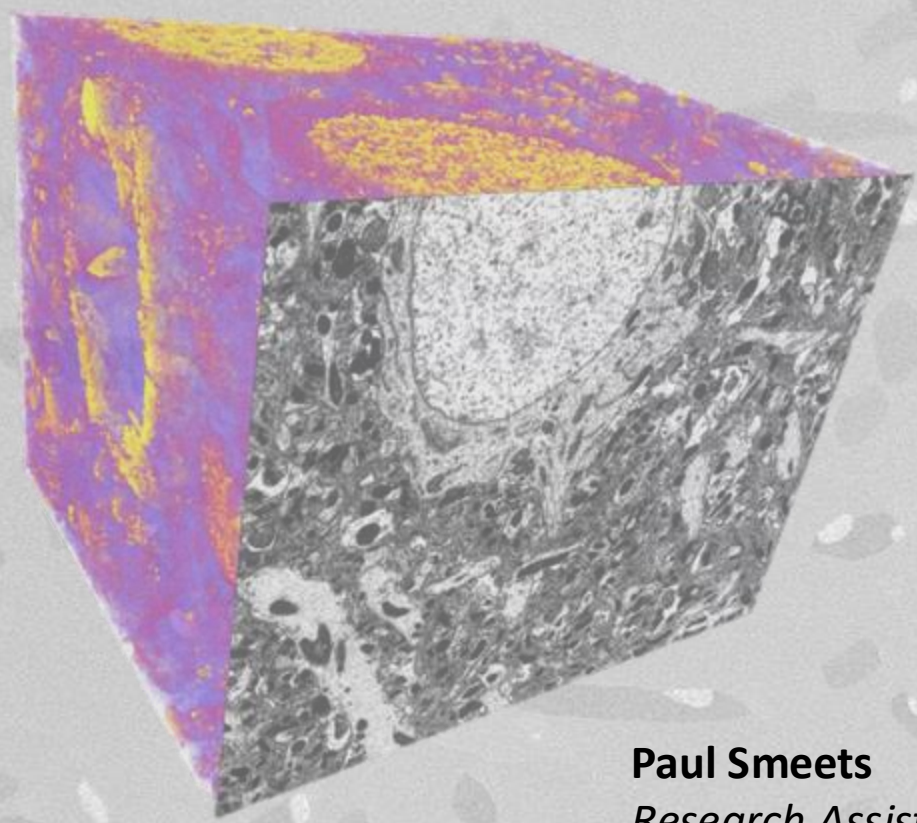
The Power of Plasma:

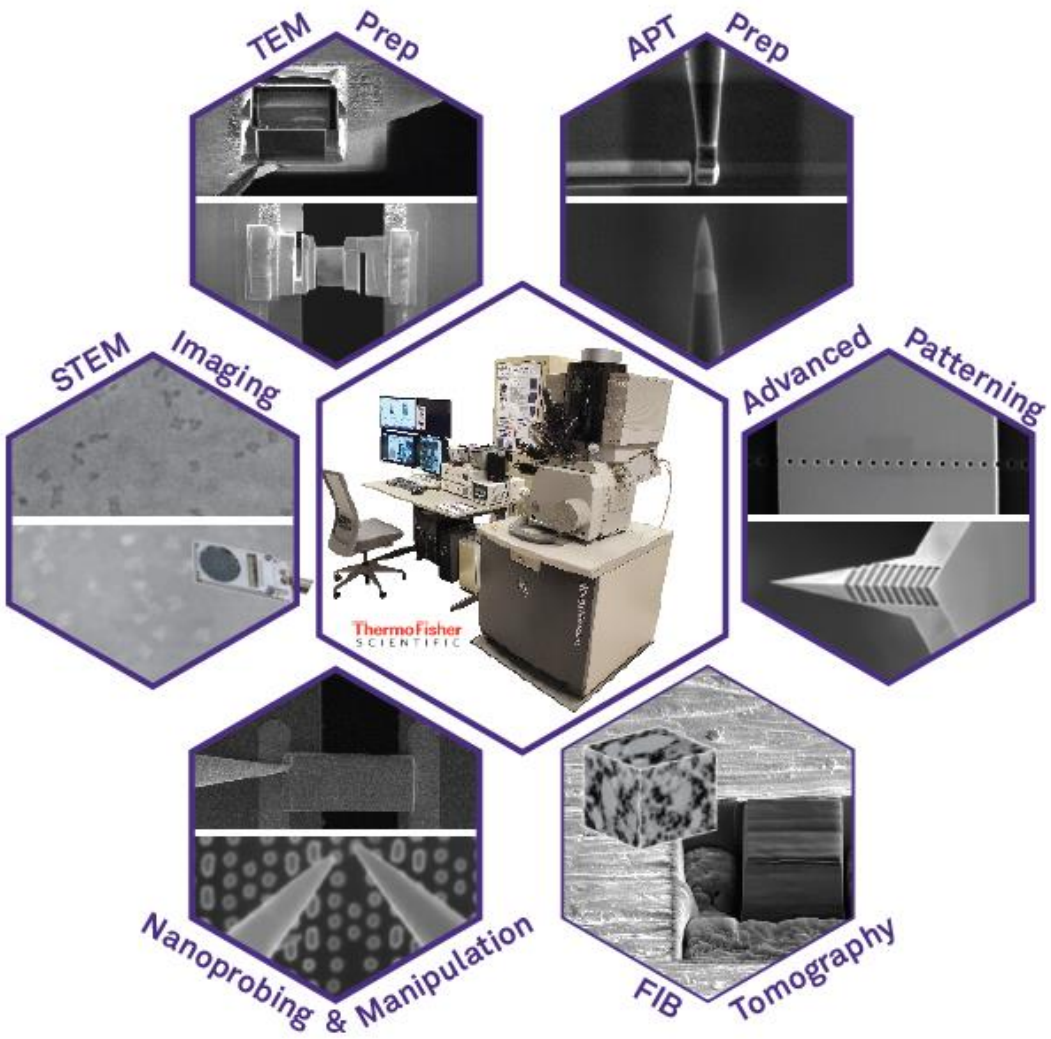
Introducing the Thermo Fisher Hydra Plasma FIB-SEM



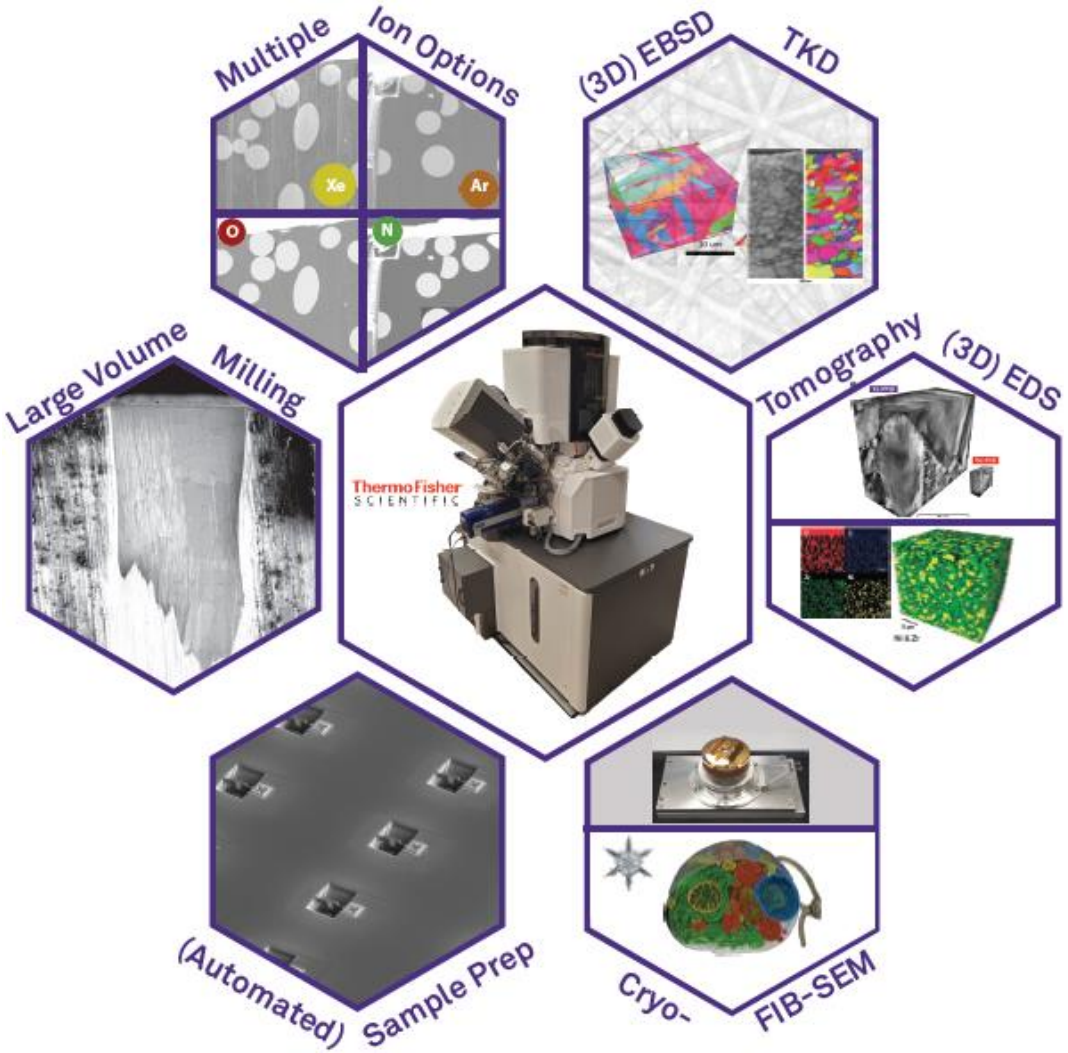
Paul Smeets
Research Assistant Professor
Materials Science and Engineering
Northwestern University

EPIC – FIB Instrumentation

FEI Helios Nanolab 600 FIB-SEM



Thermo Fisher Hydra Plasma FIB-SEM



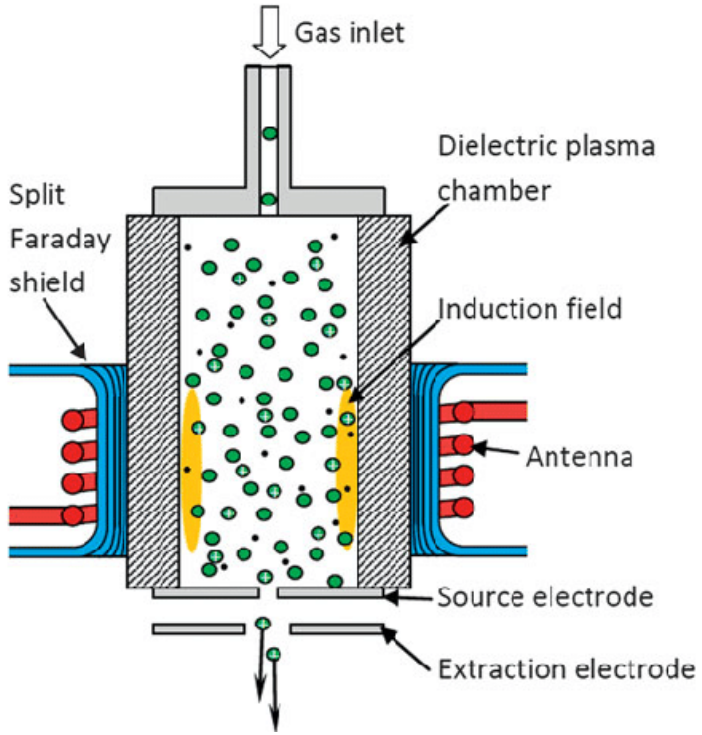
What is a Plasma?

- ‘Fourth state of matter’

- Inlet gas gets ionized by induction magnetic field

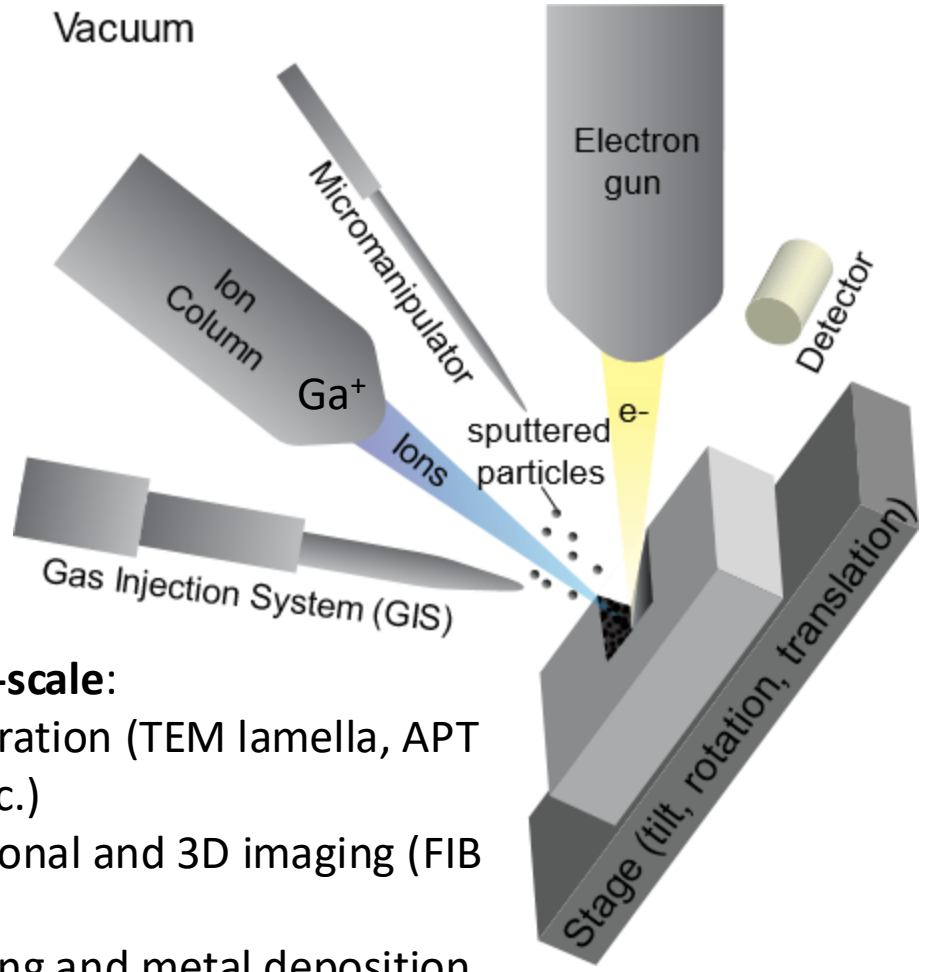
- Extraction of positive ions

$$E = -N(\Delta\phi_B/\Delta t)$$



What is a Focused Ion Beam – Scanning Electron Microscopy (FIB-SEM)?

Tech Talk – Fundamentals of FIB-SEM

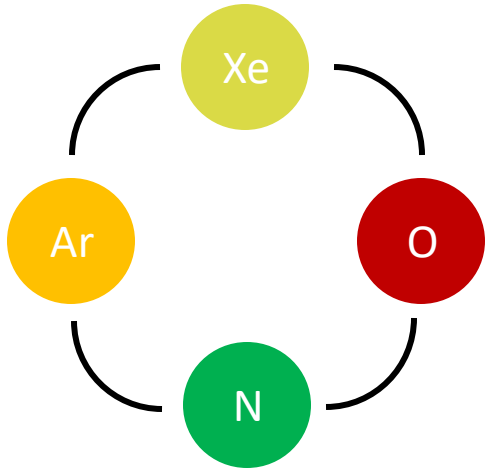


Nano- to micron-scale:

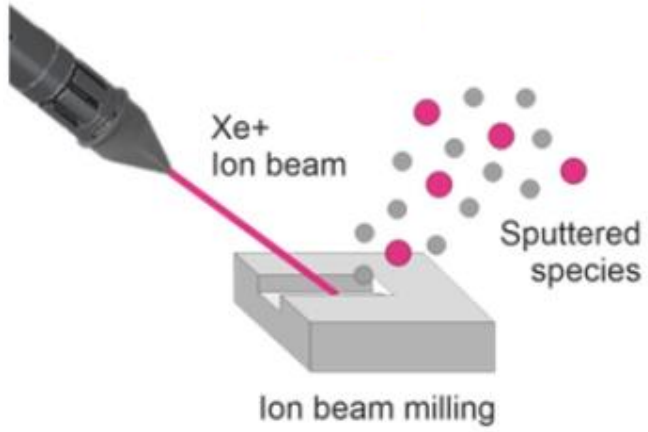
- Sample preparation (TEM lamella, APT tips, X-ray , etc.)
- 2D cross-sectional and 3D imaging (FIB tomography)
- Micromachining and metal deposition via GIS

Benefits of using Plasma in a FIB-SEM

- Four different ion species
  - Each deliver different outcomes
  - Switching species in < 10 min



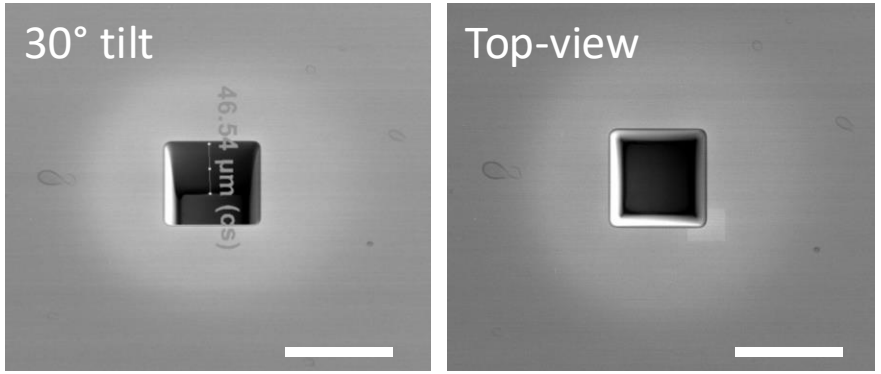
- Flexibility in sample preparation / characterization
  - Match the ion to the sample

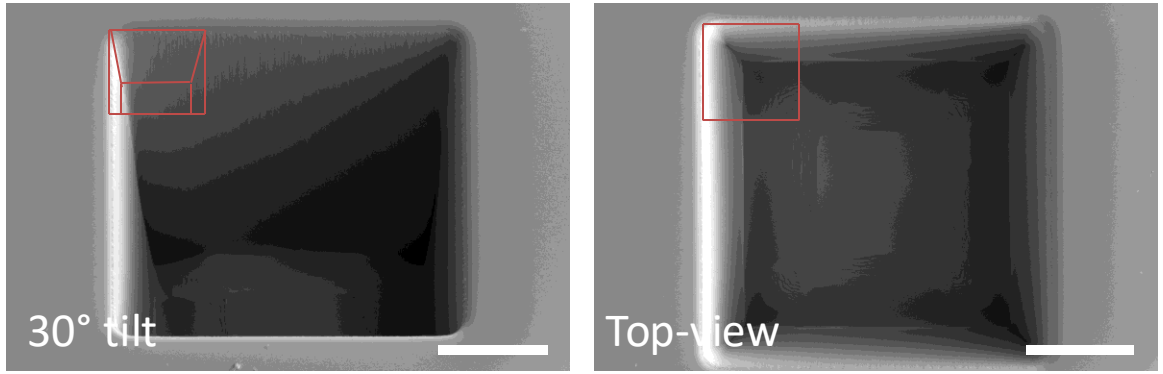


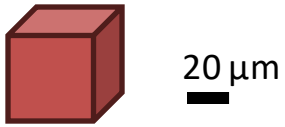
- High ion beam currents available

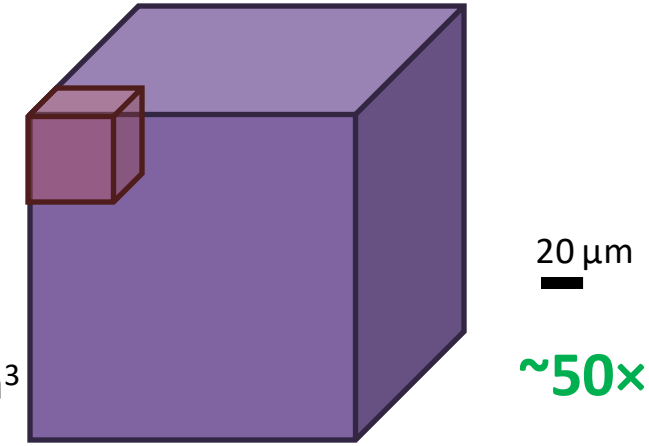
High Throughput Xe^+ Milling – Conventional FIB vs PFIB

Ion Beam Conditions	Ga^+ 30 kV; 21 nA	Xe^+ 30 kV; 2.5 µA
Instrument	Helios Nanolab 600 FIB-SEM	Hydra PFIB
Milling Time	1 hr	1 hr









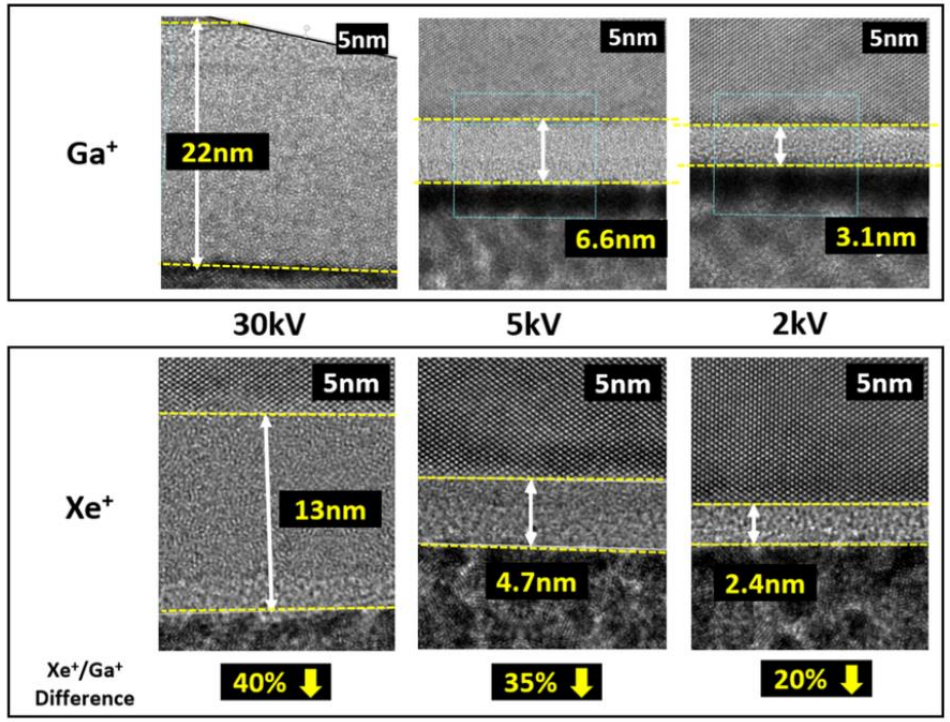
Si Volume Milled	6.4×10^4 µm^3	3.0×10^6 µm^3

~50×

PFIB Benefits over Conventional Ga^+ FIB-SEM

Less surface damage

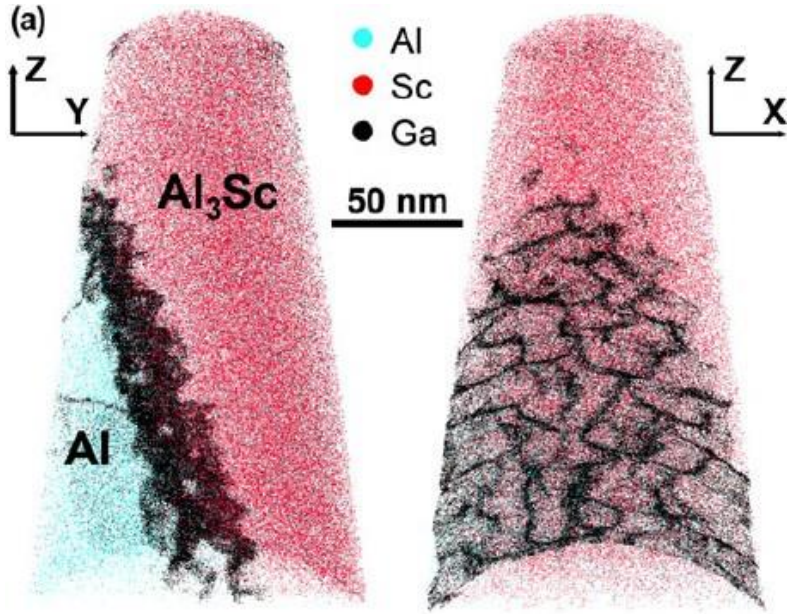
TEM lamella (Si)



Burnett, T.L. *et al.* *Ultramicroscopy* **161**, 119 (2016)

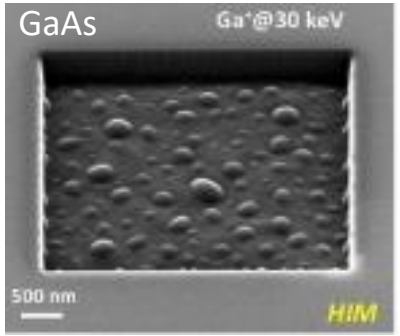
Ga^+ contamination

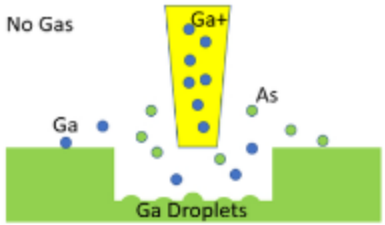
Al-based alloy using Atom Probe Tomography



Gault, B. *et al.* *Journal of Materials Research* **33**, 4018 (2018)

Milling Ga-based materials

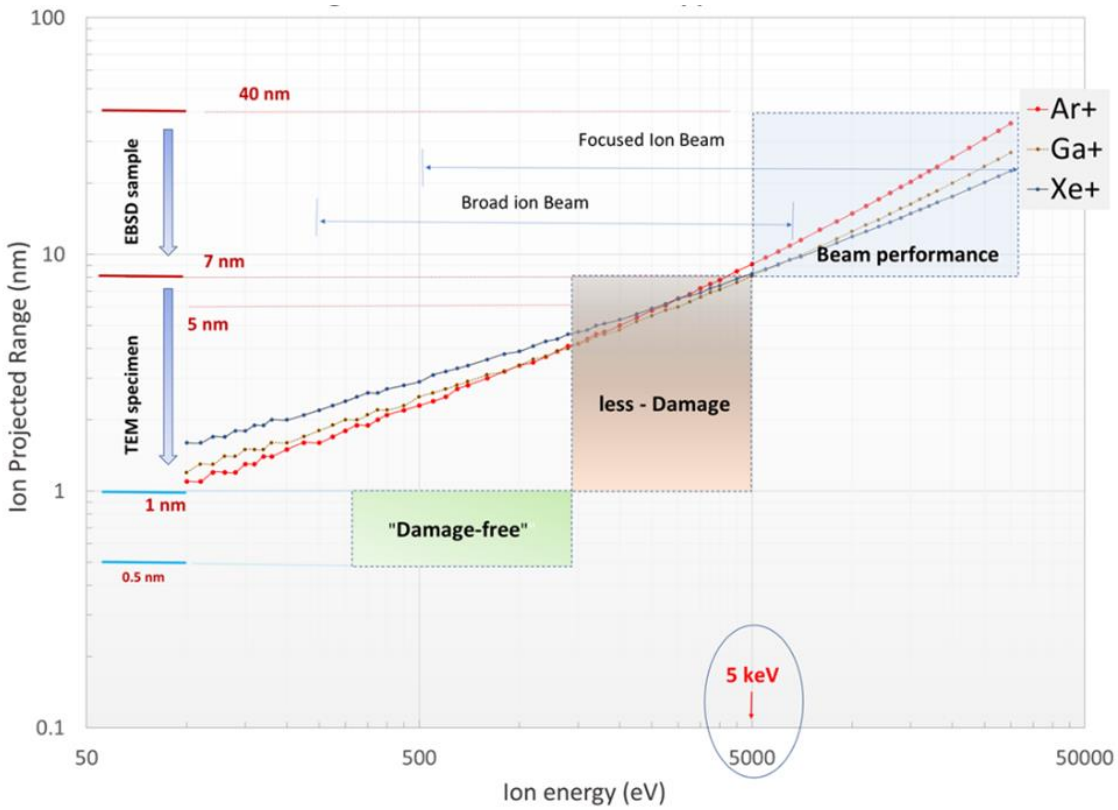


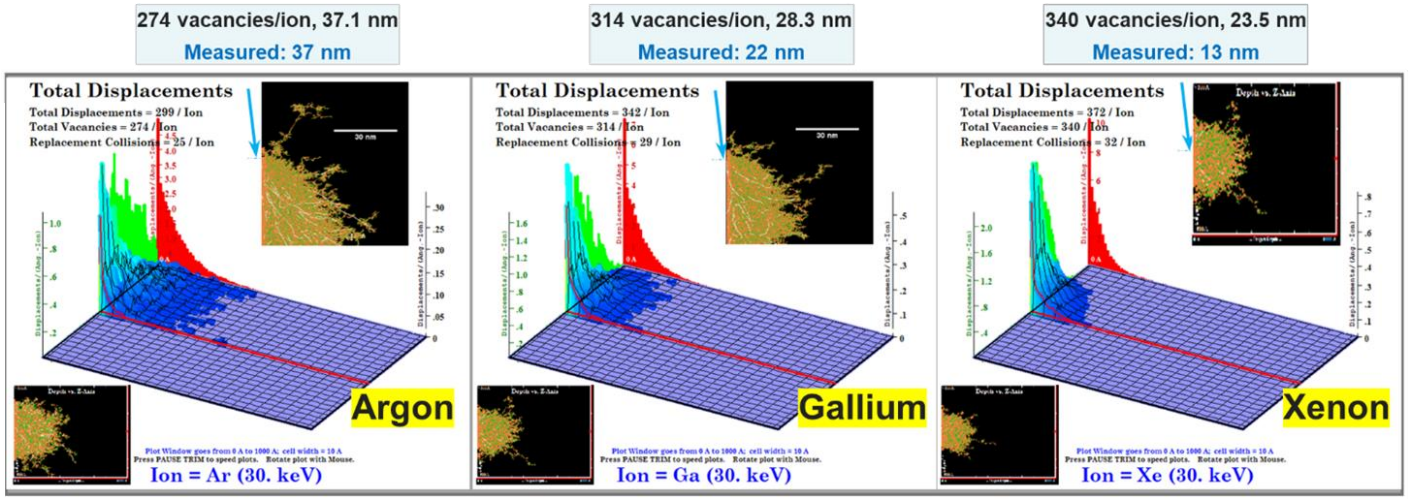


Xia, D. *et al.* *Applied Surface Science* **538**, 147922 (2021)

Exploring various Ion Interactions: Ga^+ vs Xe^+ vs Ar^+

Ion range in Si vs kV

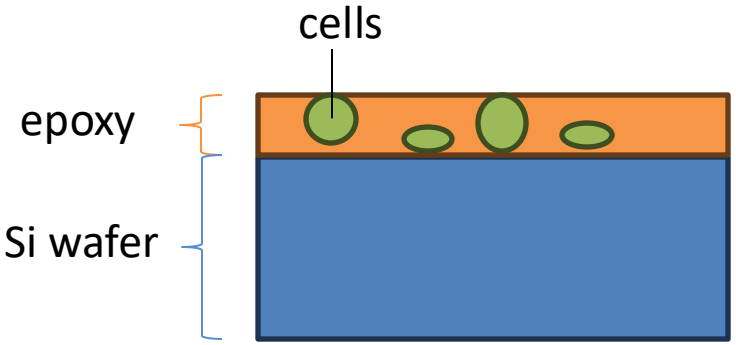


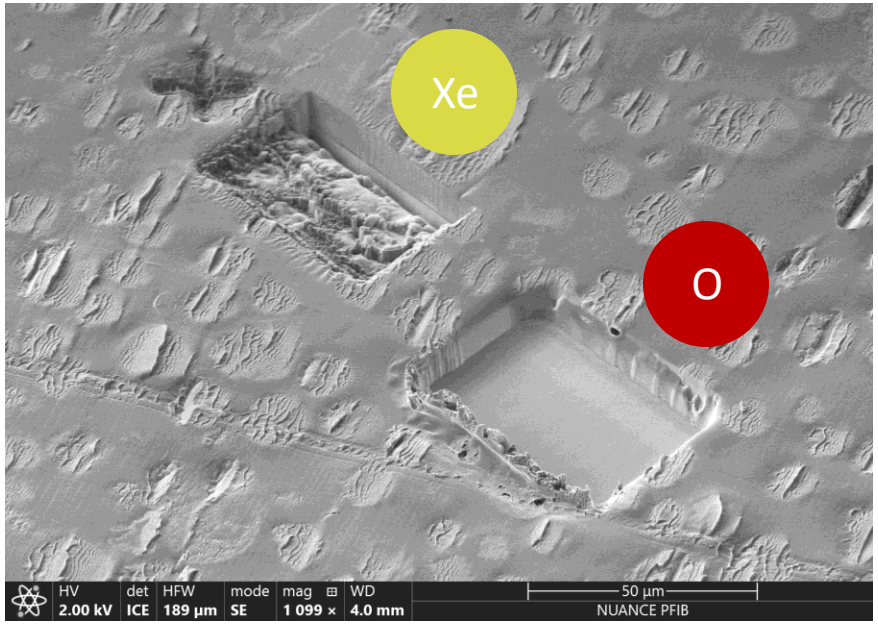


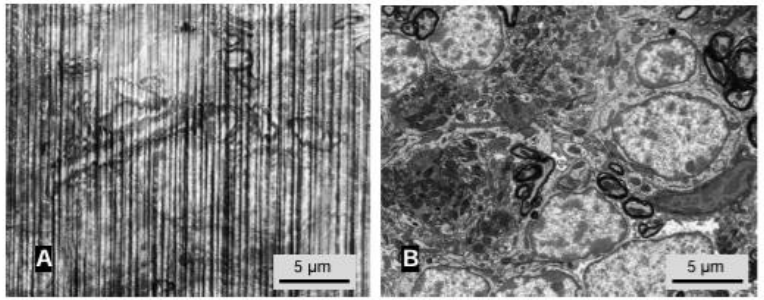
Tradeoff between Ion Range and Damage Intensity (milling rate)

Exploring Ion Interactions: Oxygen vs Xenon

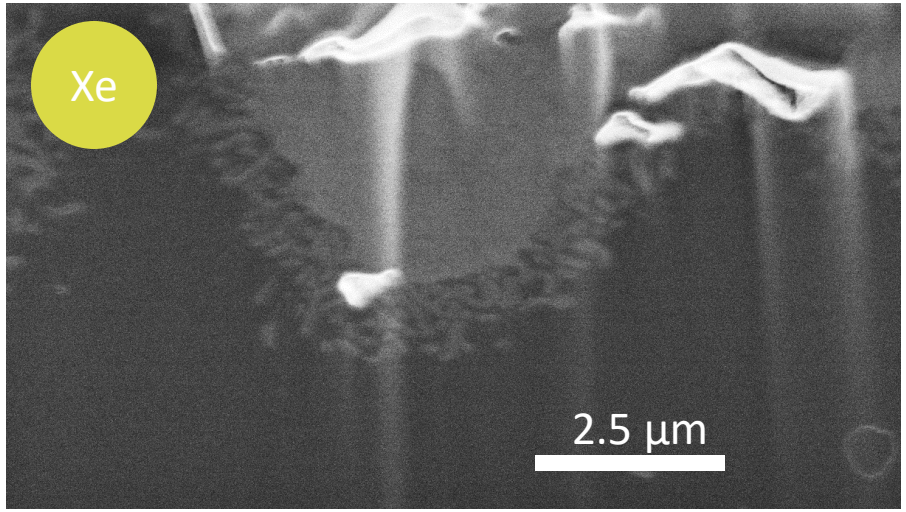
Co-culture of mouse macrophages and cancer tumor cells

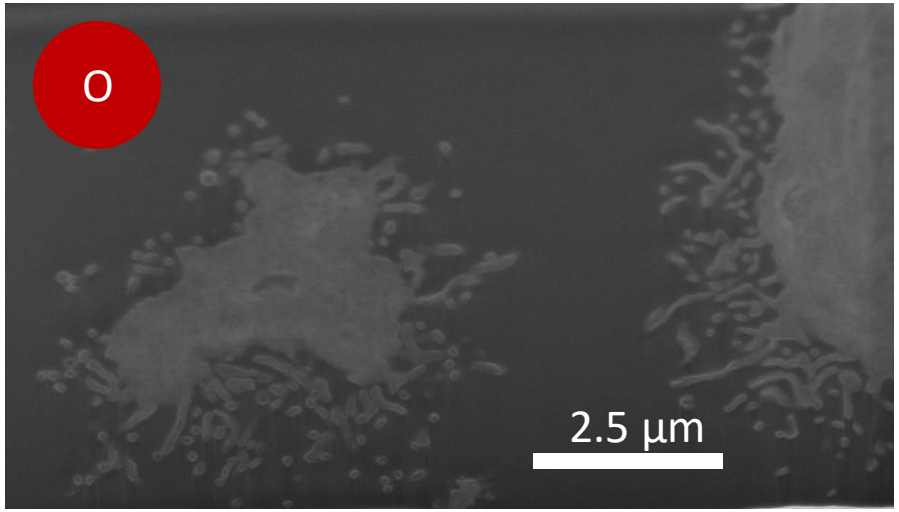






Chemically fixed mouse brain tissue embedded in LR White resin. (A) Gallium FIB milling at 30 kV, 65 nA. (B) Oxygen PFIB milling at 30 kV, 45 nA. Image taken with in-column detector at 2 kV.

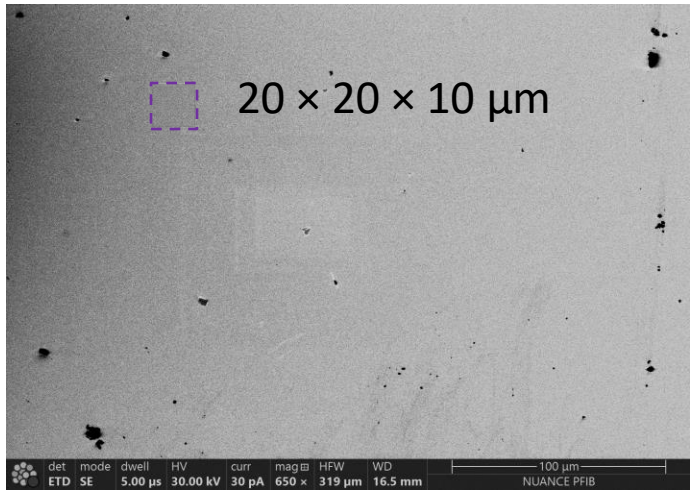




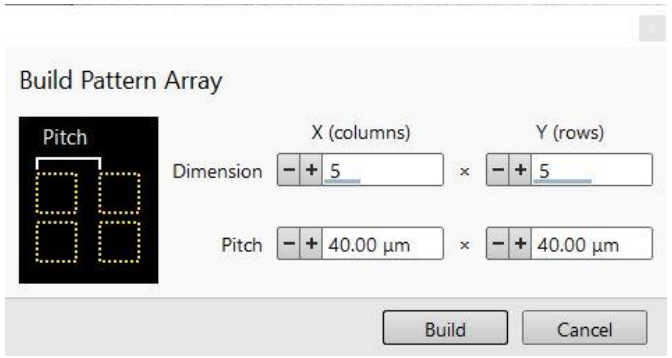
Rapid and Convenient Micromachining

Micromachining pattern array

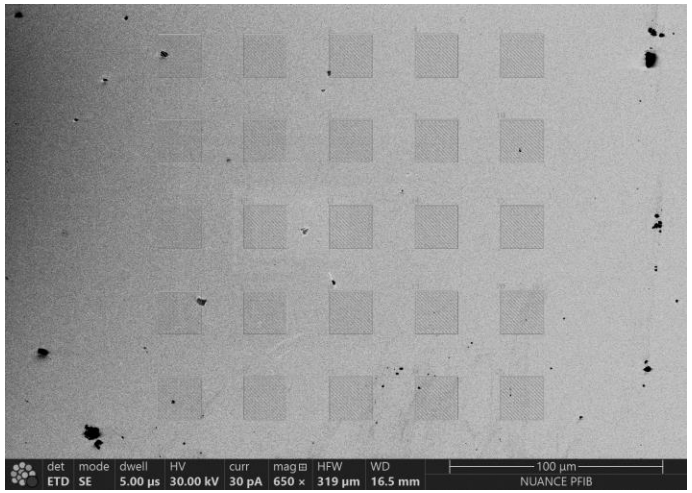
Step 1 → build pattern



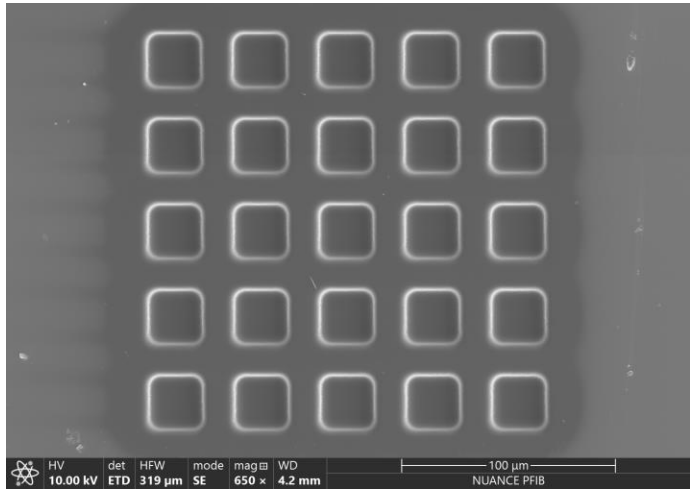
Step 2 → build pattern array



Step 3 → mill pattern array



Step 3 → final imaging result (SEM)

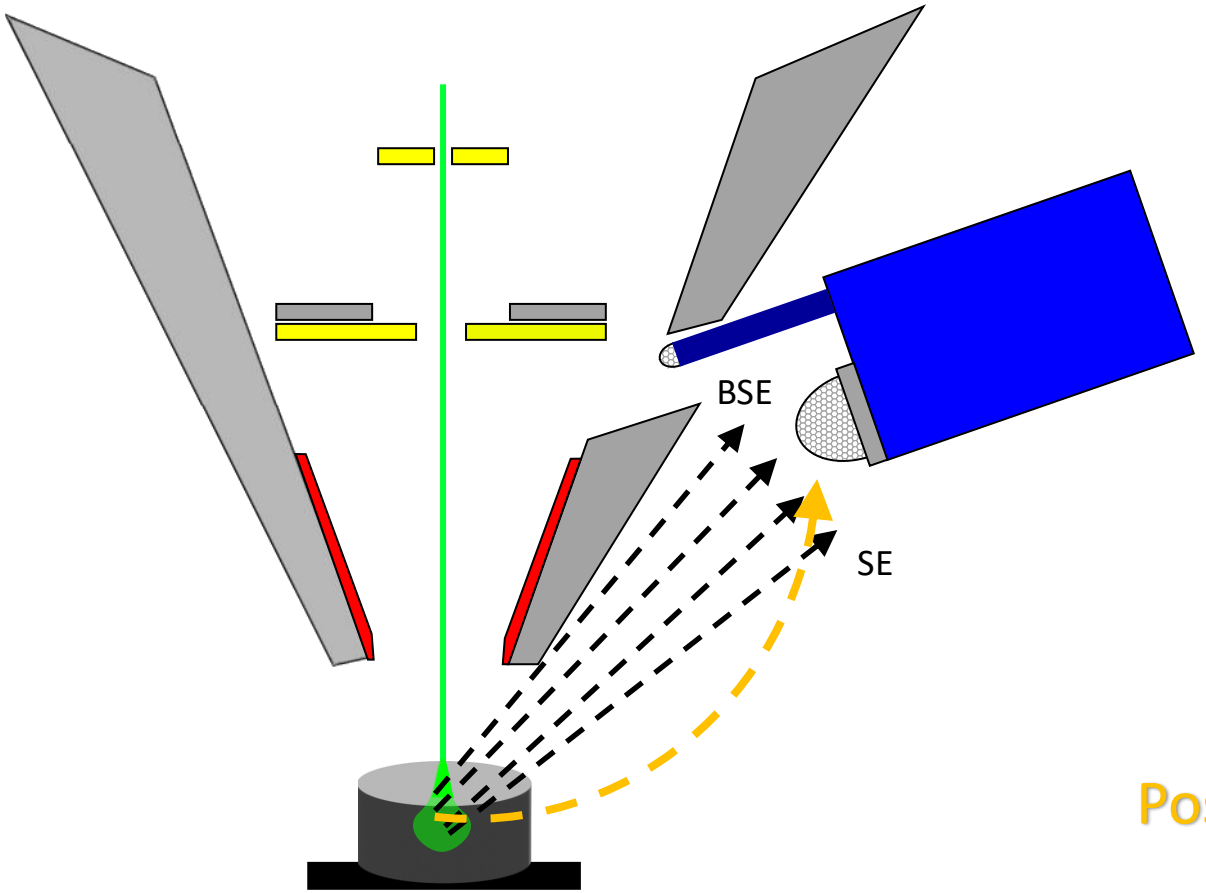


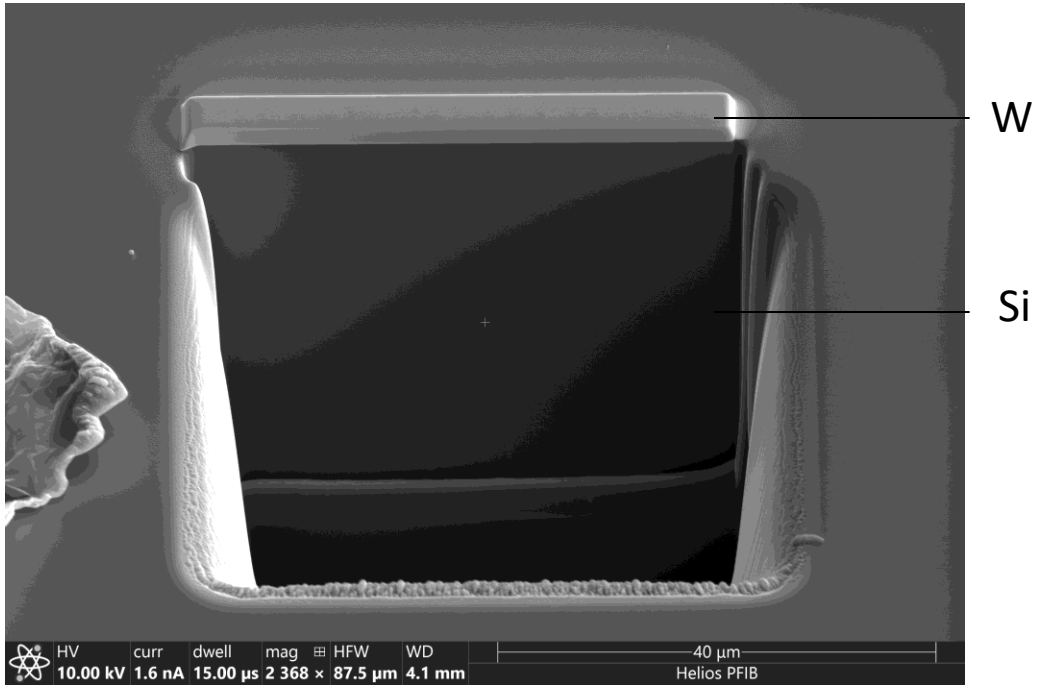
Volume milled: 10^5 µm^3
Total time: ~5 min

Imaging Detectors

1) Everhart Thornley Detector (ETD)

Secondary Electron (SE) Mode



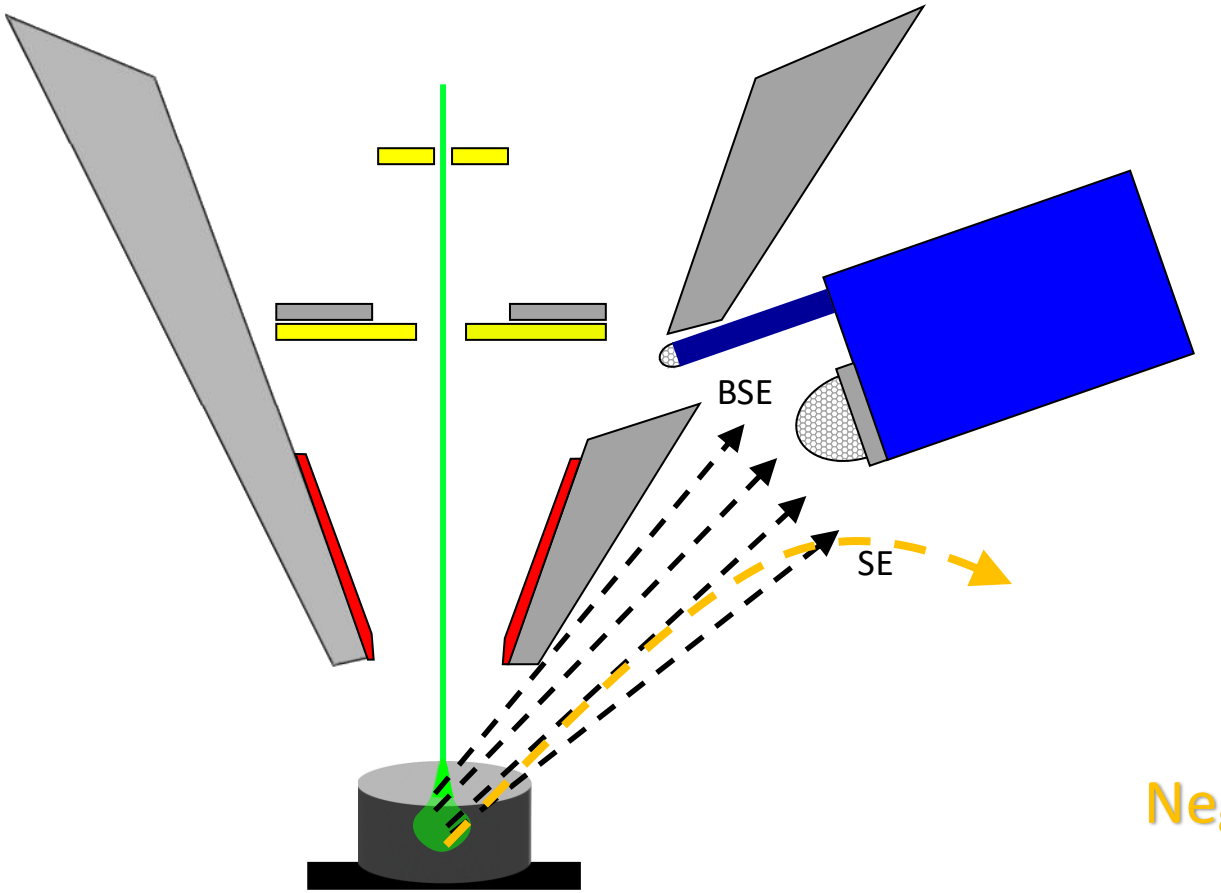


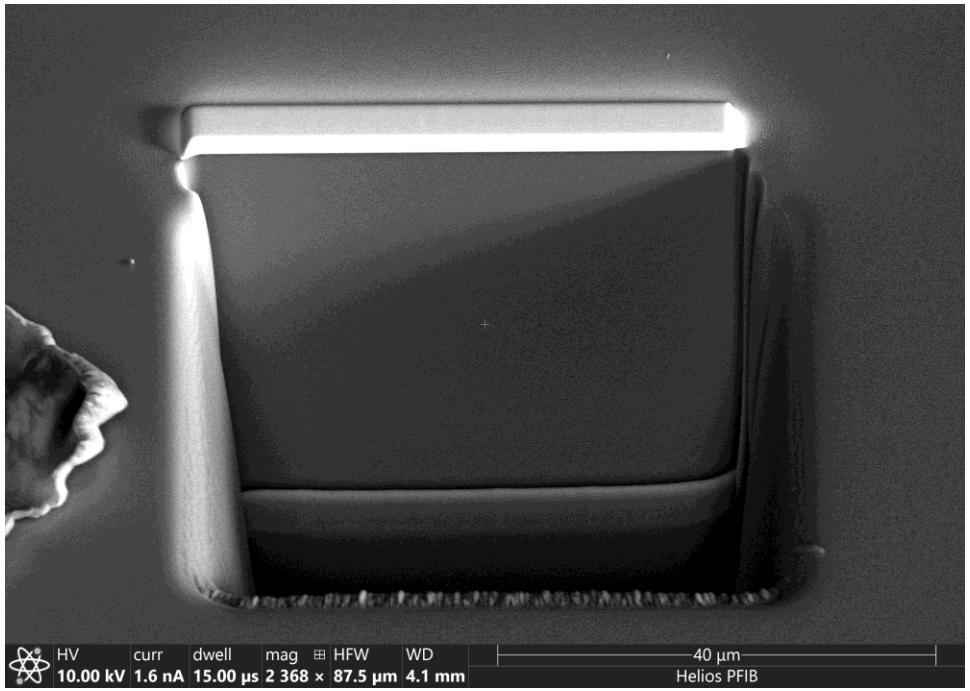
Positive grid bias to attract lower energy SEs

Imaging Detectors

1) Everhart Thornley Detector (ETD)

Backscatter Electron (BSE) Mode



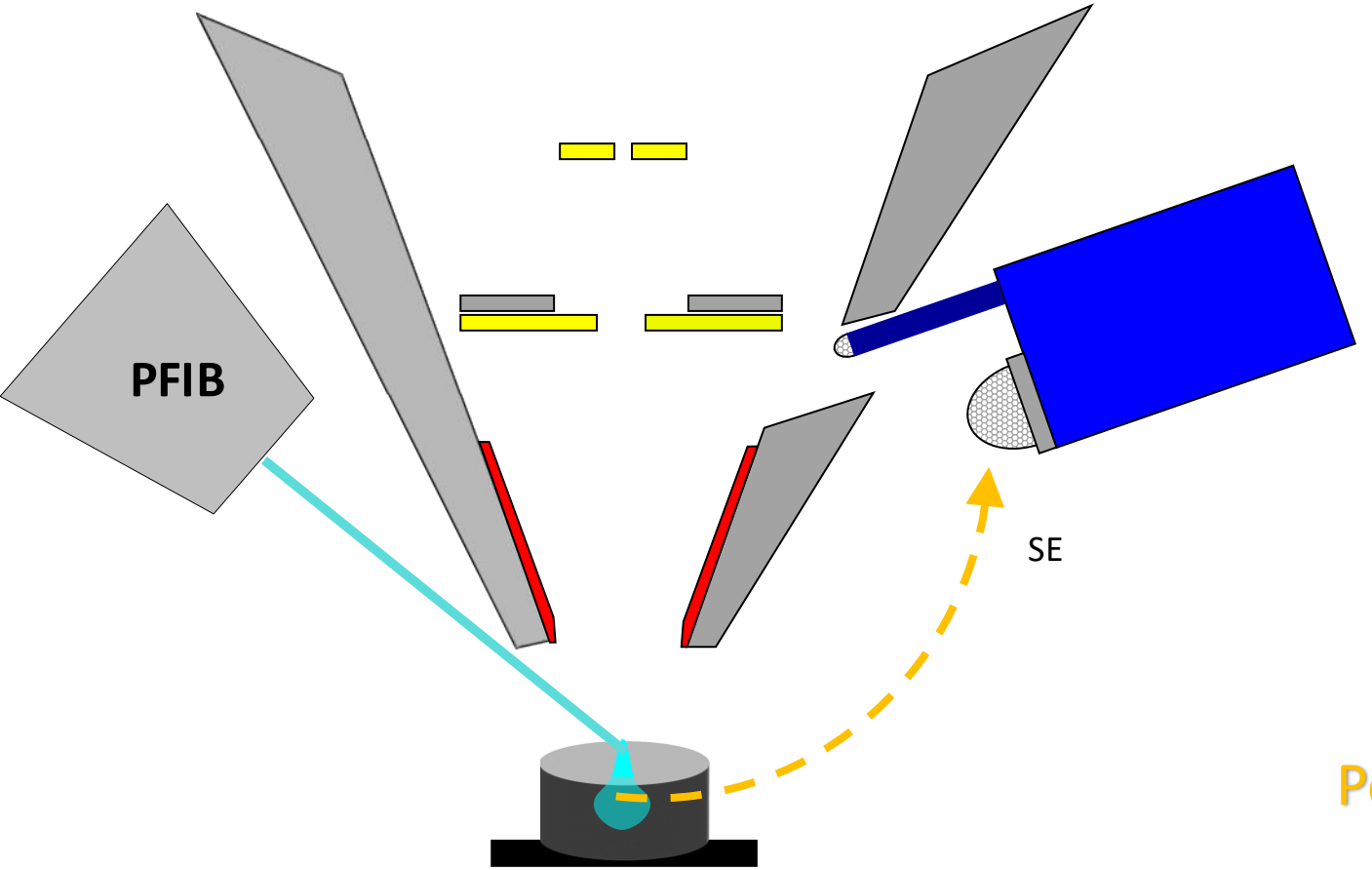


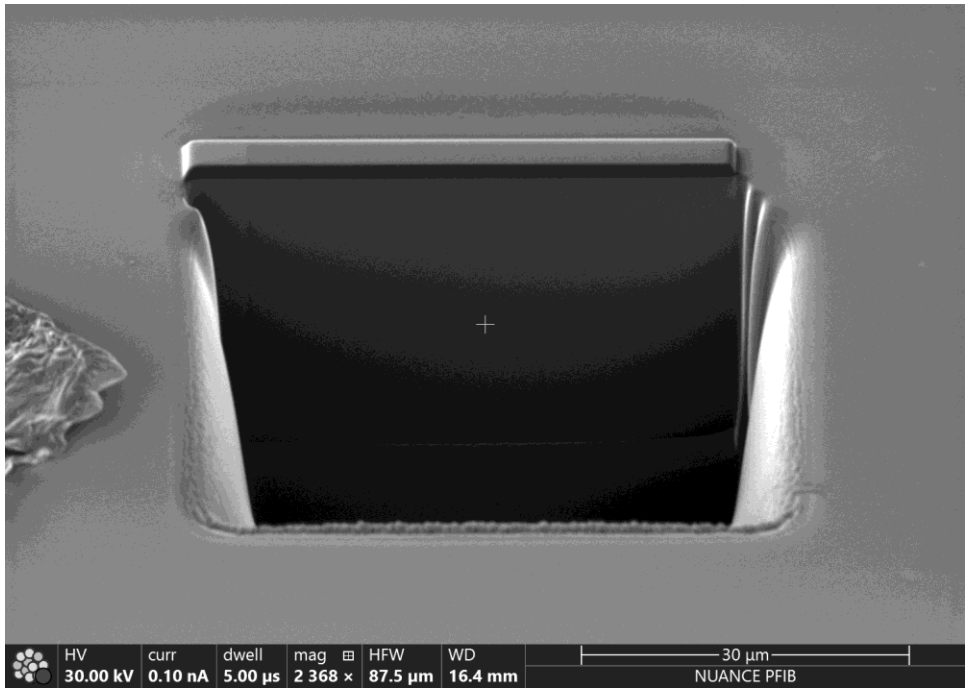
Negative grid bias to repel lower energy SEs

Imaging Detectors

1) Everhart Thornley Detector (ETD)

Ion Beam Induced (SE) Mode



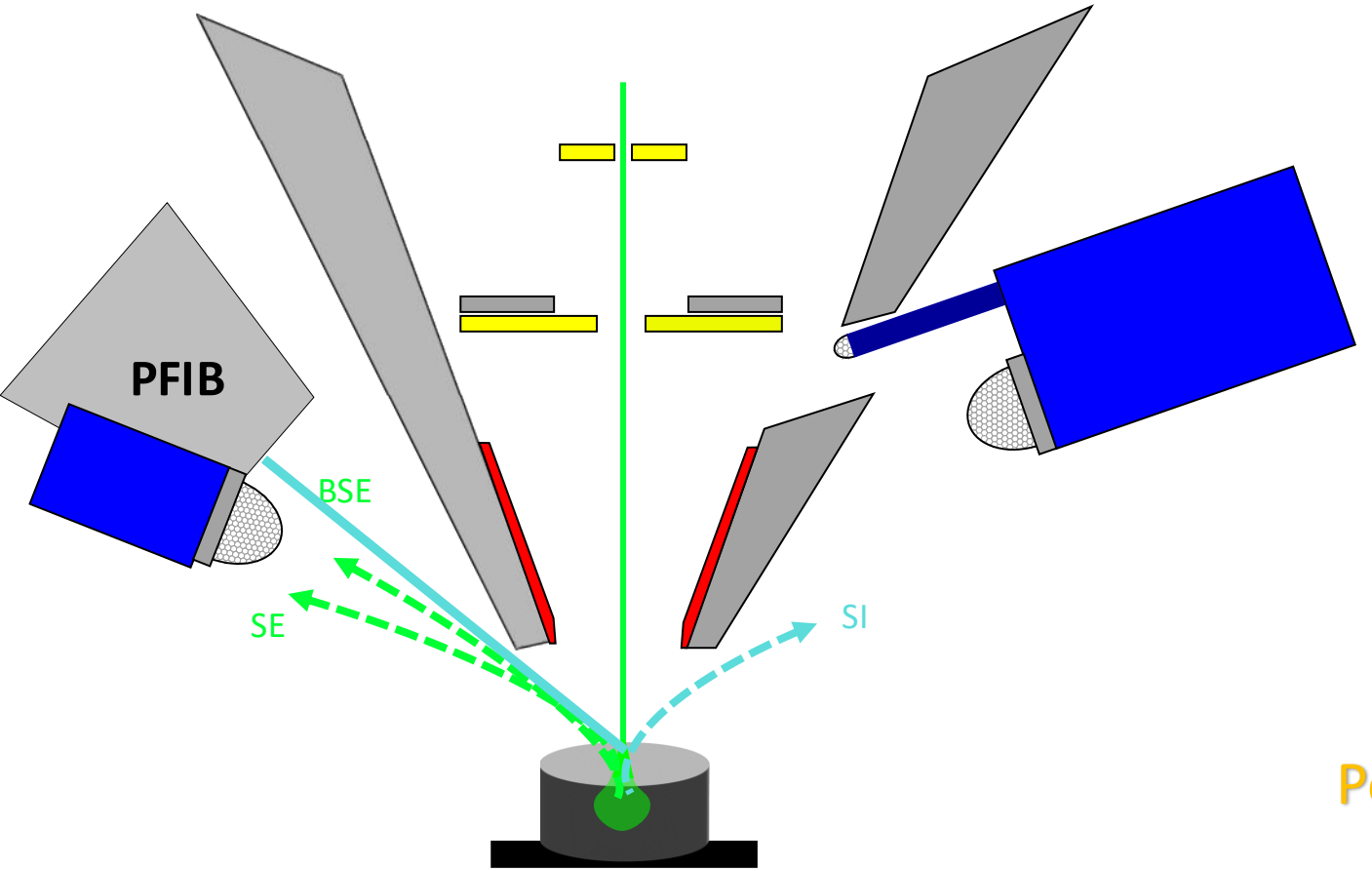


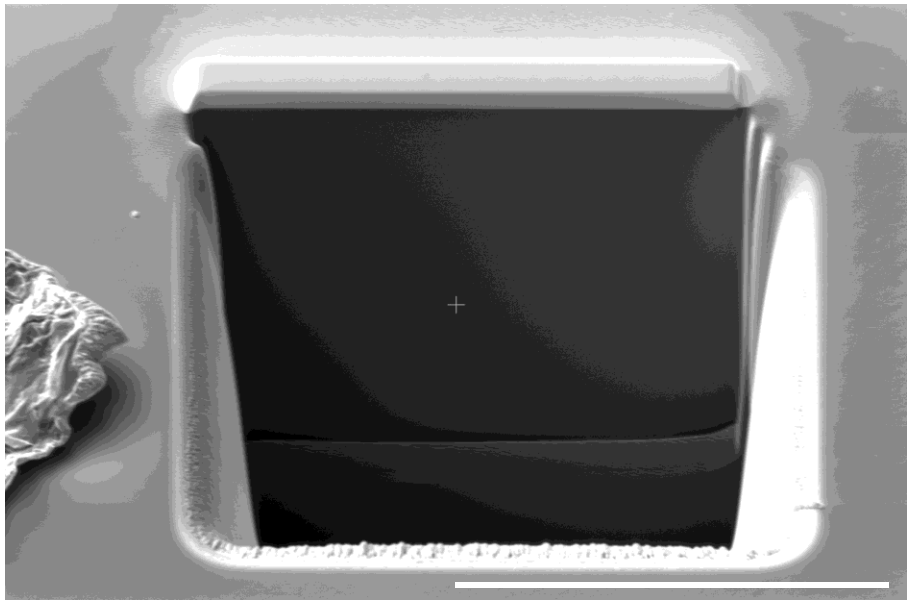
Positive grid bias to attract lower energy SEs

Imaging Detectors

2) Ion Conversion and Electron (ICE) Detector

Ion Beam Induced (SE) Mode



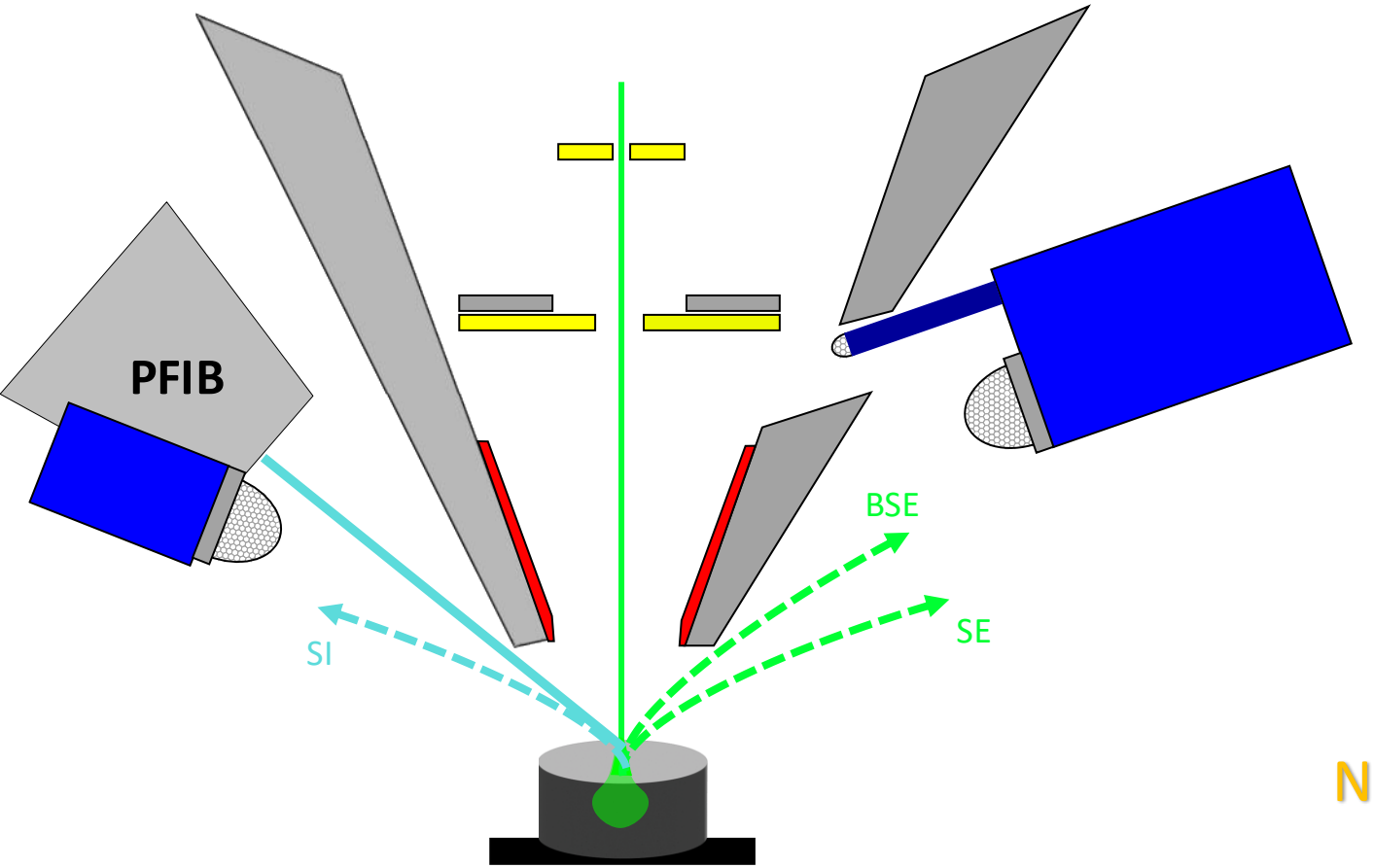


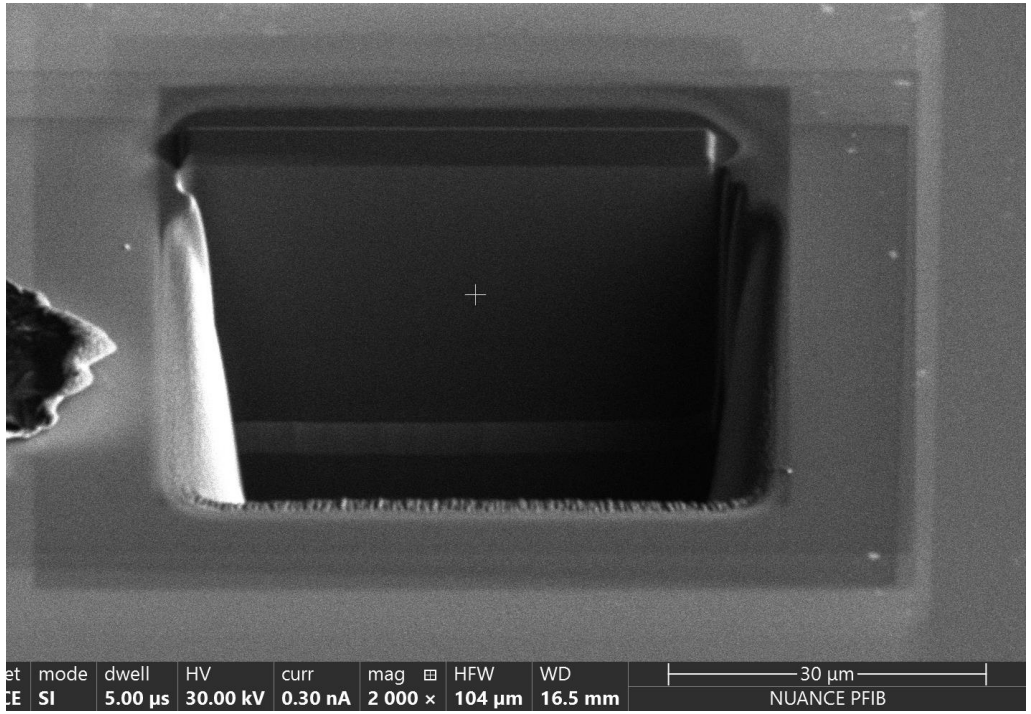
Positive grid bias to attract lower energy SEs

Imaging Detectors

2) Ion Conversion and Electron (ICE) Detector

Secondary Ion (SI) Mode

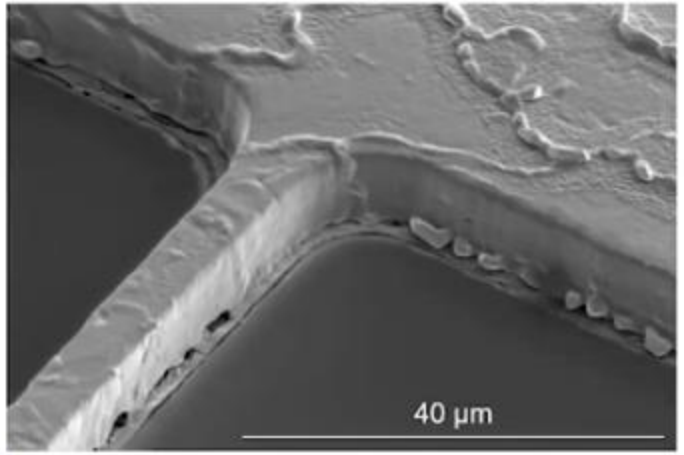
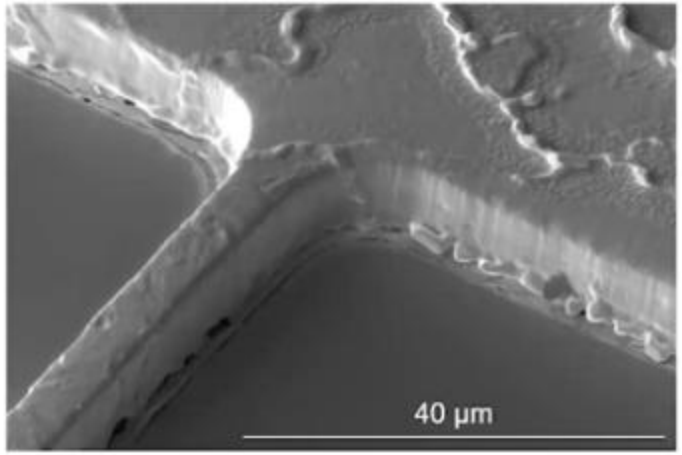
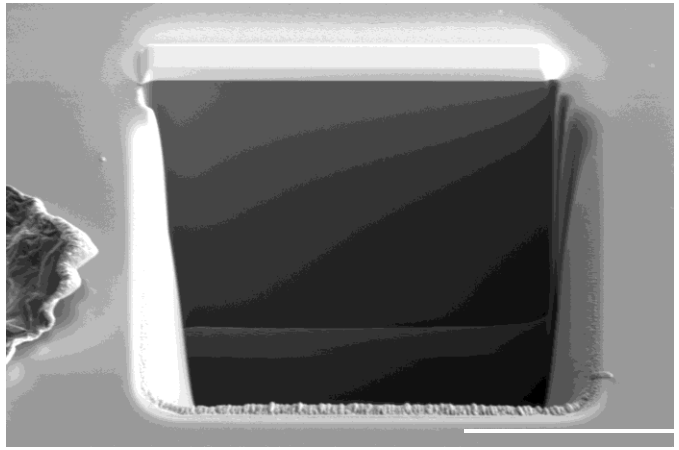
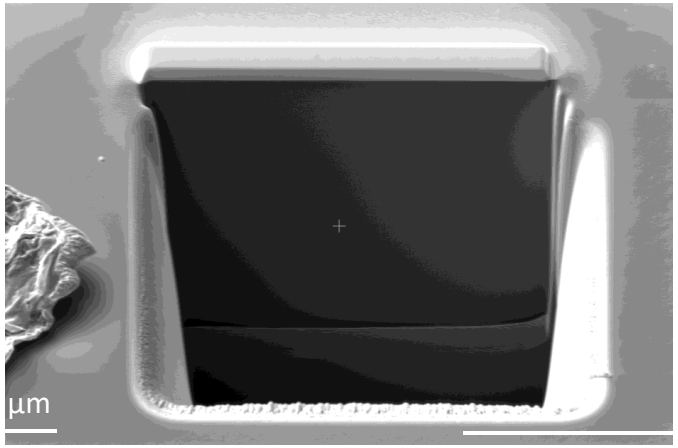




Negative grid bias to attract SIs, repel SEs

ETD vs ICE Detector

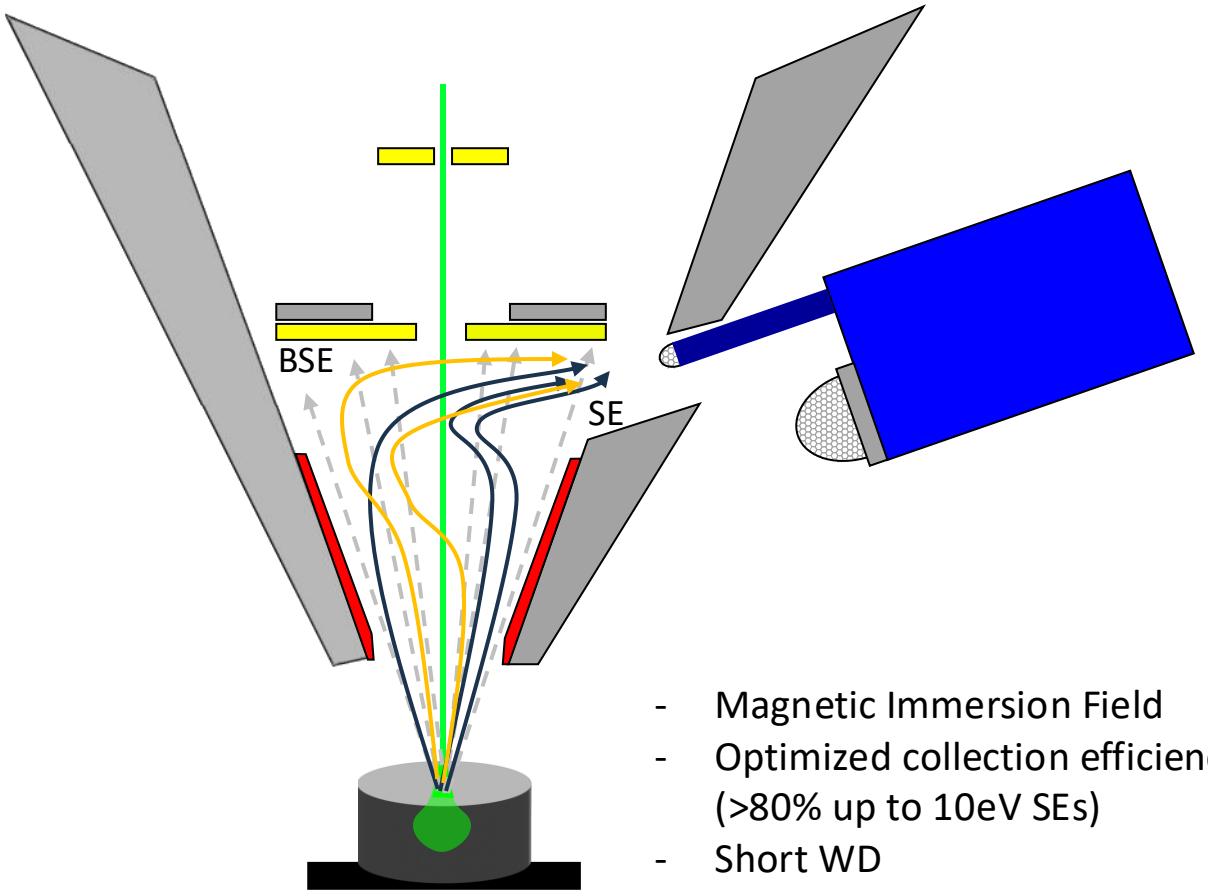
- ICE can be complementary to the ETD
- Simultaneous detector imaging

	ETD	ICE
SE mode		
		

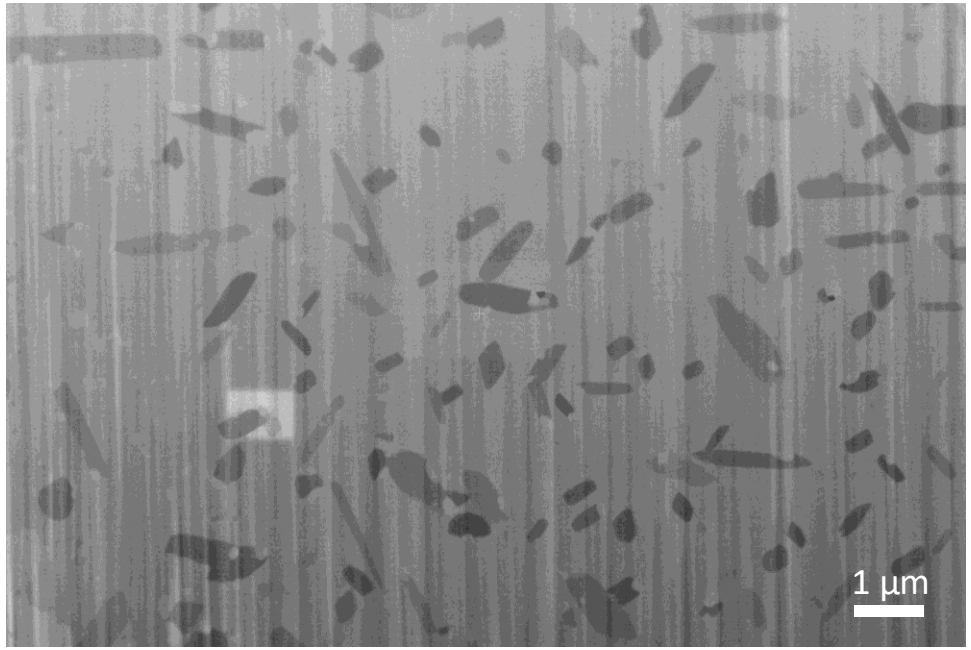
Imaging Detectors

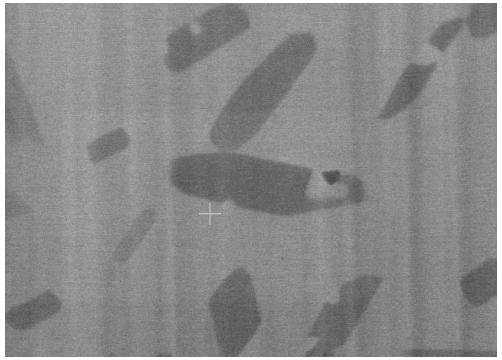
3) Through Lens Detector (TLD)

Secondary Electron (SE) Mode



- Magnetic Immersion Field
- Optimized collection efficiency (>80% up to 10eV SEs)
- Short WD



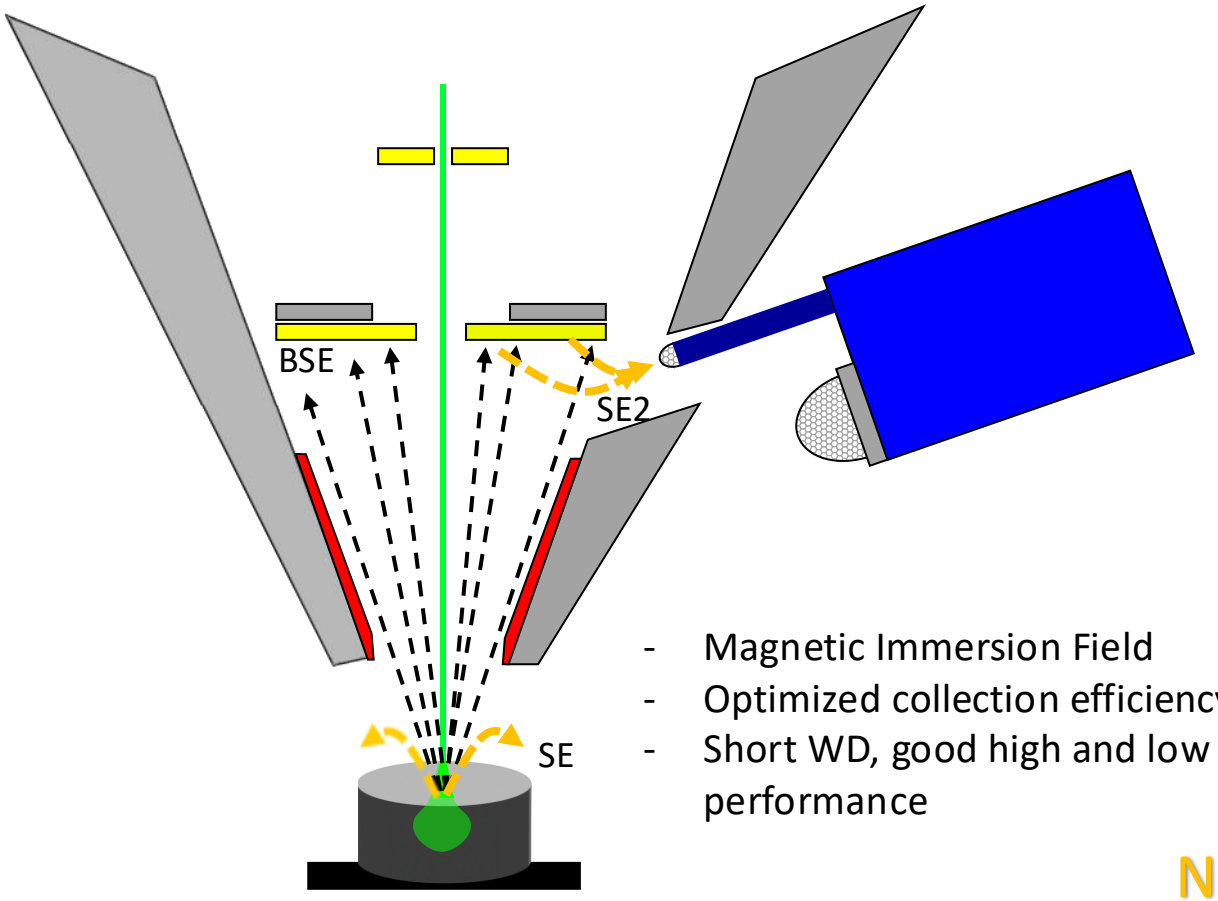


Positive suction tube bias drives SEs into TLD

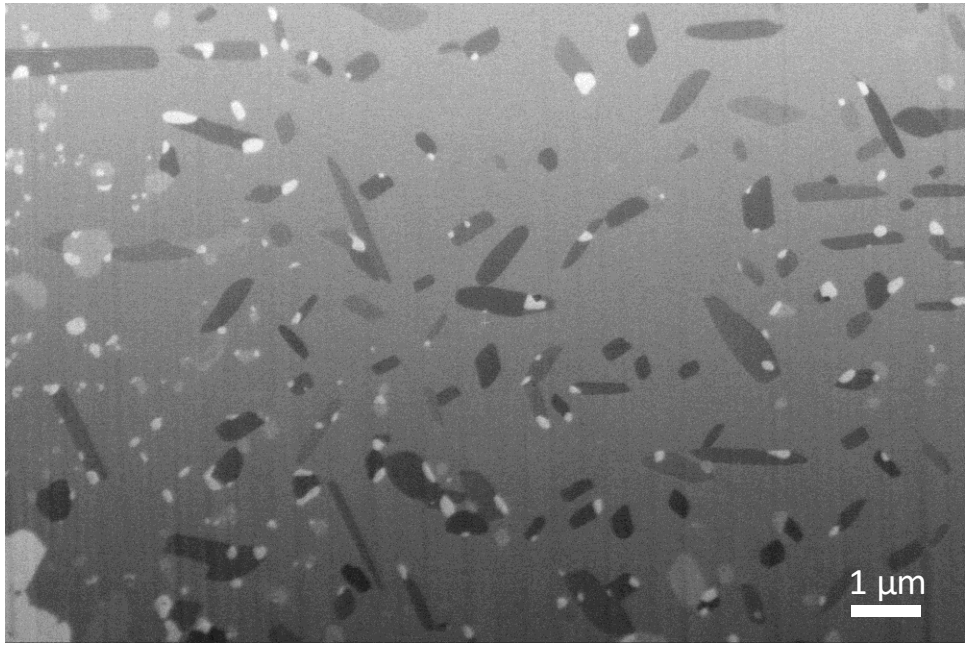
Imaging Detectors

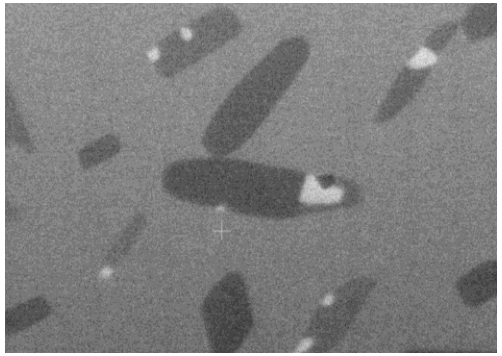
3) Through Lens Detector (TLD)

Backscatter Electron (BSE) Mode



- Magnetic Immersion Field
- Optimized collection efficiency
- Short WD, good high and low kV performance



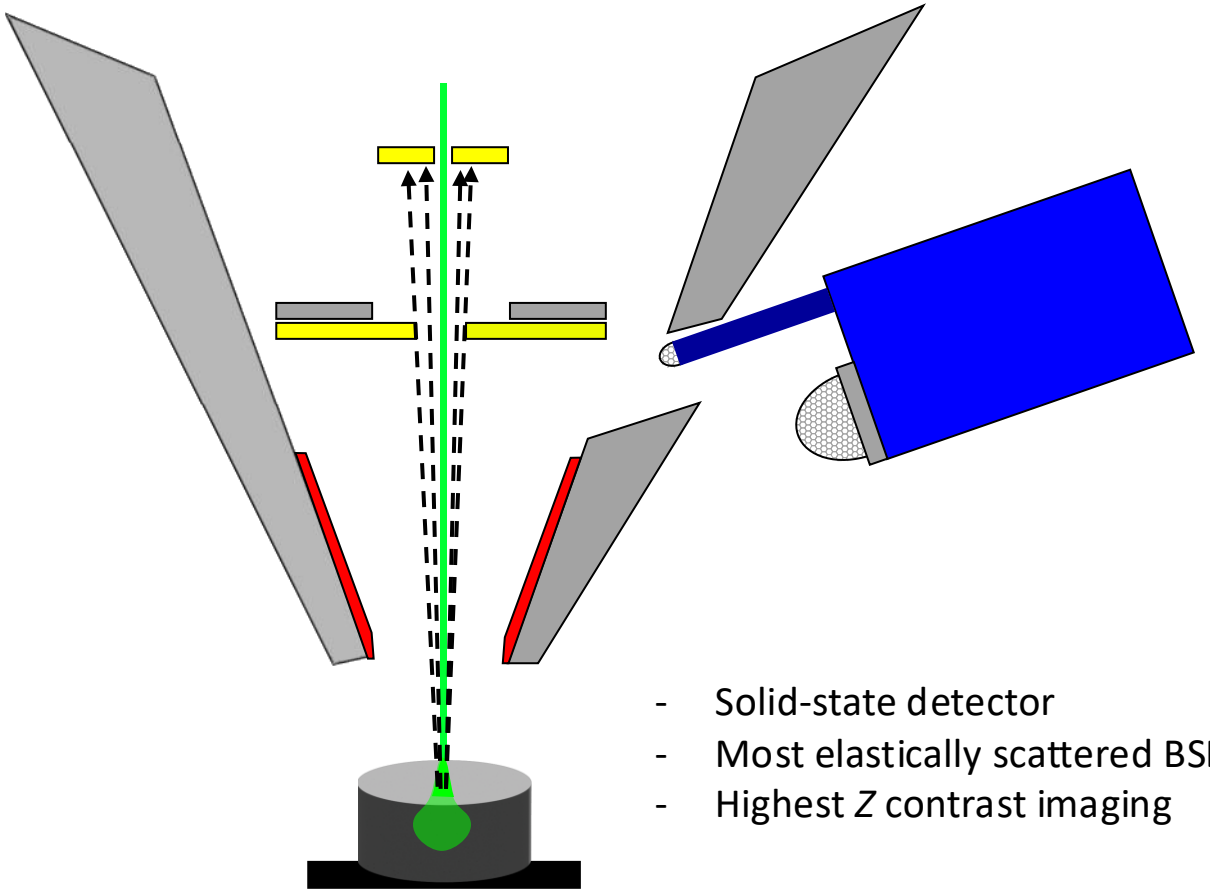


Negative suction tube bias repels SEs, SE2 into TLD

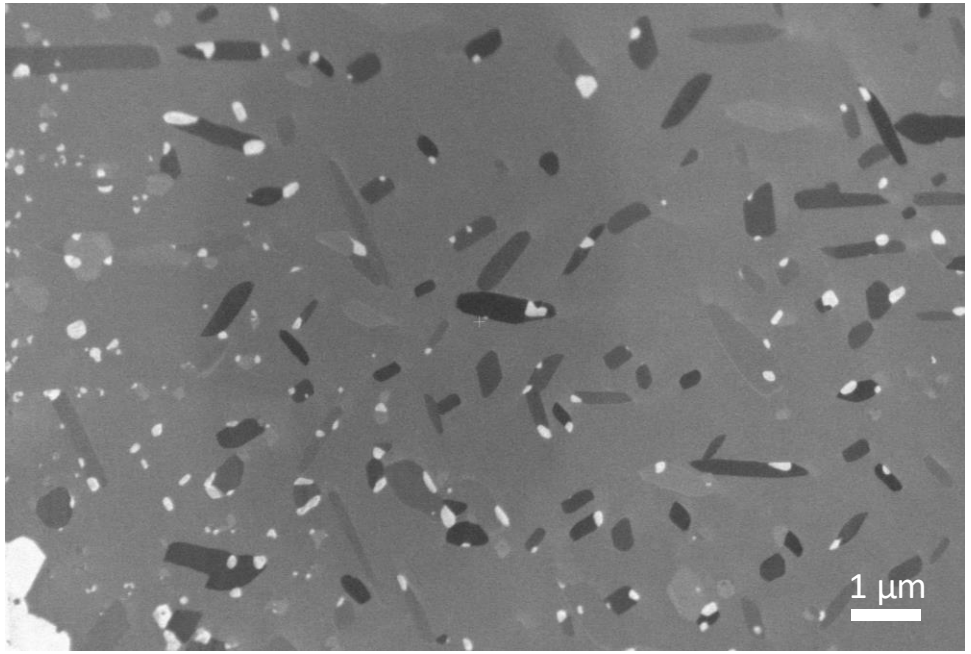
Imaging Detectors

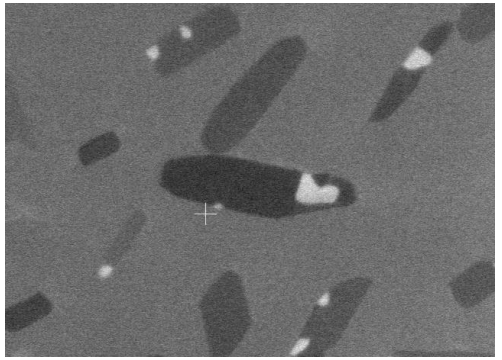
4) In-Column Detector (ICD)

Backscatter Electron (BSE) Mode

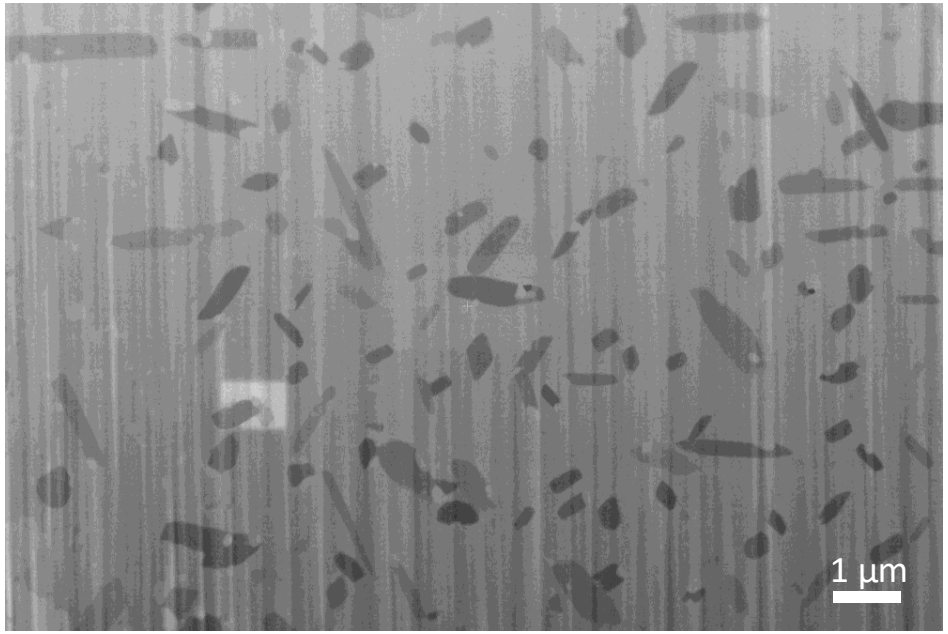


- Solid-state detector
- Most elastically scattered BSEs
- Highest *Z* contrast imaging

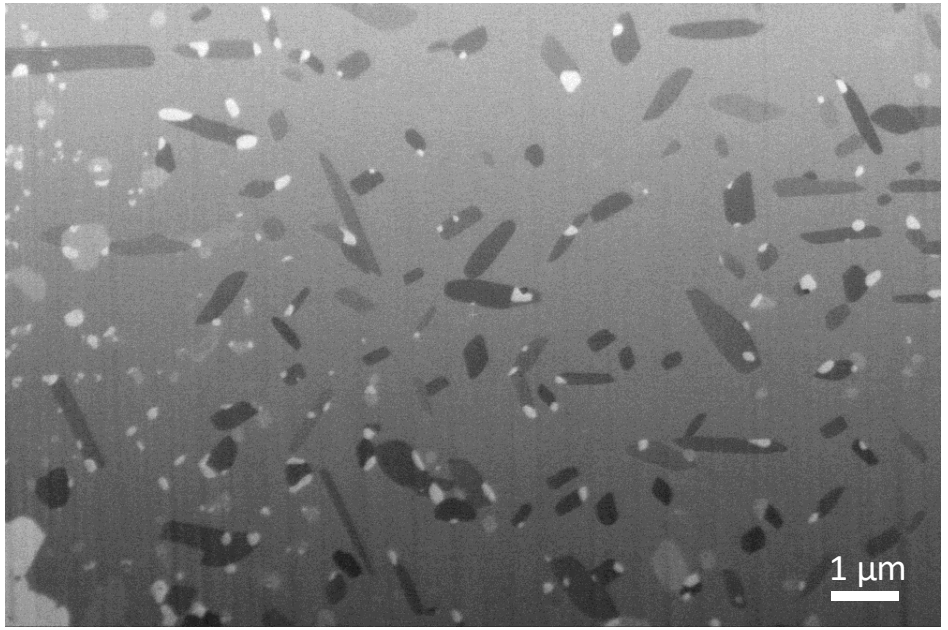




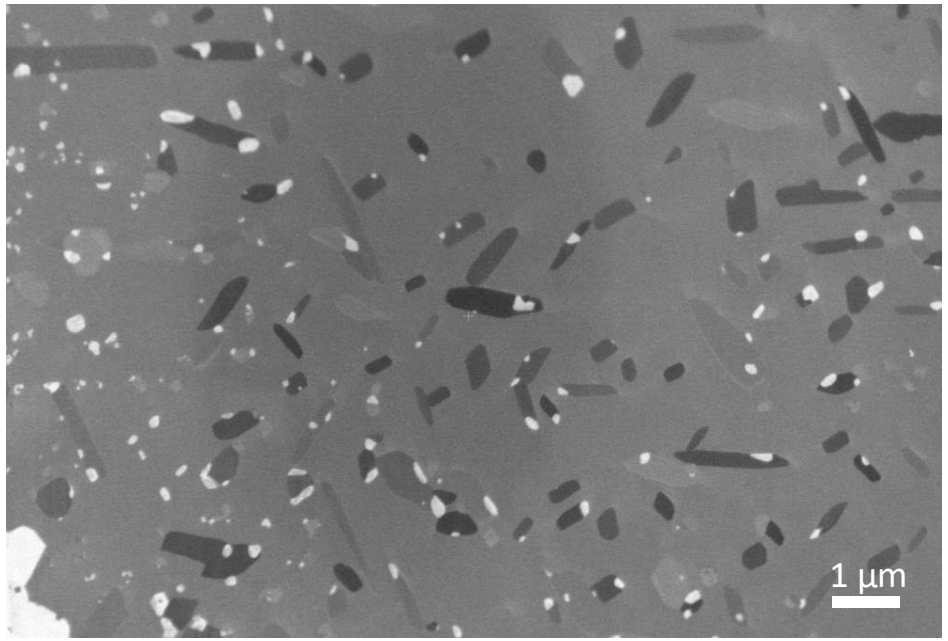
TLD vs ICD



most surface topology
No *Z* contrast imaging
Lowest SNR



least surface topology
Z contrast imaging
Lower SNR



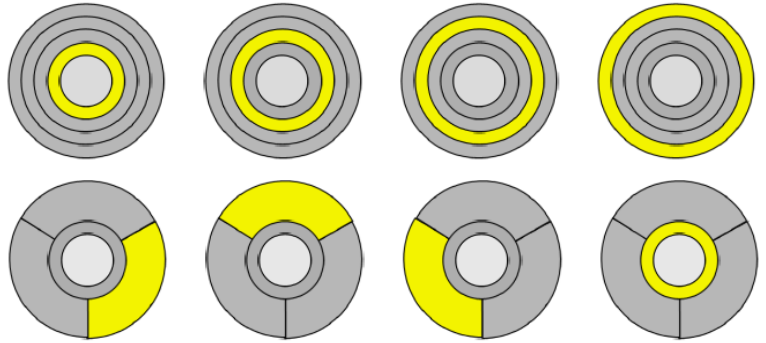
least surface topology
Z contrast imaging
Highest SNR

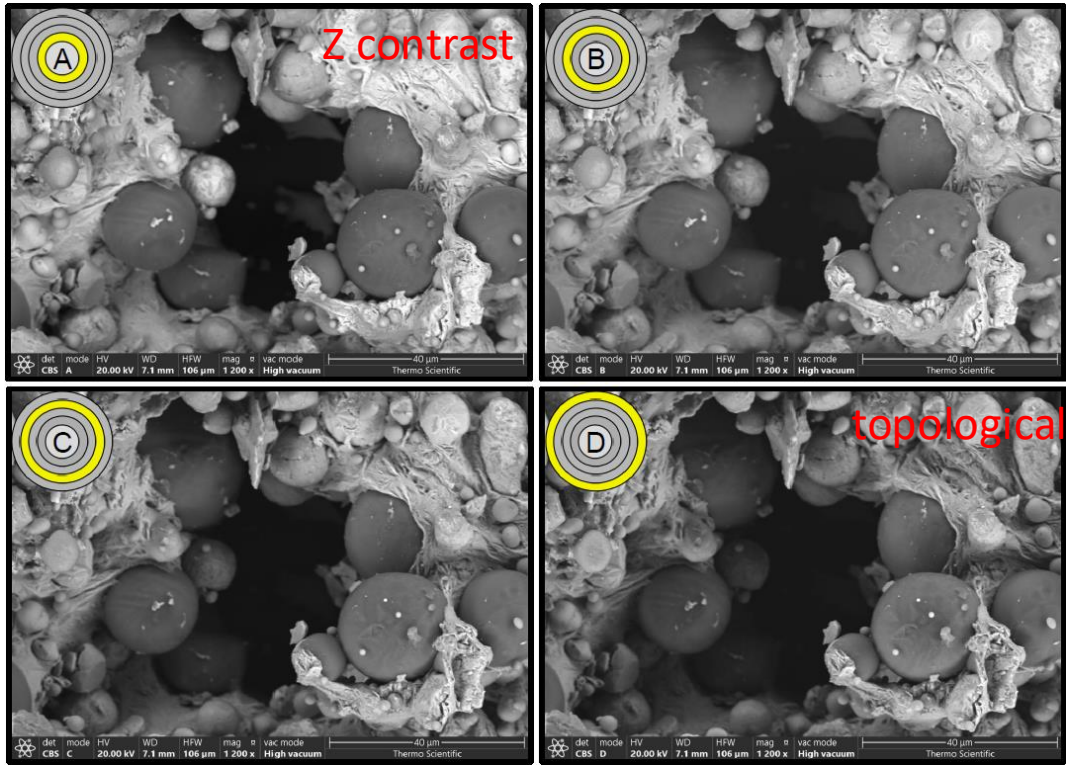
5) Retractable Directional Backscattered Detector (DBS)

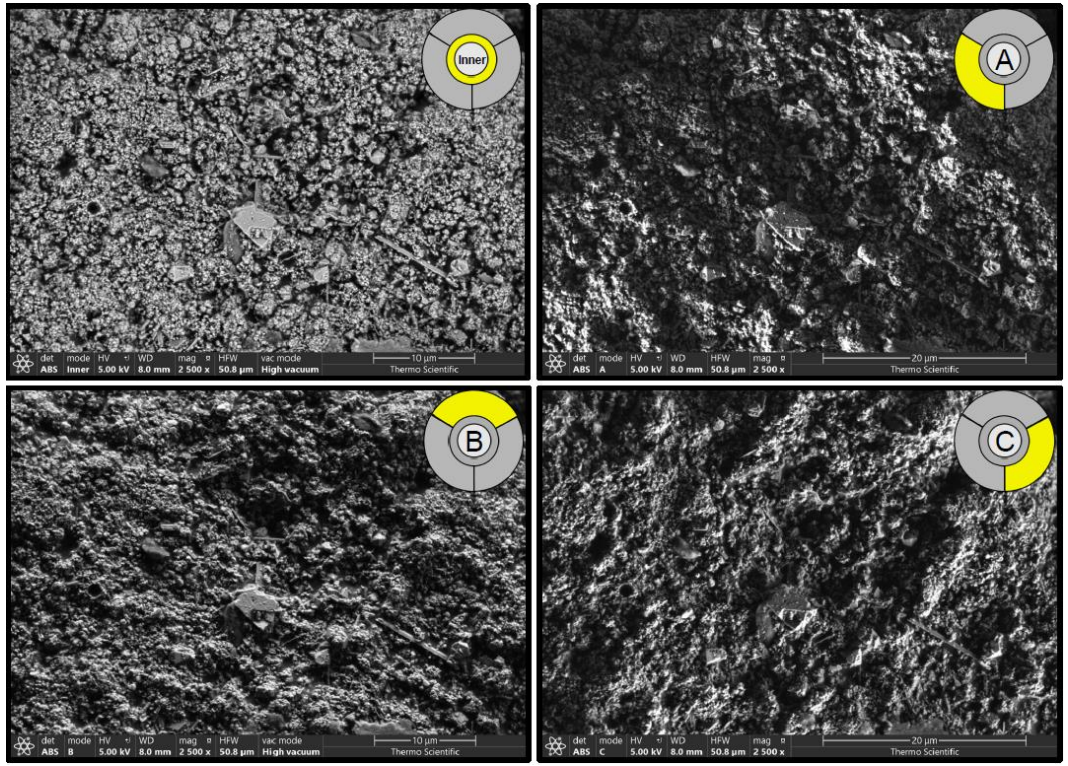
Software-based segmentation

1) Concentric Backscattered Detector (CBD)

2) Angular Backscattered (ABS) Detector

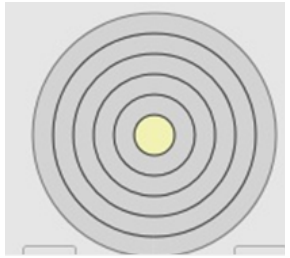




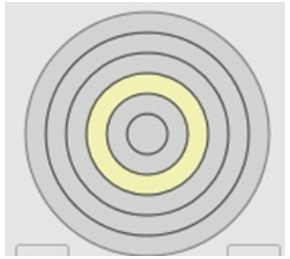


6) Retractable Scanning Transmission Electron Microscopy (STEM) Detector

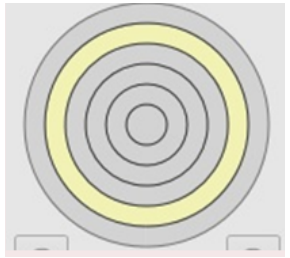
Pre-tilted row holder & STEM image



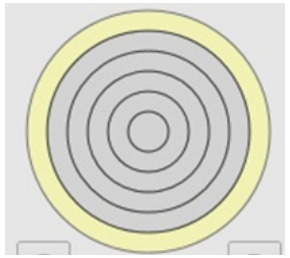
1) Bright Field (BF)



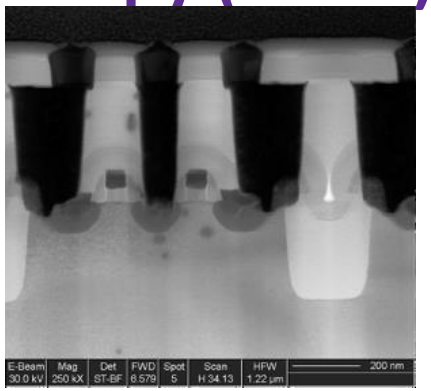
2) Dark Field 2 (DF 2)

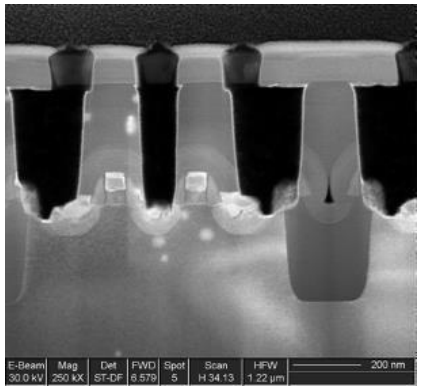


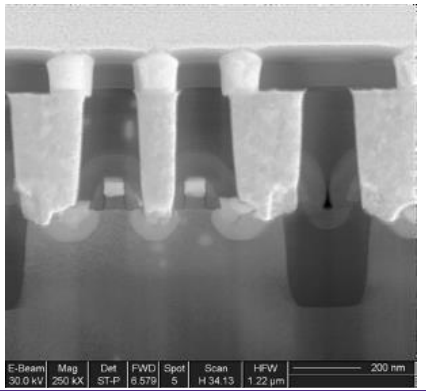
3) Dark Field 4 (DF 4)



4) High Angle Annular Dark Field (HAADF)





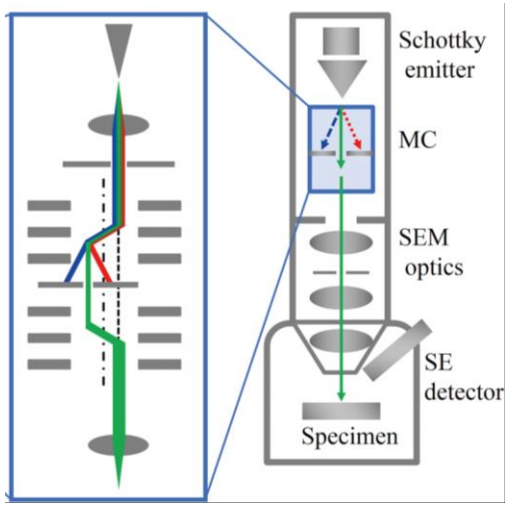


SEM Column Specs – Ultrahigh Resolution Imaging

1) UC (monochromated) mode

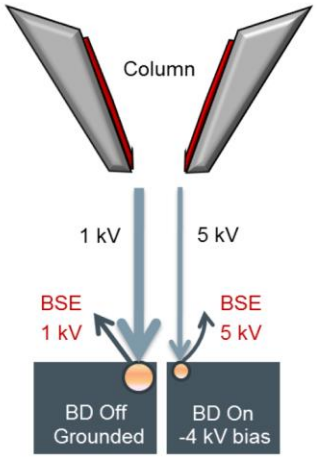
< 5 kV; < 100 pA

Energy spread
< 0.2 eV

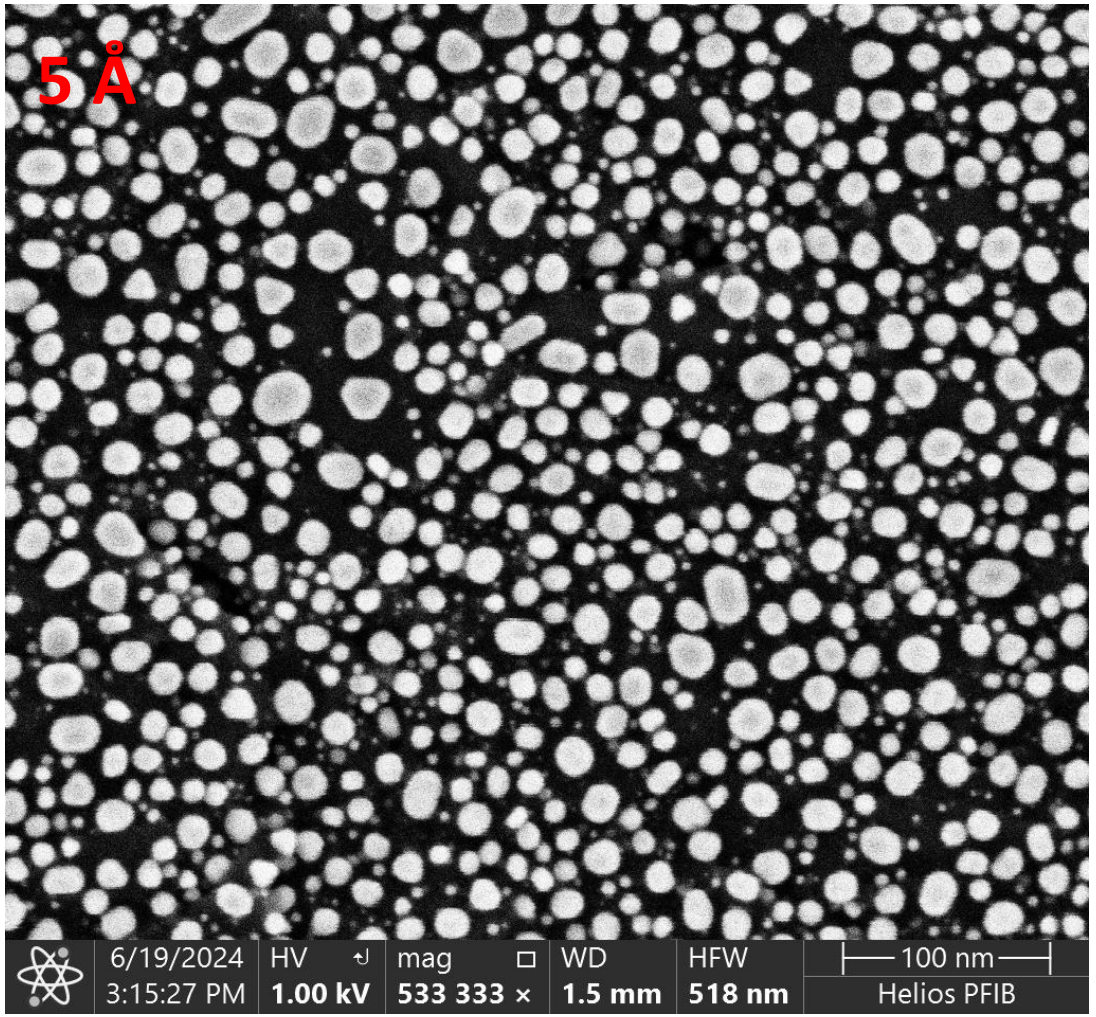


2) Beam Deceleration

Down to 50 eV

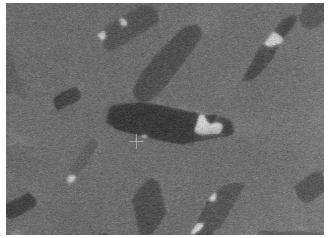


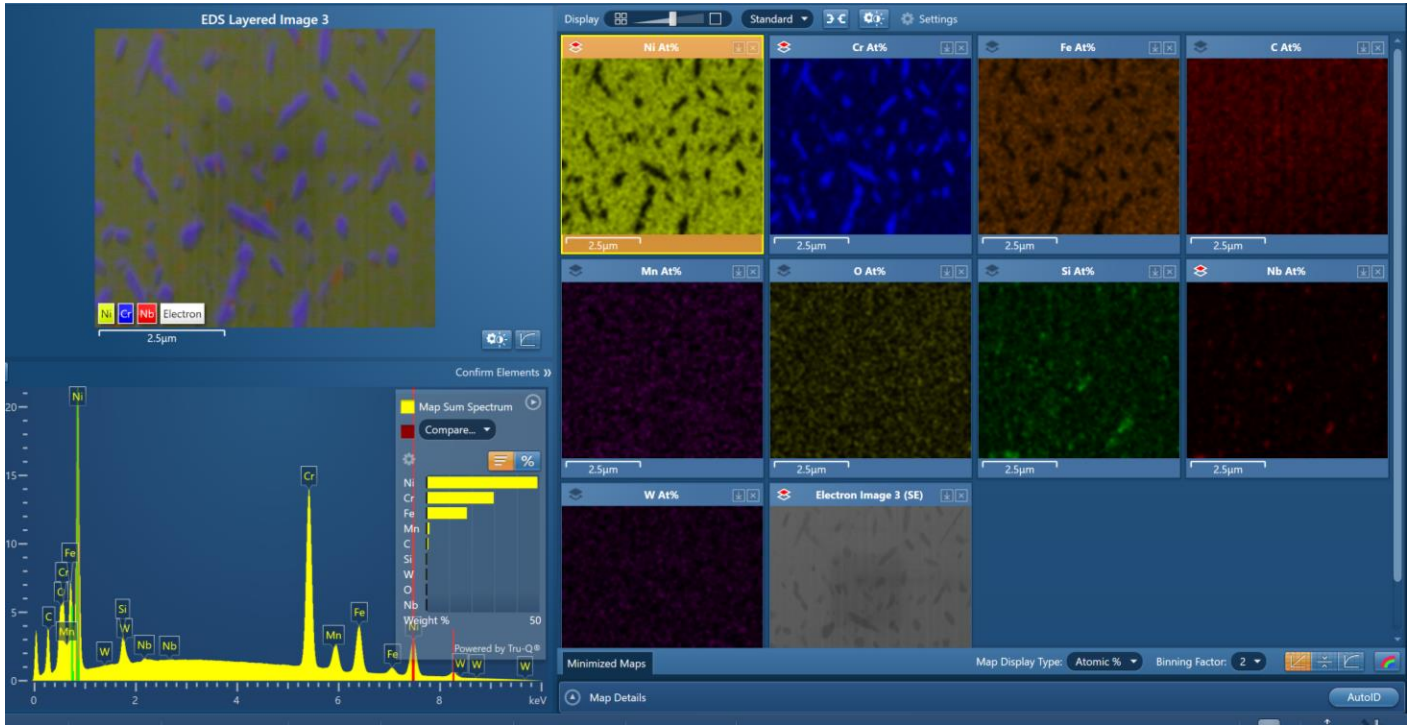
3) Immersion mode/TLD detector: Au on C



Analytical Detectors

Oxford Instruments Ultim Max 100 mm² EDS detector

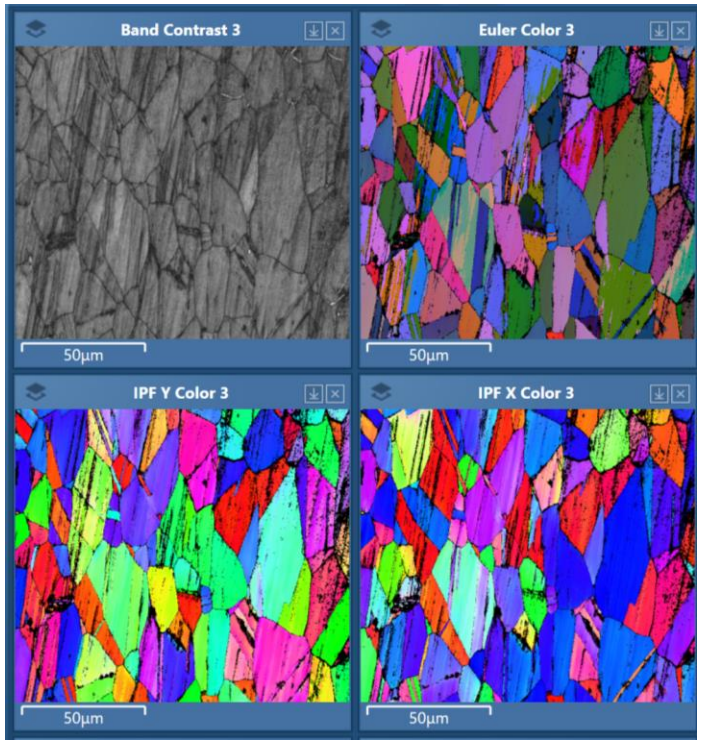




Oxford Symmetry S3 EBSD detector

High speed data acquisition:
~5800pps @ 156 × 128 px EBSP

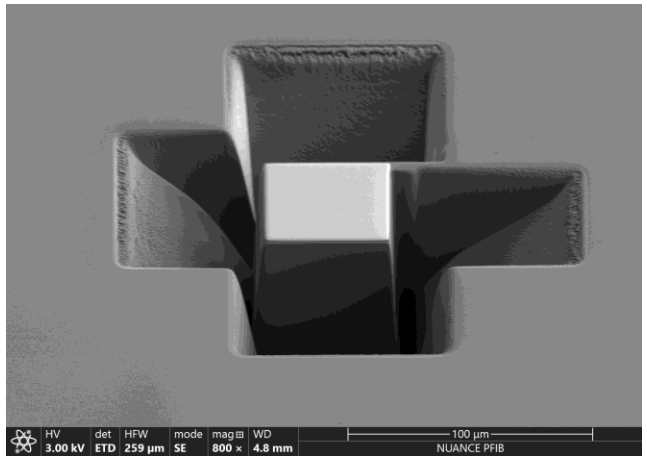
85% ESBP

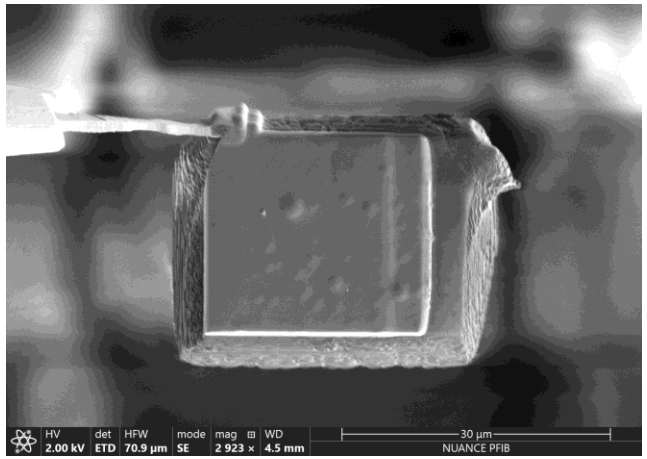


Micromanipulator (“Easylift”) with Rotation

- **Resharpen** micromanipulator

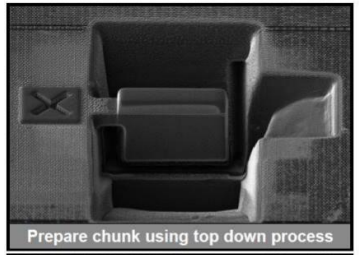
- **Liftout of large volumes**; rotation adjustment for attachment to support

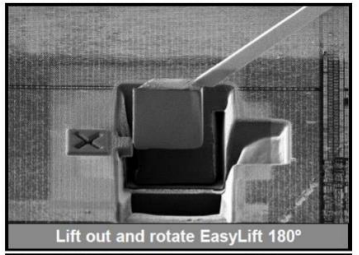


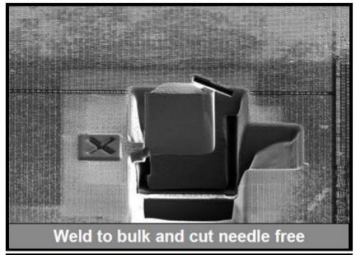


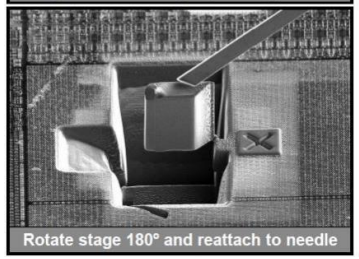
- **Flexible TEM sample prep**

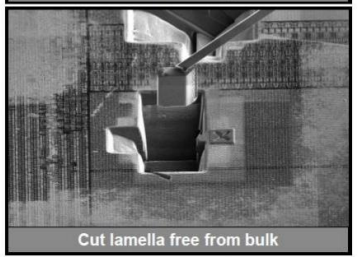
easier access to plan view sample preparation
and inverted lamella

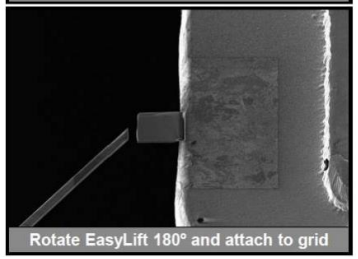






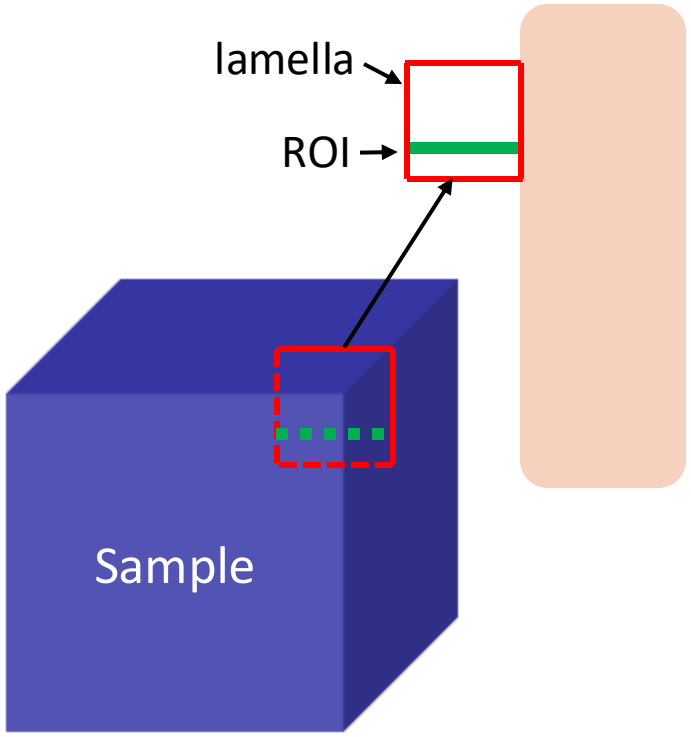






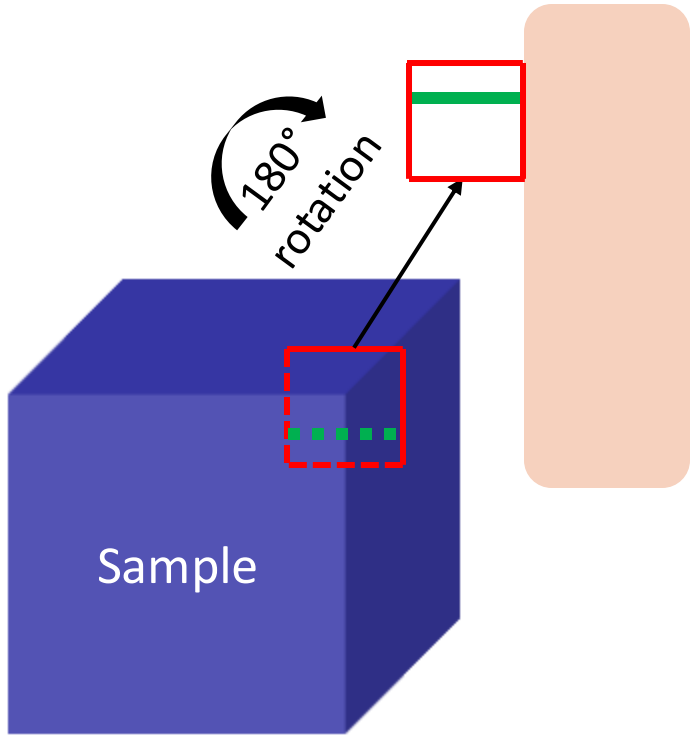
TEM Sample Prep Configurations

Conventional (Top Down)



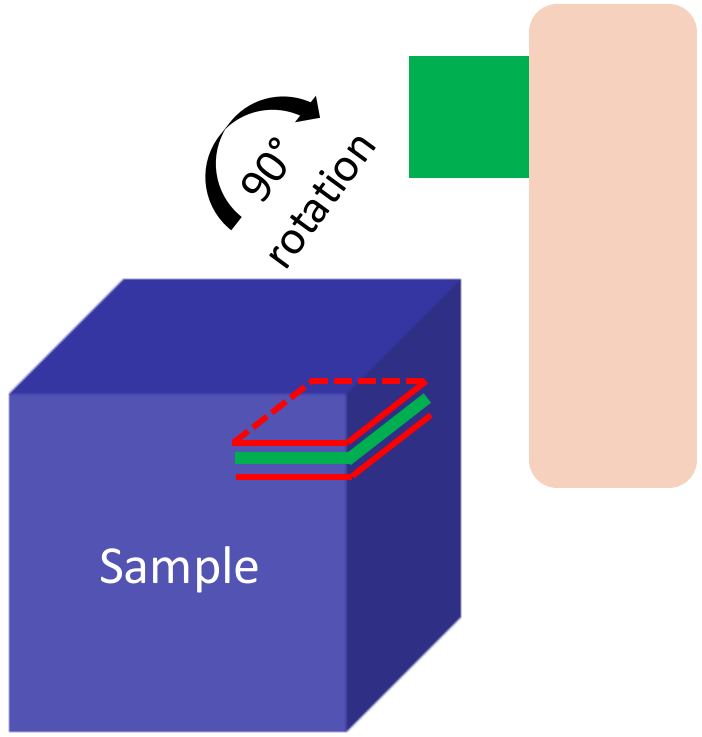
Direct transfer of lamella from bulk to grid

Inverted



Lamella flipped 'upside down' from bulk to grid

Plan View



Lamella is rotated 90˚ where ROI is paralell to bulk surface

Available Thermo Fisher Software

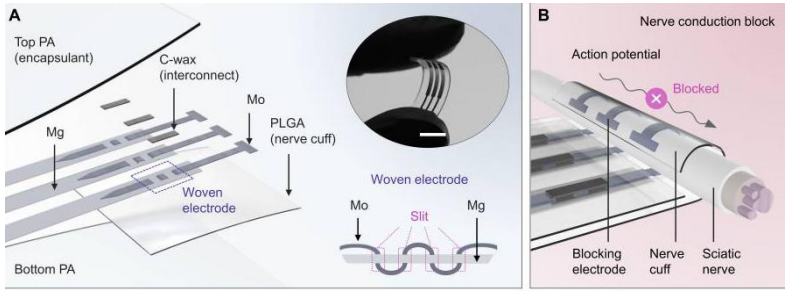
- **AutoTEM 5** – automated, high productivity creation of high quality (S)TEM sample preparation

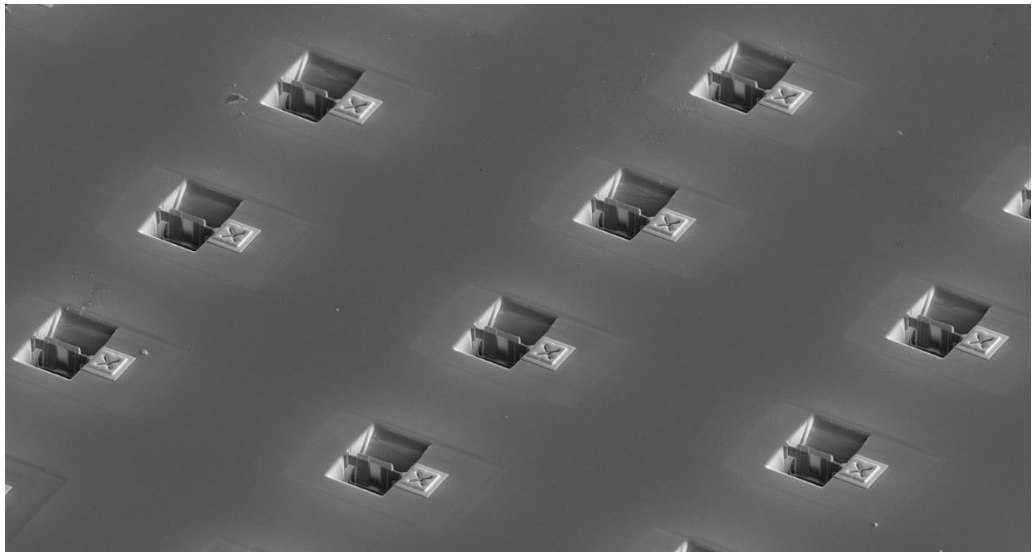
- **Auto Slice & View 5** - Automated FIB serial sectioning software with 3D EDS and 3D EBSD capability

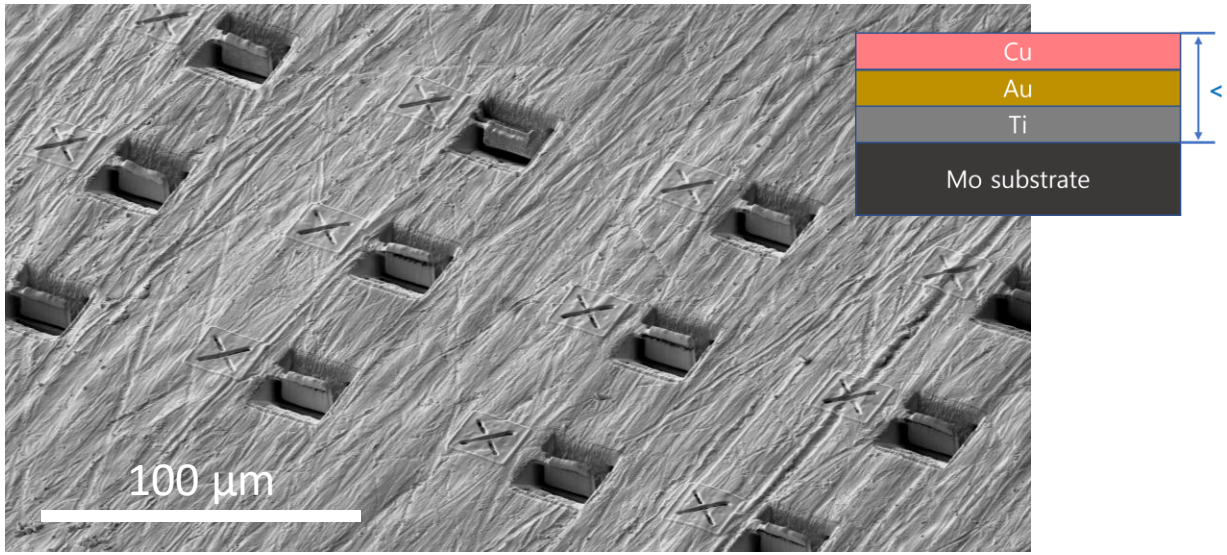
- **MAPS 3** - Correlative electron microscopy and cross-platform imaging automation software

- **Avizo 3D** - Software for microscopy image data visualization and analysis

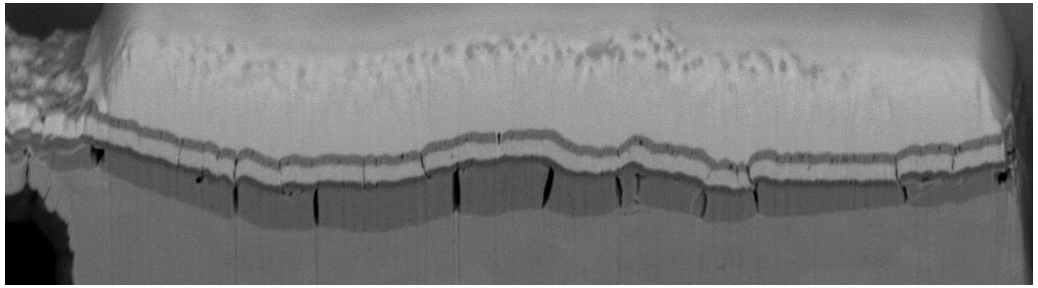
AutoTEM 5



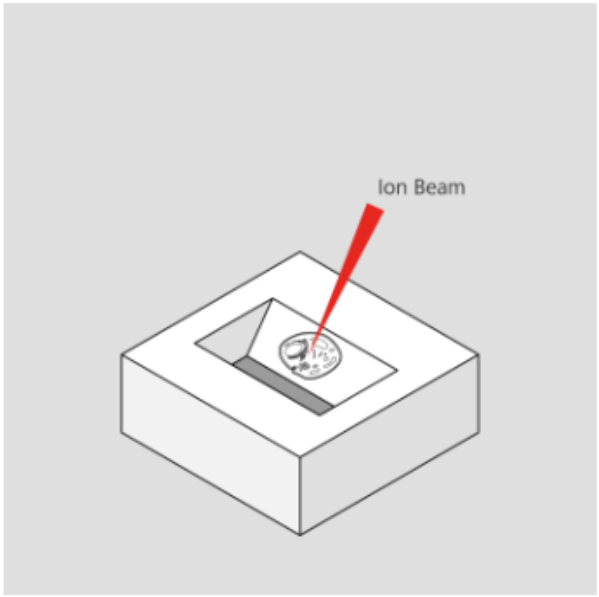


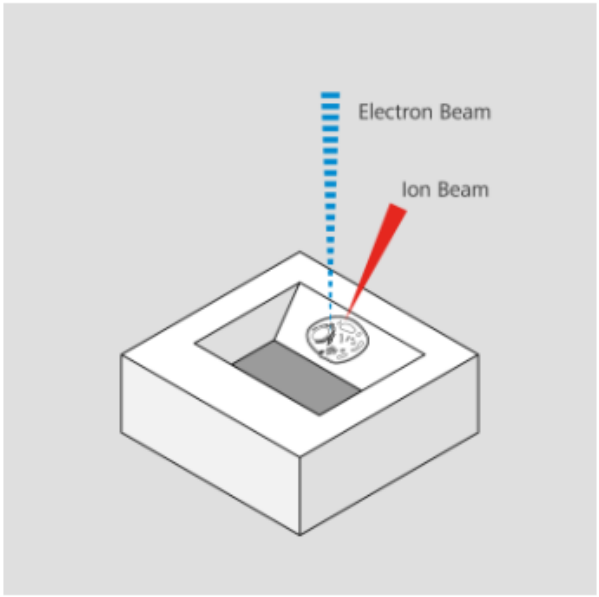


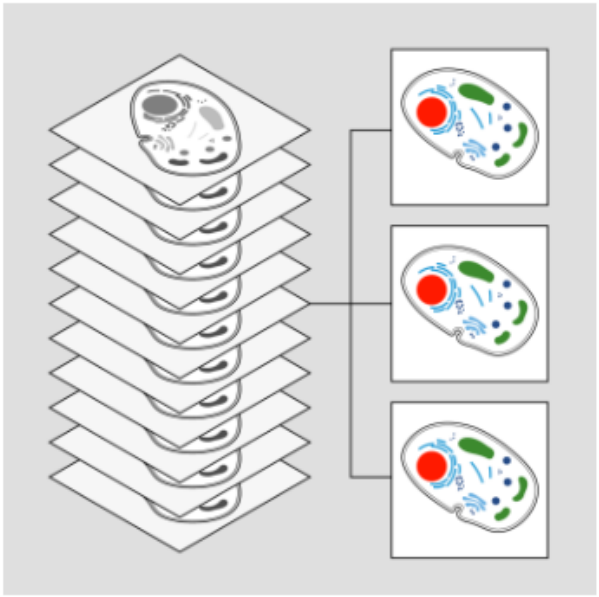
12 TEM lamella in 7 hrs



Auto Slice & View 5







1

A trench is milled into a resin-embedded sample with a focused ion beam until the structure of interest becomes visible.

2

The newly exposed sample surface of the structure of interest is imaged. This milling and imaging process is repeated until the structure is completely imaged.

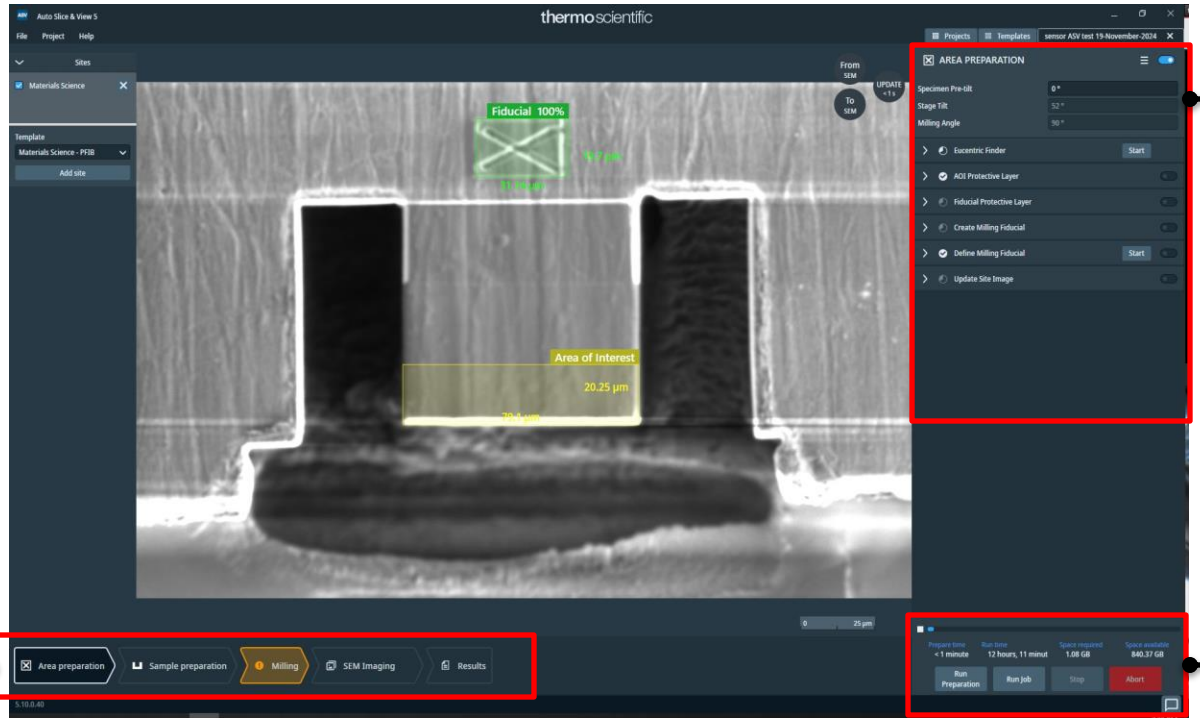
3

The acquired EM images are processed and digitally aligned into a 3D data set.
Cell compartments can be identified and segmented.

4

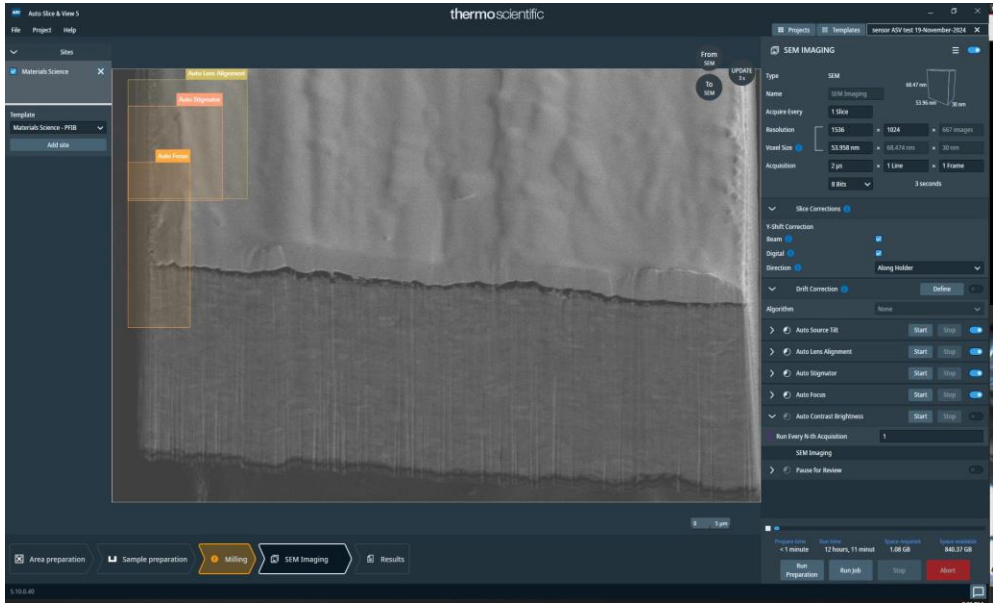
The segmented 3D data set can be visualized, investigated, and statistically analyzed.

Auto Slice & View 5



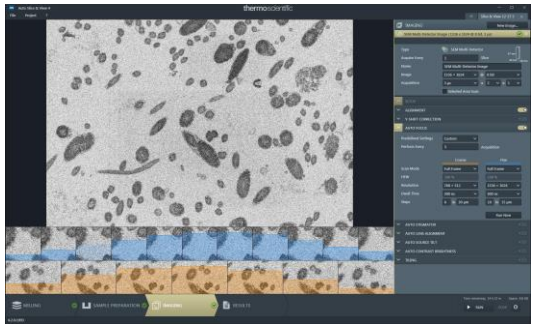
Parameters for each step

Progress

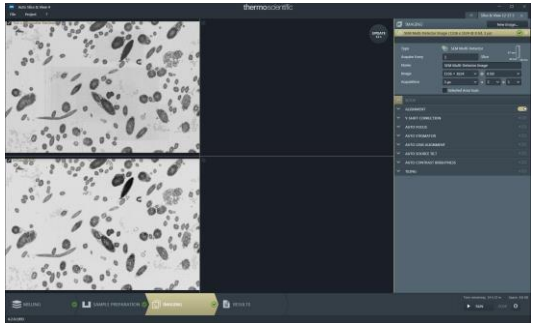


Workflow

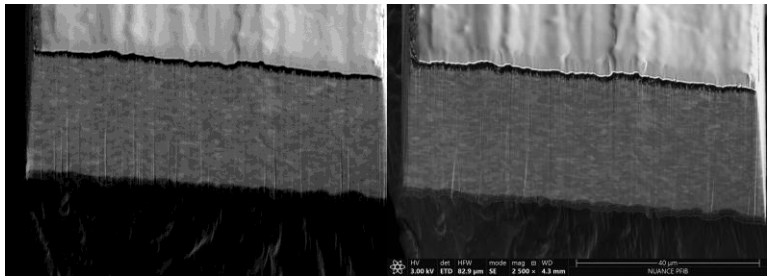
- Autofocus and autostigmation strategies



- Simultaneous Multiple detector imaging



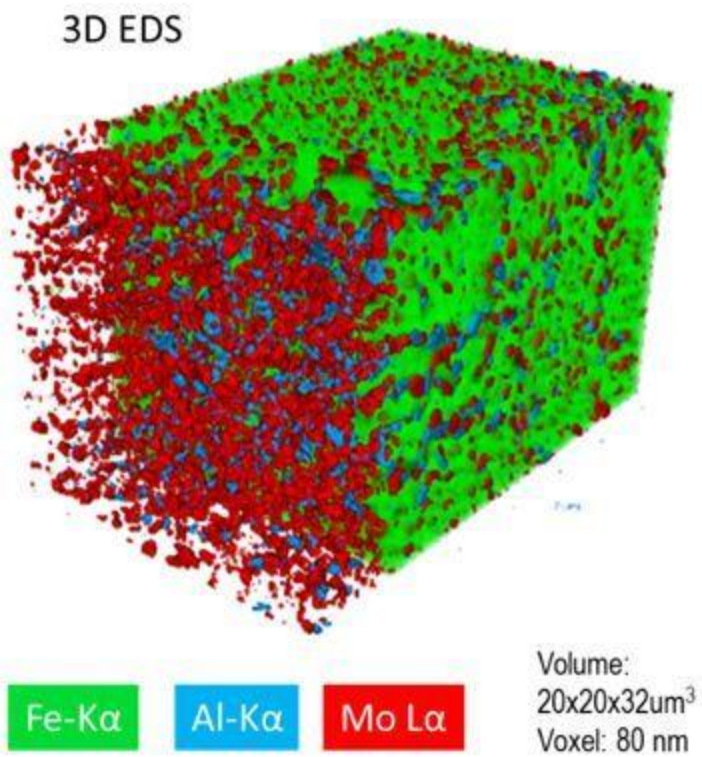
- Rocking step for decreasing curtaining

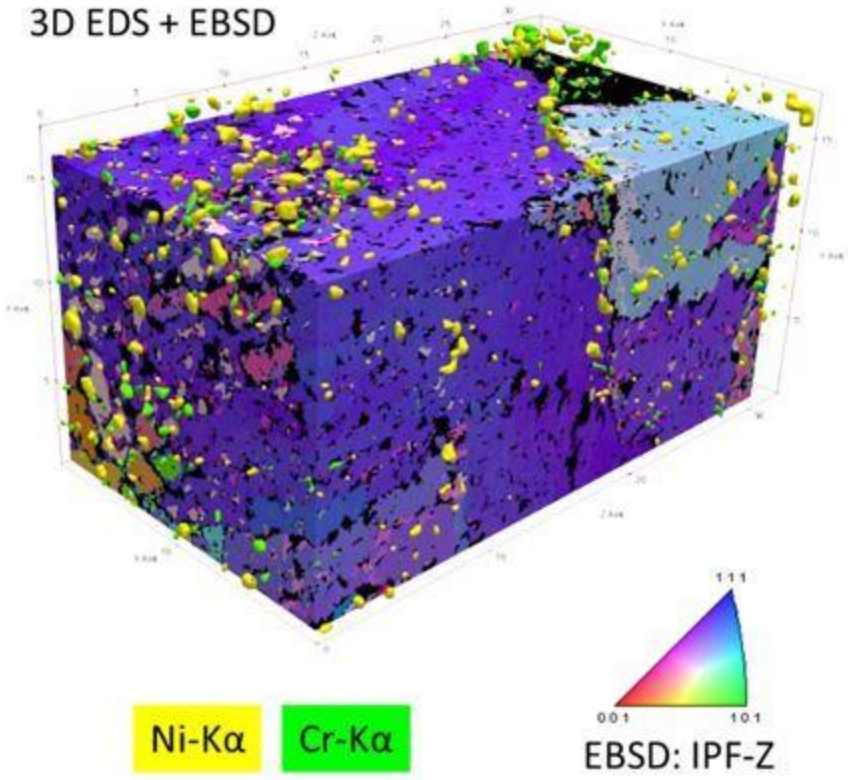


… and much more!

Auto Slice & View 5; 3D EDS & EBSD

Multimodal analysis in three dimensions

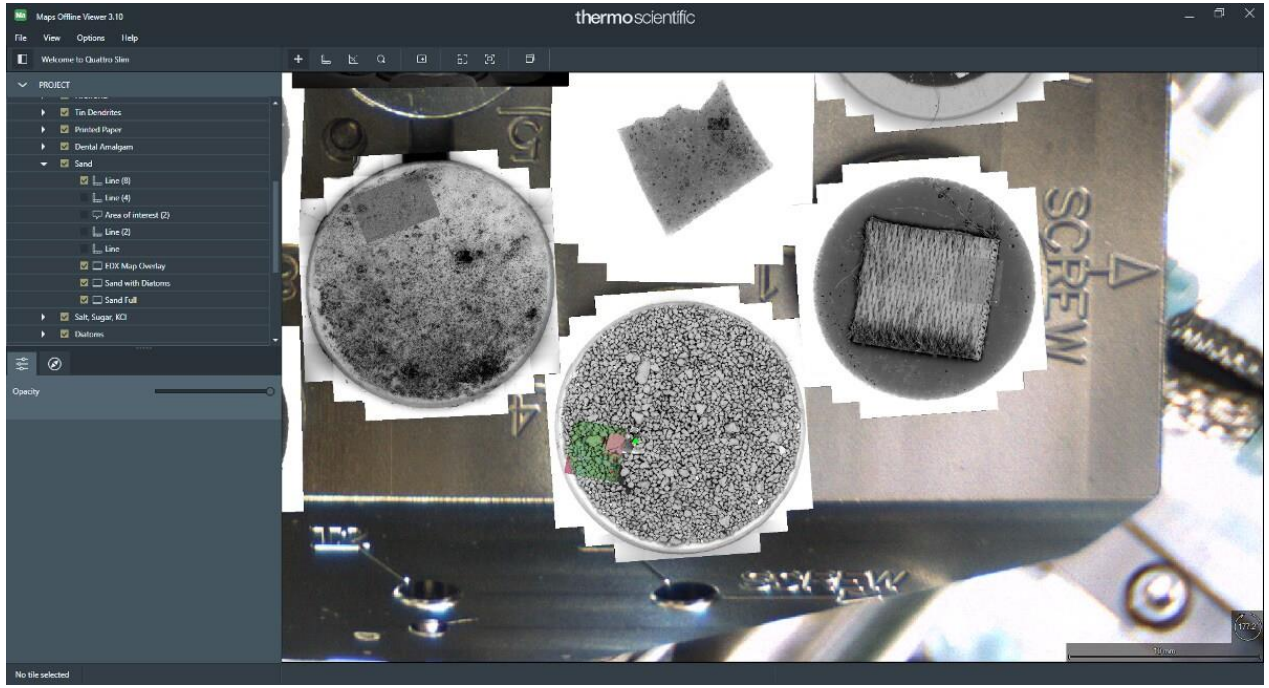


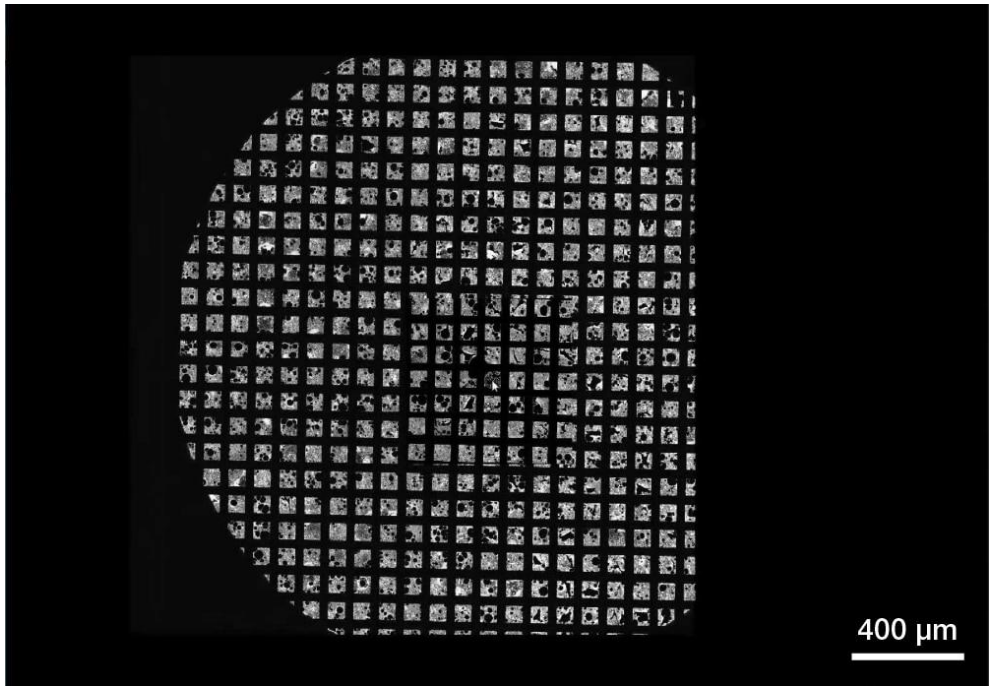


Sample from Ecole Polytechnique in Montreal

MAPS 3

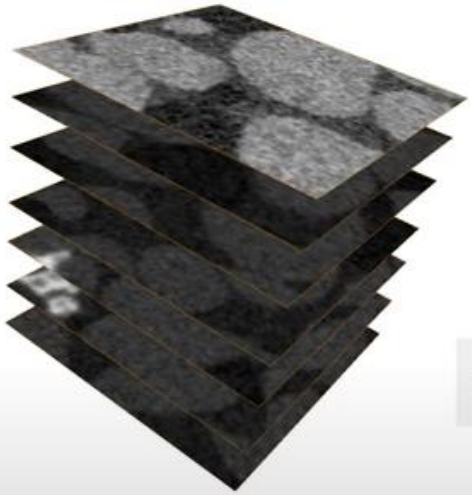
- Correlative (optical) imaging and FIB-SEM image stitching





Avizo 3D

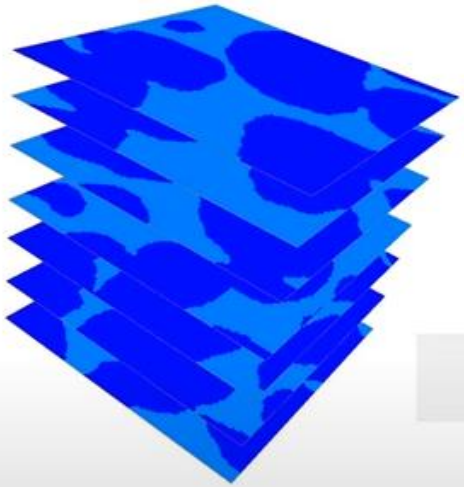
image stack



e.g., 16-bit images
(tiff, png, jpeg)

segmentation

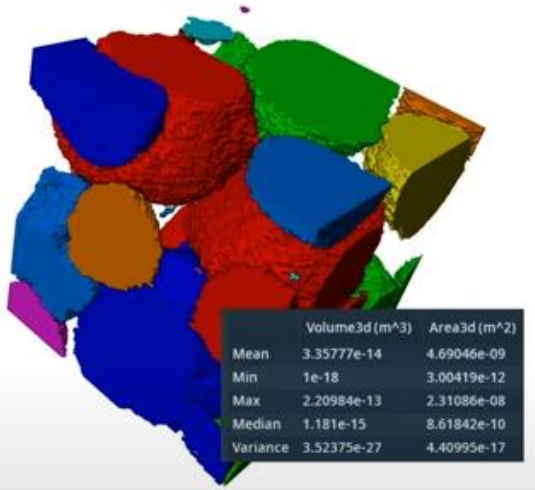
label images



e.g., 8-bit images
(tiff, png)

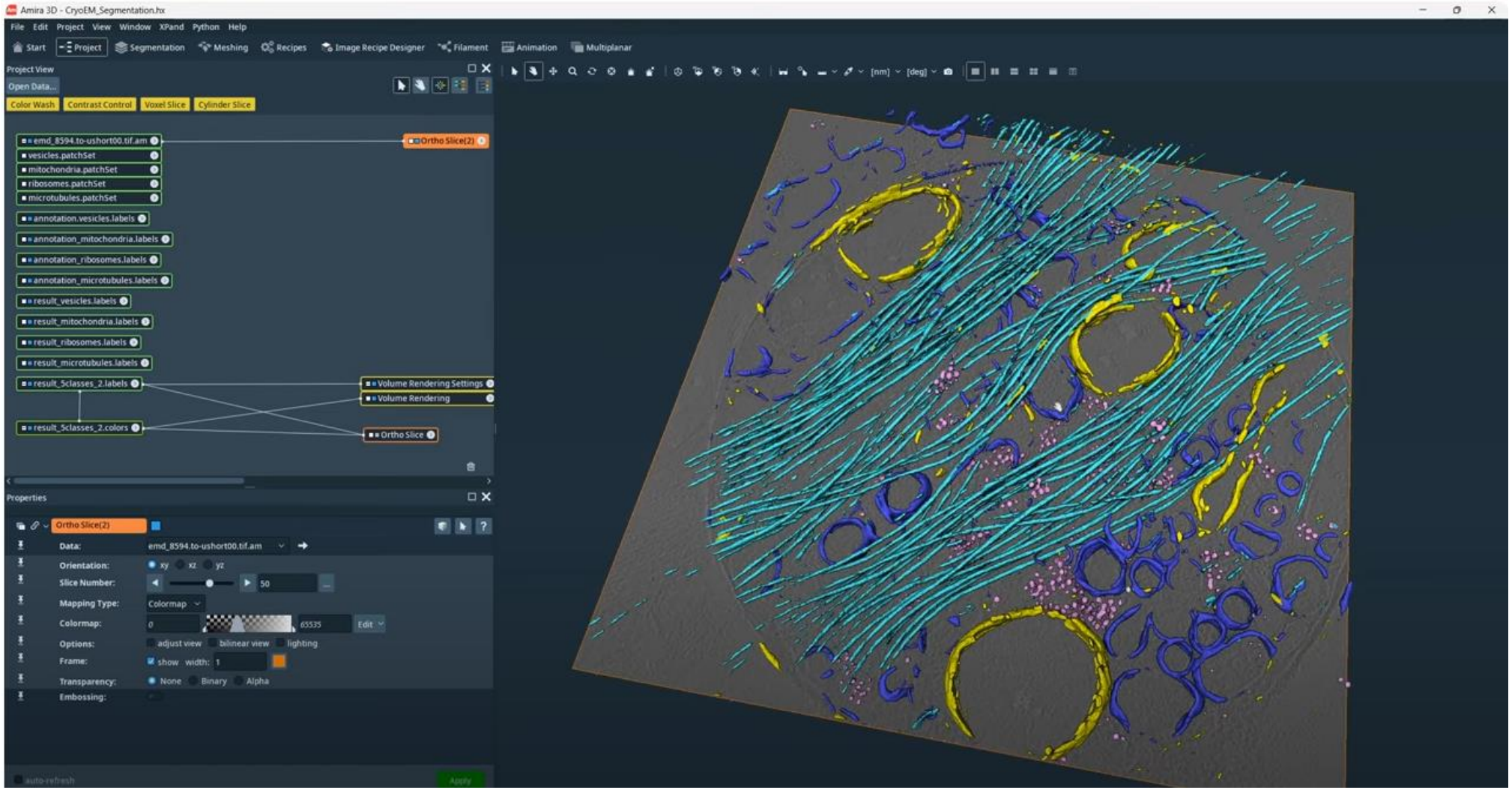
analysis

analysis results

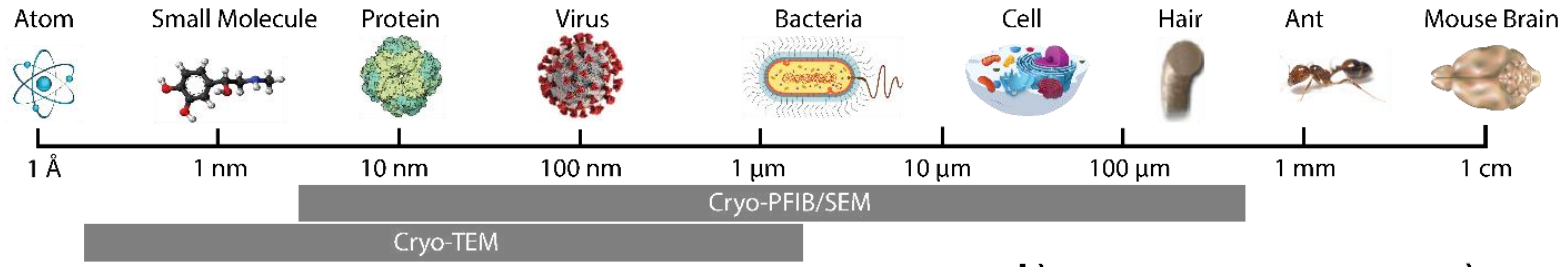


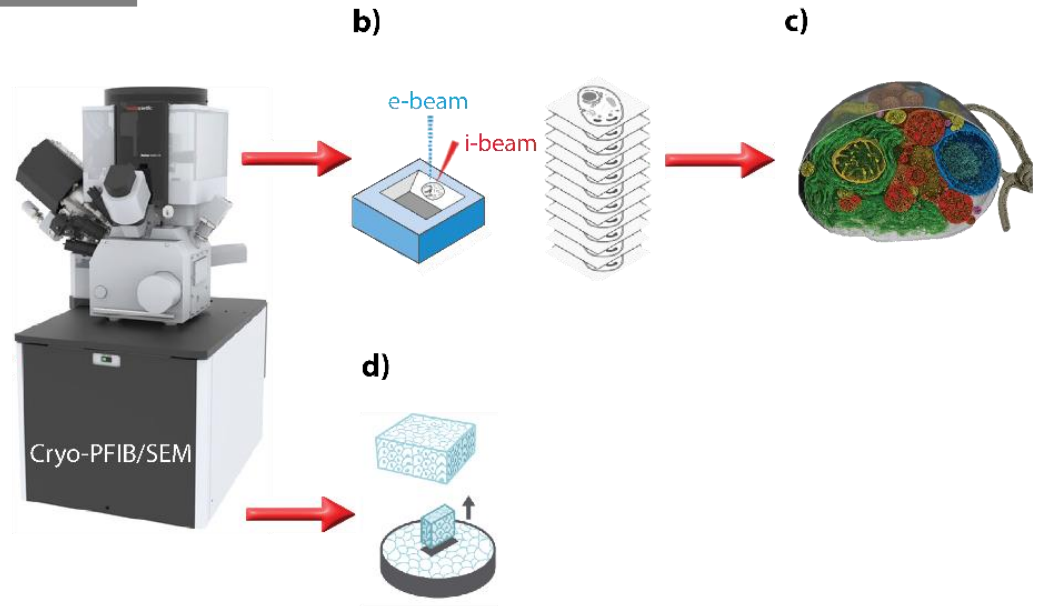
e.g., tabular data
(csv, excel)

Avizo 3D

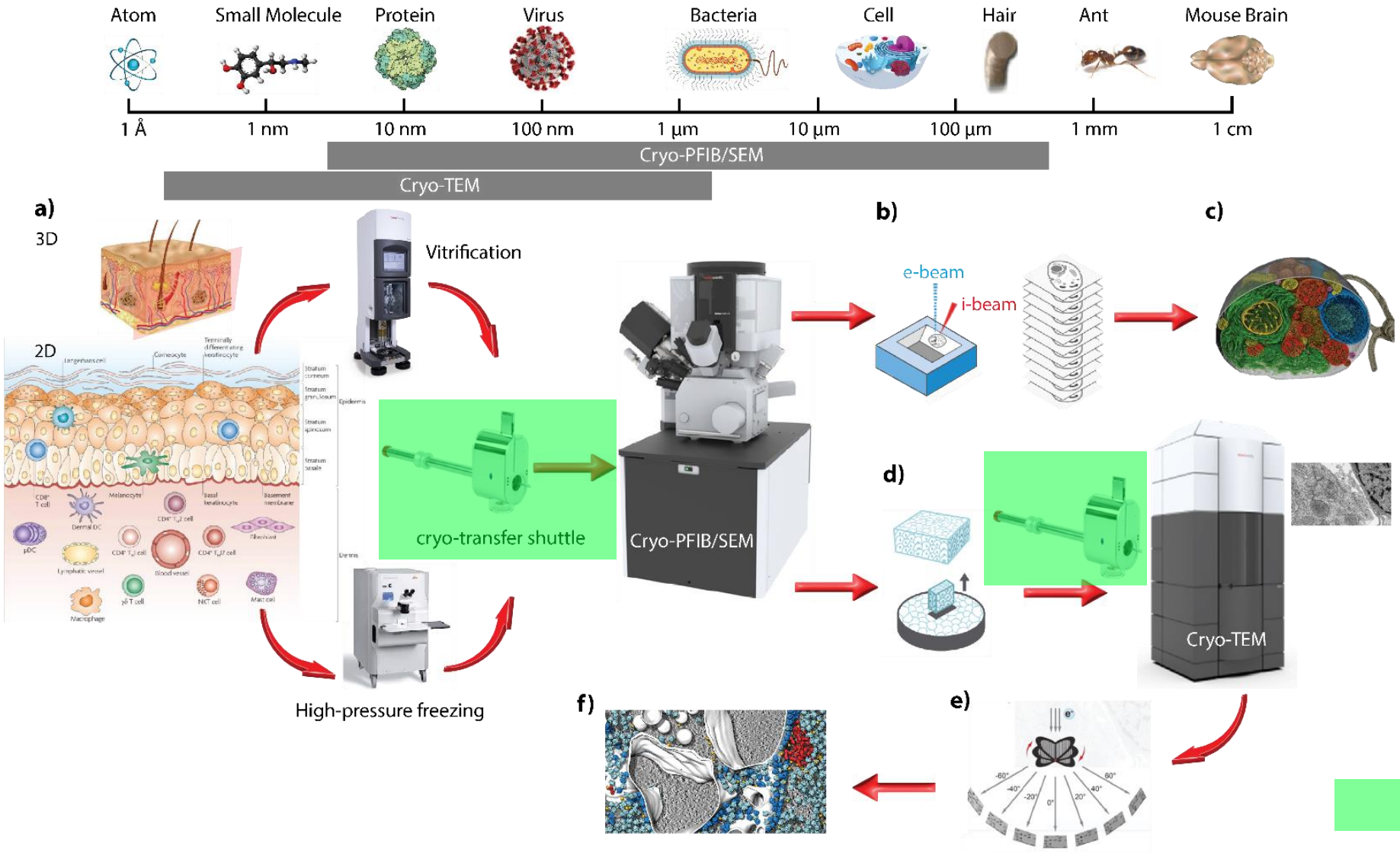

Amira 3D - CryoEM_Segmentation.hx
File Edit Project View Window XPand Python Help
Start Project Segmentation Meshing Recipes Image Recipe Designer Filament Animation Multiplanar
Project View
Open Data...
Color Wash Contrast Control Voxel Slice Cylinder Slice
emd_8594.to-ushort00.tif.am
vesicles.patchSet
mitochondria.patchSet
ribosomes.patchSet
microtubules.patchSet
annotation.vesicles.labels
annotation_mitochondria.labels
annotation_ribosomes.labels
annotation_microtubules.labels
result_vesicles.labels
result_mitochondria.labels
result_ribosomes.labels
result_microtubules.labels
result_5classes_2.labels
result_5classes_2.colors
Ortho Slice(2)
Volume Rendering Settings
Volume Rendering
Ortho Slice
Properties
Ortho Slice(2)
Data:
Orientation:
Slice Number:
Mapping Type:
Colormap:
Options:
Frame:
Transparency:
Embossing:
auto-refresh
Apply

Cryo-PFIB/SEM





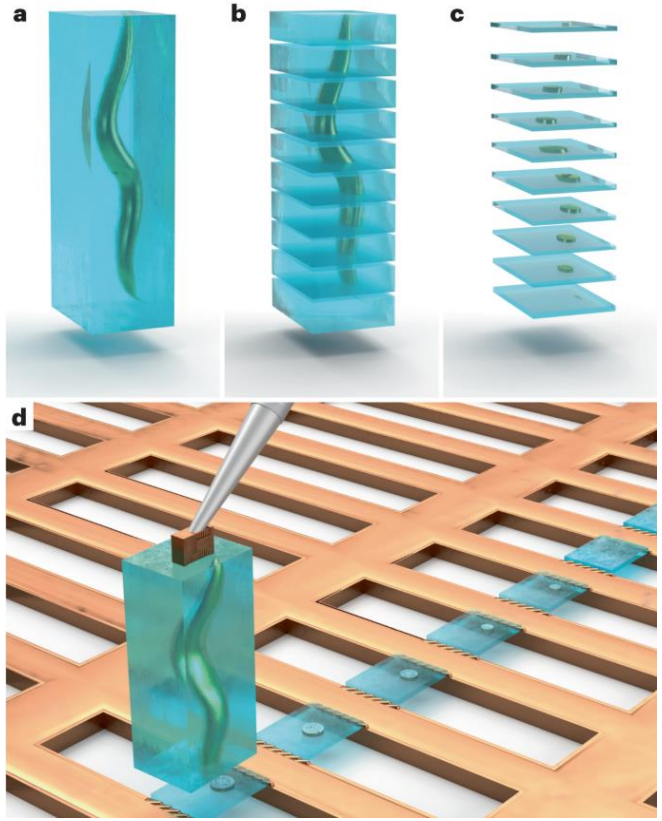
Cryo-PFIB/SEM



Anticipated future capability

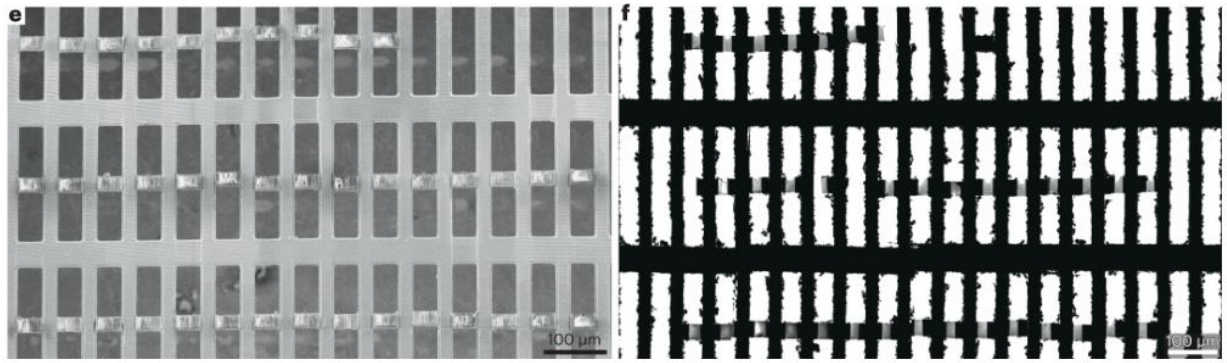
Cryo-PFIB/SEM

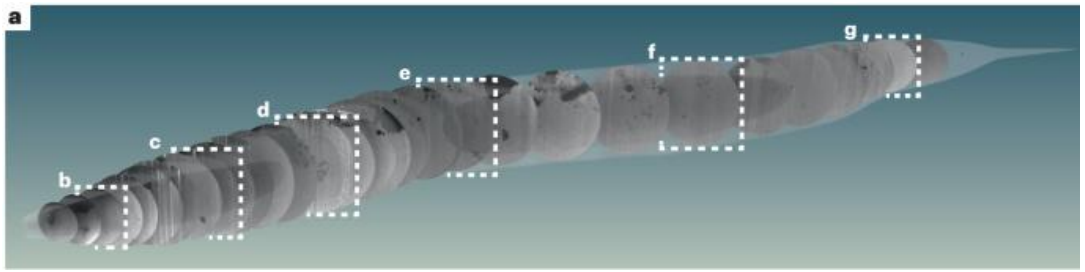
L1 larva of *C. elegans*

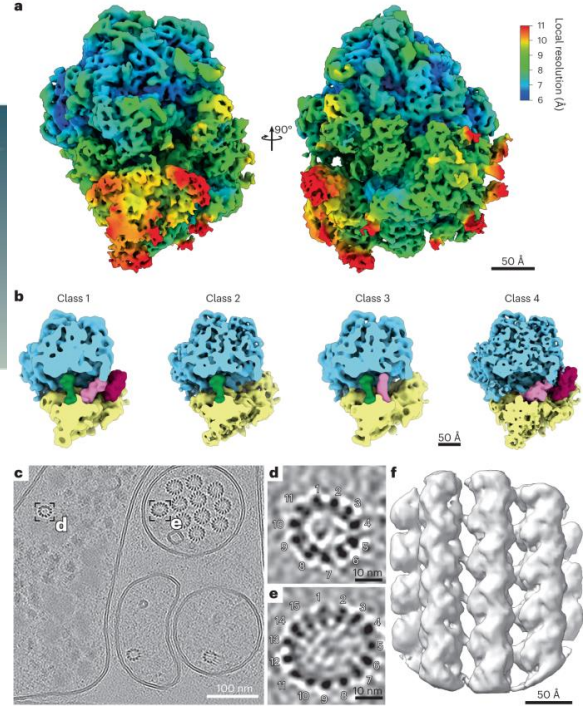


SEM

TEM







EPIC-FIB Facility

Access

to Thermo Fisher Hydra Plasma FIB-SEM

Dr. Paul Smeets

paul.smeets@northwestern.edu

Dr. Yu Wen

yu.wen@northwestern.edu

- FIB-SEM experienced users

Questions?