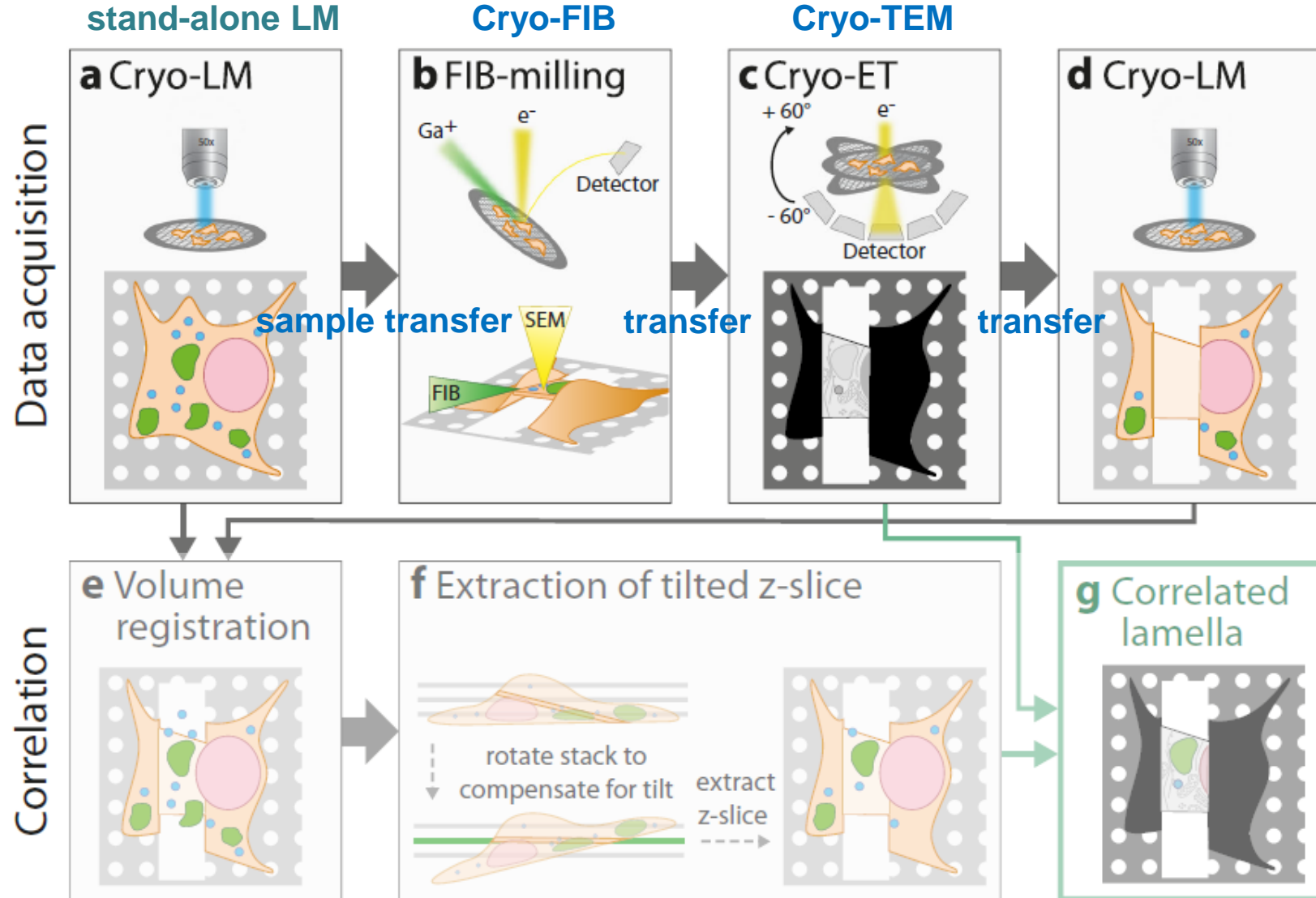
Cryo-TEM lamella overview image
of a FIB-thinned *Chlamydomonas* cell.

Data Courtesy Max Planck Institute of Biochemistry | *Miroslava Schaffer and Ben Engel.*



Cryo-tomograms = “snapshots of molecular landscapes inside the cells at the time of freezing”.*

Cryo-Correlative Microscopy (...how to identify the target in the cell volume)

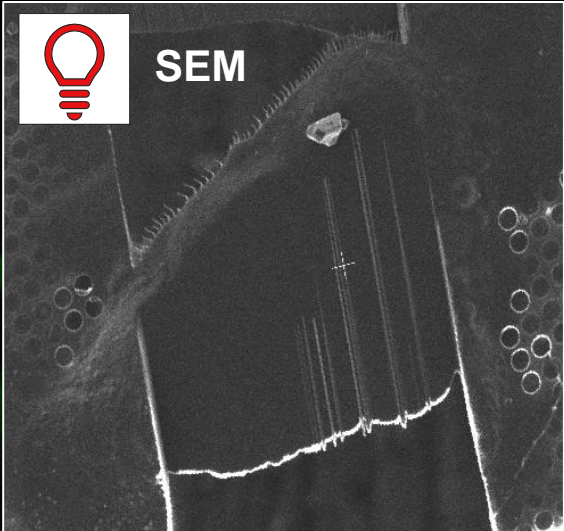
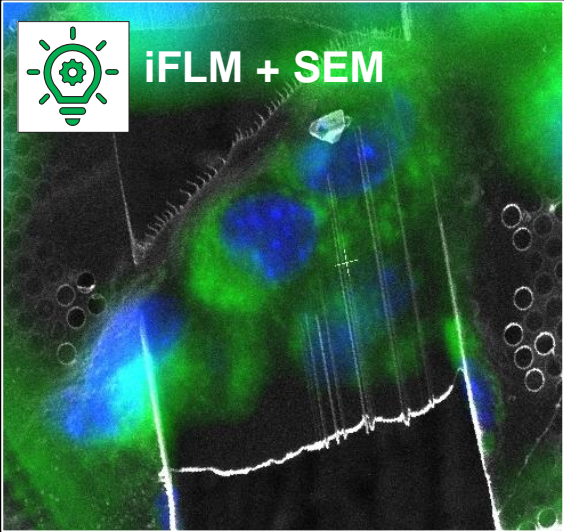
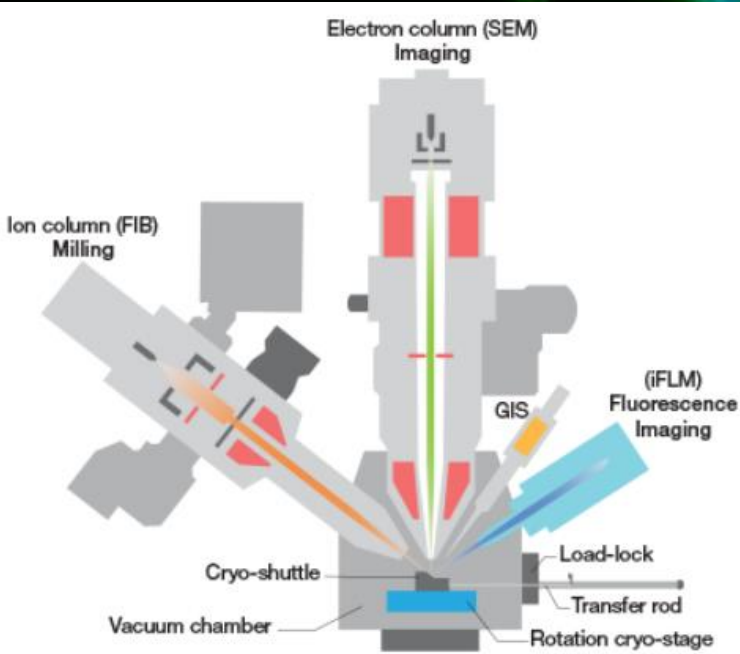


Example. Cryo-CLEM Tomography WF applied to reveal the membrane architecture of lamellar bodies.

- (“external”) FLM ~ ROI identification.
- Cryo-FIB ~ cell thinning for cryo-tomography.
- Cryo(TEM) Tomography ~ ROI architecture determination.

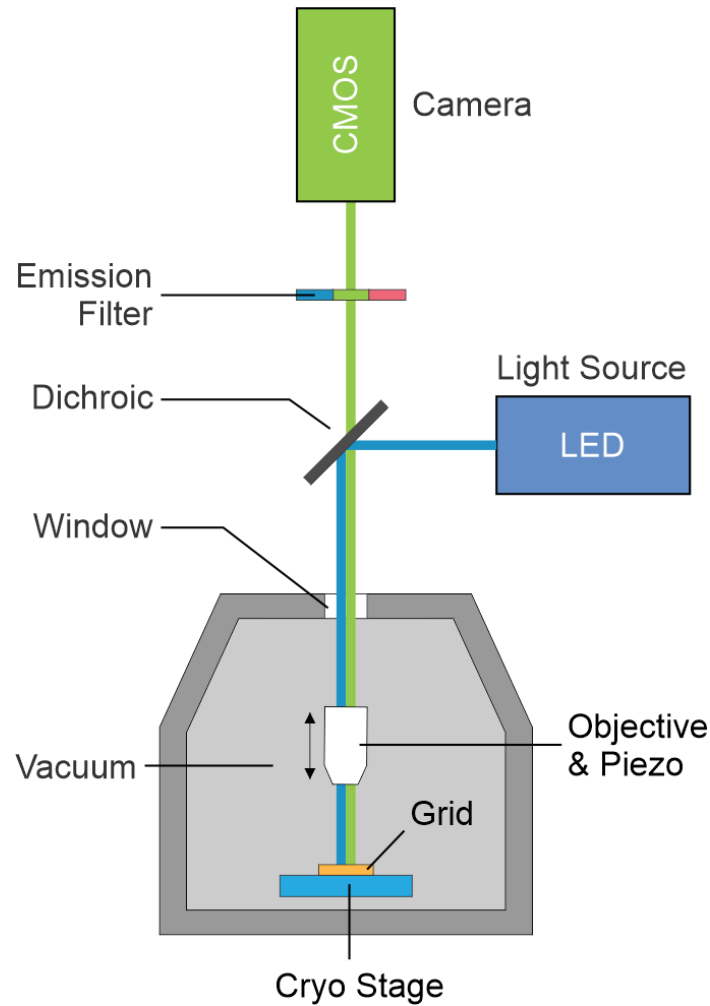
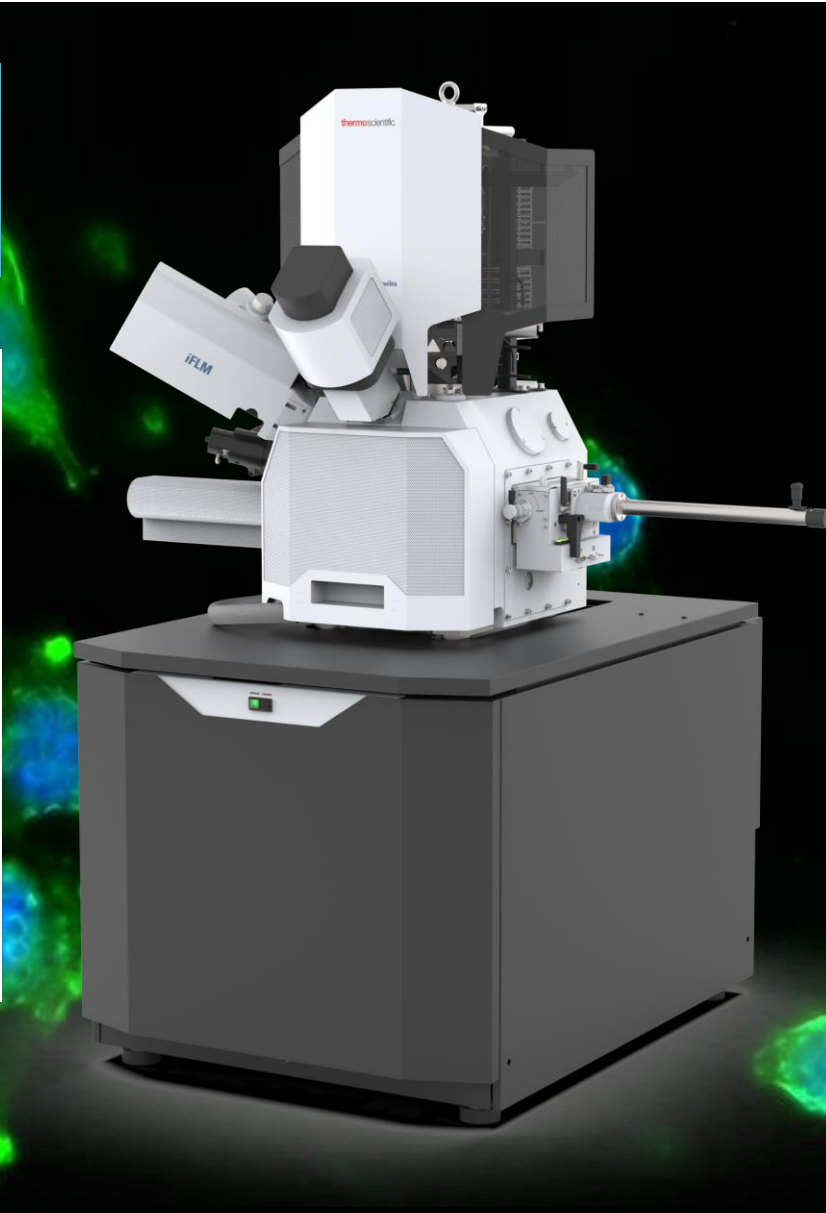
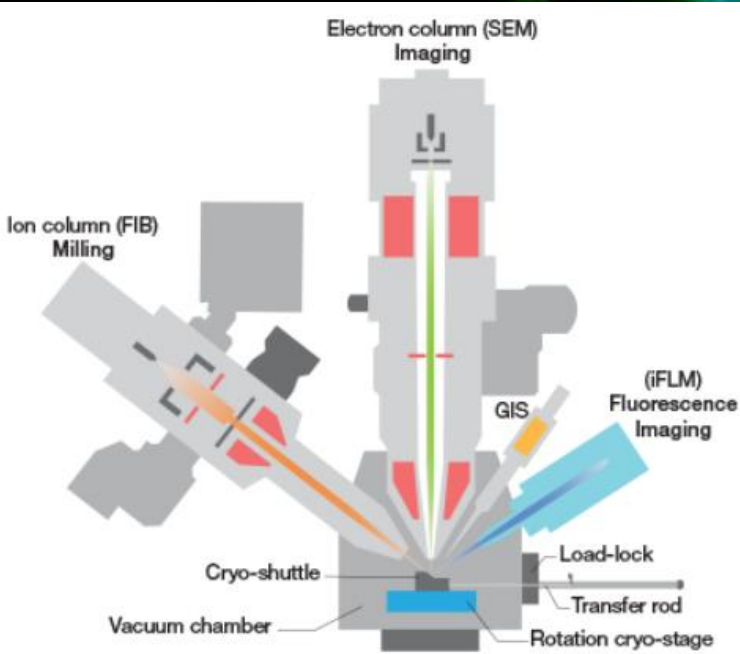
Cryo-FIB with integrated fluorescence light microscope

Aquilos 2 with Integrated
Fluorescence Light
Microscope (iFLM)

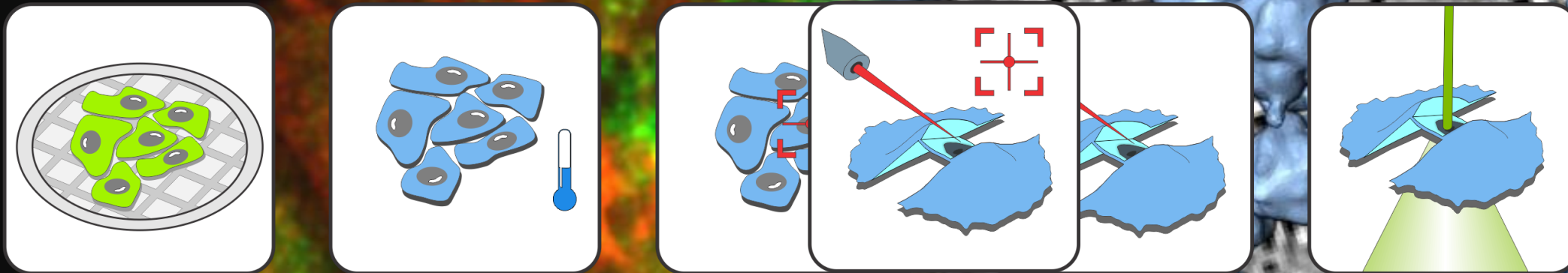


Cryo-FIB with integrated fluorescence light microscope

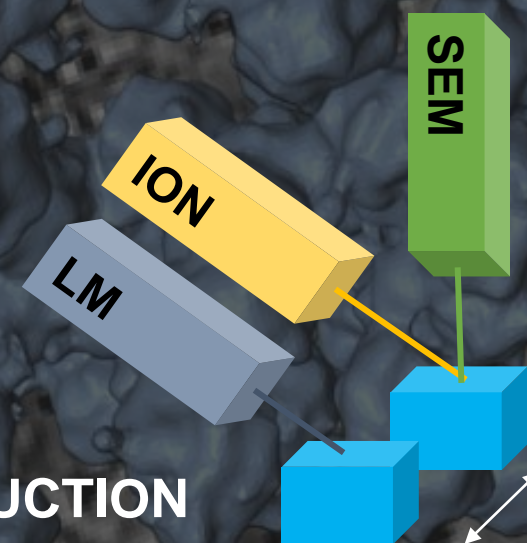
Aquilos 2 with Integrated
Fluorescence Light
Microscope (iFLM)

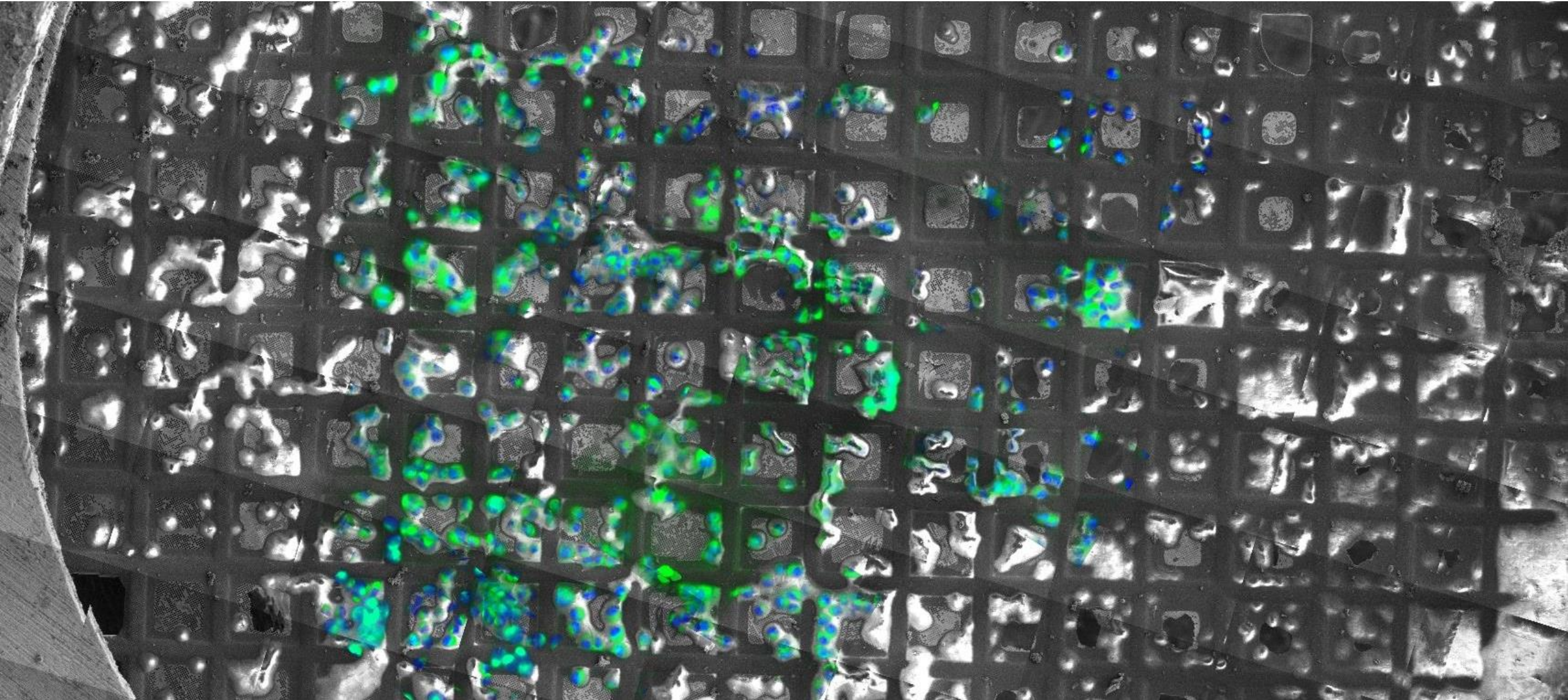


Cryo-Correlative Microscopy with iFLM



- ✓ **ELIMINATING ONE SAMPLE TRANSFER STEP**
REDUCE TRANSFER CONTAMINATION RISKS
- ✓ **SIMPLYFYING LIGHT-TO-ELECTRON CORRELATION**
CORRELATE TWO IMAGING MODALITIES WITHIN ONE SYSTEM
- ✓ **FASTER RETRIEVAL OF REGIONS OF INTEREST**
HIGHER THROUGHPUT
- ✓ **MONITORING AND VALIDATION OF LAMELLA PRODUCTION**
'CHECK-BACK' OPTION DURING LAMELLA MILLING AND TARGETING

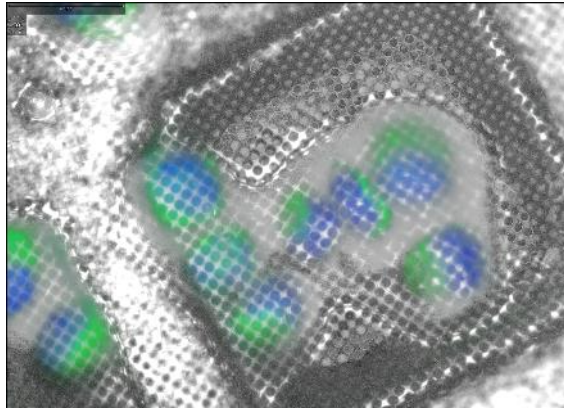




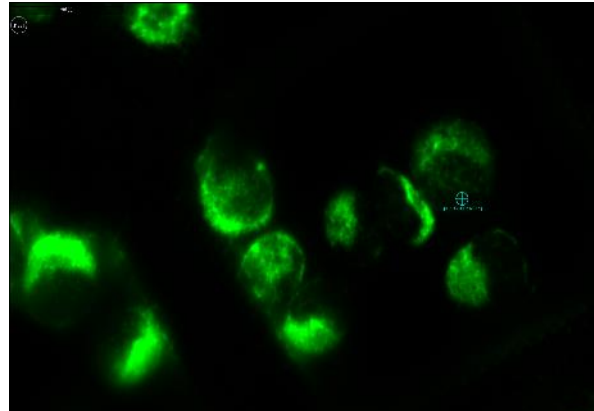
CHO cells on TEM grid, blue – nuclei, green: mitochondria. | Sample prepared by J. Moravcova, Ceitec MU.

iFLM | Reflection and Fluorescence

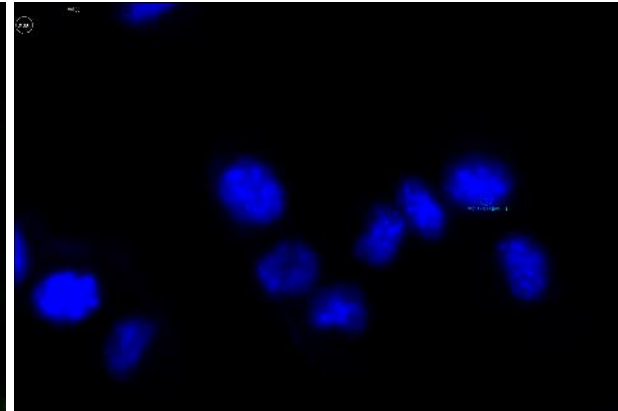
Reflection



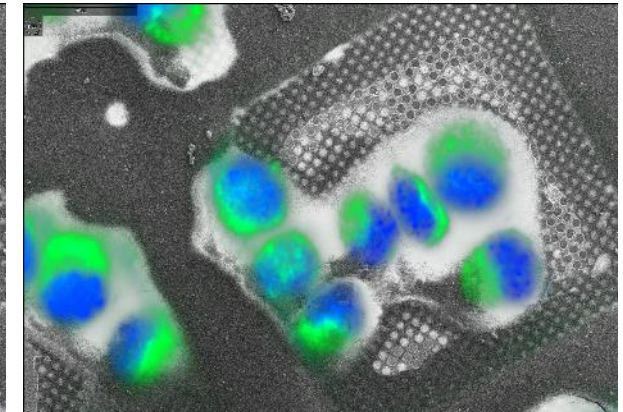
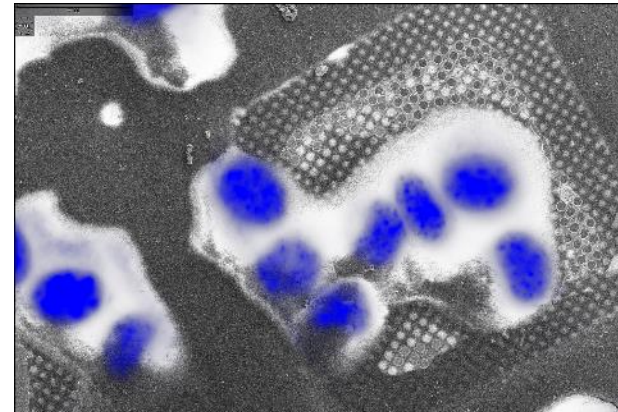
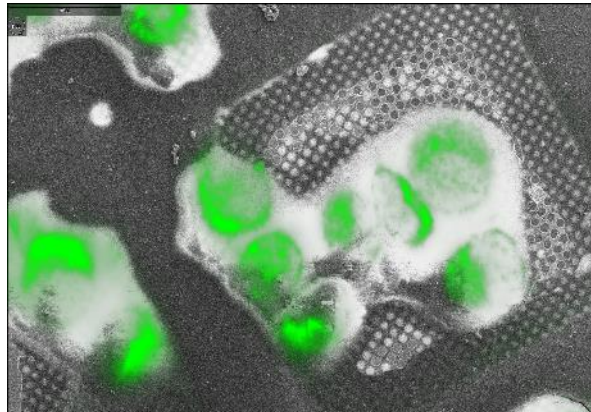
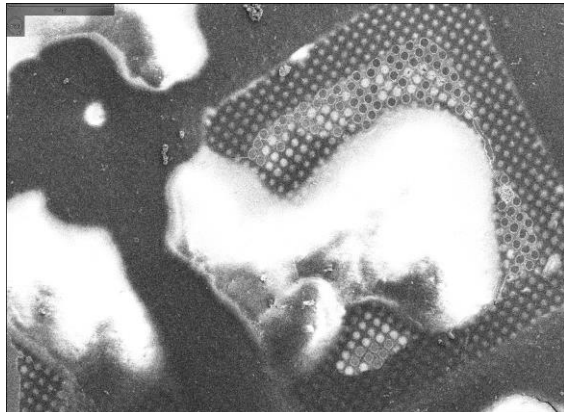
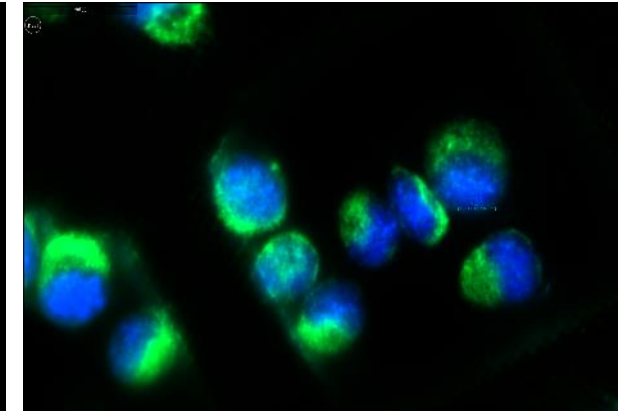
Green



Blue

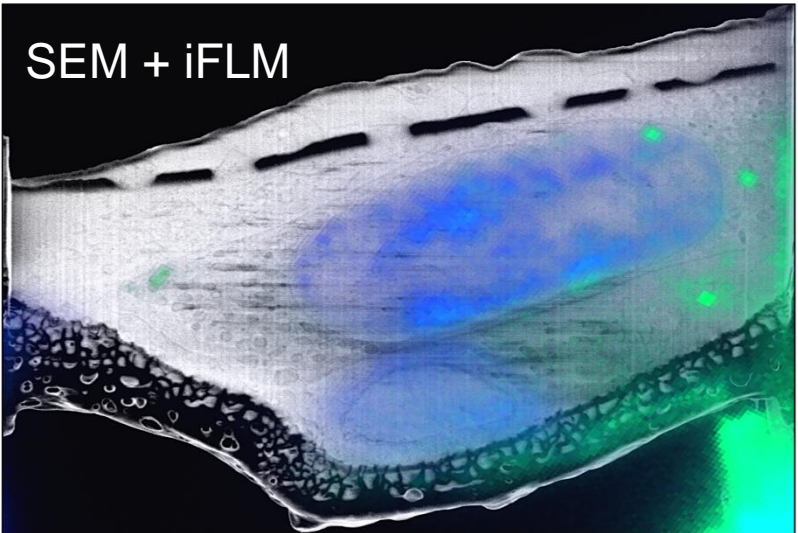
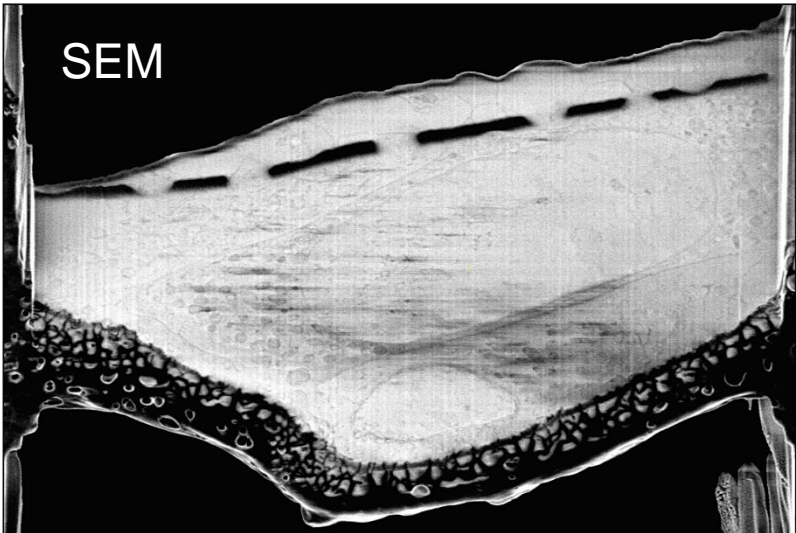
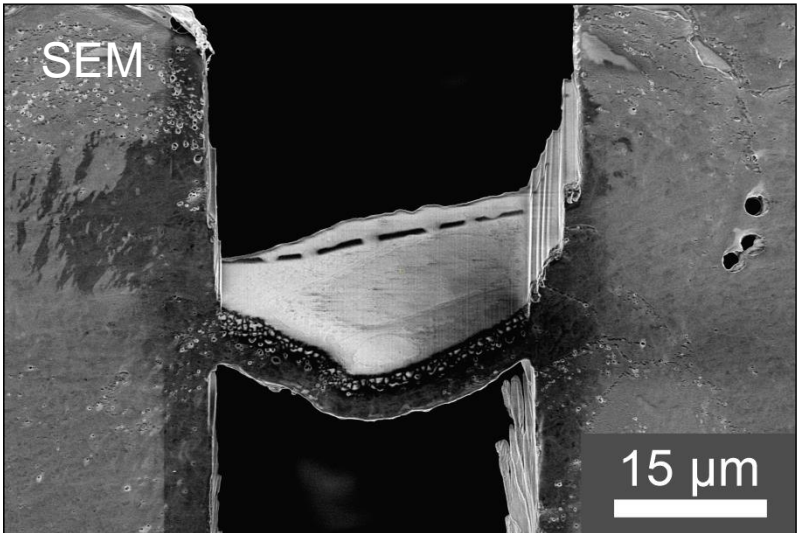
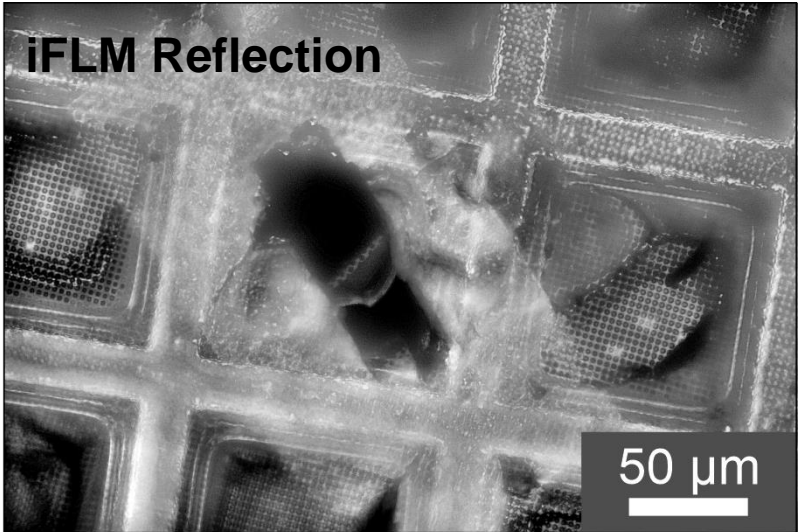
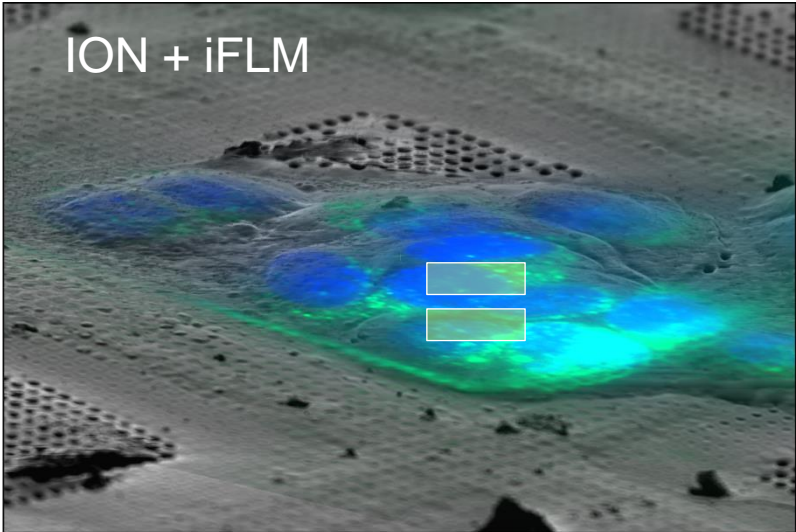
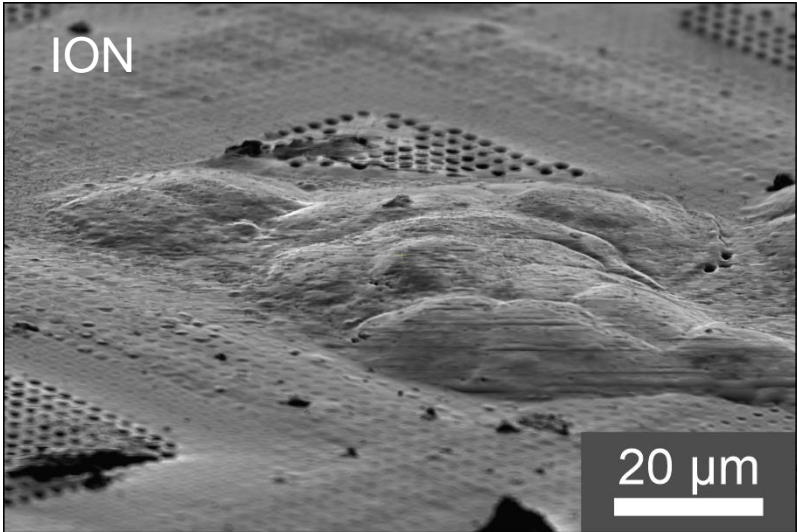


Composite

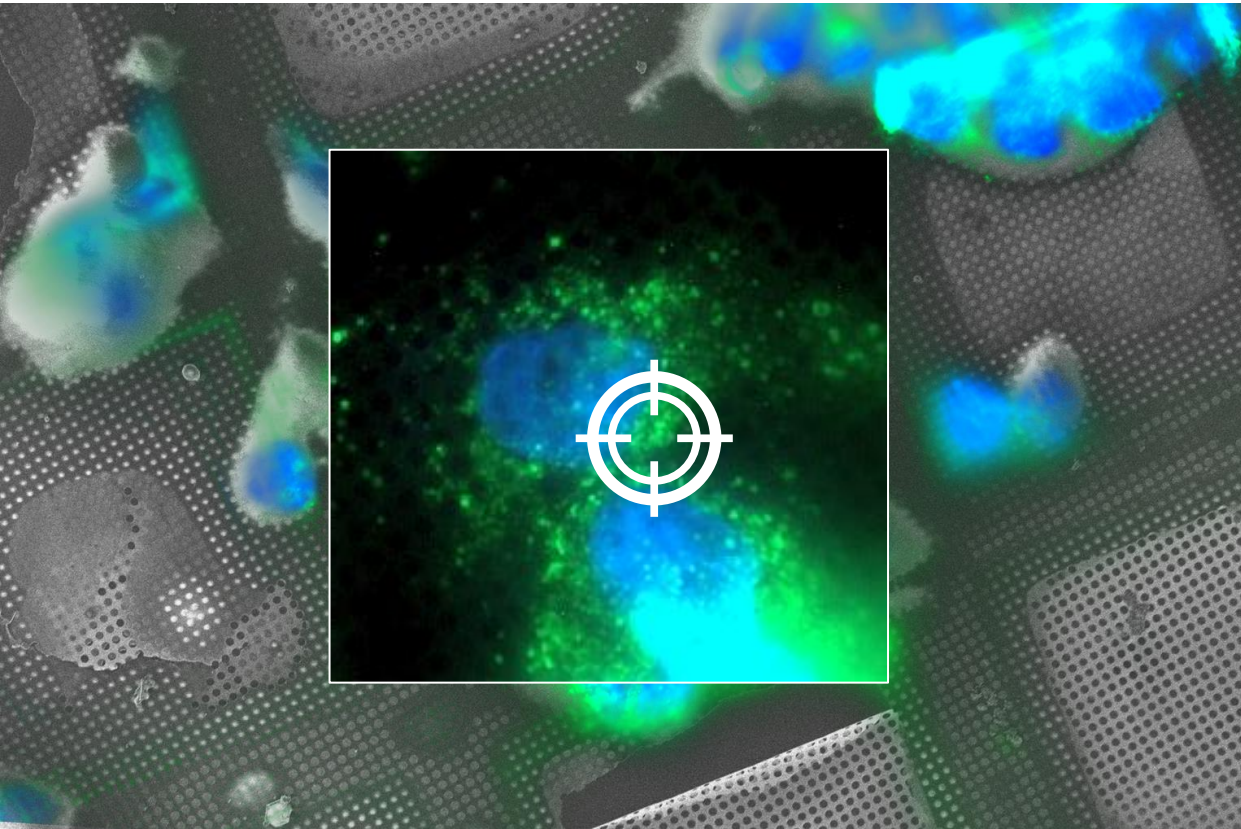


Upper row – reflection and fluorescence images.
Lower row – SEM images with overlaid fluorescence.

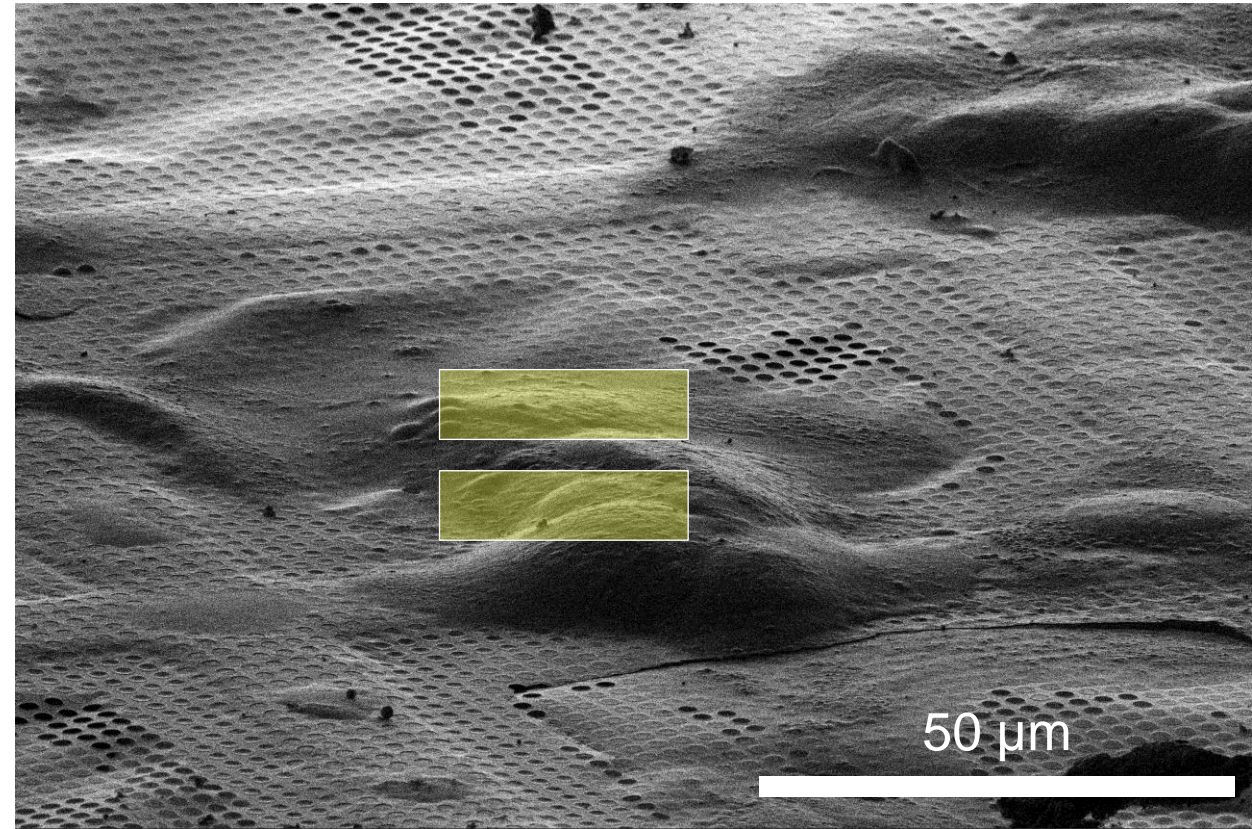
iFLM | Application examples



iFLM guided lamella milling | an example (...put all the pieces together)

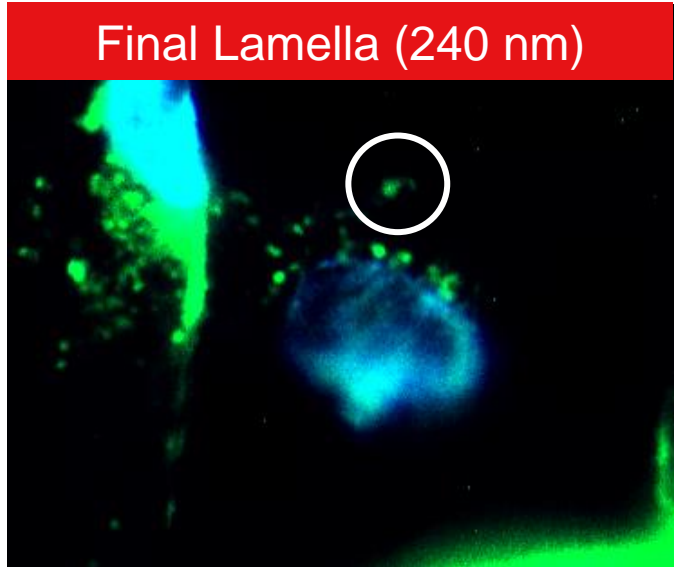
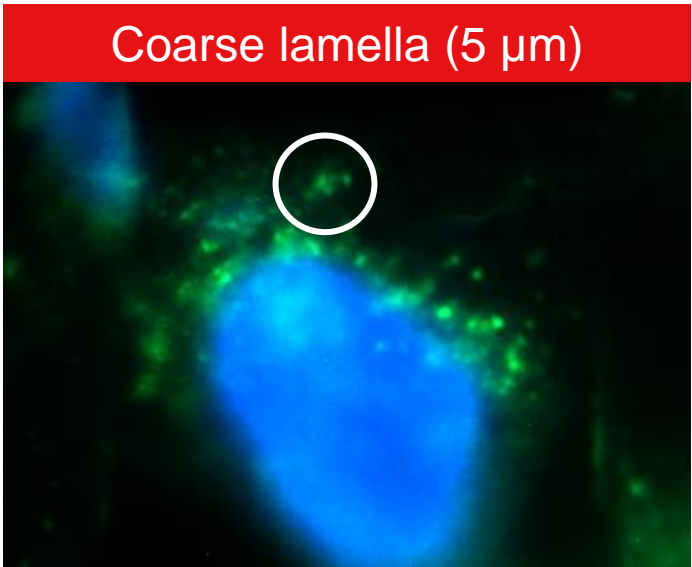
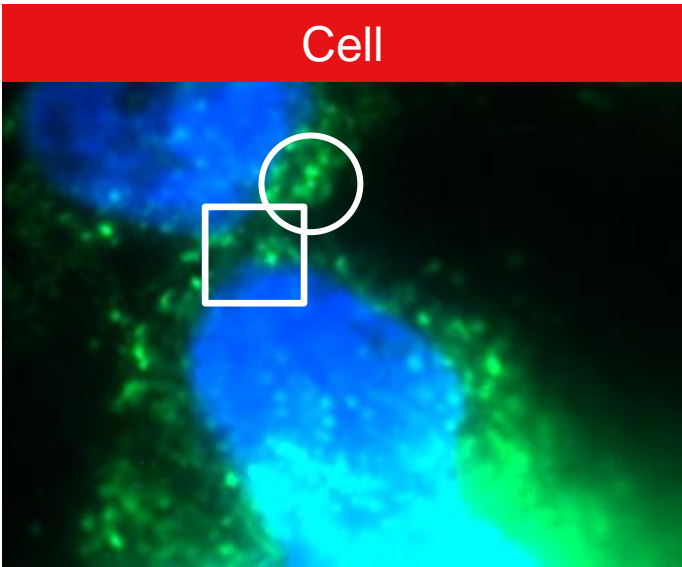
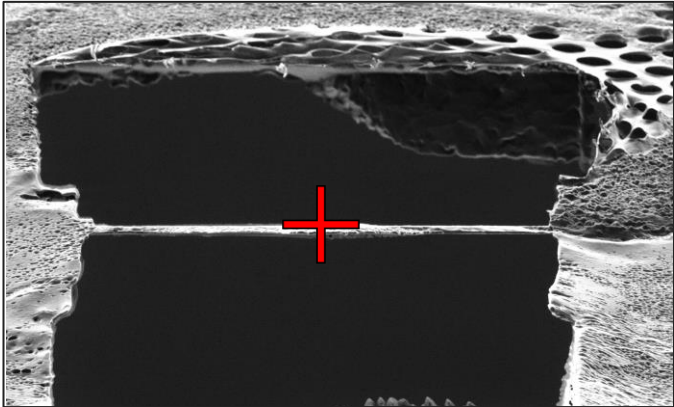
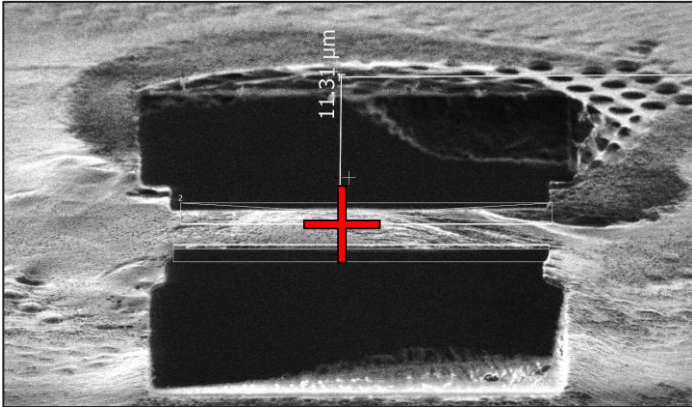
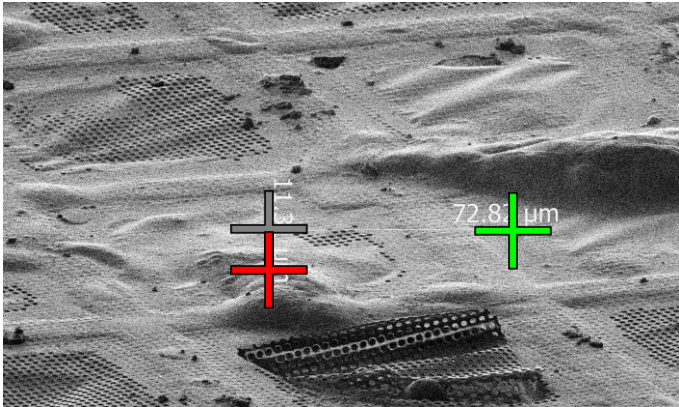


SEM
(top view)

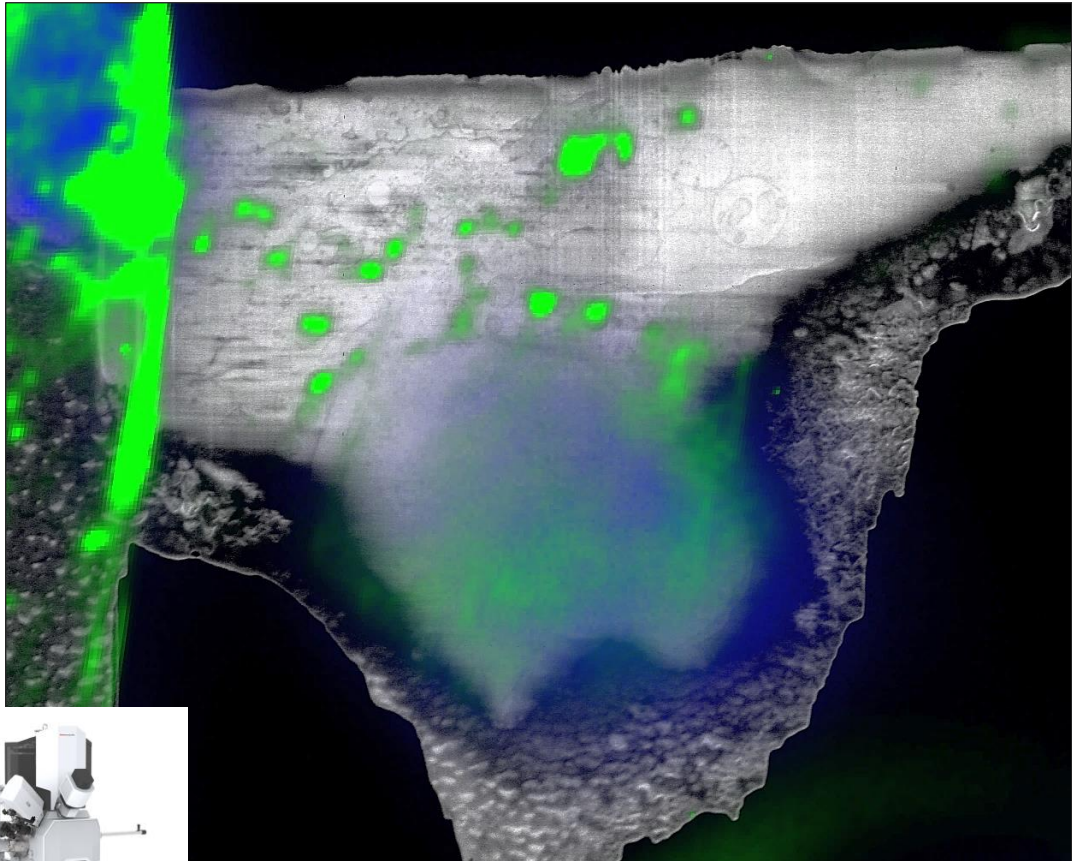


ION
(Tilted view)

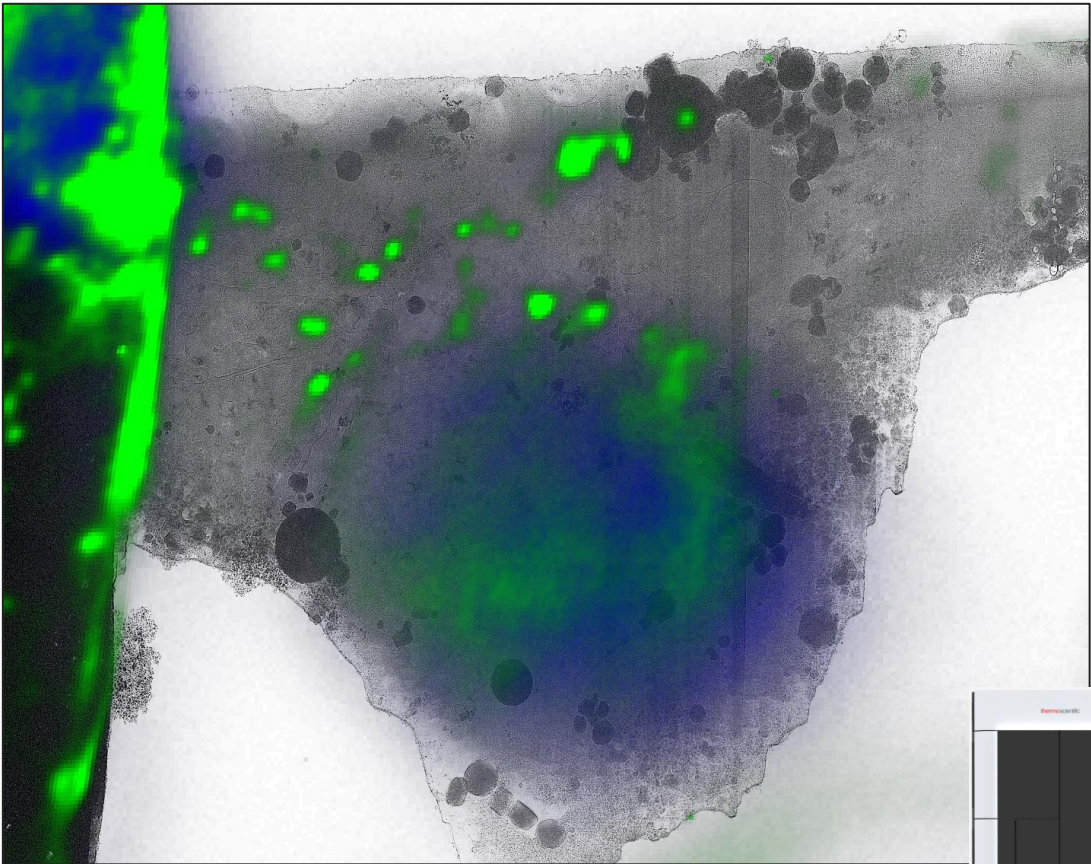
iFLM guided lamella milling check back



Final cryo-lamella fluorescence overlay



SEM



TEM

Thank you

