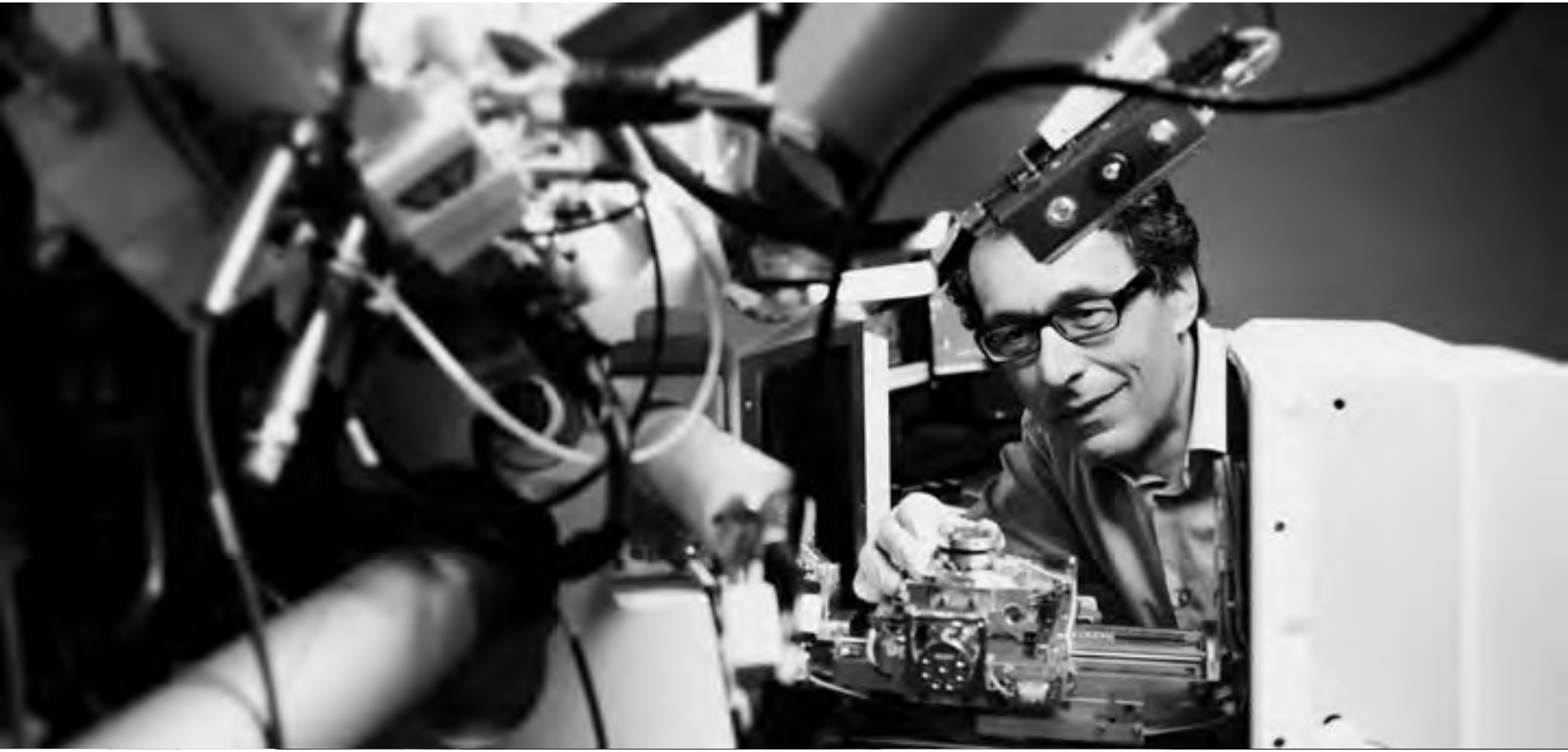
Advancements of Biological Electron Microscopy and Correlative Microscopy



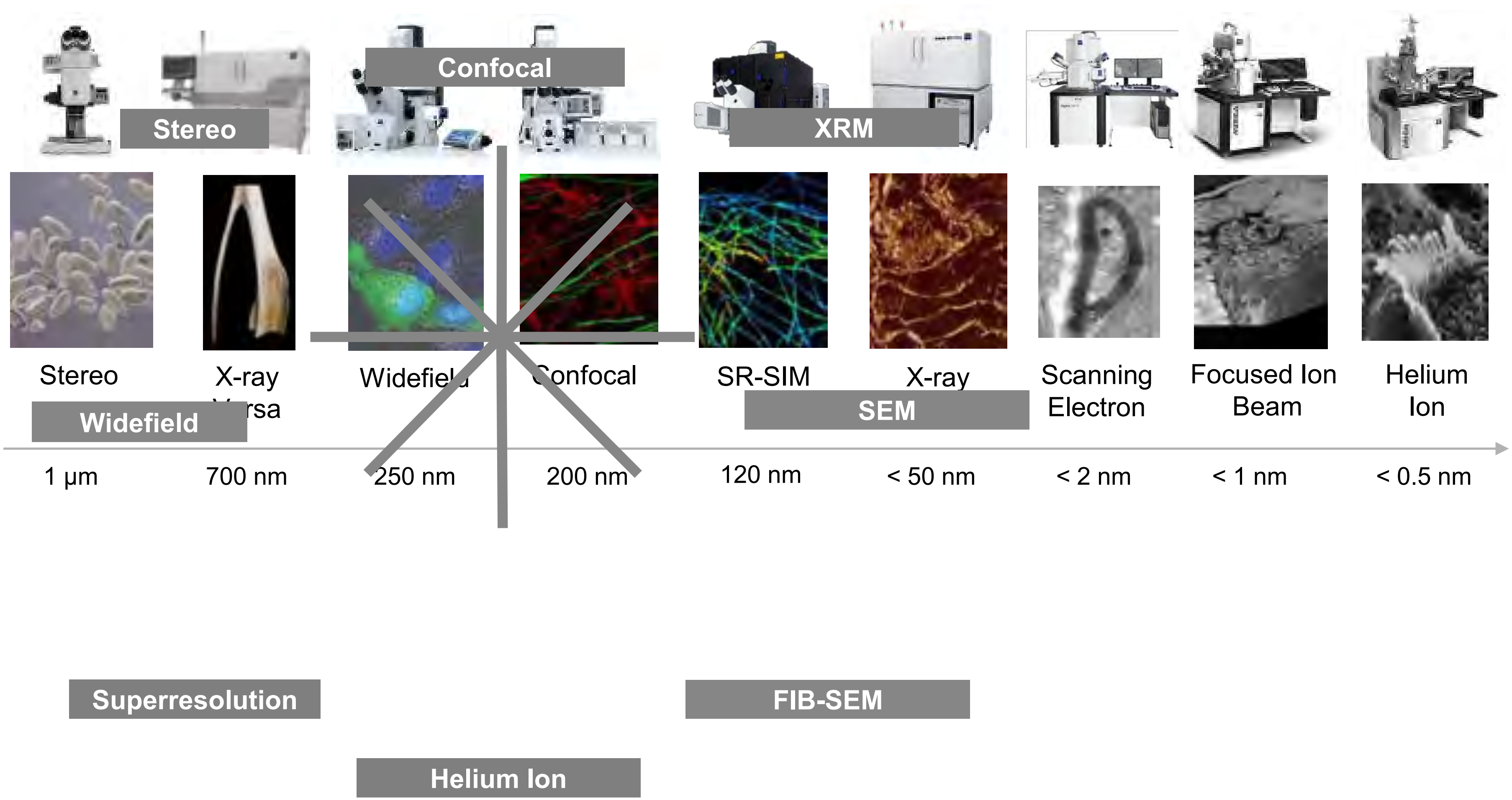
Joel Mancuso
Academia Life Science EM/XRM Specialist

Agenda

Agenda

1	Introduction and Agenda
2	Multiscale and Correlative Microscopy
3	Traditional Electron Microscopy
4	Using an SEM in place of a TEM
5	Automated & 3D EM
6	Correlative Microscopy
7	Cryo FIB SEM

The Correlative Microscopy Environment



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2. Multiscale and Correlative Microscopy
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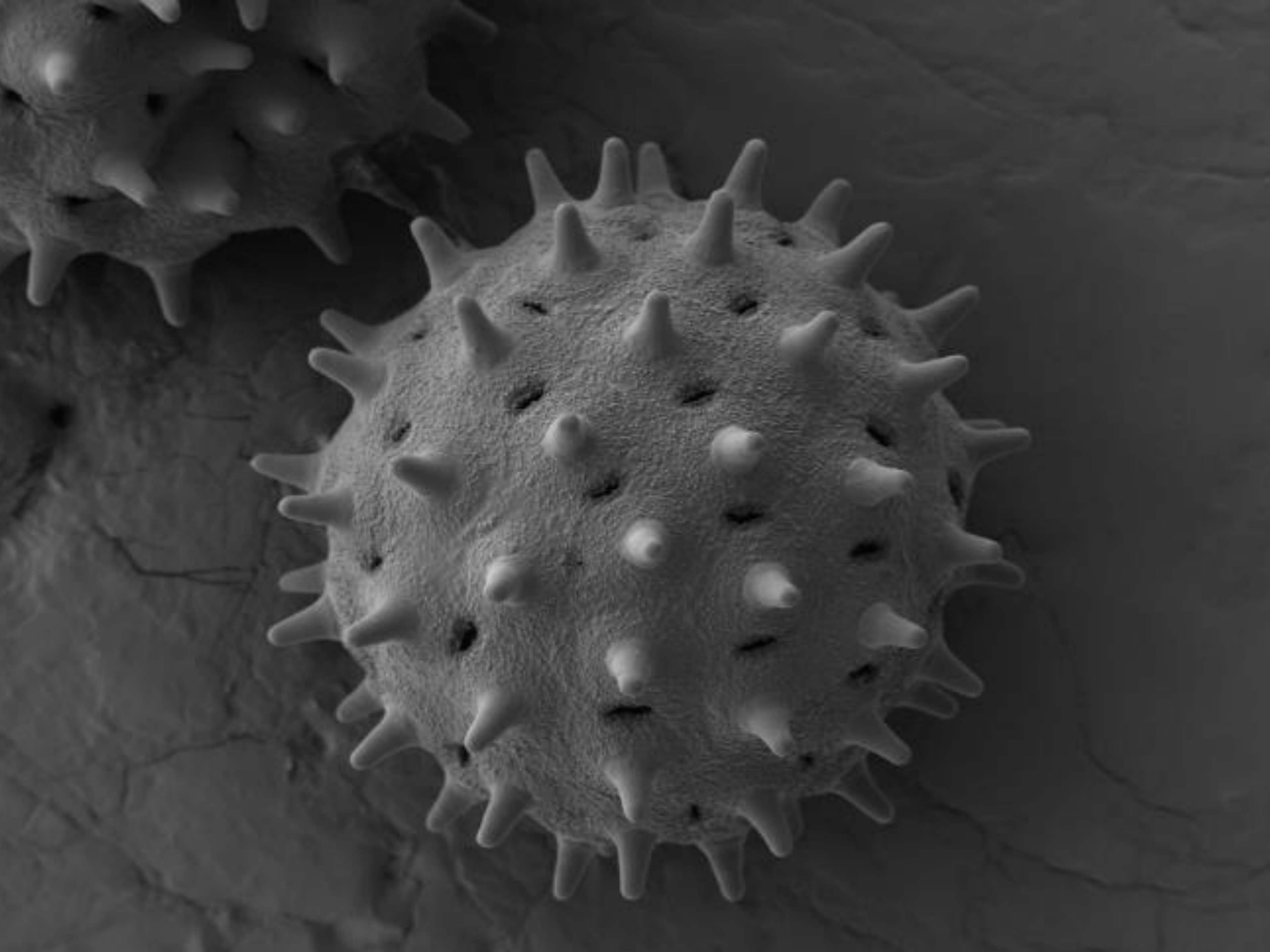
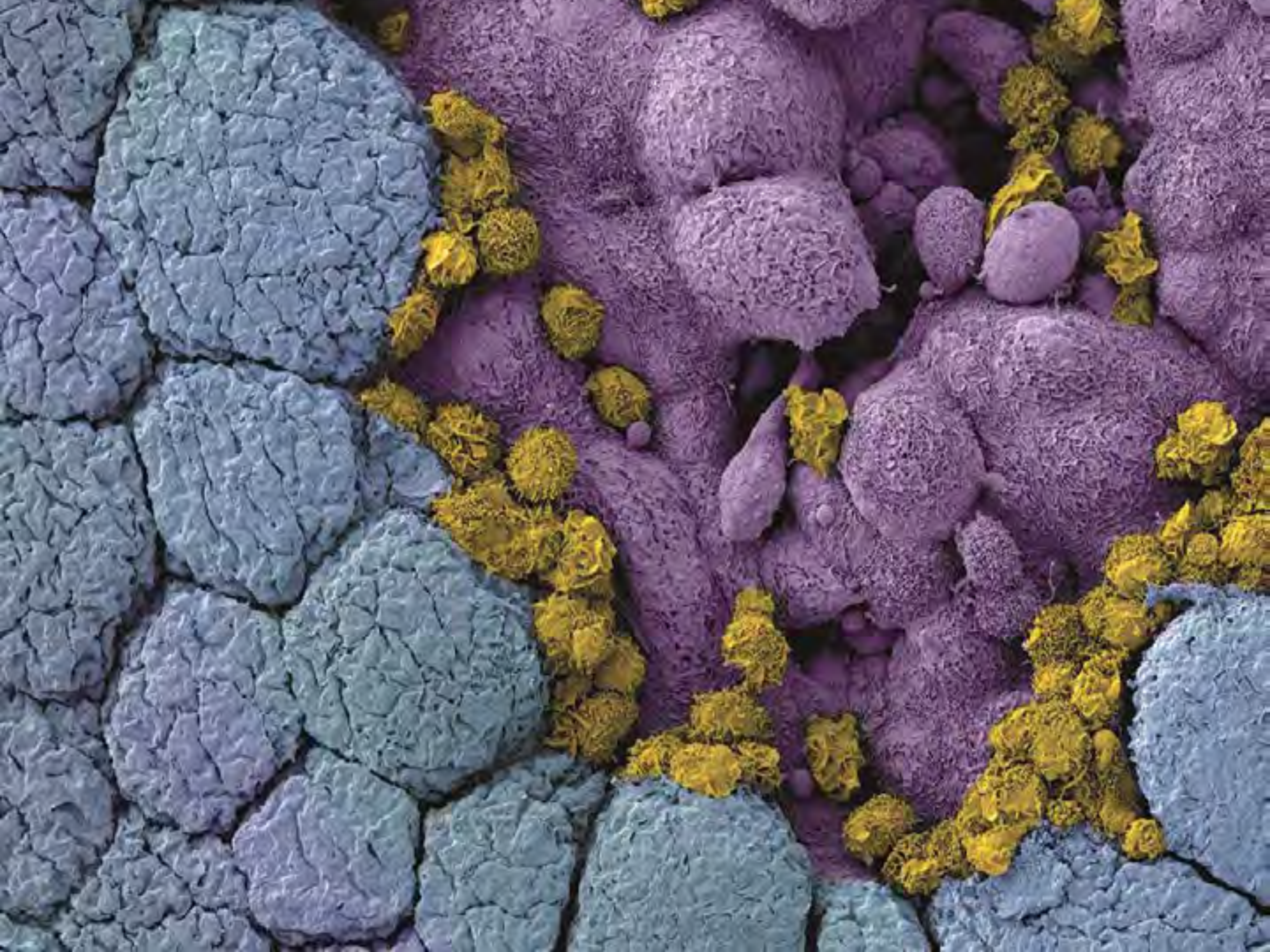
7. Cryo FIB SEM

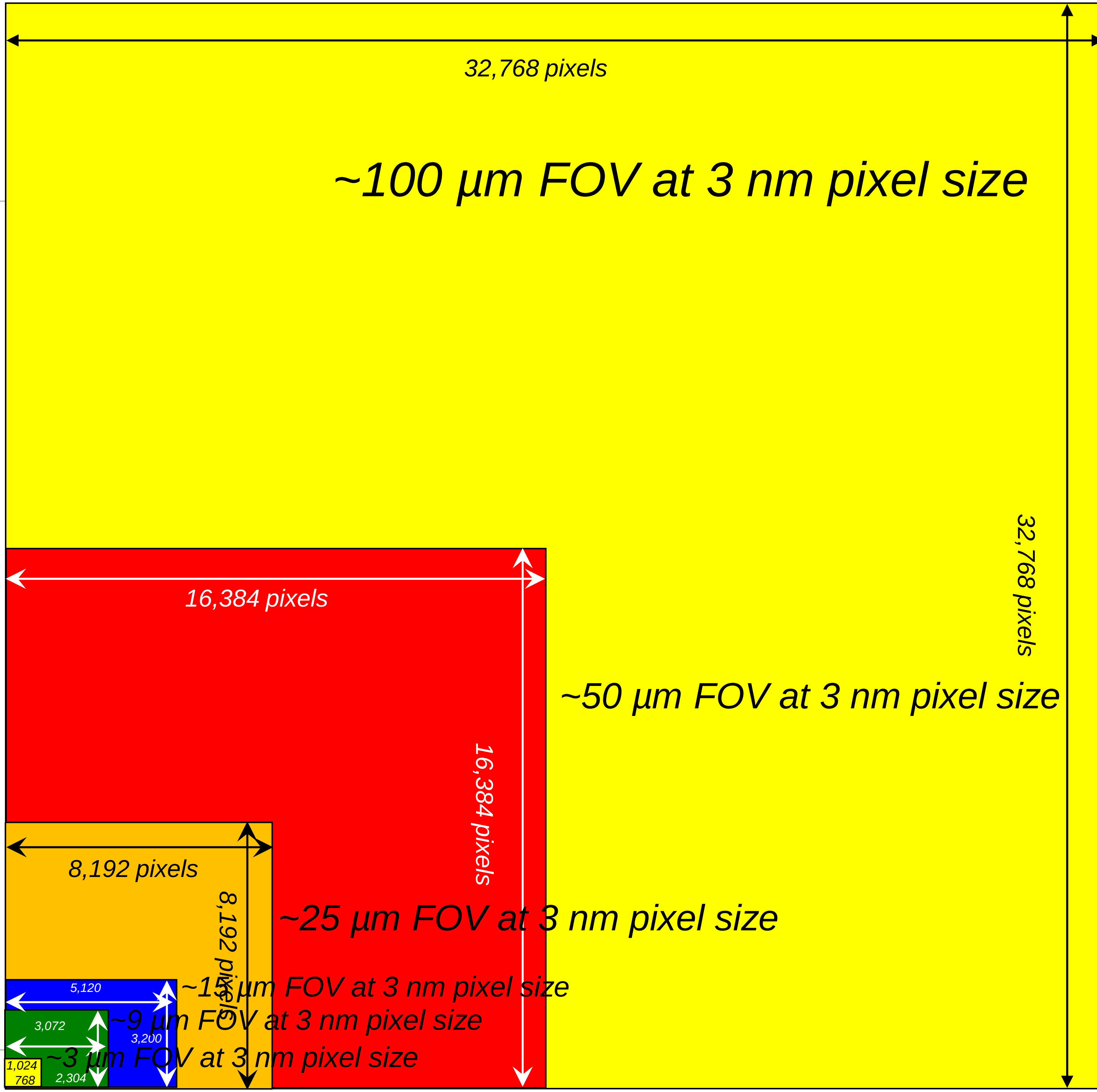




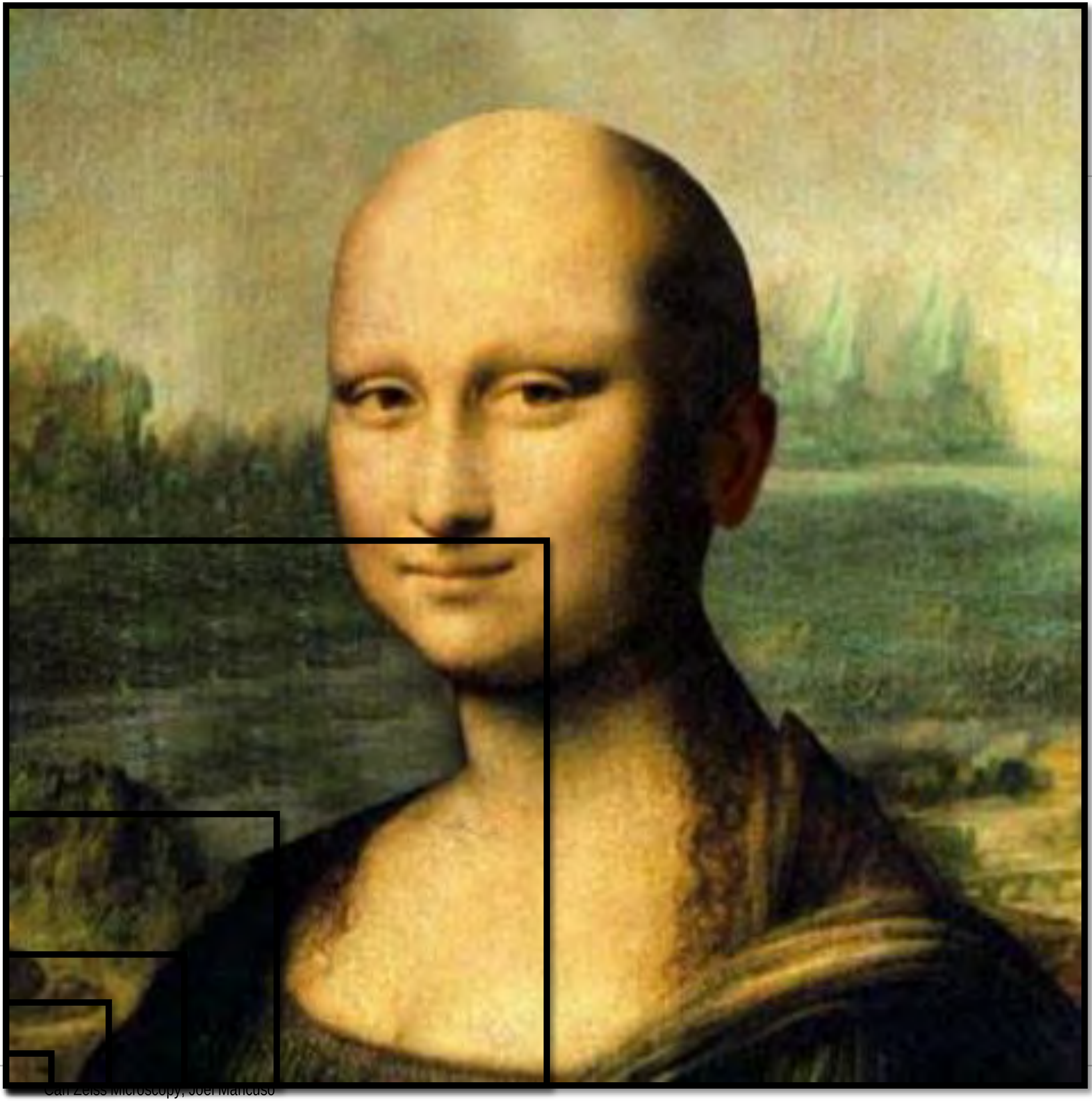
Agenda

Why use a SEM instead of a TEM?

Large frame store
and
Automation



Comparative Single Image Sizes

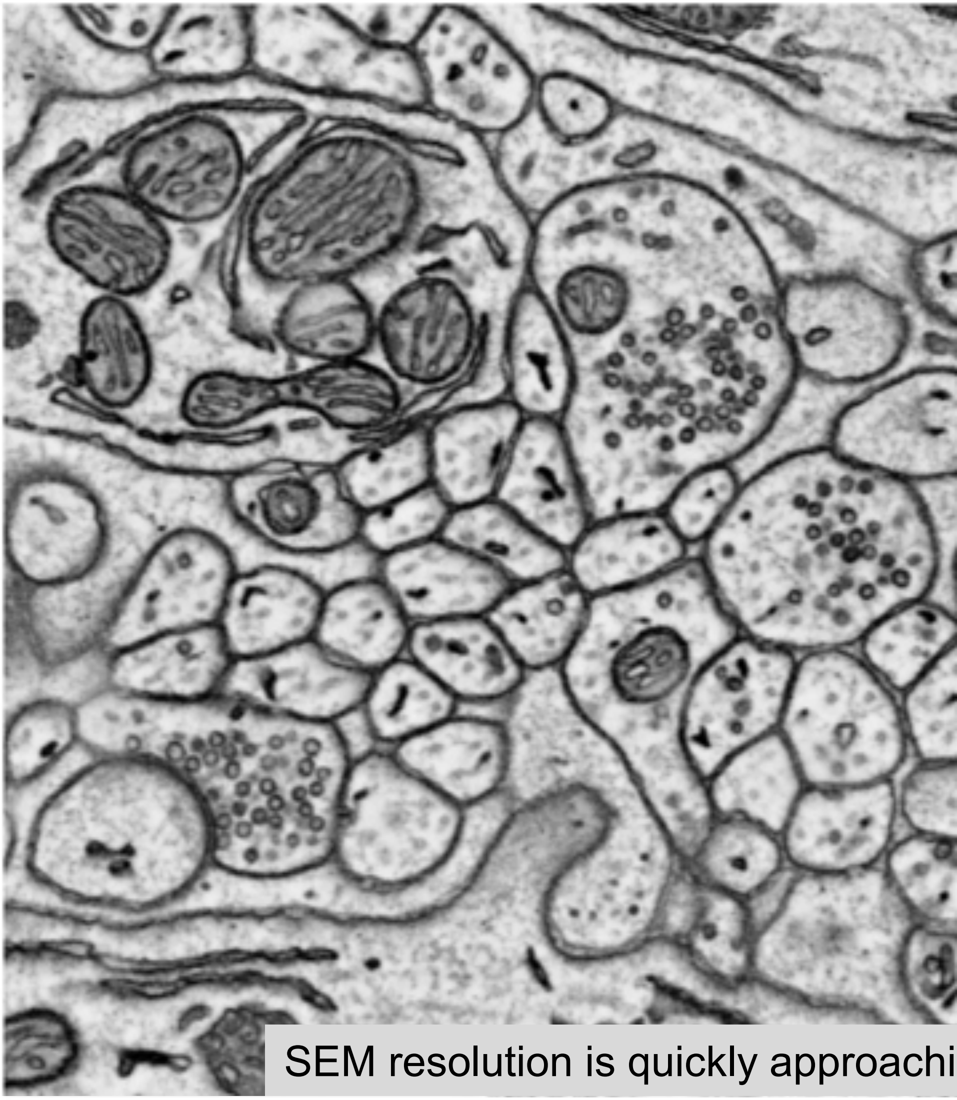


Comparative Single Image Sizes:

How much information do you want in each image?

TEM of ultrathin section versus SEM of blockface: Which is which?

SEM blockface image at 1.9 keV

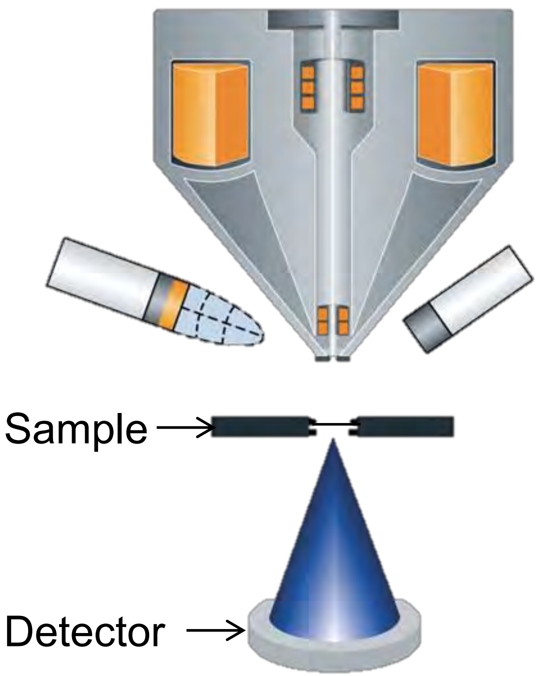


TEM section image at 80 keV

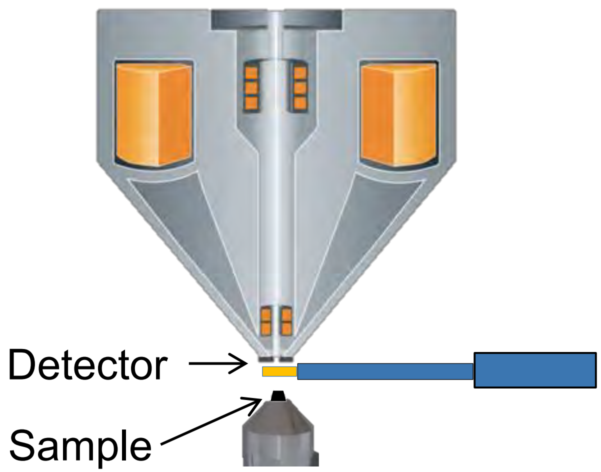
SEM resolution is quickly approaching TEM resolution of plastic sections.

TEM like imaging in the SEM

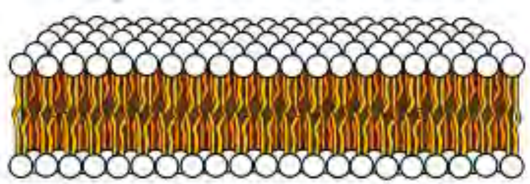
STEM

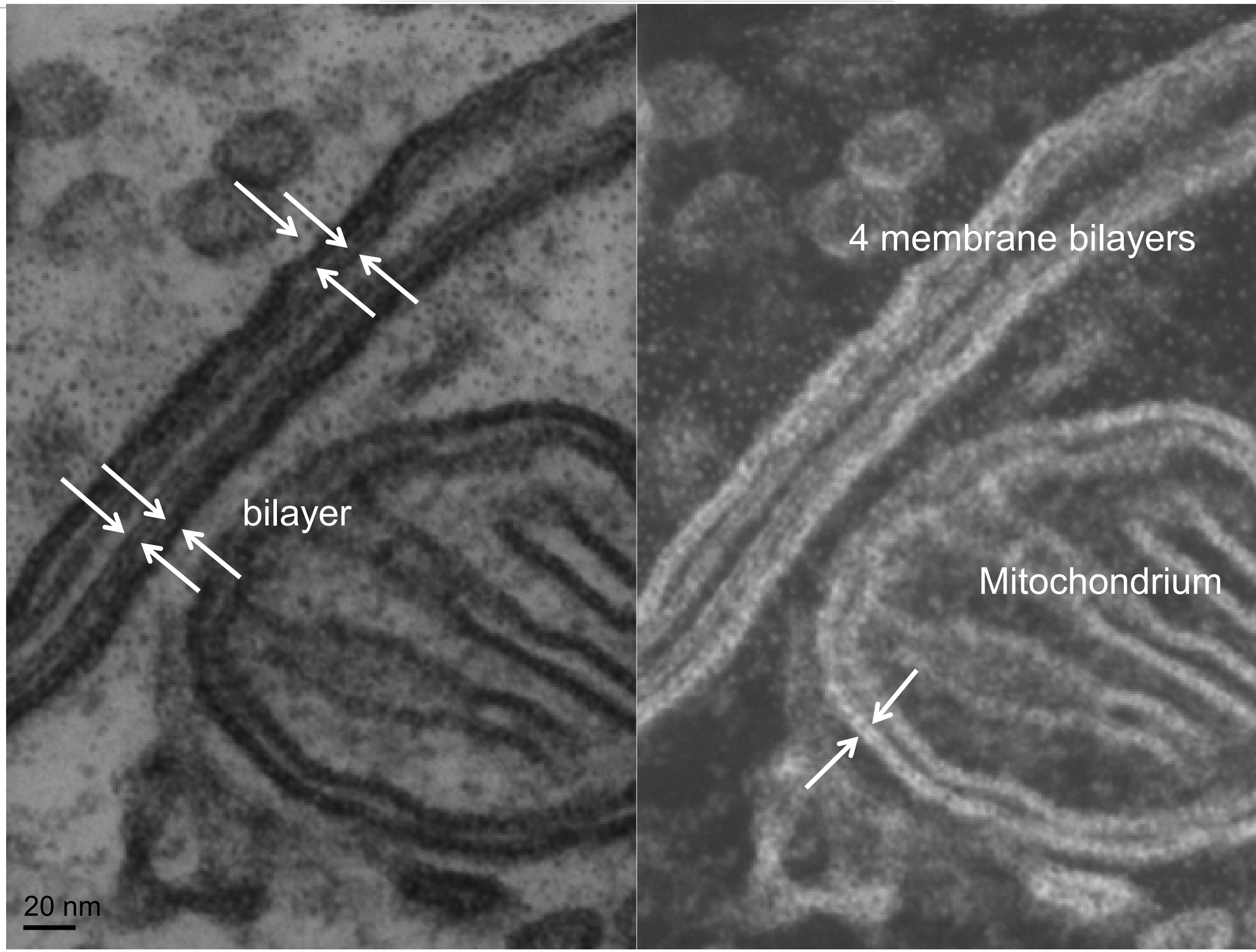


Back Scatter

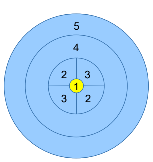


SEM Resolution Achievements

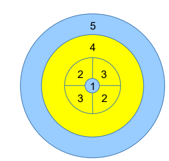




Brightfield



Darkfield

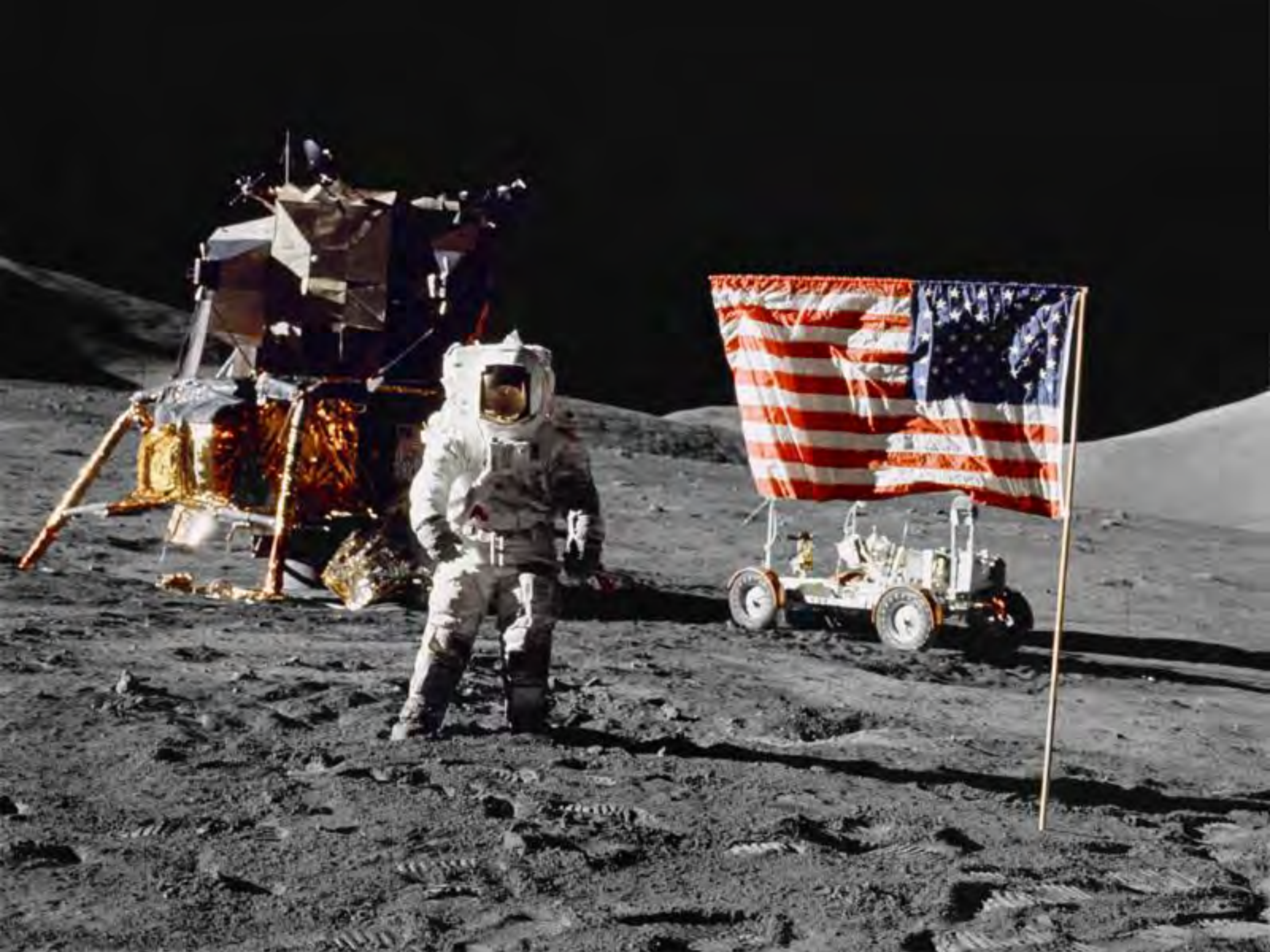


Mouse Brain, Sample courtesy of Cantoni, EPFL Lausanne.

SEM vs TEM Review

	SEM	TEM
Resolution	0.4nm (STEM)	0.2nm (STEM)
Sample types	Bulk samples – block face, whole animal mounts Thin sections – traditional TEM samples	Thin sections or thin samples ONLY
Image size	Variable – largest image size is 40k x 50k	Limited to detector or camera size usually 4k x 4k
Automation	Many Automated workflow Options including large scale 3DEM	Limited

Agenda

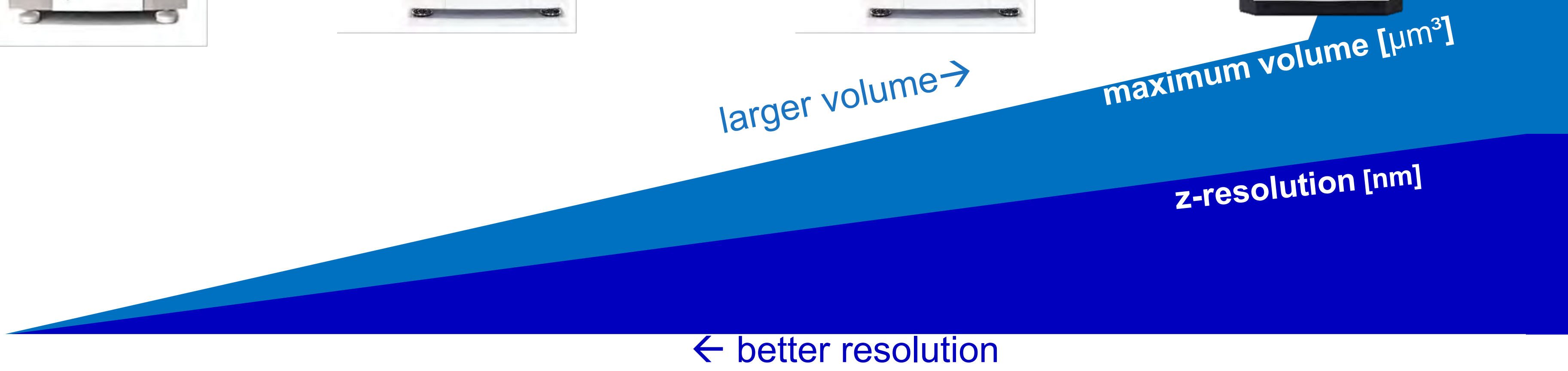


Automated 3DEM Techniques

Crossbeam
(FIB/SEM)

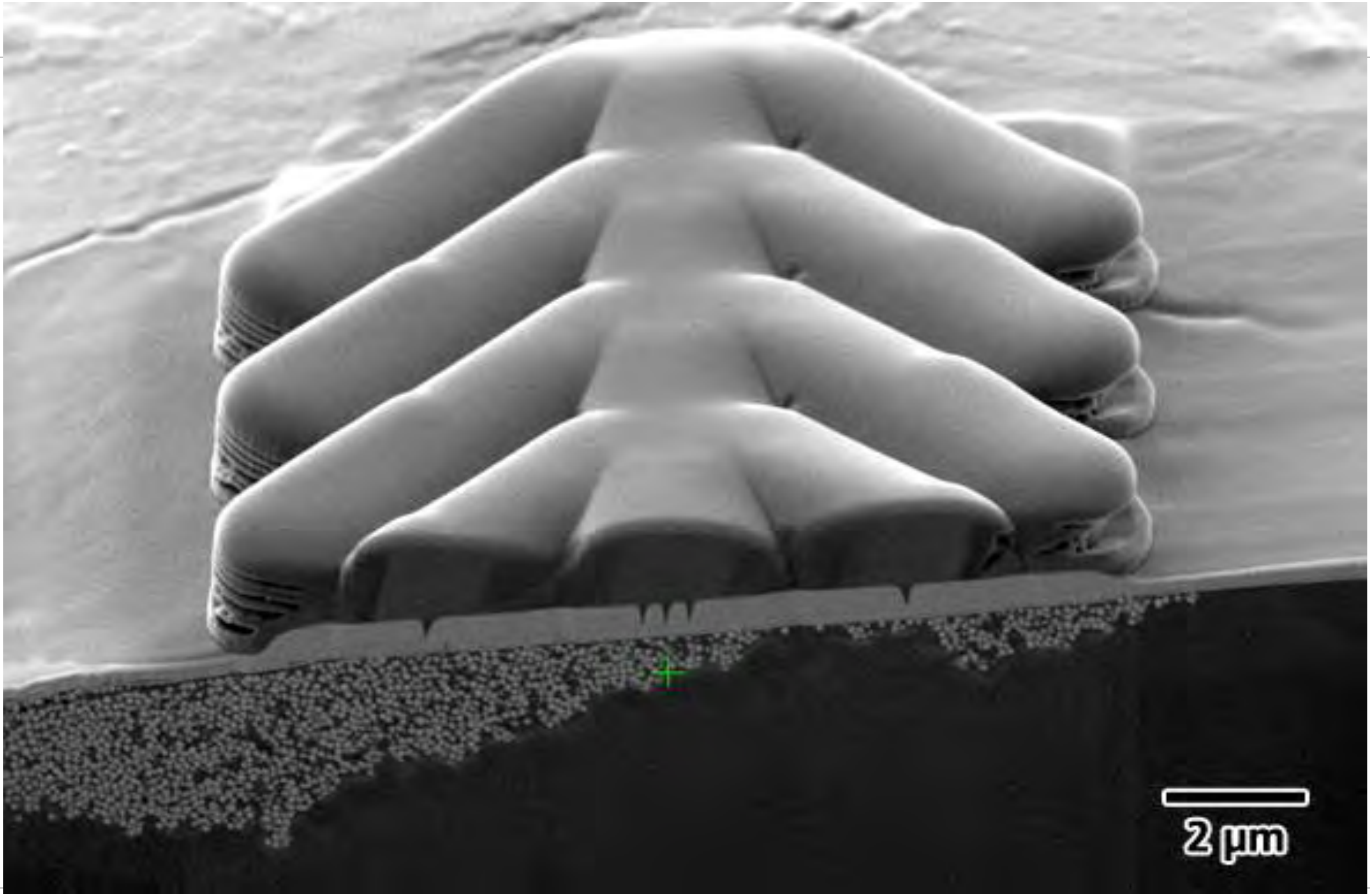
3View SEM

Array Tomography SEM

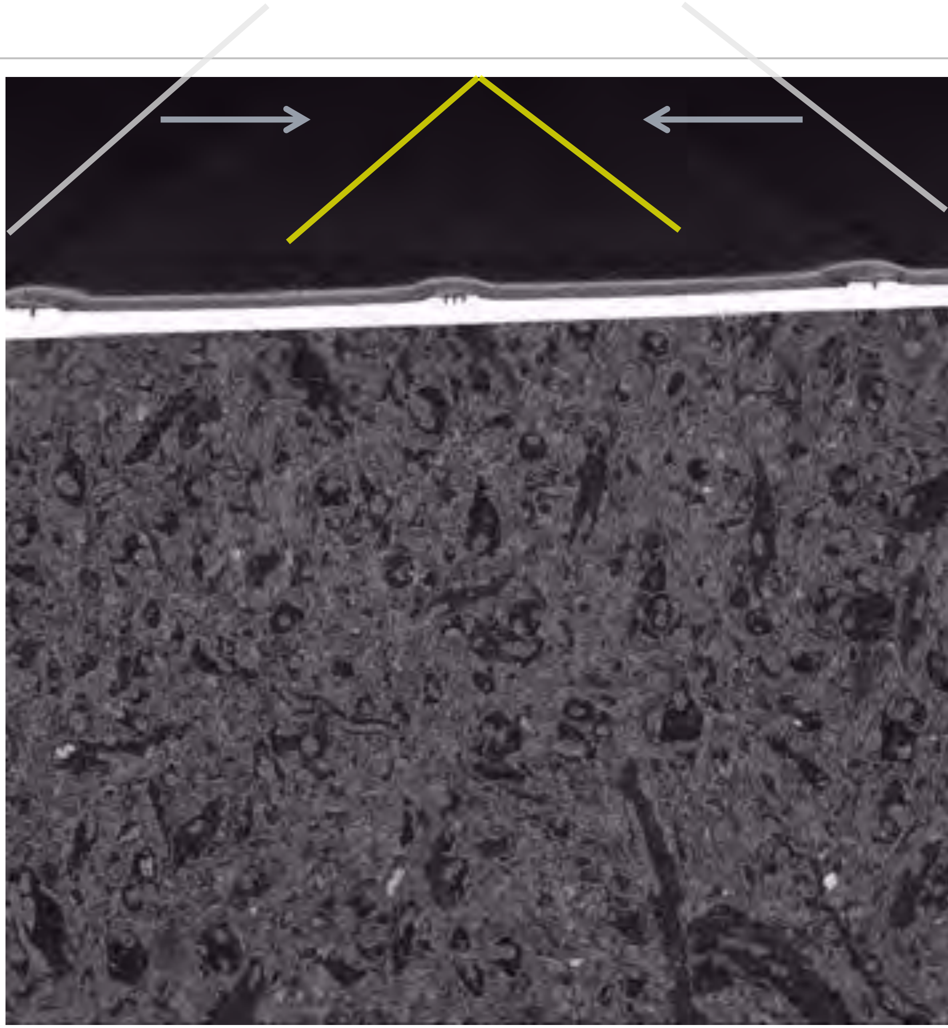


3D FIB-SEM

High resolution simultaneous milling
at the Coincidence Point

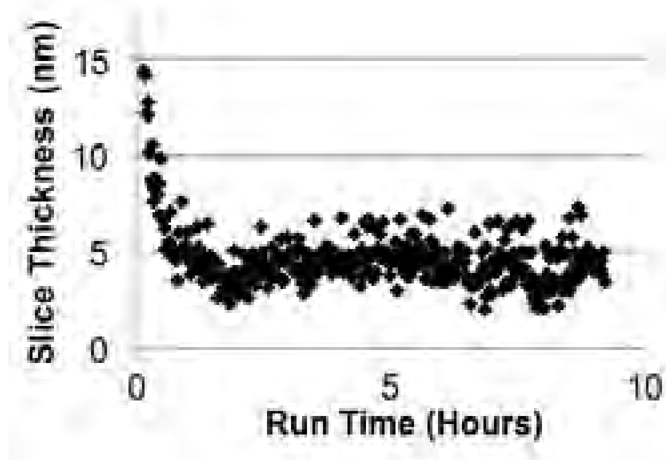

2 µm

FIB Nanotomography: Meeting the Demand for <10 nm Slice Thickness



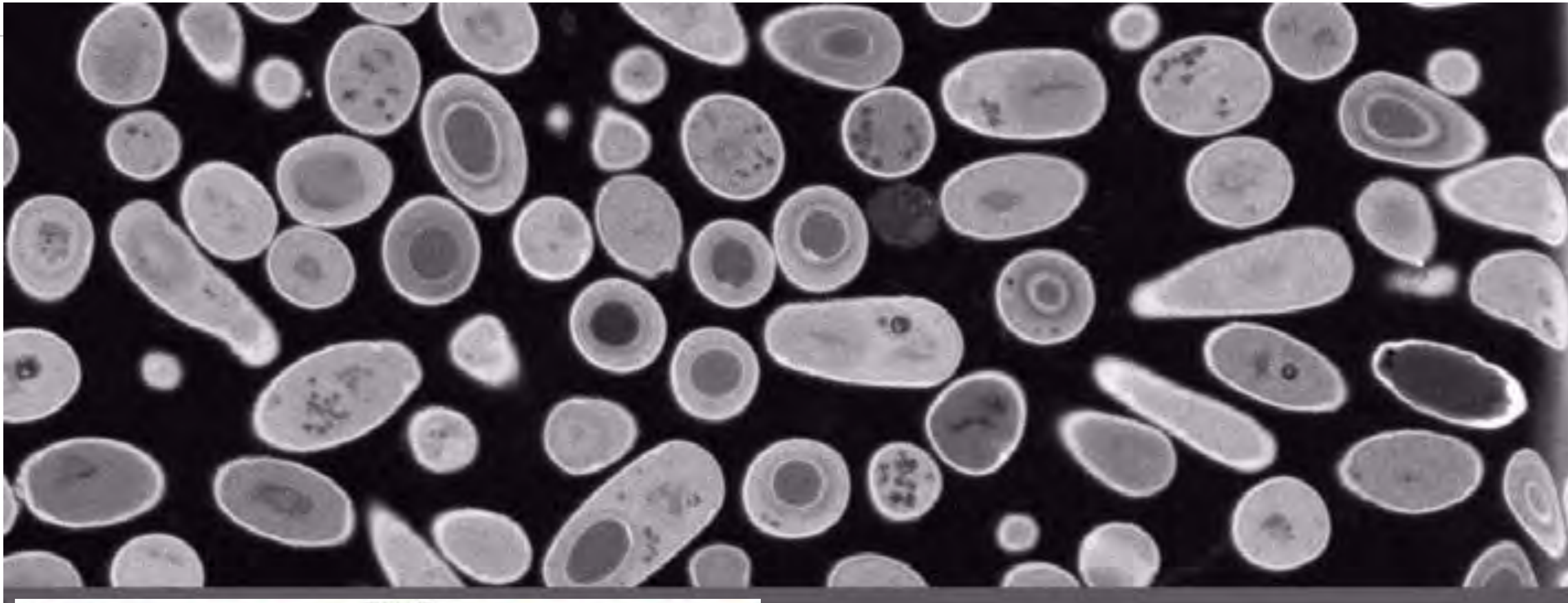
Predictive 3D Drift Tracking in Action

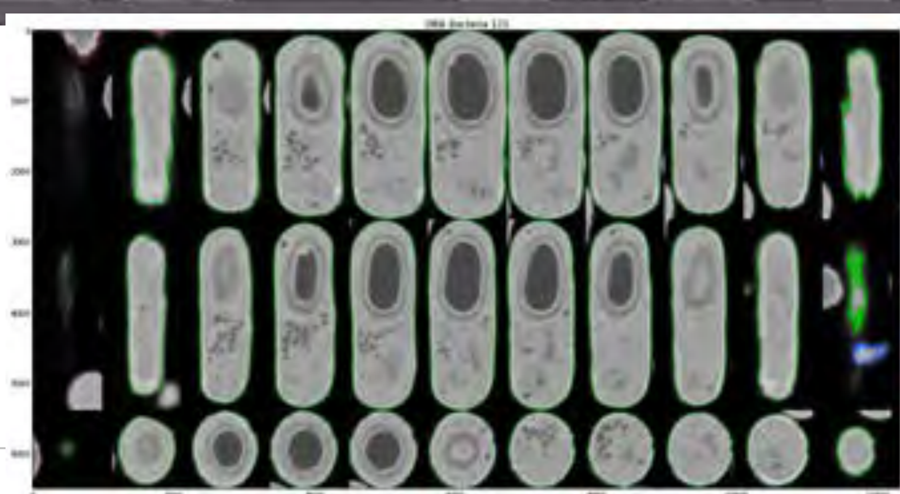
Nanofabricating "chevron" fiducials, FIB'd into a protective bilayer of "dual material contrast" allows precise tracking of the cross-section and slice thickness.



Keyframes 50 nm pixels, 30x30 μm

FIB Nanotomography: Virtual Cross Sections & The Benefit of 3 nm Voxels

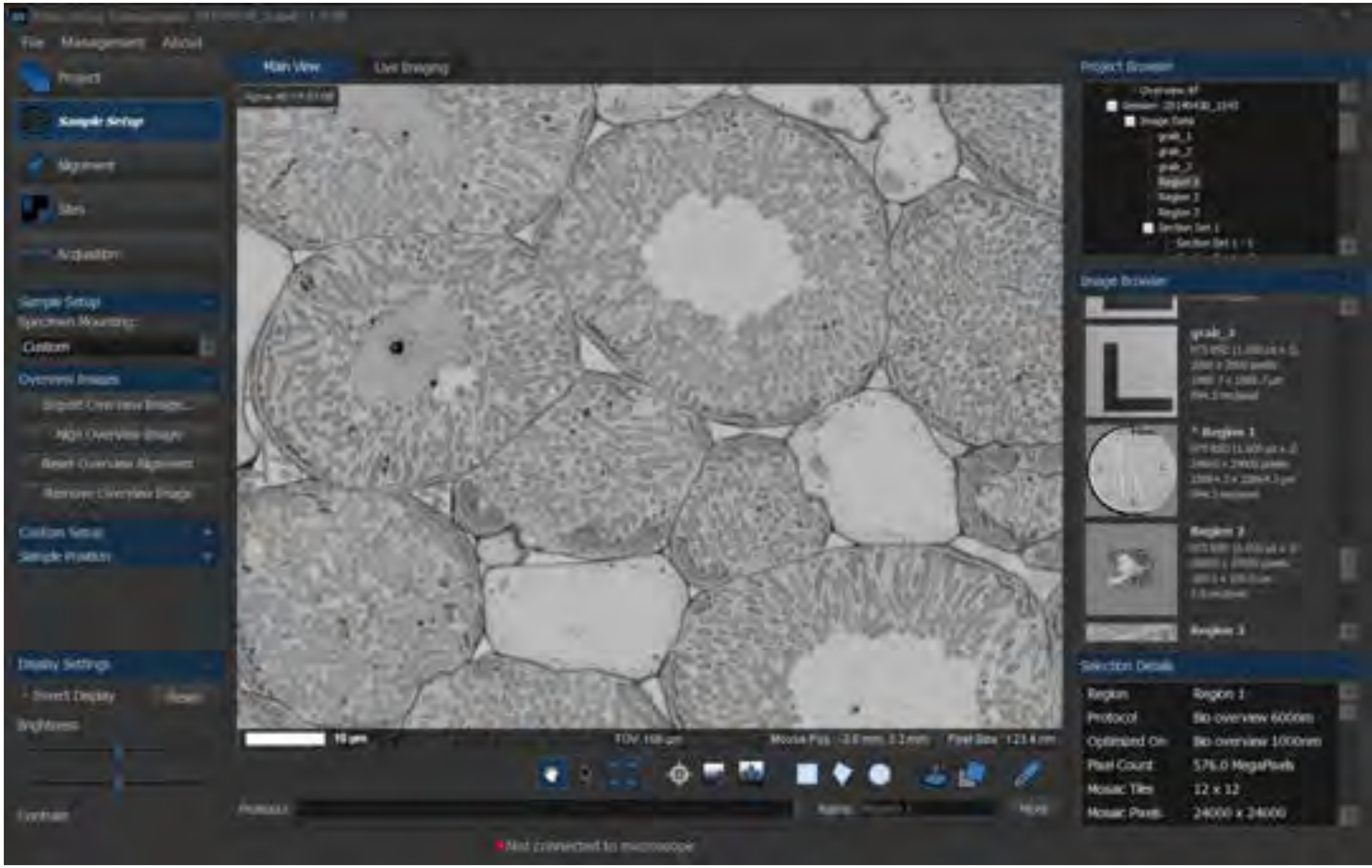




Data courtesy of Dr. Kedar Narayan,
Research Group of Dr. Sriram Subramaniam
BioPhysics Section, NCI, NIH, Bethesda MD

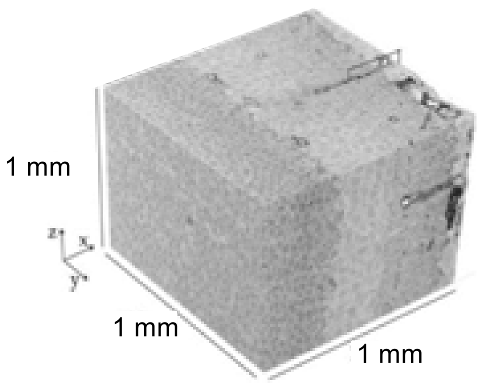
Array Tomography workflow

Ultramicrotome –
Arrays of Serial Sections

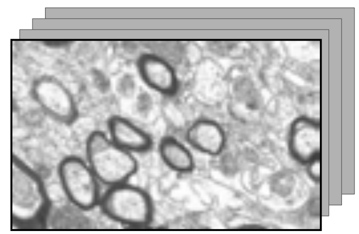


MultiSEM the Connectomics Tool
Throughput Consideration for Large Volume

1 cubic millimeter of tissue



@ 50 nm section thickness



A total area of 20,000 mm²

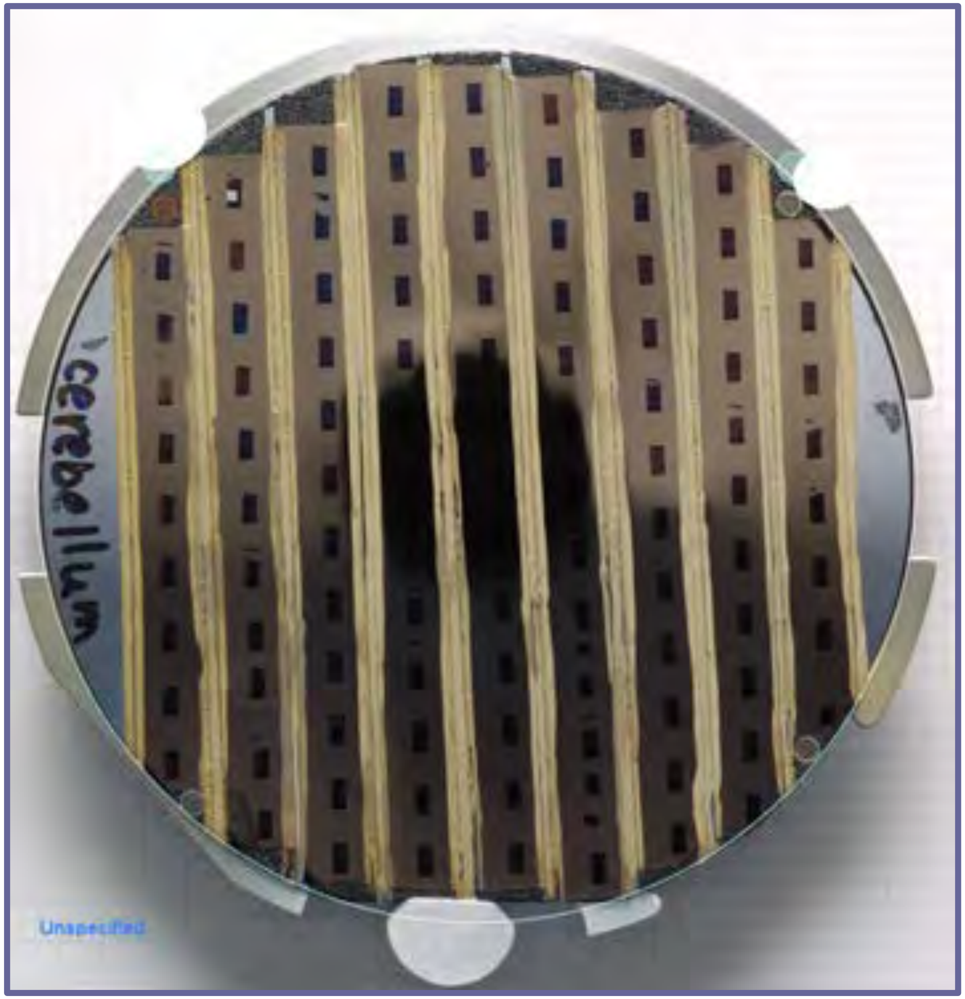
A state-of-the-art single beam SEM needs around **2.5 hours** to image an area of **1 mm²** at a pixel size of **4 nm**

→ 2.5 x 20,000 = 2083 days of imaging → almost **6 years!**

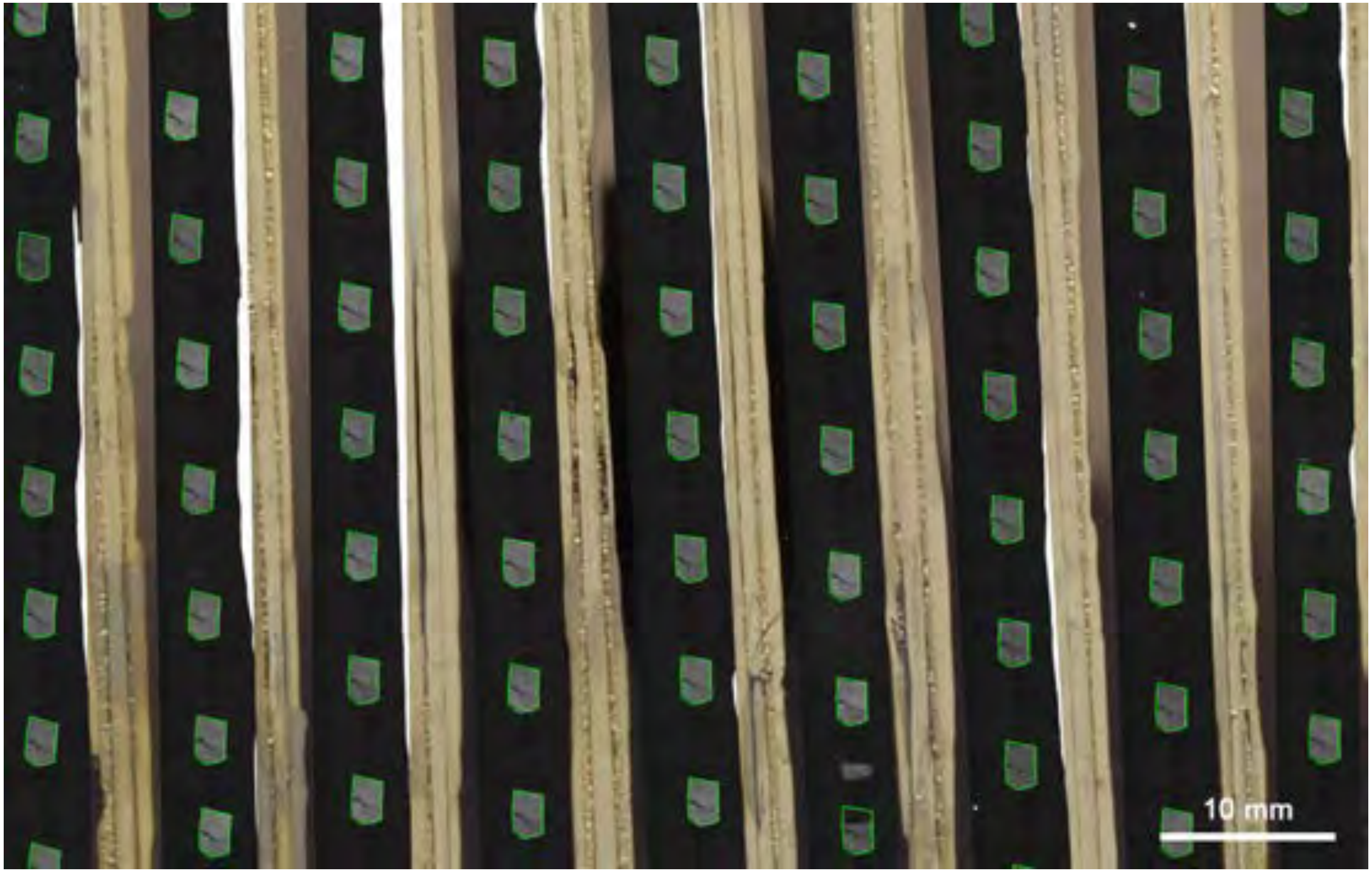
This can now be reduced!!!

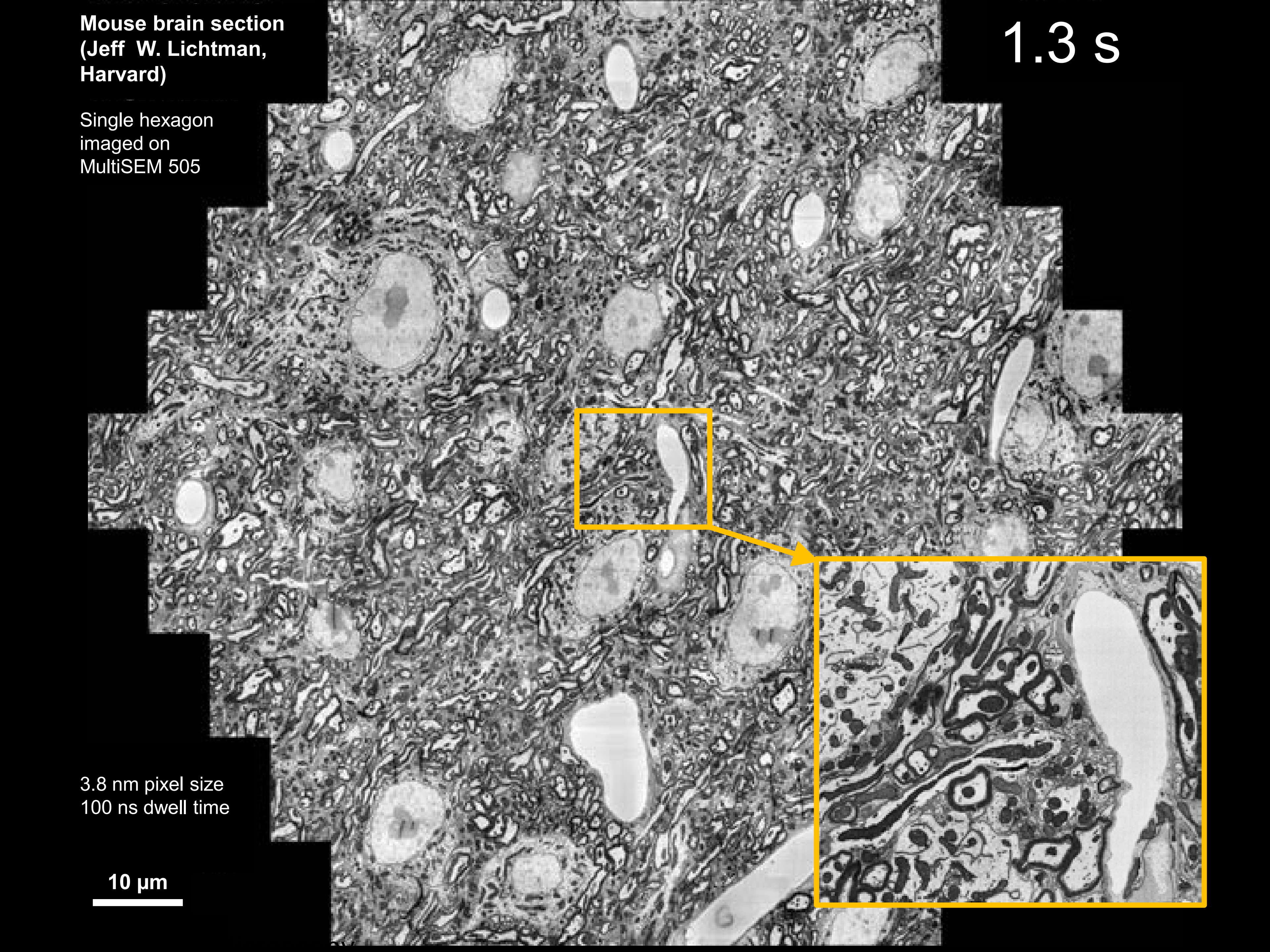
→ With parallel multi-beam image acquisition!

MultiSEM the Connectomics Tool
Throughput Consideration for Large Volume



MultiSEM the Connectomics Tool
Throughput Consideration for Large Volume





Mouse brain section (Jeff W. Lichtman, Harvard)
Single hexagon imaged on MultiSEM 505
1.3 s
3.8 nm pixel size
100 ns dwell time
10 µm

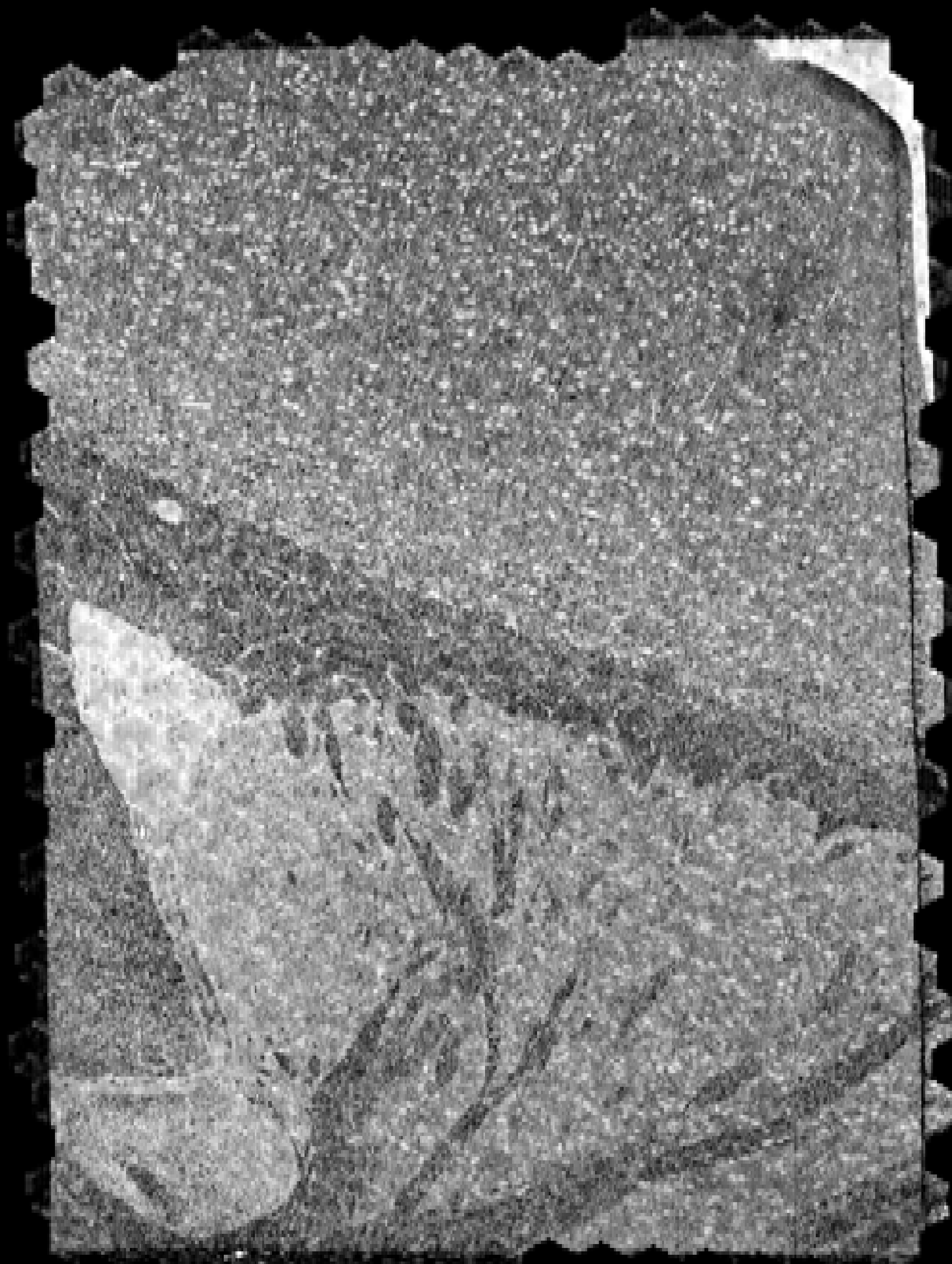
Large-Area Imaging

Fully automatic
492 individual hexagons
1,6 x 2 mm sample size
4 nm pixel size
290 GB file size
100 ns dwell time

Typical imaging time:

6.5 minutes for 1 mm x 1 mm

(→ example 1 mm² cutout from this data set on **www.zeiss.com/zen-browser**)



1 mm

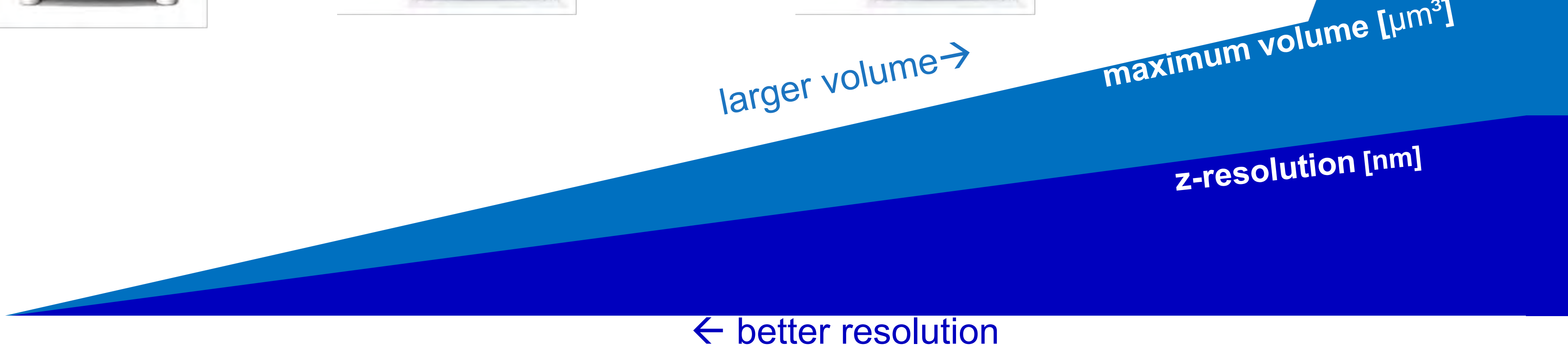
Jeff W. Lichtman, Harvard

Automated 3DEM Techniques

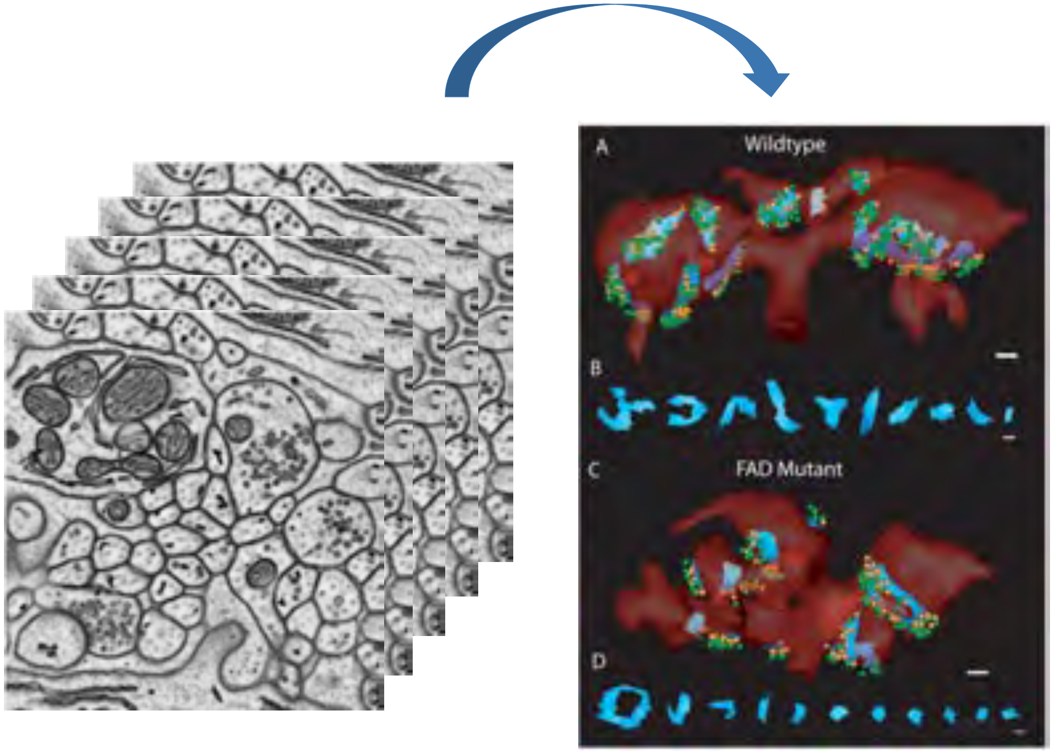
Crossbeam
(FIB/SEM)

3View SEM

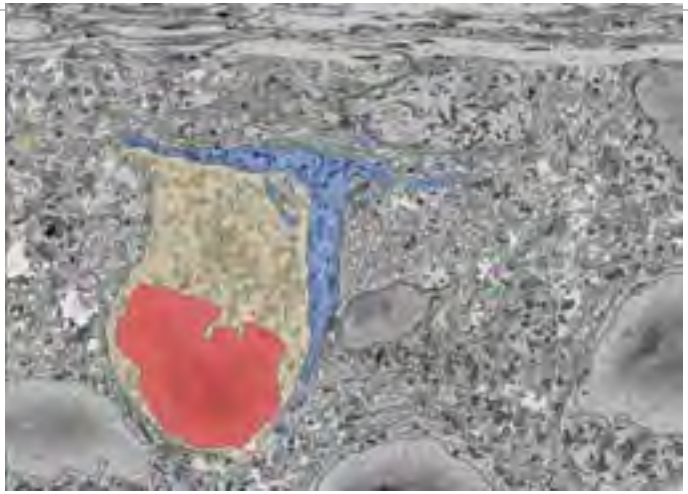
Array Tomography SEM

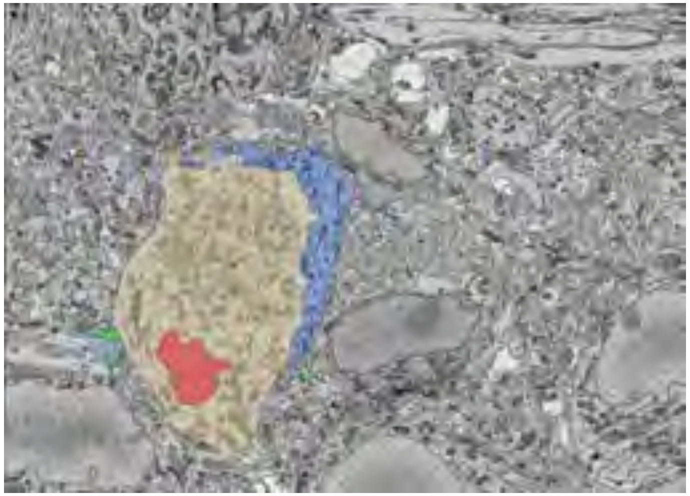


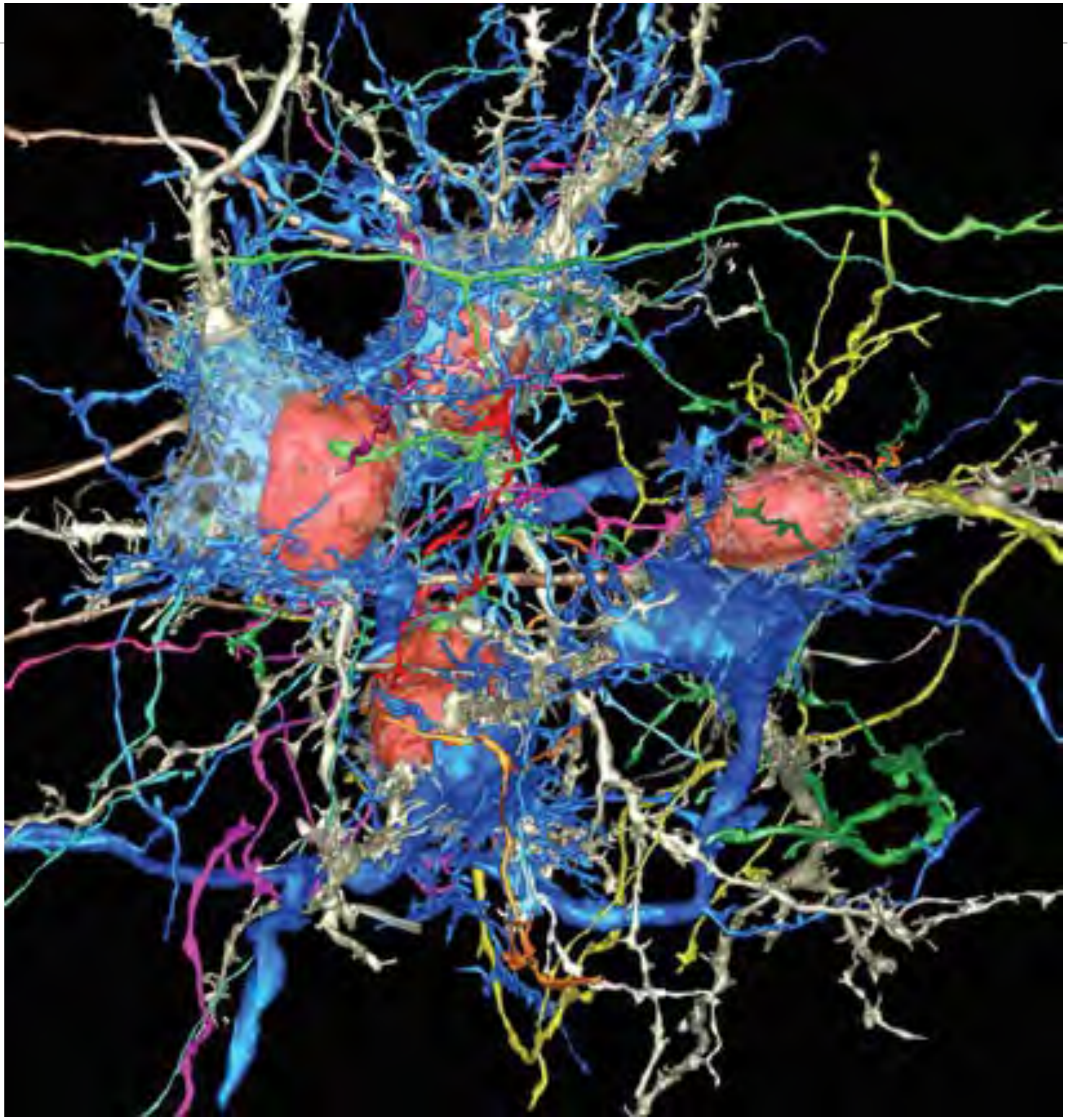
3DEM Example Data



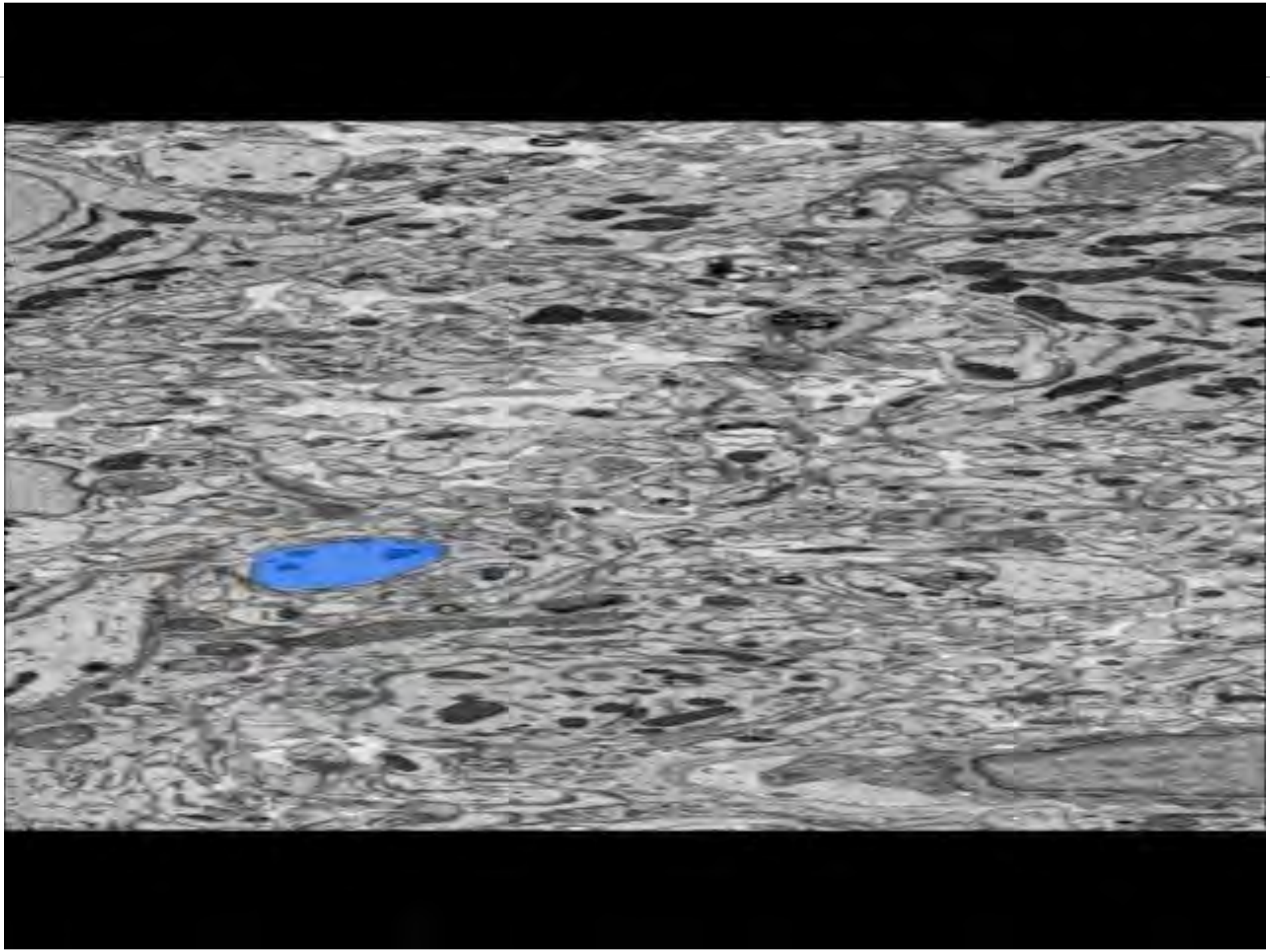
3DEM Models Created from 2D Serial Images



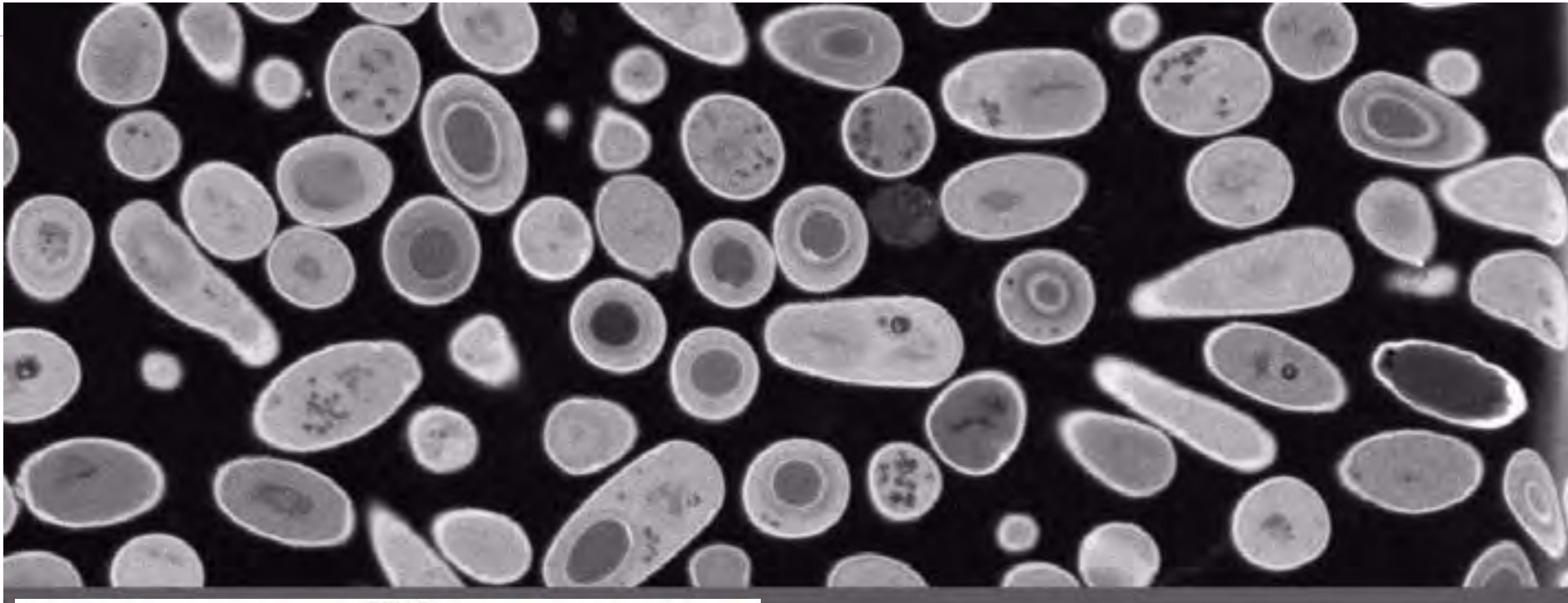


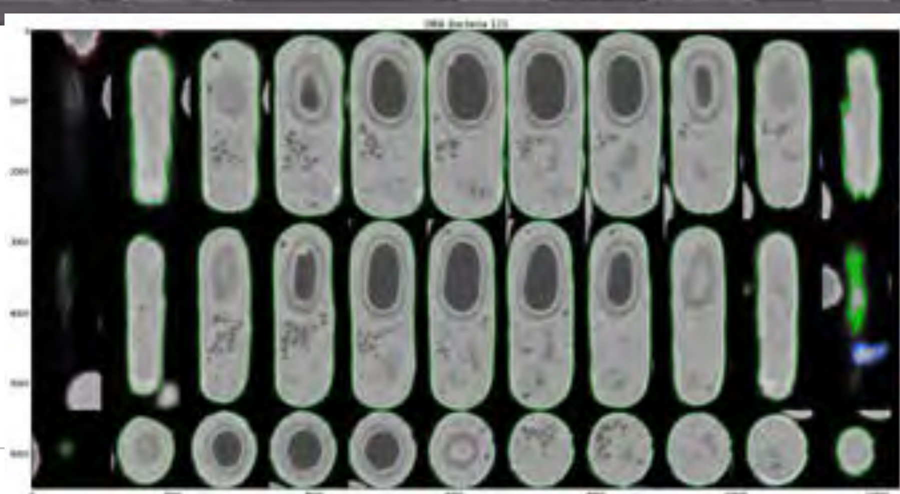


3D Segmentation



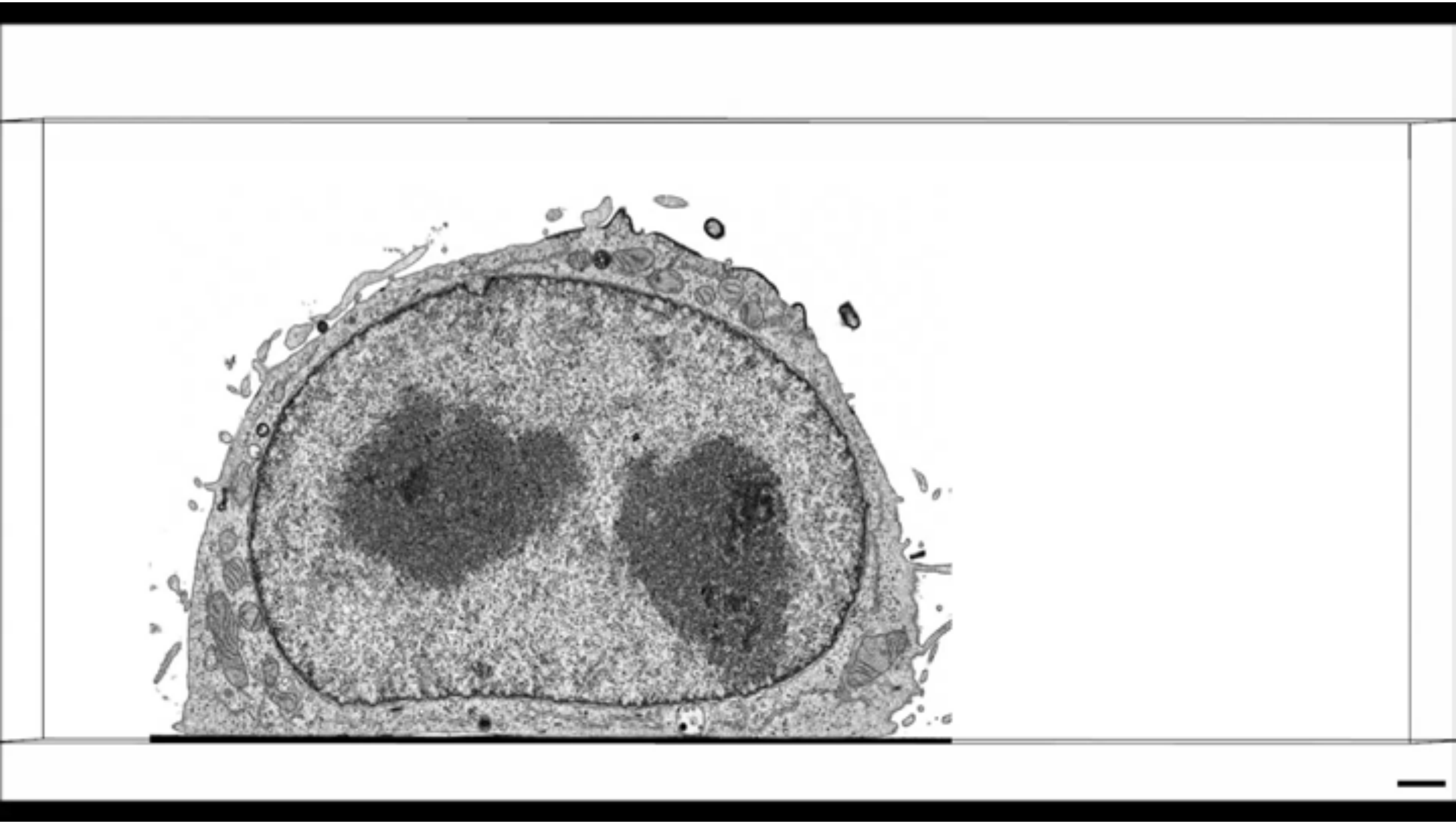
FIB Nanotomography: Virtual Cross Sections & The Benefit of 3 nm Voxels



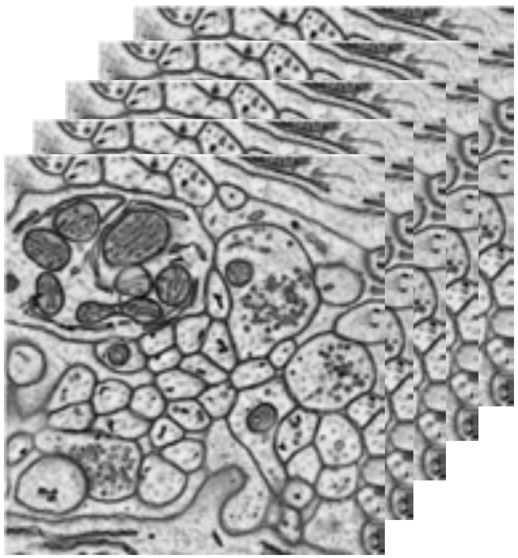


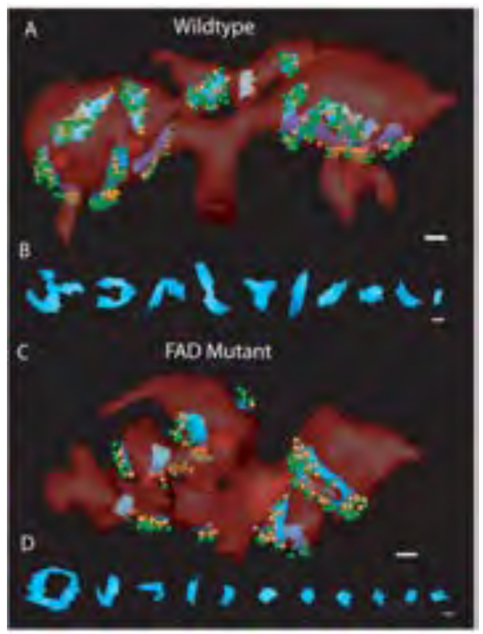
Data courtesy of Dr. Kedar Narayan,
Research Group of Dr. Sriram Subramaniam
BioPhysics Section, NCI, NIH, Bethesda MD

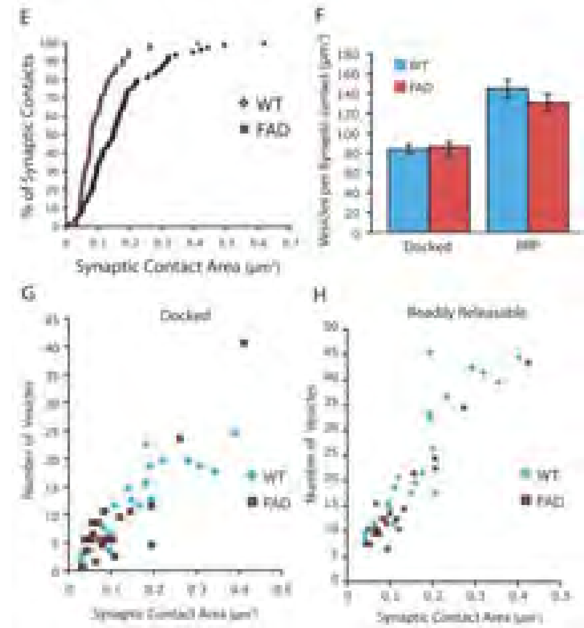
Focused ion Beam Scanning Electron Microscopy
Ultra High-Resolution 3D Cell

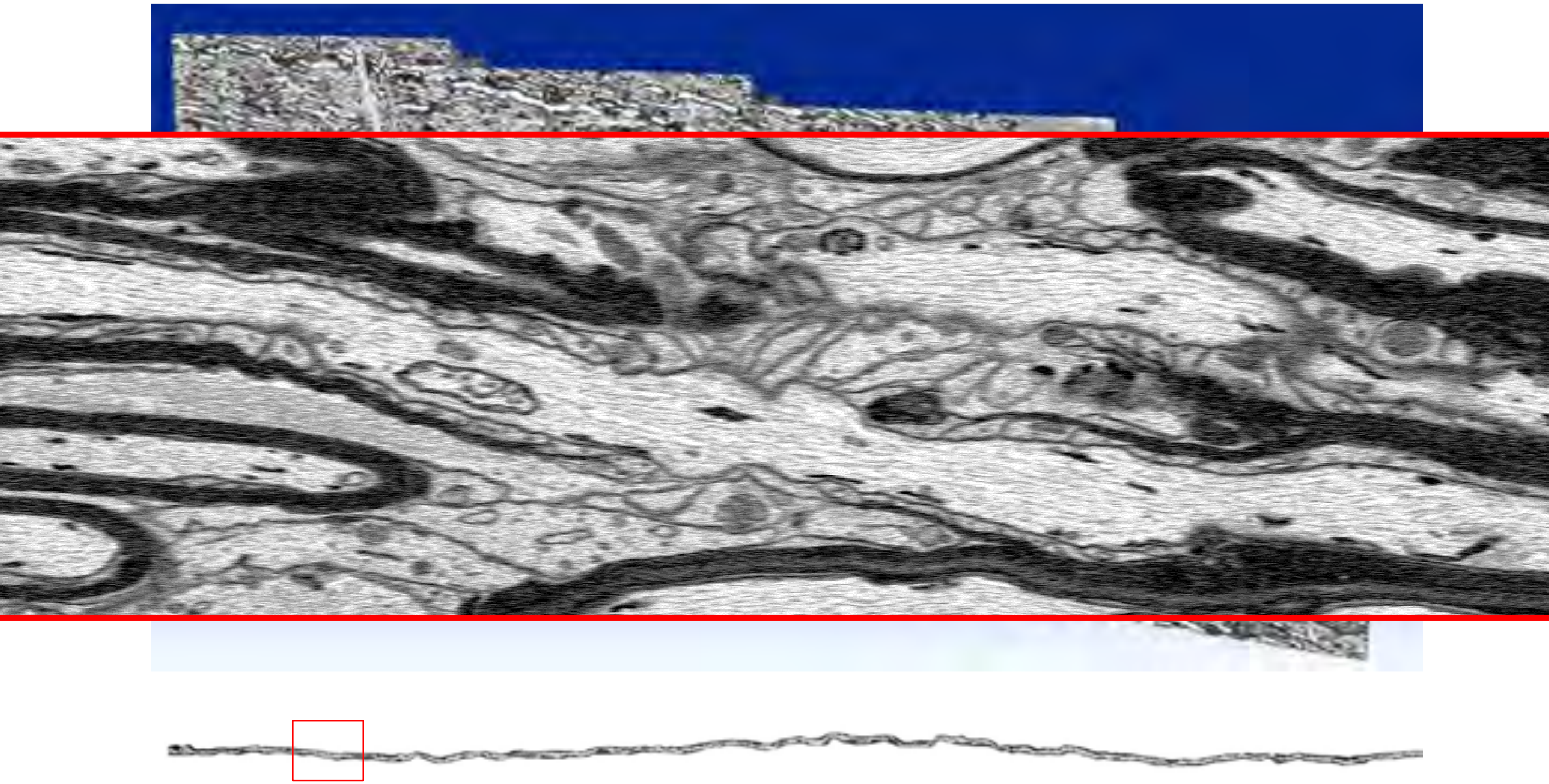


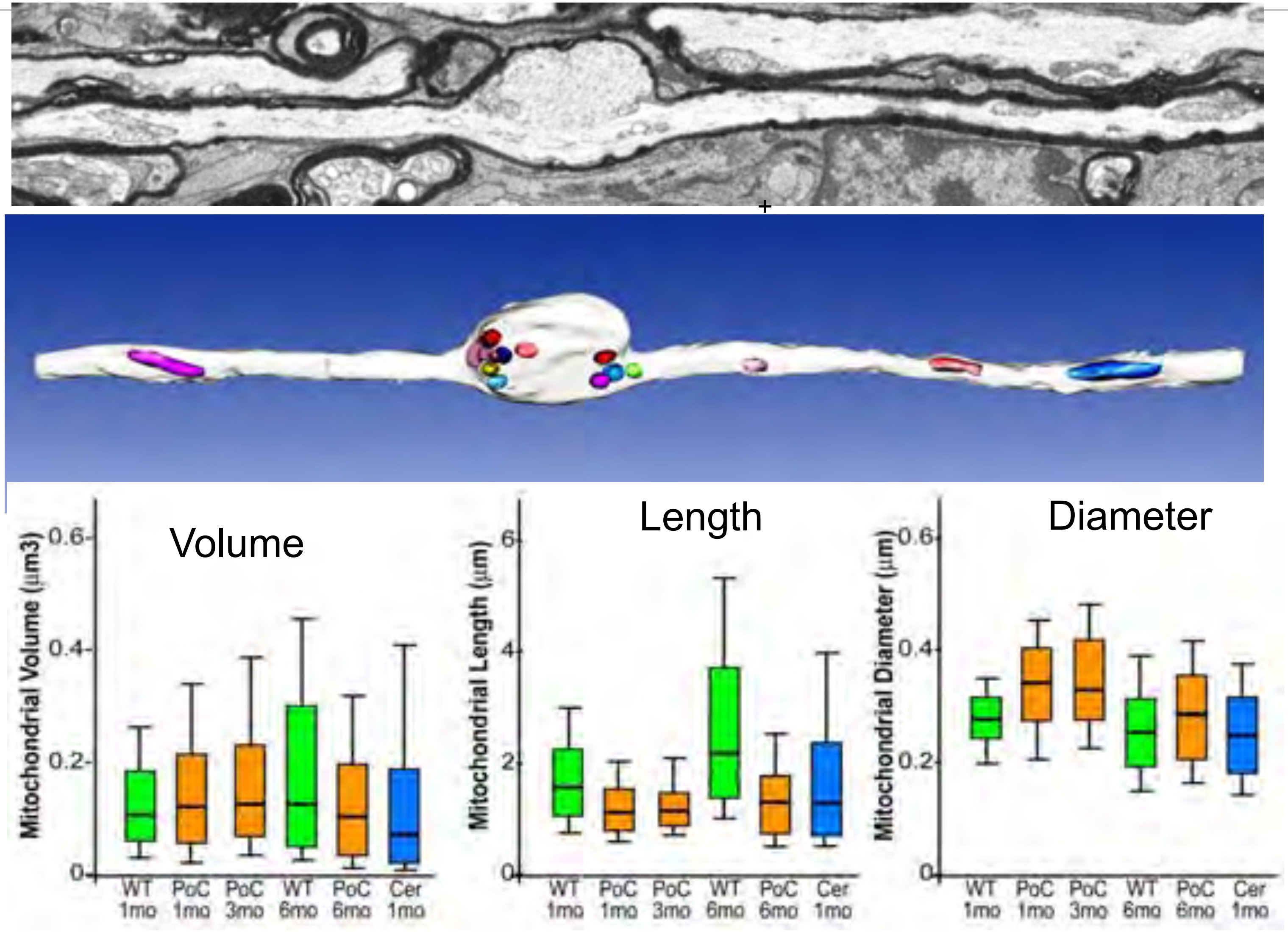
Statistical analysis from 3DEM datasets









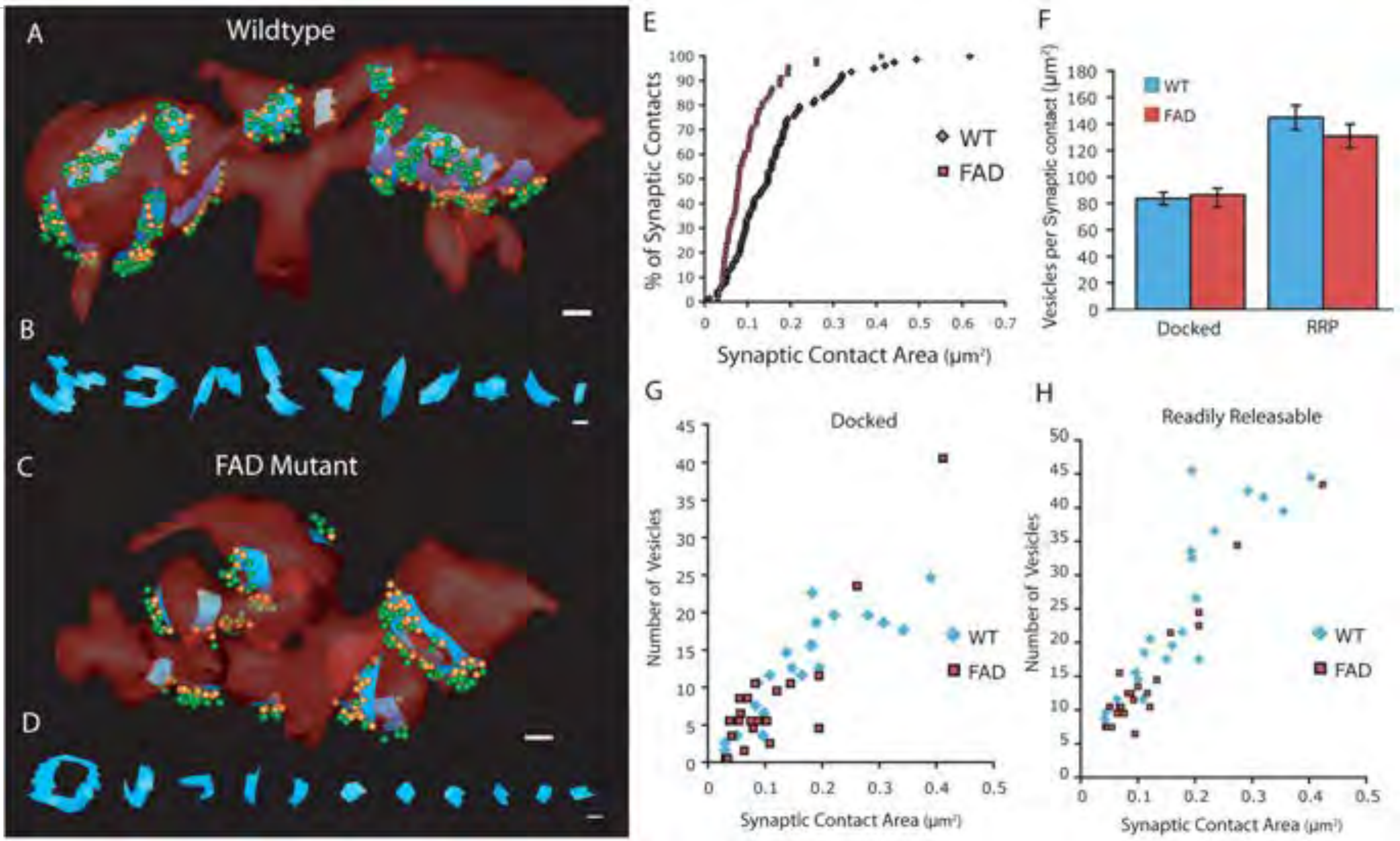


Volume
Length
Diameter
Mitochondrial Volume (µm3)
Mitochondrial Length (µm)
Mitochondrial Diameter (µm)
WT 1mo
PoC 1mo
PoC 3mo
WT 6mo
PoC 6mo
Cer 1mo

Whole cell volumetric measurements

Cellular component	Volume (µm³)	Volume Percentage	Surface Area (µm²)
Endoplasmic reticulum	0.420643	2.2%	31.403
Nuclear envelope	0.227859	1.2%	11.416
Heterochromatin	0.577432	3.0%	24.441
Euchromatin	0.459362	2.4%	20.716
Golgi equivelent	0.022677	0.1%	1.291
Mitochondria	0.299339	1.6%	6.949
Lipid droplets	0.139214	0.7%	2.692
Vesicles	0.000256	0.0%	0.025
Vacuoles	1.480174	7.8%	27.164
Cell Wall	3.017192	15.9%	67.115
Quantitative analysis of volume, volume percentage and surface area of cellular components segmented in Avizo software.			

Wei D, Jacobs S, Modla S, Zhang S, Young CL, Cirino R, Caplan J, and Czymmek K.



A
Wildtype
B
C
FAD Mutant
D
E
% of Synaptic Contacts
Synaptic Contact Area (µm²)
WT
FAD
F
Vesicles per Synaptic contact (µm²)
WT
FAD
Docked
RRP
G
Docked
Number of Vesicles
Synaptic Contact Area (µm²)
WT
FAD
H
Readily Releasable
Number of Vesicles
Synaptic Contact Area (µm²)
WT
FAD

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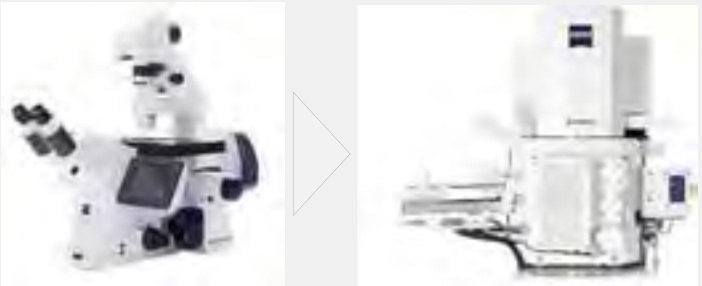
Neurosciences

The classical way of doing correlative microscopy: from LM to EM



Axio Observer.Z1
with ZEN Connect

Gemini SEM 300
with ZEN Connect

Widefieldsystem (Axio Observer.Z1):

- Acquire a fluorescence image
- Result: Fluorescence image showing the distribution of the fluorescence-labeled proteins Synapsin-I and Gephyrin in brain

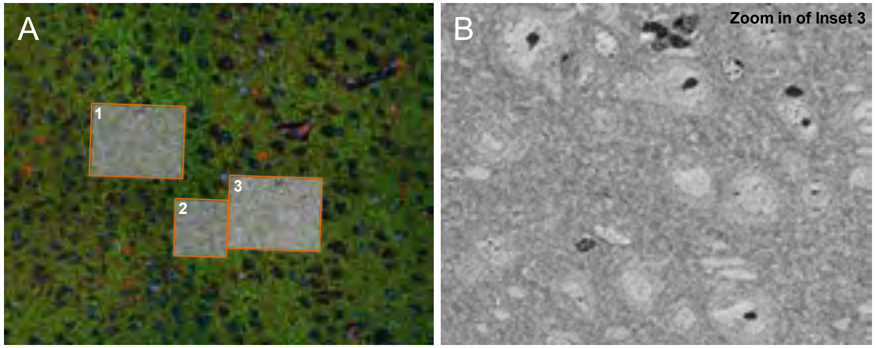
Scanning electron microscope (Gemini SEM 300):

- Acquire high-resolution images
- Result: high-resolution images with ultrastructural information

ZEN Connect:

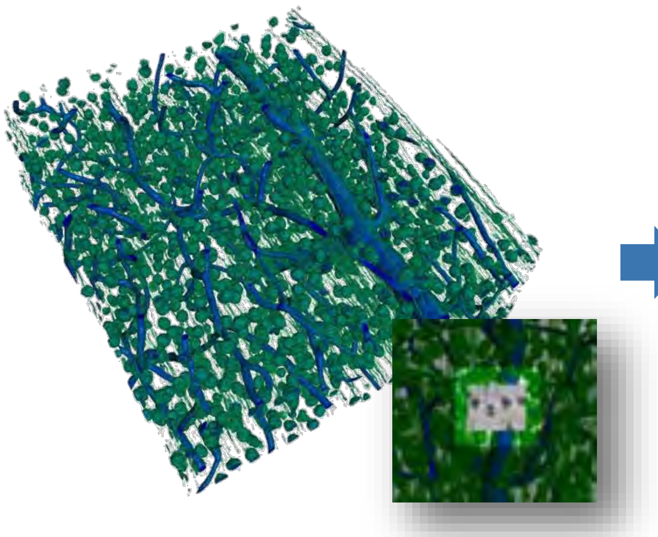
- for navigation and identification of interesting regions
- for bringing the images of different imaging modalities into context
- Result: A contextual image showing functional and structural data

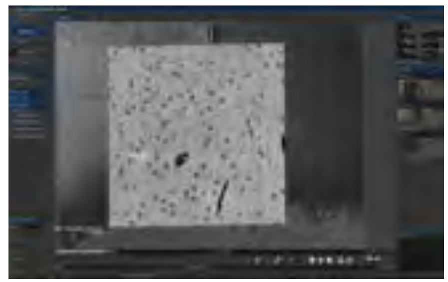
Imaging of an ultrathin mouse brain section with a fluorescence widefield system and a scanning electron microscope to investigate the neuronal network. Sample courtesy Michelle Ocana, Harvard University.

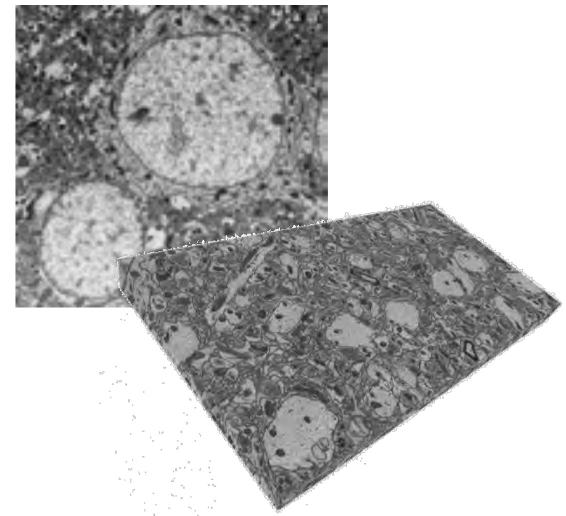


Correlating Electron and X-ray Microscopy

ATLAS 5 Correlative XRM → FIB/SEM Workflow
Stained Mouse Cortex







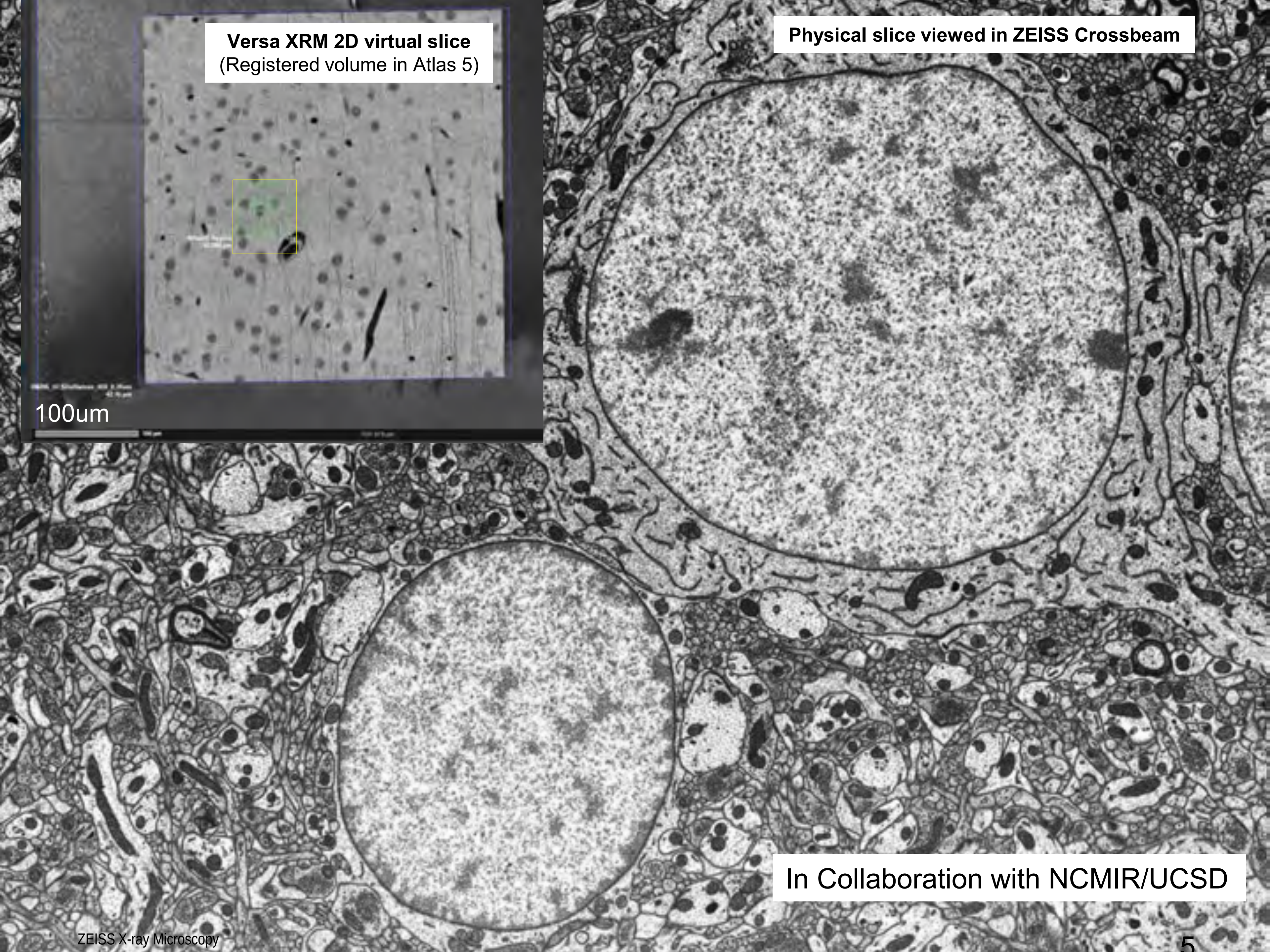
XRM (tomography)
Locate Cell Doublet (inset)

ATLAS 5
Register, Navigate and Drive Crossbeam to Cell Doublet

FIB-SEM (tomography)
Efficiently located cell doublet

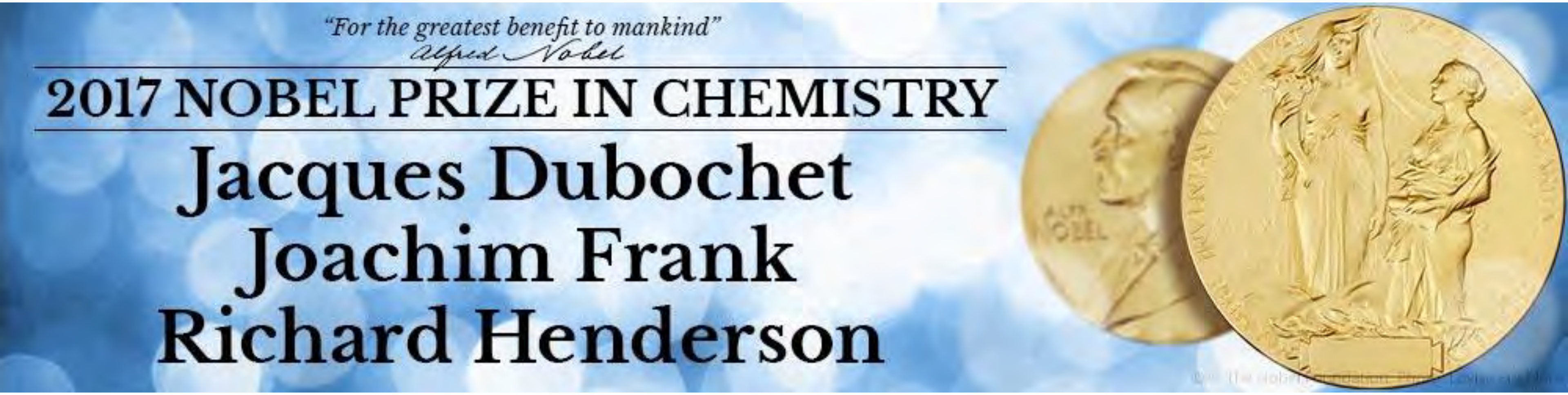
In collaboration with NCMIR @ UCSD

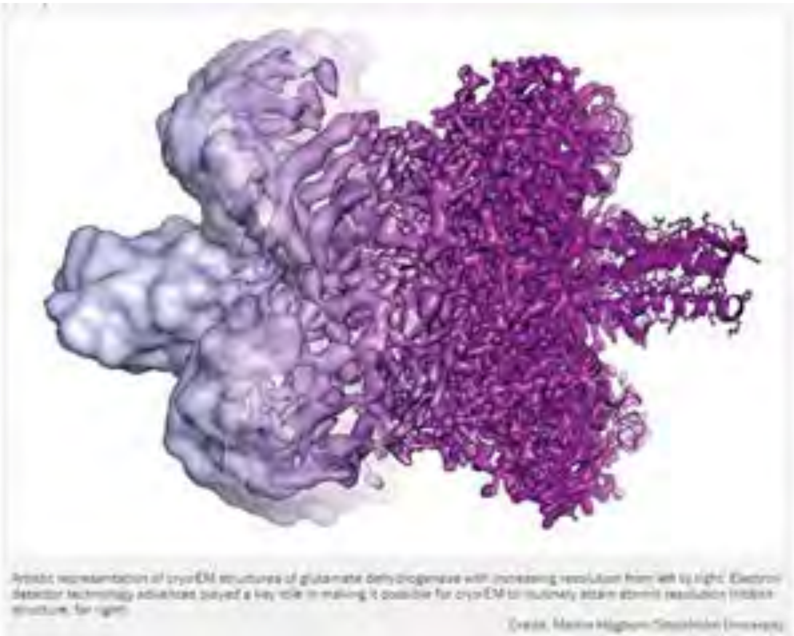
Atlas 5: Correlative Workflow Example
Using XRM to target FIB-SEM volume in Neuroscience

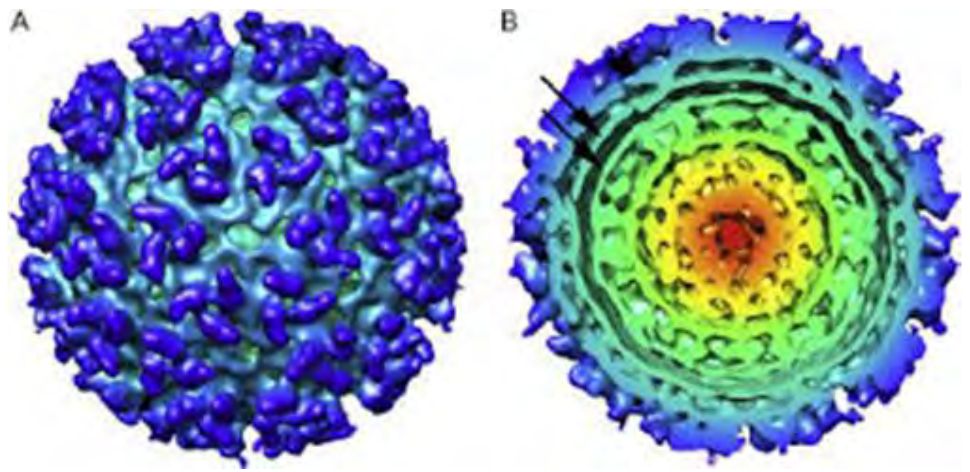

Physical slice viewed in ZEISS Crossbeam
In Collaboration with NCMIR/UCSD
Versa XRM 2D virtual slice
(Registered volume in Atlas 5)
100um

Agenda

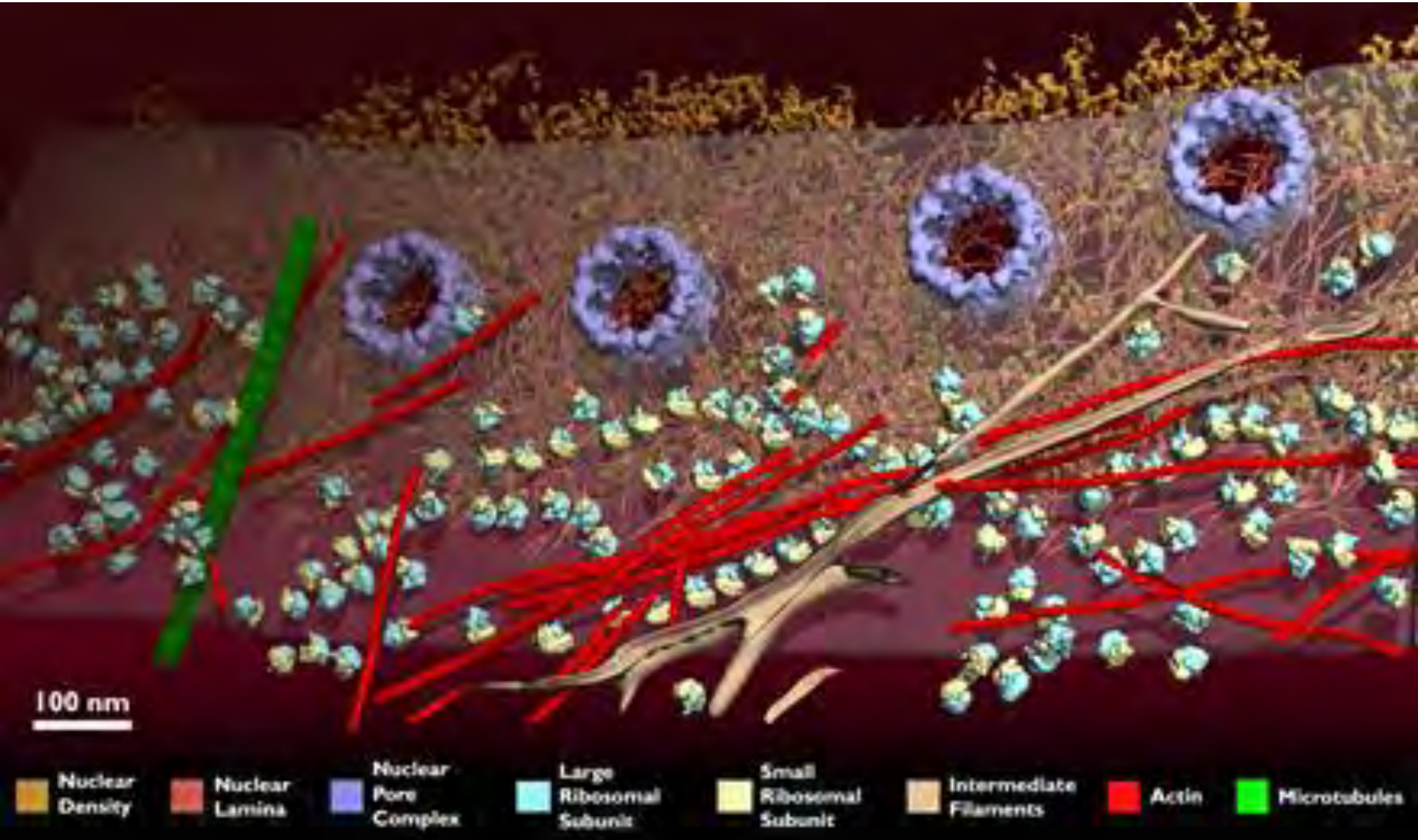
Imaging Biomolecules in 3D at High-Resolution using Cryo Electron Tomography







The Next Frontier – Cellular EM Tomography



We make it visible.