

Keep the Context of Your Experiments

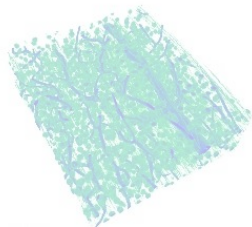
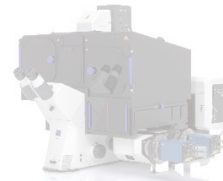
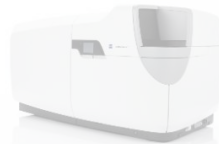


X-ray

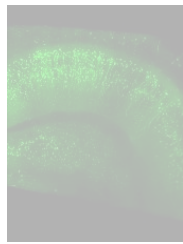
Light Microscopy

Electron Microscopy

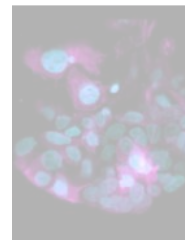
Ion



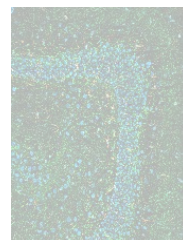
X-ray



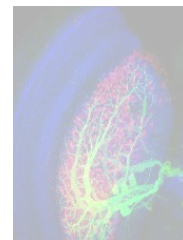
Lightsheet



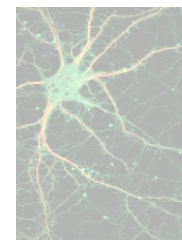
Lattice
Lightsheet



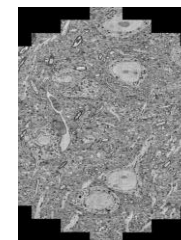
Widefield



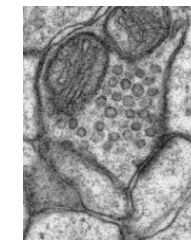
LSM
Airyscan



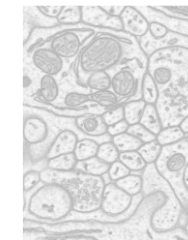
Super-
resolution



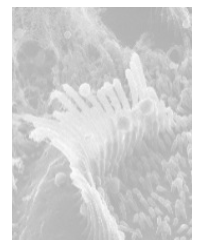
MultiSEM



Field Emission
Scanning Electron



Focused
Ion Beam



Helium Ion

700 nm

500 nm

290 nm

250 nm

120 nm

20 nm

5 nm

< 2 nm

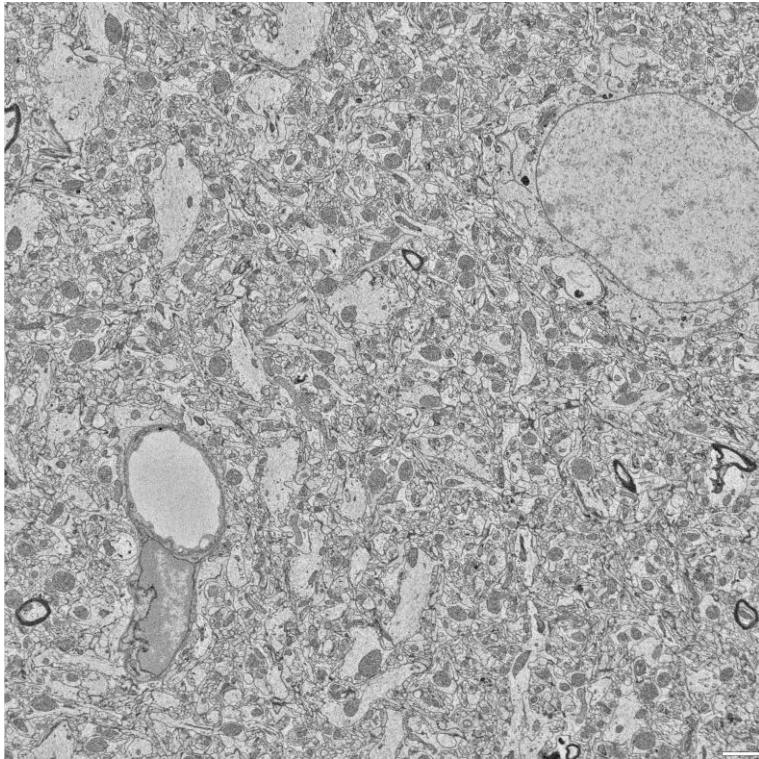
< 1 nm

< 0.5 nm

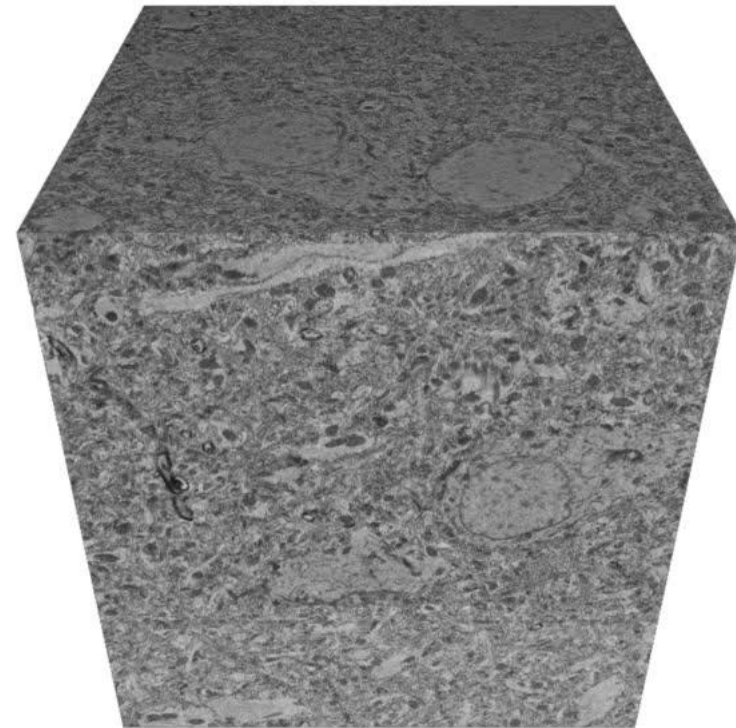


Why Volume EM?

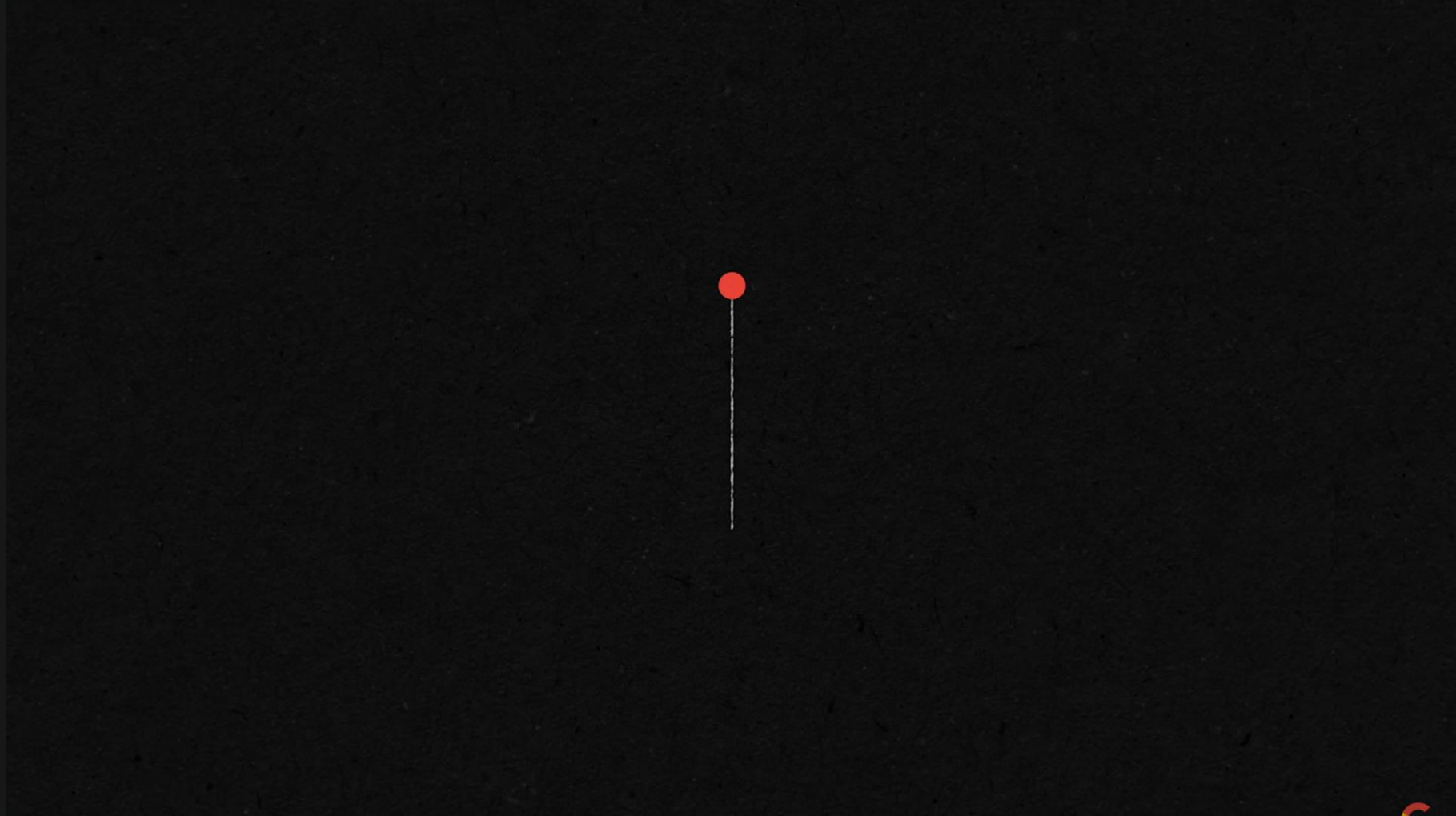
2D



3D



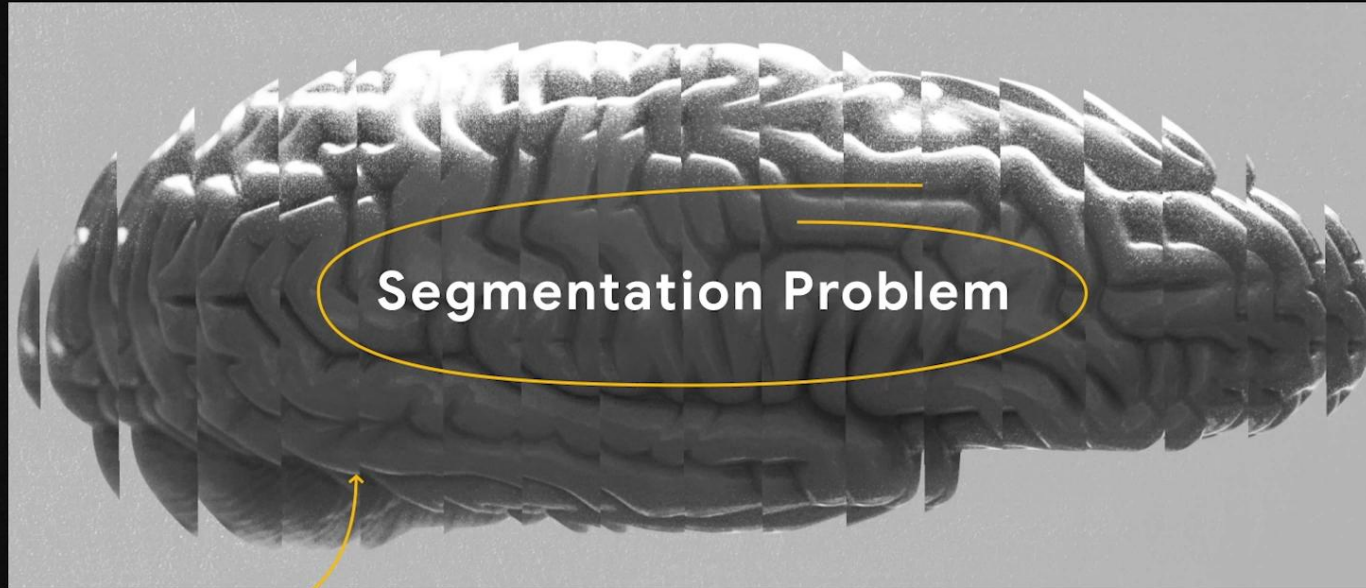
The Scientific Road of Volume EM



The Scientific Road of Volume EM



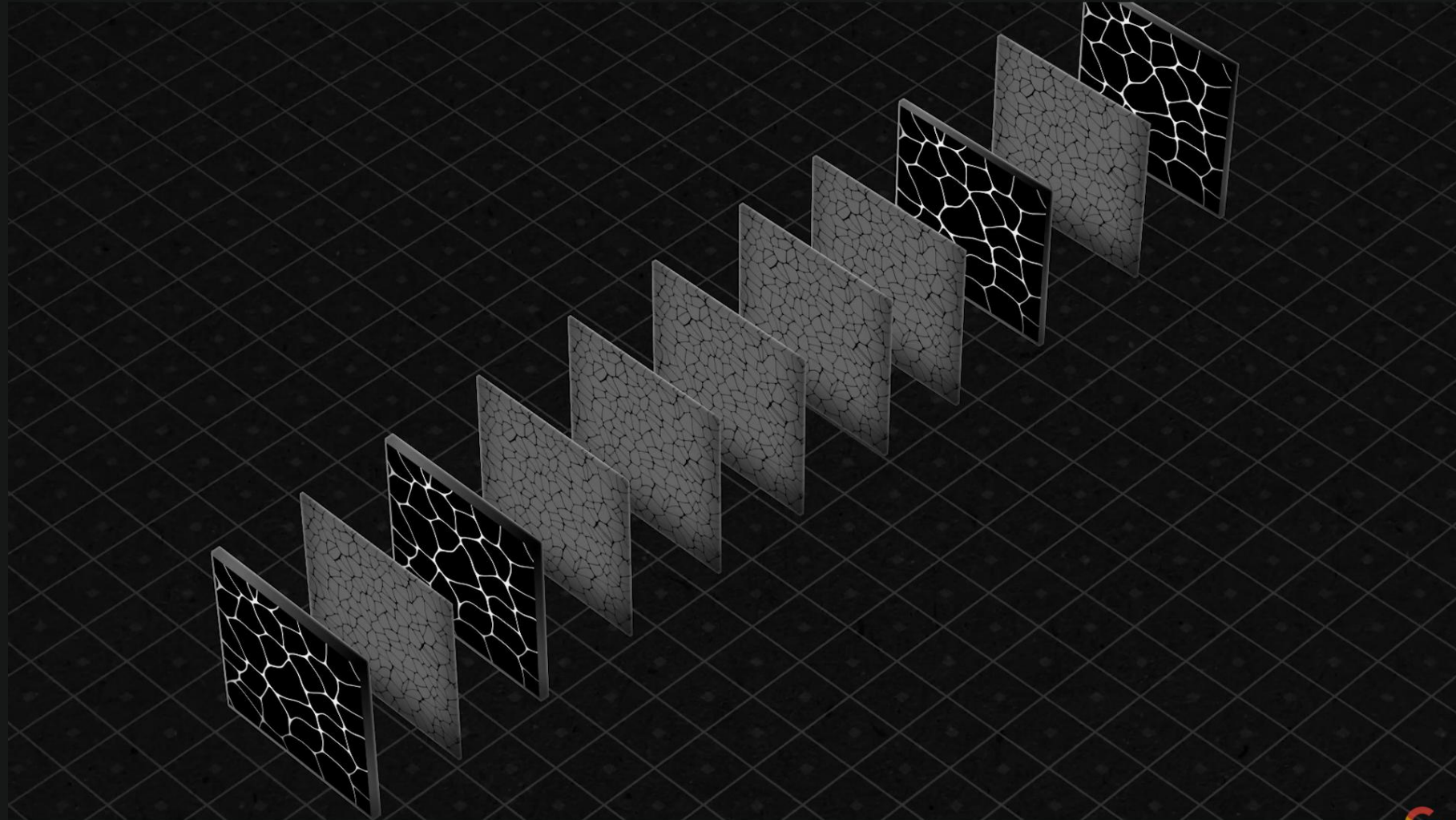
#ko-01



sample ad12



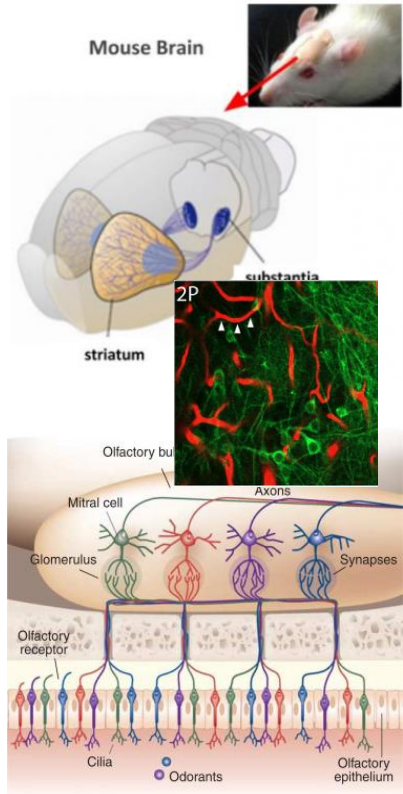
The Scientific Road of Volume EM



Volume SEM Workflow Overview

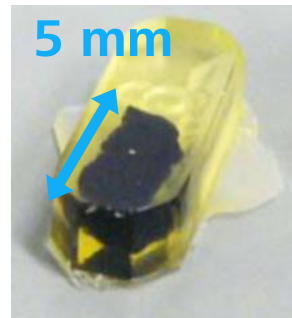
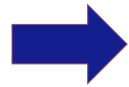


Explant Sample



1 - 3 days

Prepare Sample



2 - 3 days



1 - 2 mm



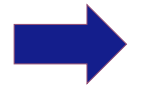
Hours

Section Sample

3D SEM imaging



days - weeks

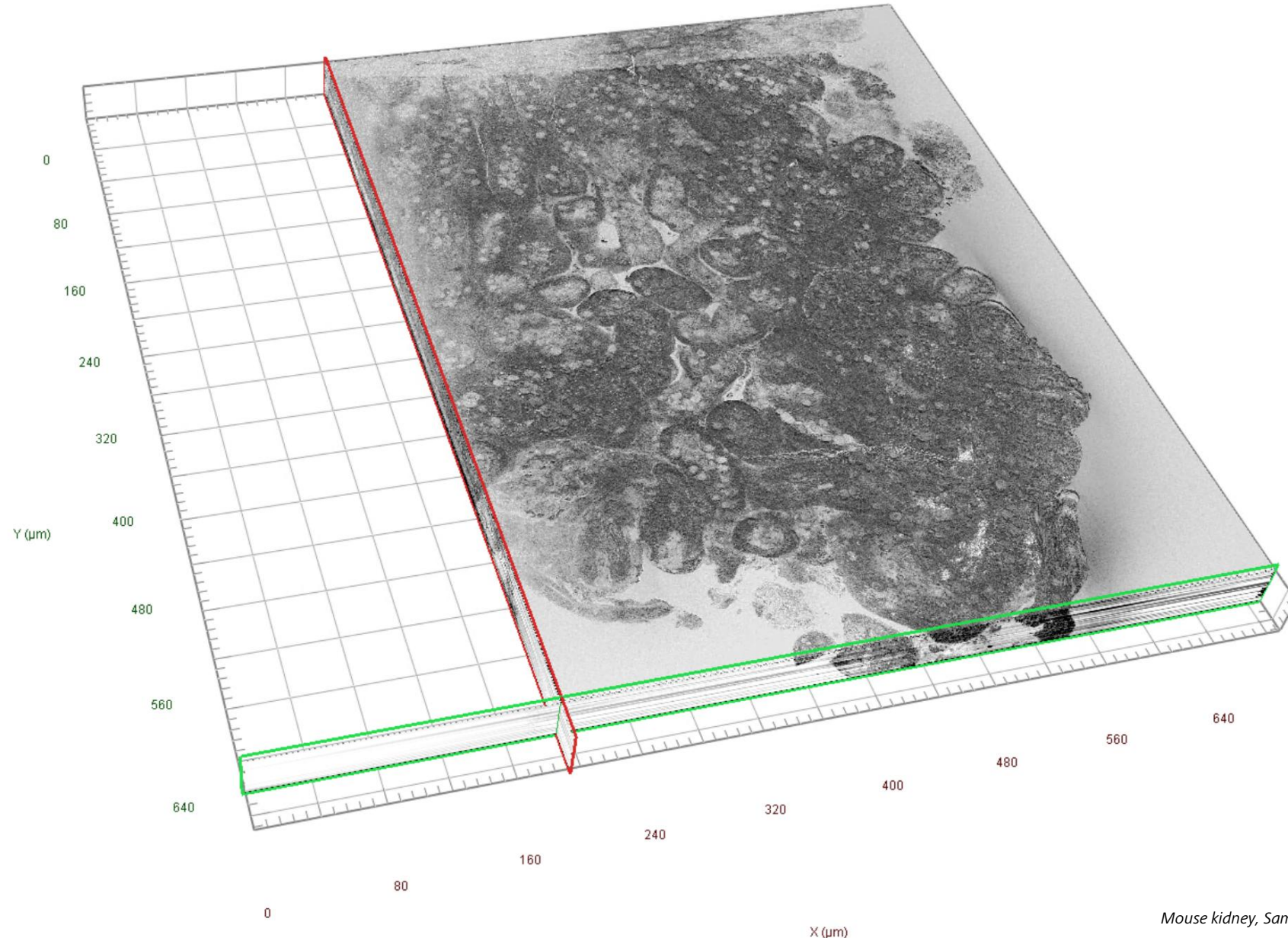


**50 - 100 μ m
weeks**

Image Analysis

Failed volume-EM experiments due to suboptimal sample preparation are costly and waste tremendous amounts of time

Large Area Imaging of a kidney sample

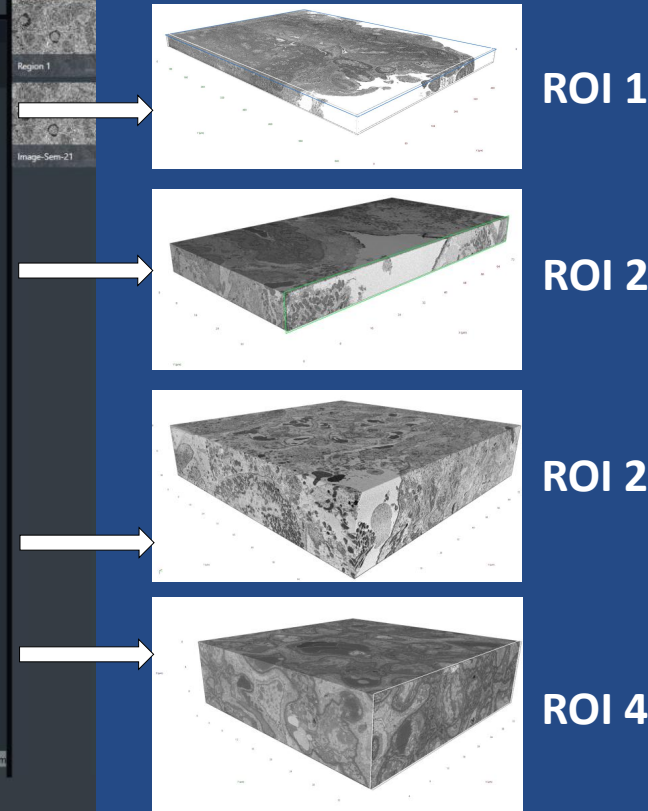
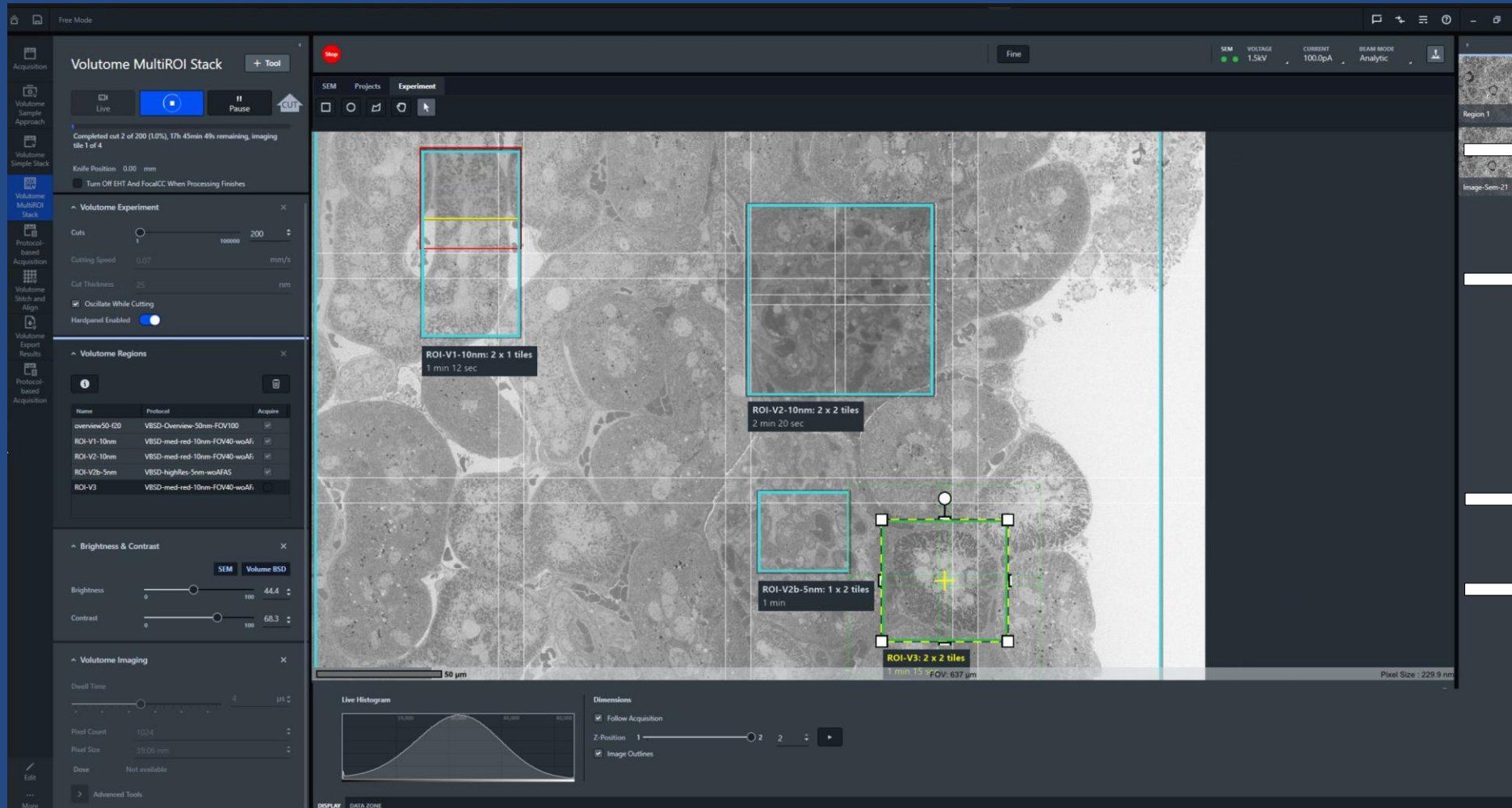


X = 680 μm
Y = 650 μm

Imaging multiple regions of interest with MultiROI Stack



Collect multiple stacks and overview images within a single Volutome run

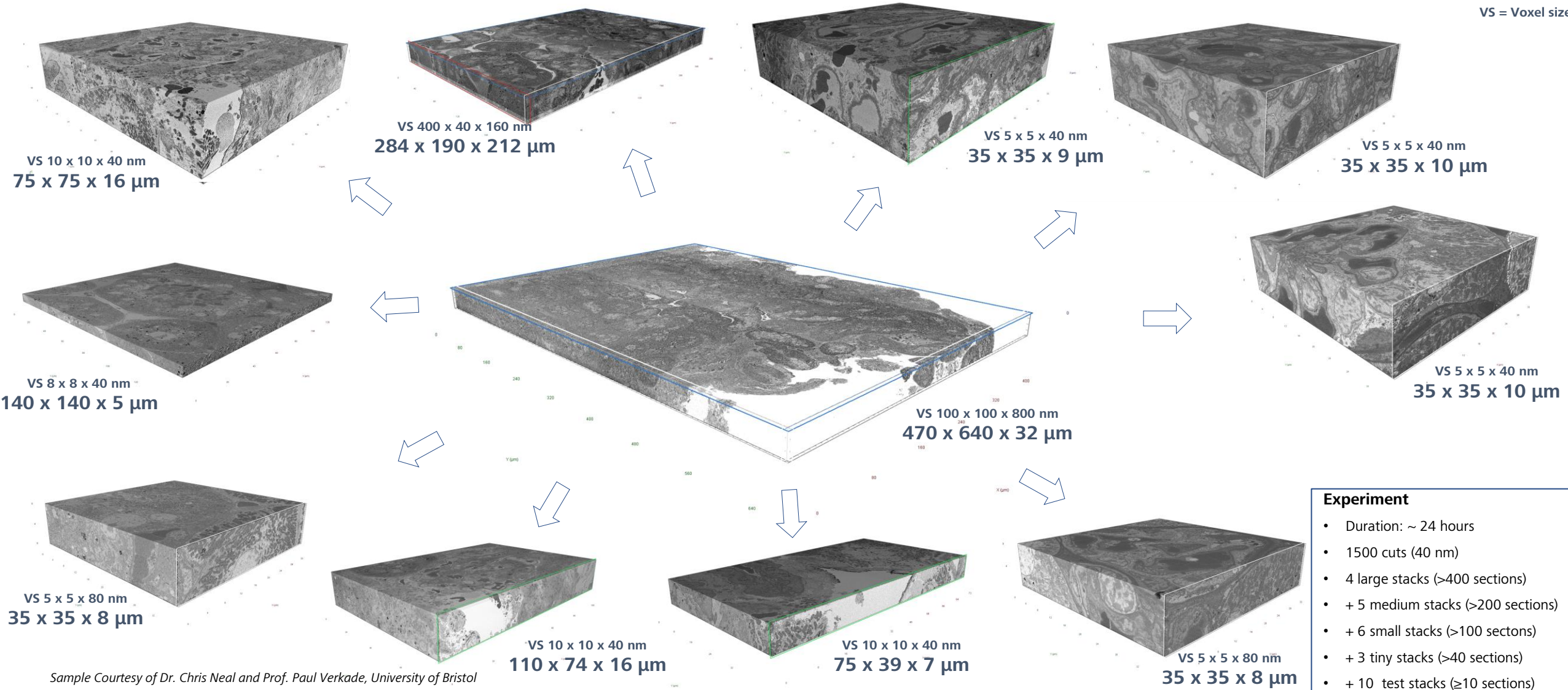


MultiROI Imaging of Mouse Kidney

One Experiment, multiple stacks with different ROIs and individual protocols

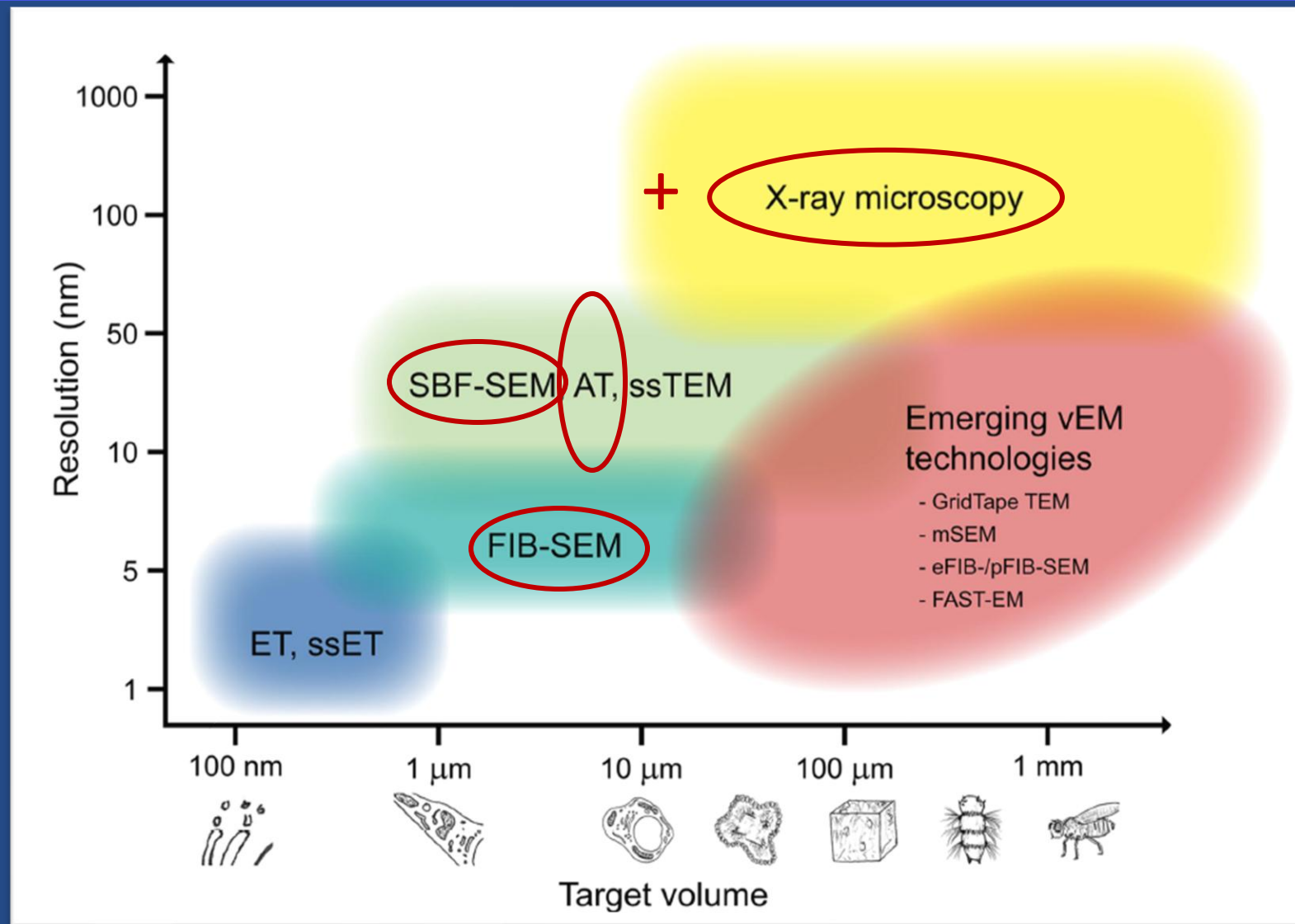


VS = Voxel size



- Experiment**
- Duration: ~ 24 hours
 - 1500 cuts (40 nm)
 - 4 large stacks (>400 sections)
 - + 5 medium stacks (>200 sections)
 - + 6 small stacks (>100 sections)
 - + 3 tiny stacks (>40 sections)
 - + 10 test stacks (≥10 sections)

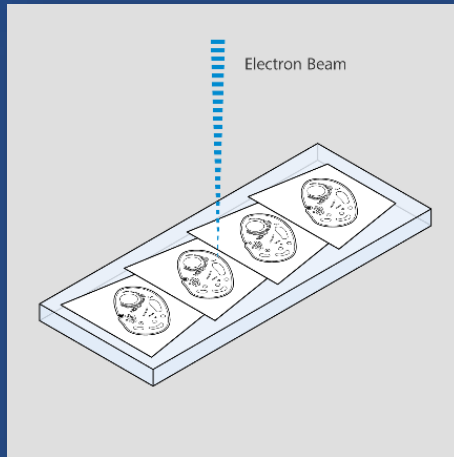
vEM as a multiscale multimodal imaging technique



Volume EM methods at a glance

Array Tomography (AT)

- Thin serial sections are cut and affixed to a sample holder.
- Serial sections are imaged by SEM.
- Serial 2D images are digitally reconstructed to create a 3D data set.

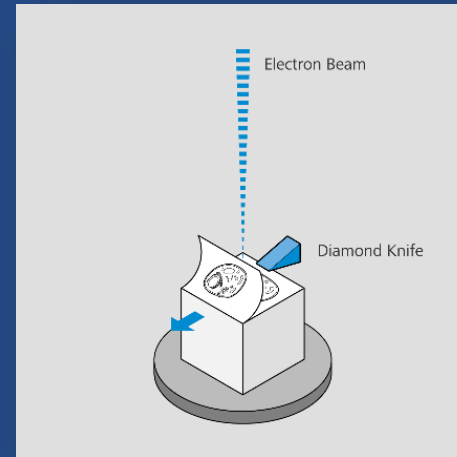


Main Benefits

- Can be performed with standard SEM
- Non-destructive imaging preserves the sample
- Particularly suitable for correlative workflows

Serial block-face SEM (Volutome)

- A resin-embedded sample is cut with an ultramicrotome mounted inside the SEM chamber.
- The newly exposed sample surface is imaged.
- This cutting and imaging process is repeated until the structure of interest is completely imaged.

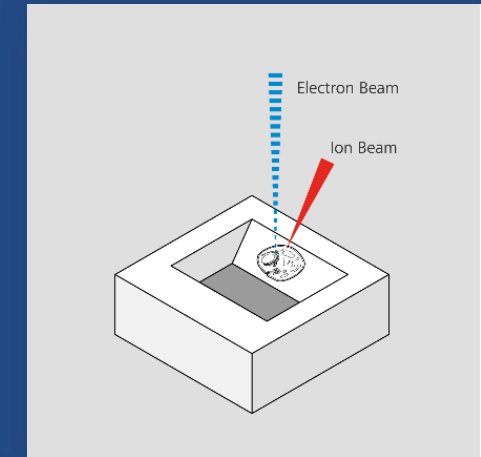


Main Benefits

- Highly automated image acquisition
- Large volume acquisition (1 mm cube)
- Higher Z-resolution than array tomography
- Focal CC for imaging charge-prone samples

FIB-SEM

- A focused ion beam mills away a layer from a resin-embedded sample as small as 3-10 nm before it is imaged by the electron beam.
- Milling and imaging is repeated until the structure of interest is completely imaged.

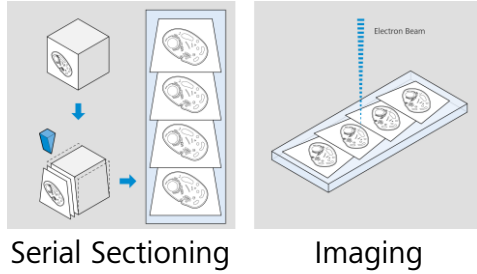


Main Benefits

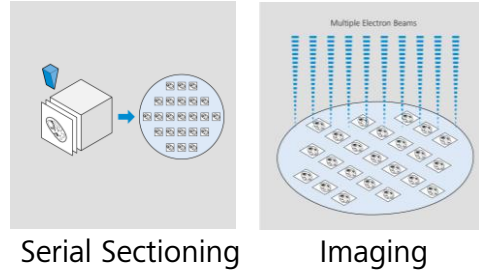
- Highest Z-resolution
- Isotropic 3D measurements
- Reconstruction of subcellular features in accurate 3D proportions

1

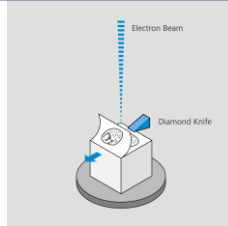
Array Tomography with single-beam SEM



Array Tomography with Multibeam SEM

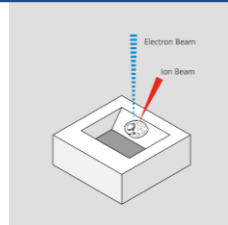


Serial block-face SEM (Volute)



Cutting inside the SEM + Imaging
Cutting & imaging cycle is repeated thousands of times

Focused ion-beam SEM

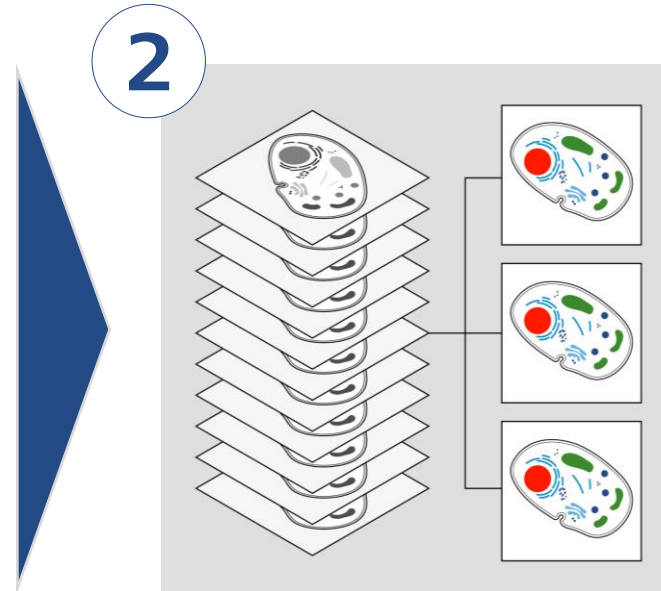


Trench Milling + Imaging
Milling & imaging cycle is repeated thousands of times

Volume EM Workflows

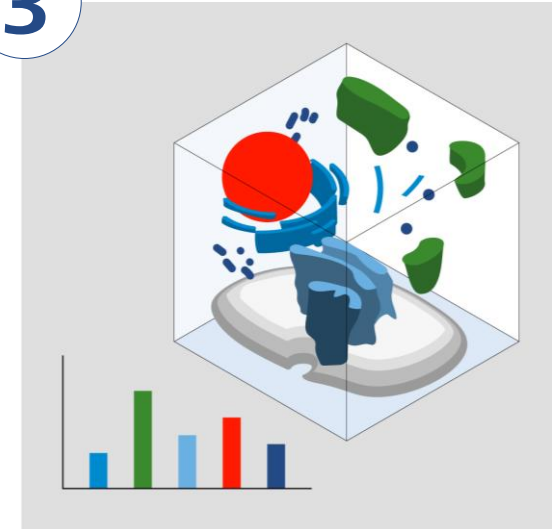


2



- Image Processing
- 3D Reconstruction
- Segmentation

3

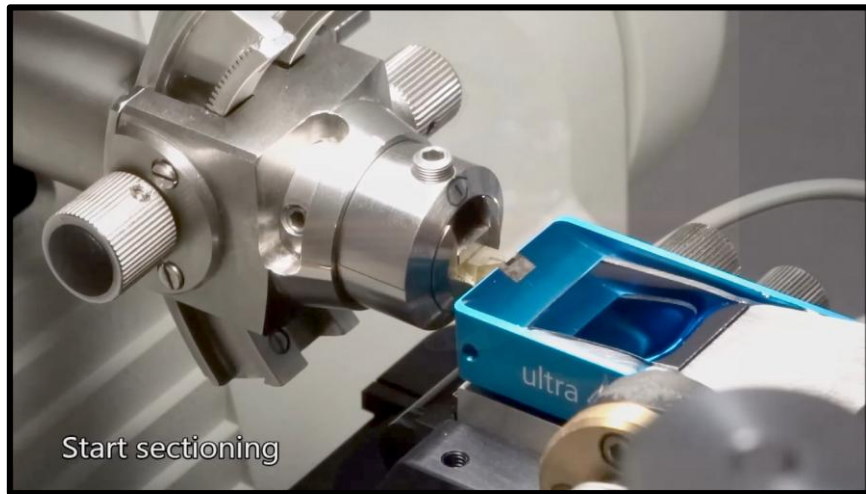


- Visualization
- Investigation
- Analysis

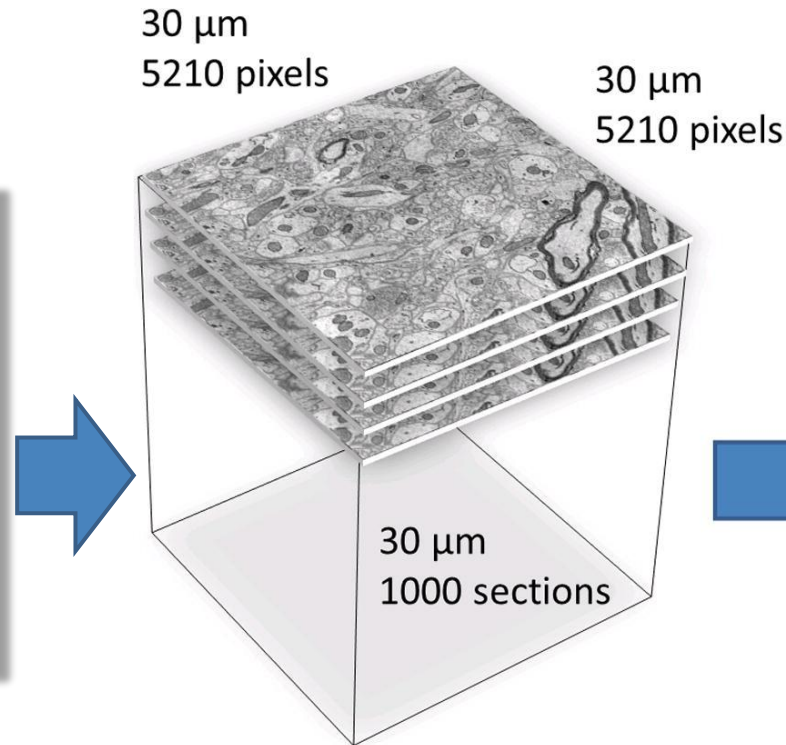
Array Tomography | Non-destructive Volume EM



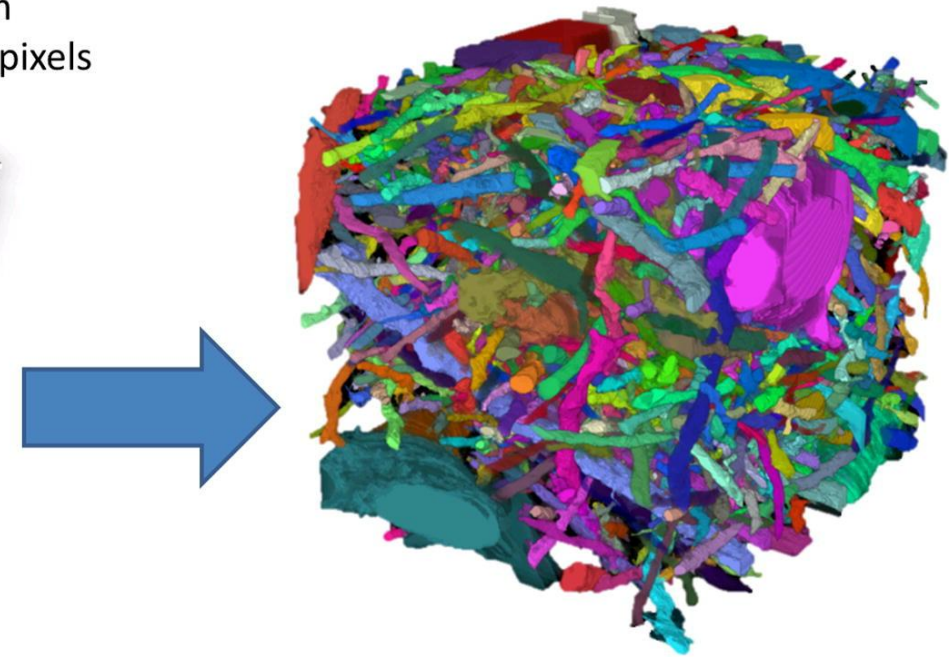
Kaynig et al. (2015) Medical Image Analysis



Serial sectioning



EM images



Reconstruction

Project

Sample Setup

Alignment

Section - Site Sets

ATLAS Acquisition

Sample Setup

Specimen Mounting:

Bare Stub holder for general u

Overview Images

Import Overview Image...

Align Overview Image

Reset Overview Alignment

Remove Overview Image

Custom Setup +

Sample Position +

Overlay -

Import 3D Overlay...

Import 2D Overlay Image...

Align 2D Overlay Image

Remove 2D Overlay Image

2D Overlay Transparency:

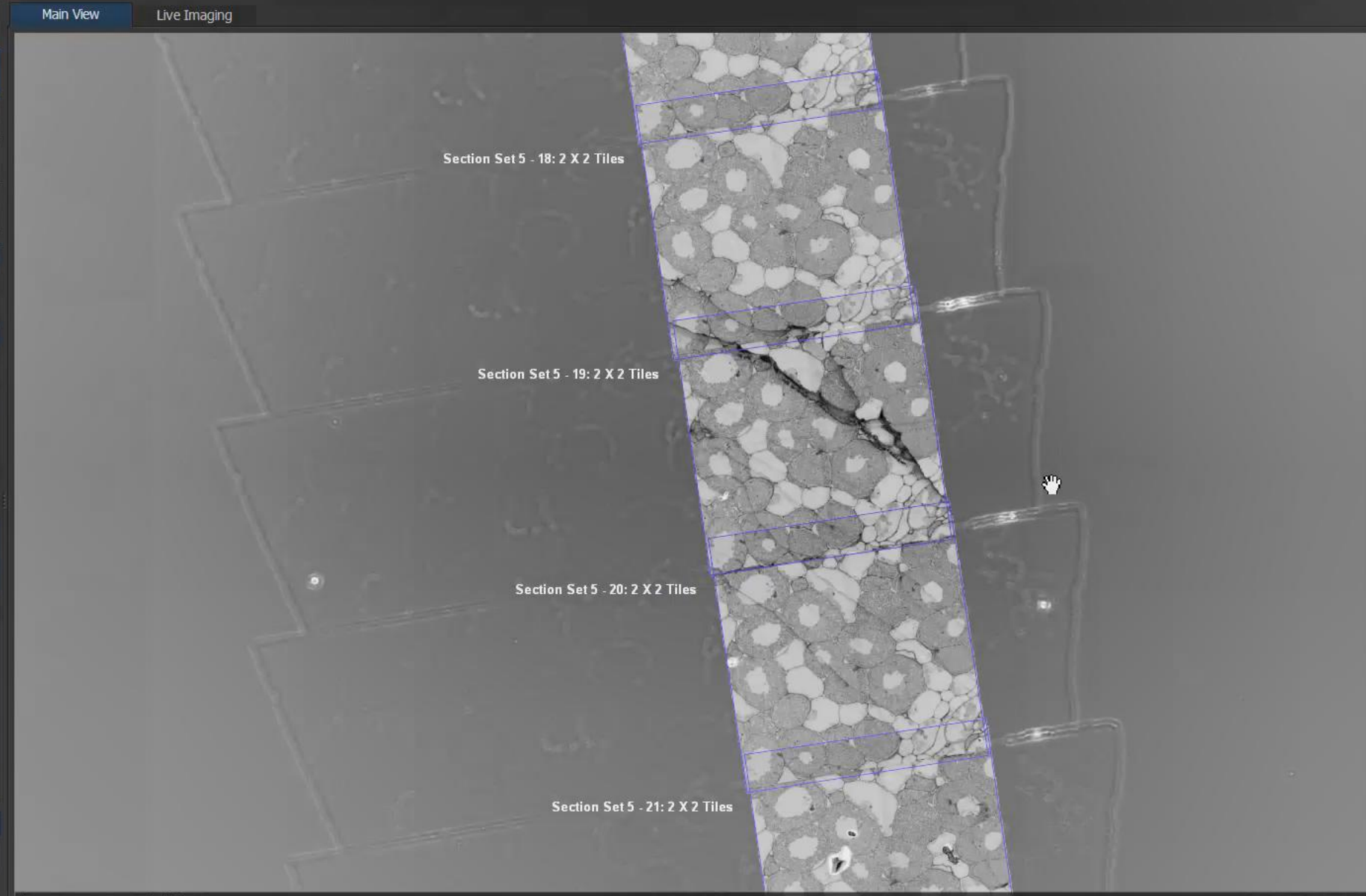
3D Overlay Transparency:

Display Settings -

Invert Display Reset

Brightness

Contrast



100 μm FOV: 1.1 mm Pixel Size : 777.5 nm

Navigation icons: Home, Pan, Zoom, Rotate, etc.

Protocol Name More

Project Browser

- Nodule Epoxy 20140513.xml
 - Overlay Images
 - 3D Volume Images
 - Overview Images
 - CAT Overview Images
 - Session: 20140513_1435
 - Session: 20140515_0918
 - Image Data

Image Browser

- hidden
- grab_3
 - NTS BSD (1.600 μs x 1)
 - 2048 x 2048 pixels
 - 1235.1 x 1235.1 μm
 - 603.1 nm/pixel
 - hidden
- grab_4
 - NTS BSD (1.600 μs x 1)
 - 2048 x 2048 pixels
 - 1235.1 x 1235.1 μm
 - 603.1 nm/pixel
 - hidden
- grab_5
 - NTS BSD (1.600 μs x 1)
 - 2048 x 2048 pixels
 - 1235.1 x 1235.1 μm
 - 603.1 nm/pixel
 - hidden
- grab_6
 - NTS BSD (1.600 μs x 1)
 - 2048 x 2048 pixels
 - 1235.1 x 1235.1 μm
 - 603.1 nm/pixel
 - hidden
- Region 1
 - NTS BSD (1.600 μs x 1)
 - 22528 x 36864 pixels
 - 13585.9 x 22231.5 μm
 - 603.1 nm/pixel
 - hidden

Selection Details

Array Tomography | Non-destructive vEM

Serial sectioning with cutting thickness down to 50 nm depends on ultramicrotome

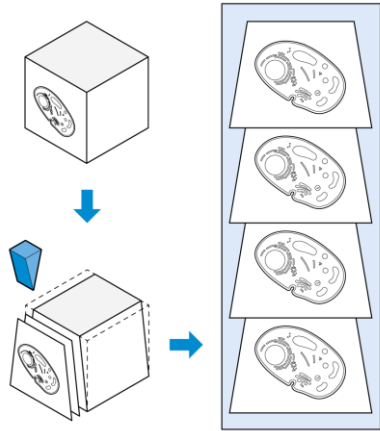
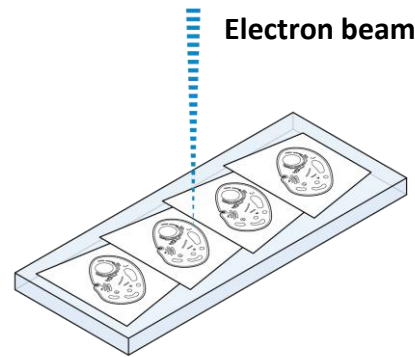


Image acquisition



Post processing

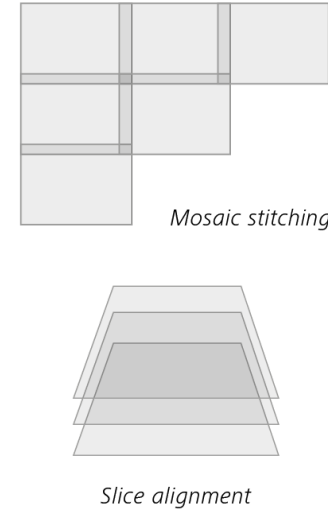
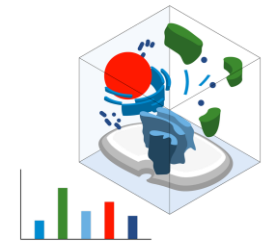
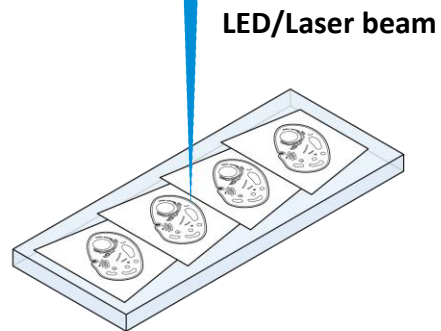


Image analysis



Can perform post-sectioning staining

Flexibility to schedule image acquisition



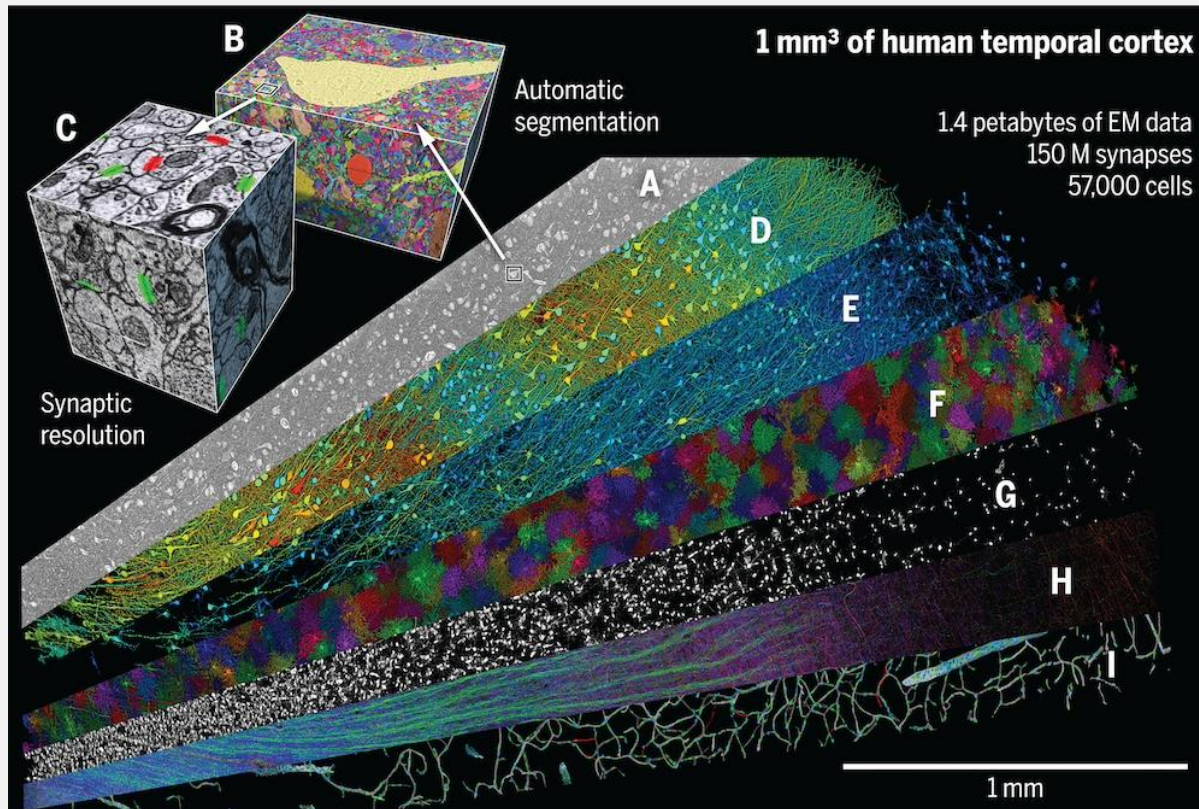
Correlative Array Tomography

Re-image

- Different resolution
- Different regions

Connect to other workflows (e.g., elemental mapping)

Mapping the Brain with Multibeam Array Tomography



A petavoxel fragment of human cerebral cortex reconstructed at nanoscale resolution

Alexander Shapson-Coe et al. (2024) Science

Harvard Researchers Create Largest-Known Map Of Portion of a Human Brain

IN THE LAB

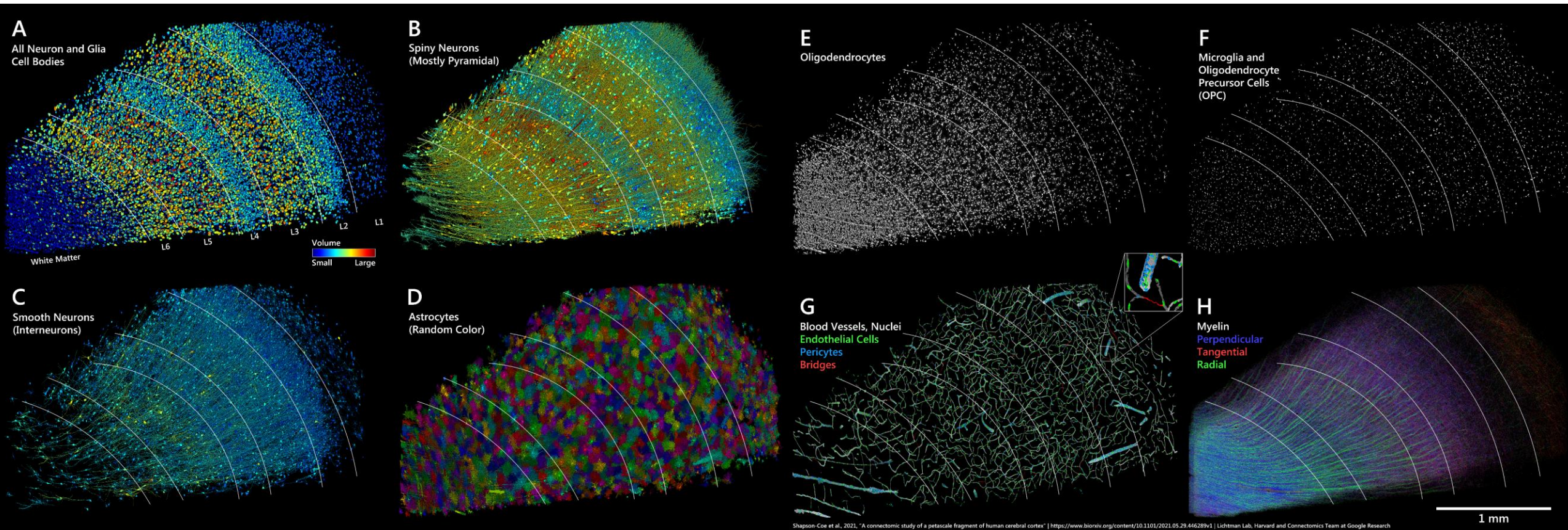
STAT+

Harvard scientists unveil the most detailed map of the brain ever: 'It's an alien world inside your own head'



Dr. Jeff W. Lichtman
Professor of Molecular and Cellular Biology, Harvard University, USA.

Mapping the Brain with Multibeam Array Tomography



A visual demonstration of various distributions among the cortical layers found in the sample.

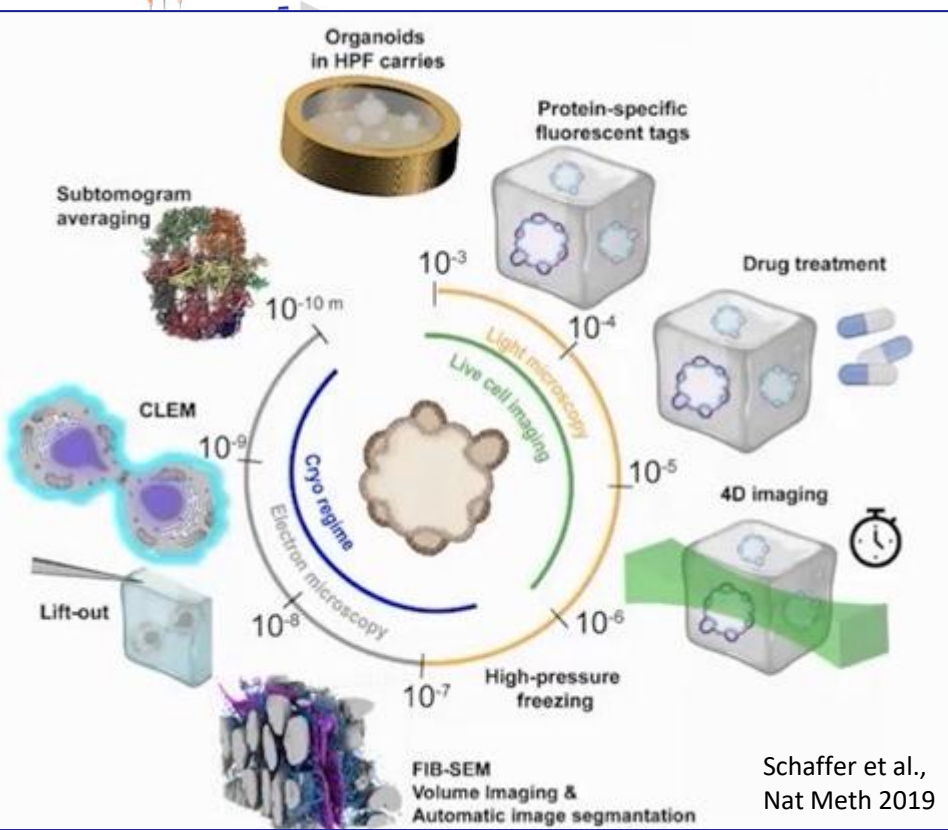
Why Correlative Microscopy ?

X-ray

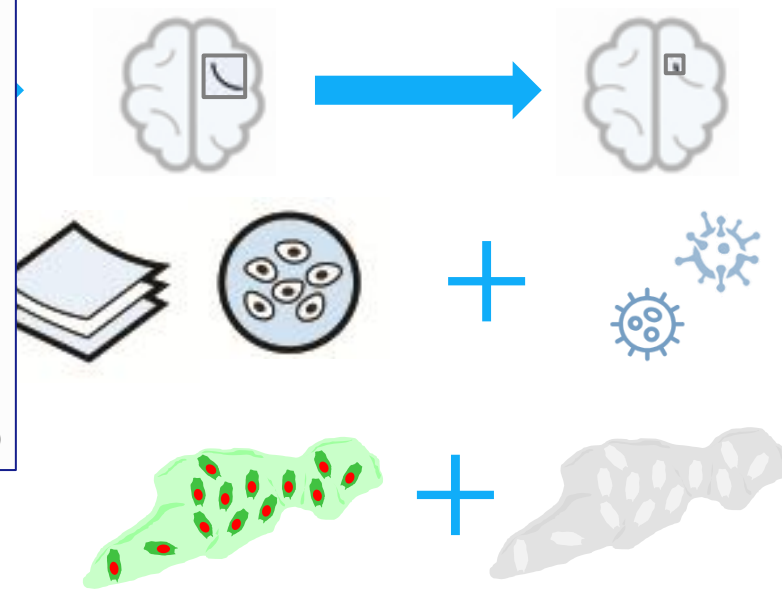
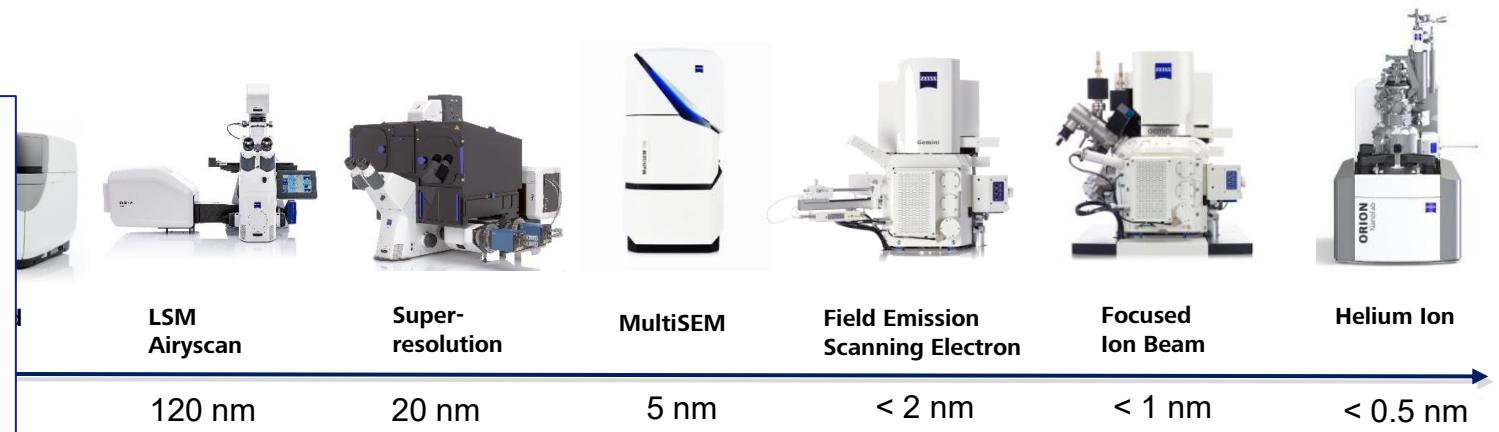
Light Microscopy

Electron Microscopy

Ion



imaging modalities



- Dive into ultrastructure
 - Neuroscience, cell biology
- Multi-dimensional research
 - Drug/vaccine research, spatial biology
- Combine fluorescence and topography
 - Biomaterial, pathology

Optimise your CLEM Workflow Using LM



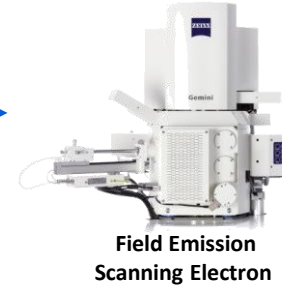
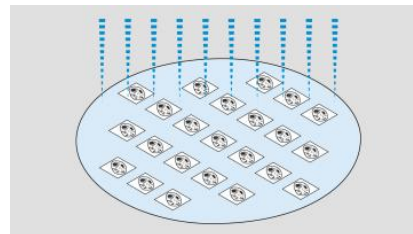
Light Microscopy

- Locate structures of interest with specific labelling
 - 3D dataset of targeted volume
 - Live cell imaging for dynamic events



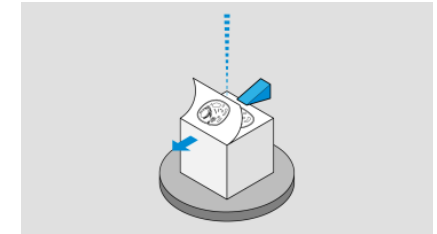
MultiSEM

**Multibeam
Array Tomography**

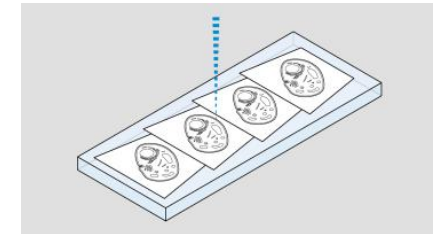


**Field Emission
Scanning Electron**

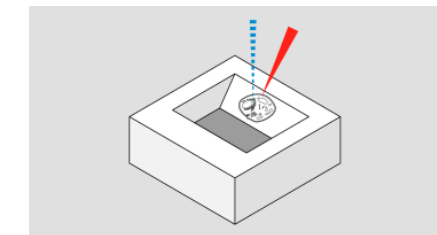
Serial Block-Face SEM



Array Tomography



Focused Ion Beam SEM (FIB-SEM)



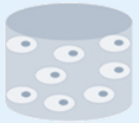
Focused Ion Beam

Combine Fluorescence Labeling and Topographic Scanning



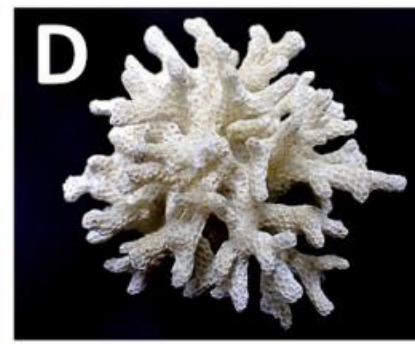
<https://doi.org/10.1016/j.matdes.2025.114610>

LSM 980 NLO x Cryo Gemini 360



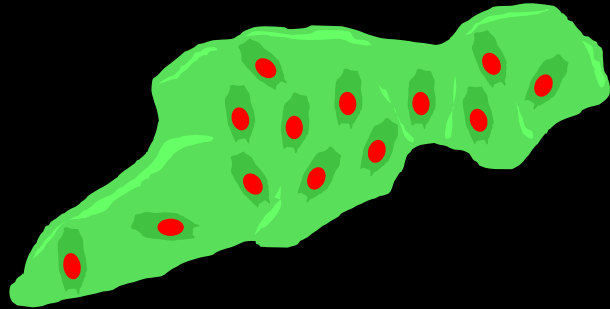
Verifying cell viability on coral substrate

PhD. Long Chang
Institute of Biopharmaceutical
Sciences, National Sun Yat-Sen
University, Kaohsiung, Taiwan

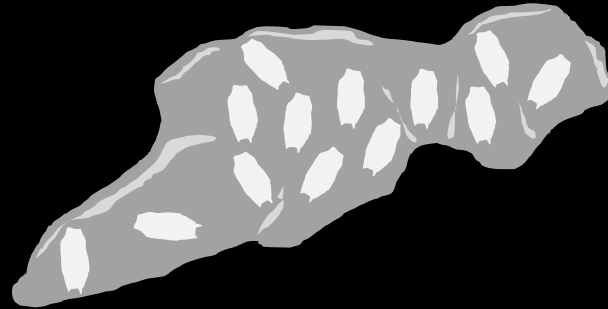


Verify bone cells on coral bone

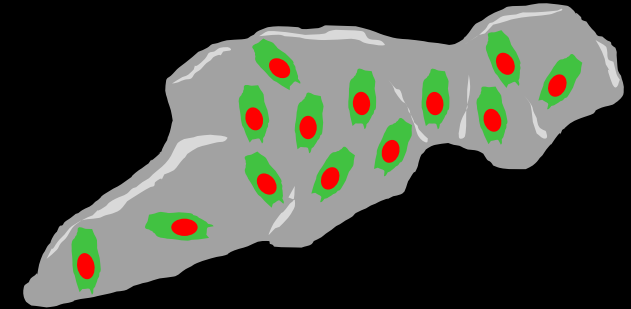
■ Coral bone ■ Bone cell



LSM 980 NLO
Localize bone cells



Cryo Gemini 360
Visualize cell morphology



ZEN connect
Verify bone cells on coral bone

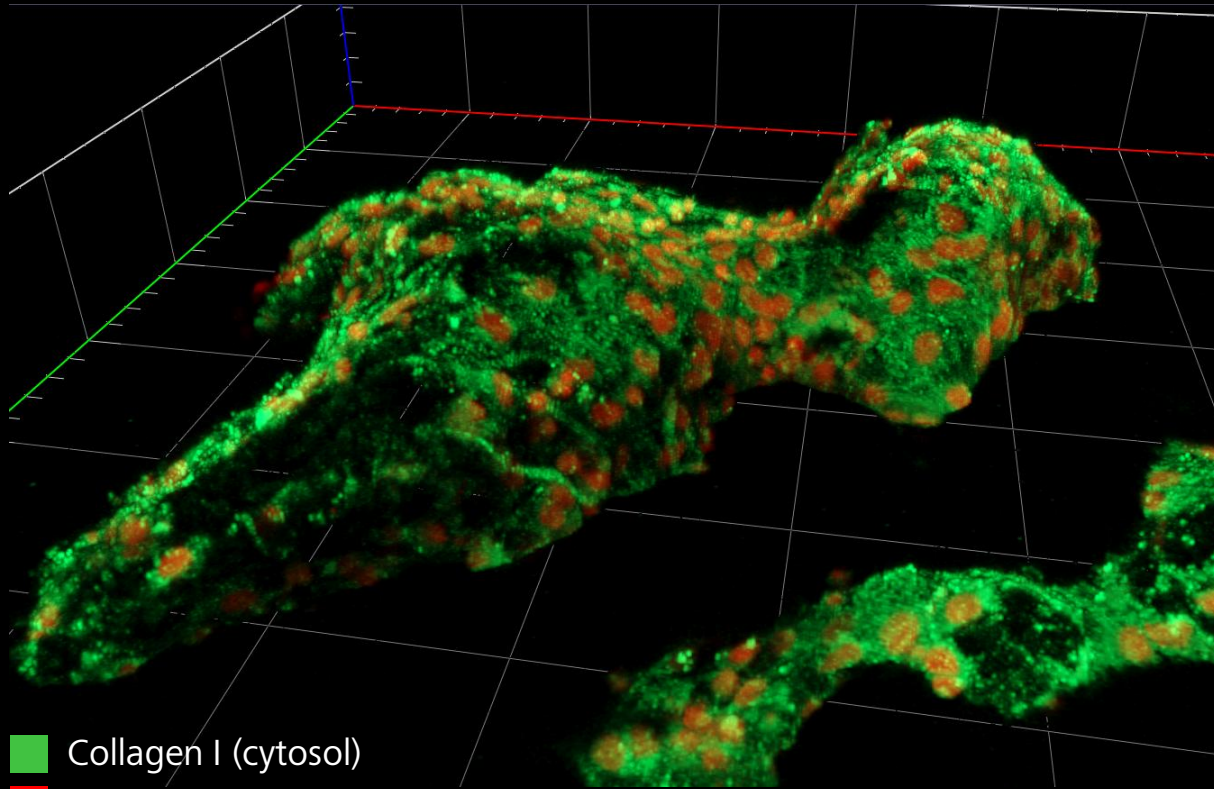


Challenges

- Autofluorescence from coral bone merged with immunolabeled bone cells.
- Bone cells showed little contrast against coral bone (SE, BSE)
- How to alleviate drifting during sample transfer

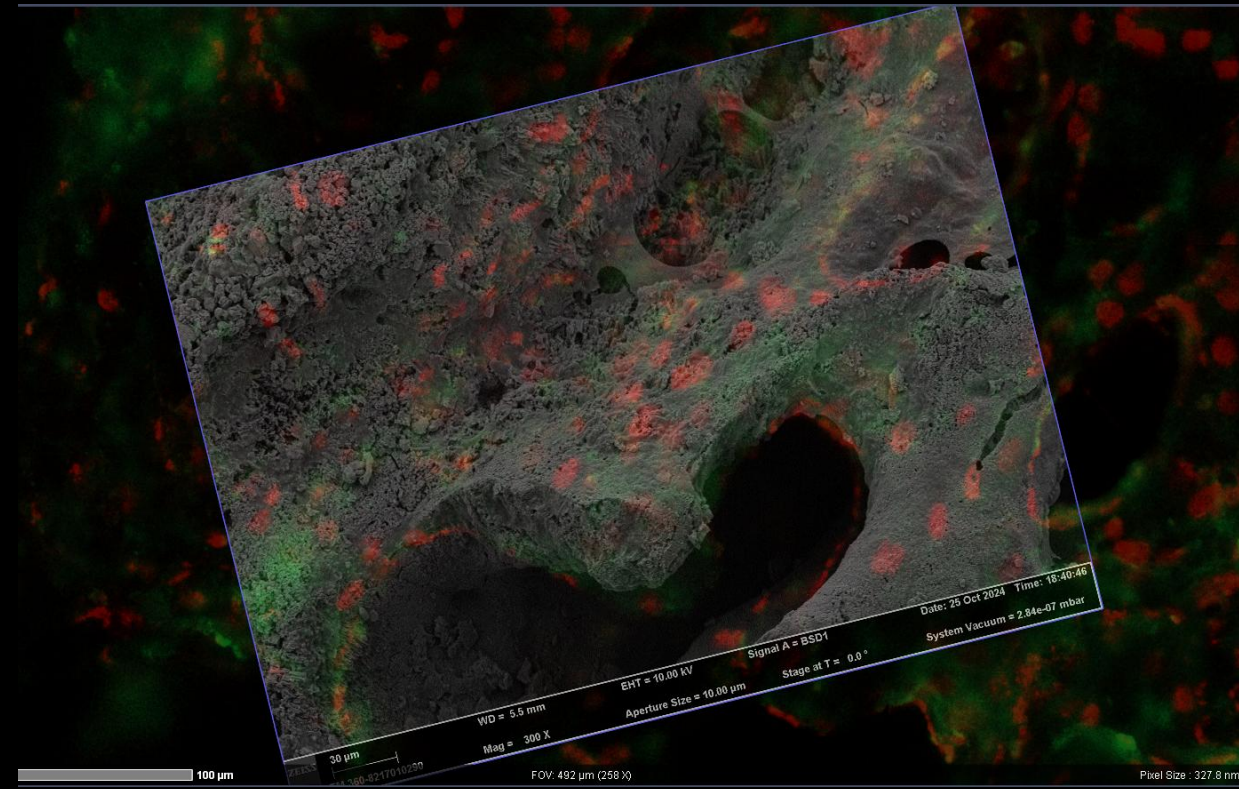
Verify bone cells on coral bone

LSM 980 NLO



■ Collagen I (cytosol)
■ Nuclei

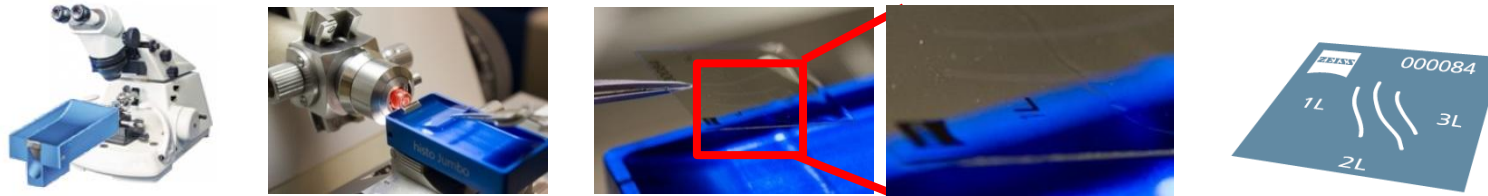
LSM 980 NLO + Cryo Gemini 360



CLEM enabled visualization of transparent targets on opaque substrate.

Correlative Array Tomography (CAT) Workflow

Ultramicrotome – Arrays of Serial Sections



Fluorescence Microscopy

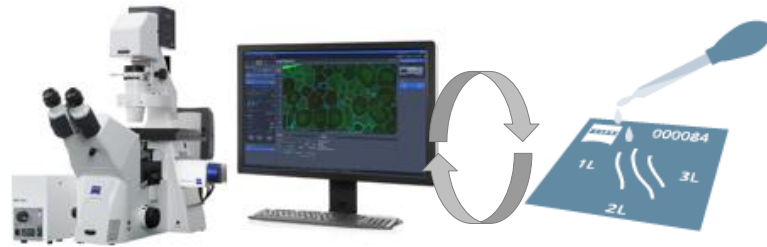
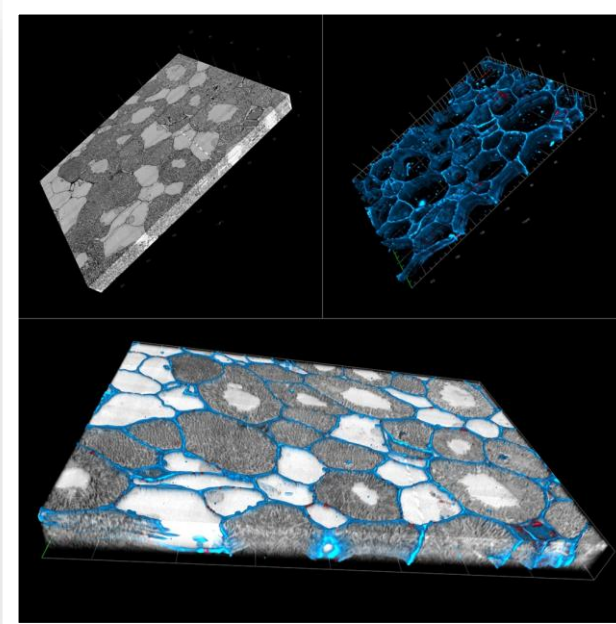


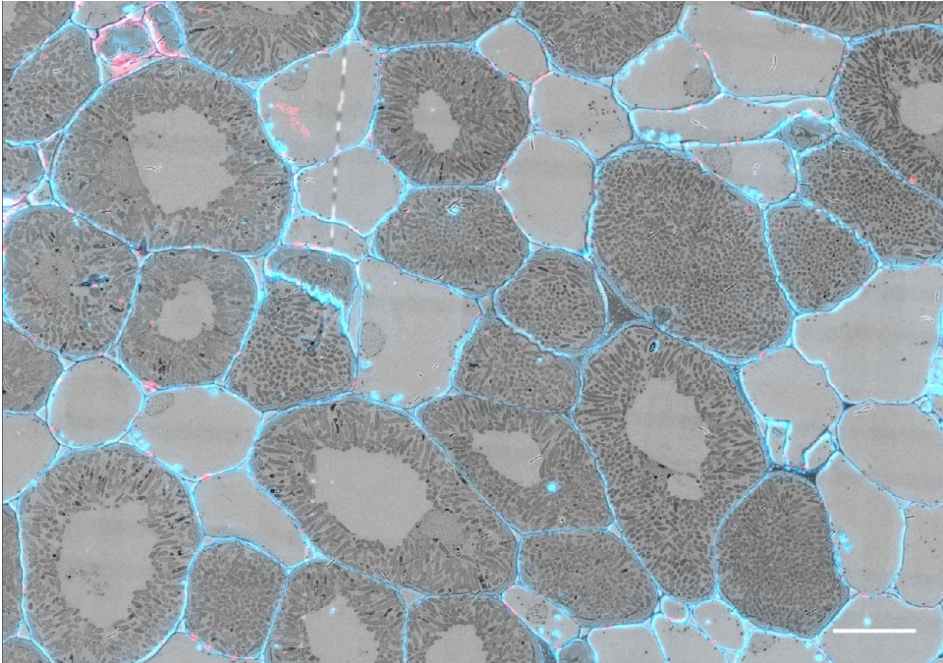
Image Processing



Scanning Electron Microscopy



Correlative Confocal and SEM of Root Nodules



■ Cell wall

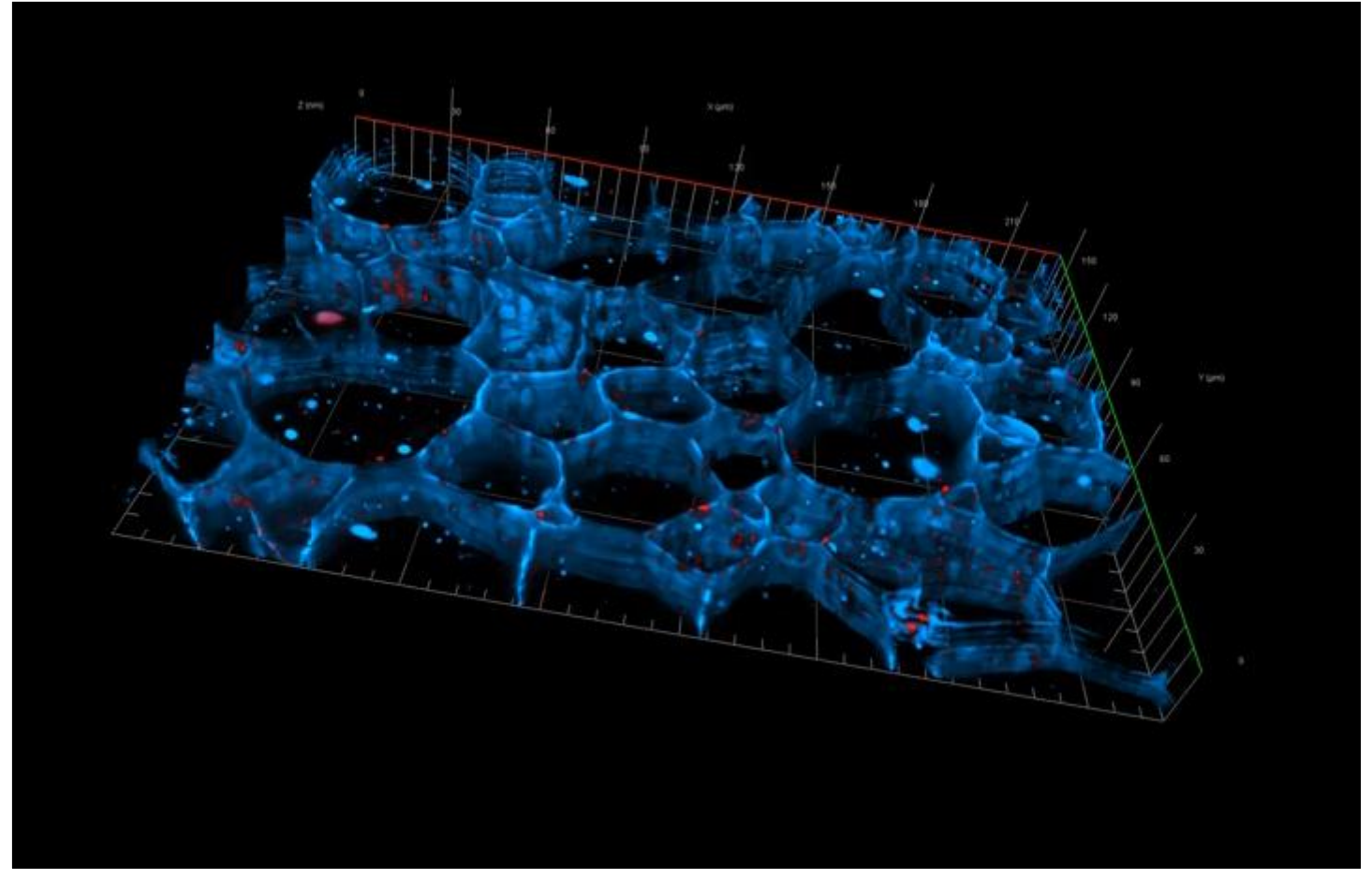
■ Plasmodesmate

AIM

Understand the symbiotic relationship of plants and bacteria in root nodules.

Method

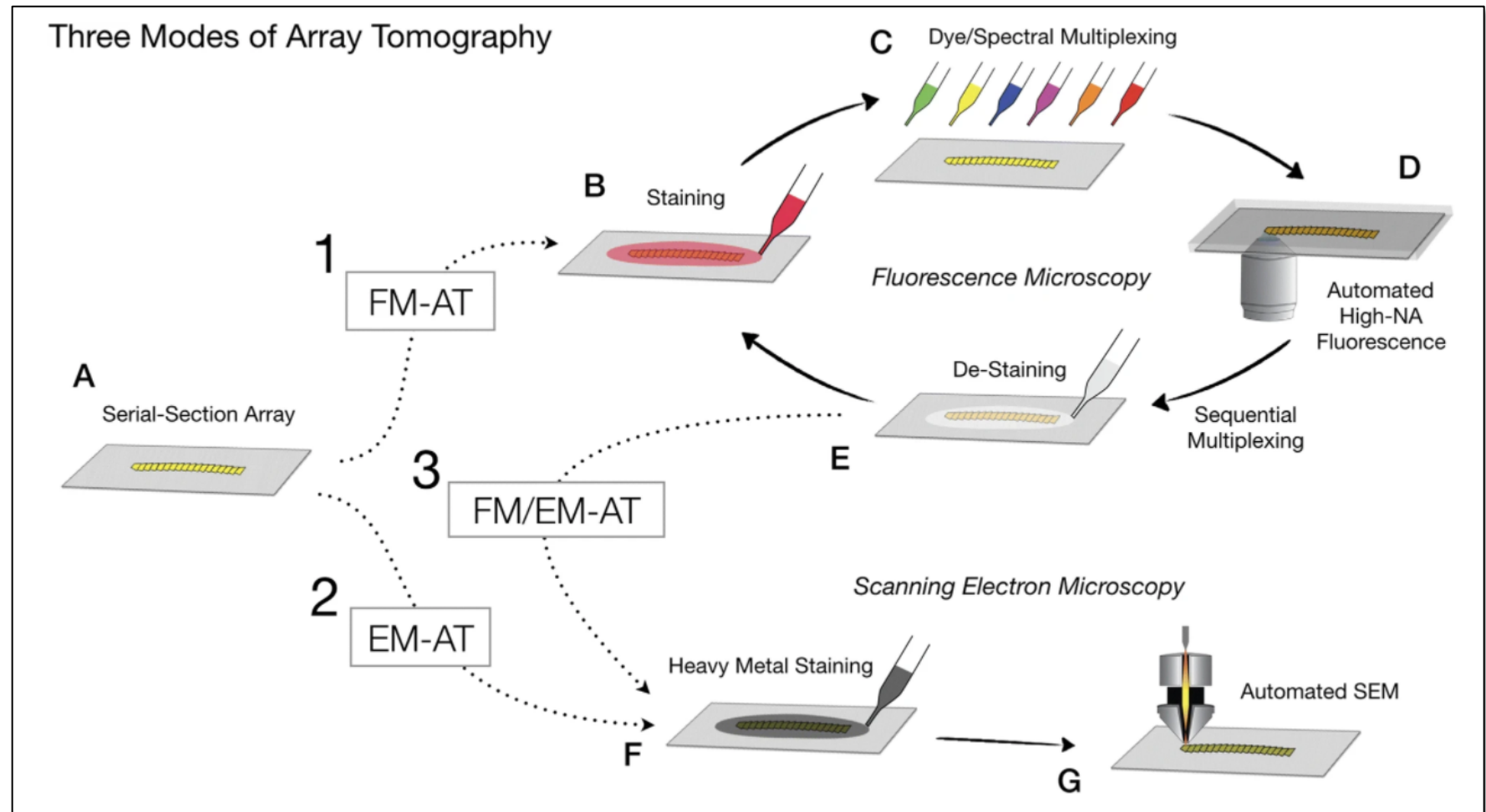
Visualize 3D distribution of plasmodesmata and bacterial infection threads in WT and mutants.



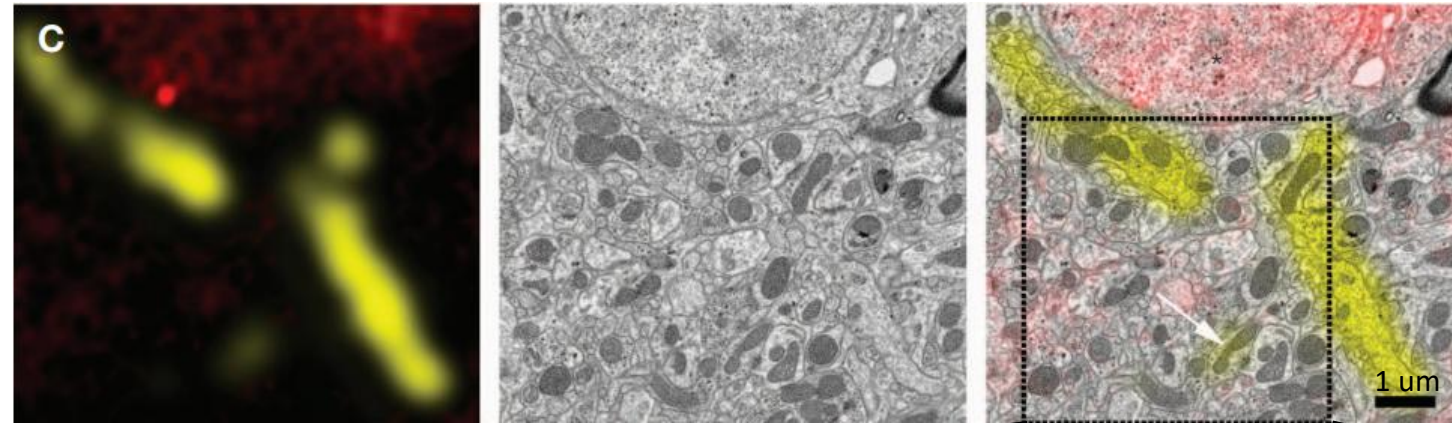
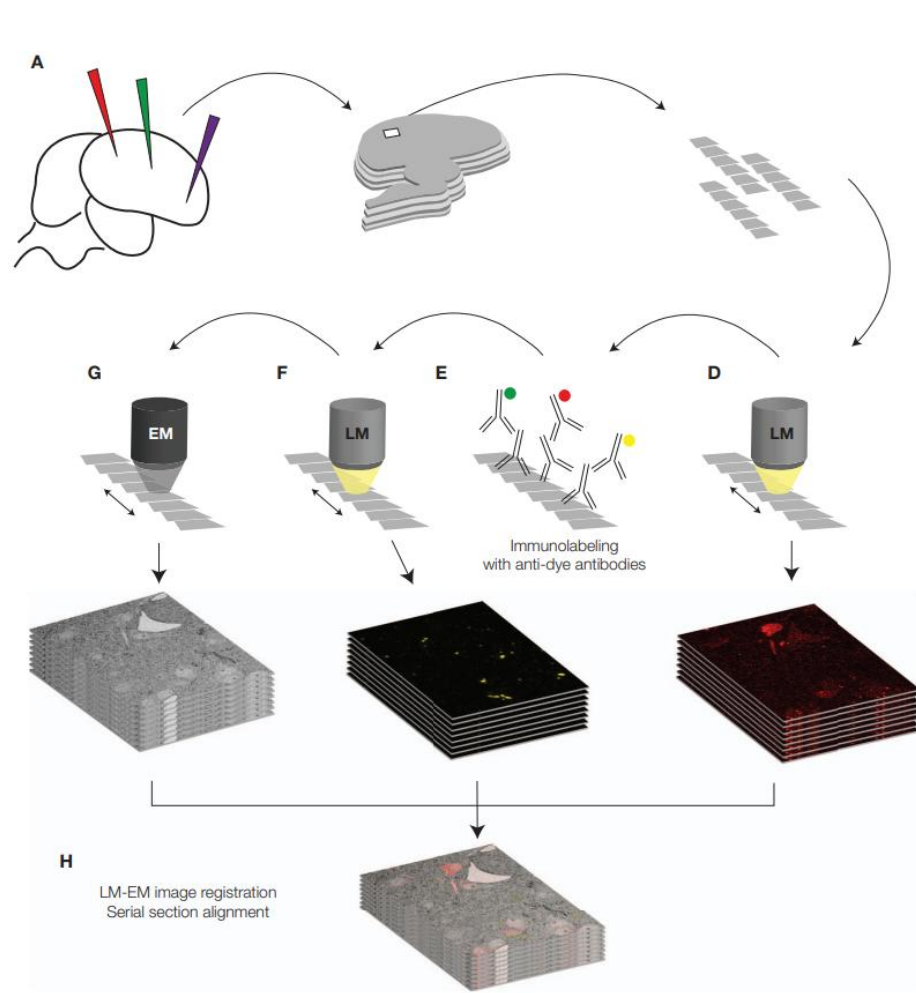
Courtesy of D. Sherrier, J. Caplan, and S. Modla, University of Delaware, USA.

Flexible Correlative Workflows

The collection of a ribbon of serial sections onto a substrate using an ultramicrotome followed by staining and imaging via LM and/or EM

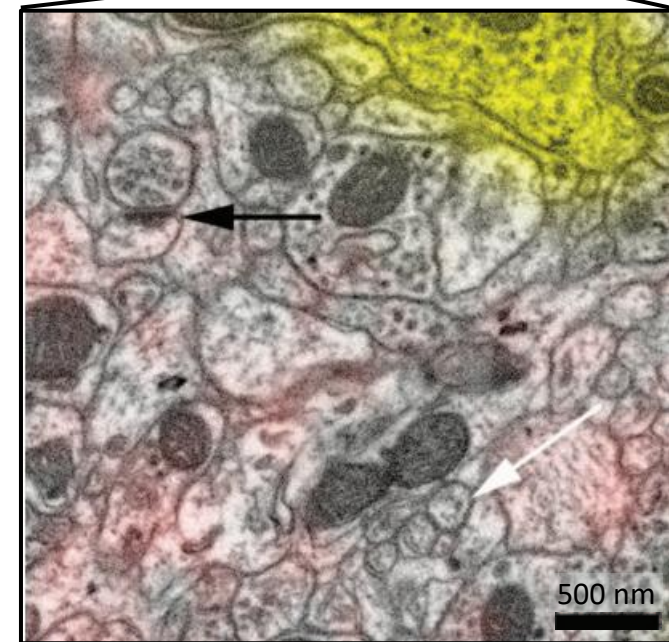


CAT for Neural Circuit Reconstruction



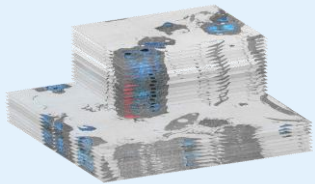
■ RA-projecting HVC neuron
■ HVC-projecting Uva neuron

Synapses are identified in EM imagery based on their morphology and ultrastructure and classified into projection neuron type based on fluorescence signal.

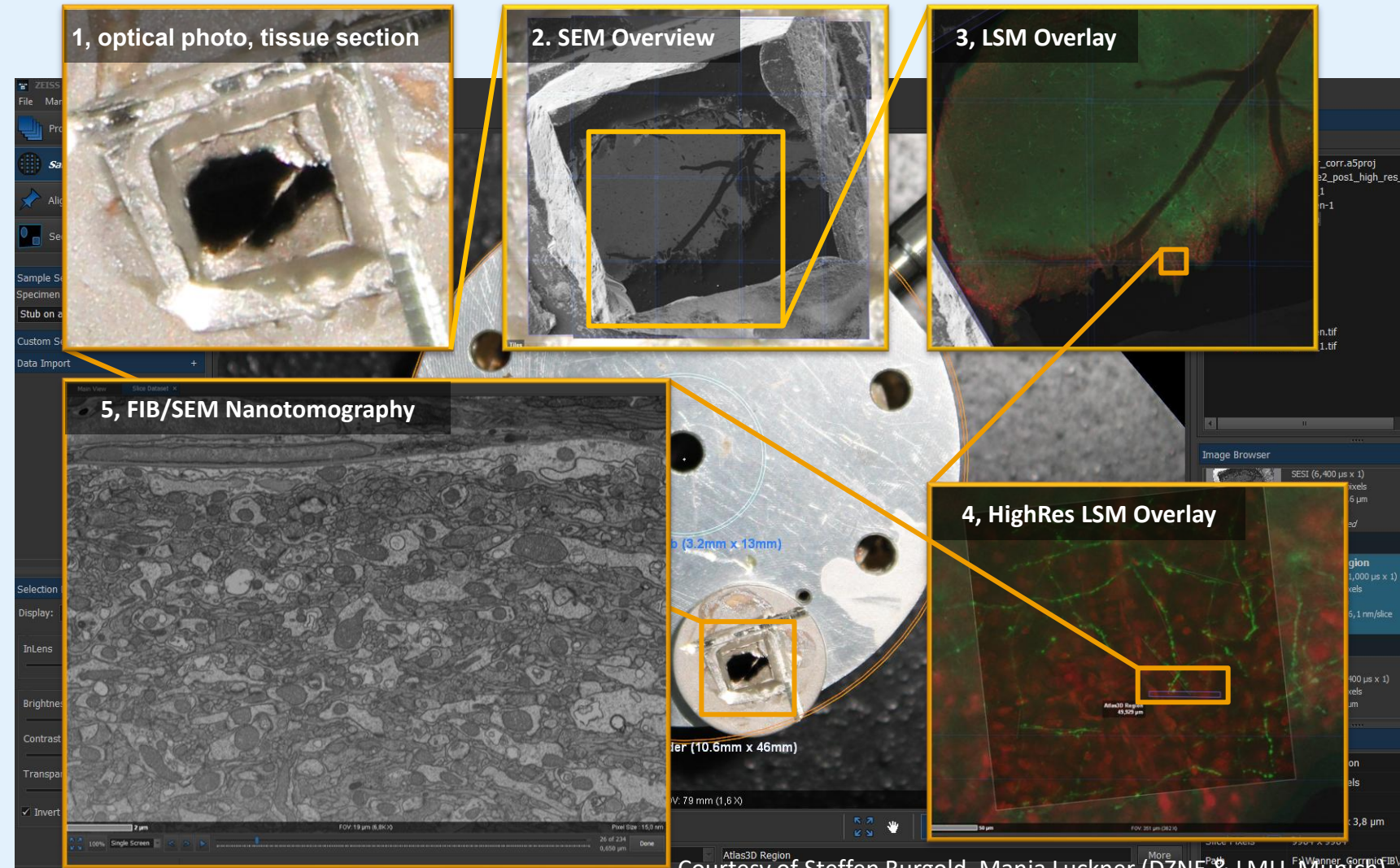


Confocal Microscope to FIB/SEM for Targeted Milling

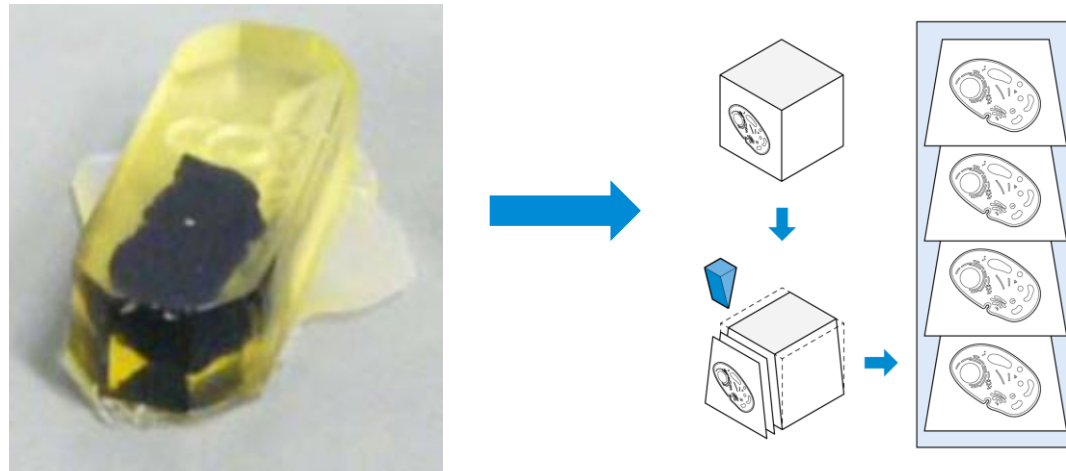
Correlative Microscopy



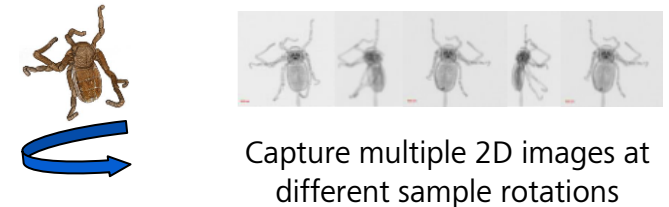
- Connect various microscopy system (i.e., LM, EM, XRM)
- Combine analytical solution (i.e., Raman, EDS)



How to observe the structure inside stained blocks?



Introducing X-ray Tomography



Calculate the 3D render of internal structure

Slice your sample in any orientation



How to evaluate the staining process?

How to localize the volume of interest?



Non-destructive 2D X-Ray Image series captured at **different sample orientations** is reconstructed into a **3D volume**

Optimise your Workflow to Volume Electron Microscopy Using X-ray Imaging



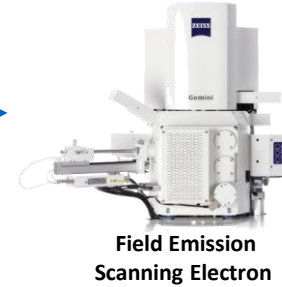
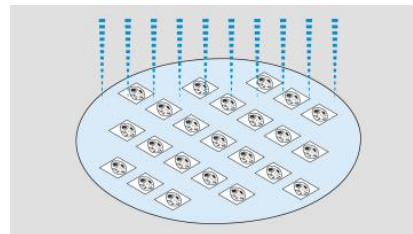
X-ray Microscopy

- Verify sample quality
- Locate structures of interest
- 3D overview dataset of the whole sample



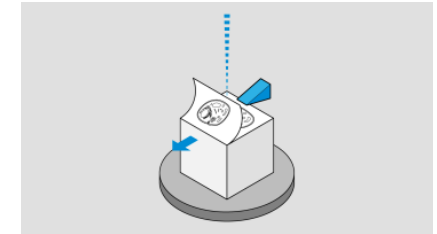
MultiSEM

Multibeam Array Tomography

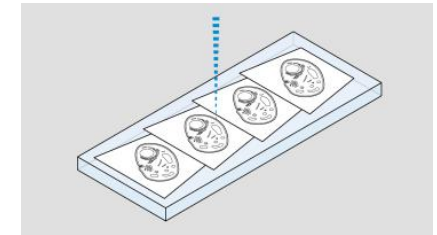


Field Emission Scanning Electron

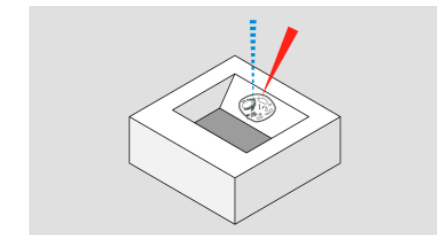
Serial Block-Face SEM



Array Tomography



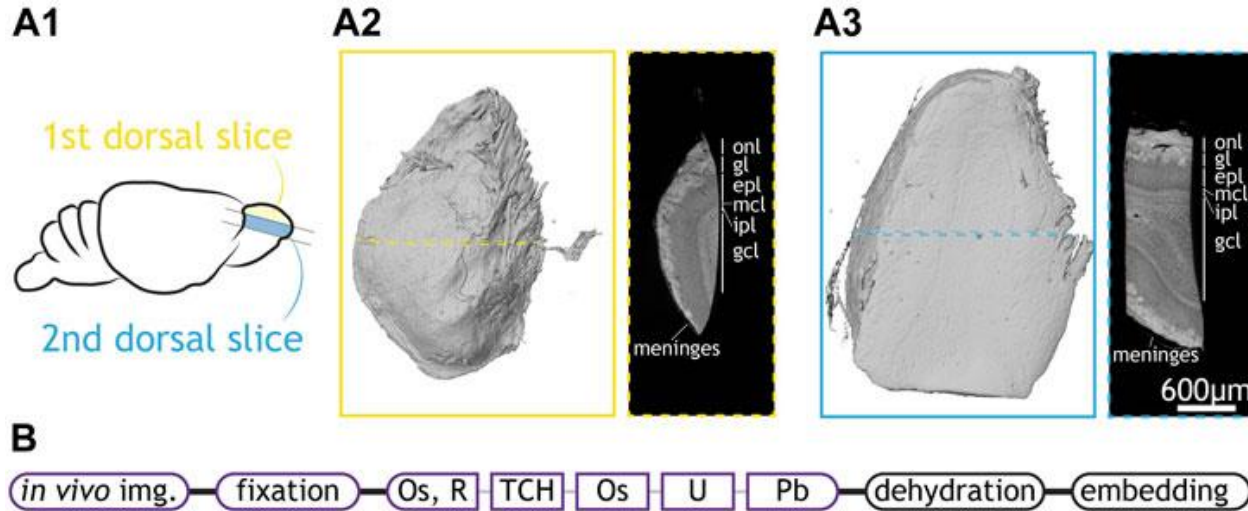
Focused Ion Beam SEM (FIB-SEM)



Focused Ion Beam

Using X-ray imaging to check staining and sample quality

Optimising EM sample preparation of mouse olfactory bulb

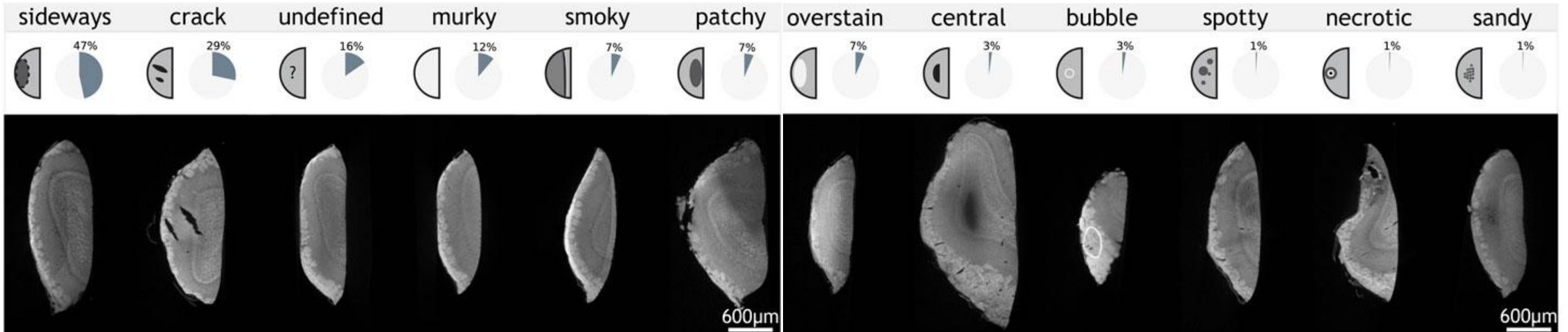


frontiers | Frontiers in Cell and Developmental Biology

Sample Preparation and Warping Accuracy for Correlative Multimodal Imaging in the Mouse Olfactory Bulb Using 2-Photon, Synchrotron X-Ray and Volume Electron Microscopy

Yuxin Zhang^{1,2}, Tobias Ackels^{1,2}, Alexandra Pacureanu^{1,2,3}, Marie-Christine Zdora^{4,5,6,7}, Anne Bonnin⁷, Andreas T. Schaefer^{1,2*} and Carles Bosch^{1*}

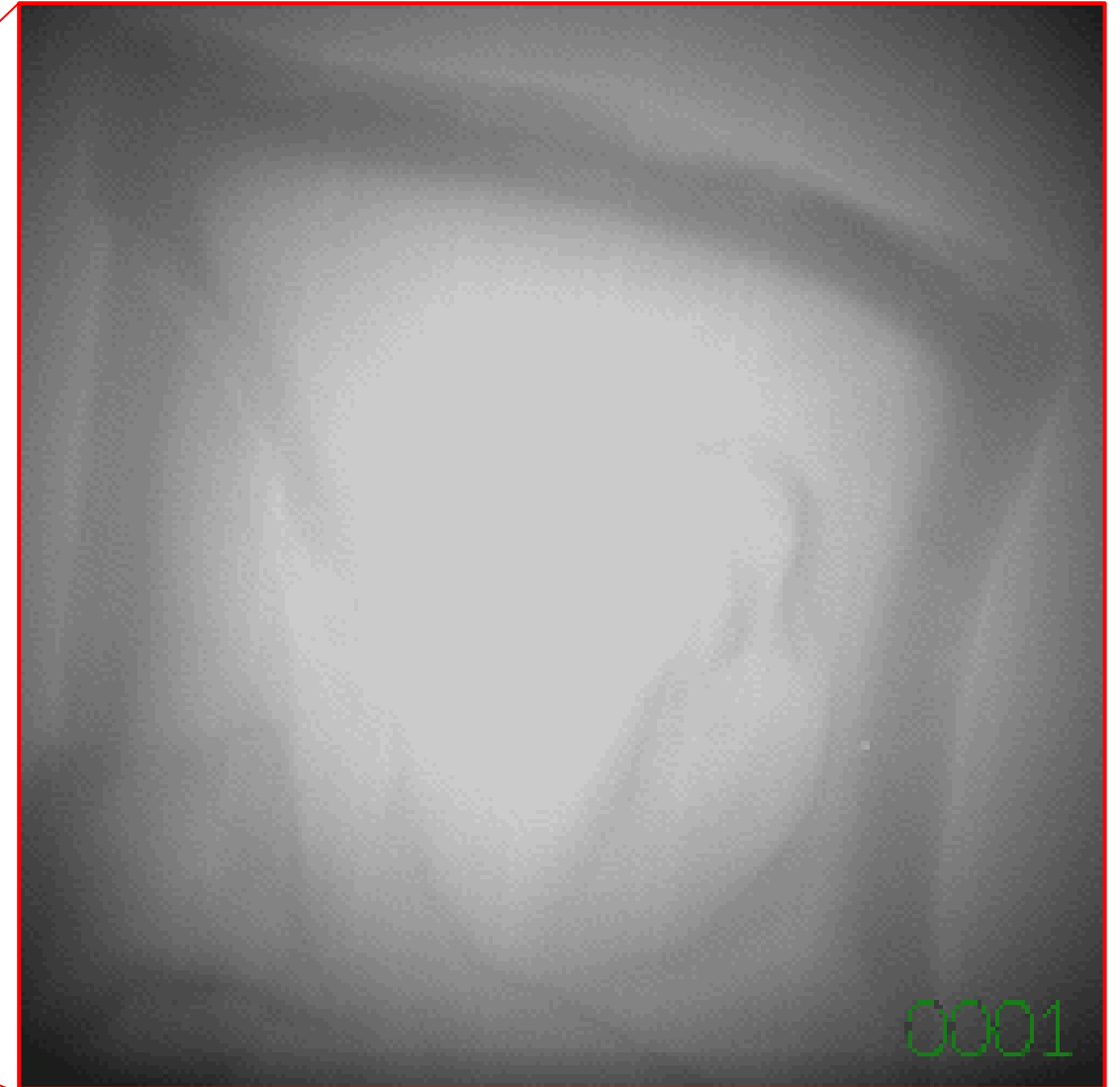
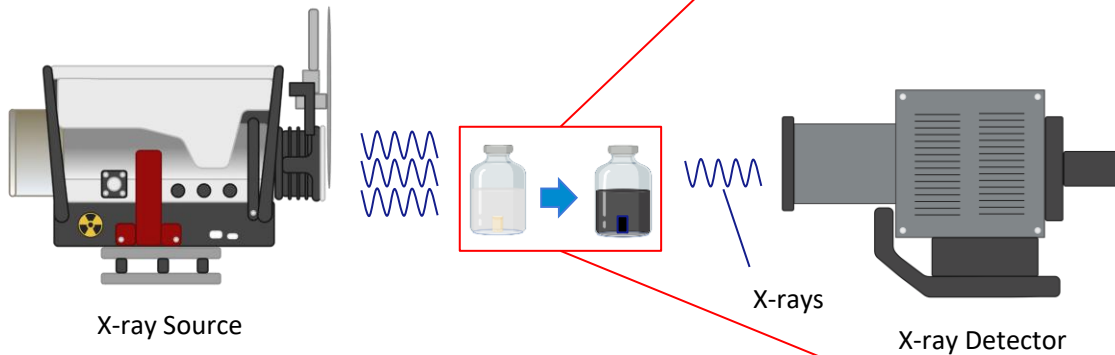
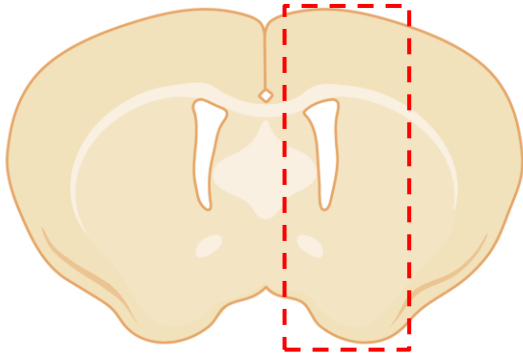
published: 08 June 2022



Sample quality verification

How many things can go wrong? *Optimise staining 'live' in the Versa*

4 mm Cylinder of Brain Tissue

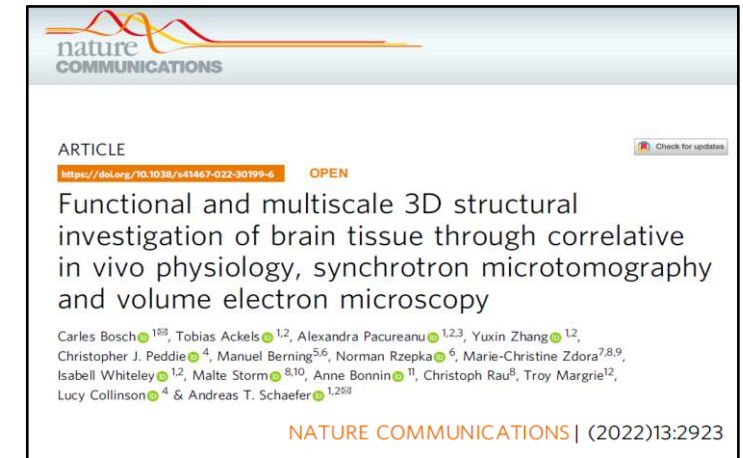
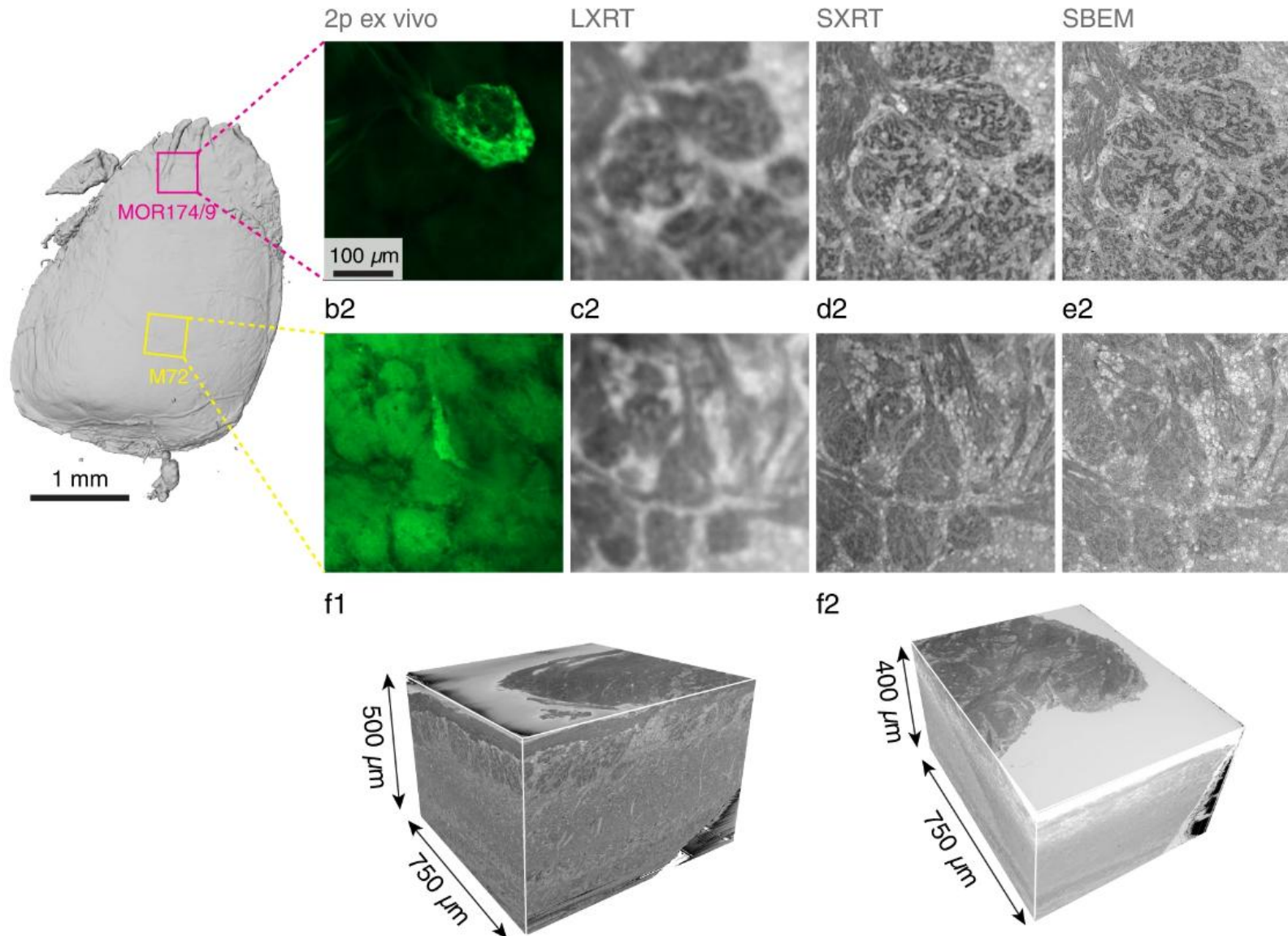


S. Ströh, E. Hammerschmith *et al* (2022) **In situ X-ray-assisted electron microscopy staining for large biological samples** *eLife* 11:e72147

<https://doi.org/10.7554/eLife.72147>

Time Lapse of Osmium Incubation

Correlative workflow of X-ray microscope and SBF-SEM



Multiscale structural investigation of brain tissue

Proactive Manage Multimodal Data

Integrated software solution for every steps



Image Management

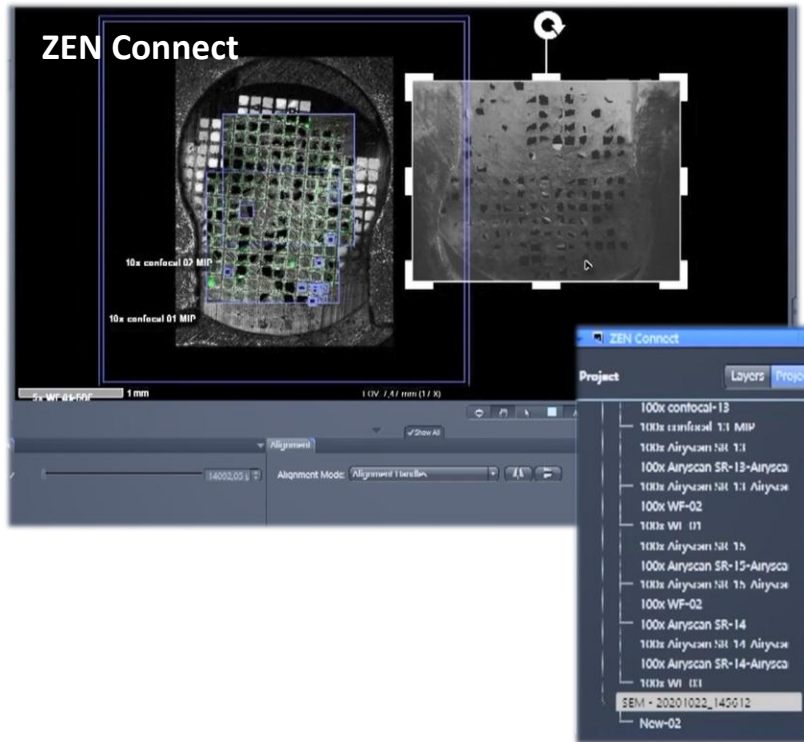


Image Optimisation

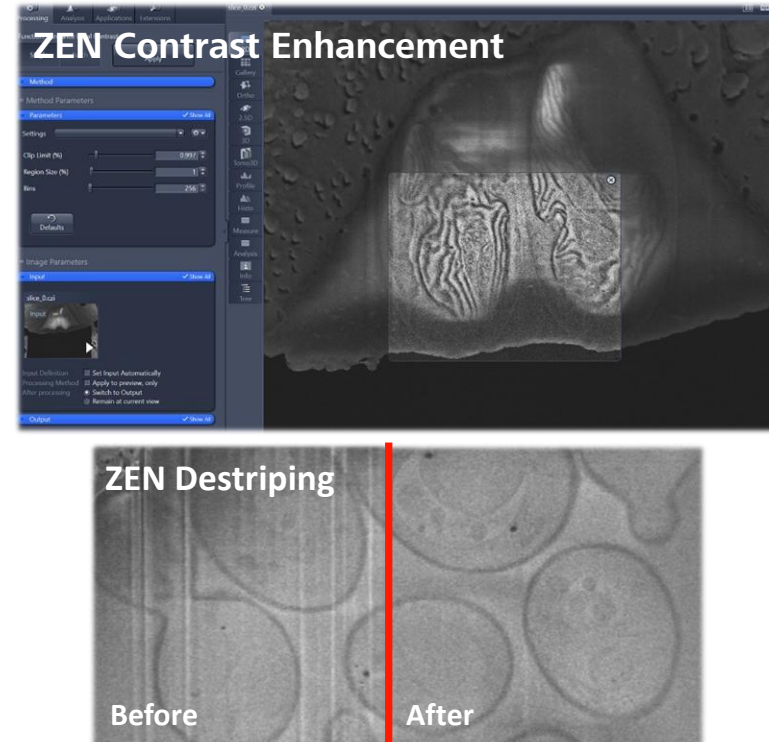
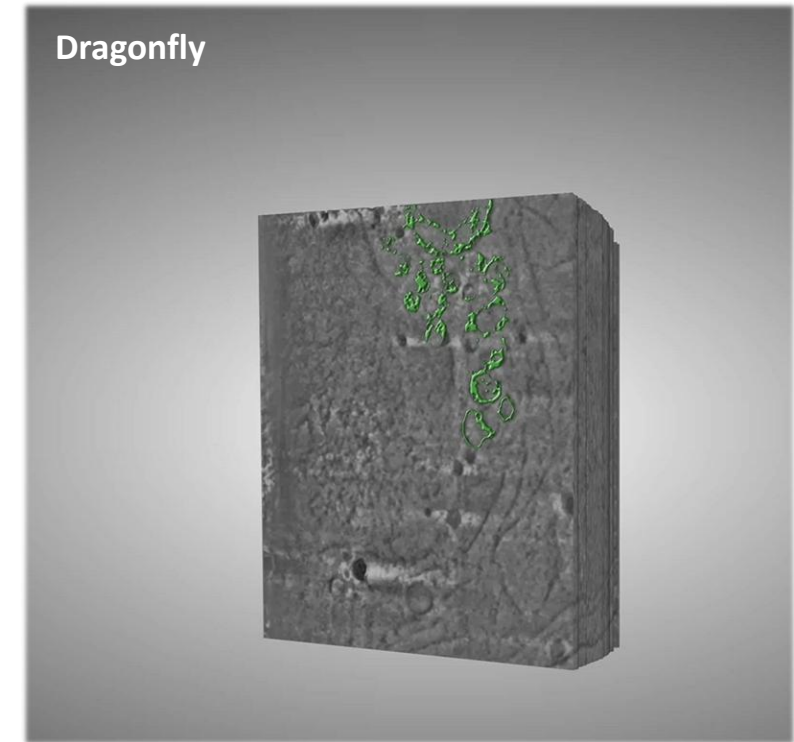


Image Visualisation



Structure datasets & metadata from multiple instruments with ZEN Connect

Remove Stripes and enhance contrast with ZEN EM Processing Toolbox

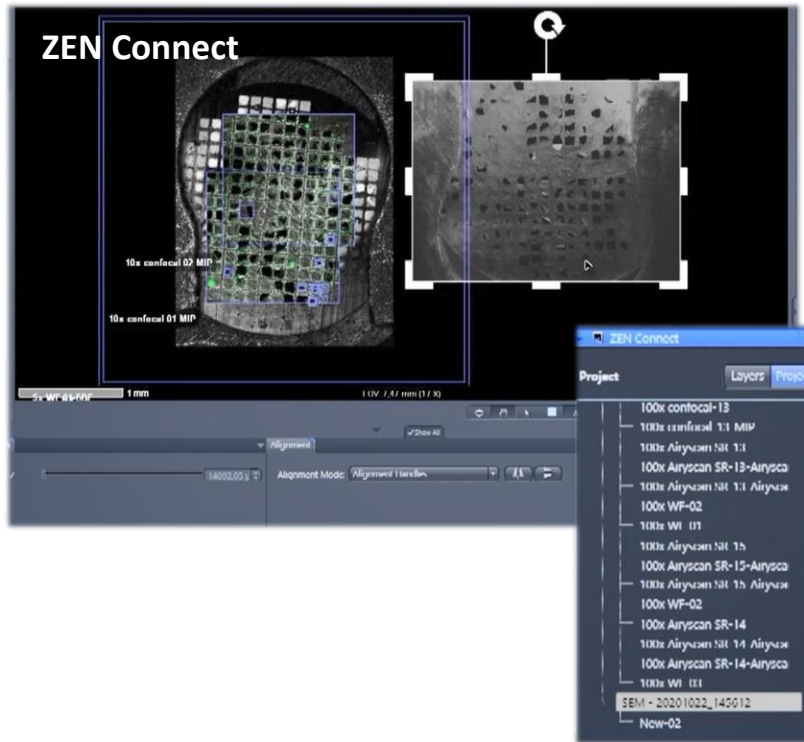
Visualise your 3D SEM data with ZEN, arivis or Dragonfly

Proactive Manage Multimodal Data

Integrated software solution for every steps

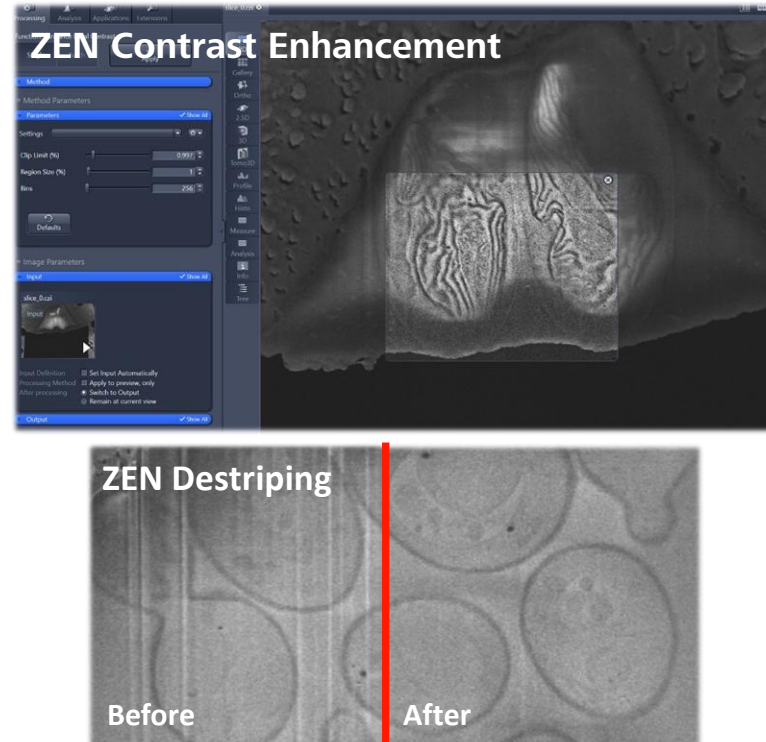


Image Management



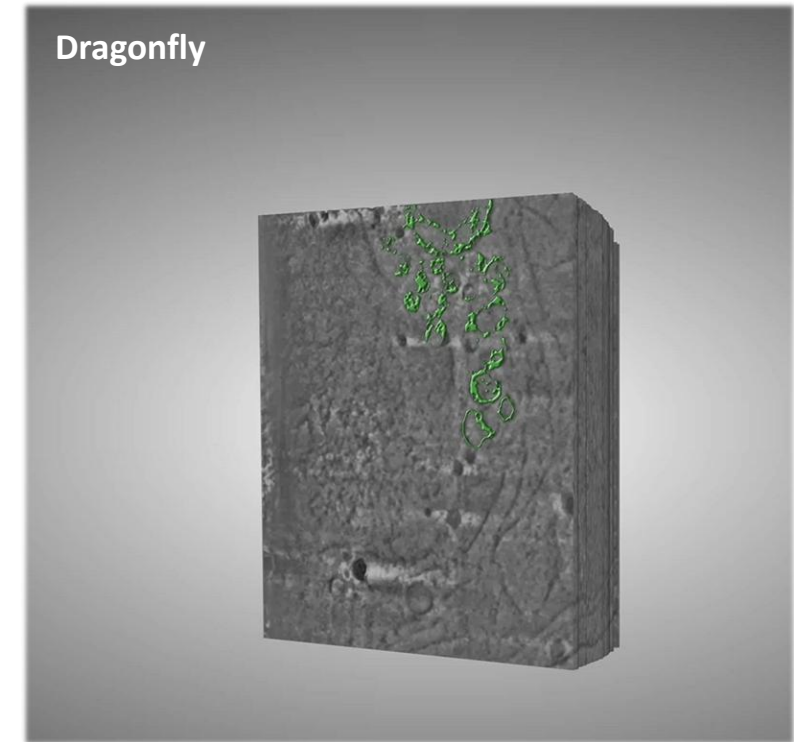
Structure datasets & metadata from multiple instruments with ZEN Connect

Image Optimisation



Remove Stripes and enhance contrast with ZEN EM Processing Toolbox

Image Visualisation



Visualise your 3D SEM data with ZEN, arivis or Dragonfly

ZEN EM toolbox

Image (stack) processing to facilitate its analysis

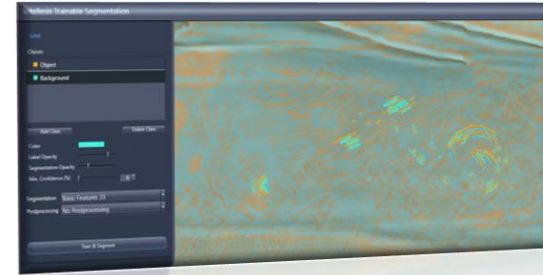
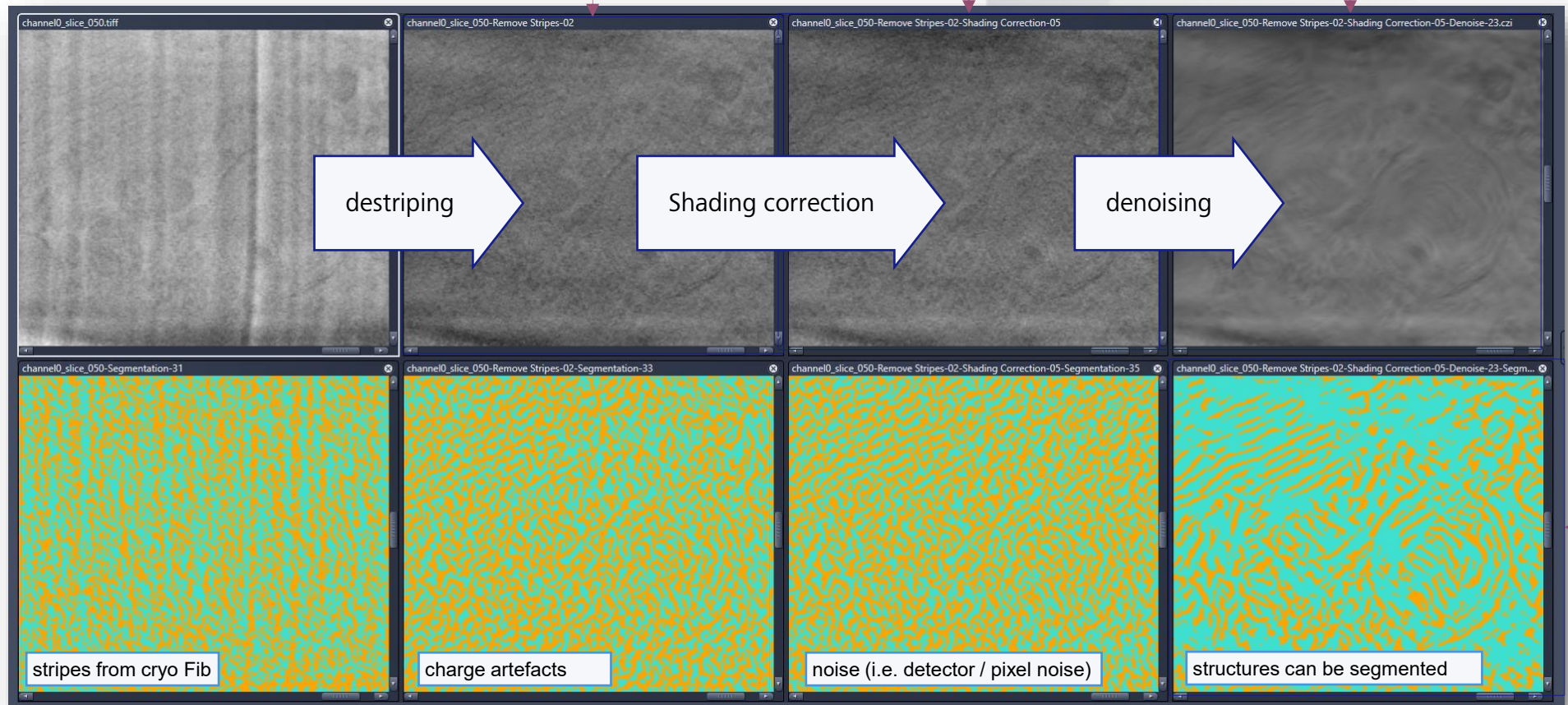
ZEISS Help Viewer



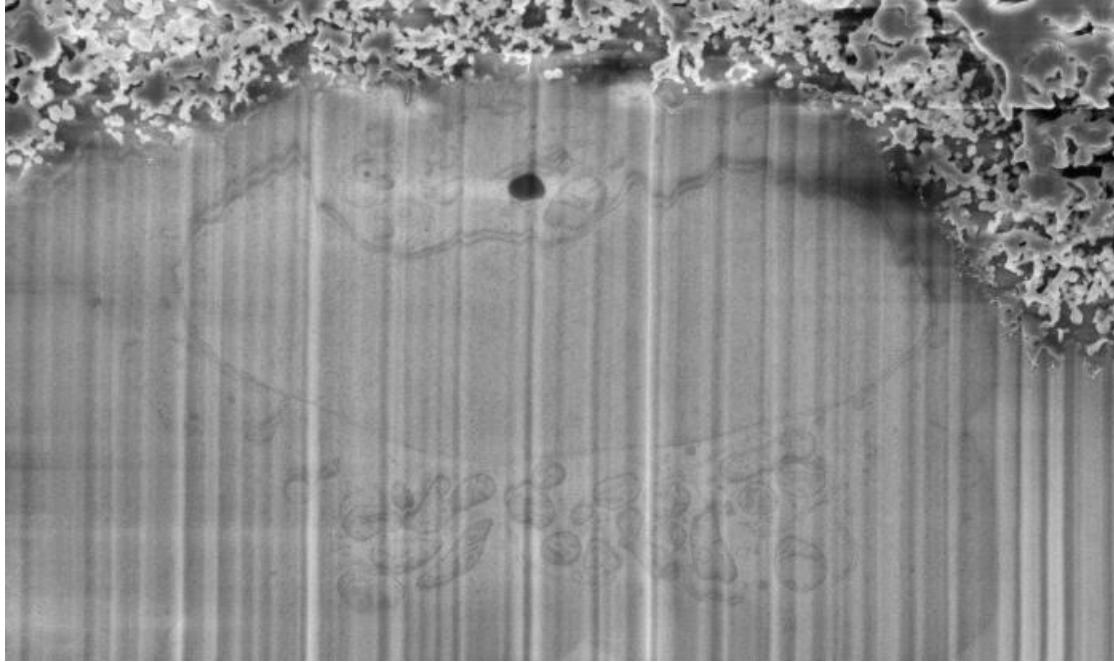
ZEN 3.9 > Basic Concepts > Image Processing > Image Processing Functions

EM Processing

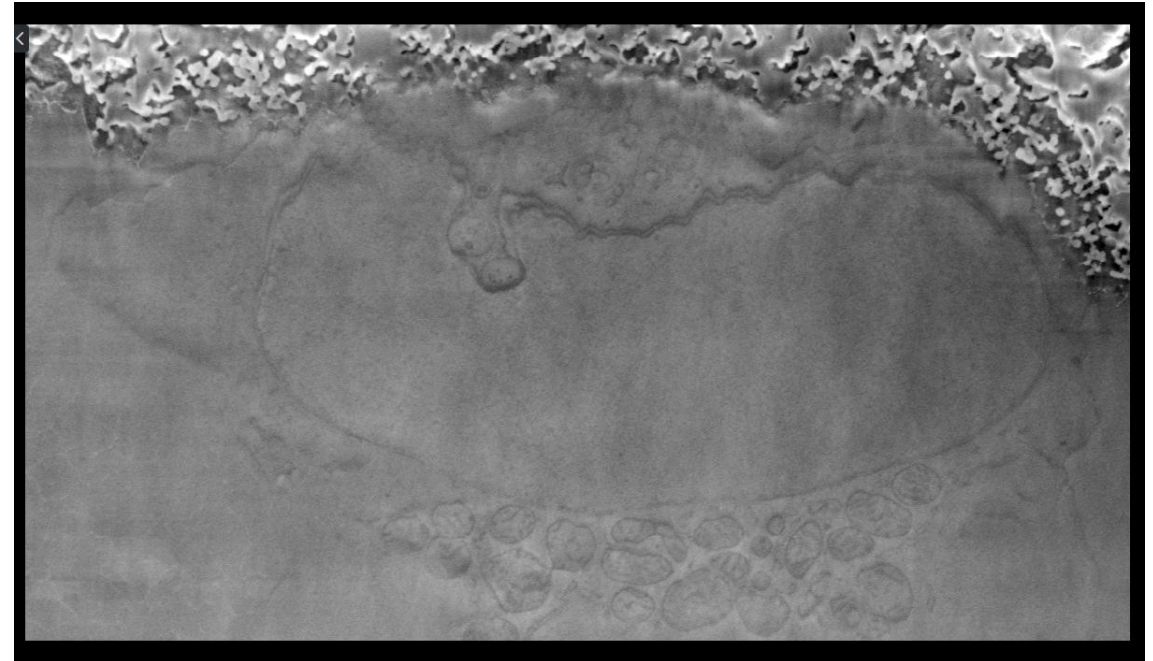
- Change Pixel Type
- Coarse Z-Stack Alignment
- Create Image Subset
- Cut Out Regions
- Denoise
- Enhance Local Contrast
- Gauss
- Highpass
- Import SmartFIB TIFFs
- Median
- Not
- Remove Stripes
- Shading Correction
- Slices Replacement
- Sort SmartFIB TIFFs
- Z-Stack Alignment with ROI
- Z-Stack Equalization



ZEN: EM processing toolbox



raw .tif images from customer



in ZEN (EM Toolbox) processed data: stack import, destriping, AI denoising, z-alignment, ROI cropping

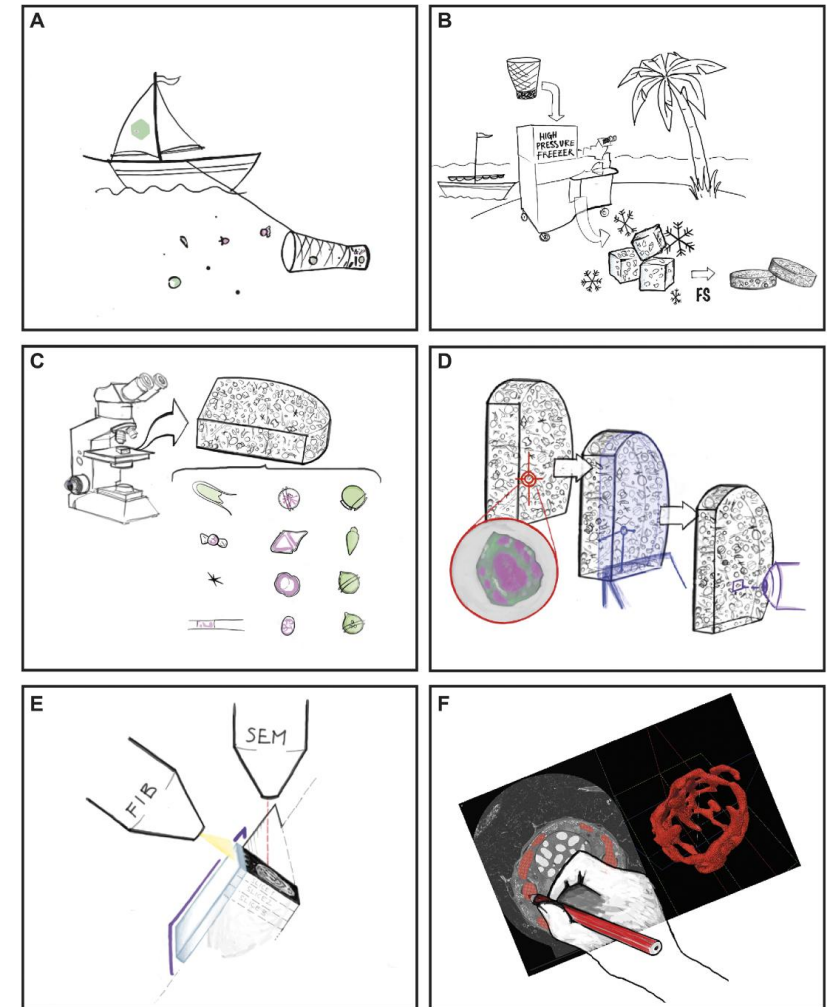
A.I. assisted volume rendering training users how to train models



EMBL



Mocaer, K. et al., Targeted volume correlative light and electron microscopy of an environmental marine microorganism.
J. Cell Sci. (2023). doi:10.1242/jcs.261355



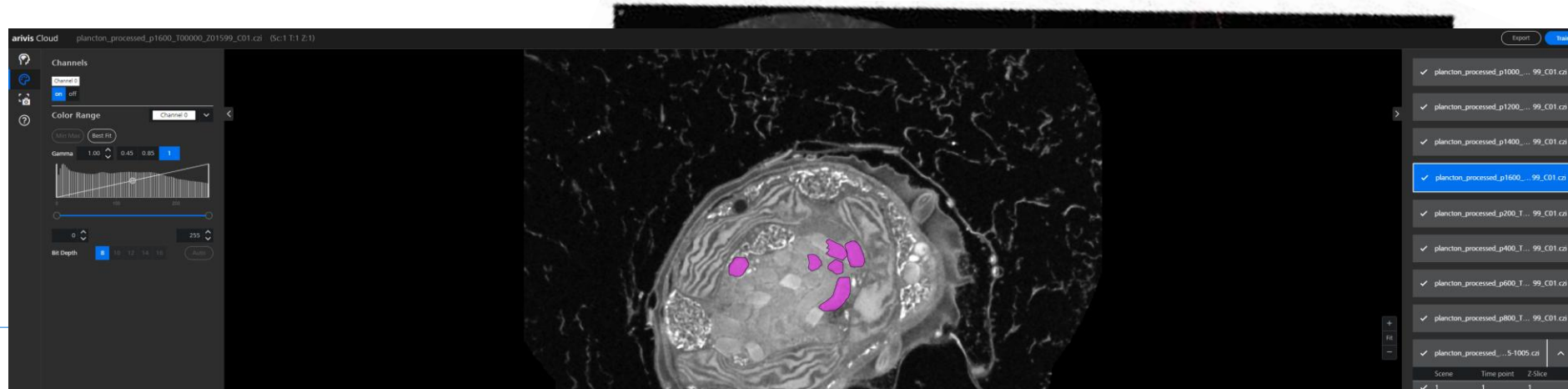
Workflow of the study. (A) Sample collection in the Villefranche-sur-Mer bay area, Mediterranean sea, France. (B) High-pressure freezing in close proximity to the sampling site, later followed by freeze substitution (FS) and resin embedding. (C) Mapping of the block using confocal microscopy and identification of various microorganisms present in the heterogeneous sample. (D) Targeting of a specific cell and determination of its x, y and z coordinates (red target), followed by ultramicrotome trimming to approach the cell of interest in z (in blue) and finally laser branding to mark the cell position in x and y on the block surface (in purple). (E) FIB-SEM acquisition of the cell of interest. (F) Segmentation of the organelles of the targeted cell. Here, the chloroplast is shown during segmentation and after rendering in red.

A.I. assisted volume rendering

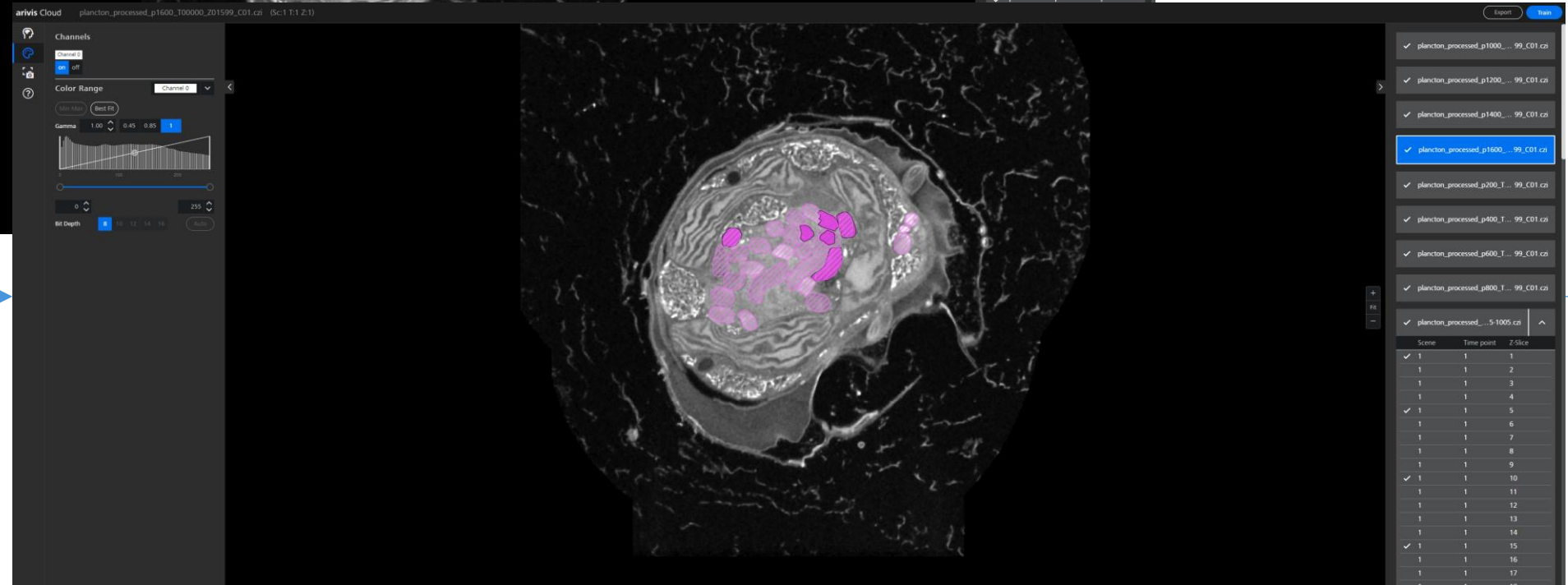
training users how to train models



EMBL

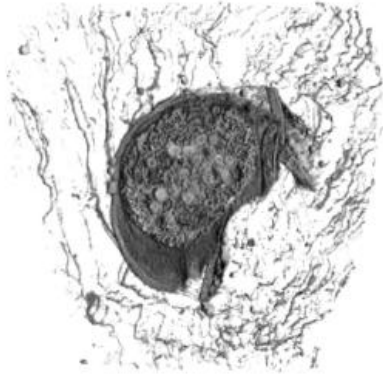


draw annotations

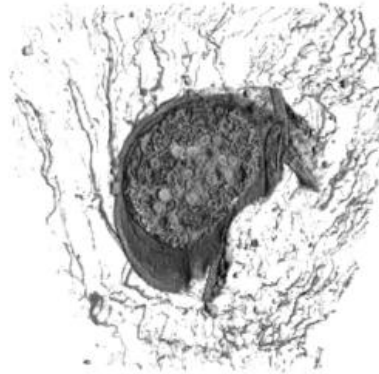


check predictions

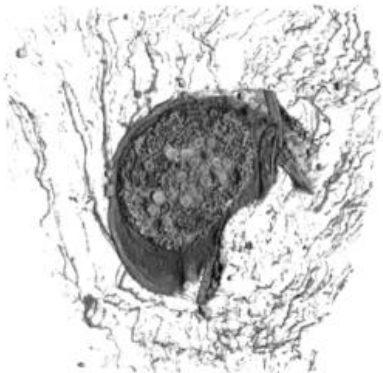
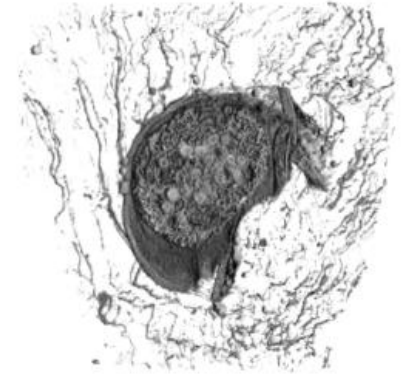
Thresholding:
condensed chromosomes



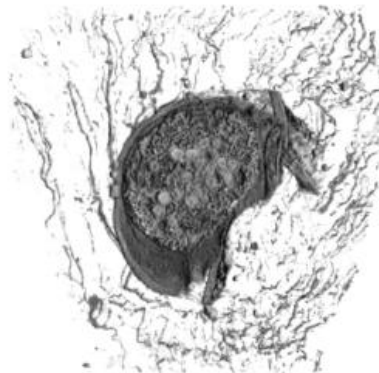
ML pixel classification:
Chloroplast / thylakoids



DL instance segmentation:
thrycocysts



condensed chromosomes
+ thrycocysts



condensed chromosomes
+ thrycocysts
+ Chloroplast / thylakoids

Volume 136 (2023) August 2023
**Journal of
Cell Science**

Dataset from:
Mocaer, K. et al.

Targeted volume correlative
light and electron microscopy of
an environmental marine
microorganism

J. Cell Sci. (2023),
doi:10.1242/jcs.261355



Streamlined Array Tomography Workflow



Collection System

SYNTEK Cuesta & Noventa
Serial Sectioning



**Field Emission
Scanning Electron**

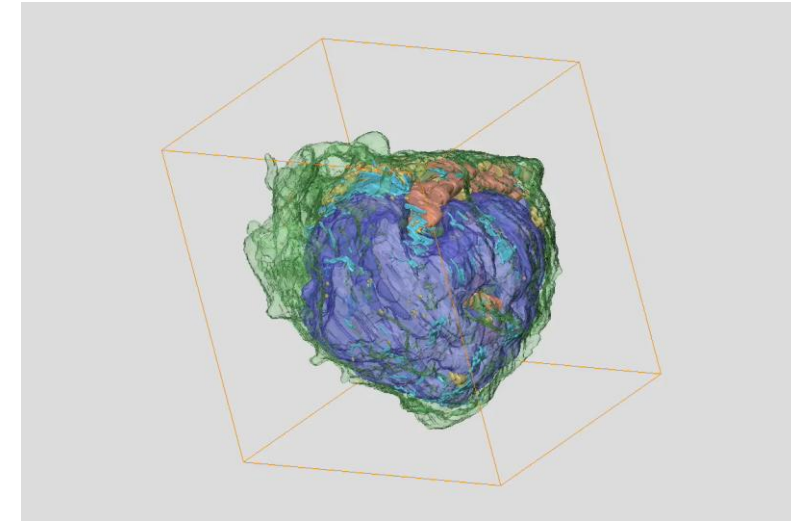
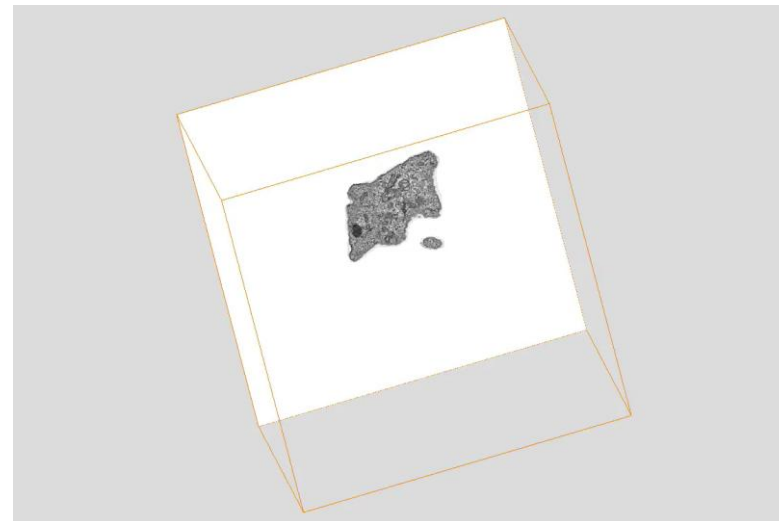
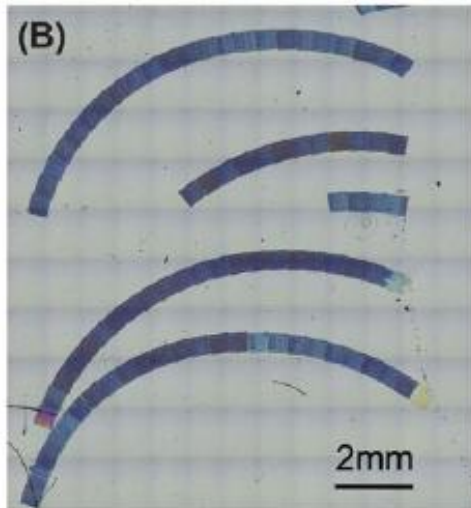
FE-SEM

ZEISS Sigma/Gemini SEM
Array Tomography



3D Software

ZEISS aravis
3D reconstruction





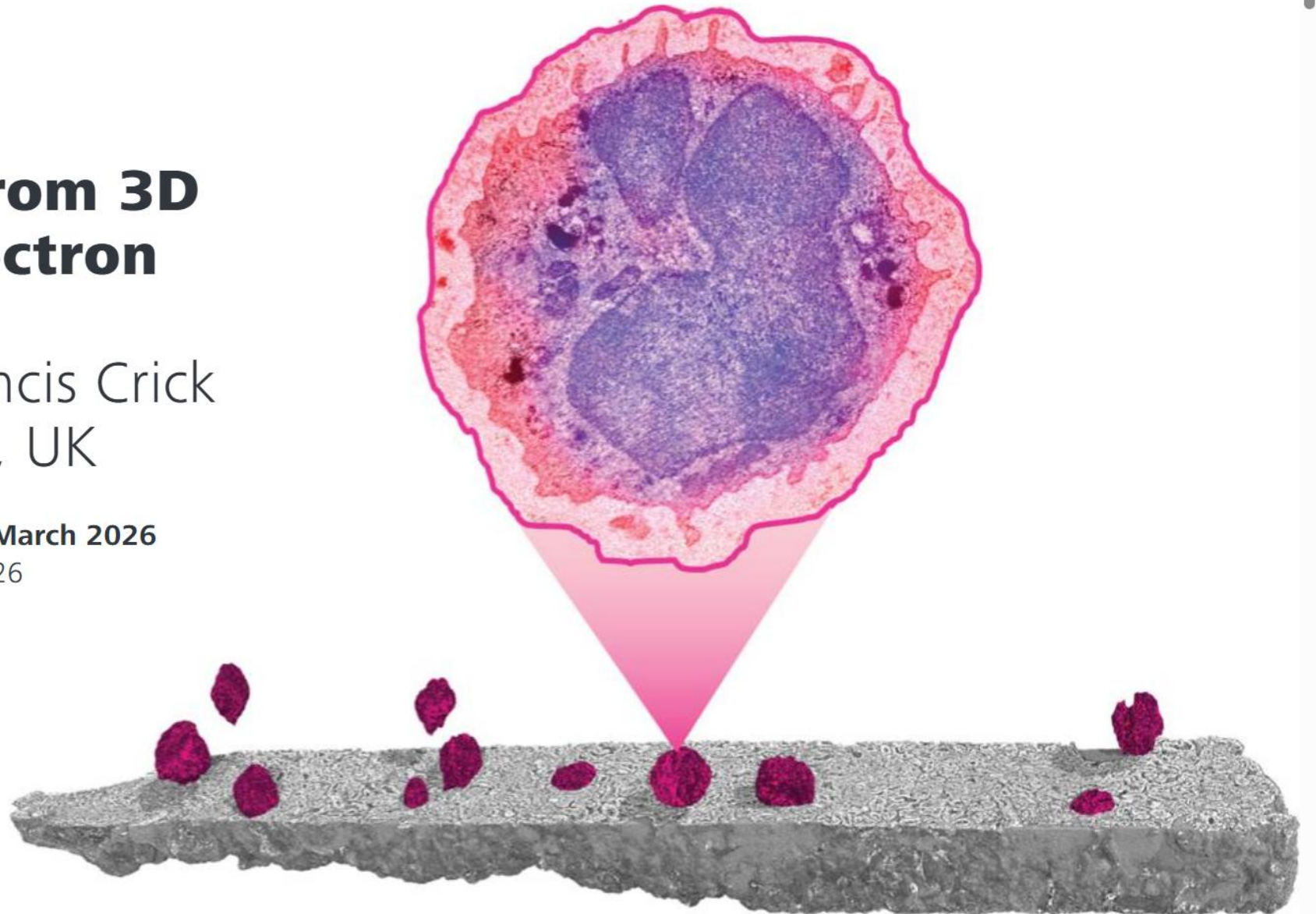
Symposium "From 3D Light to 3D Electron Microscopy"

Hosted at the Francis Crick Institute | London, UK

Sun, 22 March 2026 - Wed, 25 March 2026

Abstract deadline: January 15th, 2026

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Seeing beyond