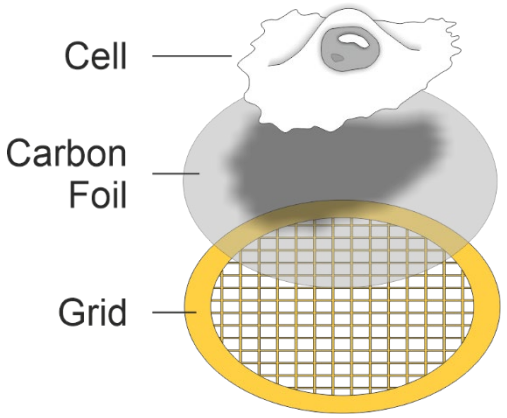
ThermoFisher SCIENTIFIC

Cryo-ET Sample Prep: Defining, optimizing, and creating ideal lamellae

Geoff Perumal
Sr. Sales Development Rep., Electron Microscopy

The world leader in serving science

From cell culture to subcellular 3D data

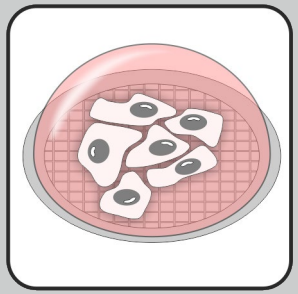


Vitrobot
(Plunge-Freezing)

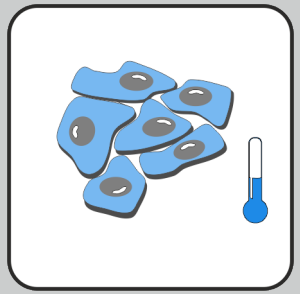
Or High Pressure Freezer
(HPF-Freezing)

Aquilos/Hydra/Arctis
Cryo-FIB

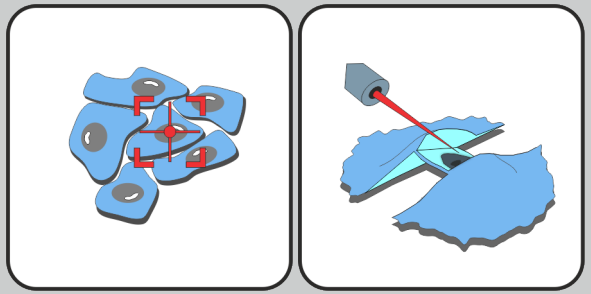
Krios
Cryo-TEM



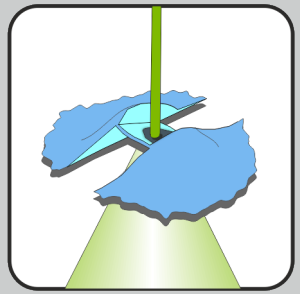
Cell Culture



Vitrification



Lamella Making
+ Correlative LM Targeting



Tomography

Analysis
Sub-Tomo Averaging
3D Visualization

Range of samples accessible with tomography

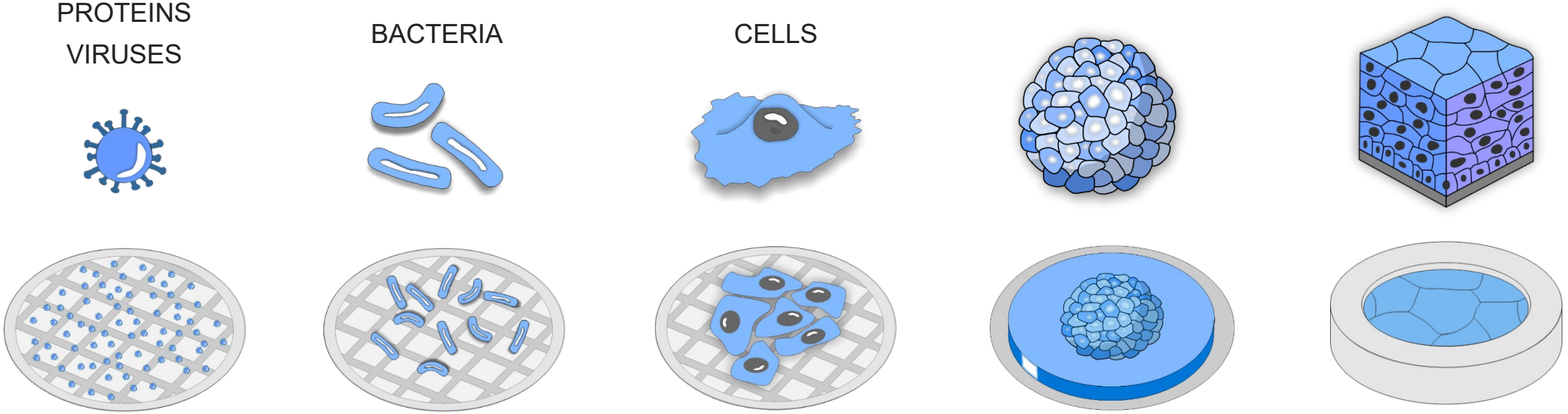
PROTEINS
VIRUSES

BACTERIA

CELLS

ORGANOIDS
TISSUE CELLS

BULK TISSUE
SMALL ORGANISMS



IN SITU LAMELLA MILLING — WAFFLE LAMELLA METHOD — CRYO-LIFT-OUT LAMELLA

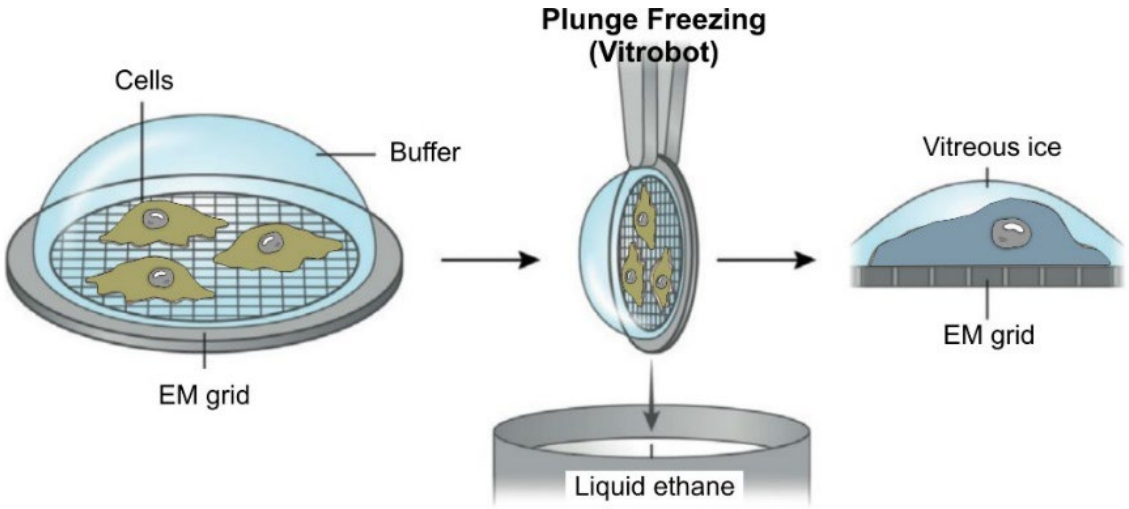
Plunging (5-10 µm)

High-Pressure-Freezing (> 200 µm)

CRYO-FIB MILLING

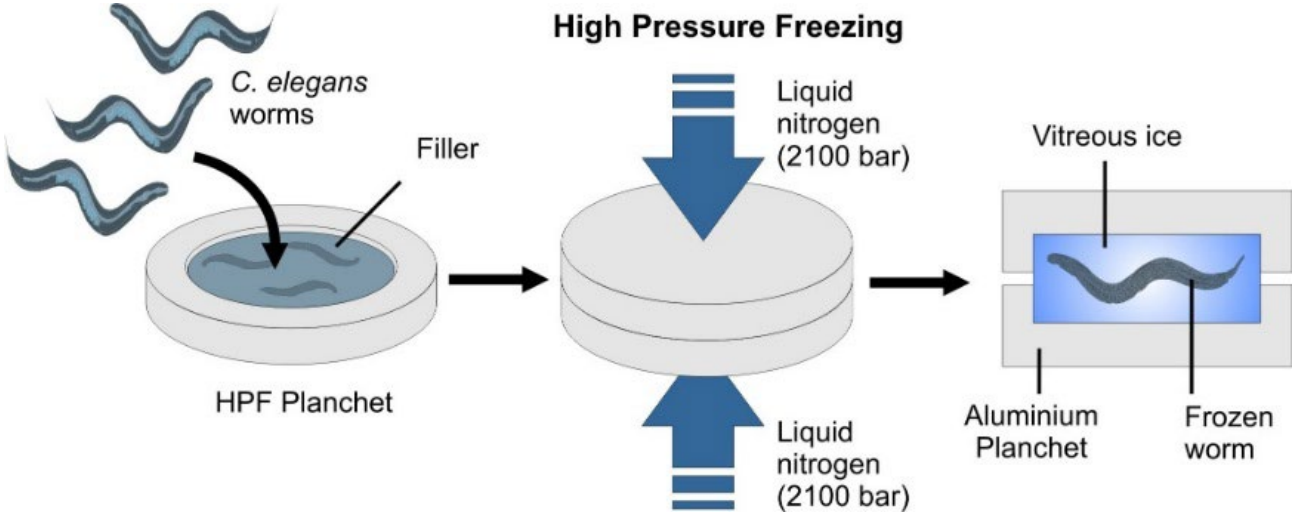
Sample vitrification methods

Proteins or Cells



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

Small organisms or tissue biopsies

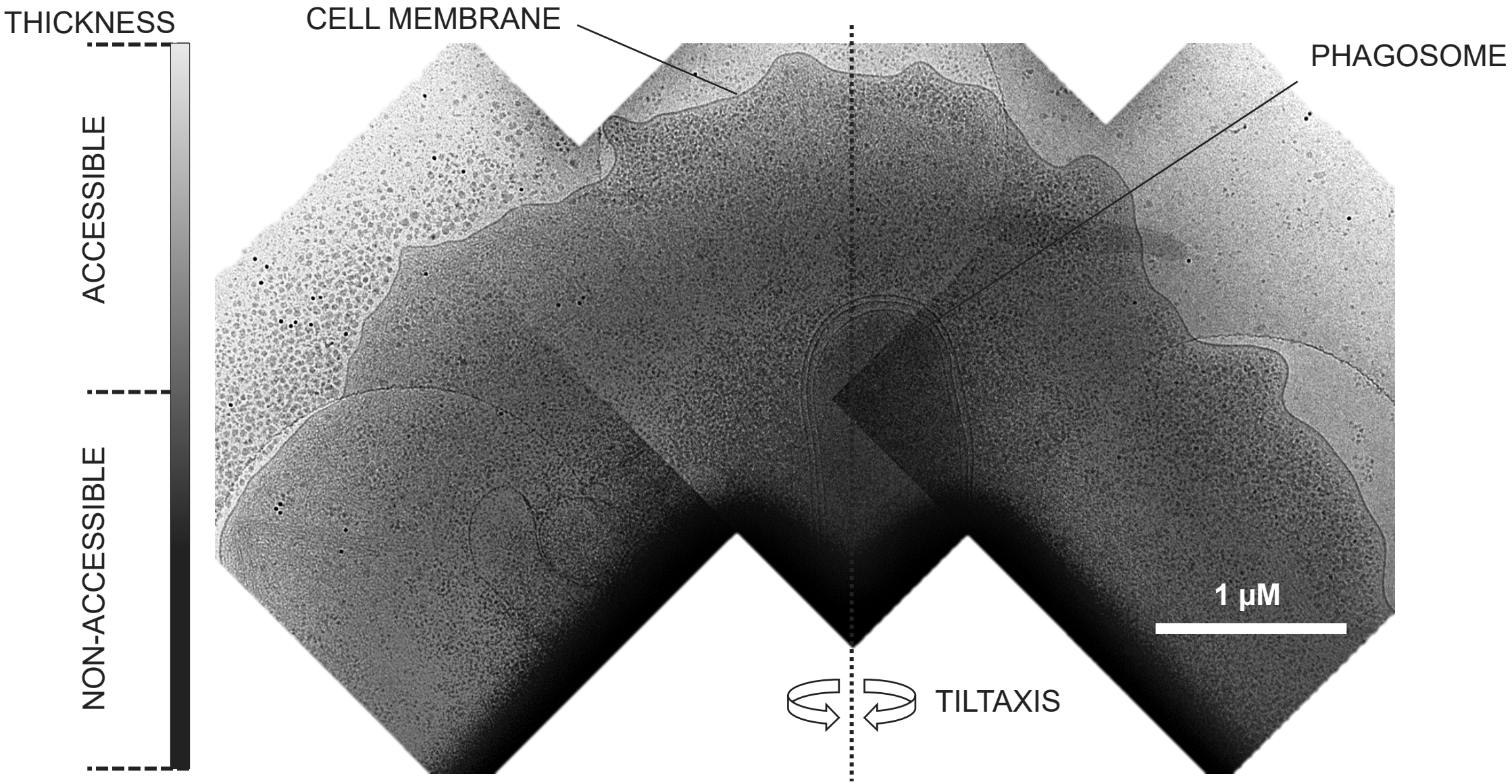


Thin-film vitrification
(< 5 µm)

High-pressure freezing
(< 200 µm)

Cell preparation

Rationale for cryo-FIB thinning

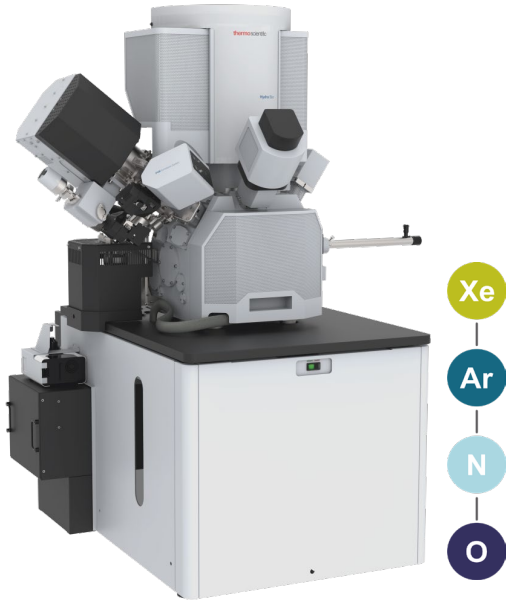


Cryo-FIB-SEM Systems for Life Sciences

Cryo-EM and **Volume-EM** applications

AQUILOS 2 Cryo-FIB

Pioneering Cryo-Lamella Prep



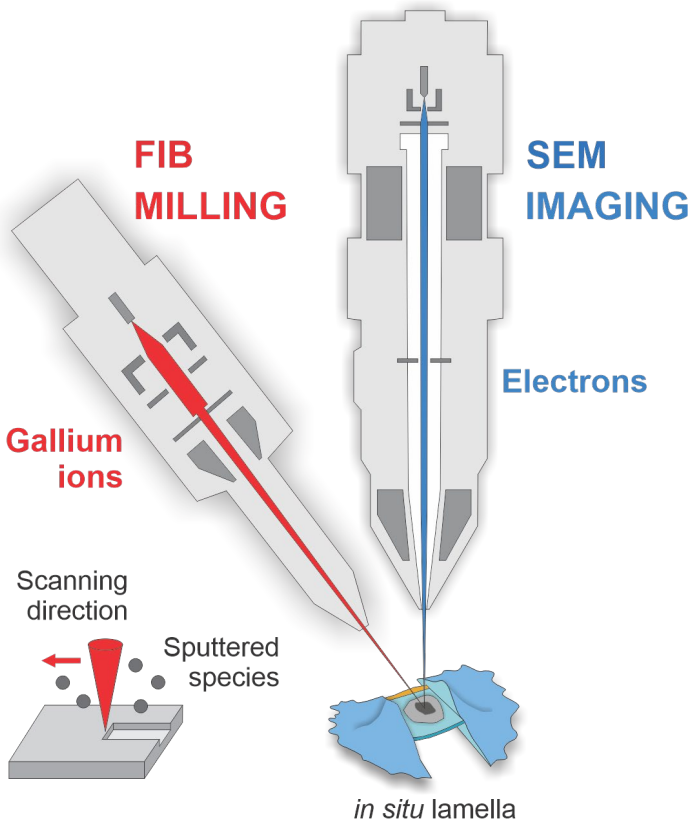
HYDRA Bio Cryo-PFIB

Multi-application Versatility

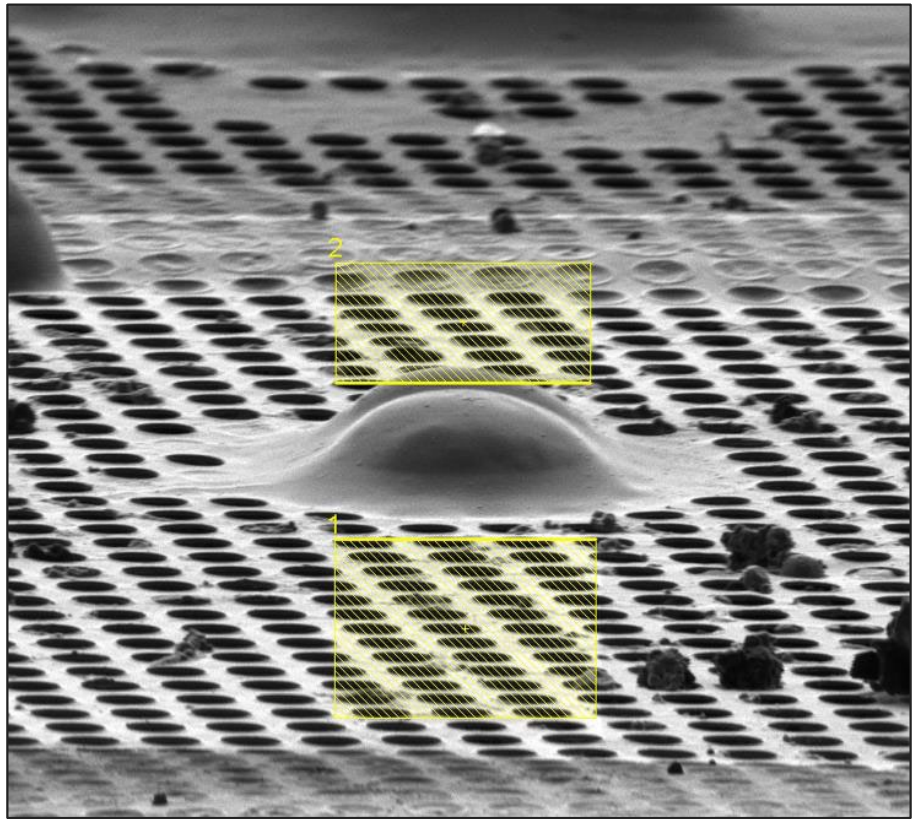
ARCTIS Cryo-PFIB

Automation & Connectivity

FIB-SEM Principle

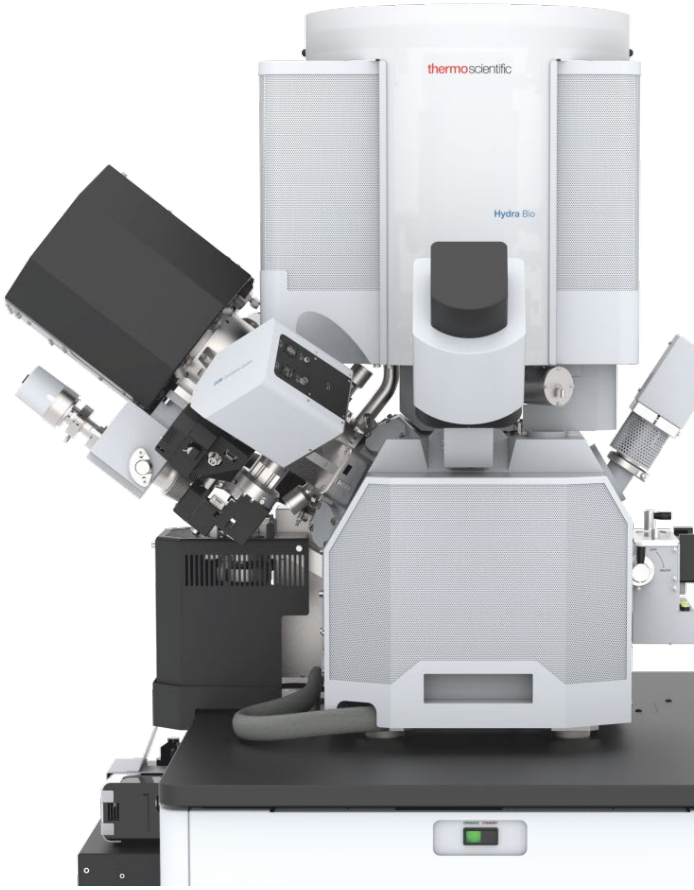


FIB-SEM Principle



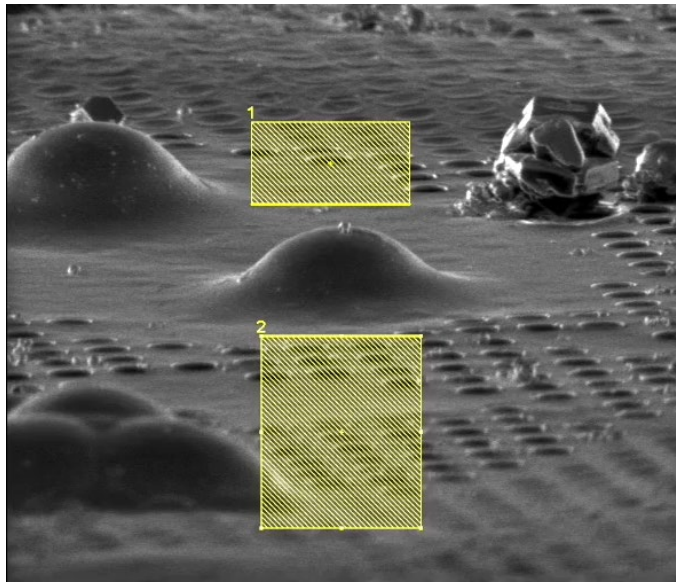
'On-the-grid' *in situ* lamella preparation by Cryo-FIB

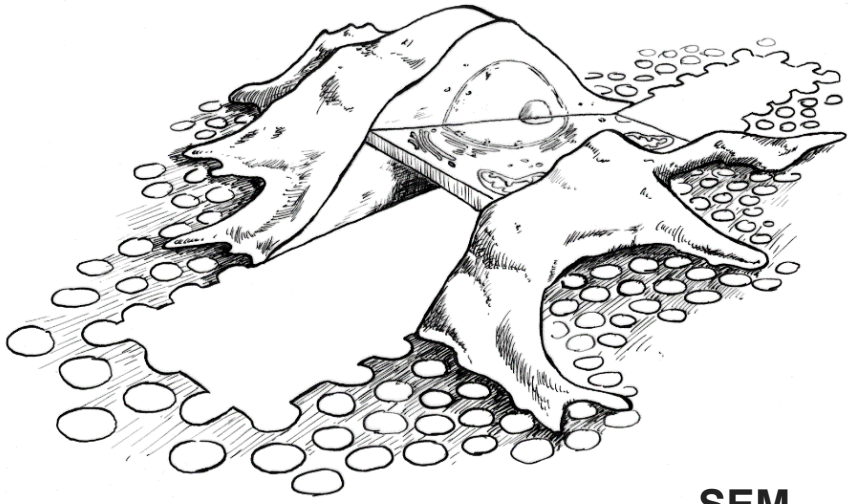
Data Courtesy Max Planck Institute of Biochemistry
(*Miroslava Schaffer and Ben Engel)*



FIB-SEM Microscope

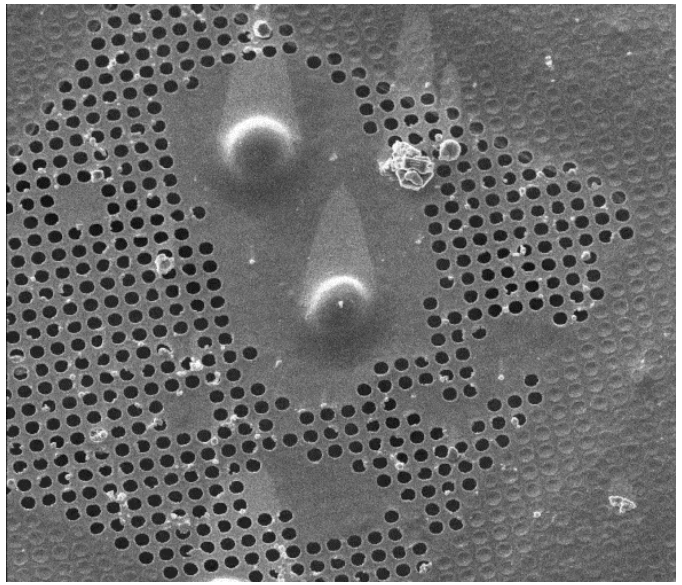
Producing thin cryo-lamellae from whole cells





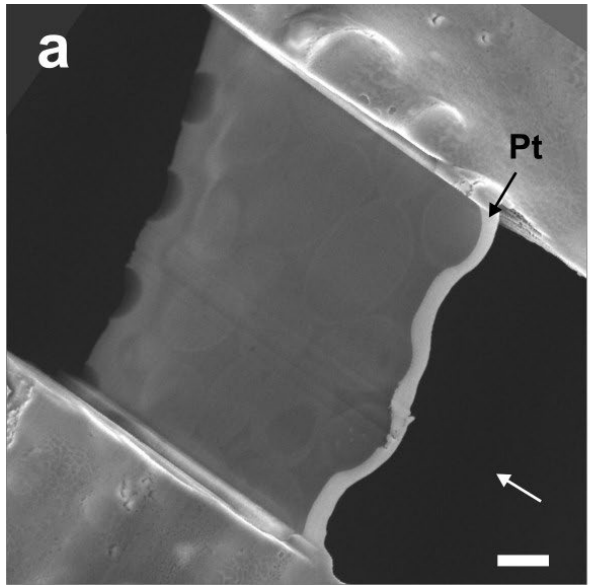
© Juergen Plitzko

'On-the-grid' *in situ* lamella preparation by Cryo-FIB

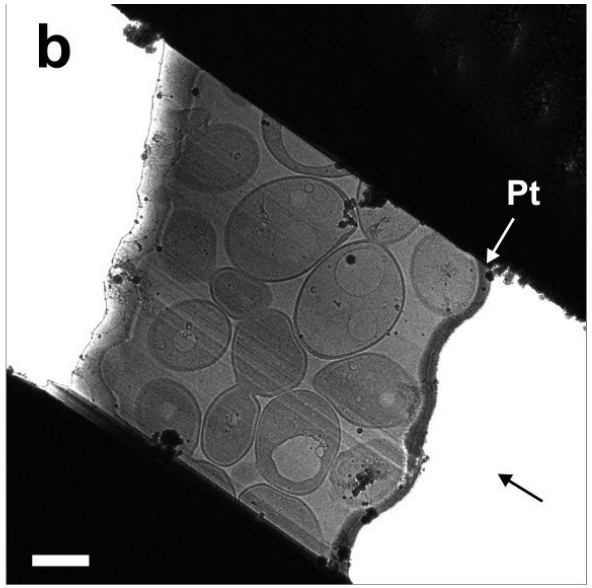


Data Courtesy
Max Planck Institute of Biochemistry
(*M.Schaffer)*

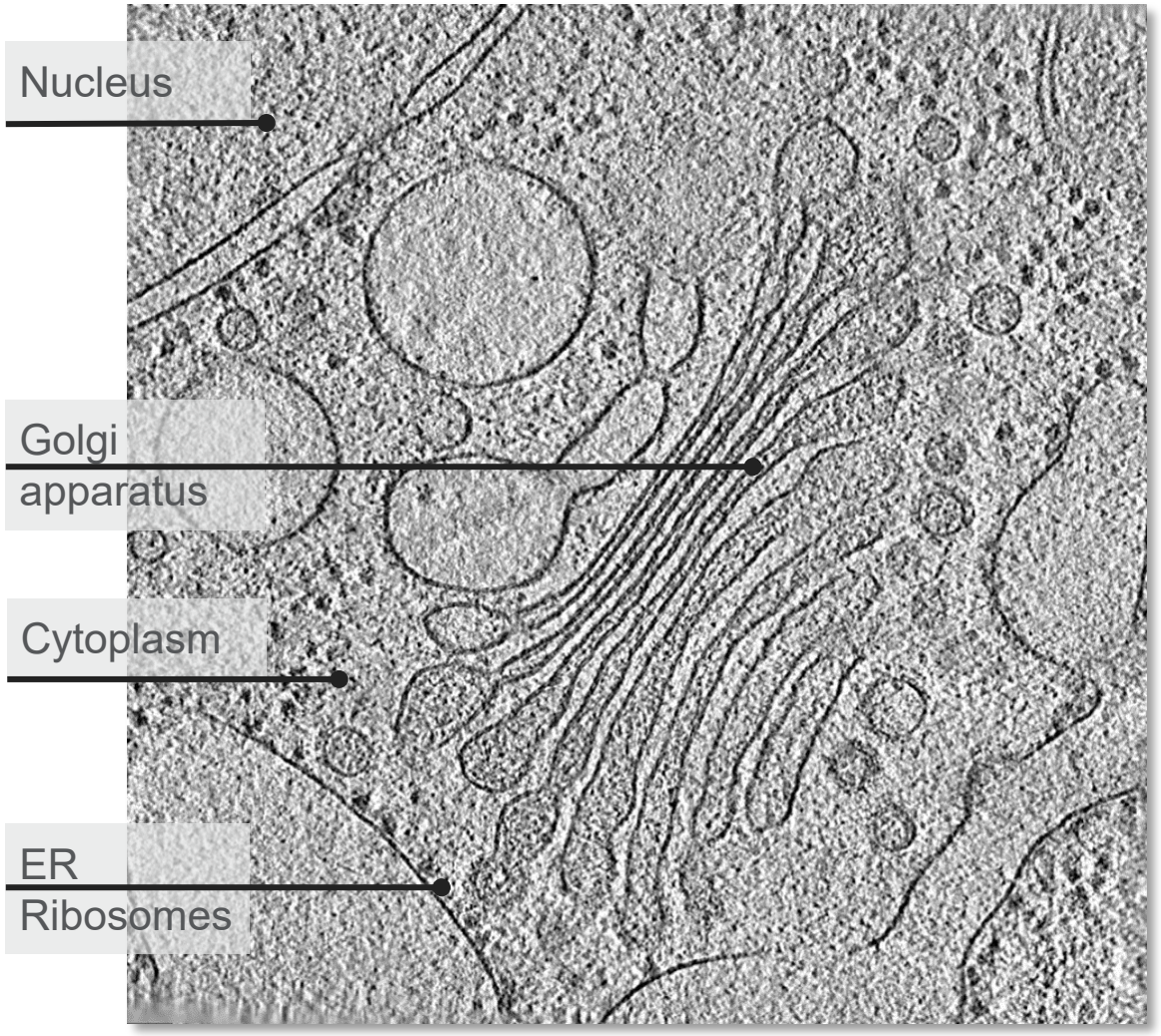
SEM



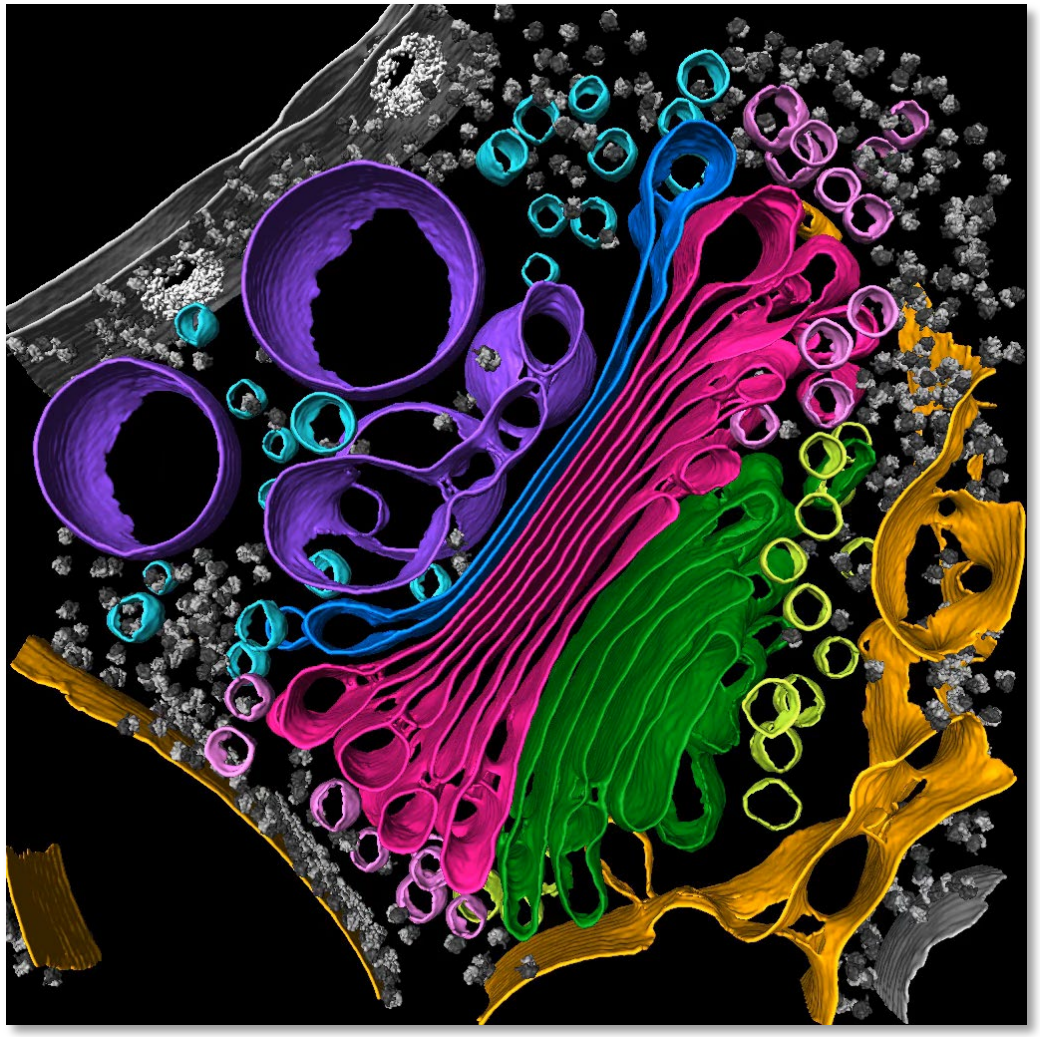
TEM



What image data does tomography provide?



TOMOGRAM



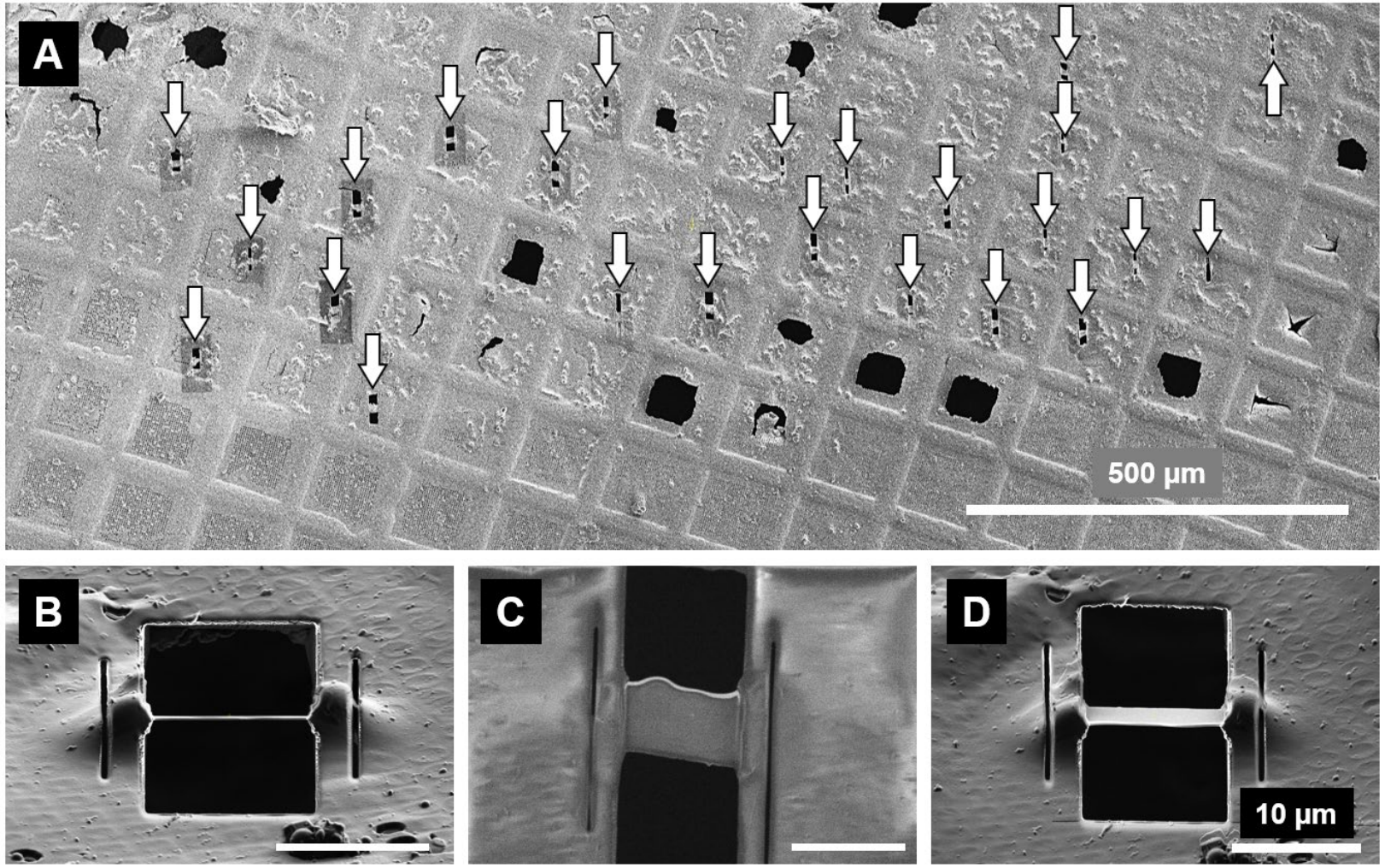
VISUALIZATION

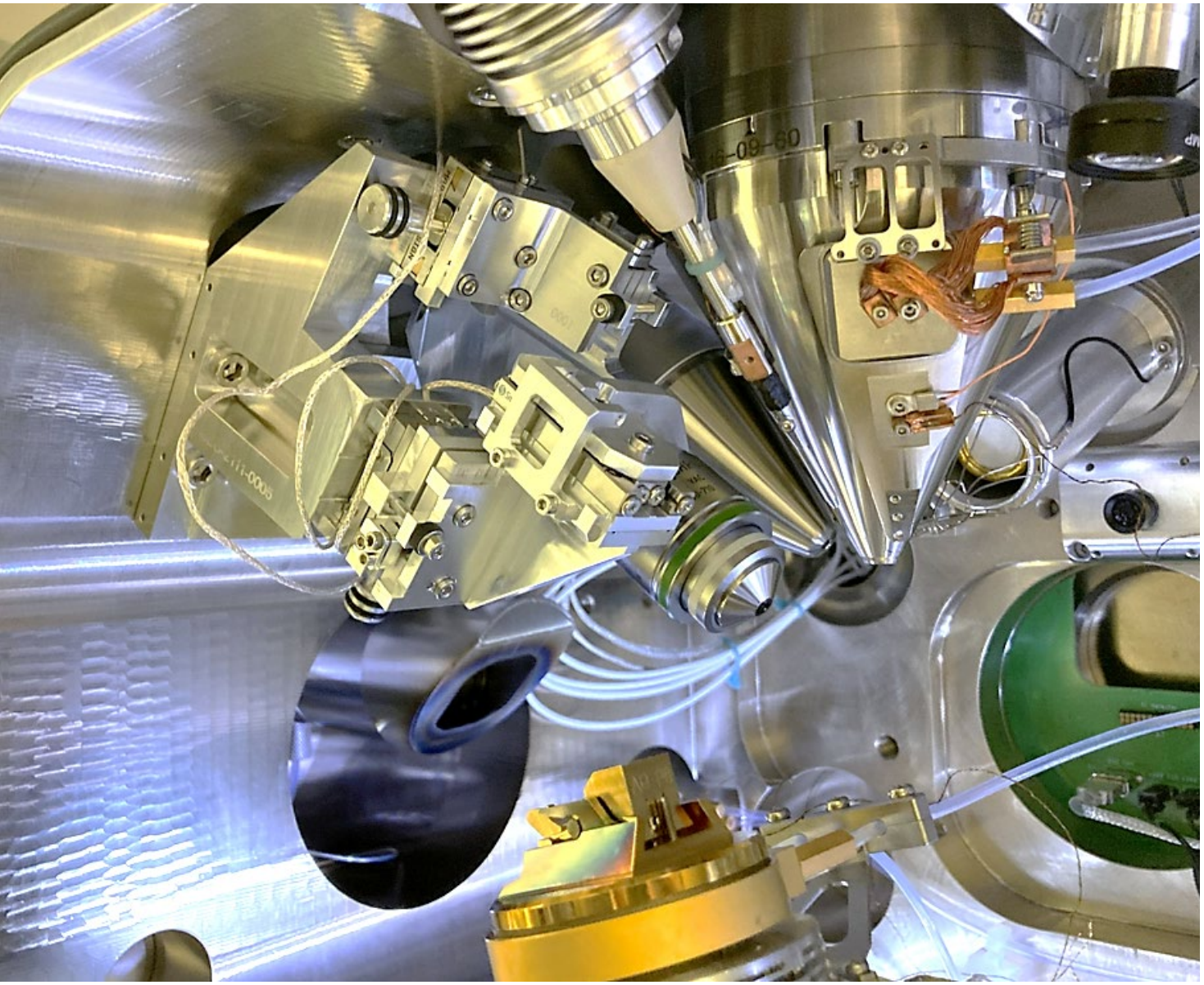
Data Courtesy Max Planck Institute of Biochemistry | *Bykov et al., eLife 2017*

Aquilos 2 Cryo-FIB | Automation capabilities

LAMELLA MILLING AUTOMATION

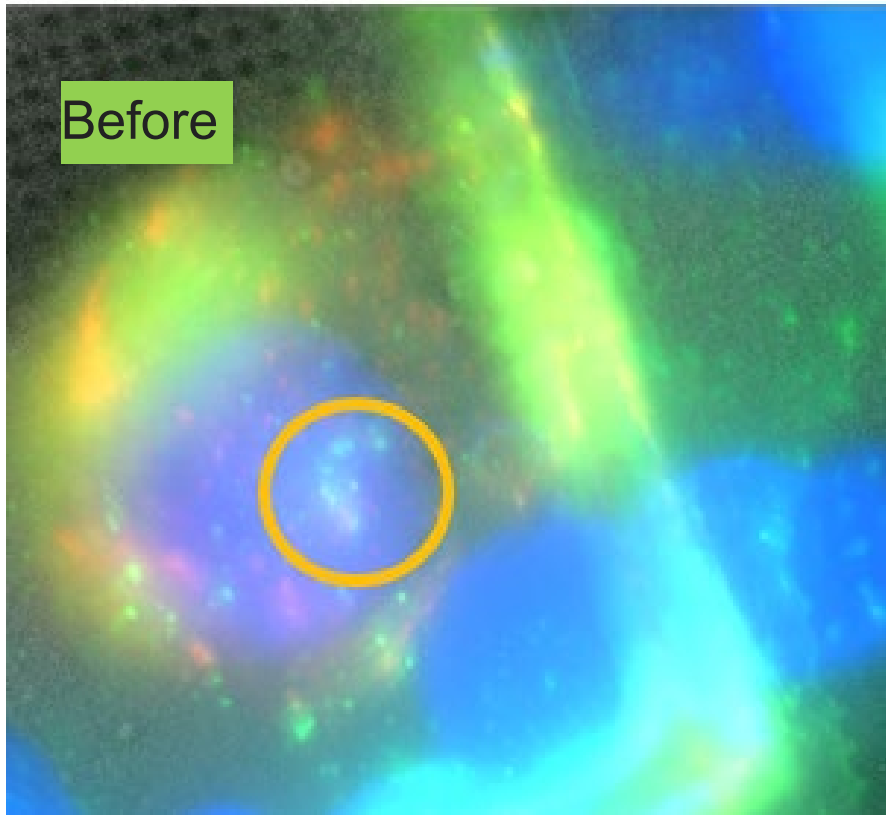
- Proven **AutoTEM Cryo** software for automated milling of cryo-lamellae
- Targeting in combination with Maps software
- Increasing milling throughput and efficiency

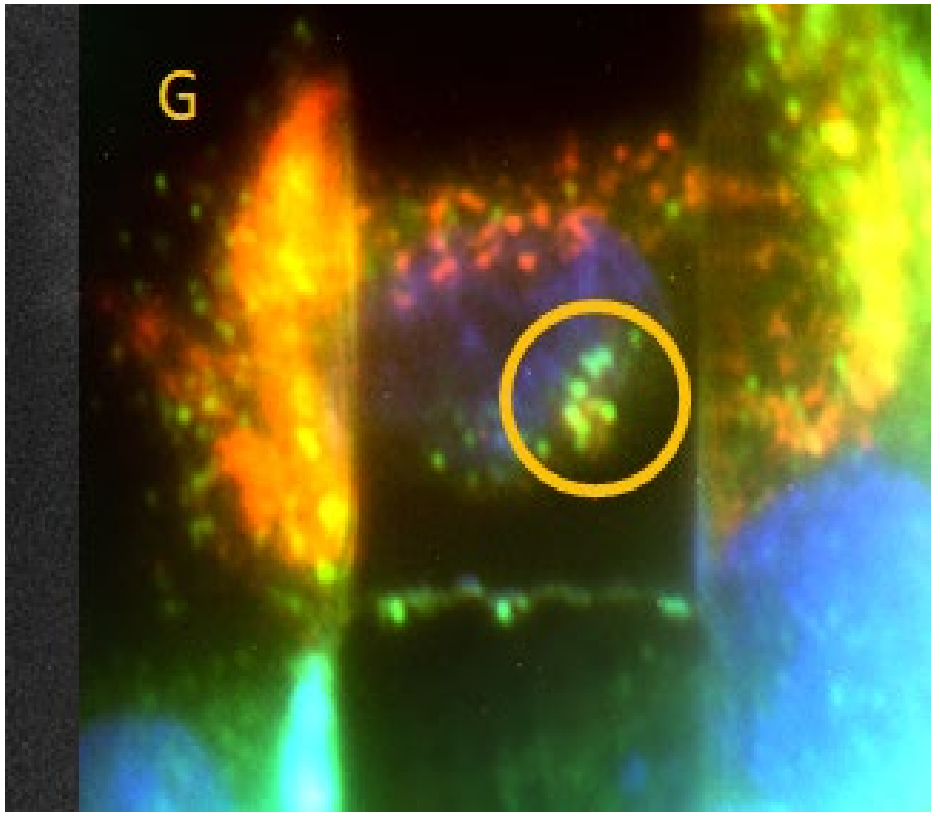




Targeting Example – Using Maps and xT

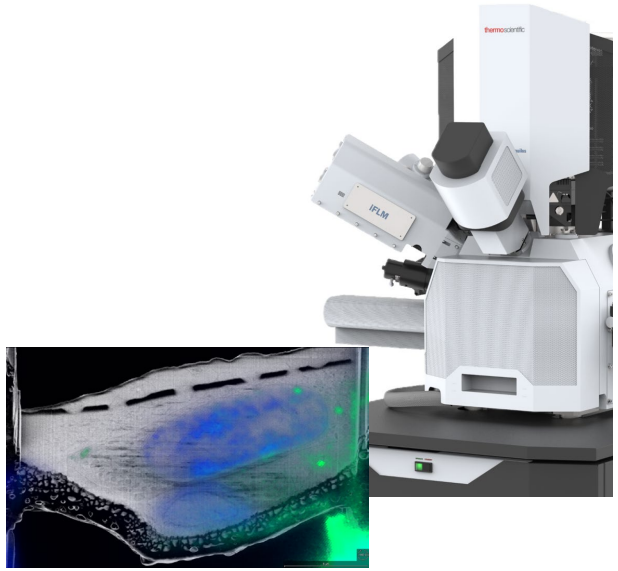
Triple Stained Chinese Hamster Ovary (CHO) Cell – Actin Red, Mitochondria (Green dots – Target Yellow Circle), Nucleus Blue

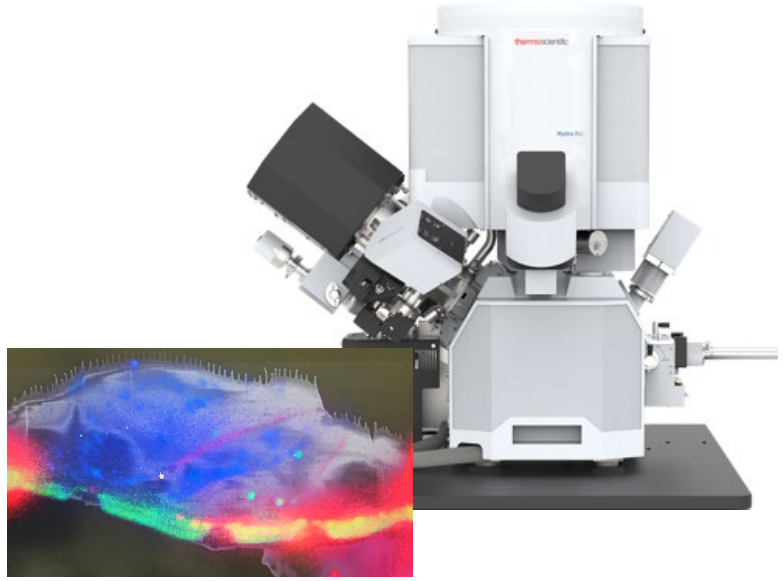




The Evolution of Integrated Fluorescence

From a novel concept, to a publication, to an integral part of cryo tomography





2019: Alex DeMarco PIEscope

- Homebuilt iFLM on Helios Hydra DualBeam
- Set standard for productization

2020: The first iFLMs in the field

- Based on DeMarco design
- Exploring ideal combinations of objective, filter, camera

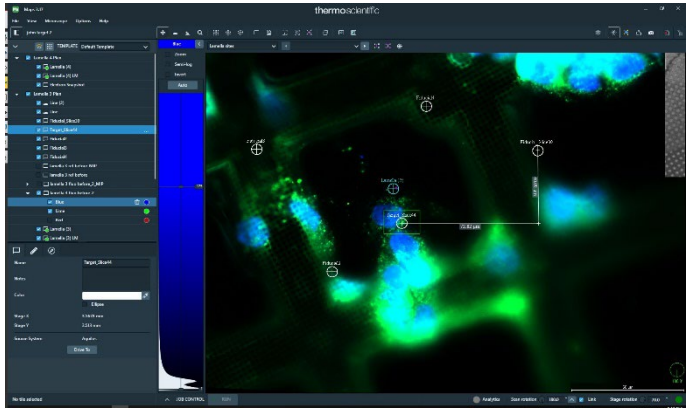
2021: Final Design iFLM in field

- Redesign of the first iFLM
- Tested and optimized objective, filter, camera

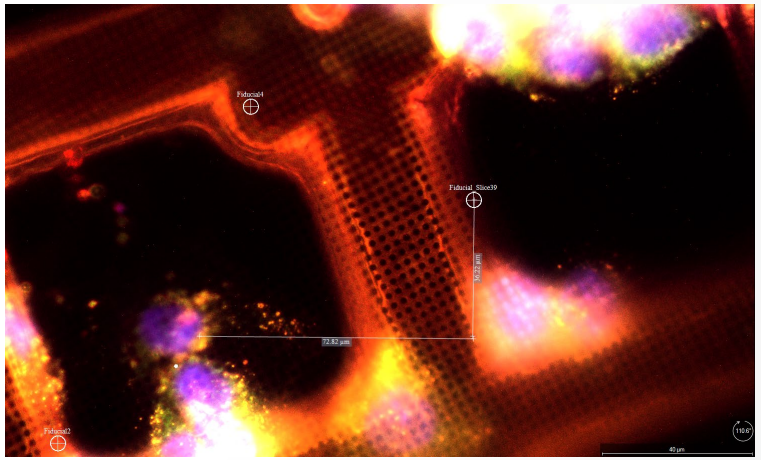
PIEscope Publication: https://elifesciences.org/articles/45919

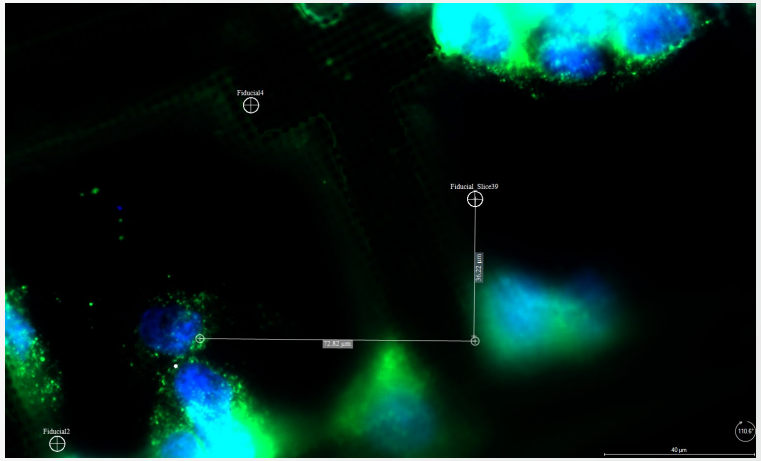
Manual Targeting

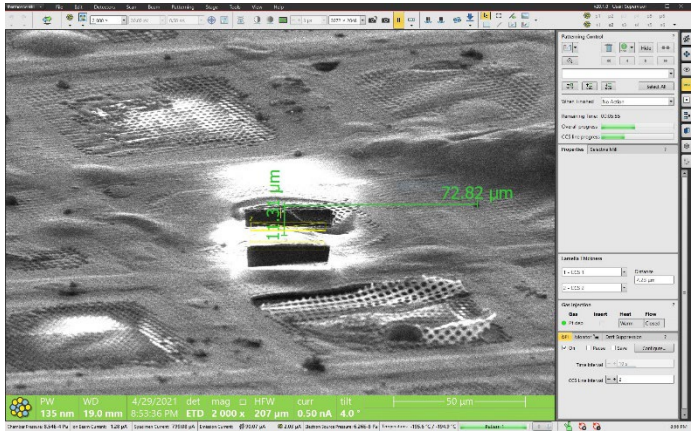
CHO with Mitotracker and Hoechst – Aim for Mitochondrial Cluster



Maps







xT UI – ion view

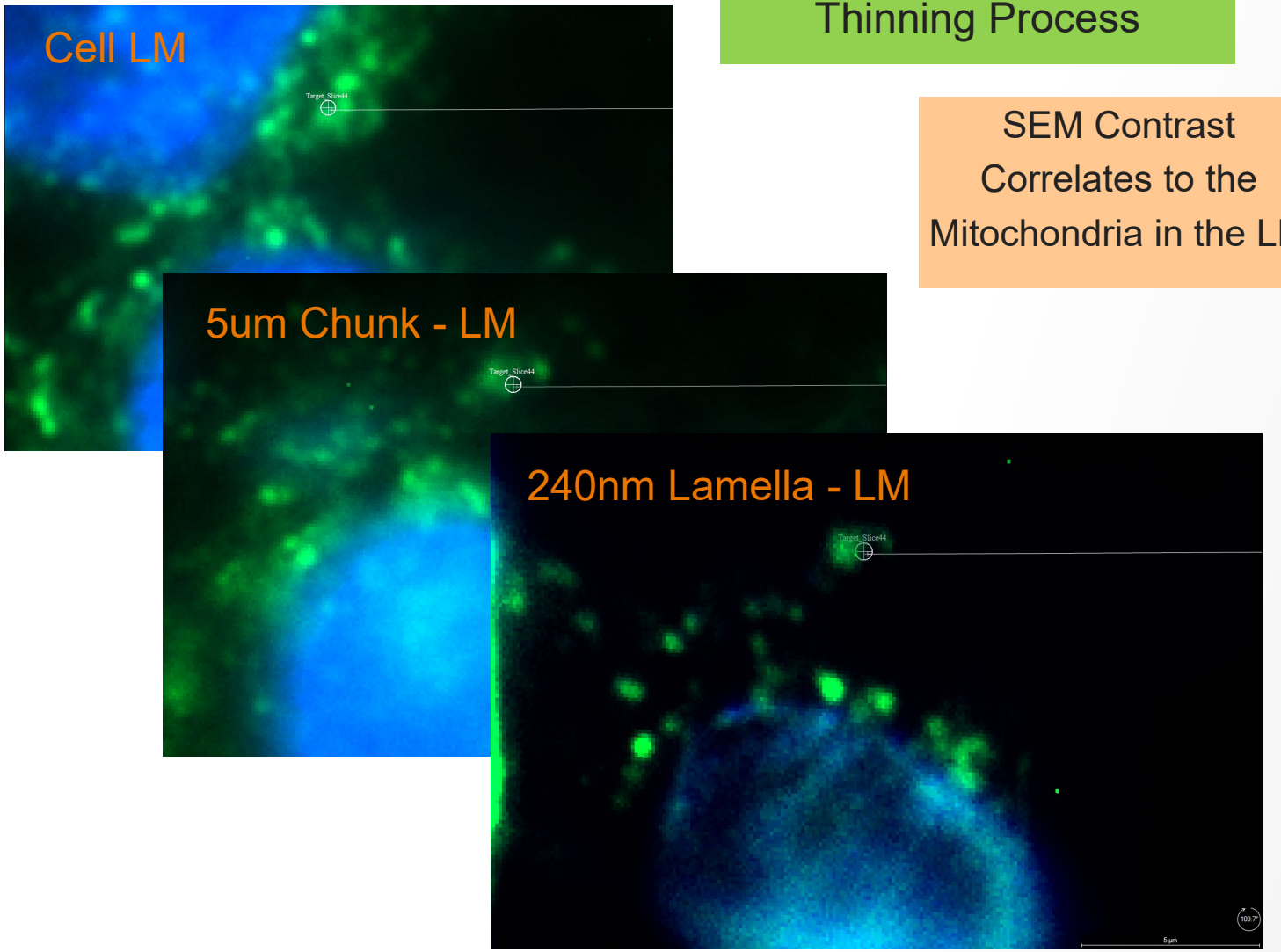
Target in Slice 44, Measure X_{CLEM} 72.82um, Measure Y_{CLEM} 36.2um, Target in Slice in 39, Difference in z is 5 slices (400nm slice = 2um), red channel was used to identify the fiducial, its not needed for the cell.

Check

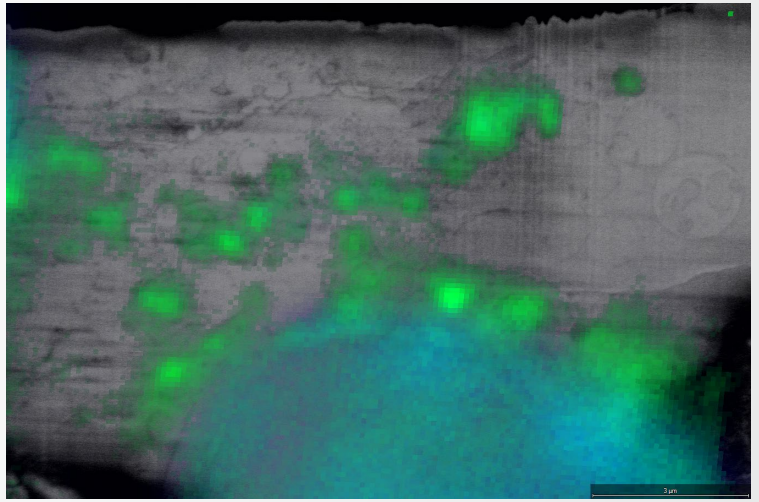
Is the target in the lamella?

Target on Track Throughout the Lamella Thinning Process

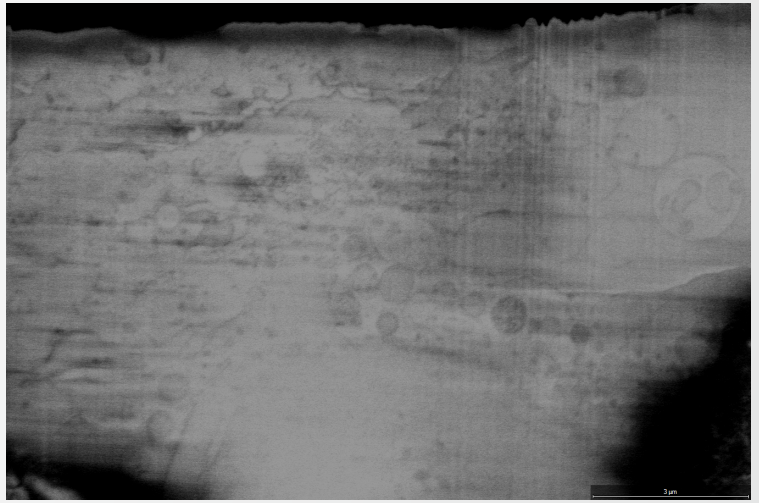
SEM Contrast Correlates to the Mitochondria in the LM



240nm Lamella – Combined SEM/LM

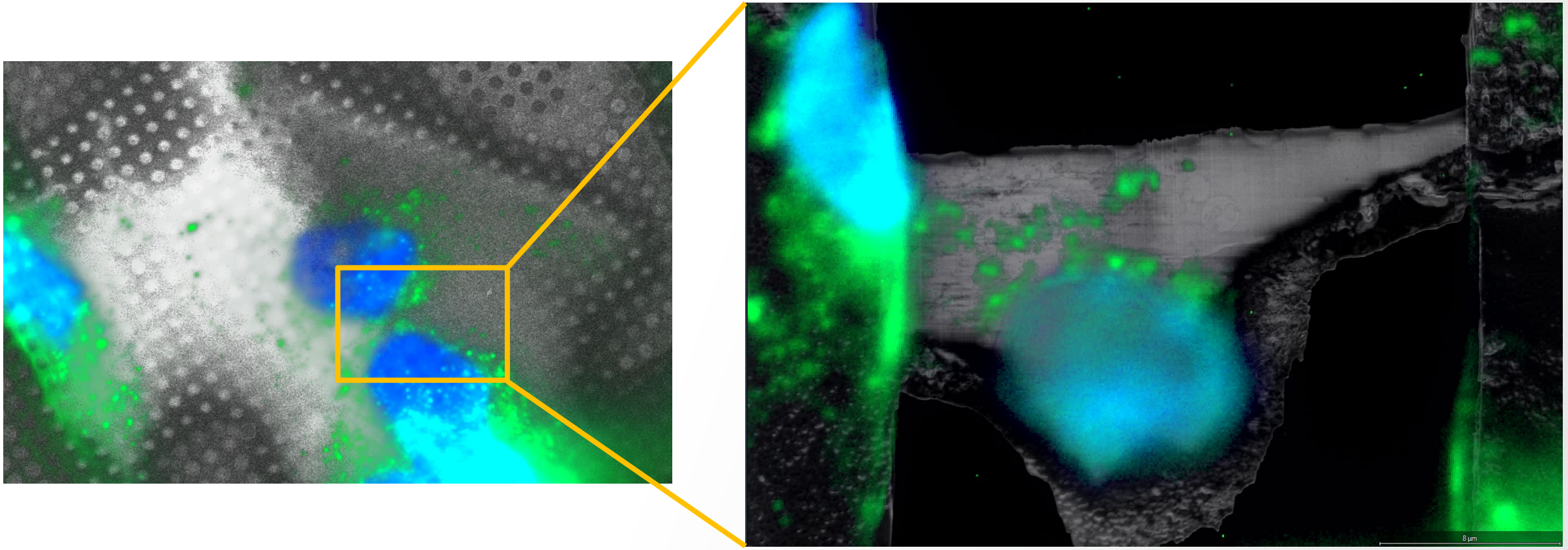


240nm Lamella – SEM



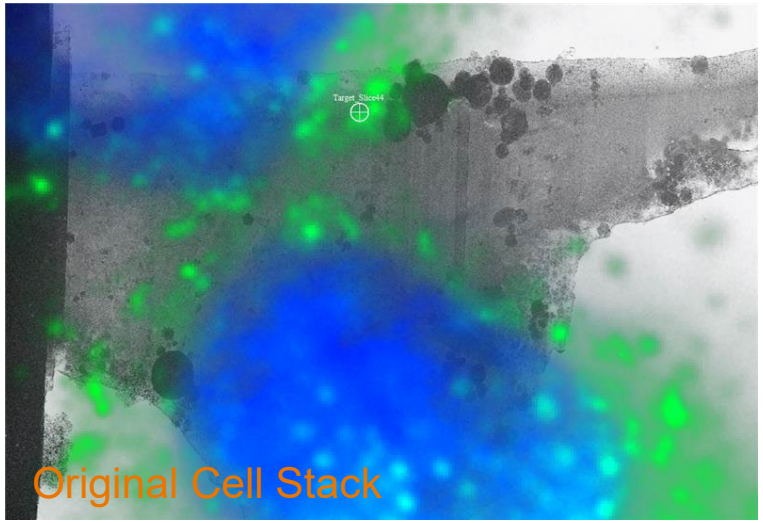
Go to TEM

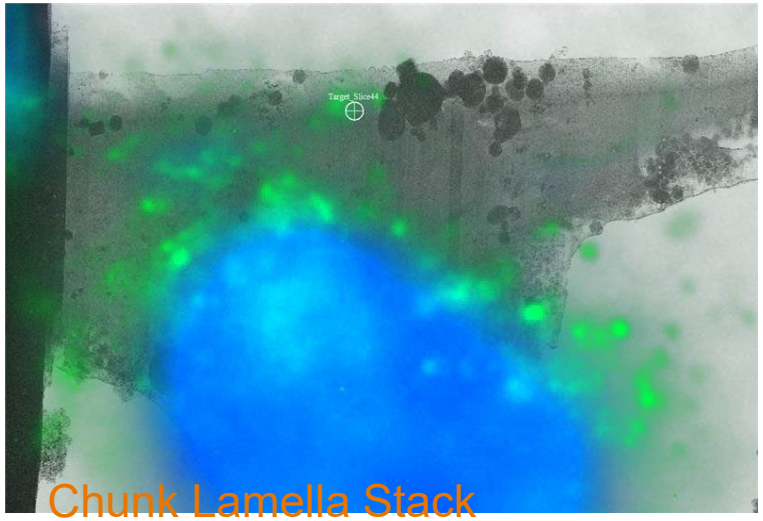
Take your project and information for down stream TEM/Processing

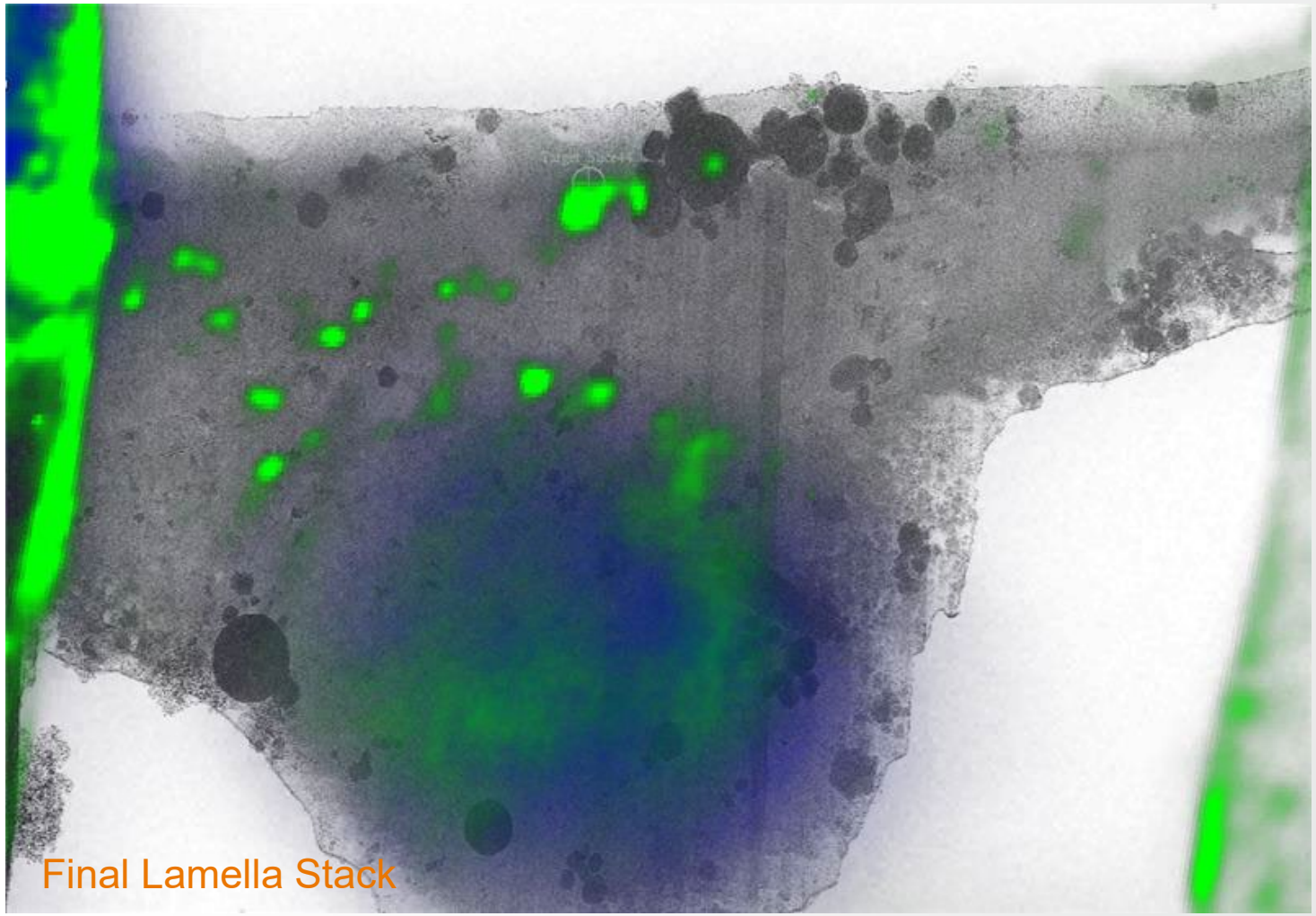


TEM

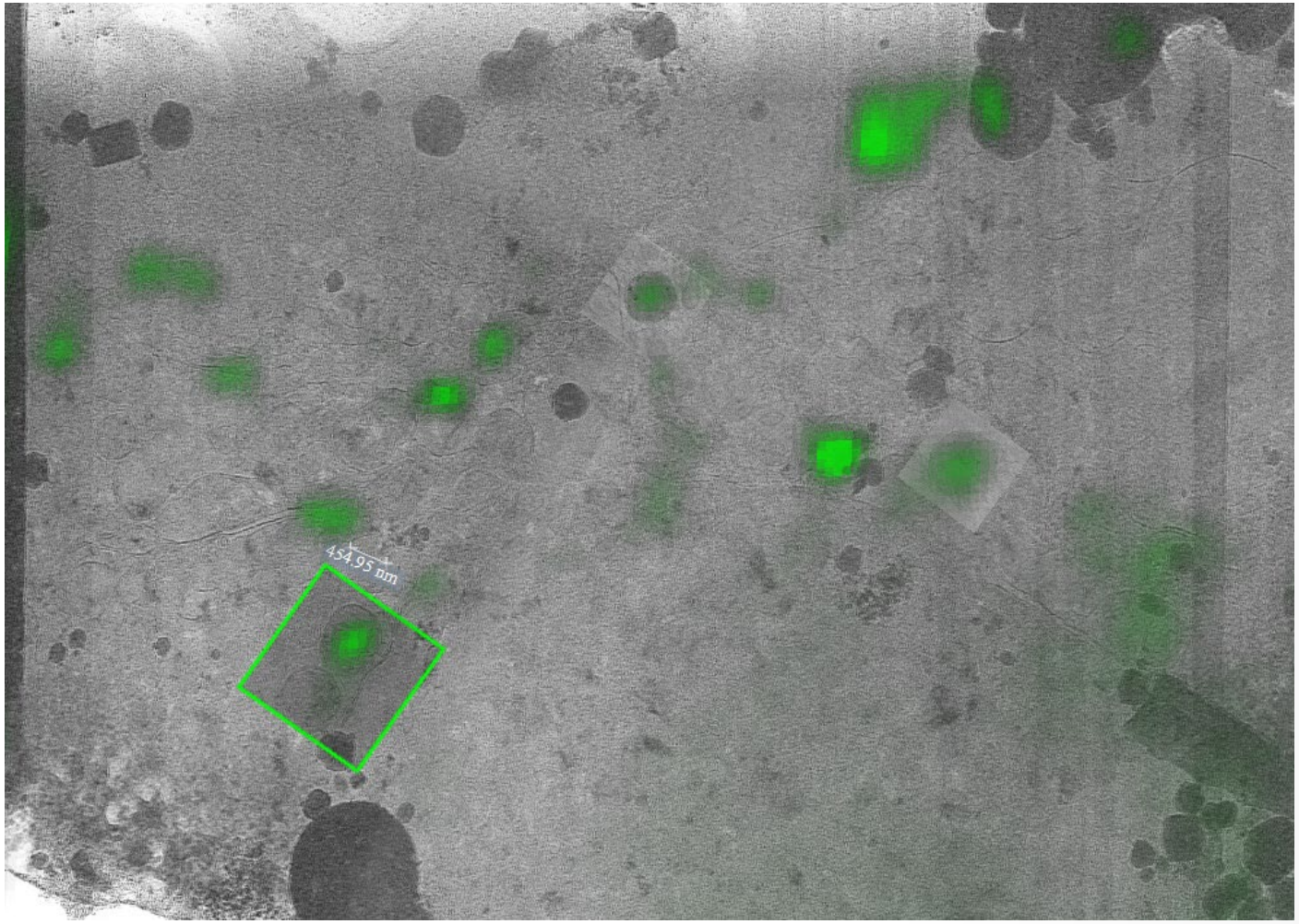
Overlay to TEM

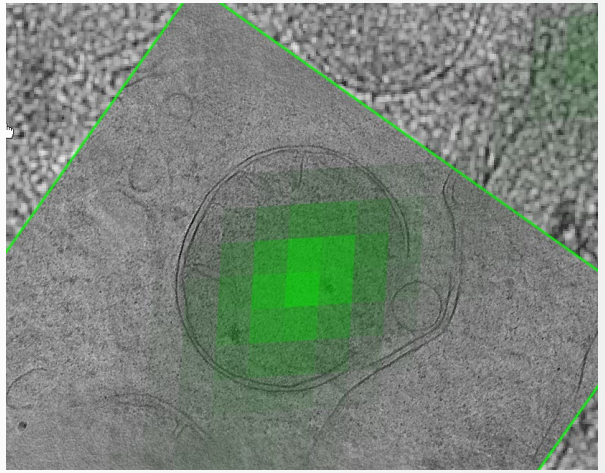


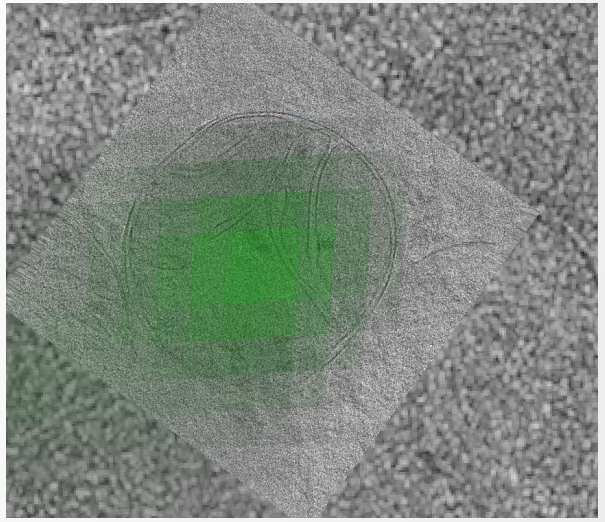


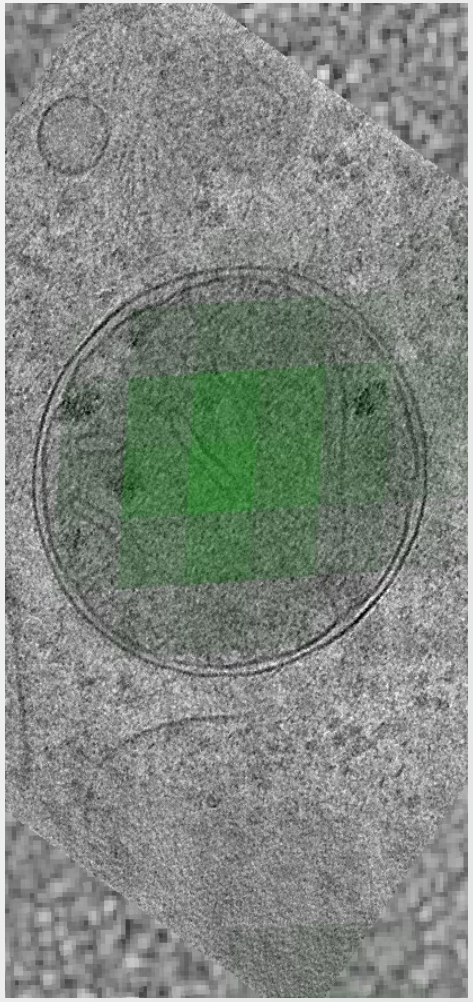


TEM Tomo

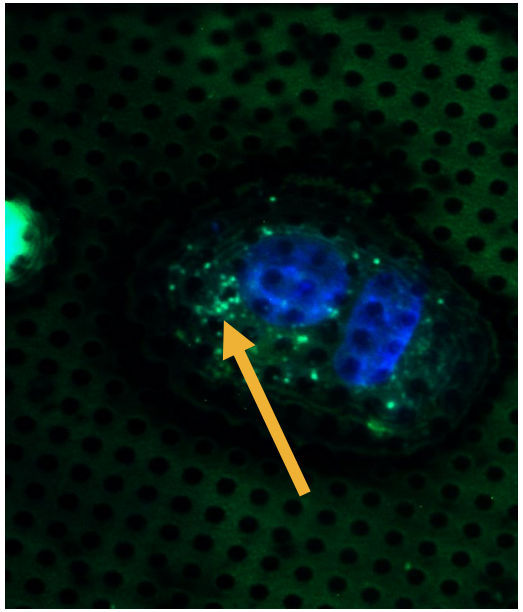
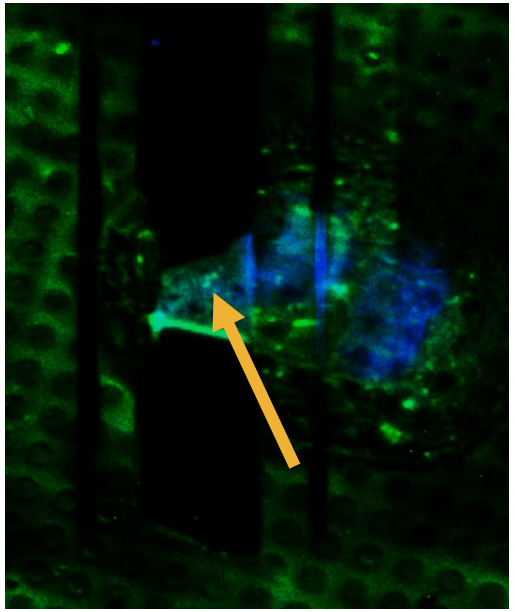
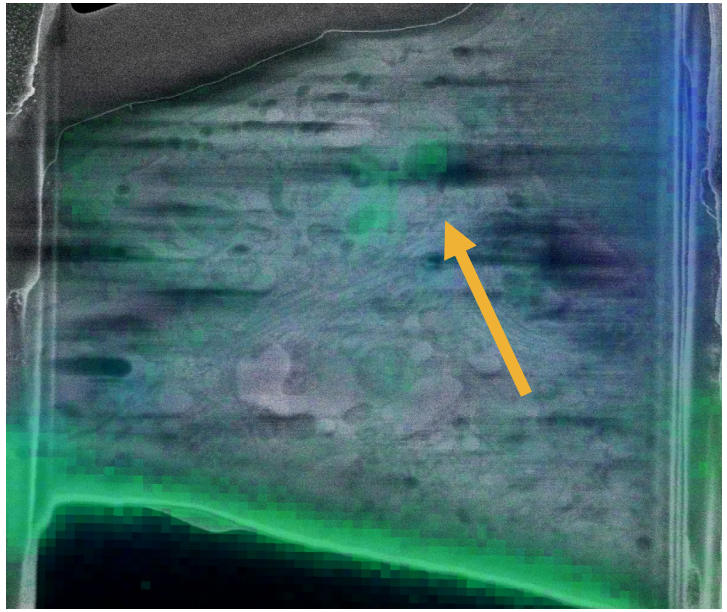


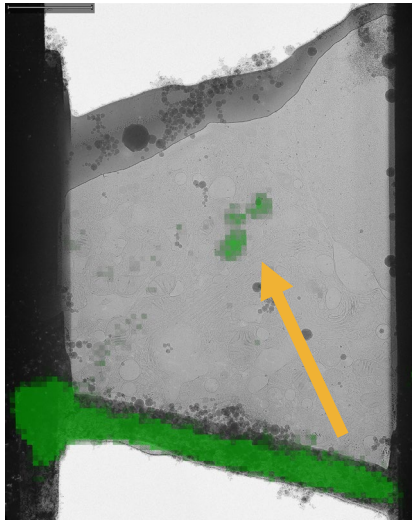
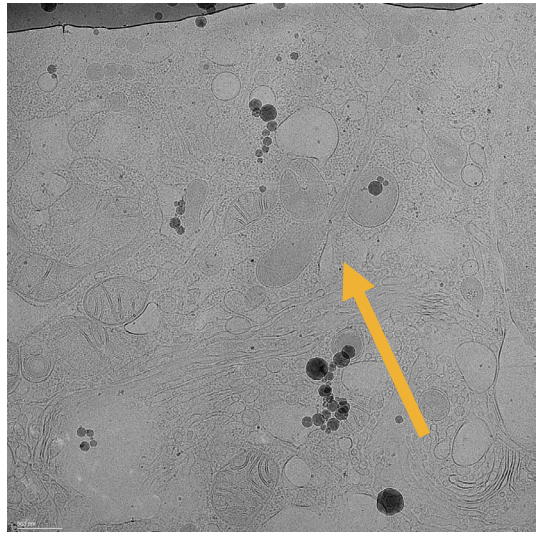
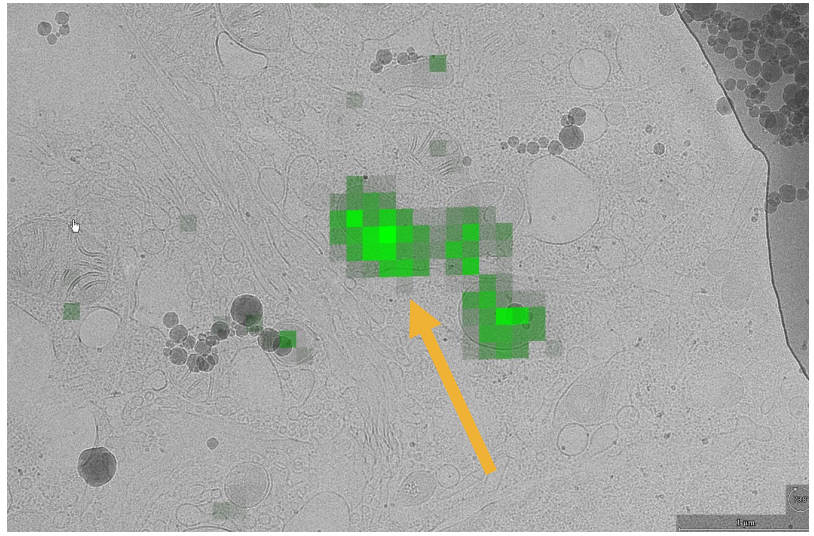




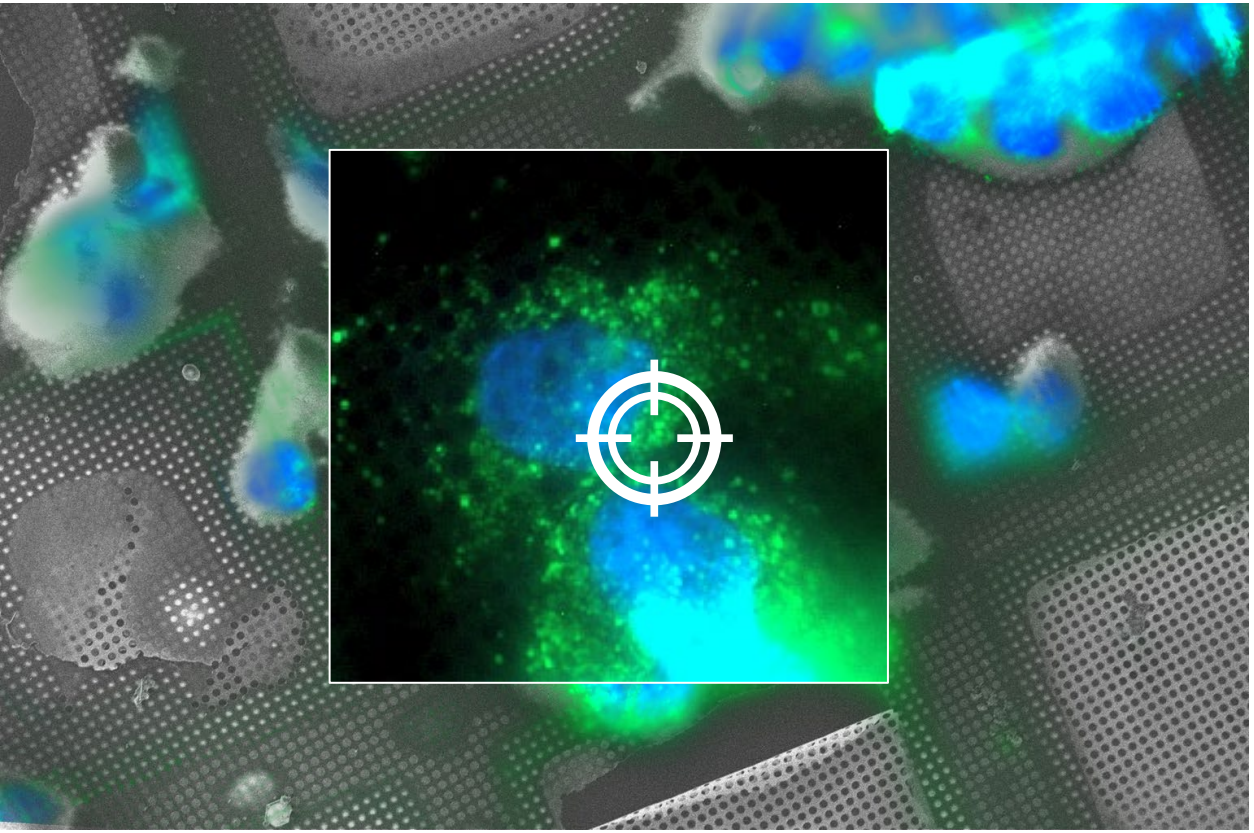


Insulinoma Cells with FITC labelled insulin

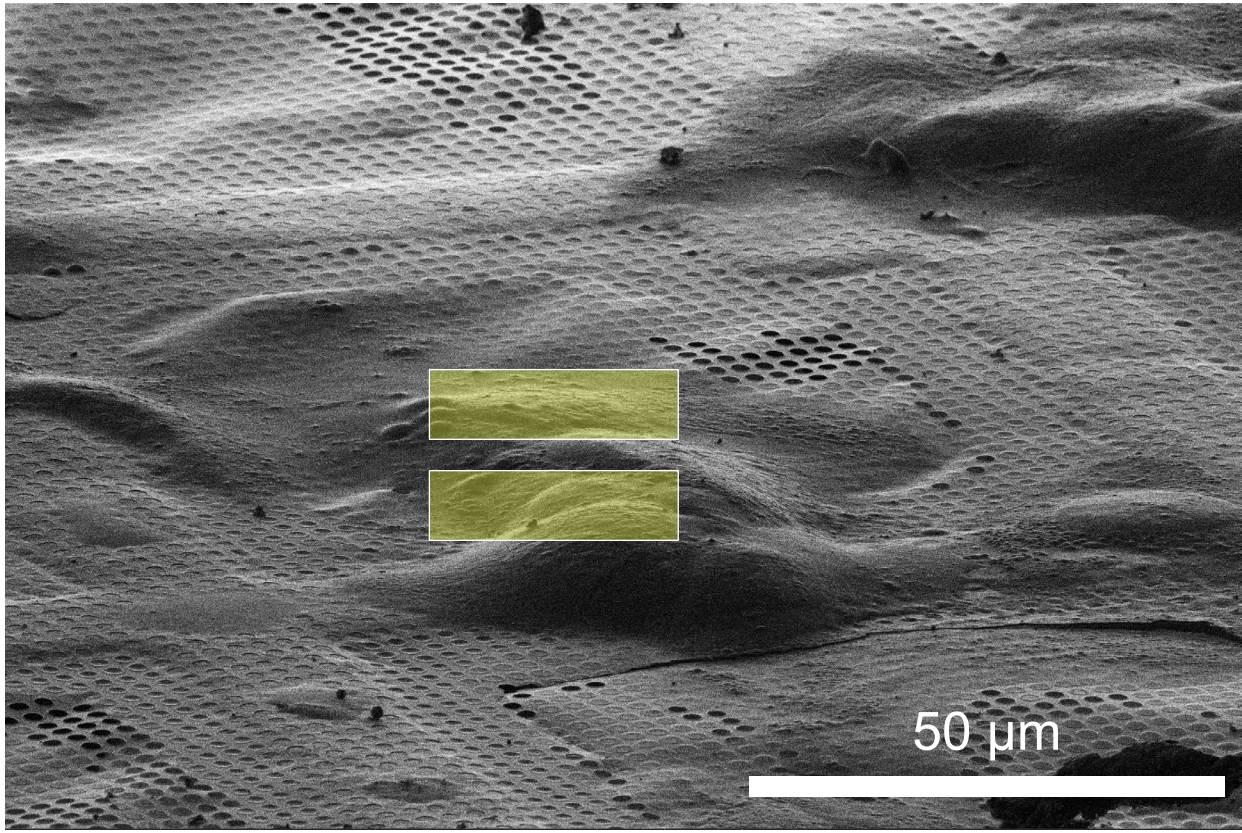





iFLM guided lamella milling



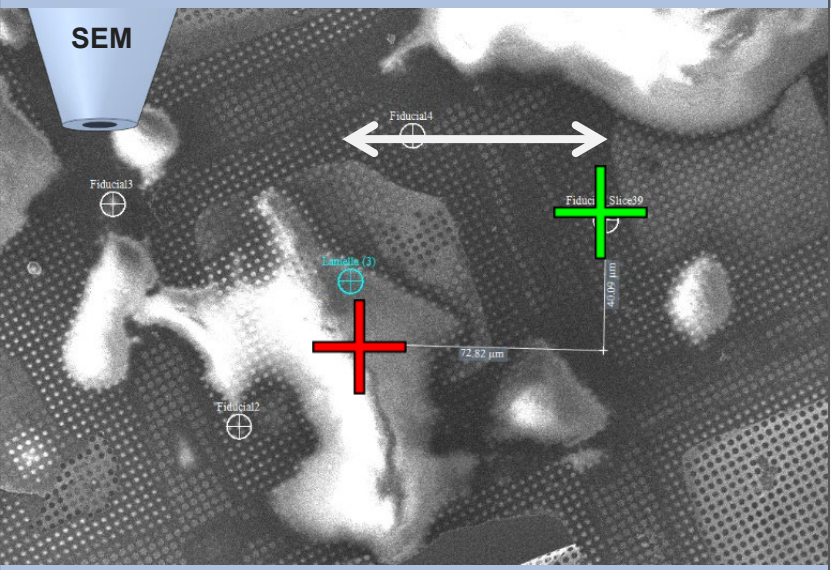
SEM
(top view)



ION
(Tilted view)

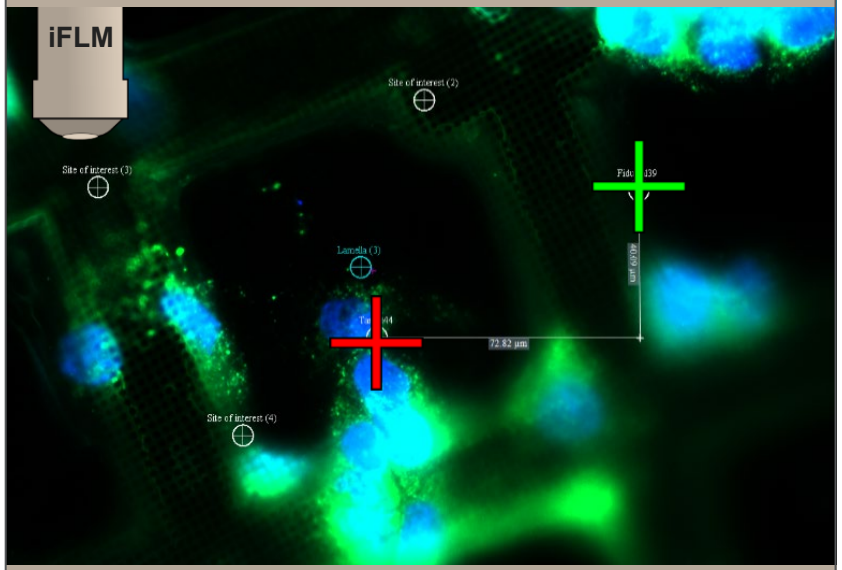
iFLM | Guided lamella milling | 3D Transformation

SEM

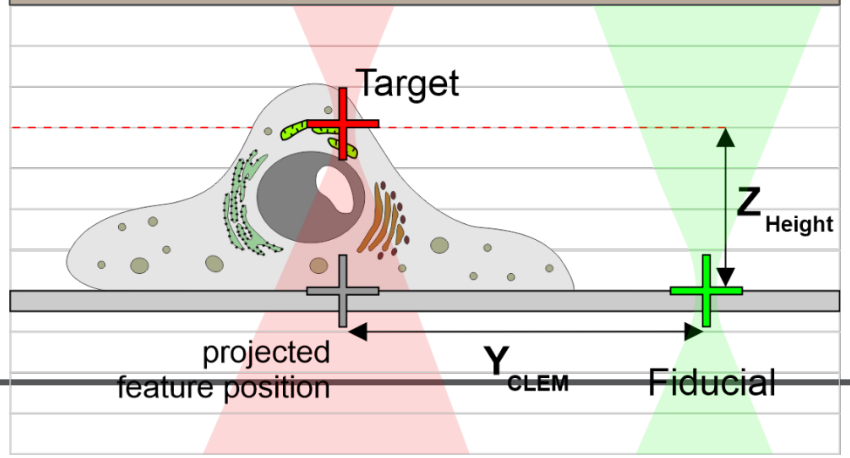


Distance to fiducial

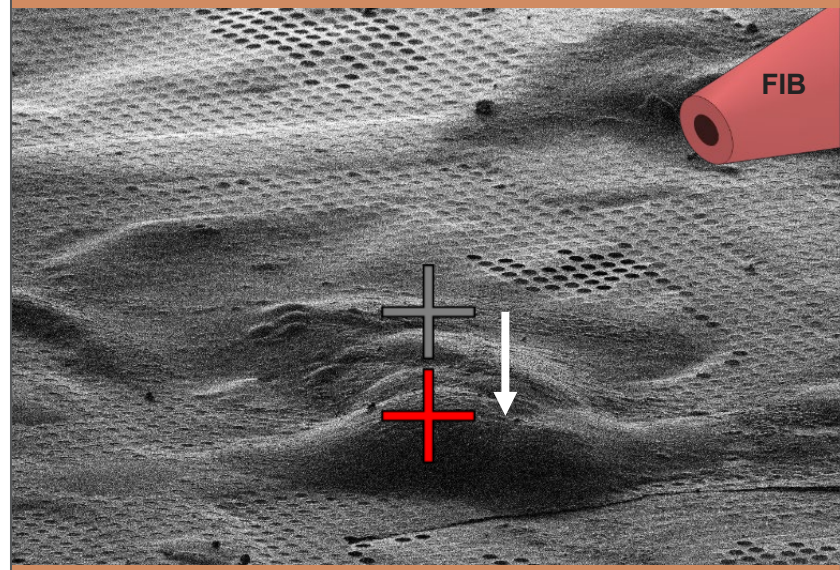
iFLM



Z-height for feature & fiducial



FIB



Calculate milling position

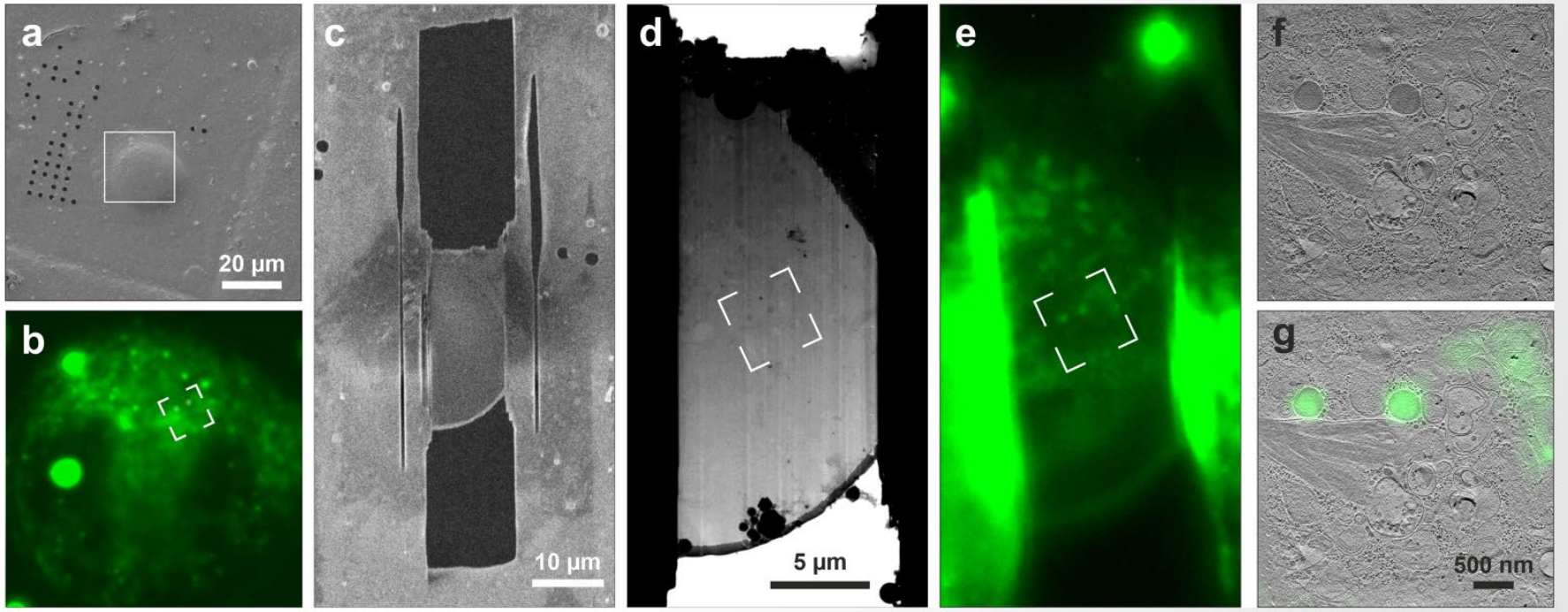
ThermoFisher SCIENTIFIC

Precise 3D Localization by Integrated Fluorescence Microscopy (iFLM) for Cryo-FIB-milling and In-situ Cryo-ET

Jae Yang, Veronika Vrbovská, Tilman Franke, Bryan Sibert, Matt Larson, Tom Coomes, Alexander Rigort, John Mitchels, Elizabeth R Wright ✉

Microscopy and Microanalysis, Volume 29, Issue Supplement_1, 1 August 2023, Pages 1055–1057, https://doi.org/10.1093/micmic/ozad067.541

Published: 22 July 2023



Range of samples accessible with tomography

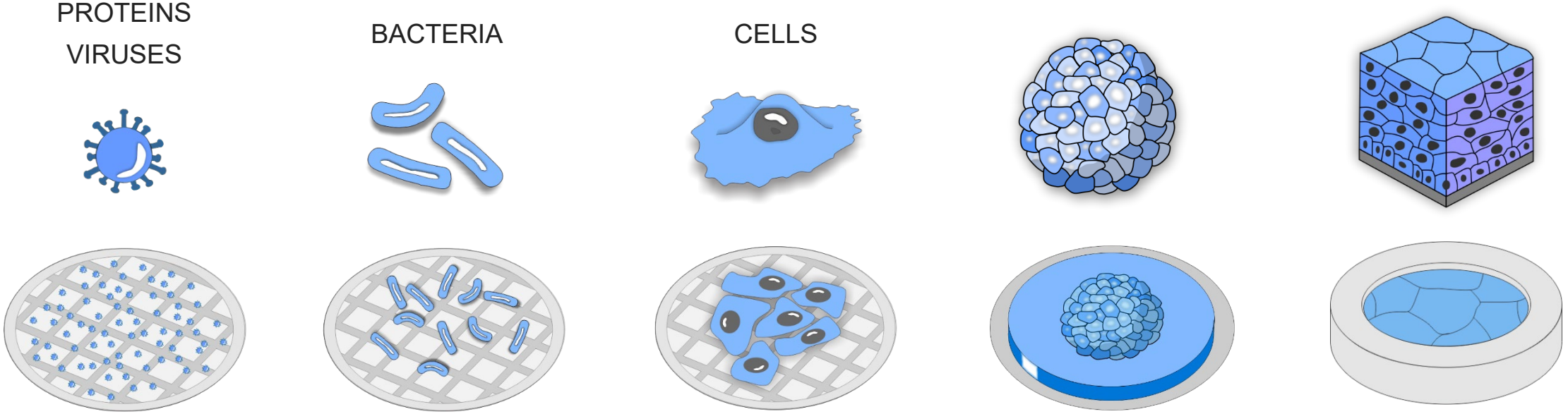
PROTEINS
VIRUSES

BACTERIA

CELLS

ORGANOIDS
TISSUE CELLS

BULK TISSUE
SMALL ORGANISMS



IN SITU LAMELLA MILLING — WAFFLE LAMELLA METHOD — CRYO-LIFT-OUT LAMELLA

Plunging (5-10 µm)

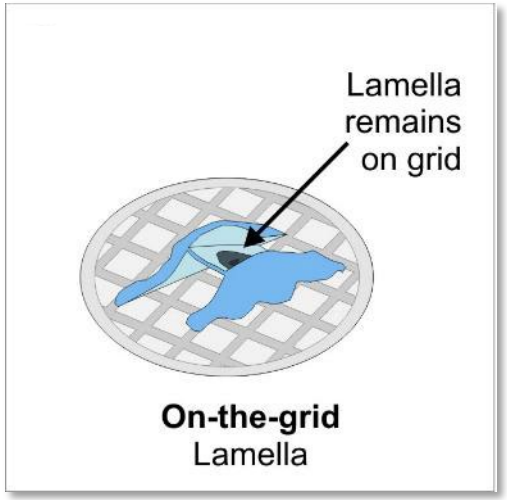
High-Pressure-Freezing (> 200 µm)

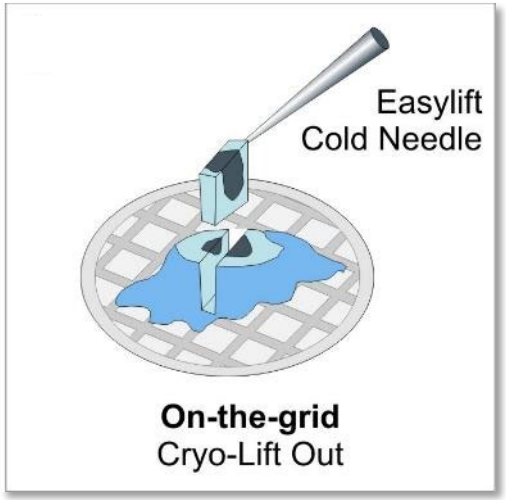
CRYO-FIB MILLING

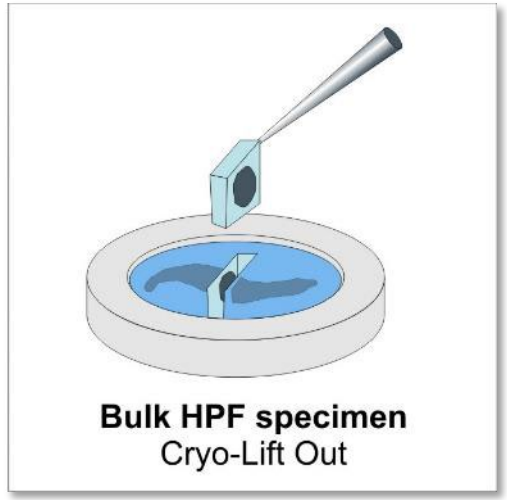
Aquilos 2 Cryo-FIB | Cryo-Lift-Out

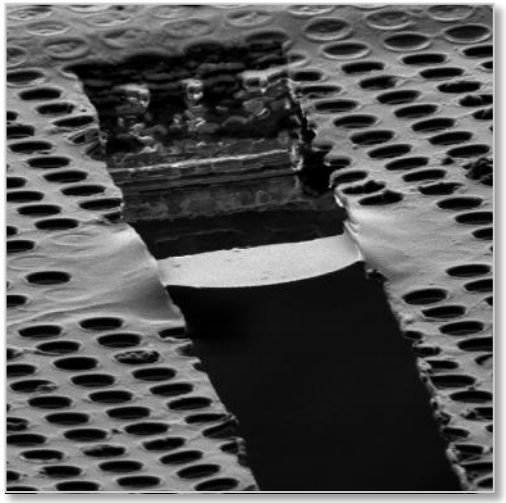
CRYO LIFT-OUT

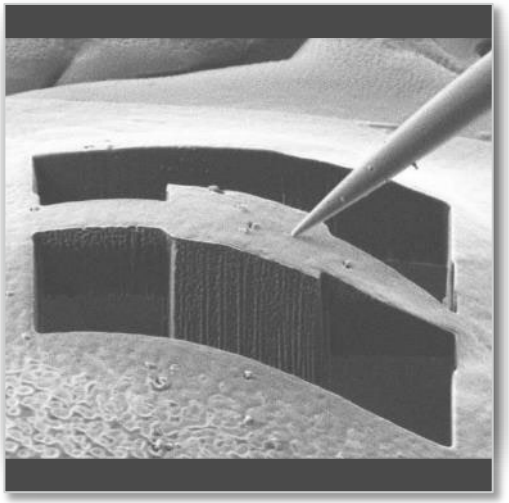
- Proven **EasyLift Cryo** lift-out system
- Supports lift-out from on-the-grid and HPF samples
- Full integration in cryo-FIB control software
- Proven best-practice procedure(s)

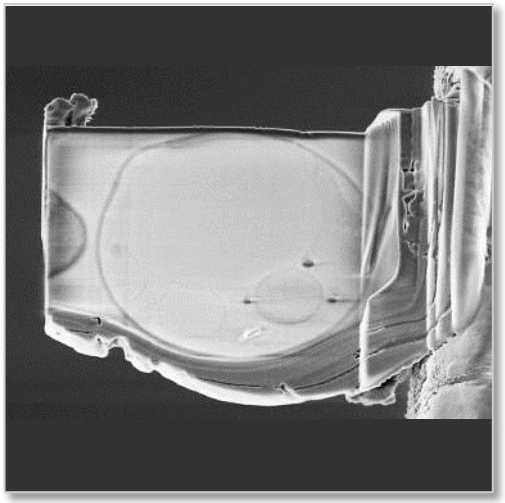




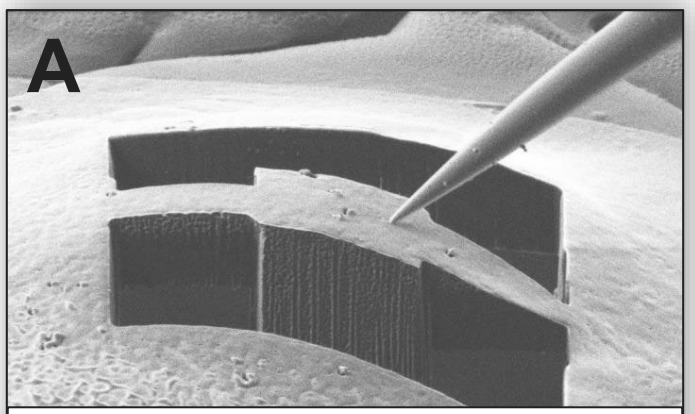




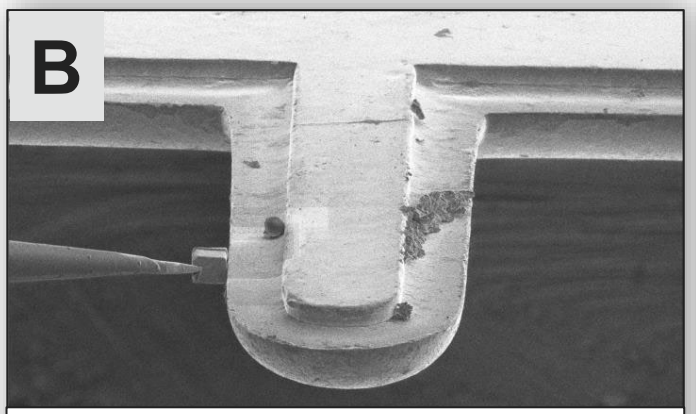




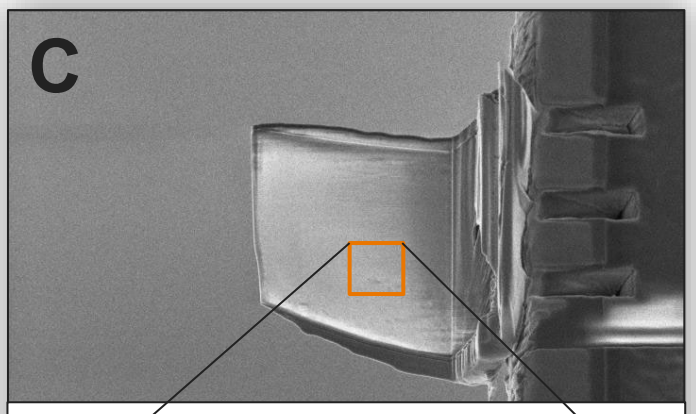
Cryo-lift-out



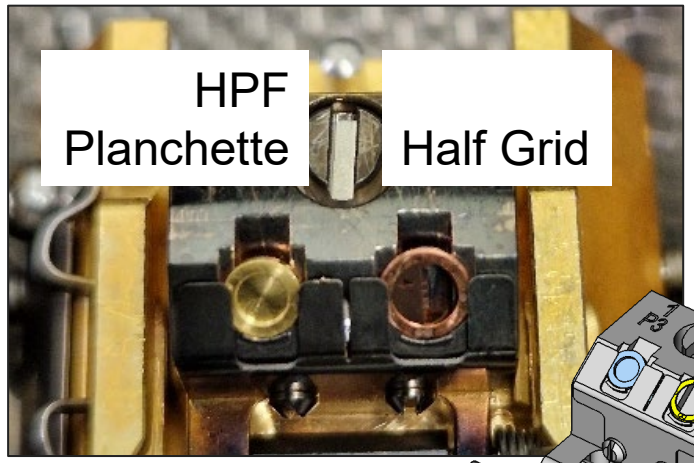
Cryo-lift-out

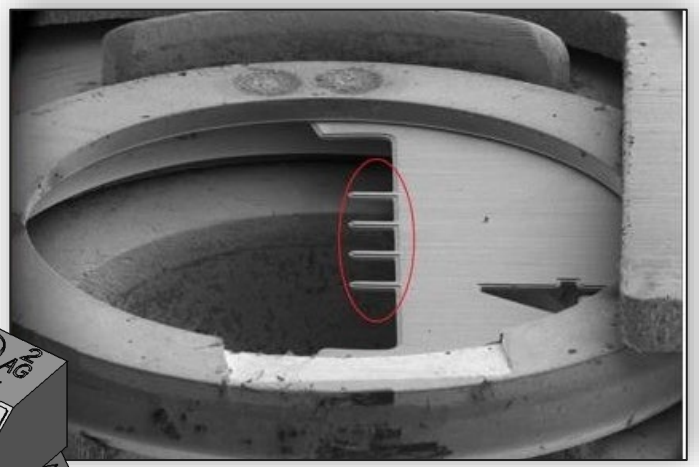


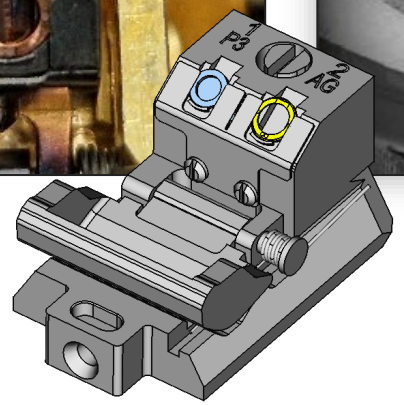
Attachment to Half Grid

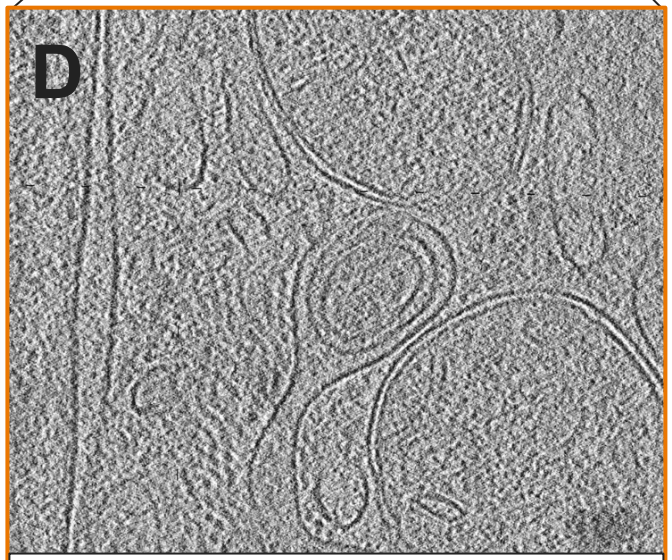


Lamella Polishing



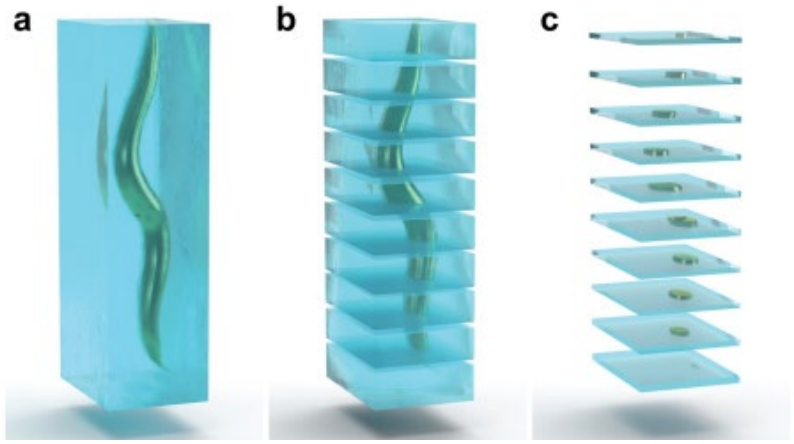


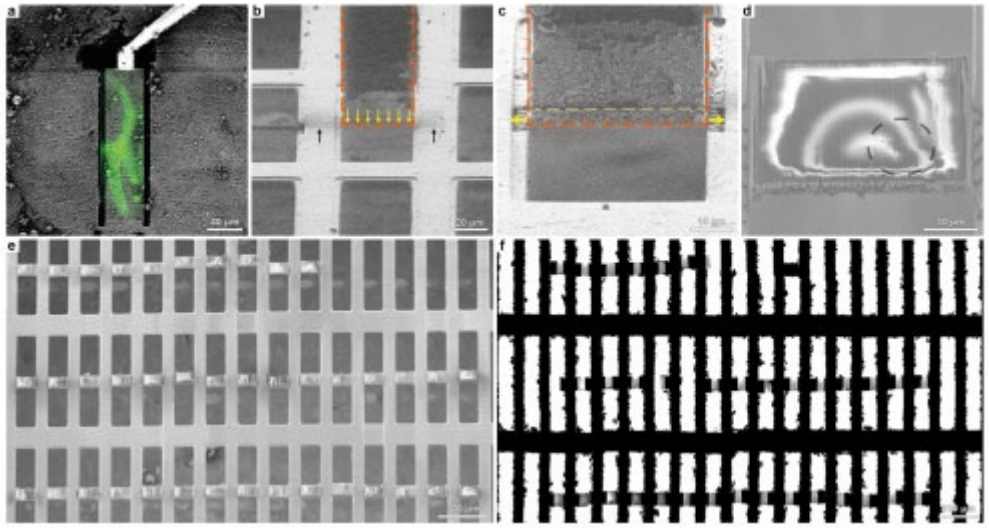


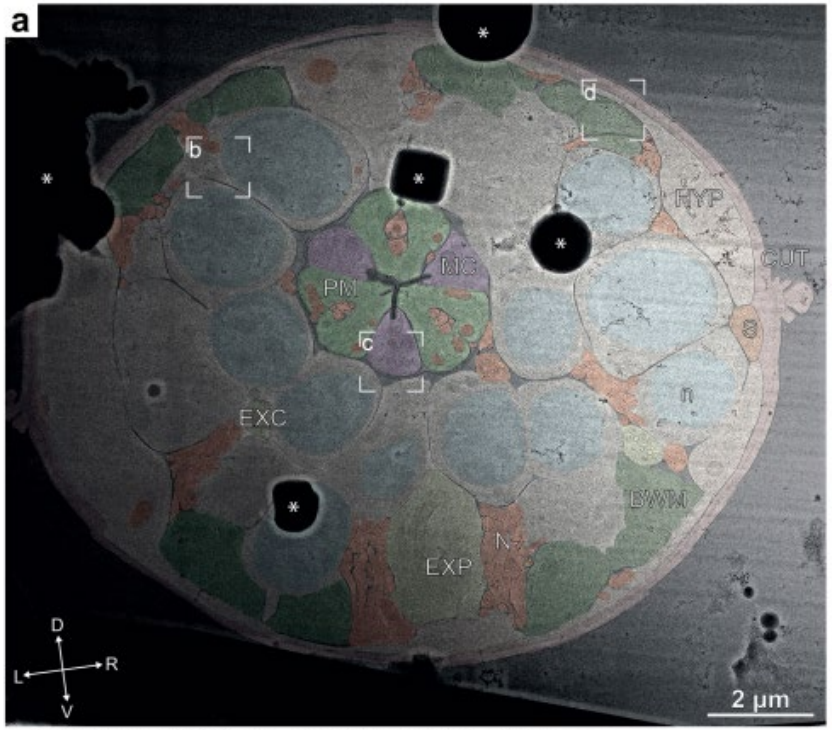


Cryo-Tomography

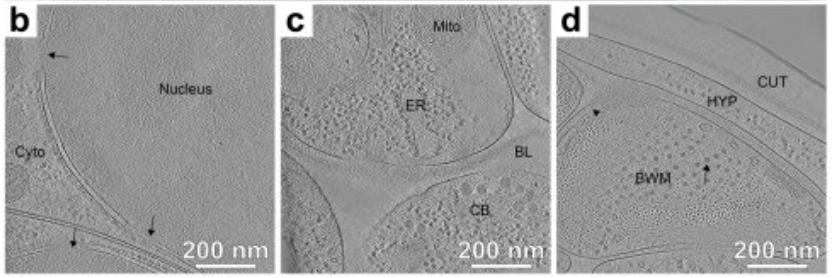
Cryo lift out - recent pre-print - serial lift out

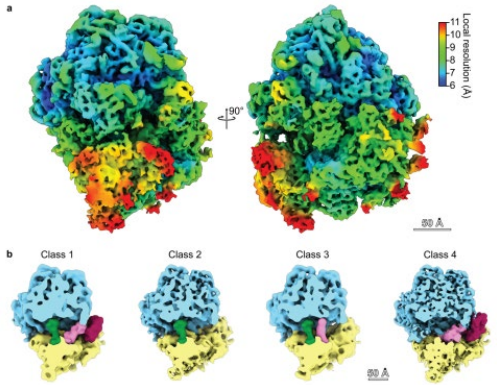






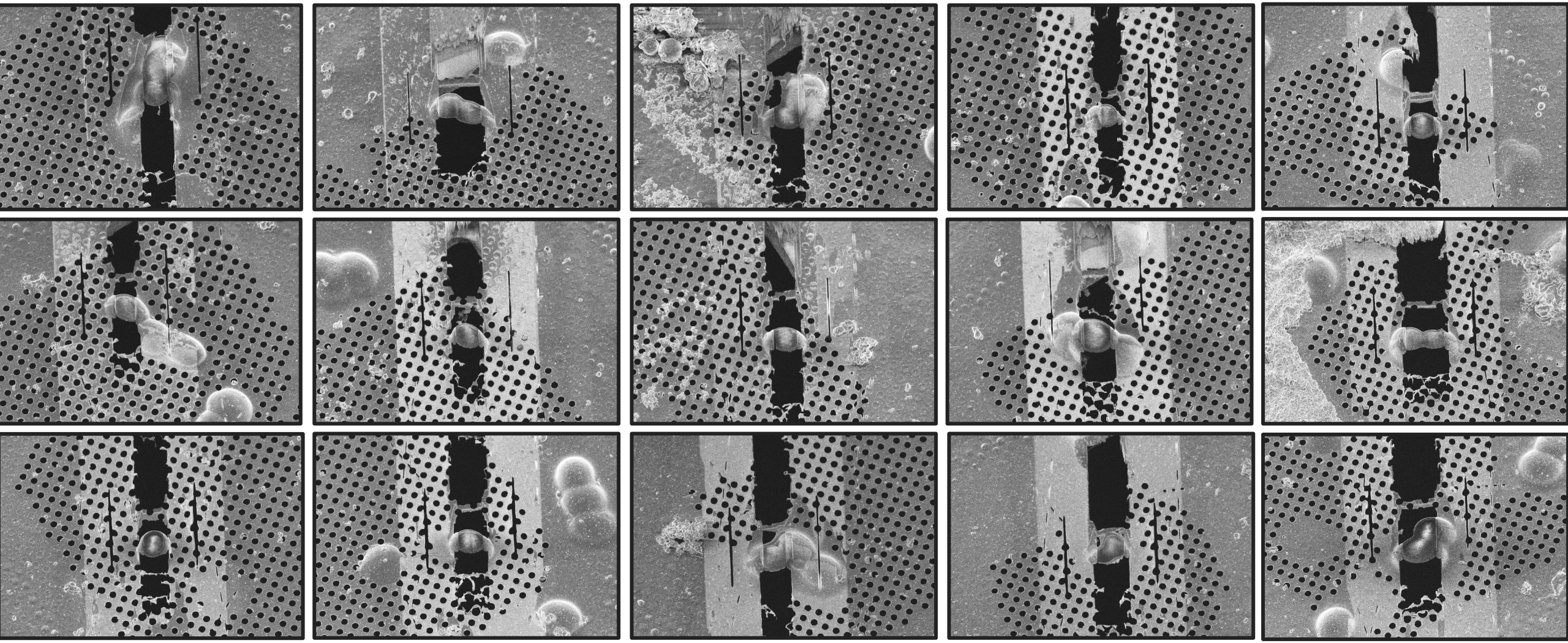
- ✓ Combines Liftout with TEM vEM
- ✓ HPF samples suitable for STA





Serial Lift-Out – Sampling the Molecular Anatomy of Whole Organisms - Max Planck Institute - from Oda Helene Schiøtz *et al.* bioRxiv 2023

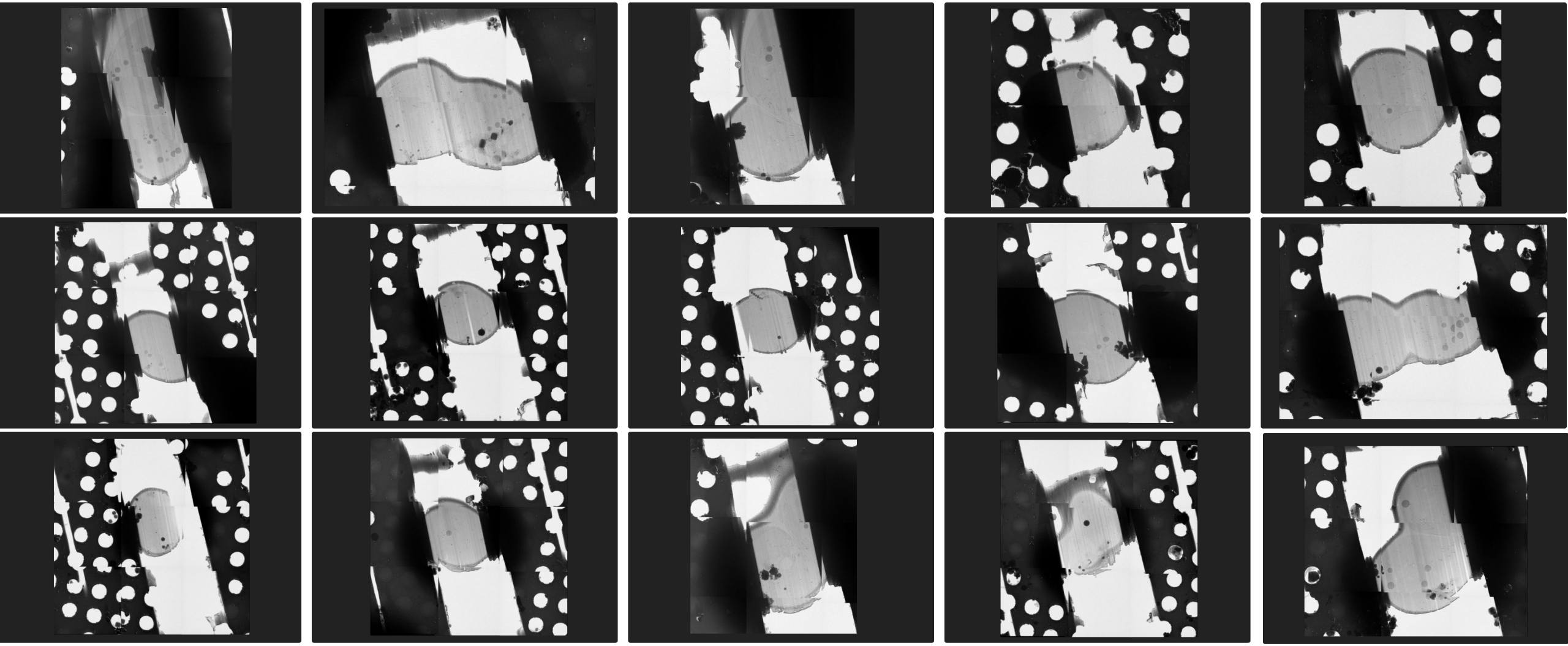
Arctis | Automated lamella prep



Fully automated cryo-lamellae production | SEM overviews

(including final polishing steps; Lamellae prepared with AutoTEM Cryo on Arctis; Sample: Chlamydomonas)

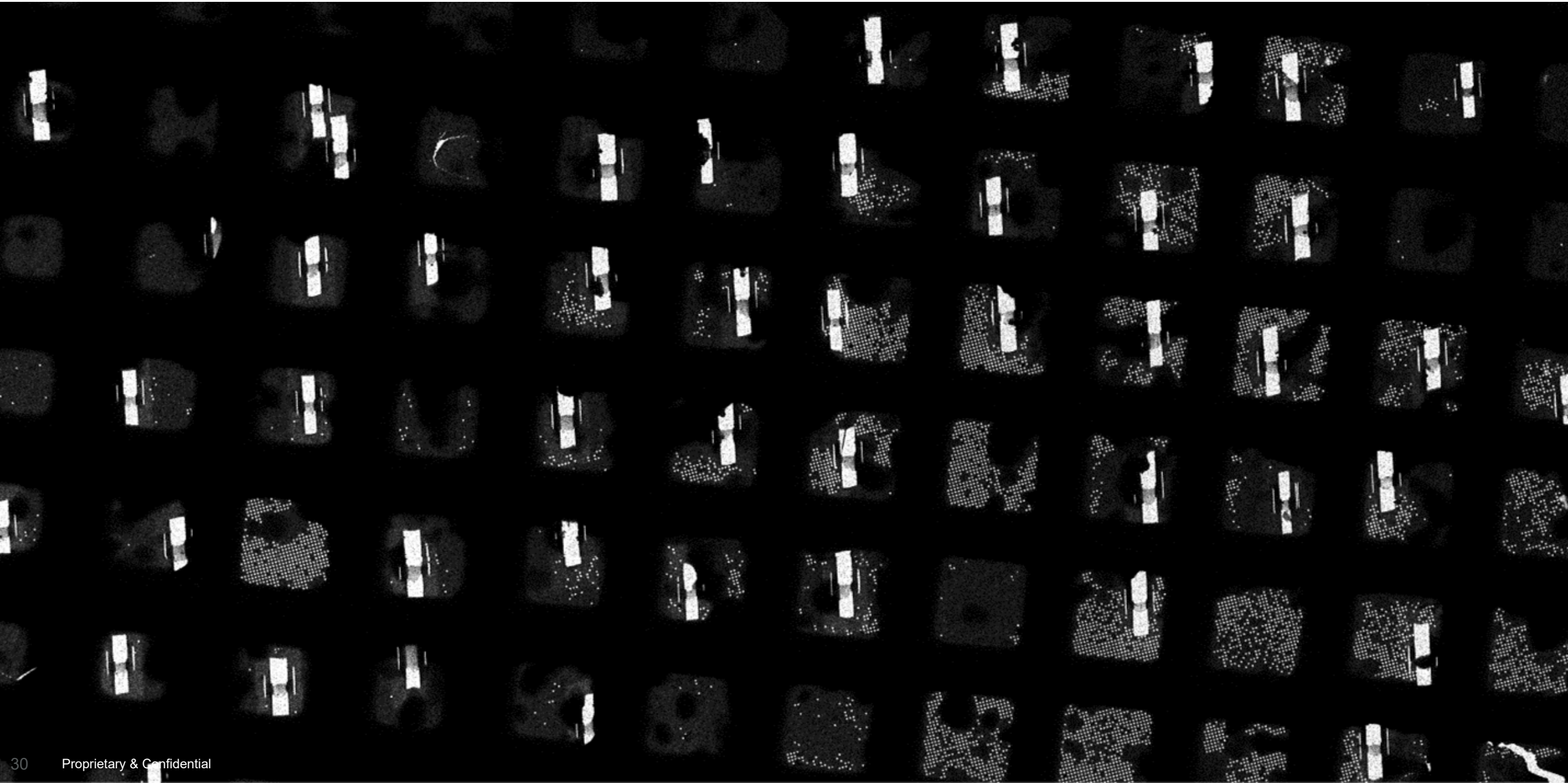
Arctis | Automated lamella prep



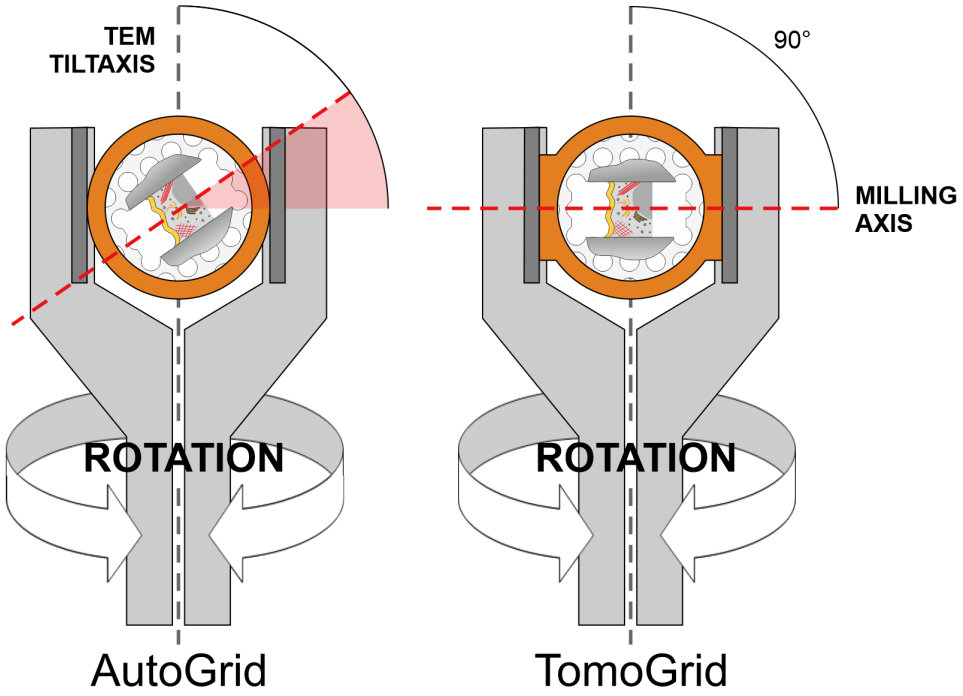
Fully automated cryo-lamellae production | TEM overviews

(including final polishing steps; Lamellae prepared with AutoTEM Cryo on Arctis; Sample: Chlamydomonas)

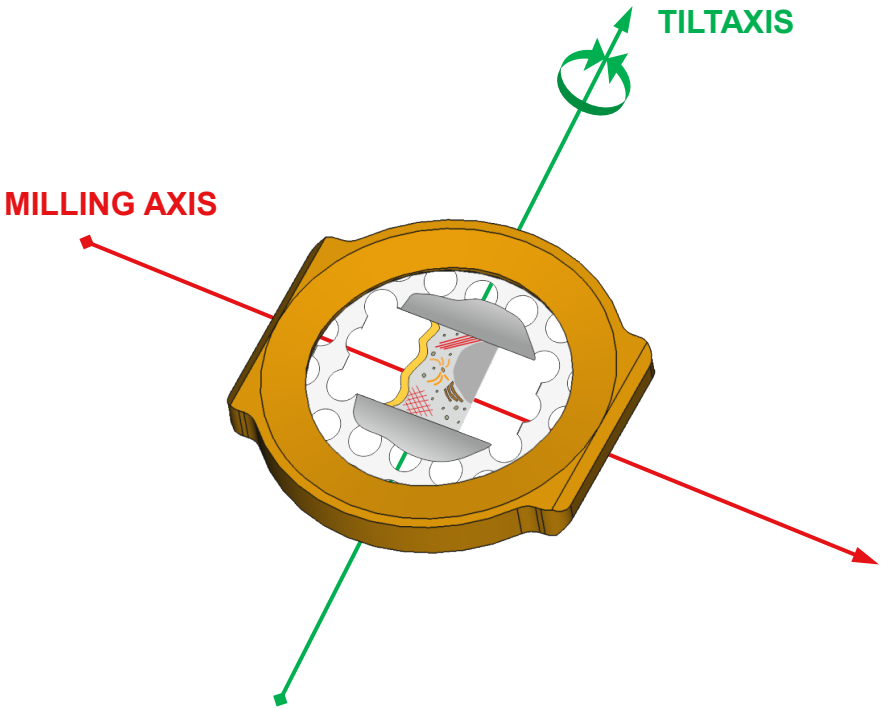
Arctis | Automated lamella prep 47 lamella in ~48hrs



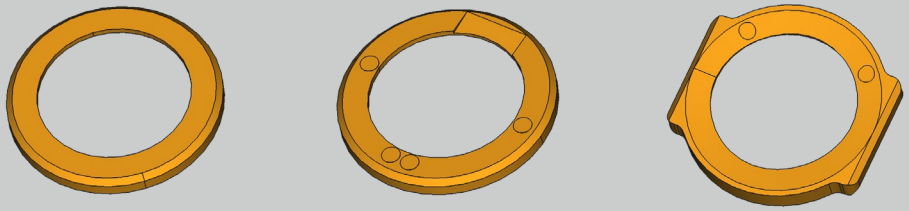
Arctis | New TomoGrid Concept



- Prone to rotation misalignment
- Assures correct alignment to TEM tilt axis

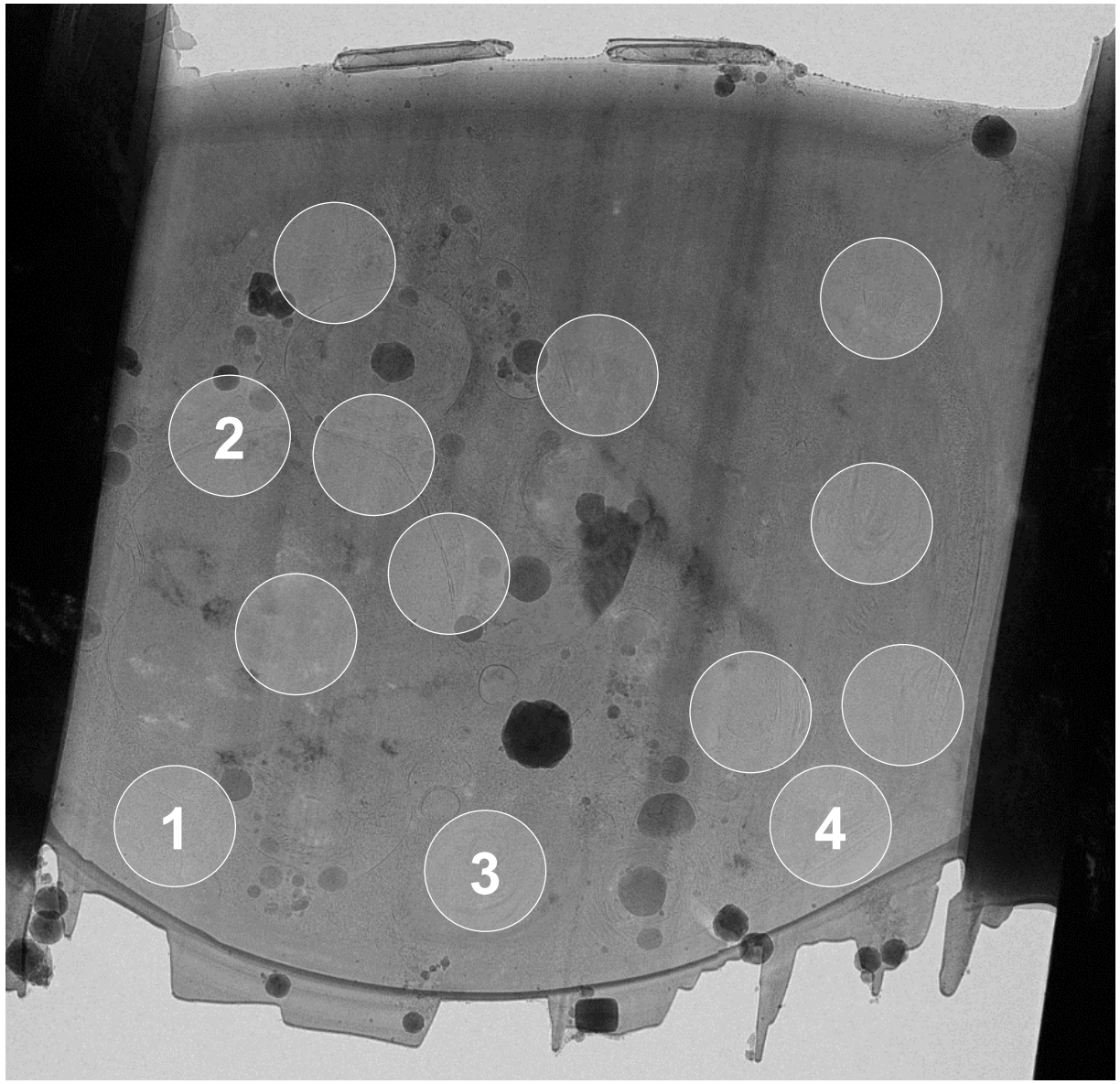


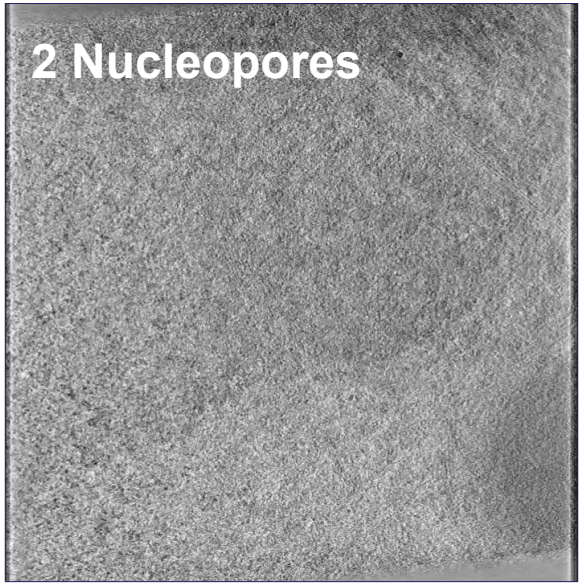
Autoloader-compatible grid-carriers:

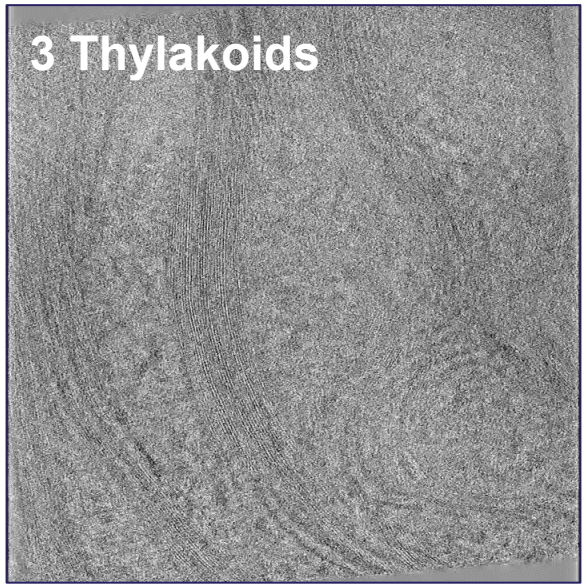


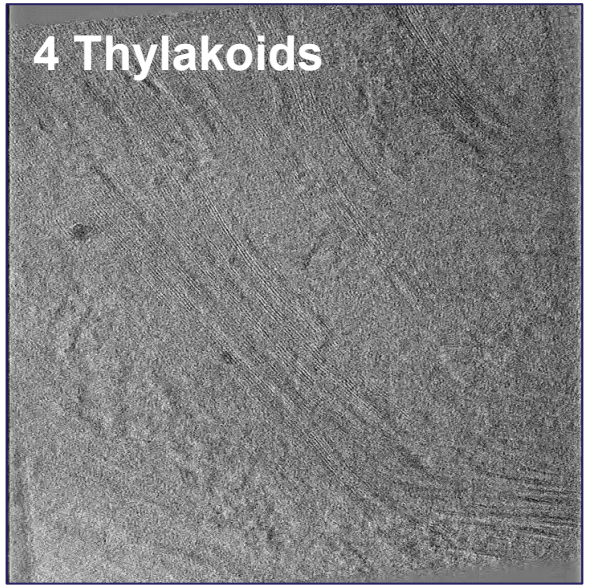
AutoGrid FIB-AutoGrid TomoGrid

Capturing multiple Tomography areas per lamella









Arctis | … in the EM-facility

Thank you