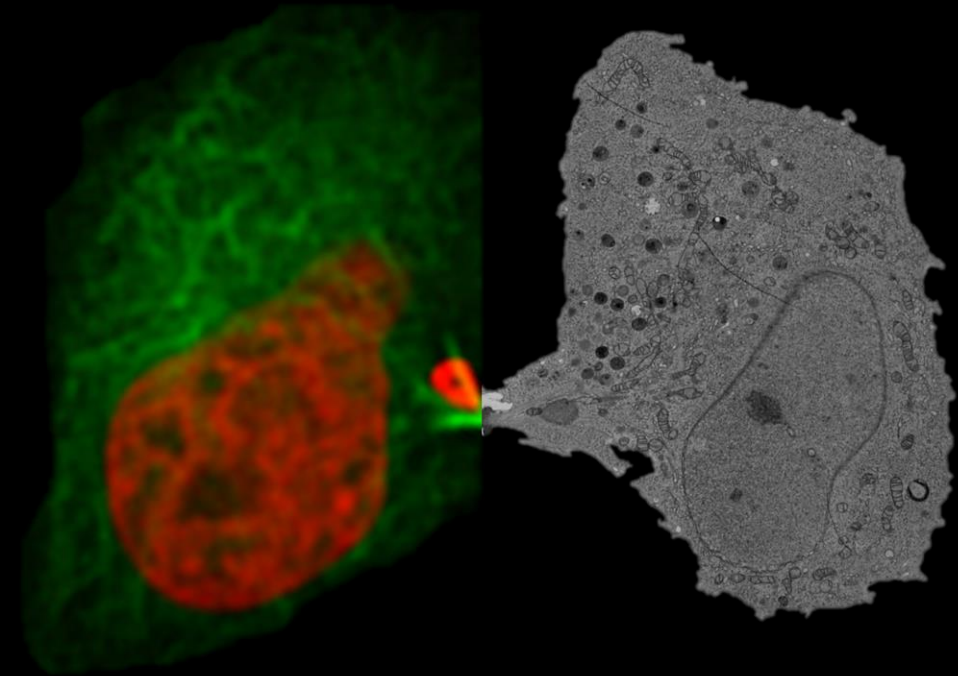


Routes to Correlative Light Electron Microscopy and latest developments

Dr. Andreia Pinto & Dr. Martin Fritsch

Advanced Workflow Specialists for EMEA

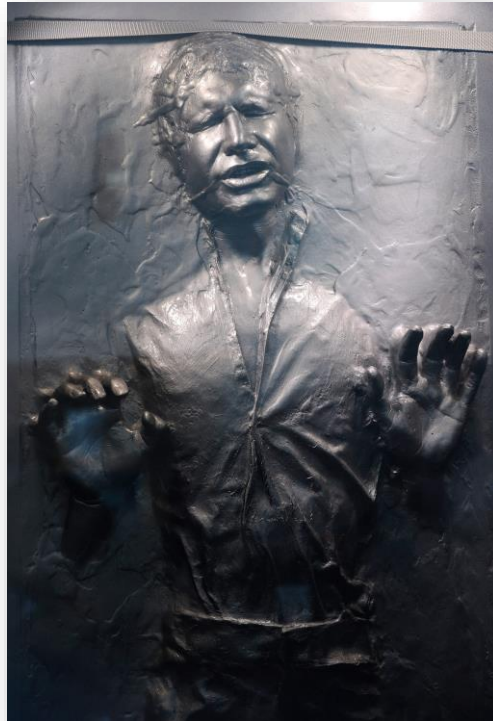
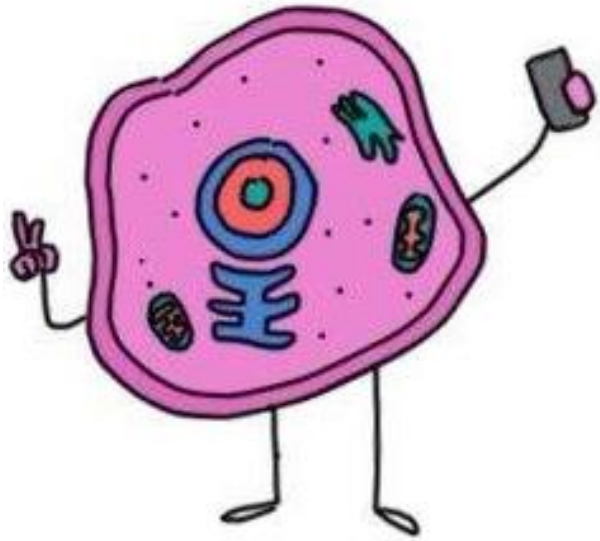


From Eye to Insight

What is your sample?

What is your goal?

What is your endgame?



Cryo TEM

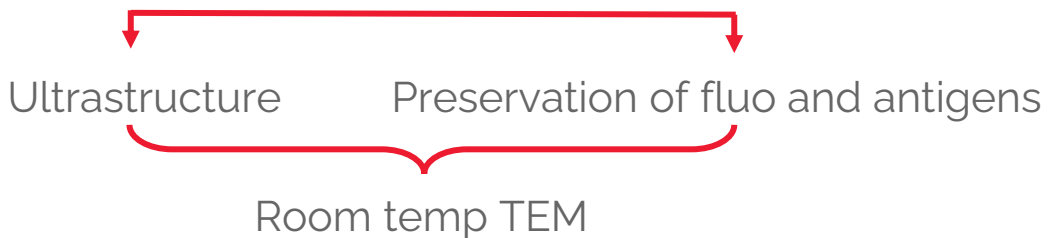


RT TEM

DECISION TIME: CORAL LIFE VS CORAL CRYO

High Pressure Freezing route [Hybrid WF]

- Sample size (shouldn't be thicker than 200 µm)
- Type of sample
 - ✓ Cells
 - ✓ Small organisms
 - ✓ Organoids
 - ✓ Thin slices of organs < 200µm
 - ✓ Plants
- Automatic freeze substitution



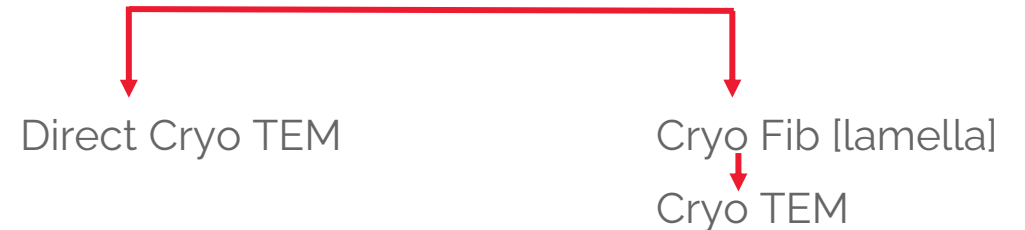
Come backs:

- Non-native state
- Access to ultrastructure and morphology
- Spaciotemporal EM
- Volume EM
- Non-destructive

Laborious – weeks of work

Plunge Freezing route [Full-Cryo WF]

- Sample size (shouldn't be bigger than 10 µm)
- Type of sample
 - ✓ Cells (animal cells and plant cells adherent or in suspension and funghi)
 - ✓ Single particles (proteins, virus)

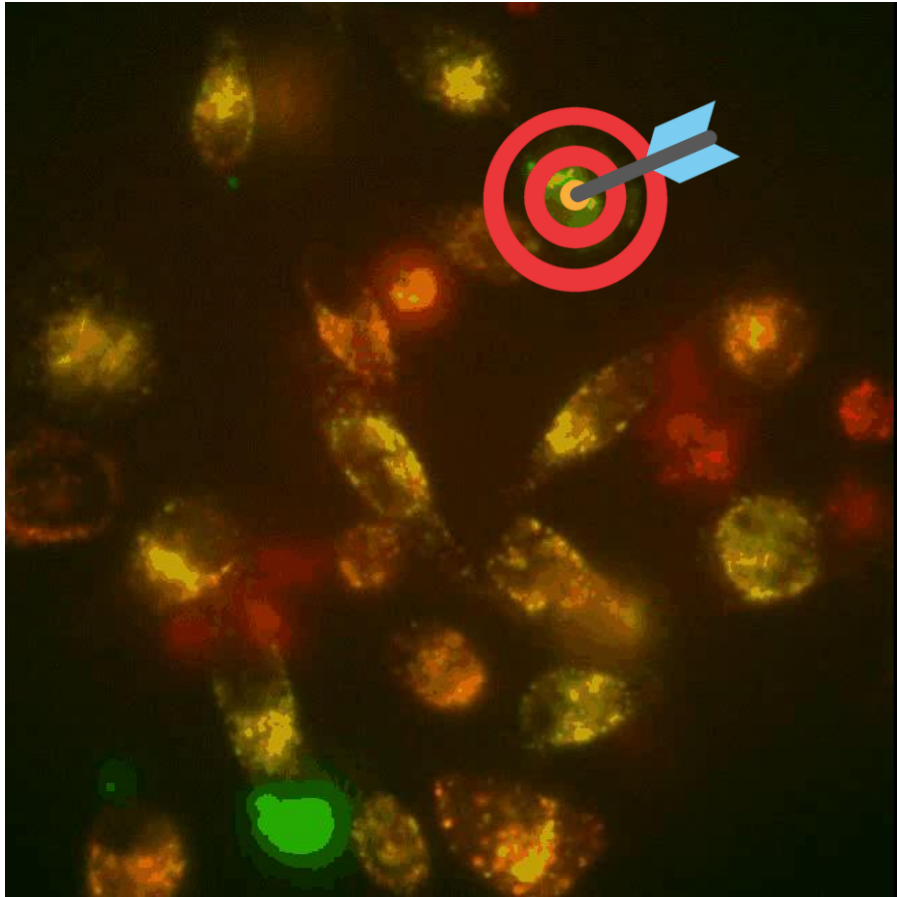


Come Backs:

- Native state
- High resolution tomography
- Access to structure
- Destructive (FIB)
- Small volumes
- Targeted correlation

Fast turnaround time

Spatiotemporal TEM



IDENTIFY



IMMOBILIZE



SCREEN

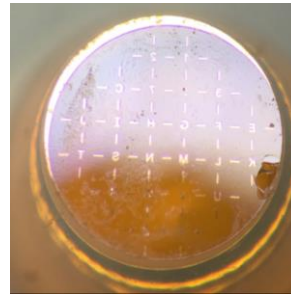
Automatic Freeze Substitution | Embedding | Sectioning



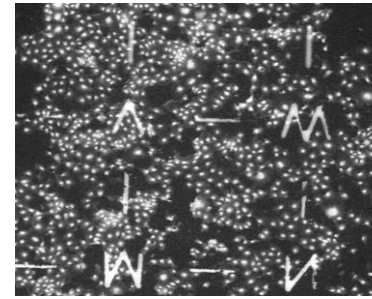
1
EMBEDDED SAPPHIRE



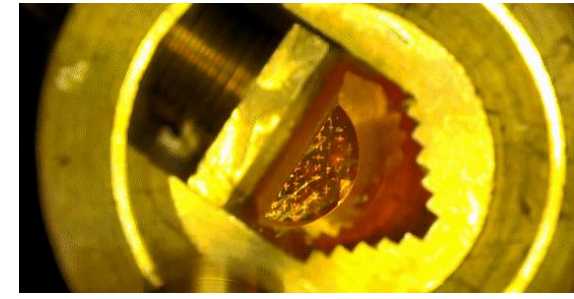
2
EMBEDDED SAPPHIRE



3
RELEASED SAPPHIRE



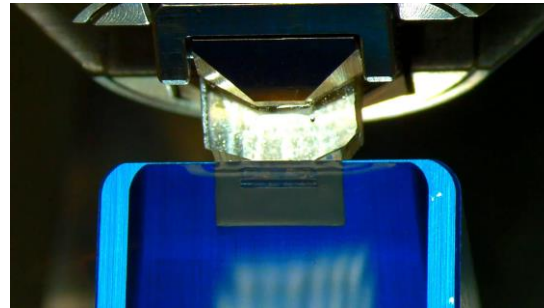
4
SEM IMAGE SURFACE



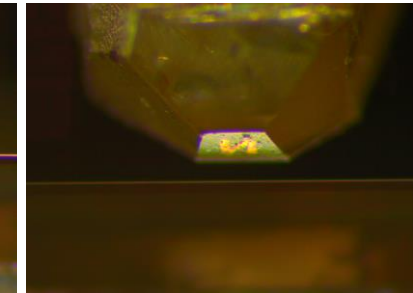
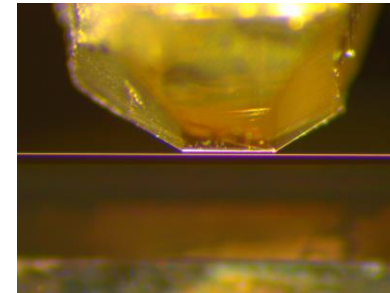
5
TRIMMING



Staining and
imaging

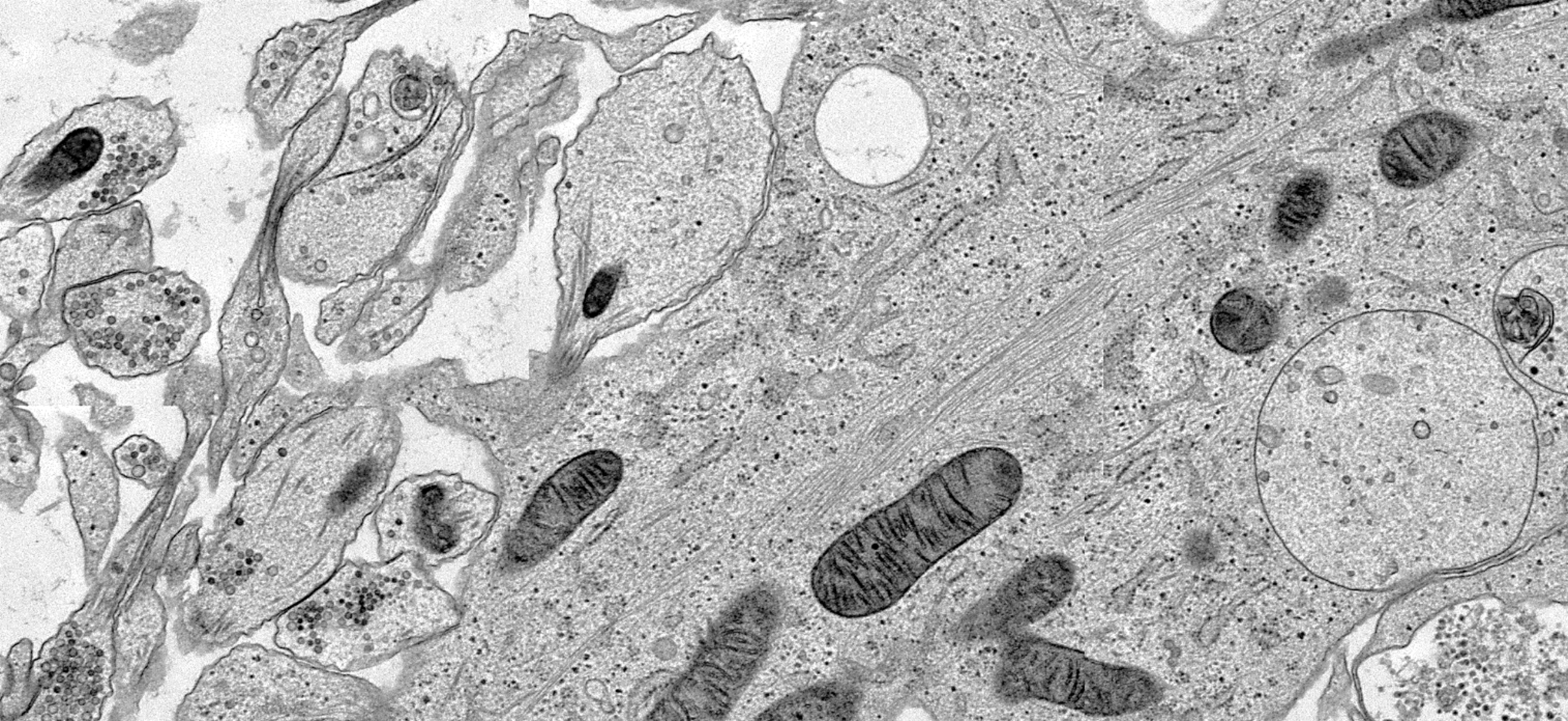


7
SECTIONING & PICK UP



6
ALIGNING

Processing: Freeze Substitution



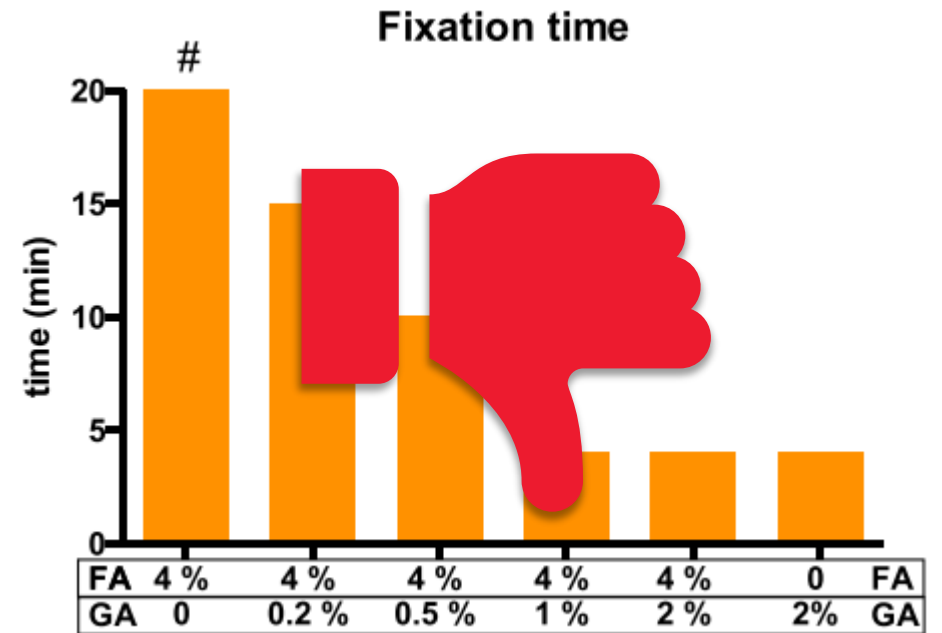
Specimen courtesy and sample preparation by G. Bosshard and S. Tyagarajan, Institute of Pharmacology and Toxicology Andres Käch, ZMB, University of Zurich, Switzerland

Strictly confidential | Dr. Andreia Pinto | 2023-06-27

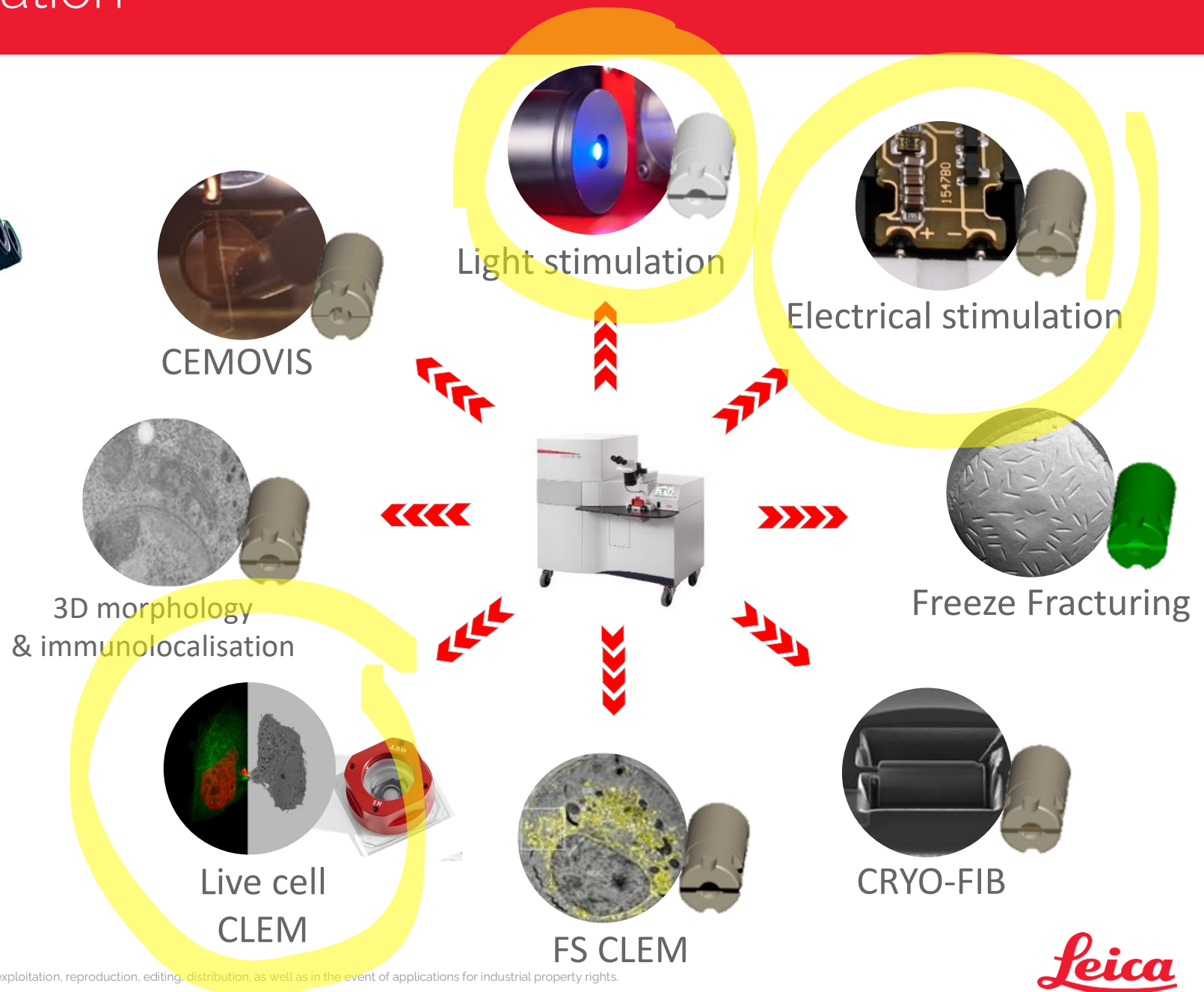
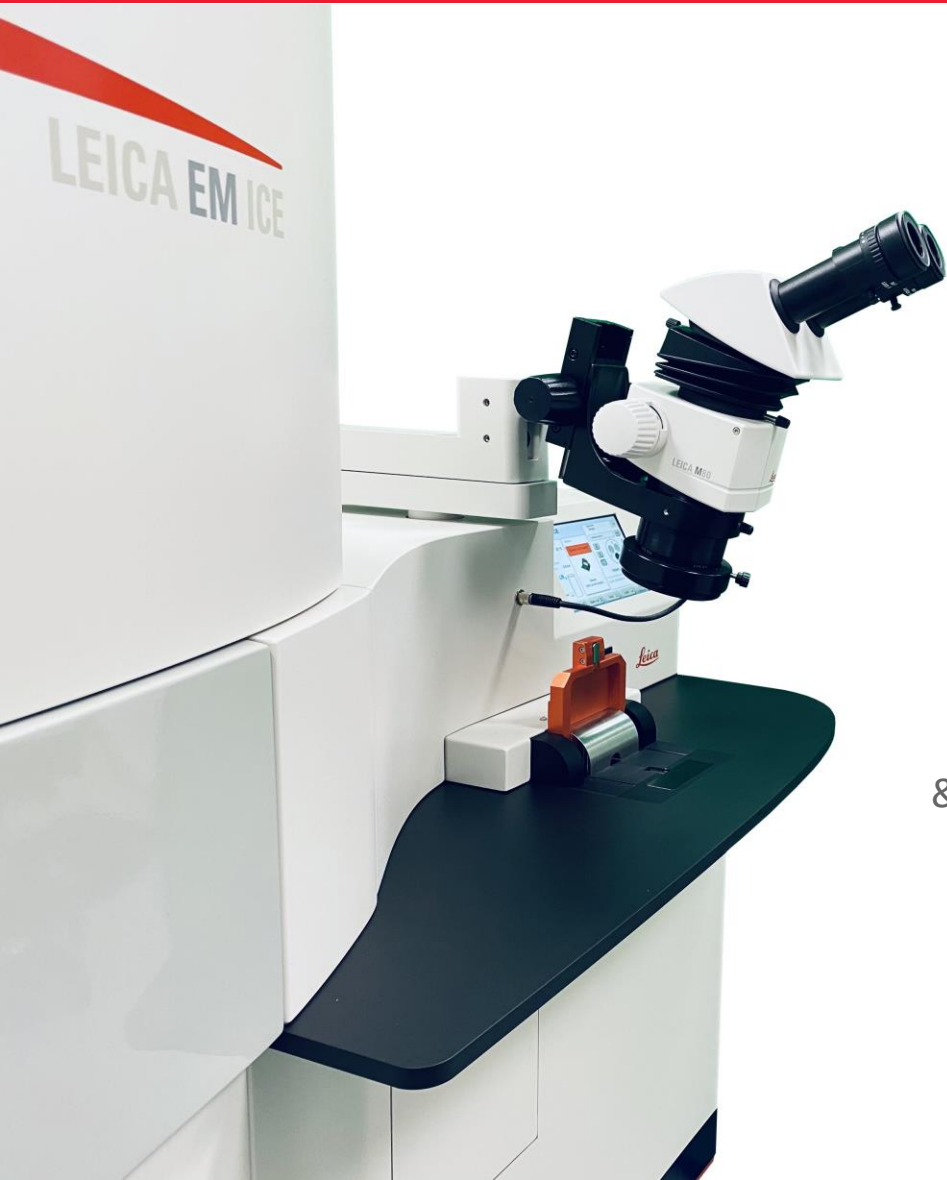
© Leica Mikrosysteme Vertrieb GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.

Leica

Adding time into the equation

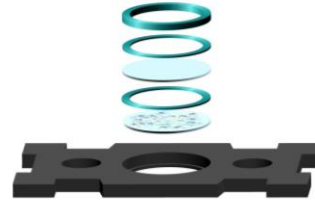


Adding time into the equation

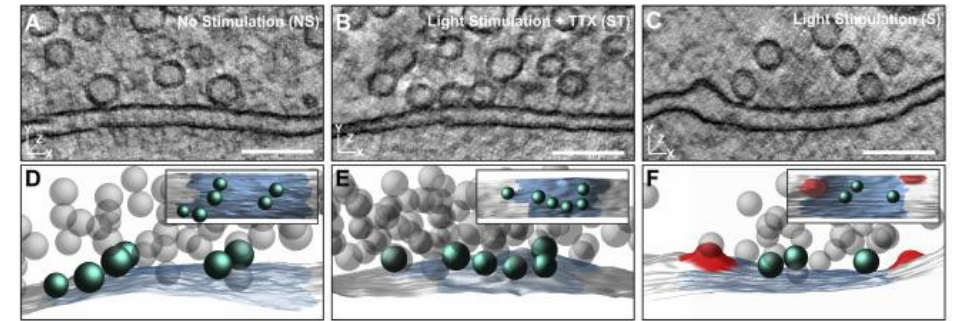


Adding time into the equation

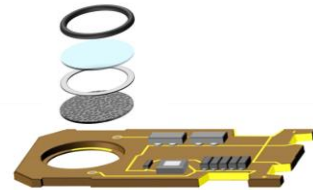
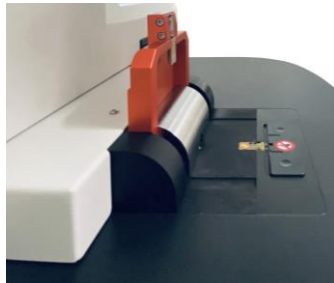
Light Stimulation



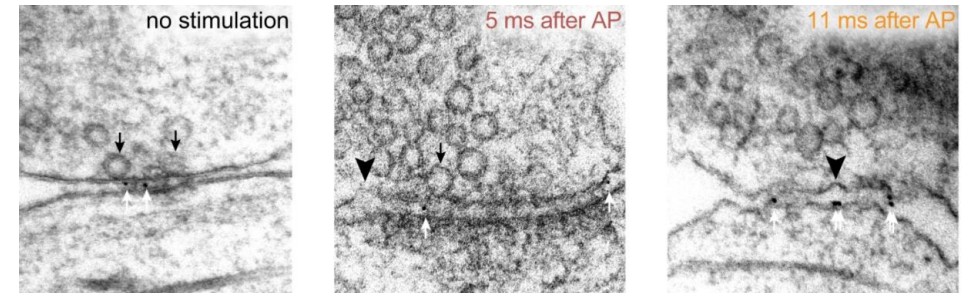
~ 5-10 ms



Electrical Stimulation



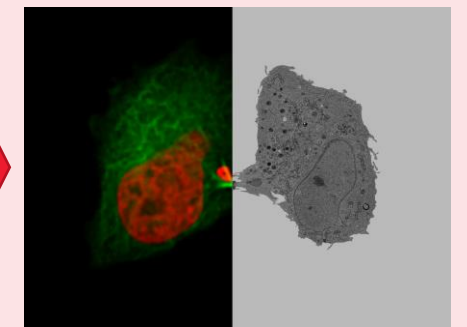
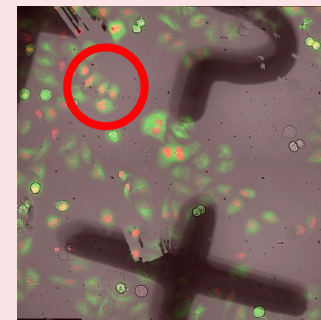
~ 1 ms



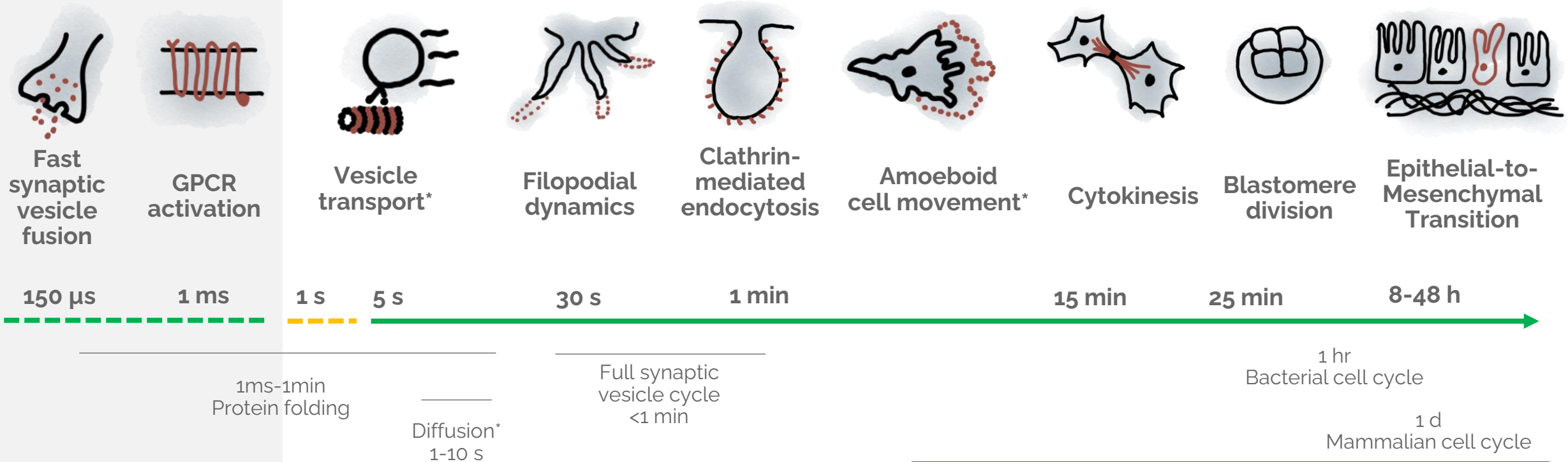
Live-Cell CLEM



~ 2-10 s



Timescales of Biological Processes and accessibility by Live-cell CLEM workflow



EM ICE with Light and/or Electrical stimulation

***over a 10 μ m distance**

[https://zfin.org/zf_info/zfbook/stages/Copos et al. Volume 112, Issue 12, 20 June 2017, Pages 2672-2682n](https://zfin.org/zf_info/zfbook/stages/Copos%20et%20al.%20Volume%20112,%20Issue%2012,%2020%20June%202017,%20Pages%202672-2682n)
https://en.wikipedia.org/wiki/Synaptic_vesicle

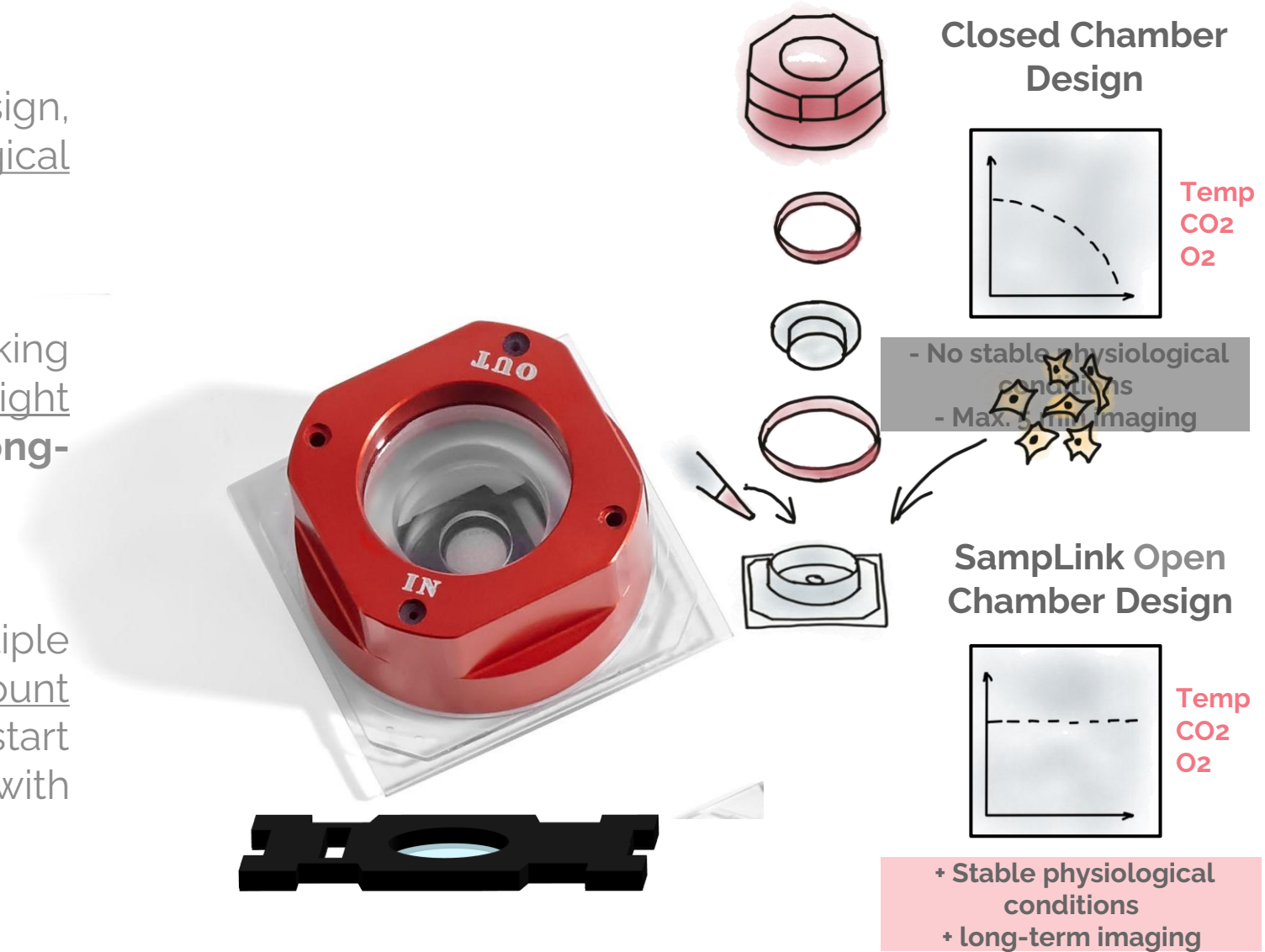
Sabatini and Regehr Nature 1996 Nov 14;384(6605):170-2. doi: 10.1038/384170ao.

Coral Life workflow - Samplink Chamber

SampLinks open chamber design, ensures stable physiological conditions.

Enjoy peace of mind while looking for the right cell at the right moment and benefit from **long-term live imaging**.

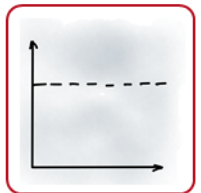
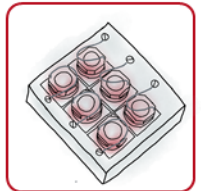
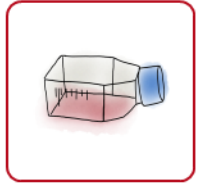
Perform live imaging on multiple cells without the need to remount the sample to ensure you start your **live cell CLEM** workflow with the “best” cell.



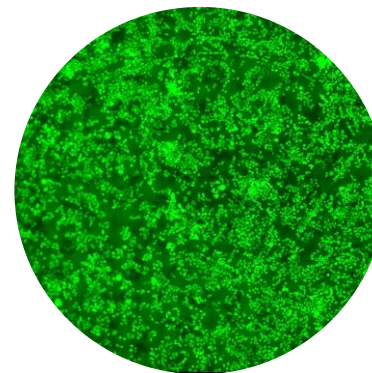
Oxygenie Incubator



- **Enables transport** between labs and institutes without compromising the samples
- **Multi sample imaging**
- **16 h temperature, CO₂ and O₂ control**
 - Temperature controller
 - 2x gas bottle connection
 - Space for 6 sample chambers



Thunder Imager

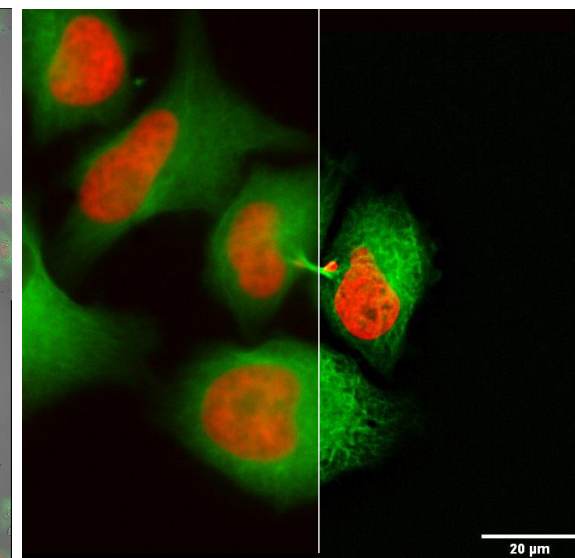
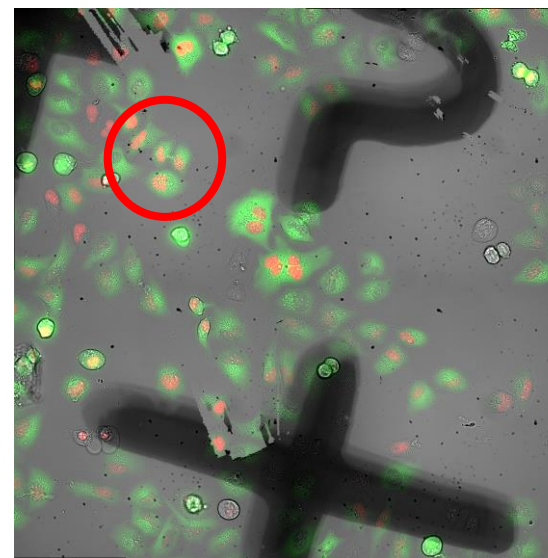
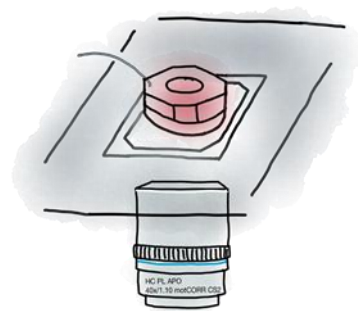


Overview image fluorescence with transmission light



Sapphire corrected objective

40x/1.1 NA W mot CORR CS2



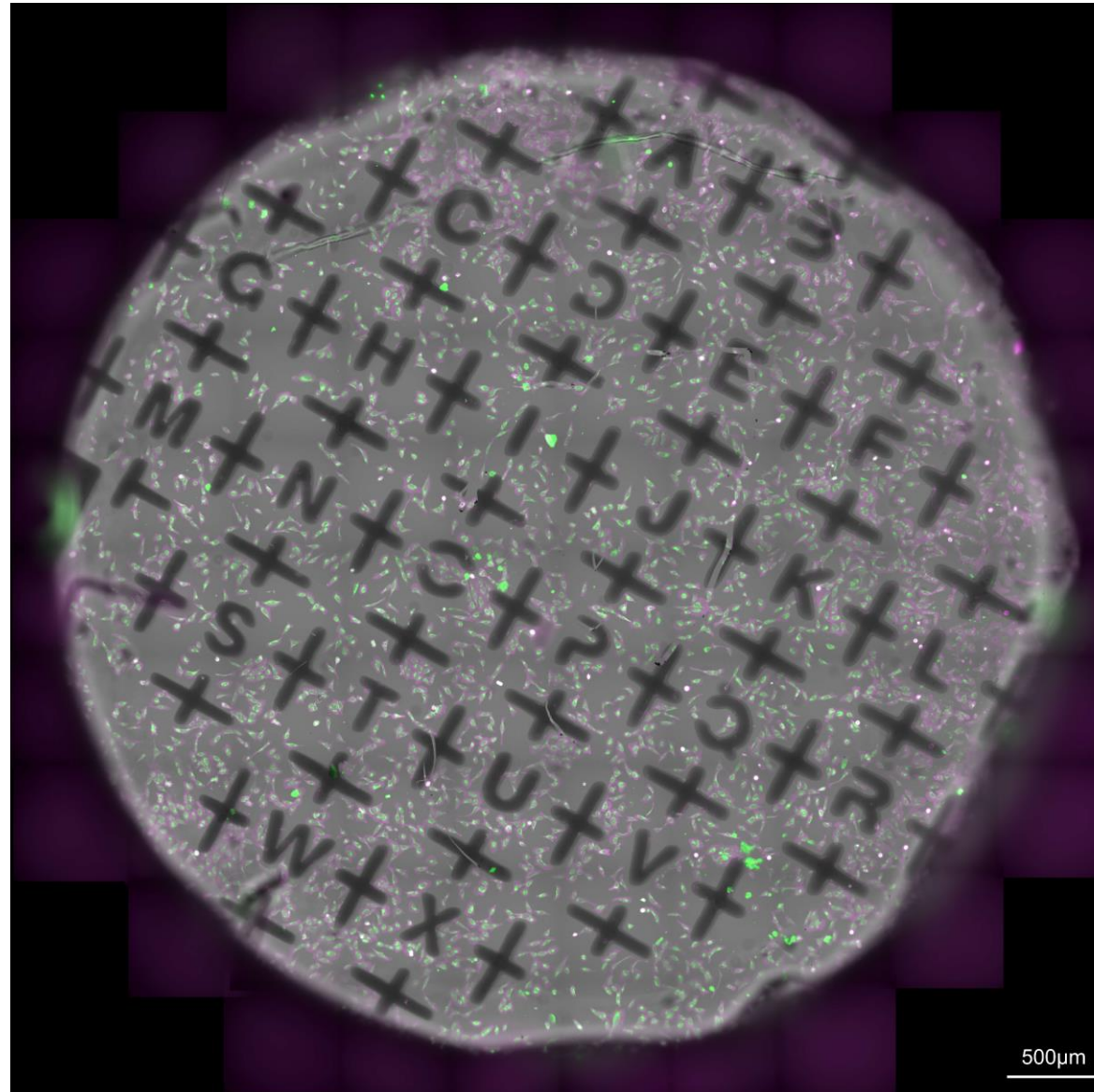
Widefield imaging

THUNDER Imager
with 40x/1.1 sapphire objective



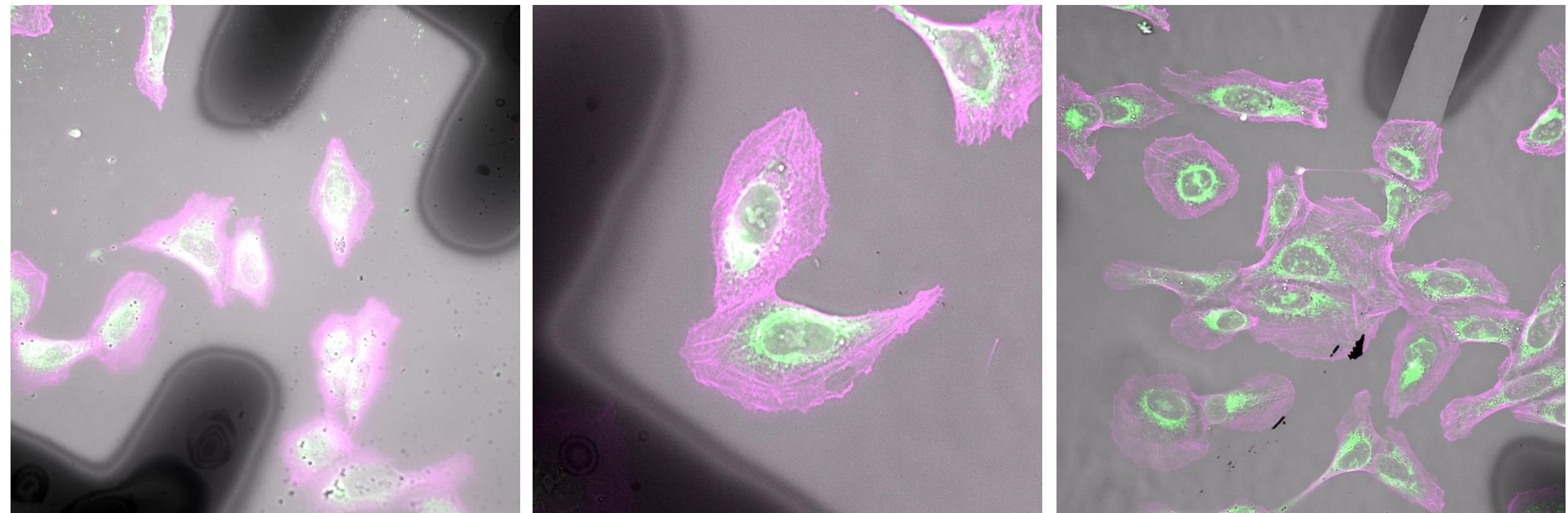
Thunder Imager – Samplink complete Sapphire Screen

Pattern coated sapphire disc



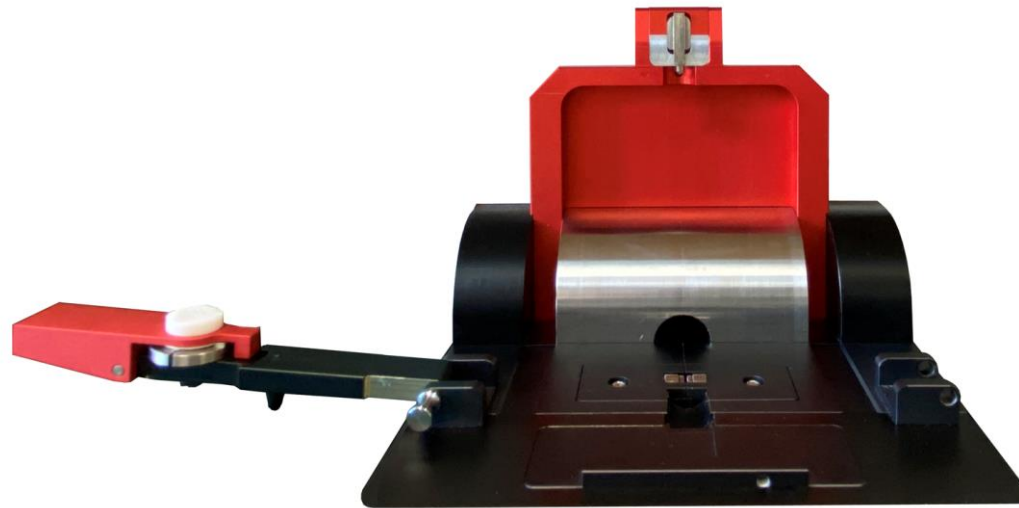
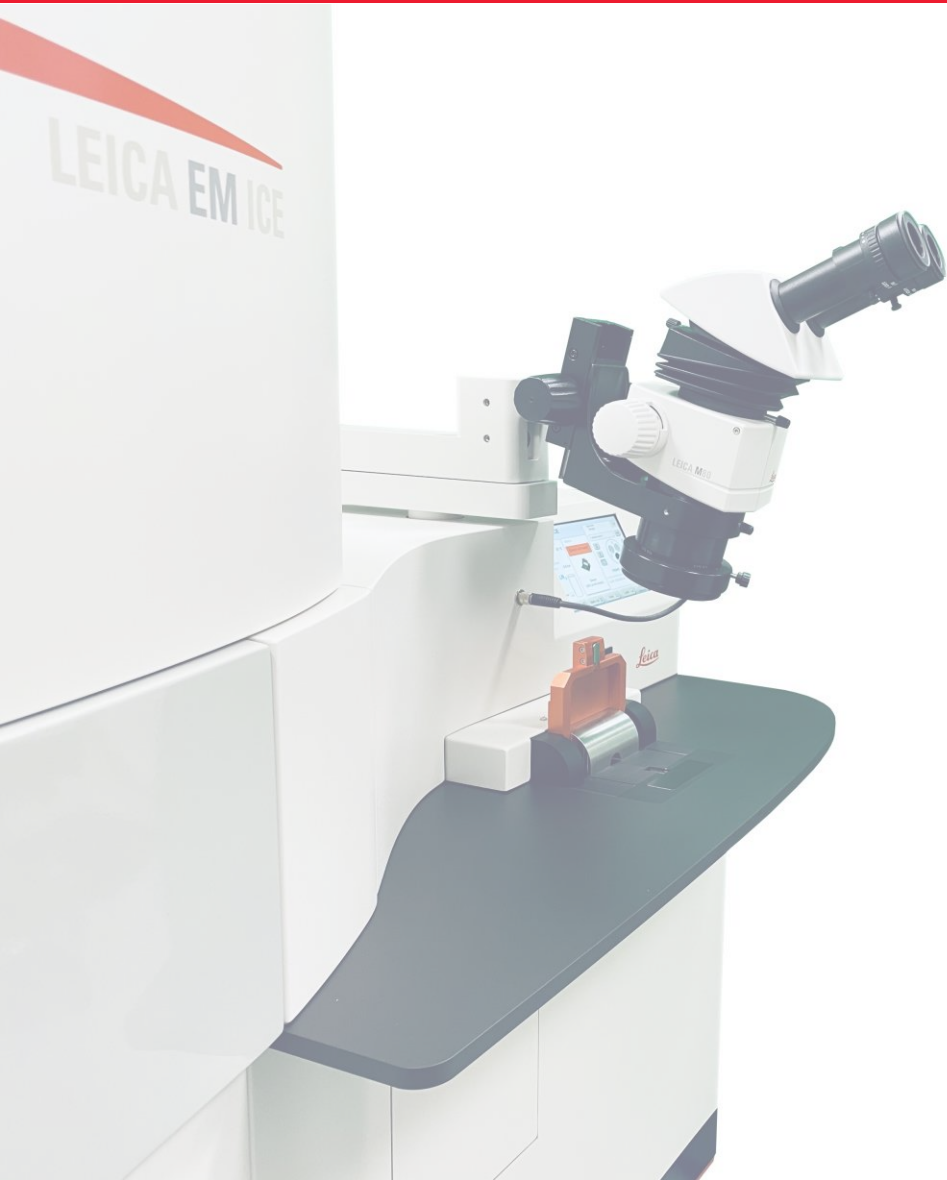
Overview image:
Mitochondria, **actin** and
brightfield channel

Thunder Imager – Samplink complete Sapphire Screen

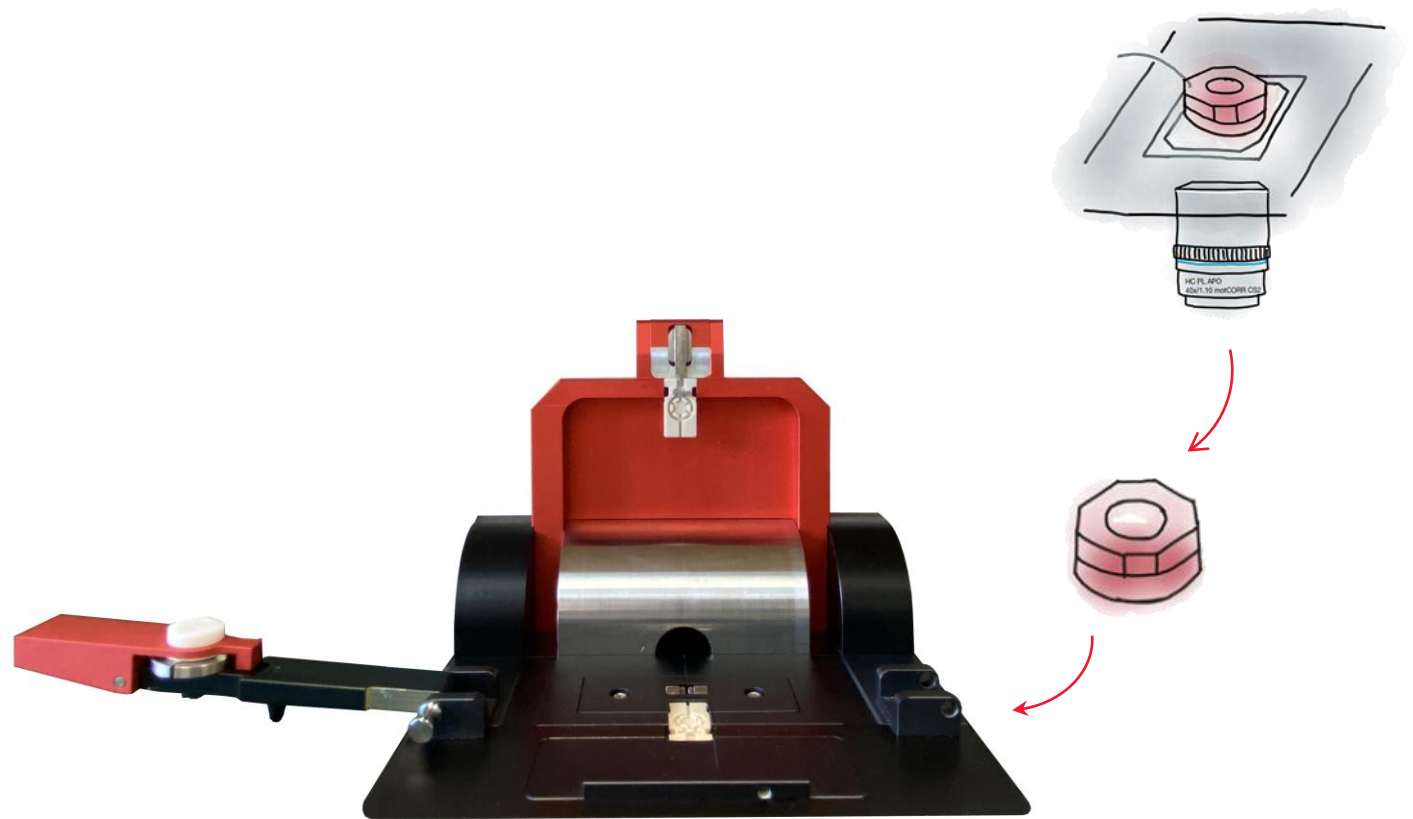
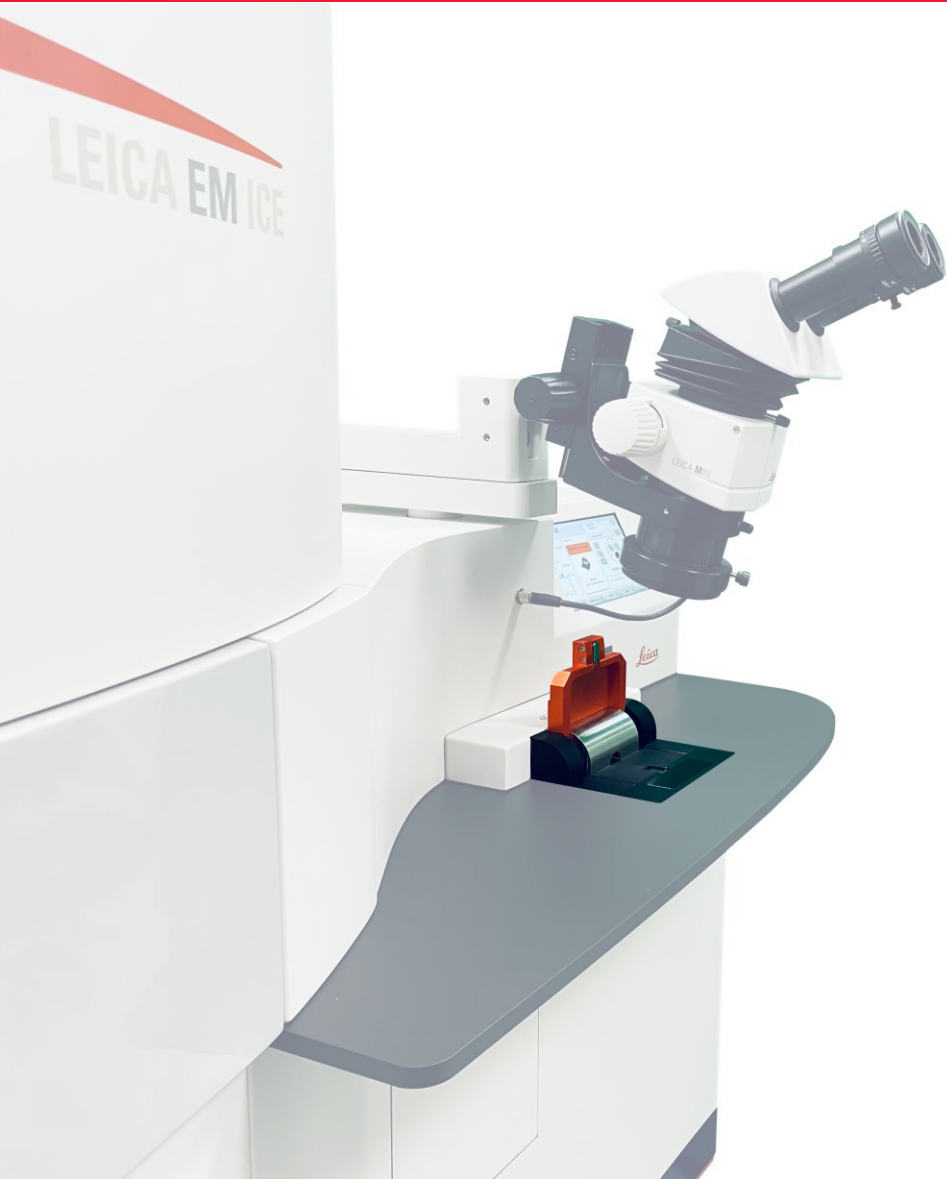


Mitochondria, actin and
brightfield channel

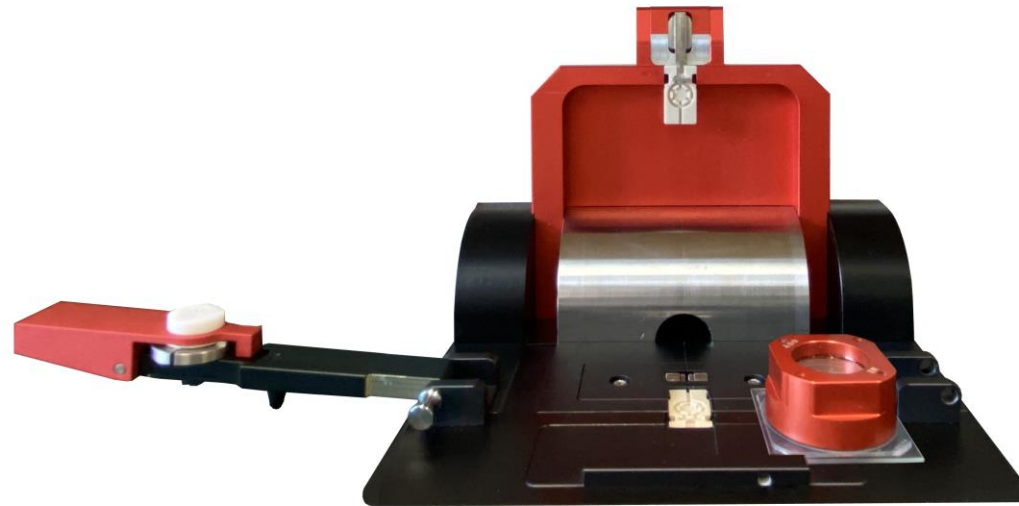
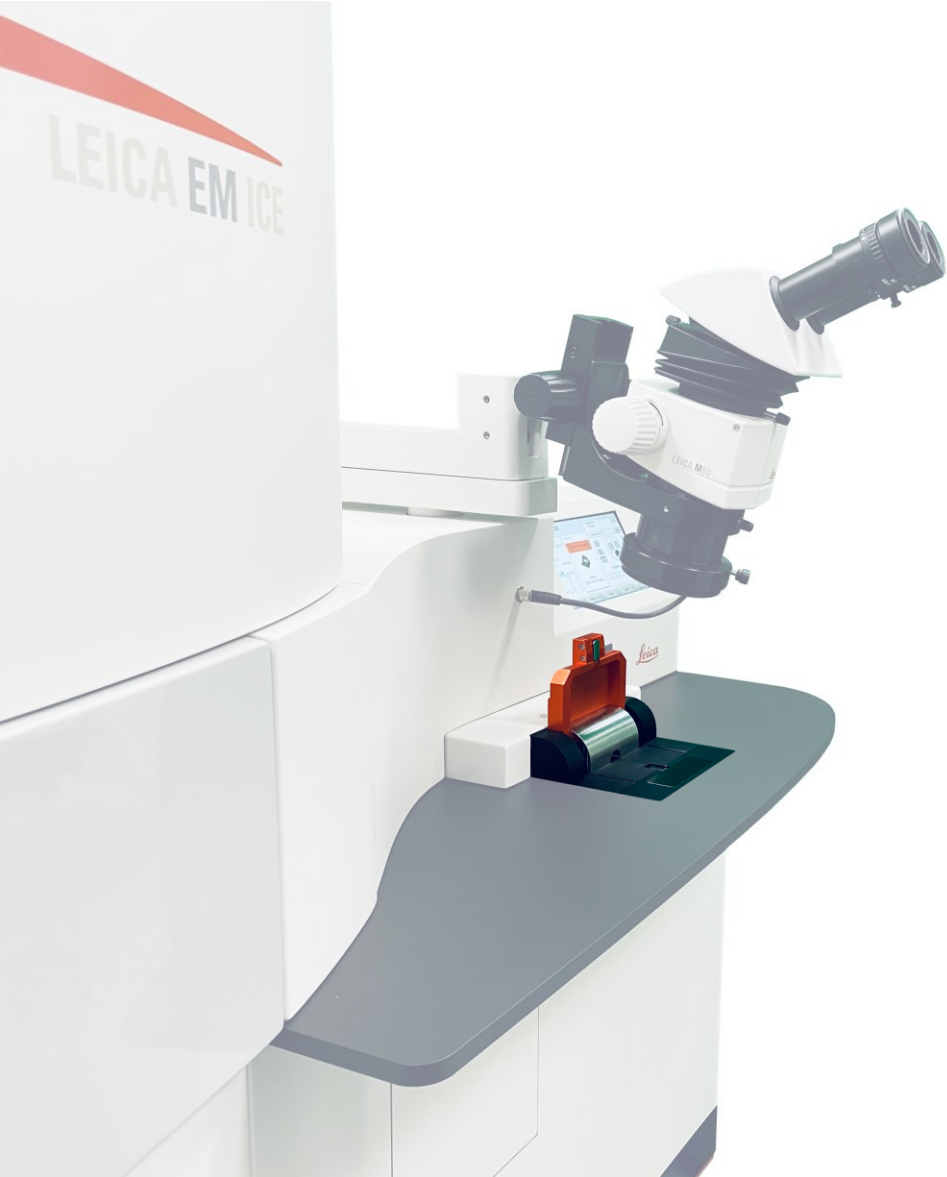
NANO Workflow



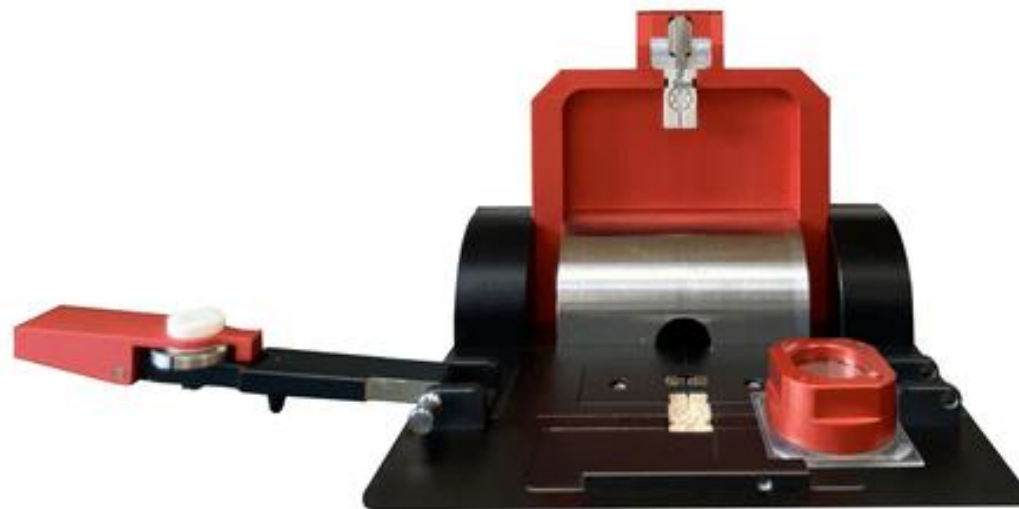
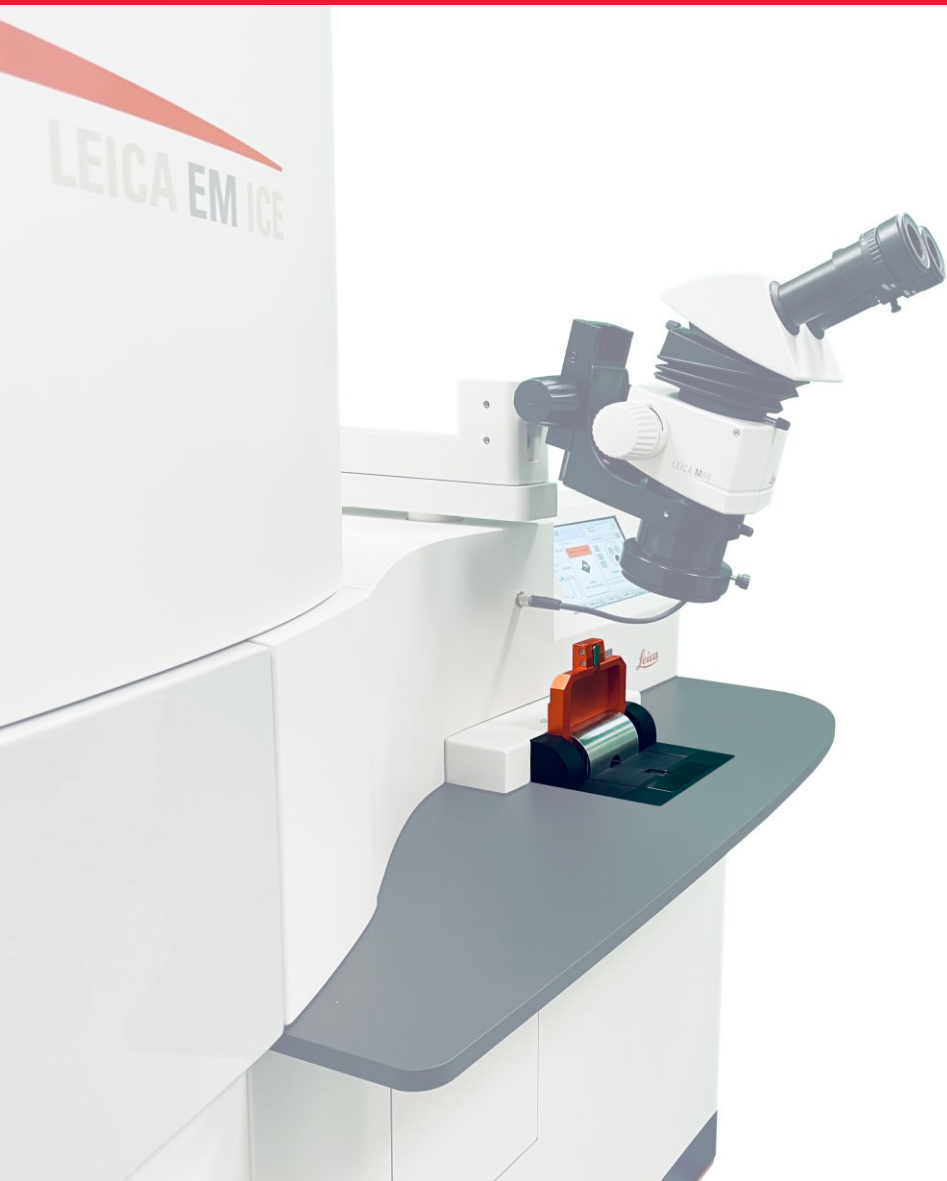
NANO Workflow



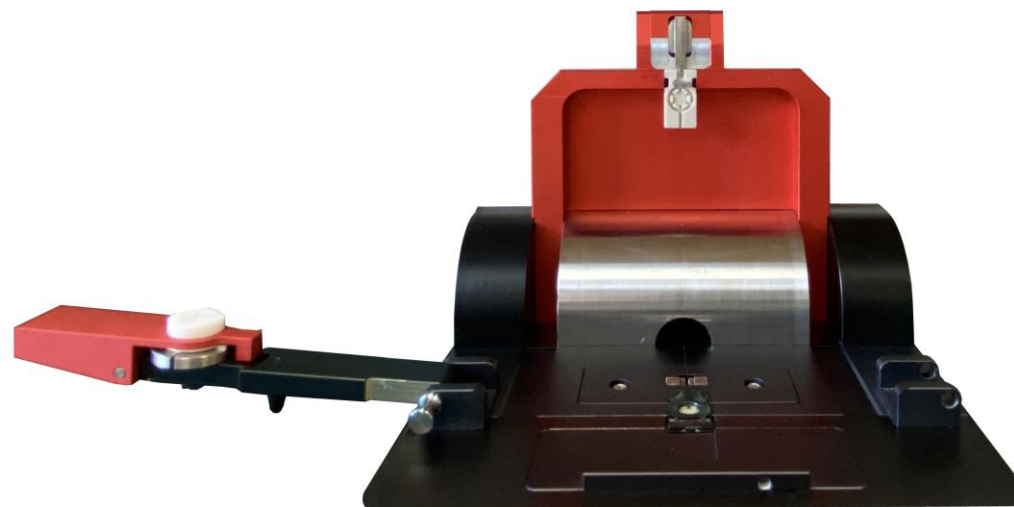
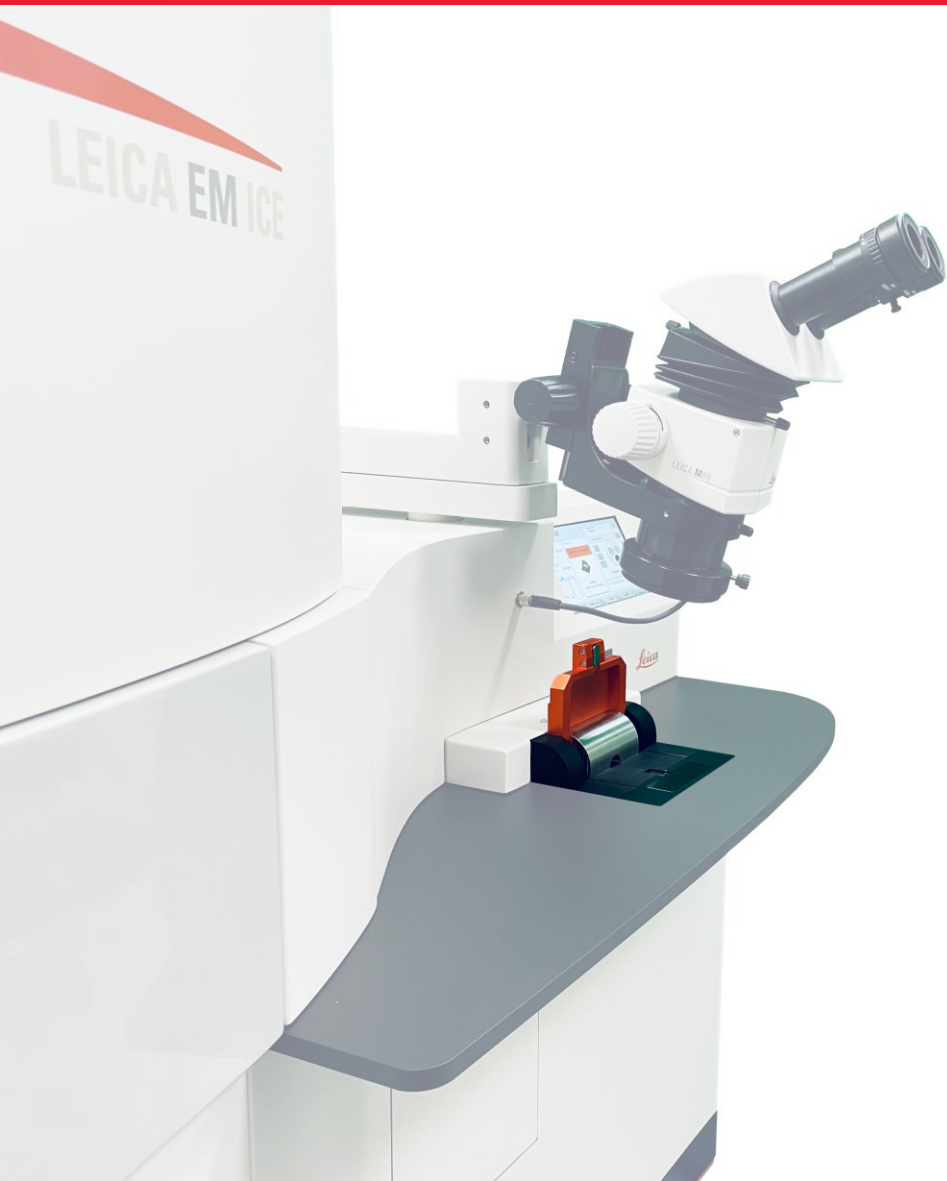
NANO Workflow



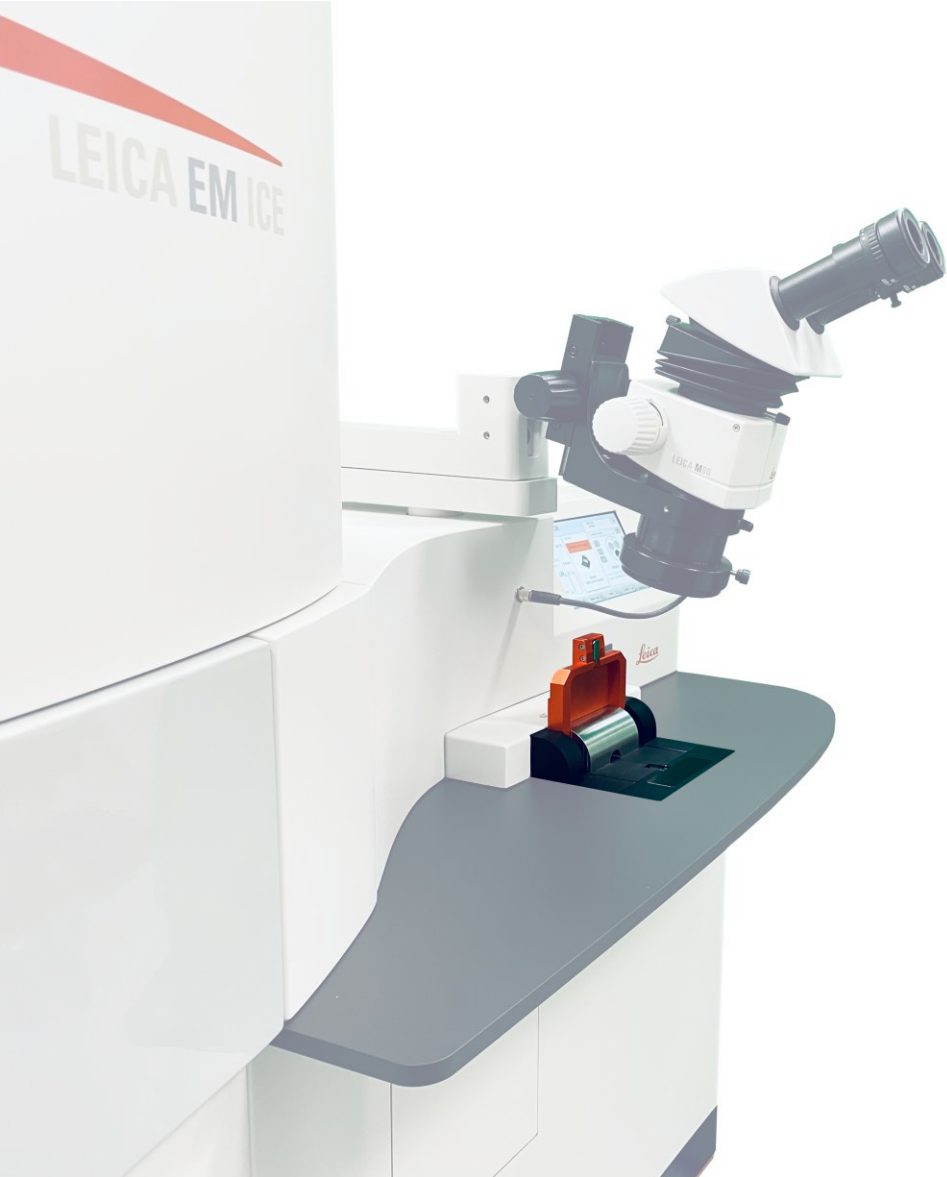
NANO Workflow



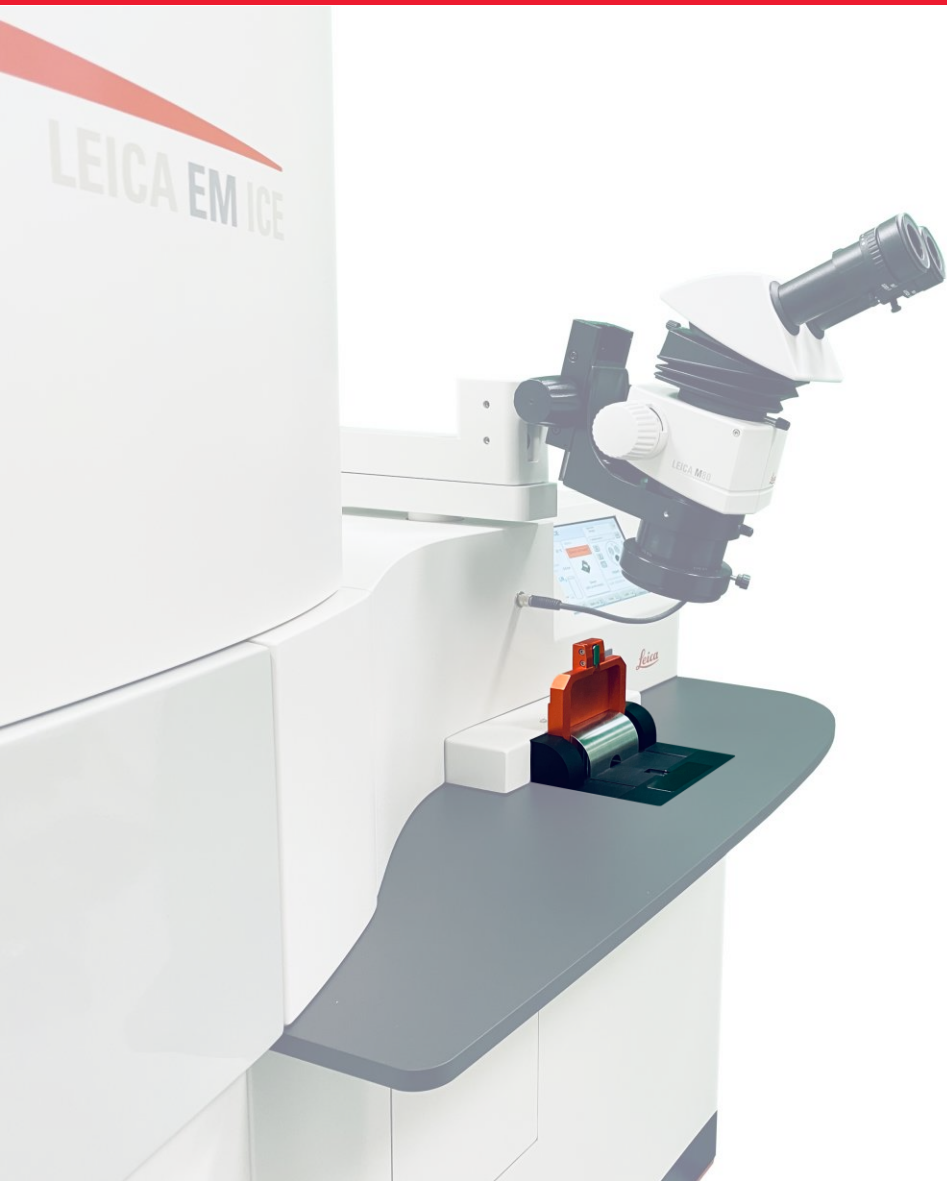
NANO Workflow



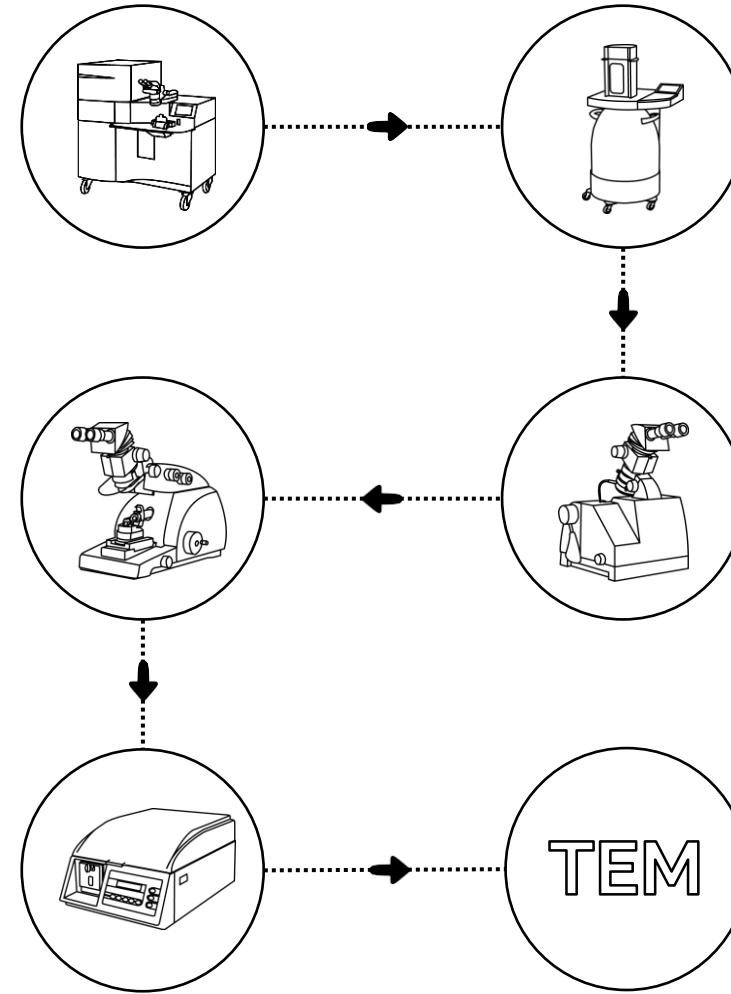
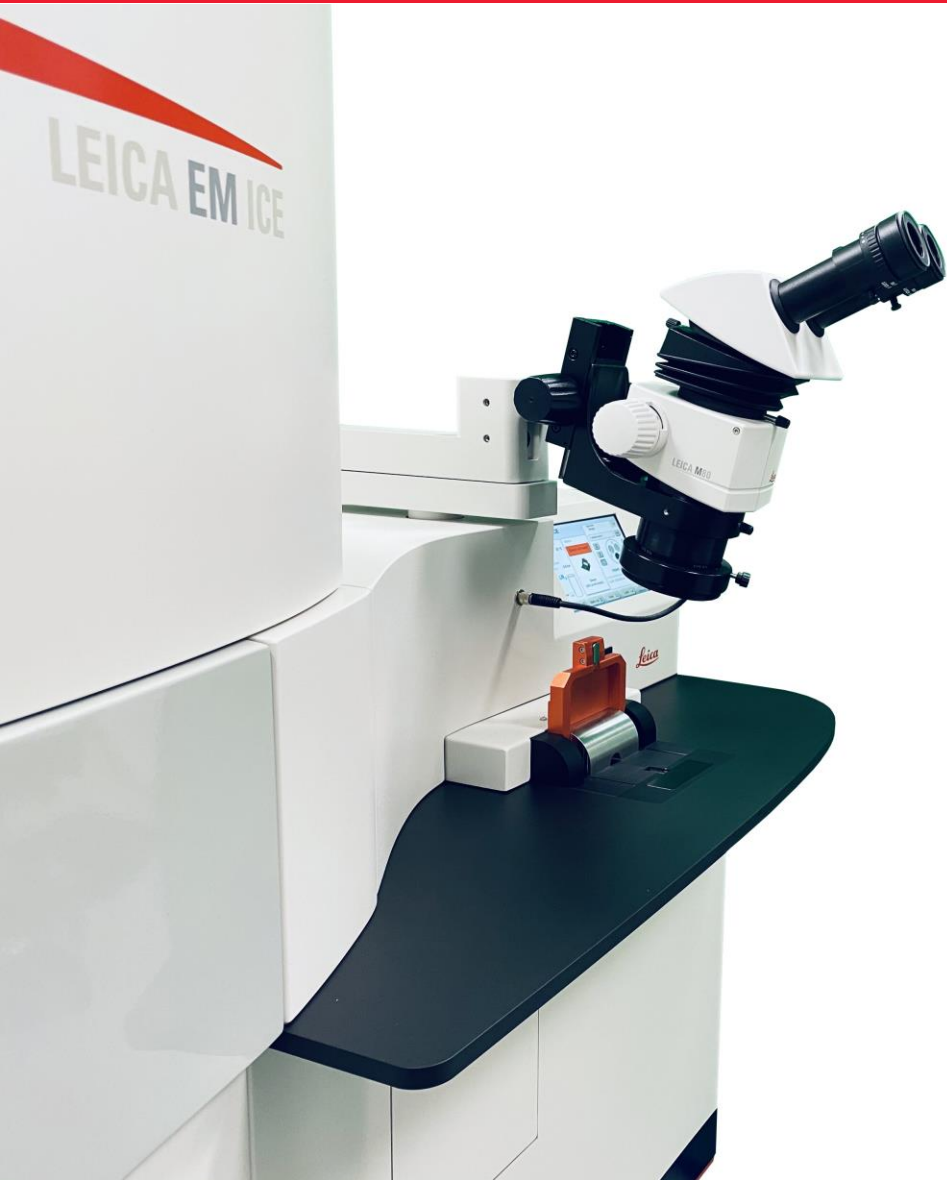
NANO Workflow



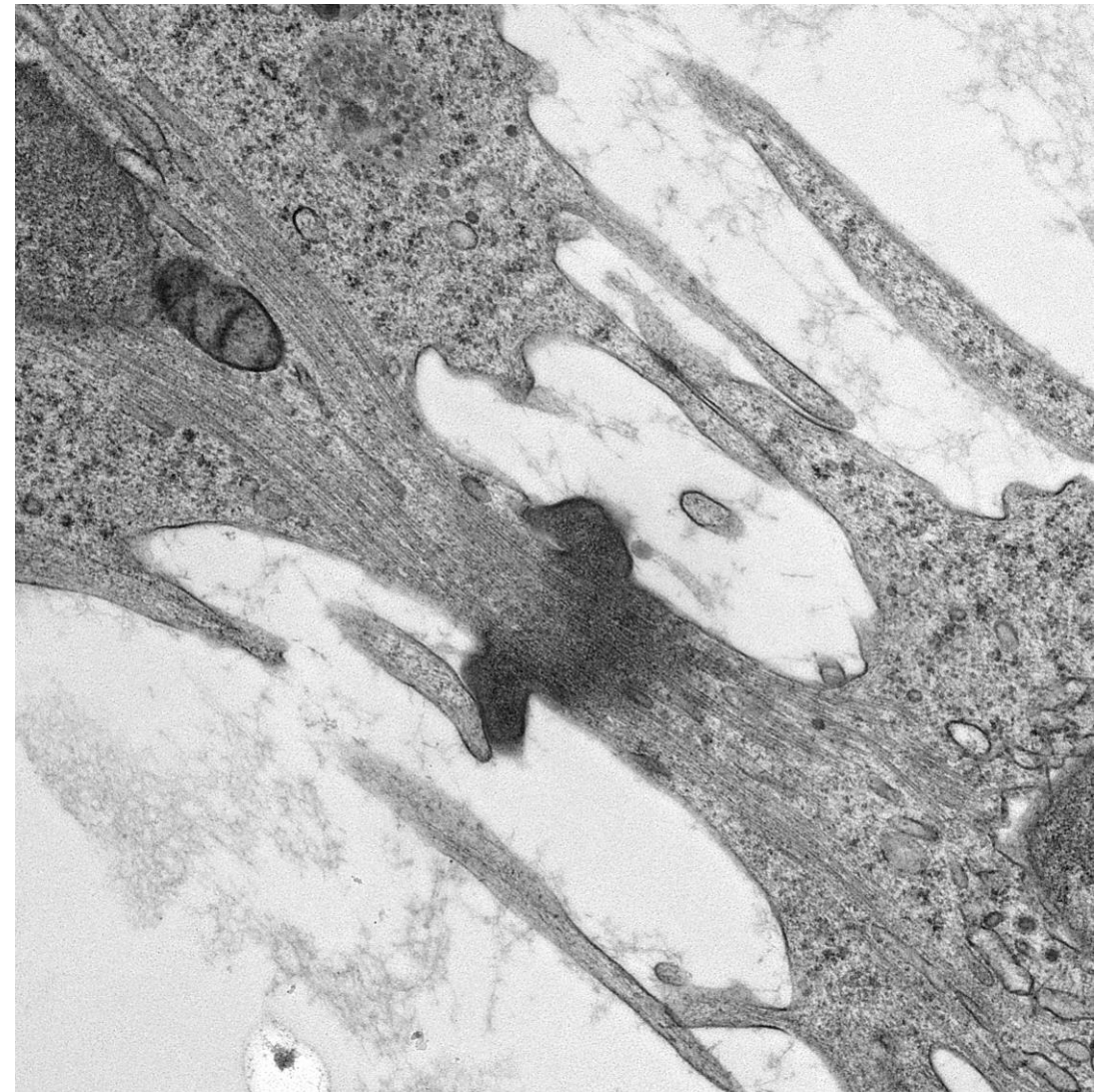
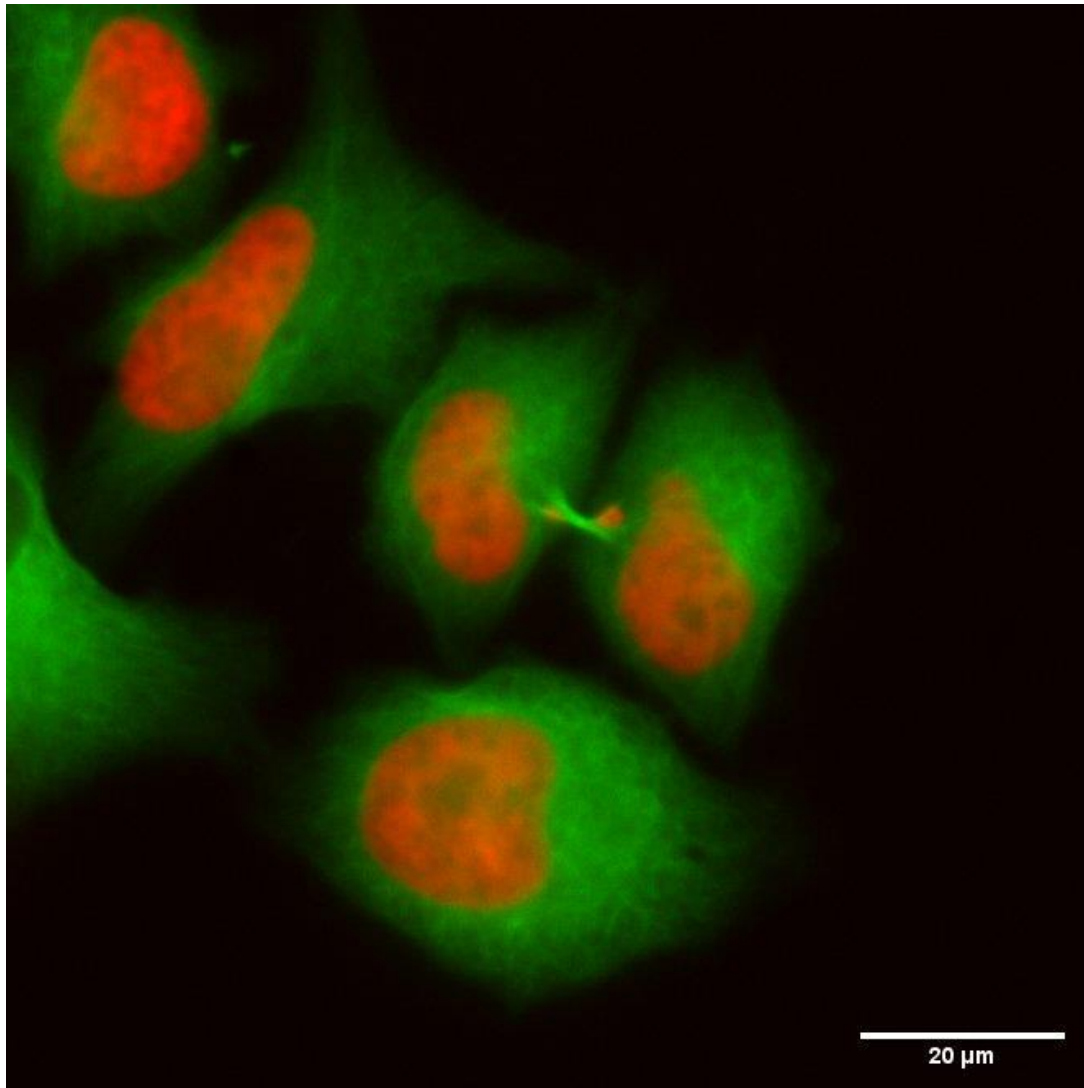
NANO Workflow



NANO Workflow



Correlation

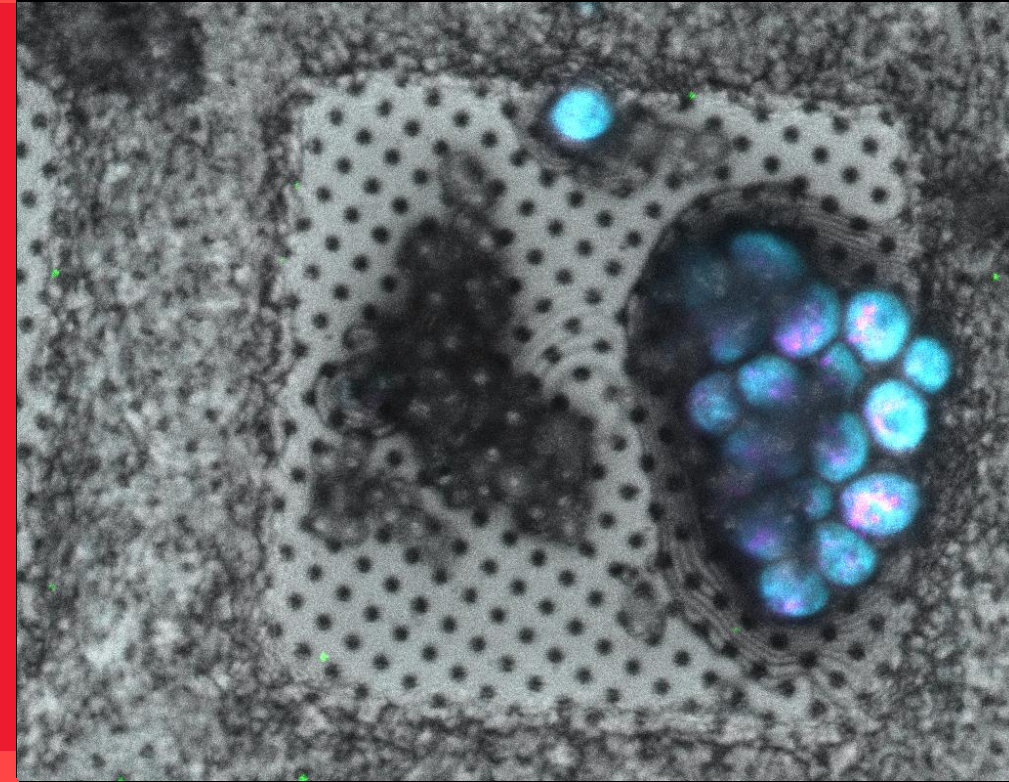


4 Major benefits of the Leica Coral Life Workflow

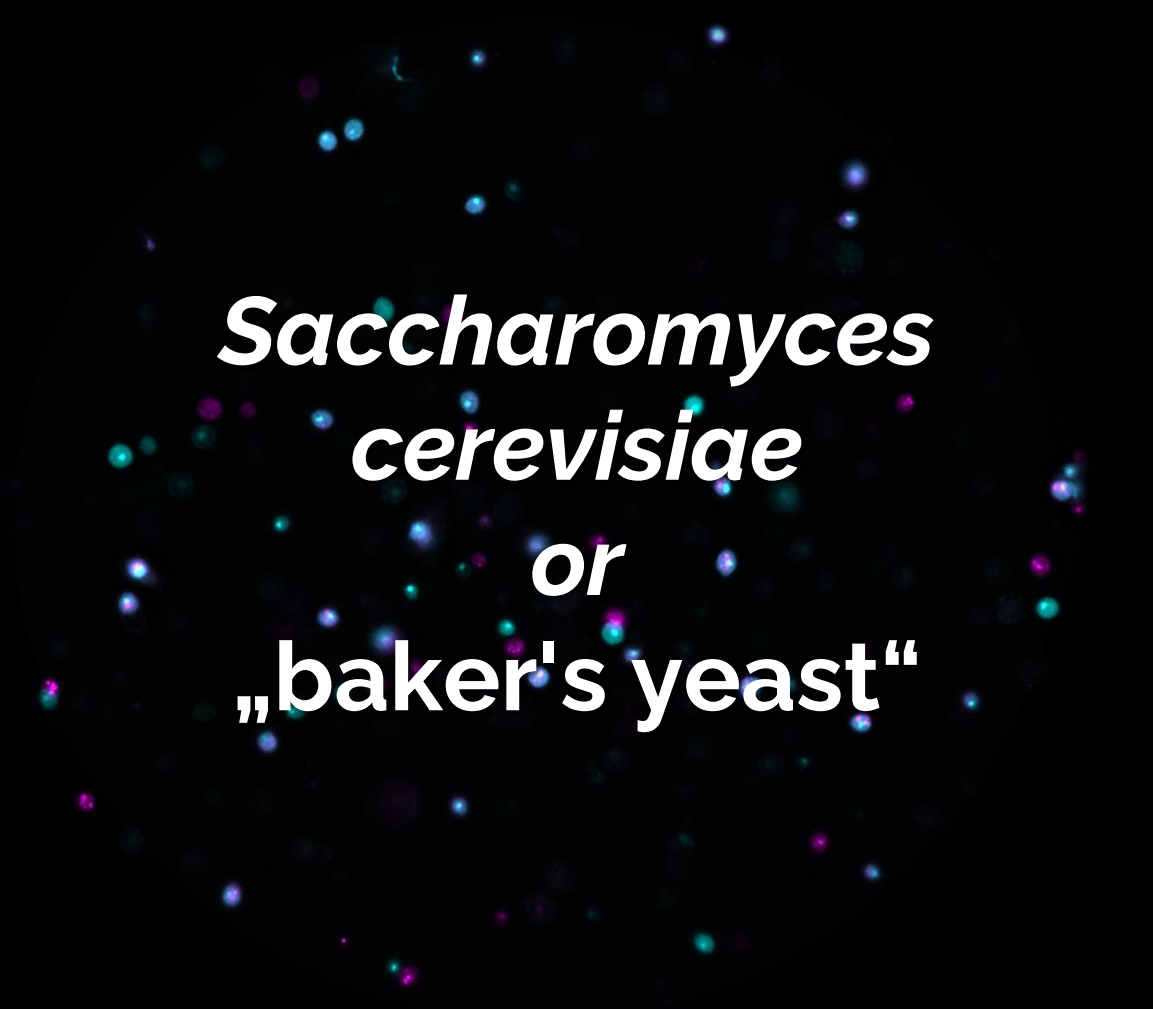
- 1 Keeping the sample in physiological conditions at all times.**
- 2 Capturing of fast cellular events.**
- 3 Obtaining excellent fixation results.**
- 4 Reliable retrieval of the event of interest in the electron microscope.**

3D Fluorescent Structures Targeting Solution for Cryo-Electron Tomography

Latest developments in Correlation Light Electron
Microscopy



A CRYO CLEM walk-through with,



*Saccharomyces
cerevisiae*
or
„baker's yeast“

Sample glow discharge and plunge freezing



ACE200



Glow discharging



GP2 loading forceps

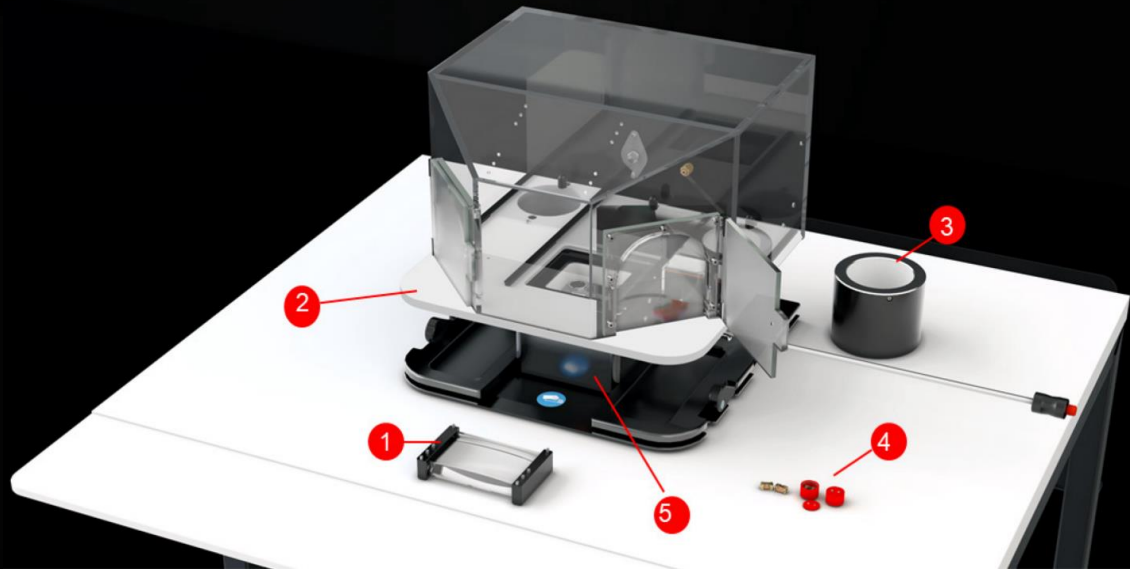


GP2 loading sample



GP2

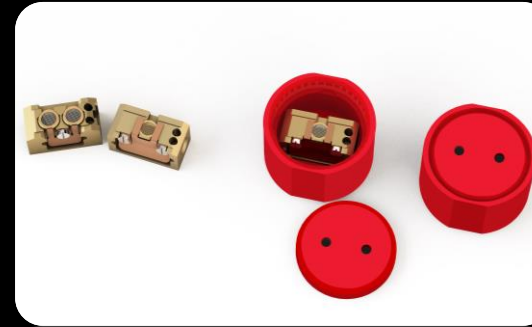
Grid loading and transfer – Cover Box



Cover Box

(for grid loading under constant / better humidity conditions)

Magnifying glass (1), cover Box (2), 1 of 2 dewars (3), grid storage boxes (4)



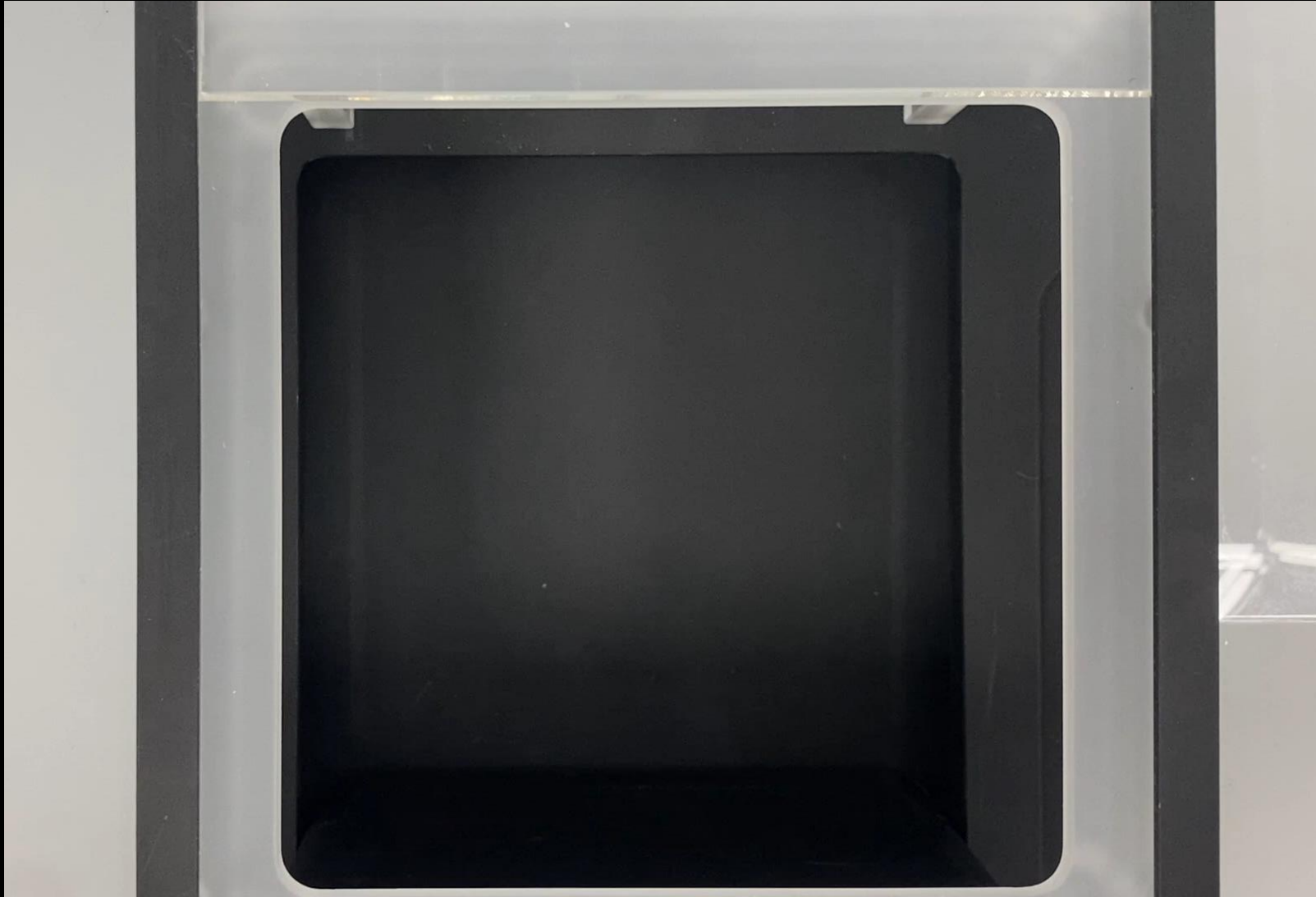
- Bare grid cartridges
- Auto grid cartridges
- (silicon wafer cartridges)

Grid cartridges

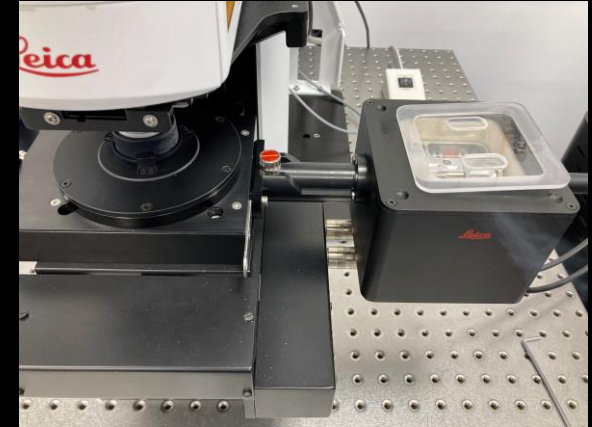


Cryo transfer shuttle

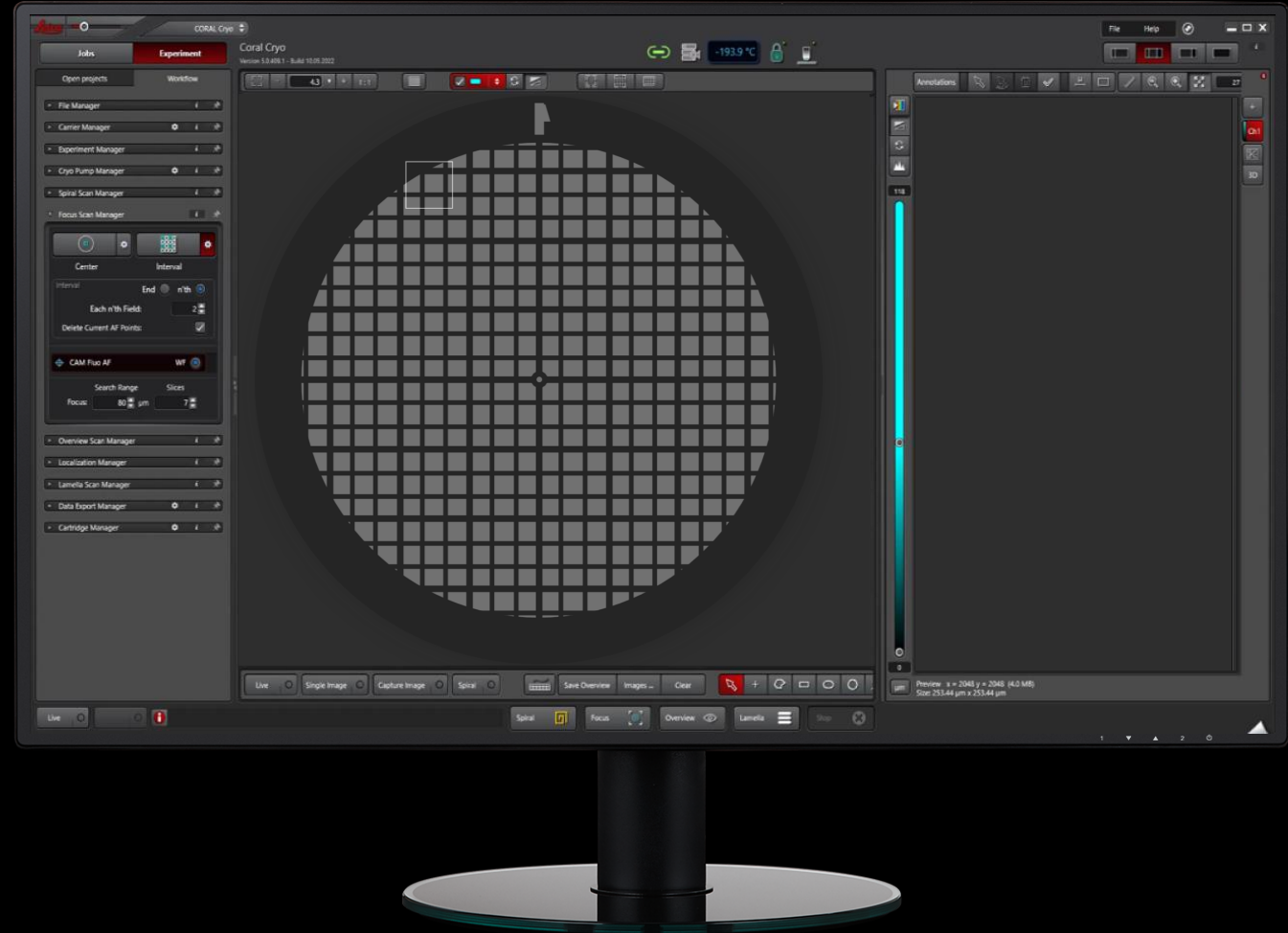
Grid loading and transfer – Shuttle loading



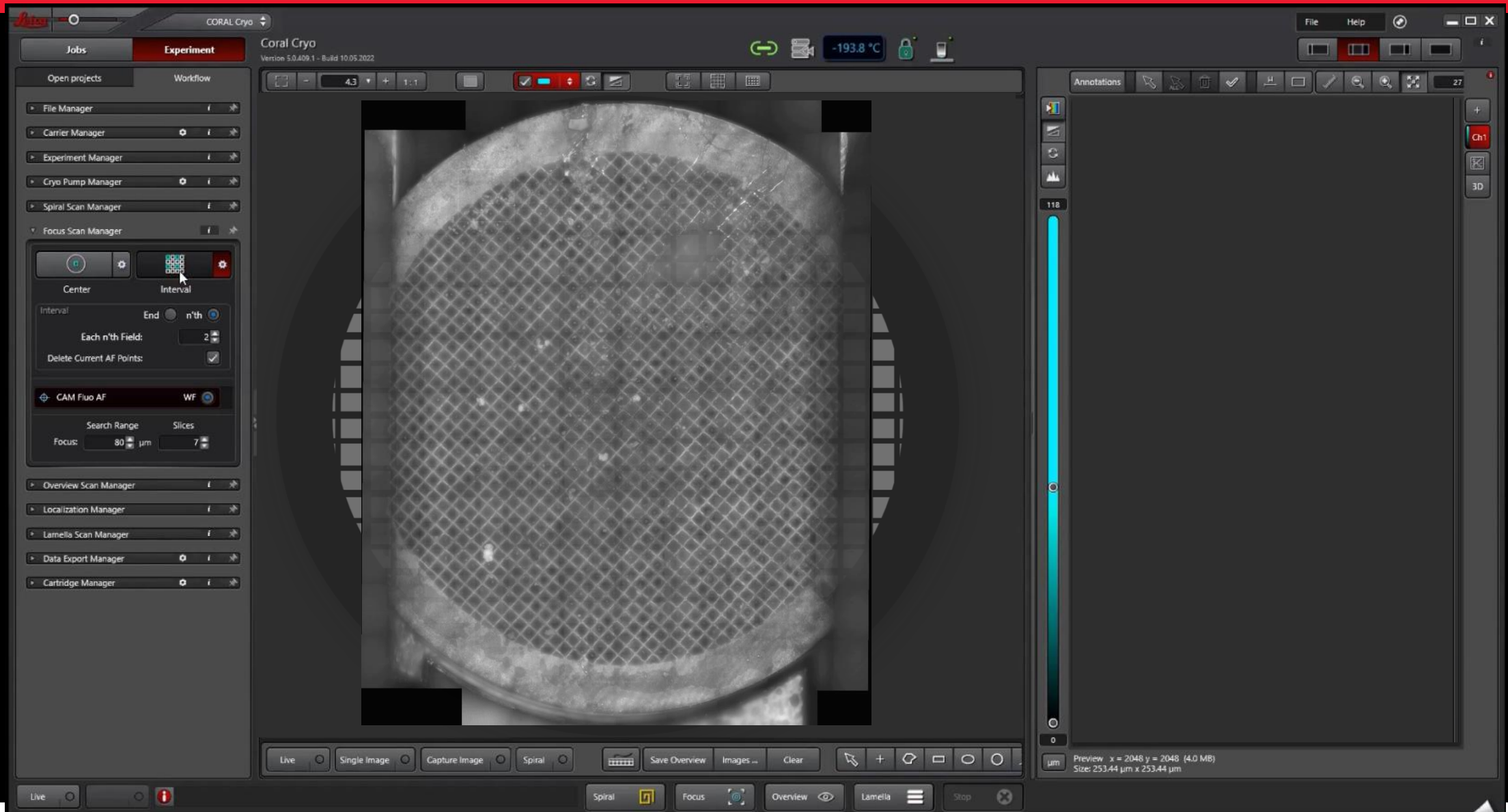
Grid loading and transfer – Cryo-stage loading



Grid loading and transfer - LAS X Coral Cryo



LAS X Coral Cryo image acquisition



LAS X Coral Cryo image acquisition – focused ROI imaging

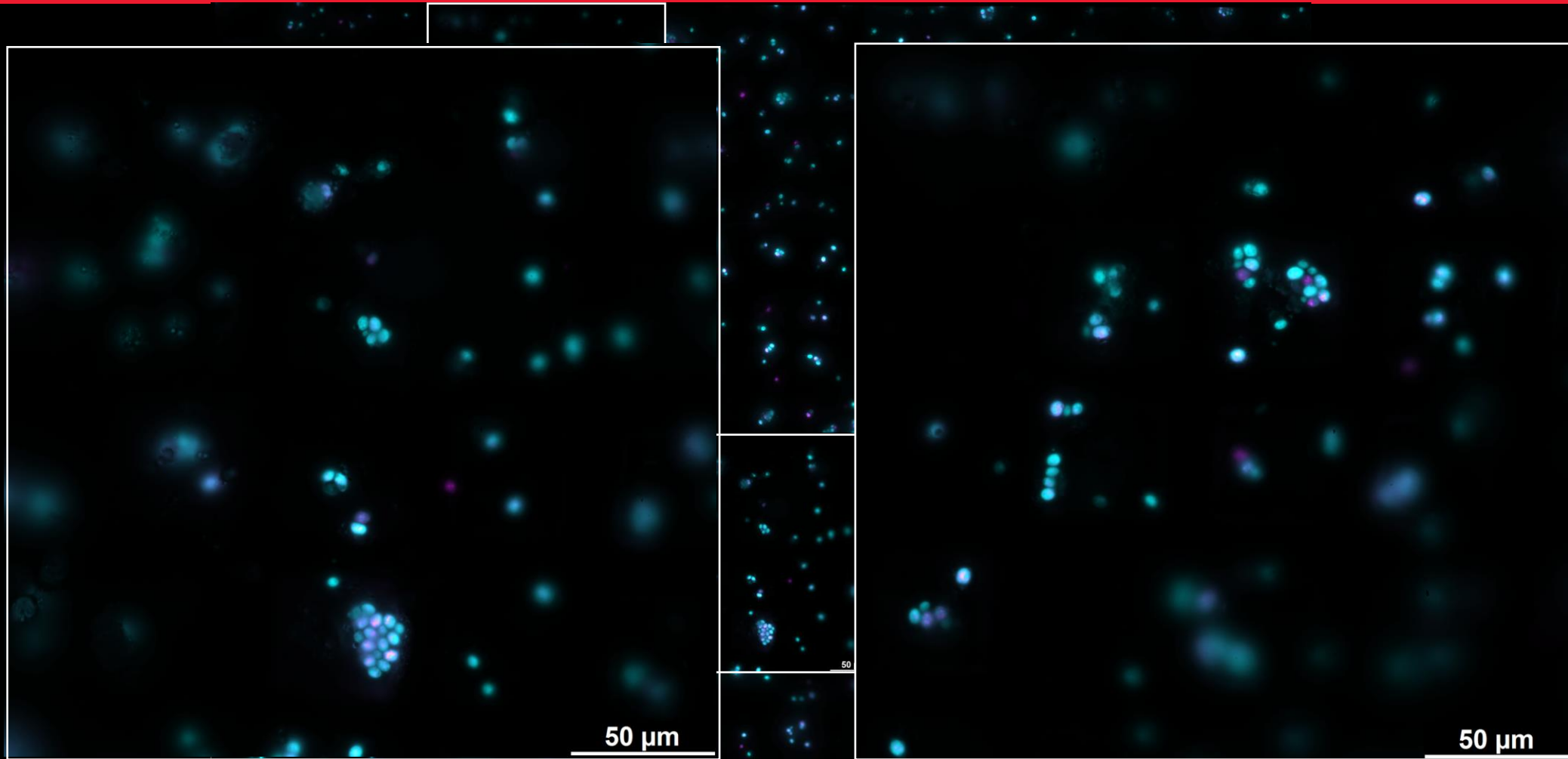
The screenshot displays the Leica LAS X Coral Cryo software interface. The main window shows a cryo-electron microscopy setup with a grid of focus points. The temperature is set to -194.1 °C. The interface includes a left sidebar with various managers (File, Carrier, Experiment, Cryo Pump, Spiral Scan, Focus Scan, Overview Scan, Localization, Lamella Scan, Data Export, Cartridge) and a right sidebar with a table of coordinates and focus points.

Table of Coordinates and Focus Points:

	X	Y	Z	AFC
☒	6.88 mm	4.50 mm	7758.13 μm	X
☒	7.13 mm	4.50 mm	7748.13 μm	X
☒	7.37 mm	4.50 mm	7738.13 μm	X
☒	7.61 mm	4.50 mm	7738.13 μm	X
☒	6.88 mm	4.74 mm	7748.13 μm	X
☒	7.13 mm	4.74 mm	7744.13 μm	X
☒	7.37 mm	4.74 mm	7741.73 μm	X
☒	7.61 mm	4.74 mm	7739.93 μm	X
☒	6.88 mm	4.98 mm	7738.13 μm	X
☒	7.13 mm	4.98 mm	7739.57 μm	X
☒	7.37 mm	4.98 mm	7739.71 μm	X
☒	7.61 mm	4.98 mm	7739.82 μm	X

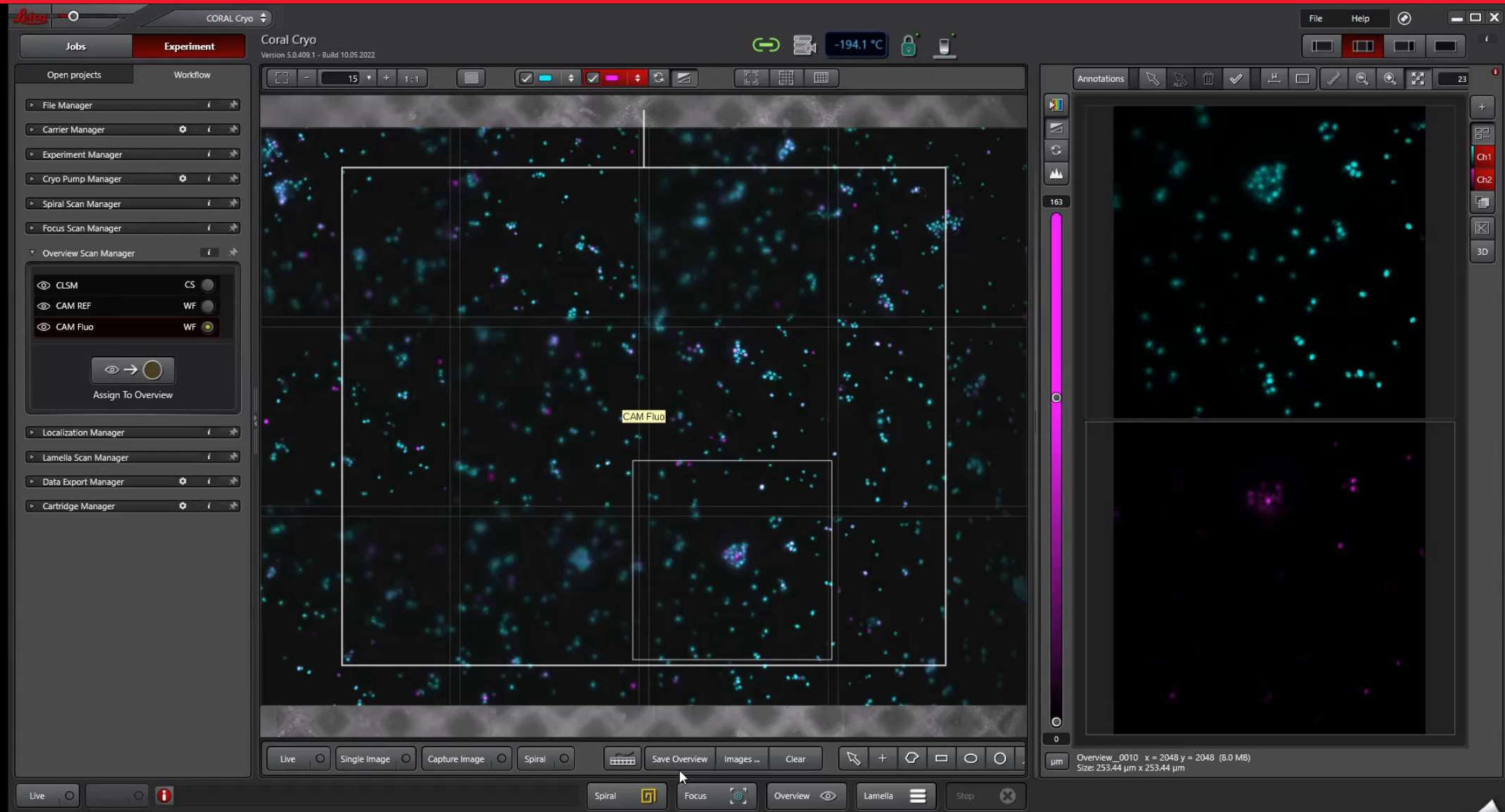
Buttons: Remove All, Clear Z, Previous, Next, Set Z, Set by Autofocus, Find all by Autofocus, Shift focus map to current Z, Close

LAS X Coral Cryo image acquisition – focused ROI imaging

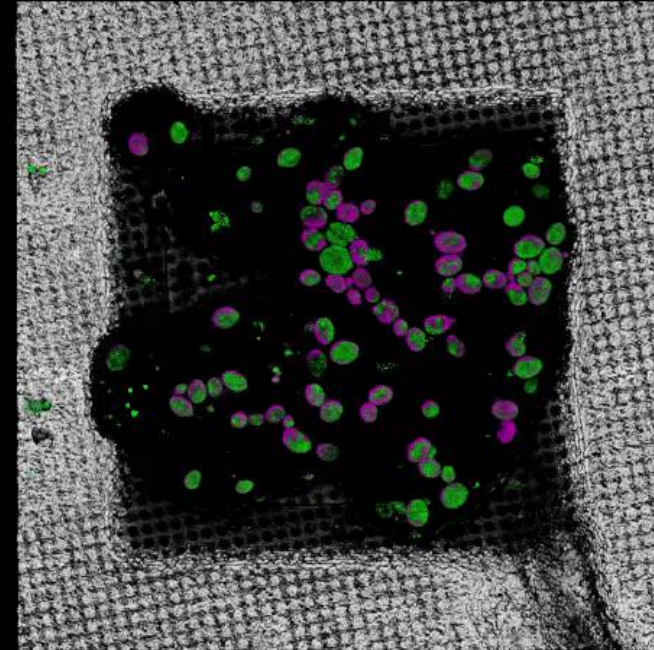
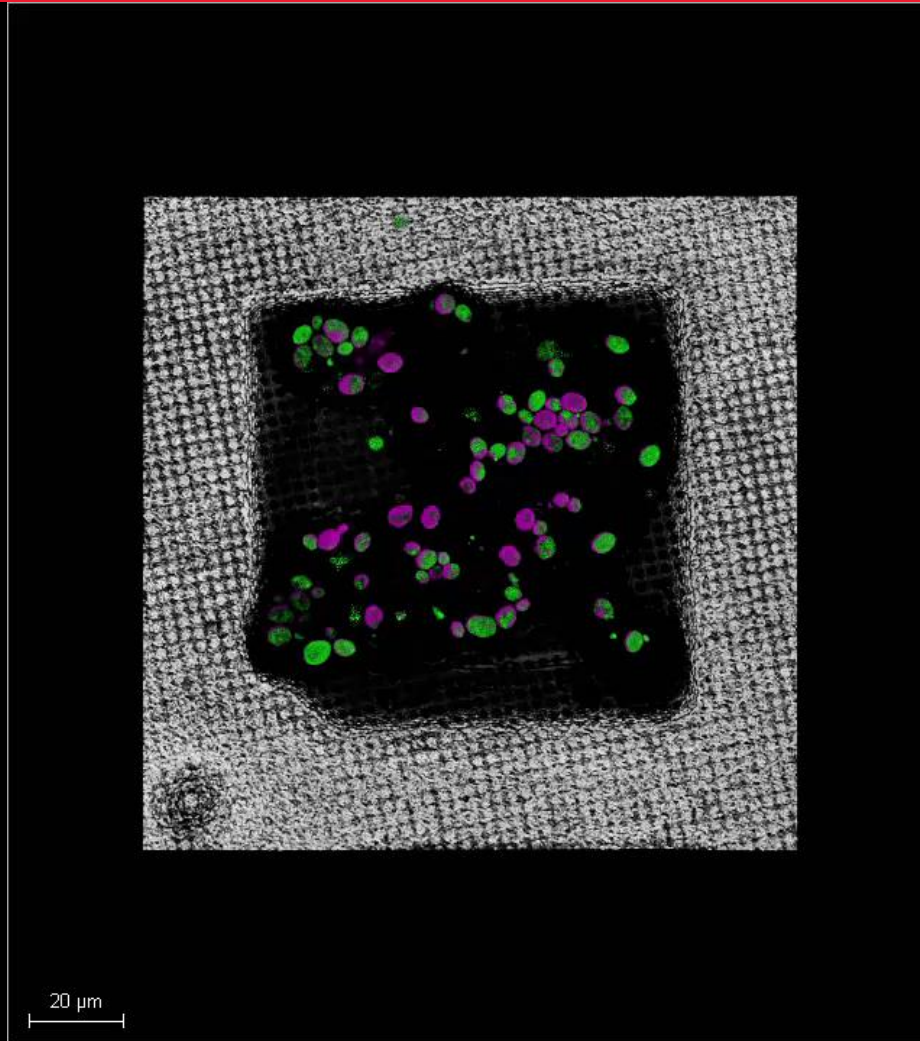


50x/0.90 dry cryo: Widefield:
2048 x 2048 x 15 voxel; 295 x 295 x 14 µm; Nuclei - Actin - Reflection

LASX Coral Cryo image acquisition – Lamella positioning



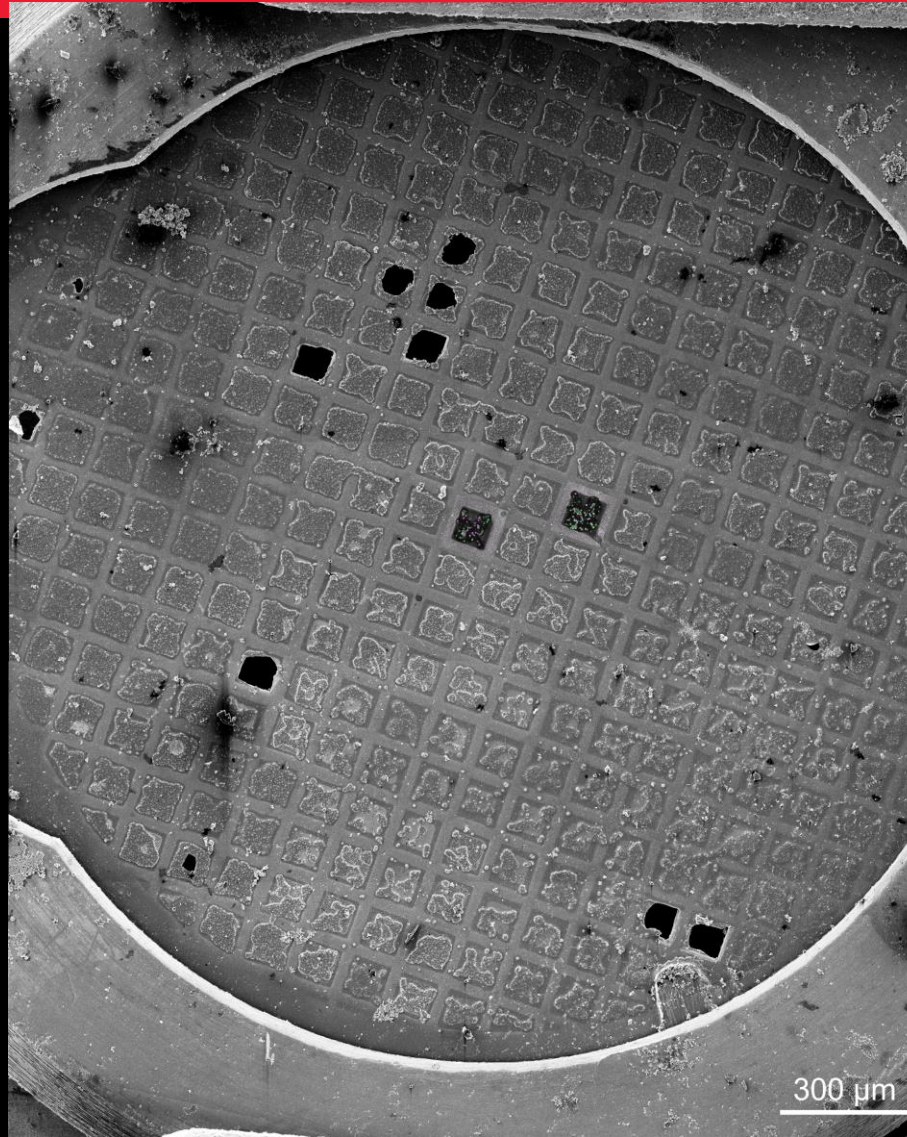
LAS X Coral Cryo image acquisition – high resolution FOV's



50x/0.90 dry cryo: 1440 x 1440 x 28 voxel; 132 x 132 x 13 μm;
MitoTracker Green Mitochondria; SIR 700 –membrane; Grid reflection

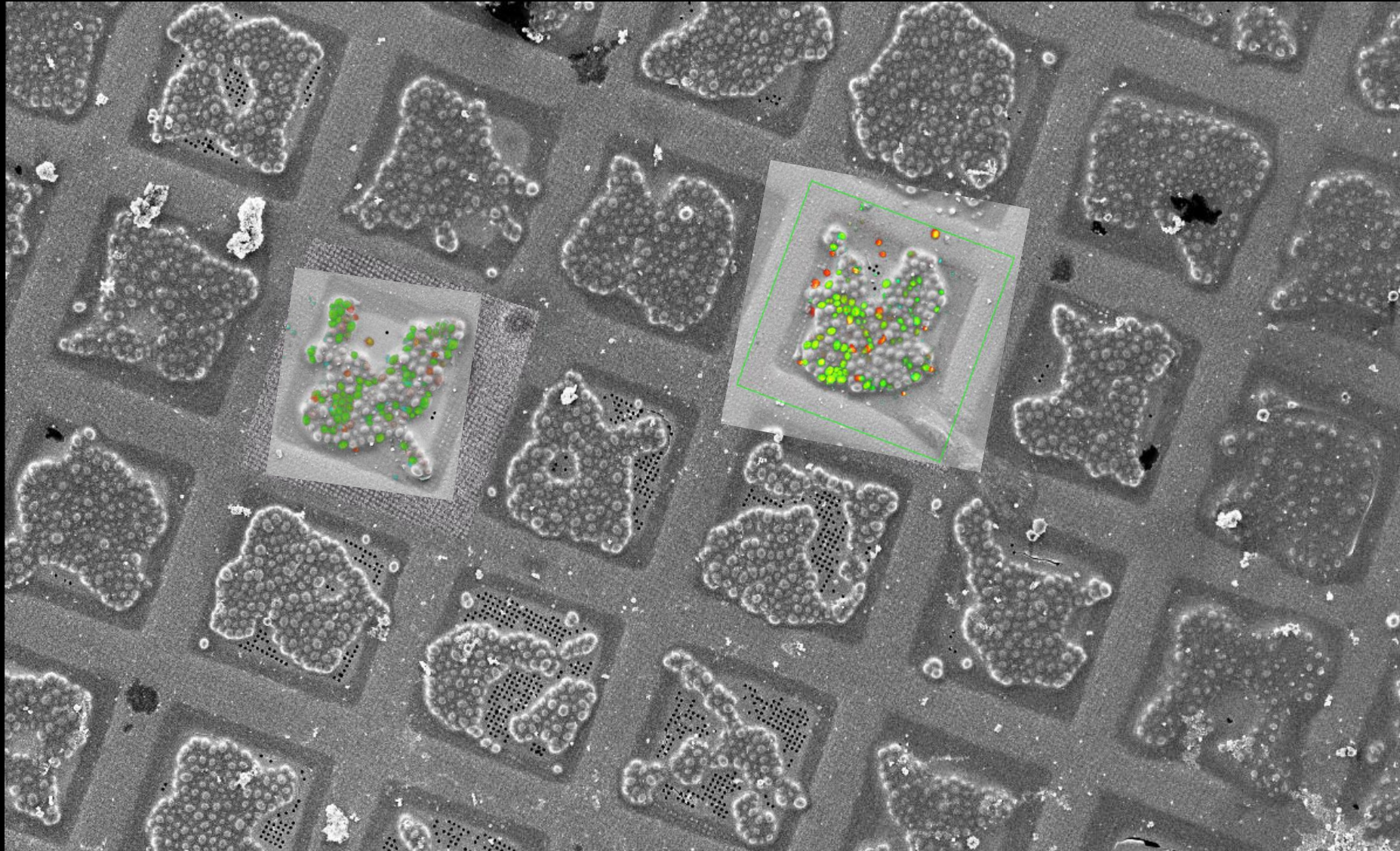
Max. projection

Cryo-FIB Cryo workflow – XY-Correlation



SEM + CLSM combo

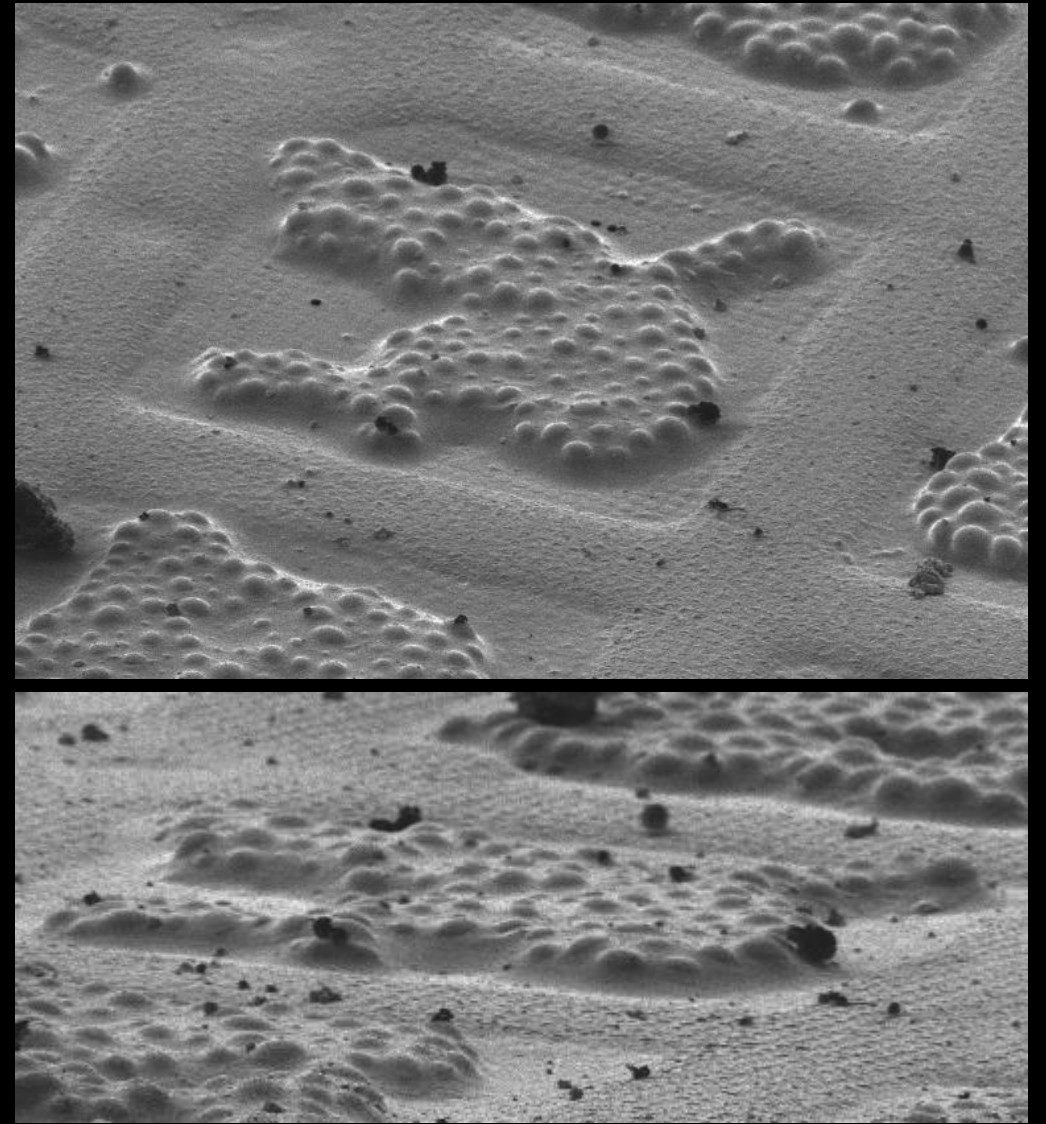
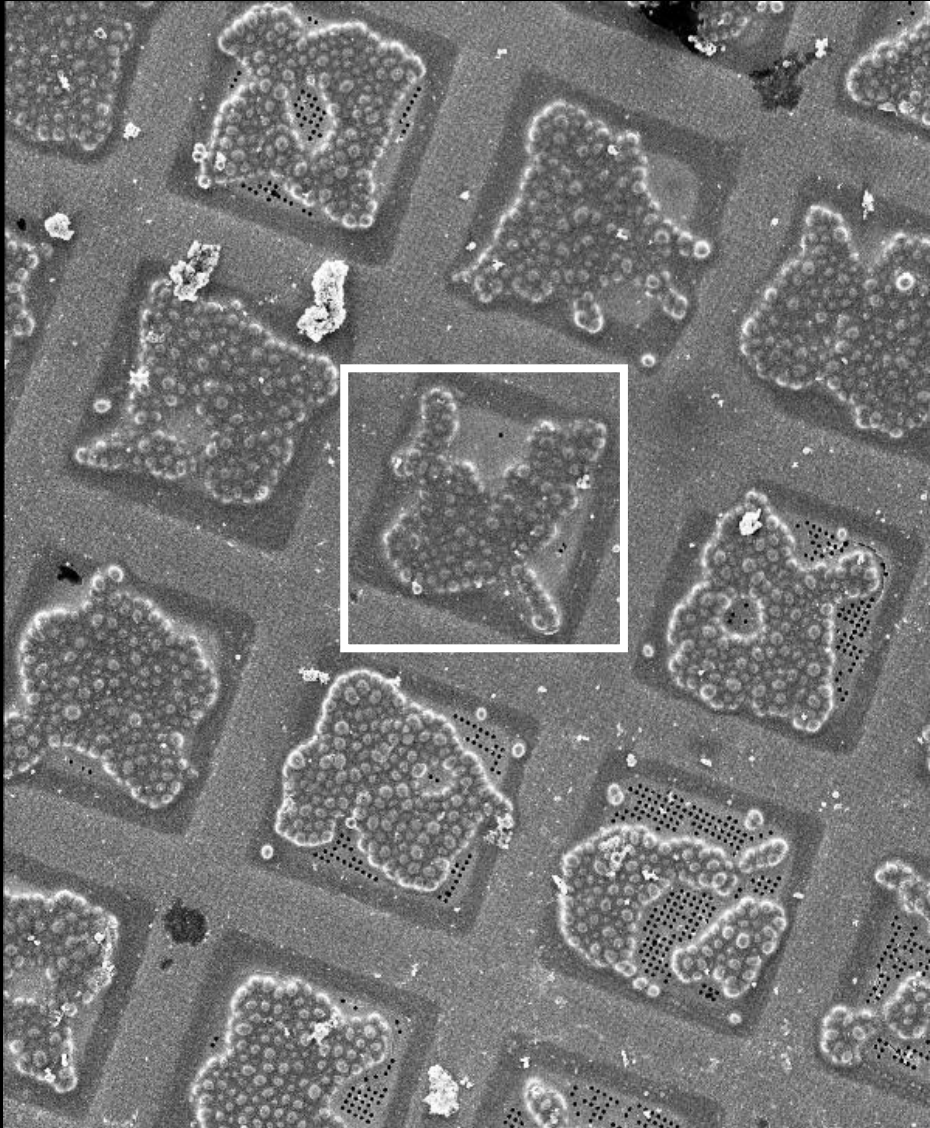
Cryo-FIB Cryo workflow – XY-Correlation



SEM +
CLSM
combo

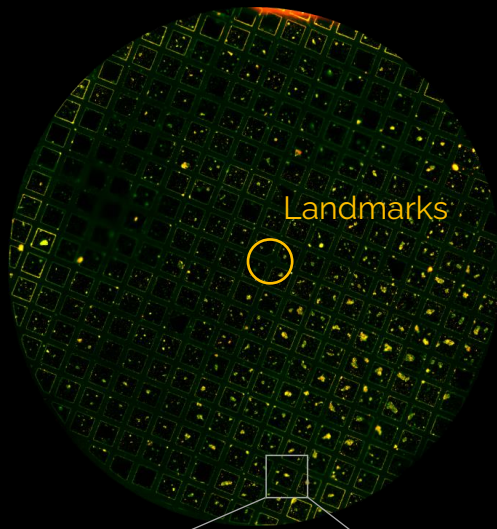
Cryo-FIB Cryo workflow – XY-Correlation

FIB SEM:
Lamella
positioning

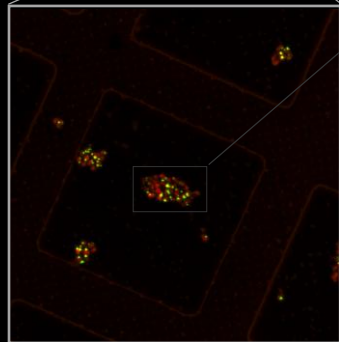


Cryo workflow – XYZ-Correlation in LASX Coral Cryo

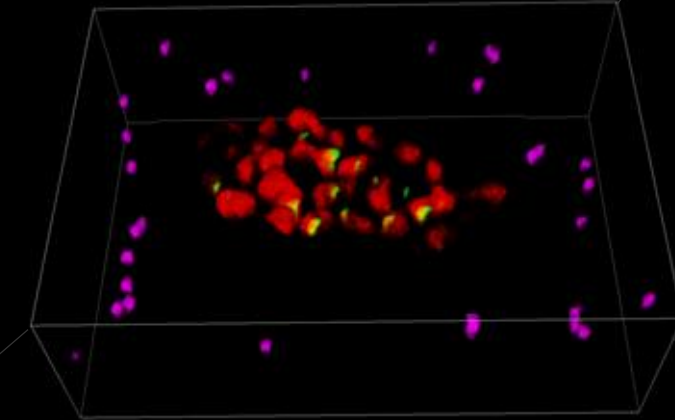
Camera Overview



Landmarks



Confocal z-stack

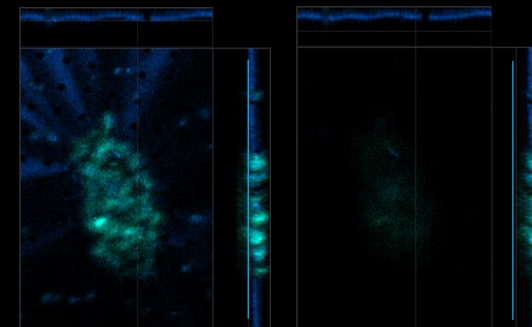
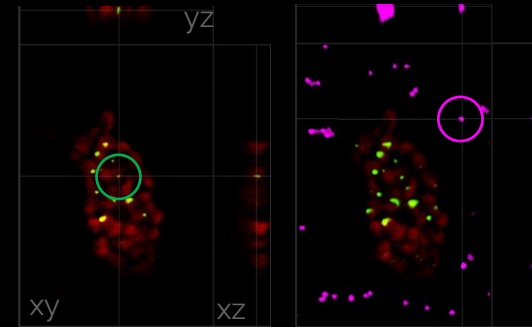


Confocal 3D

Subpixel targeting (pat. pend.!)

Lamella target

Beads



Bottom

Top

All coordinates necessary to retrieve the potential lamella target

Localization Manager

Align Lamella Bead

Lamella Marker

	X [μm]	Y [μm]	Z [μm]	ZT [μm]	ZB [μm]
L 1	3260.82	3408.77	7826	4.43	9.84
	0	0.33	1.91		
L 2	3699.23	3647.11	7826		
	0	0	0	0	0
L 3	3352.03	4038.45	7826		
	0.55	0	8.19	4.43	6.64

Delete All Delete Selected Assign Job

Fluo Bead Marker

	X [μm]	Y [μm]	Z [μm]	ZT [μm]	ZB [μm]
B 1.1	3201.97	3364.63	7826		
	0	0	0	0	0
B 1.2	3325.55	3358.75	7826		
	-60.38	50.02	7.97	4.43	6.64
B 1.3	3263.76	3476.45	7826		
	-2.97	-40.37	6.42	4.43	6.64

Delete All Delete Selected

Data Export Manager

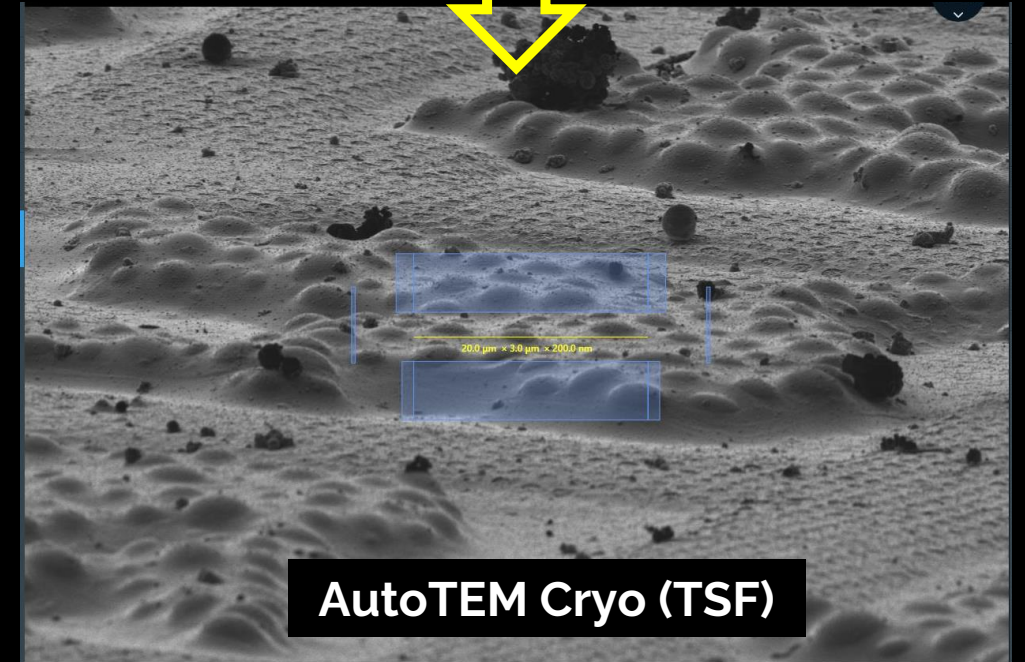
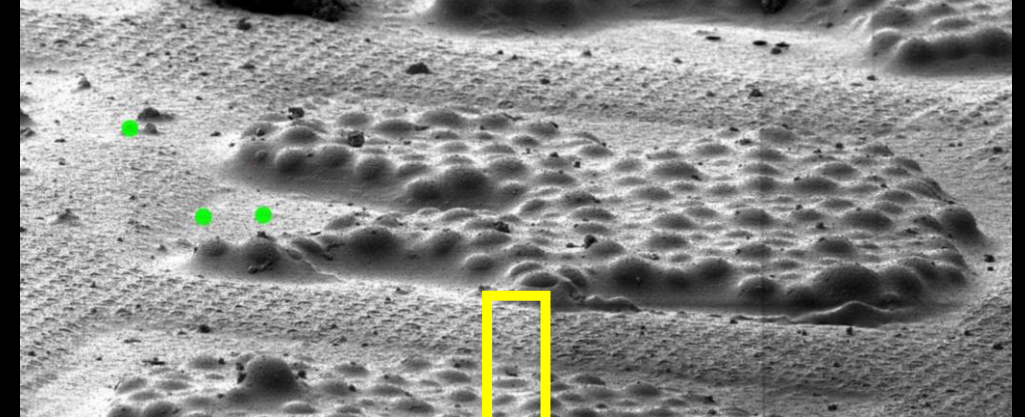
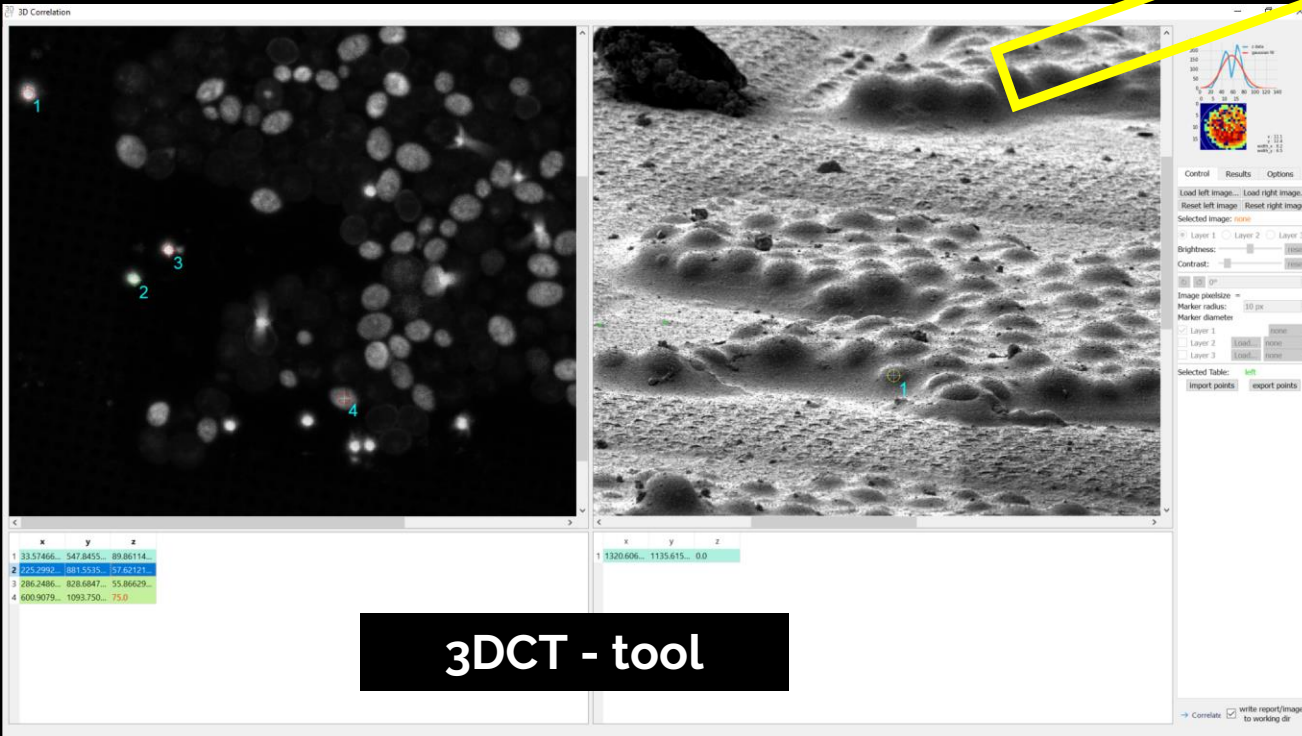
Save EM Marker File

XYZ coordinate handling – freeware 3DCT

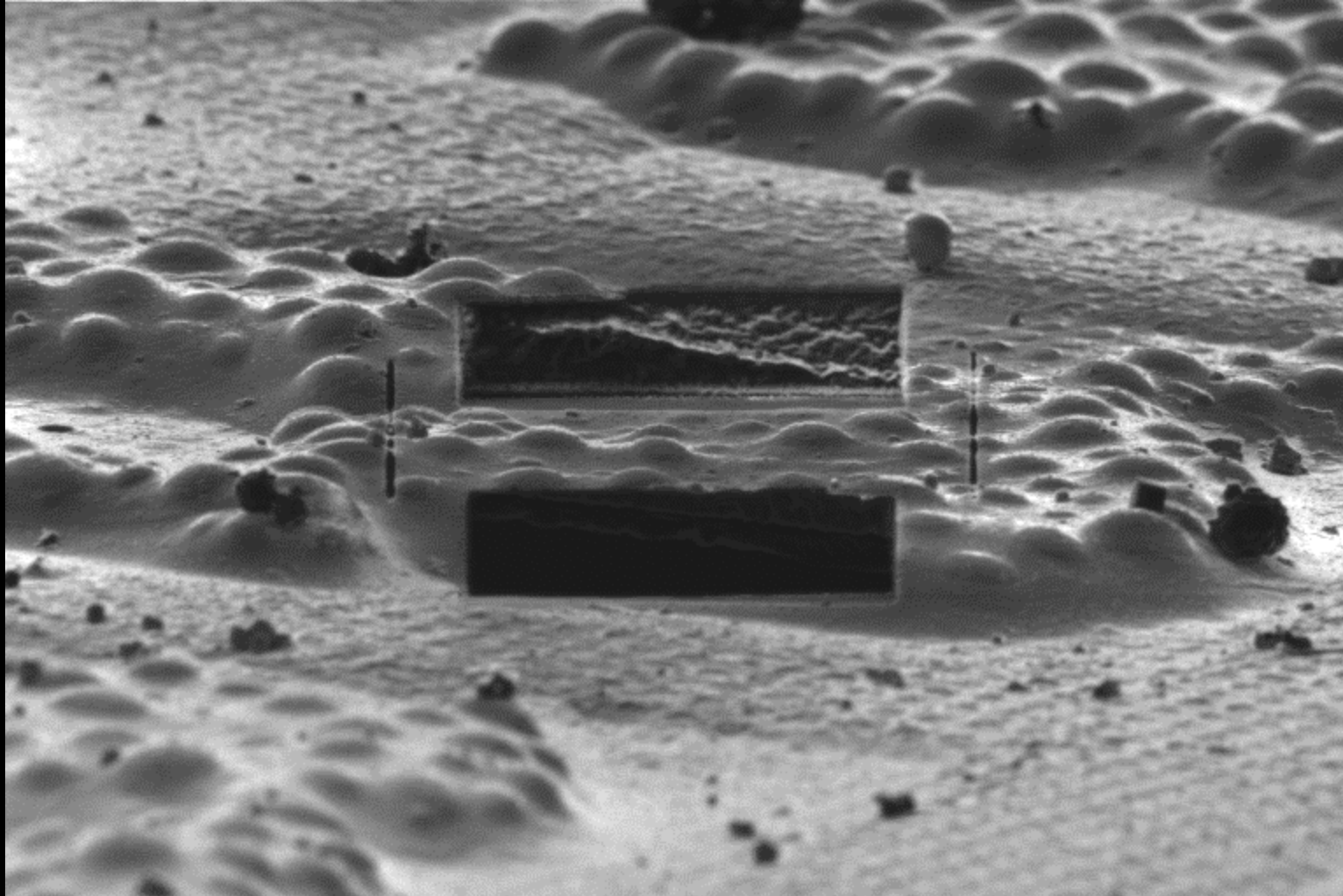
3DCT | 3D Correlation Toolbox

Developed for easy to use 2D to 3D correlation in CLEM
at the Max Planck Institute of Biochemistry, Department of Molecular Structural Biology

<https://3dct.semper.space>

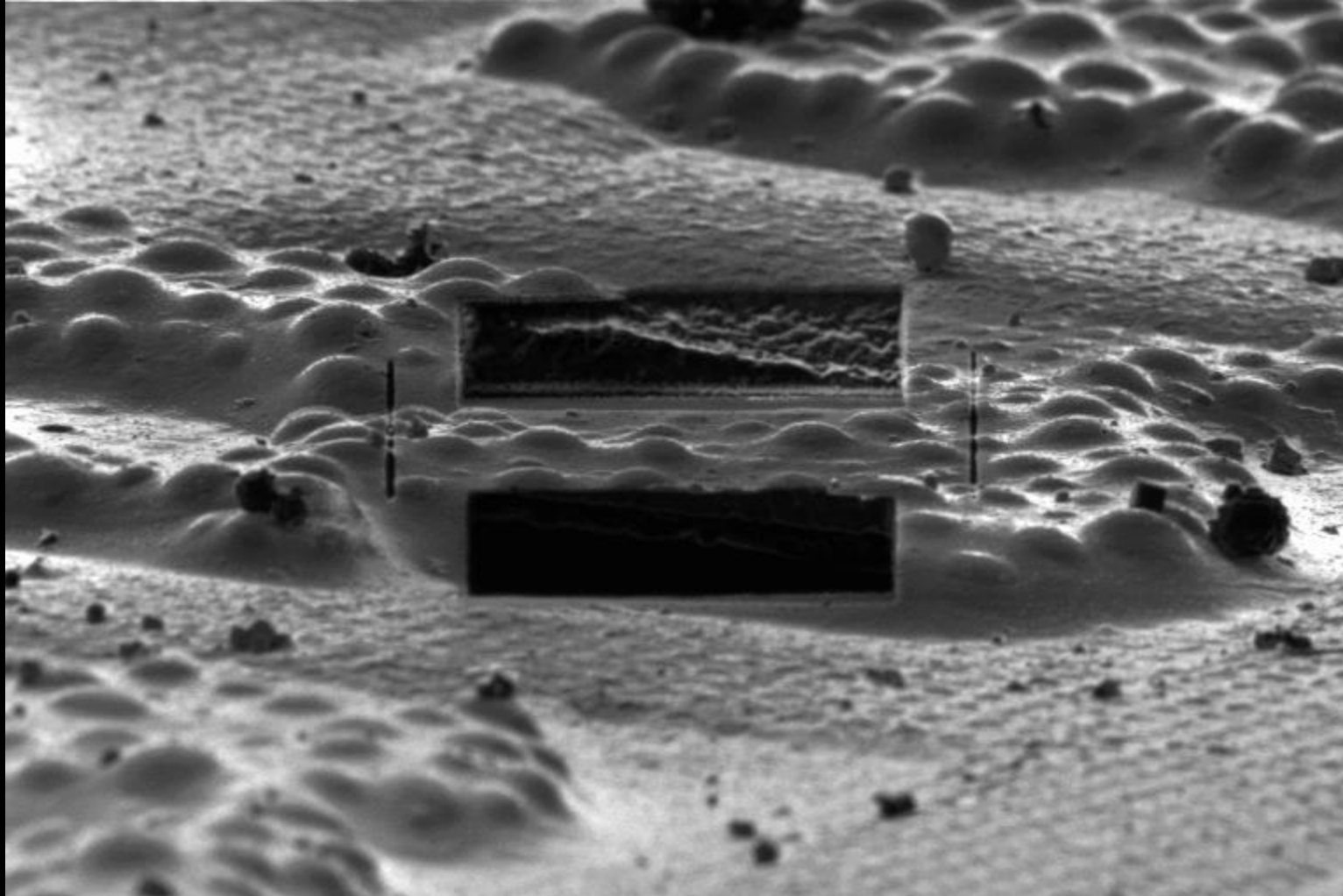


Cryo workflow (Aquilos 2 Thermo Fischer)



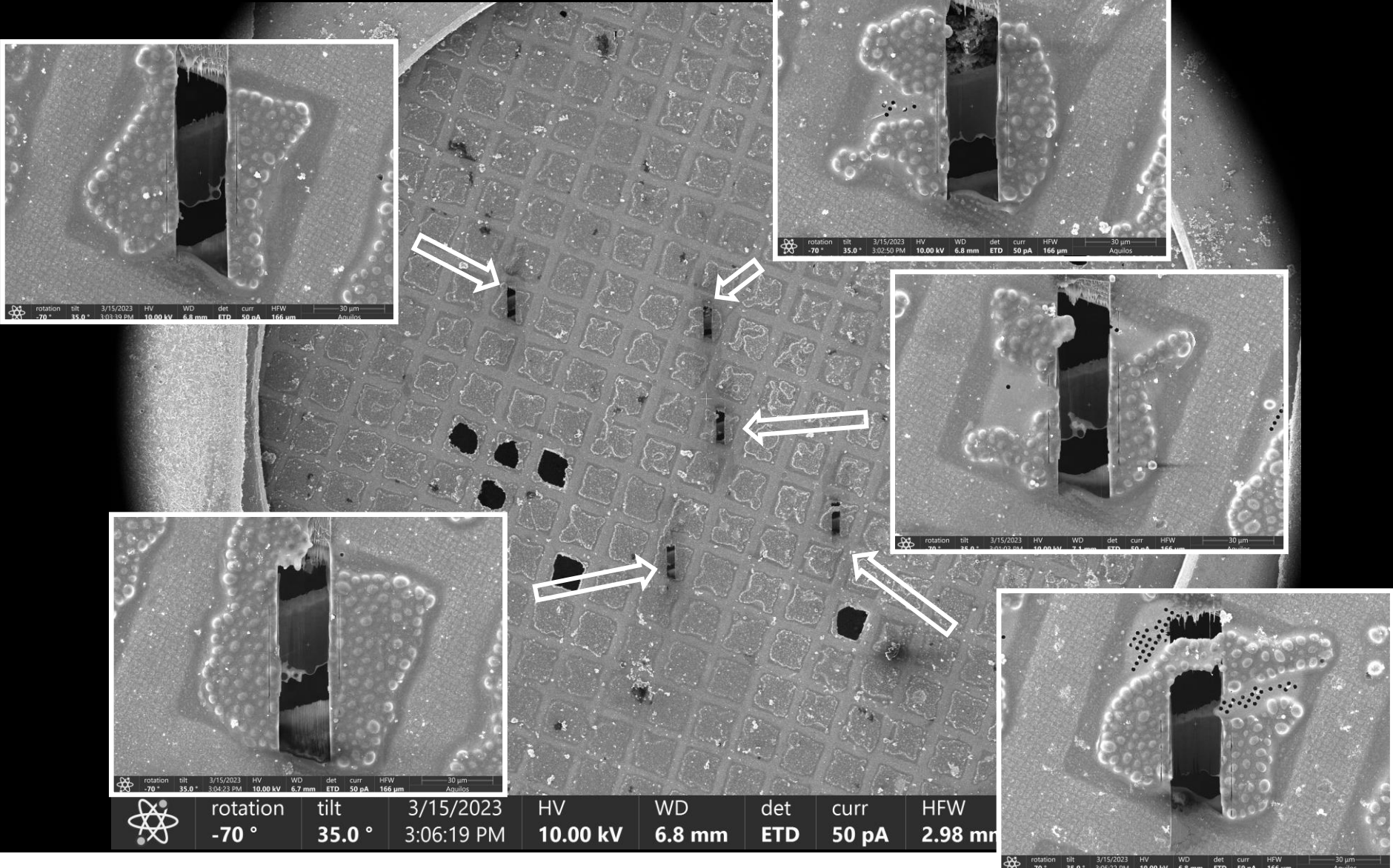
**FIB SEM:
Lamella
positioning**

Cryo workflow – lamella process



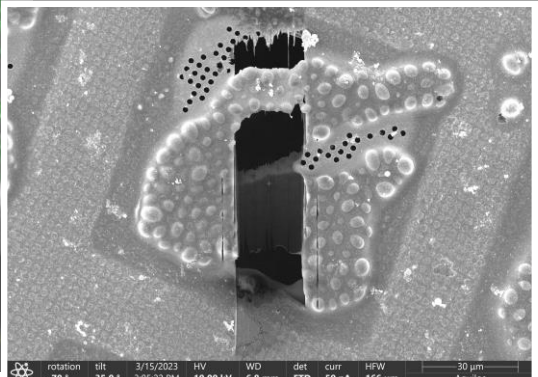
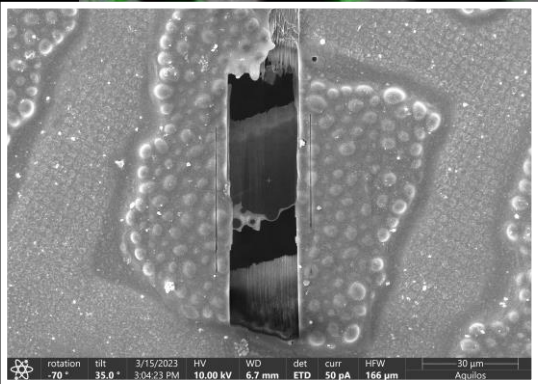
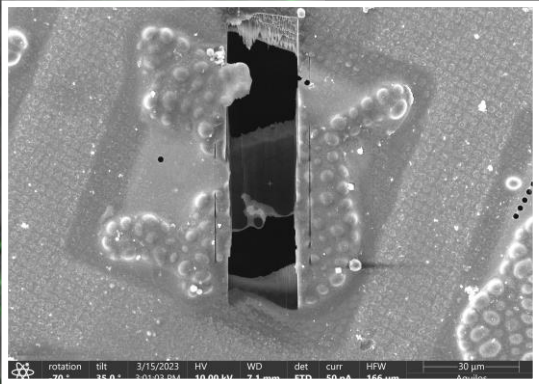
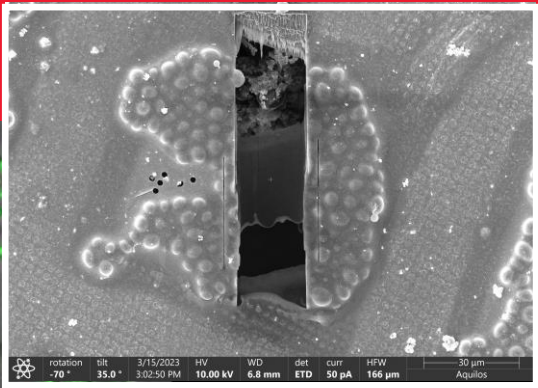
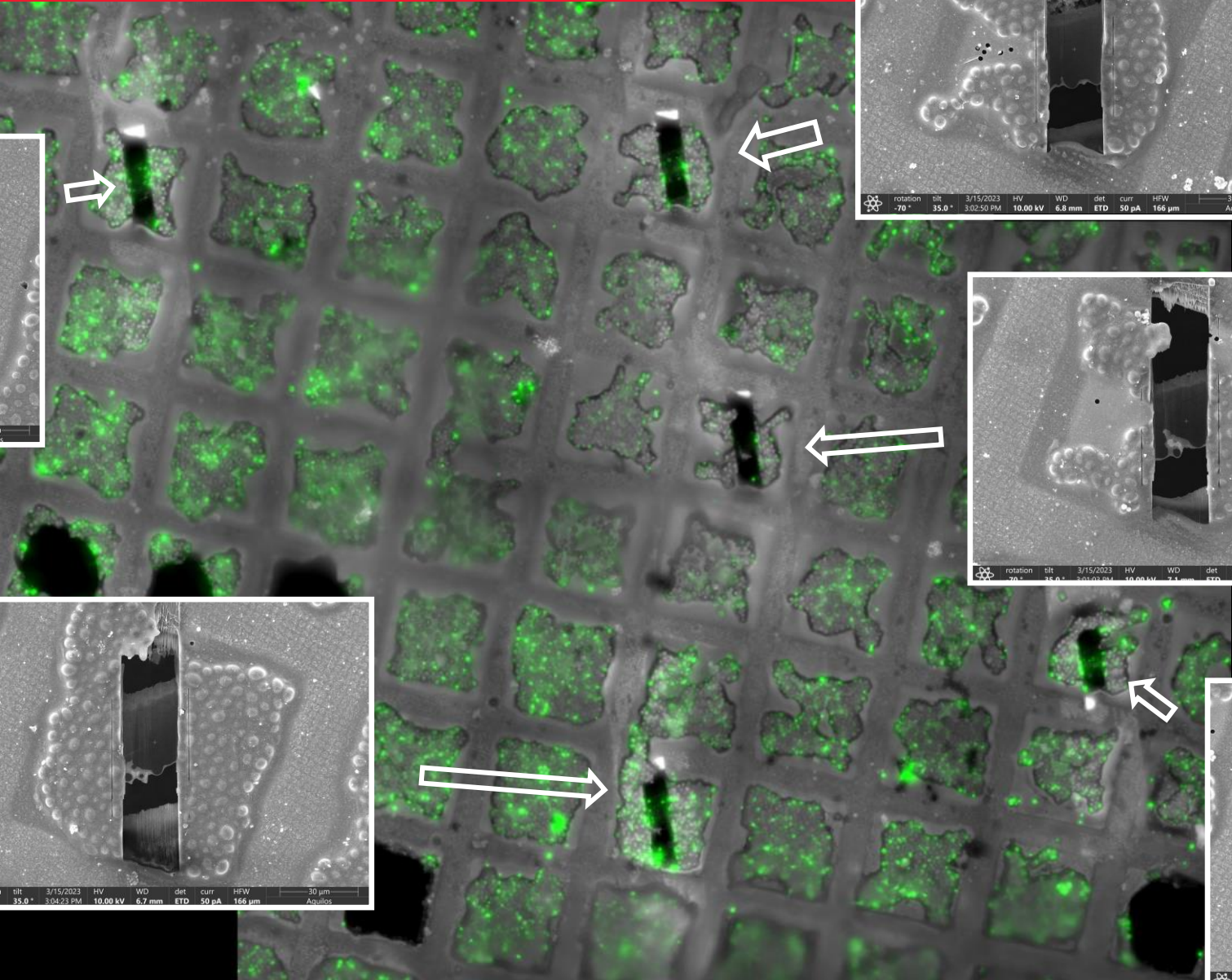
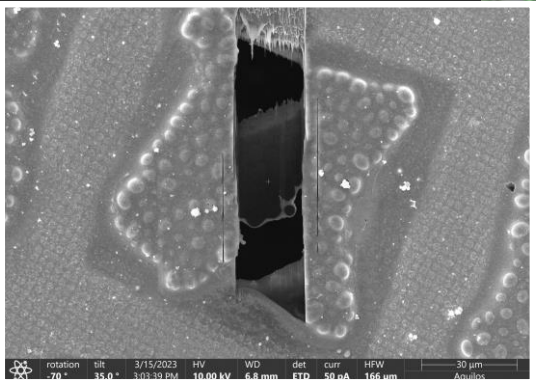
FIB SEM:
Lamella
positioning

Cryo workflow – SEM details of the lamellae sites

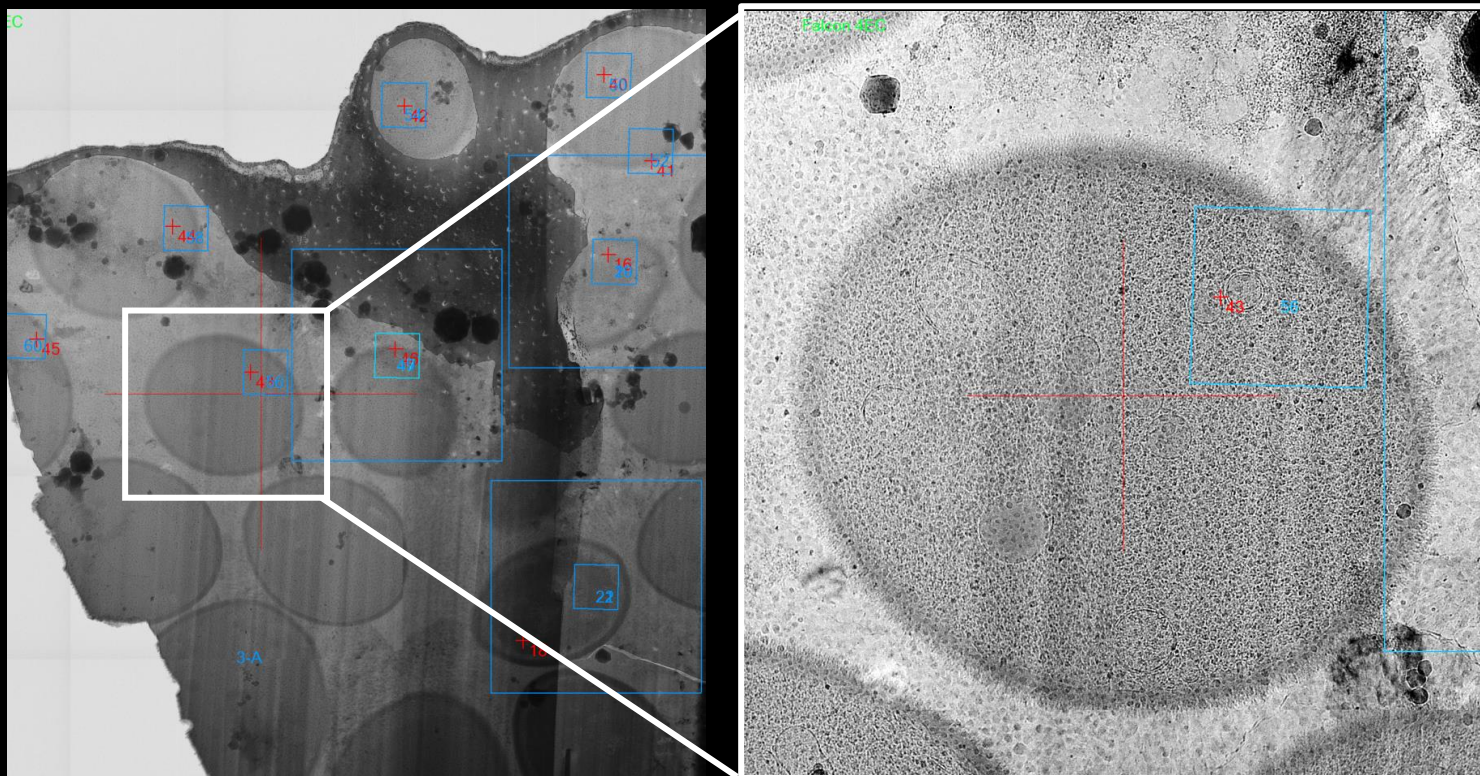


FIB SEM:
Lamella
positioning

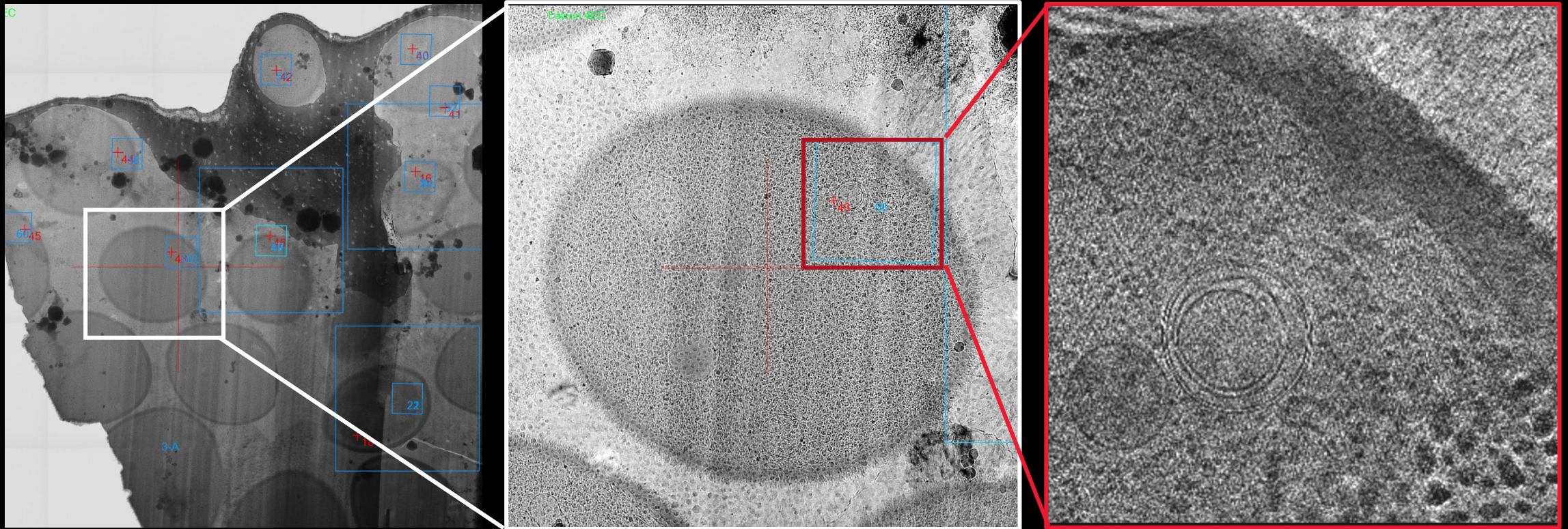
Confocal imaging of lamellae sites



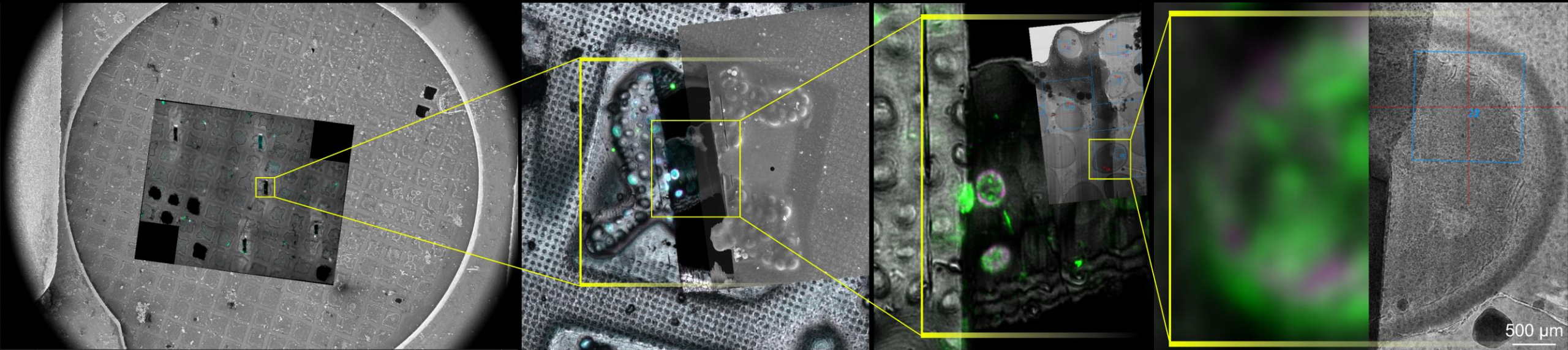
Cryo-TEM tilt series acquisition (Titan Krios Thermo Fischer)



Cryo-TEM tilt series acquisition (Titan Krios Thermo Fischer)



From the bench to the Beam!



SEM +
CLSM
combo

Acknowledgment



Leica Lab - EMBL IC Heidelberg

Special thank you to

Zhengyi Yang: (cryo tomography specialist at Imaging Centre, EMBL)

- **FIB-SEM imaging, EM – LM image alignment, Lamella and TEM acquisition**



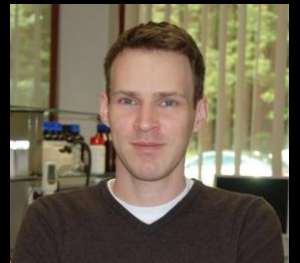
Martin Fritsch: (advanced workflow specialist; Leica Microsystems)

- **Coral cryo, confocal microscopy, 3DCT**



Frederic Leroux: (advanced workflow specialist, manager; Leica Microsystems)

- **Providing EM sample prep. images and animations**



Leica



Thank You!

Want to know more?

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 @Cellfie3

 Andreia Pinto



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