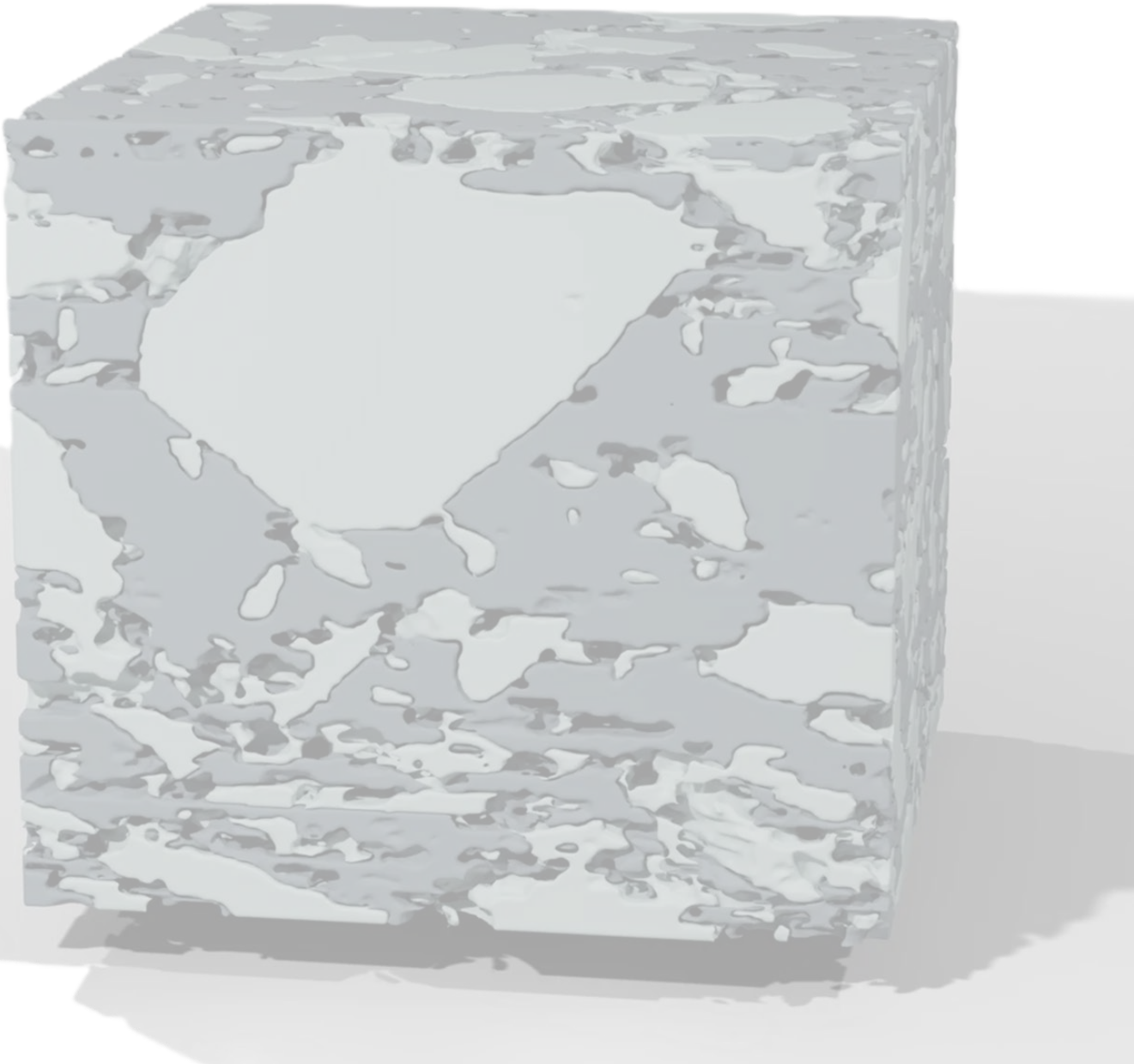




Below the Surface

Materials Characterization in 3D using a Slice-by-Slice Approach

08-14-2026

Paul Smeets

Research Assistant Professor
EPIC Facility Manager FIB & TEM

.. Let's start our journey in the kitchen

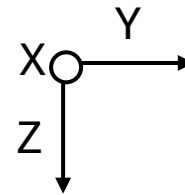
Steps to observe the layered structure on the inside of a cabbage

We could

1. Use a knife to cut away a piece
2. Look/take an image at the newly exposed surface
3. ...
4. Repeat until the cabbage is gone

Instead of a

- Cabbage → your solid sample of interest
- Phone camera → scanning electron microscope
- Knife → Focused beam of ions (Ga^+ , Xe^+ , etc.)



.. Let's start our journey in the kitchen

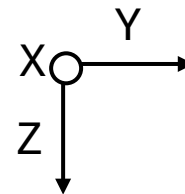
Steps to observe the layered structure on the inside of a cabbage

We could

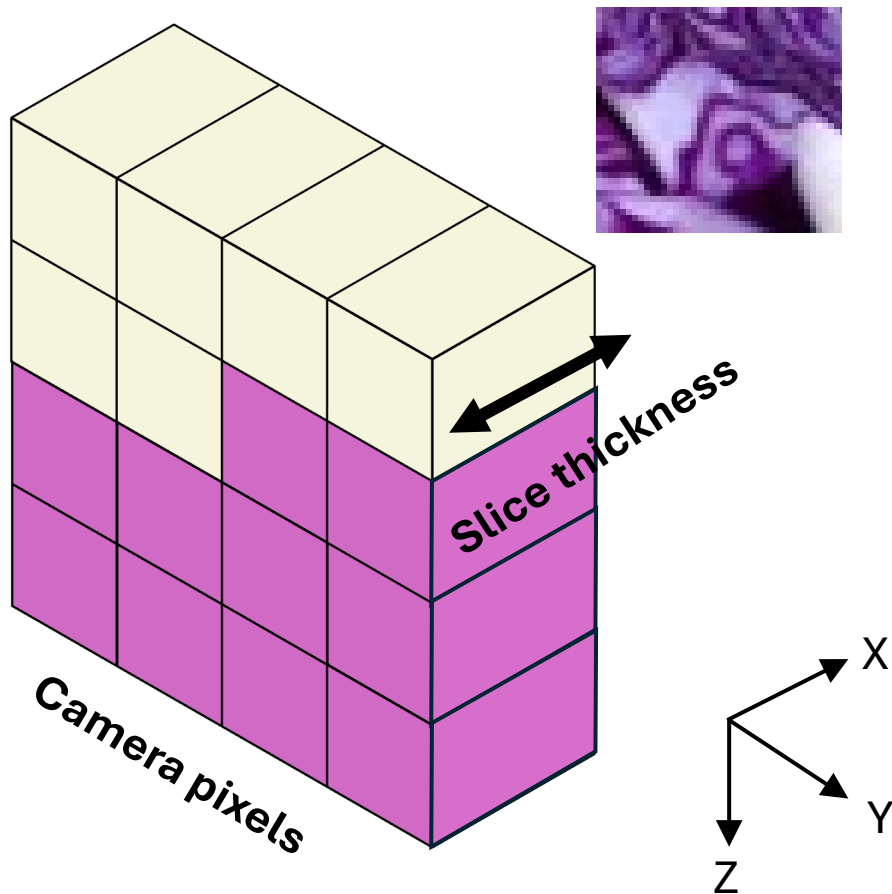
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Instead of a

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From Pixels (2D) to Voxels (3D)

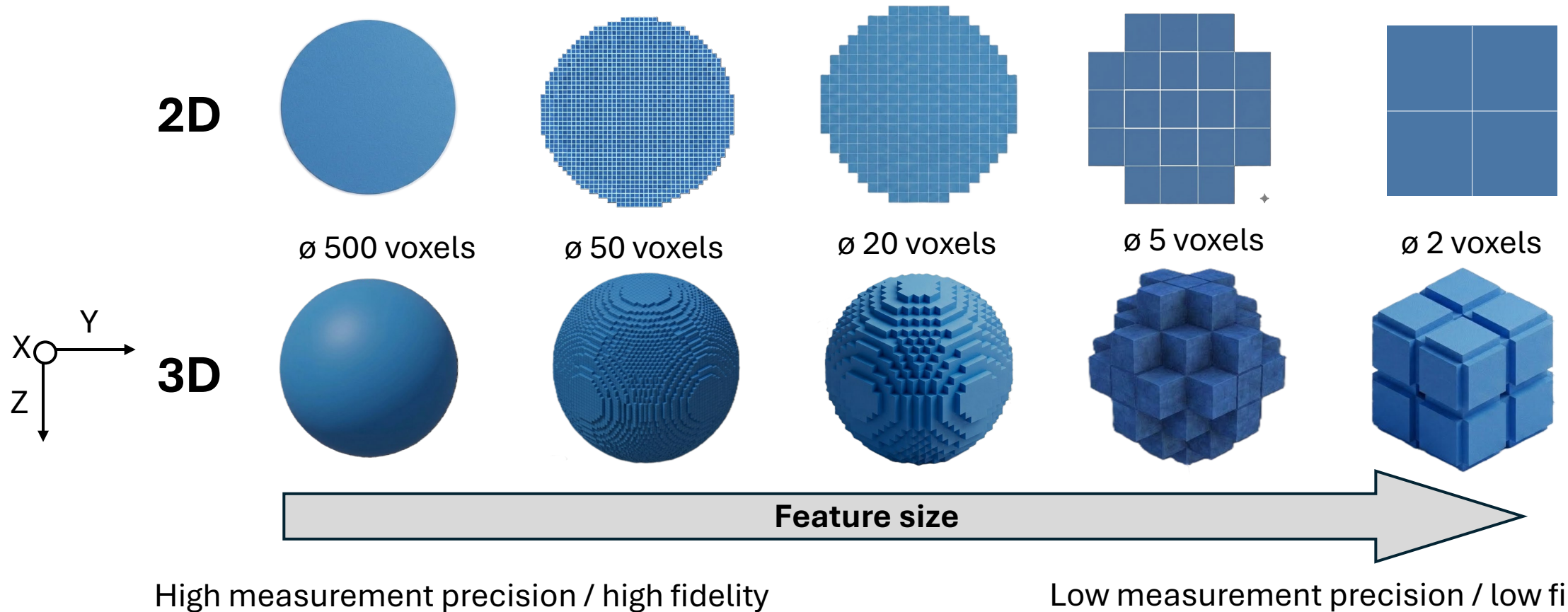


Important considerations for 3D acquisition

- **Feature size**
 - **Voxel size**
 - **Volume**
-
- **Feature size** determines required **voxel size** (~3–10x smaller than feature), and voxel size caps the maximum **imaged volume** due to processing time/data limits
 - **High resolution** demands small voxels, which forces a trade-off into a smaller overall acquired volume (small FoV in 2D)

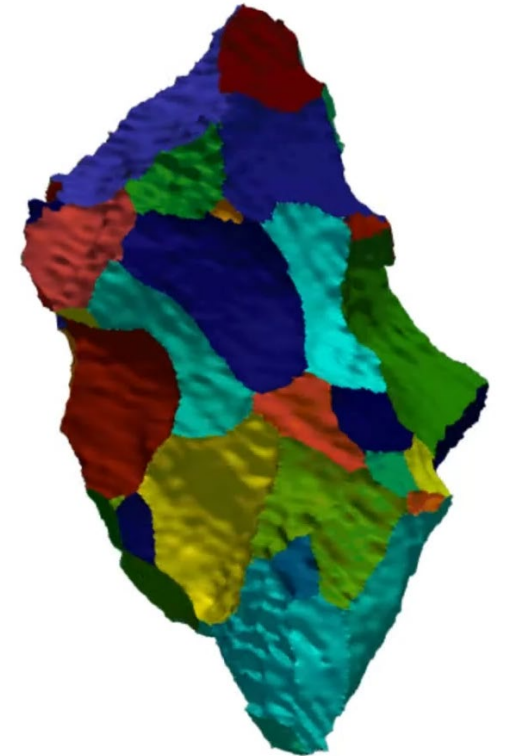
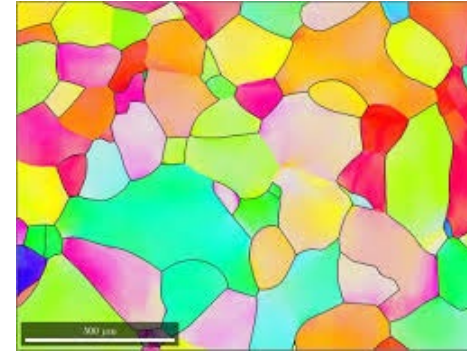
Imaging Resolution Impact on Image Fidelity

- X direction - thickness **Voxel size ↓ = Fidelity↑ = Tomo acquisition time↑**



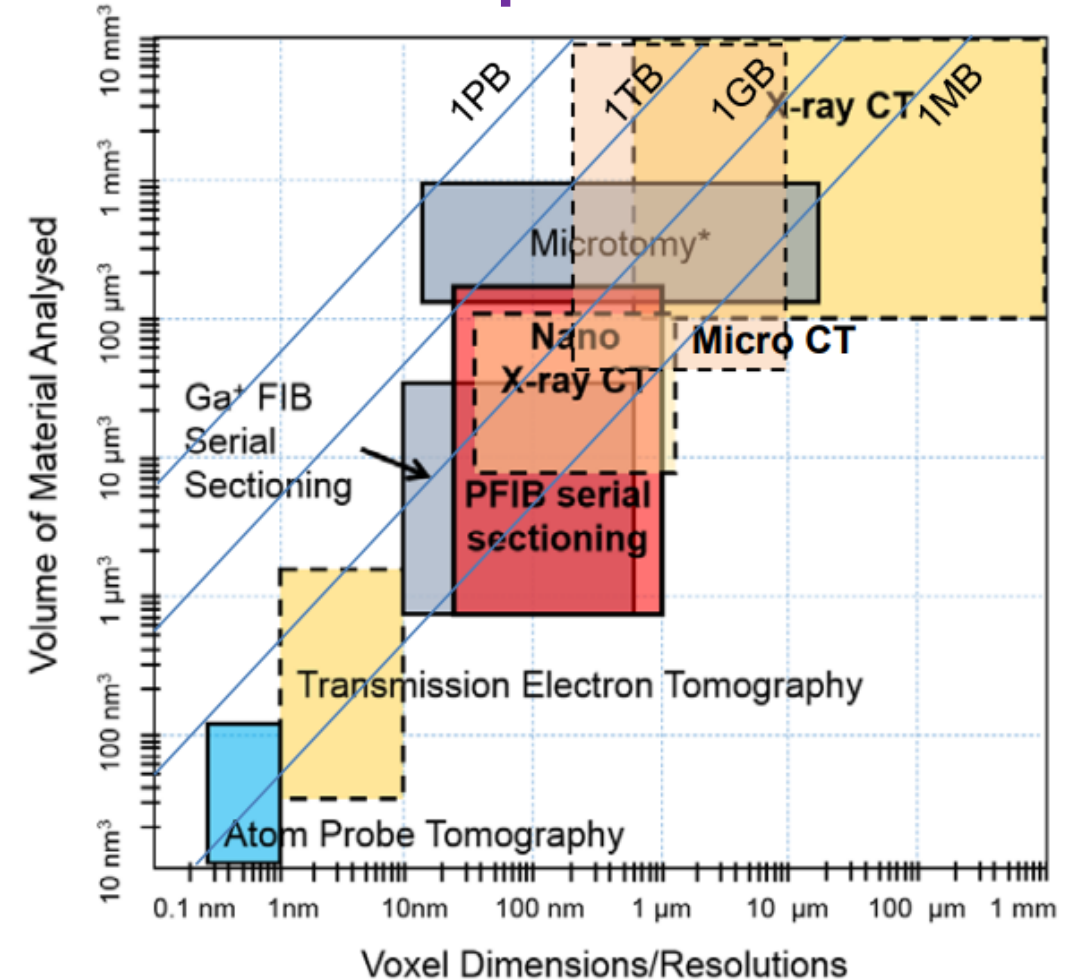
Why do we need 3D Materials Characterization?

- Failure analysis of buried features
- Quantification of features
 - **Materials Science:** Grain size analysis, thickness deviations in layered films, porosity fractions, localizing subsurface defects...
 - **Life Sciences:** Revealing cellular ultrastructure, mapping neural circuits...



3D (P)FIB-SEM vs other 3D Techniques

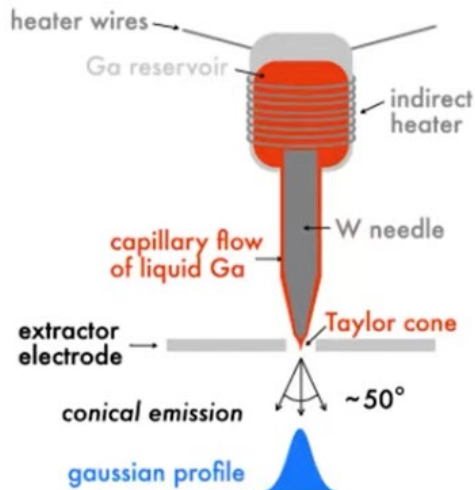
- Generally, when the amount of material analyzed increases, the spatial resolution drops
- (P)FIB-SEM tomography provides a good balance between bulk volume and spatial resolution
- However: destructive technique



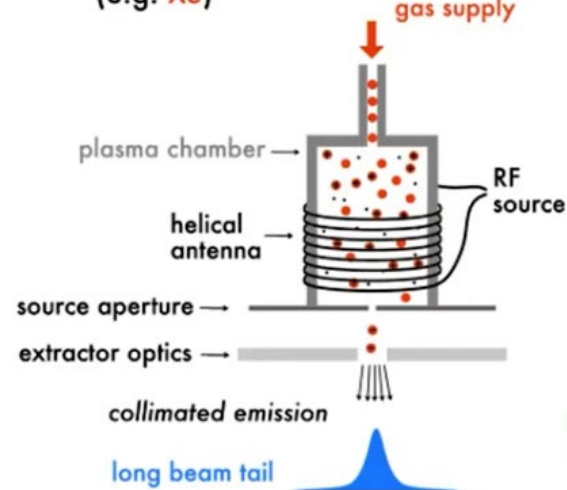
How is Matter Removed in (P)FIB-SEM?

- Ion sources: LMIS vs inductively coupled plasma source

a liquid metal ion source (e.g. Ga)



b inductively coupled plasma source (e.g. Xe)



Low Temperature Ion Source Inductively Coupled Plasma Source Gas Field Ionization Source Liquid Metal and Alloy Ion Source																	
hydrogen 1 H 1.0079																	
lithium 3 Li 6.941	beryllium 4 Be 9.0122																
sodium 11 Na 22.990	magnesium 12 Mg 24.305																
potassium 19 K 39.098	calcium 20 Ca 40.078																
rubidium 37 Rb 85.468	strontium 38 Sr 87.62																
cesium 55 Cs 132.91	barium 56 Ba 137.33																
francium 87 Fr 223																	
scandium 21 Sc 44.956	titanium 22 Ti 47.867	vanadium 23 V 50.942	chromium 24 Cr 51.996	manganese 25 Mn 54.938	iron 26 Fe 55.845	cobalt 27 Co 58.933	nickel 28 Ni 58.693	copper 29 Cu 63.546	zinc 30 Zn 65.39	gallium 31 Ga 69.723	germanium 32 Ge 72.61	arsenic 33 As 74.922	selenium 34 Se 78.96	bromine 35 Br 79.904	krypton 36 Kr 83.80		
yttrium 39 Y 88.906	zirconium 40 Zr 91.224	niobium 41 Nb 92.906	molybdenum 42 Mo 95.94	technetium 43 Tc 98	ruthenium 44 Ru 101.07	rhodium 45 Rh 102.91	palladium 46 Pd 106.42	silver 47 Ag 107.87	cadmium 48 Cd 112.41	indium 49 In 114.82	tin 50 Sn 118.71	antimony 51 Sb 121.76	tellurium 52 Te 127.60	iodine 53 I 126.90	xenon 54 Xe 131.29		
lanthanum 57 La 138.905	cerium 58 Ce 140.12	praseodymium 59 Pr 140.908	neodymium 60 Nd 144.24	promethium 61 Pm 145	samarium 62 Sm 150.36	europium 63 Eu 151.96	gadolinium 64 Gd 157.25	terbium 65 Tb 158.93	dysprosium 66 Dy 162.50	holmium 67 Ho 164.93	erbium 68 Er 167.26	thulium 69 Tm 168.93	ytterbium 70 Yb 173.05	lutetium 71 Lu 174.967	hafnium 72 Hf 178.49	tantalum 73 Ta 180.948	
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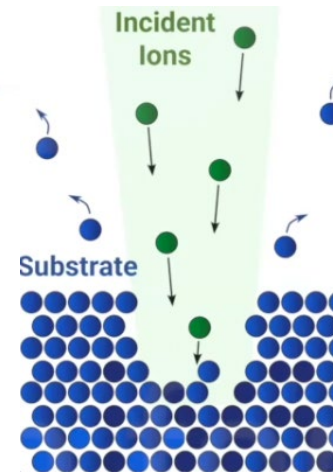
* Lanthanide series

57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb
89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No

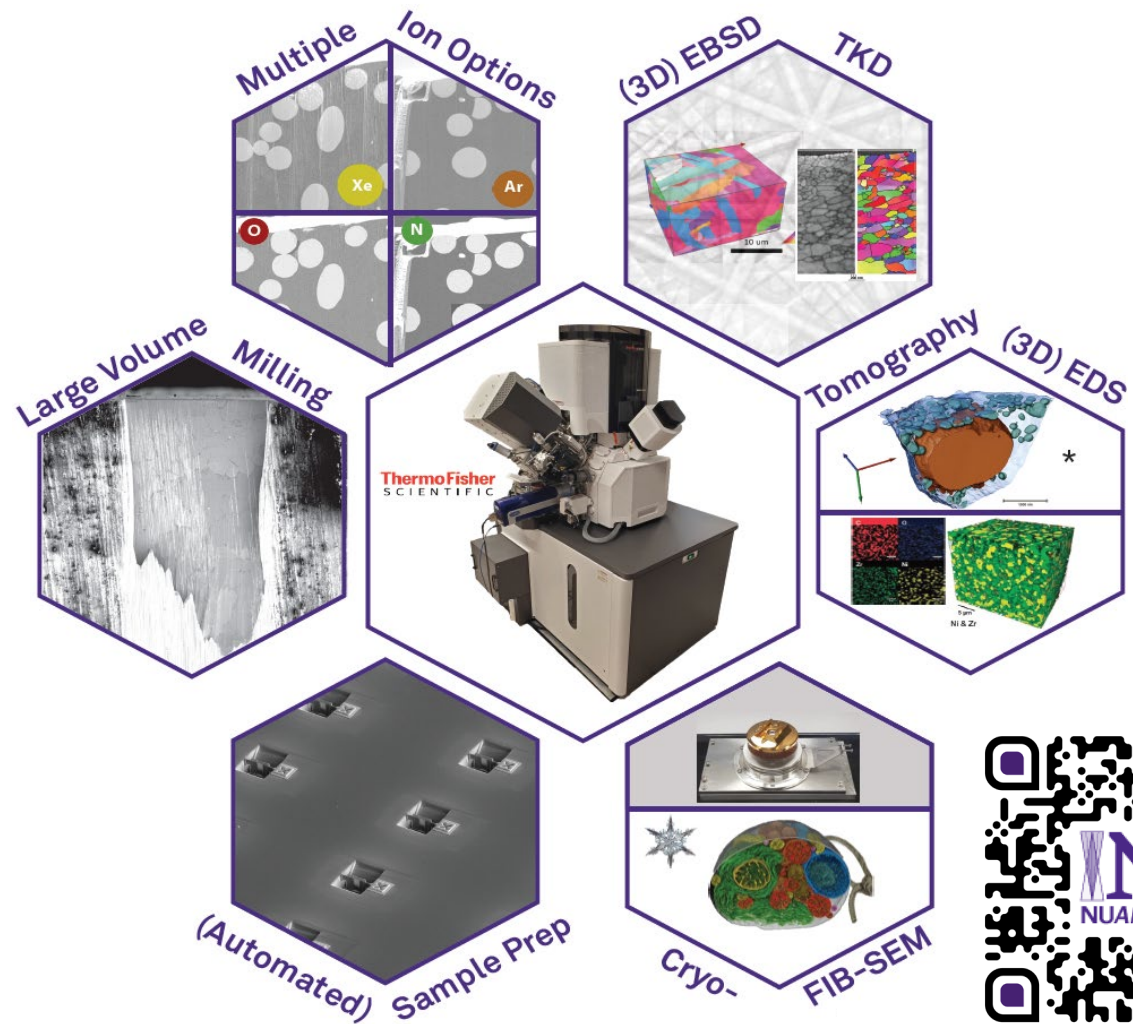
** Actinide series

- Milling (sputtering)

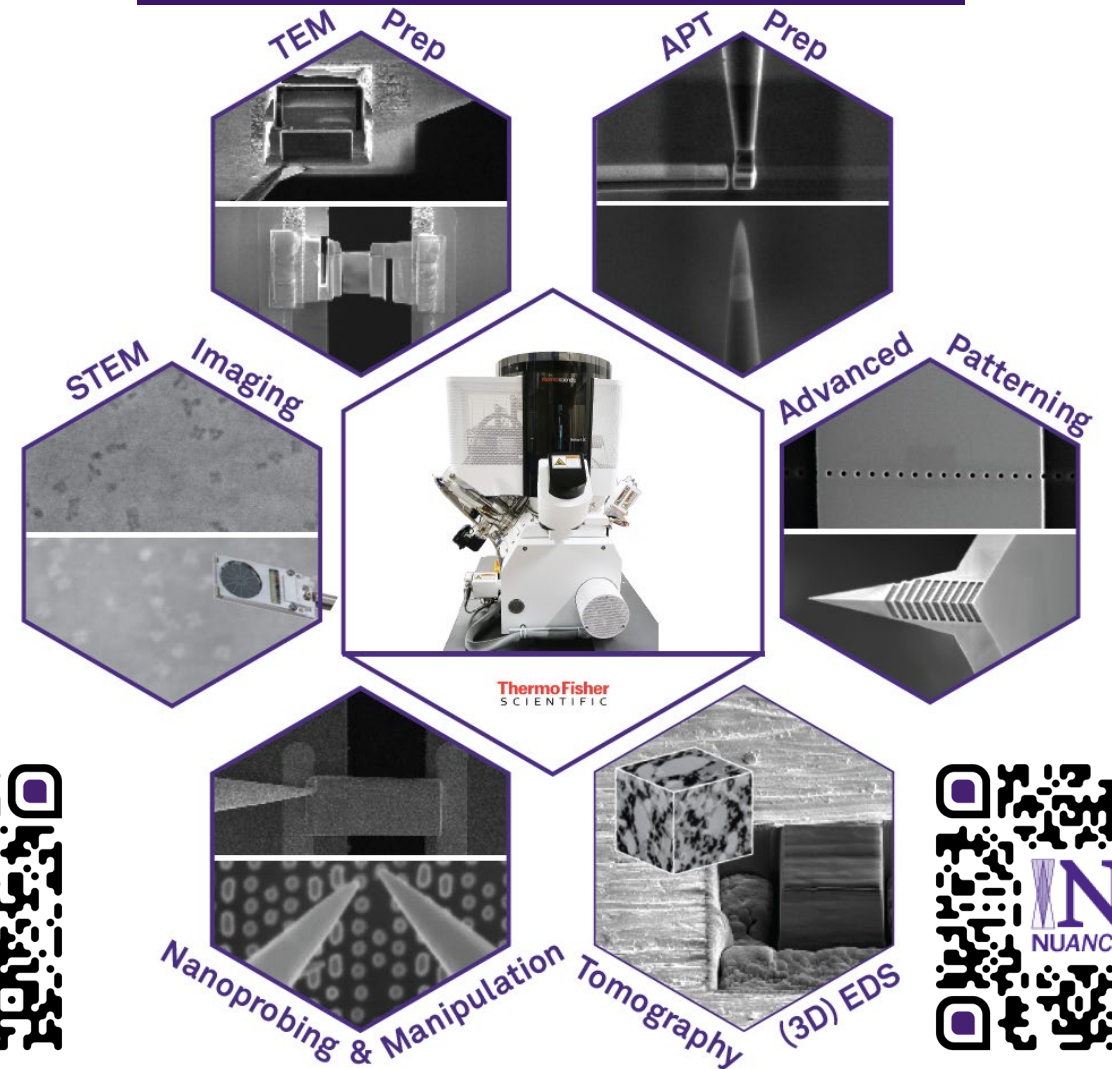
- 5 ion sources
Ga, Xe, Ar, O, N



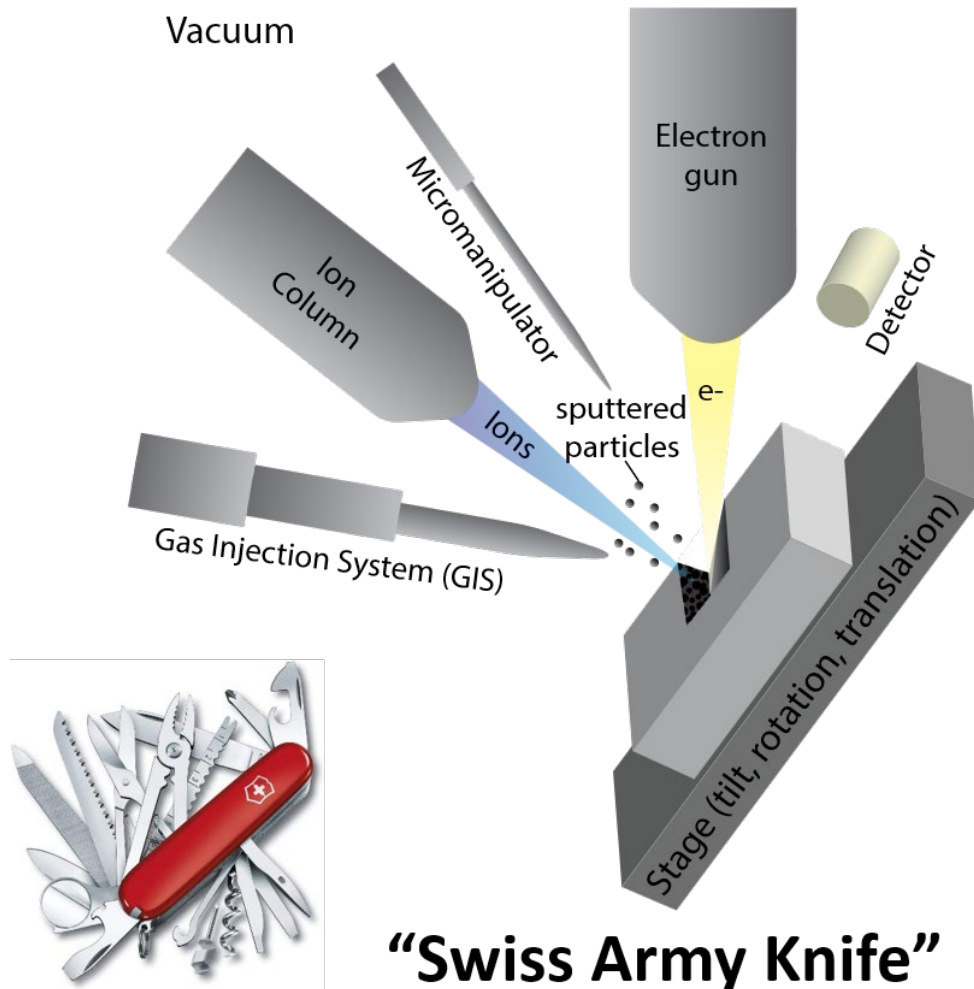
Helios 5 Hydra CX Plasma DualBeam



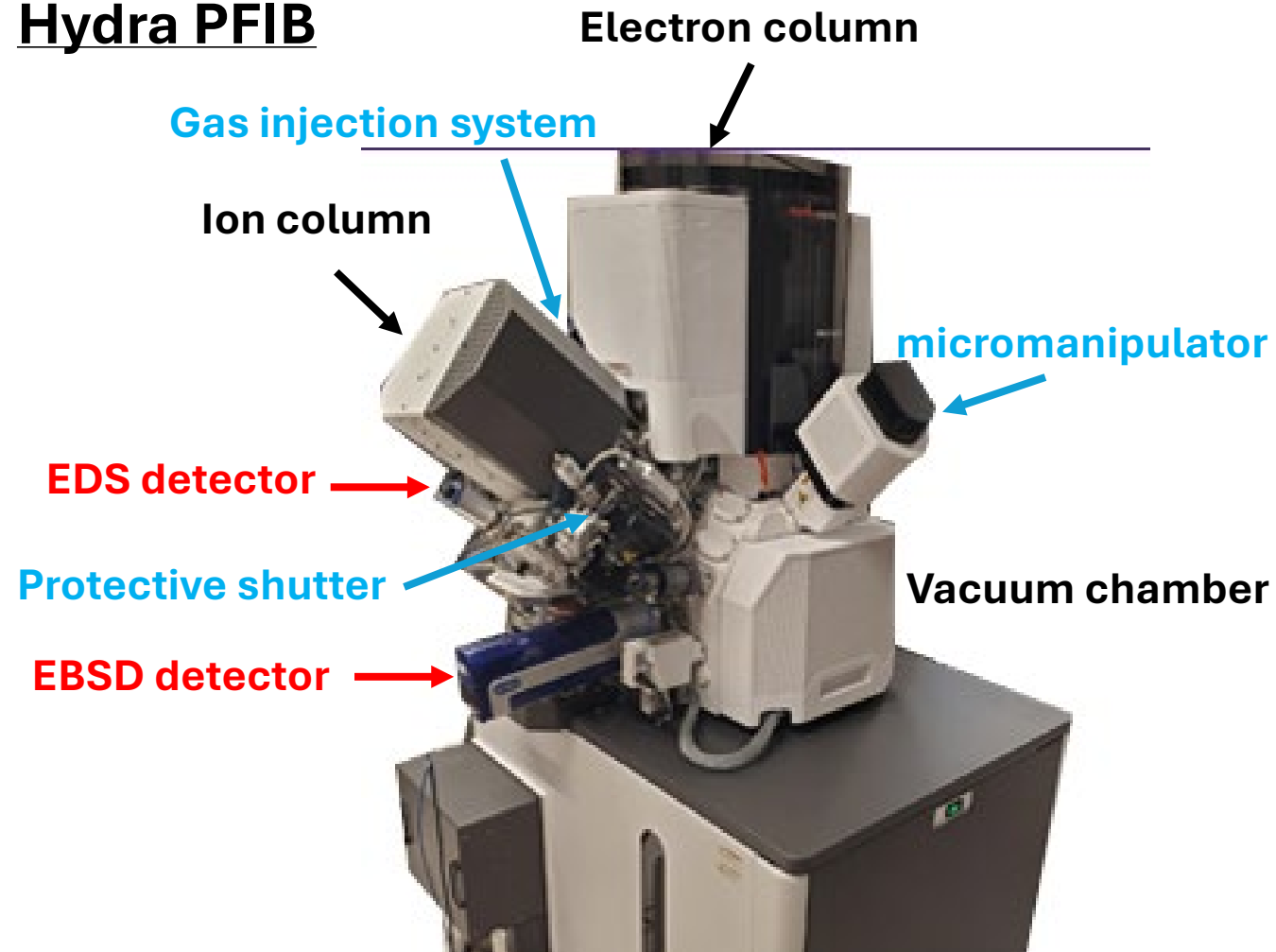
Helios 5 UC Ga DualBeam



Configuration of the (P)FIB Instrument



Hydra PFIB



FIB-SEM Tomography Configurations

1. Default – In Bulk



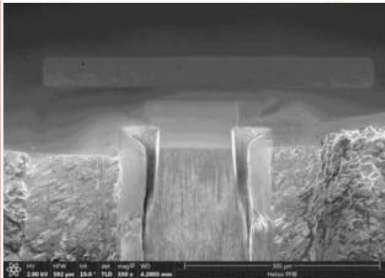
Stage tilt: 52°



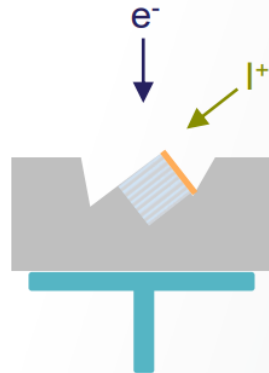
2. Default – At Edge



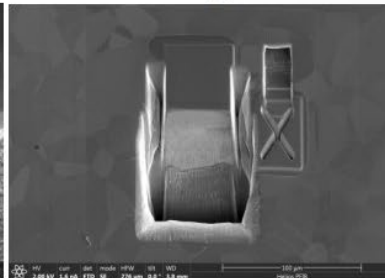
Stage tilt: 52°



3. Zero Degree Tilt



Stage tilt: 0°



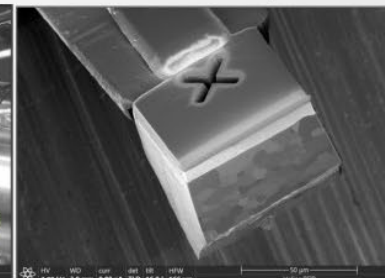
4. Pre-Tilted – At Edge 5. Chunk Liftout (Pre-Tilted)



Stage tilt: 16°
Pre-tilt: 36°



Stage tilt: 16°
Pre-tilt: 36°



6. Spin Mill (planar milling)



NUANCE
Learning Exchange

Seminars
Workshops
Demos

Optimized 3D FIB-SEM Workflows for
Multimodal Materials Characterization and
VolumeEM: Spin Mill Method

DATE / TIME / LOCATION

Seminar:

August 18, 2026
Ford Hive, Room #2350
9:00 - 11:00 AM

In-Person Demos:

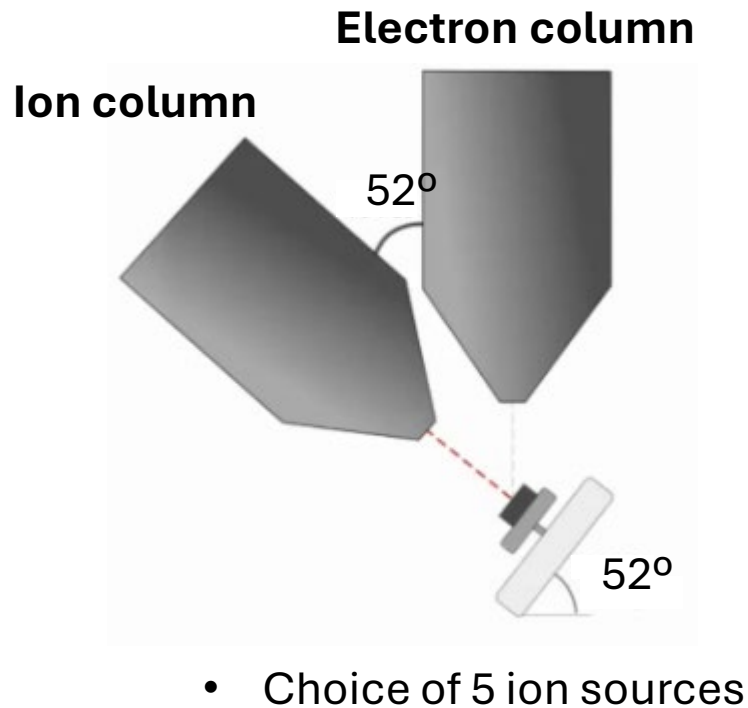
August 18-20, 2026
Tech AG98



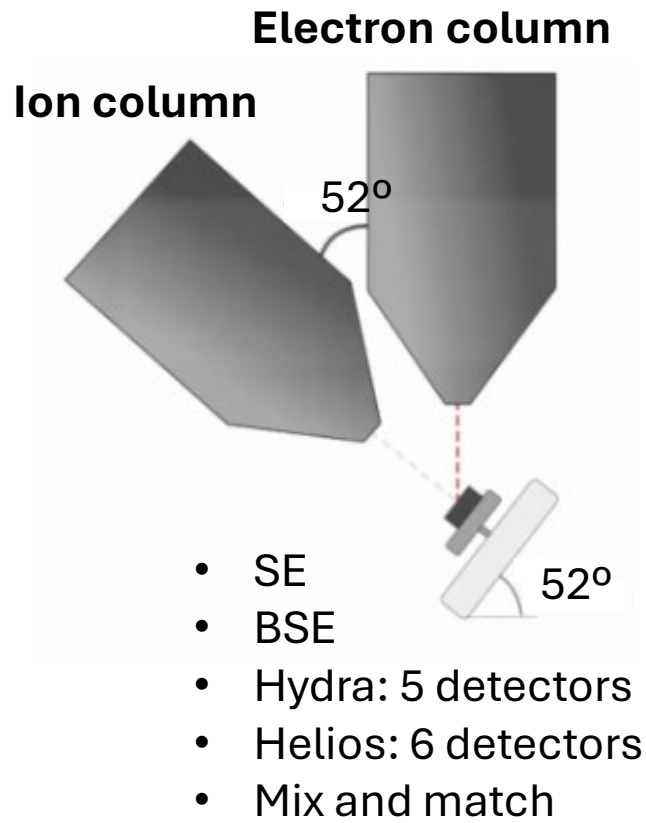
VIEW SCHEDULE
REGISTER

In-Bulk Tomography Configuration

(P)FIB Milling

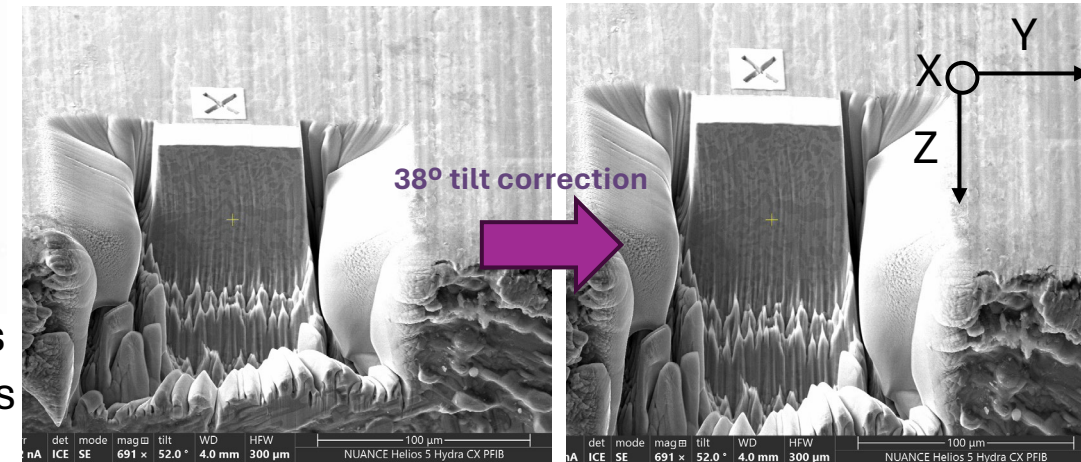


SEM Imaging



Sample does not move (or minor movements) between imaging and milling positions (faster)

Newly sliced cross-section imaged at tilted angle from SEM
→ digital tilt correction



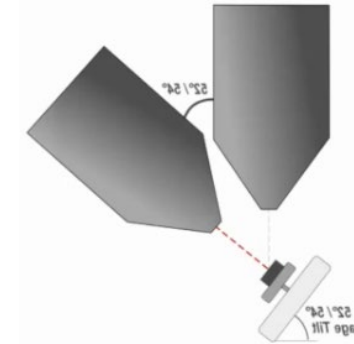
ROI Preparation for In-Bulk Tomography

1. Prepare a protective cap on the region of interest

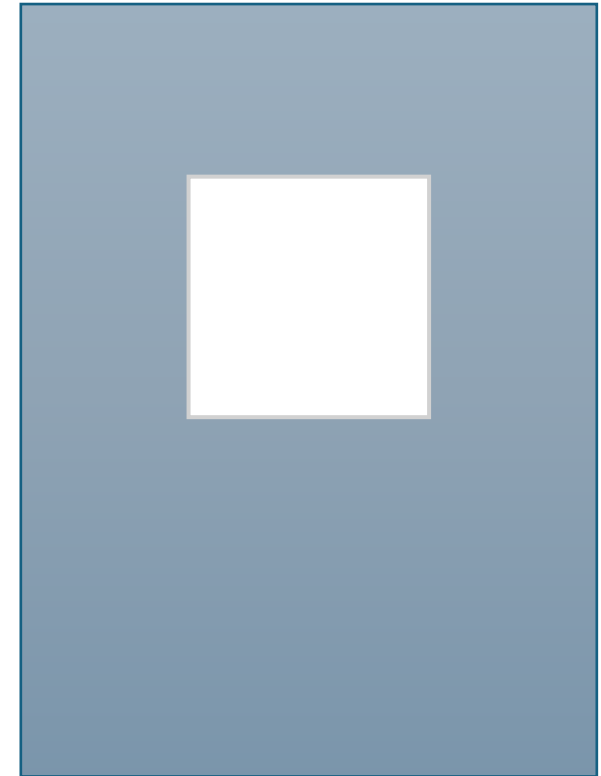
- Gas precursors available:

- Tungsten
- Carbon
- Platinum

- Cap layer can vary ~ 500 nm to ~ 4 μm , mostly depending on volume removal requirements



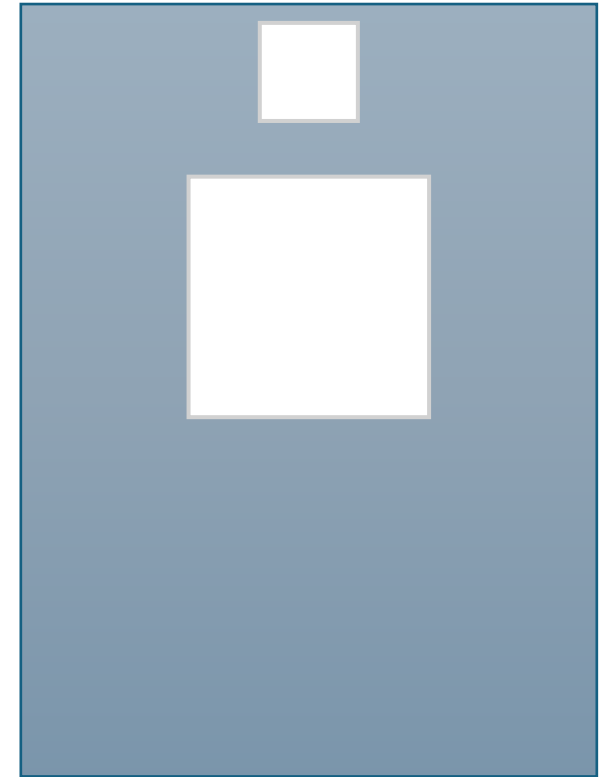
As viewed by FIB:



ROI Preparation for In-Bulk Tomography

1. Prepare a protective cap on the region of interest
2. Create a second protective layer next to ROI
 - ~2 μm height, ~ 4-5 μm away from first protective cap
 - Tracking marker to position for slice milling

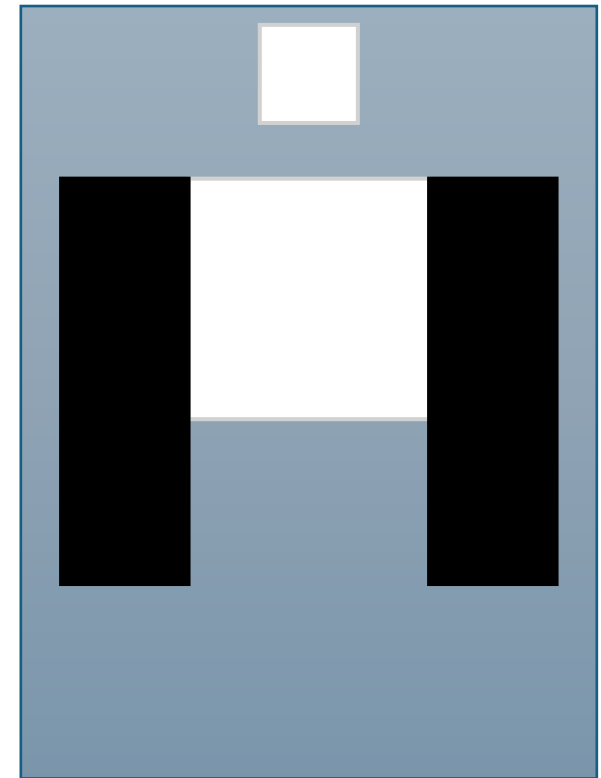
As viewed by FIB:



ROI Preparation for In-Bulk Tomography

1. Prepare a protective cap on the region of interest
2. Create a second protective layer next to ROI
3. Mill material on left and right sides of ROI
 - Prevent 'shadowing' of SEM/BSE images
 - Cavity creation where redeposited material can accumulate (i.e. no further obstruction volume of interest in subsequent slicing)
 - **Best practice:** ~33% width of ROI

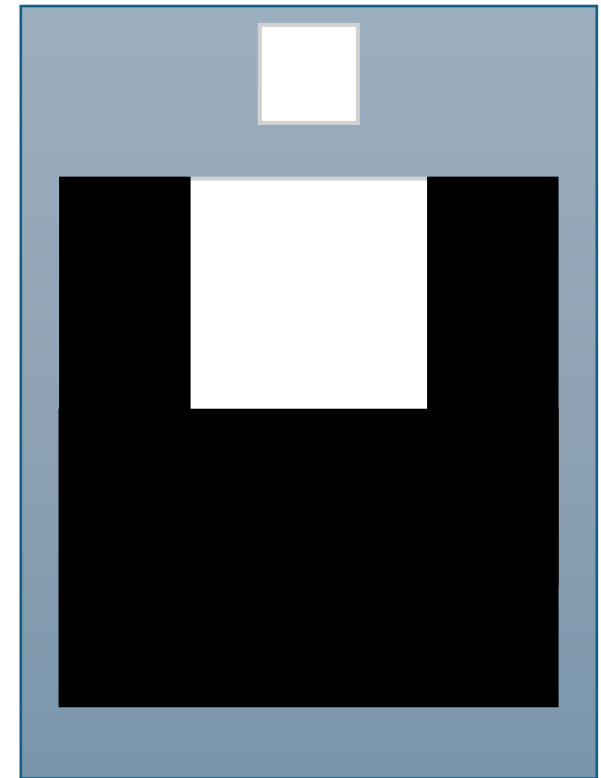
As viewed by FIB:



ROI Preparation for In-Bulk Tomography

1. Prepare a protective cap on the region of interest
2. Create a second protective layer next to ROI
3. Mill material on left and right sides of ROI
4. Mill material in front of ROI
 - Creates surface allowing to observe the cross-section
 - **Best practices:**
 - 1) Y direction ~ 1.5 -2 times the depth you wish to image
 - 2) Stepwise approach with graded usage of currents (high to low)

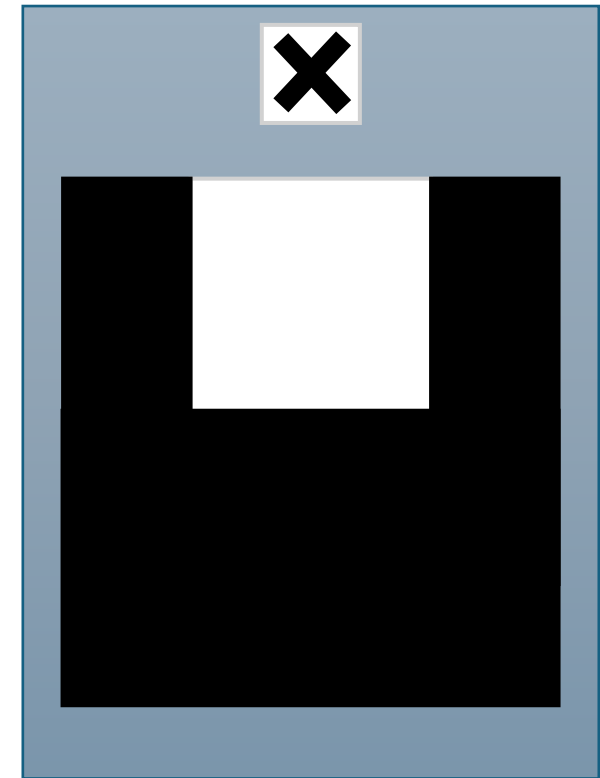
As viewed by FIB:



ROI Preparation for In-Bulk Tomography

1. Prepare a protective cap on the region of interest
2. Create a second protective layer next to ROI
3. Mill material on left and right sides of ROI
4. Mill material in front of ROI
5. Mill fiducial shape into second deposition pad
 - **Best practices:**
Mill slightly deeper than the pad depth for best contrast

As viewed by FIB:



What Parameters for Automated Tomo Acquisition?

Ion Beam

- 30 kV → highest resolution
- Current ↓ = Probe Diameter ↓ = Milling Precision ↑ = Acquisition Time ↑

Electron Beam

- Higher kV = Larger interaction volume
- Lower kV better for small slice thicknesses

Auto-alignment functions for Imaging

- Auto stigmation: every 25 slices
- Auto focus: every slice to every 10 slices
- Auto contrast-brightness: every 25 slices
- Drift correction: digital vs beam shift

8 – 24+ hrs

Voxel size (nm)	Ion Current
2-8	40 pA – 500 pA
8-12	300 pA – 1.5 nA
12-20	700 pA – 5 nA
20-30	4 nA – 20 nA
>60	12 nA to 2.5 μ A

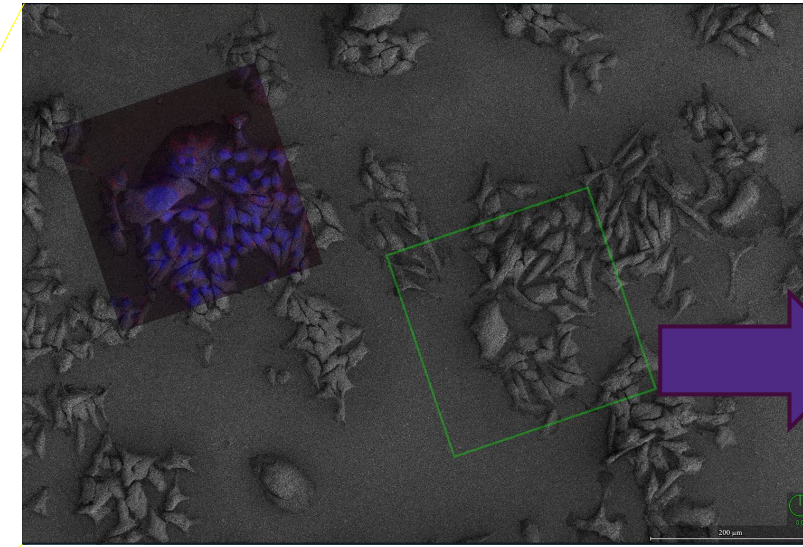
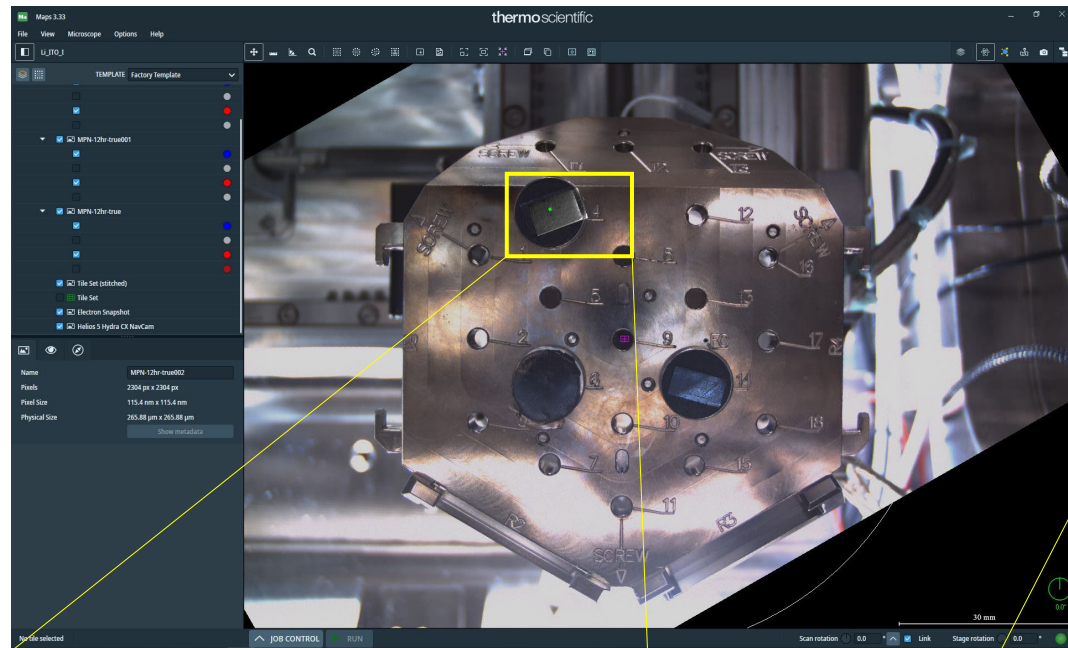


Correlative (P)FIB Tomography Workflows

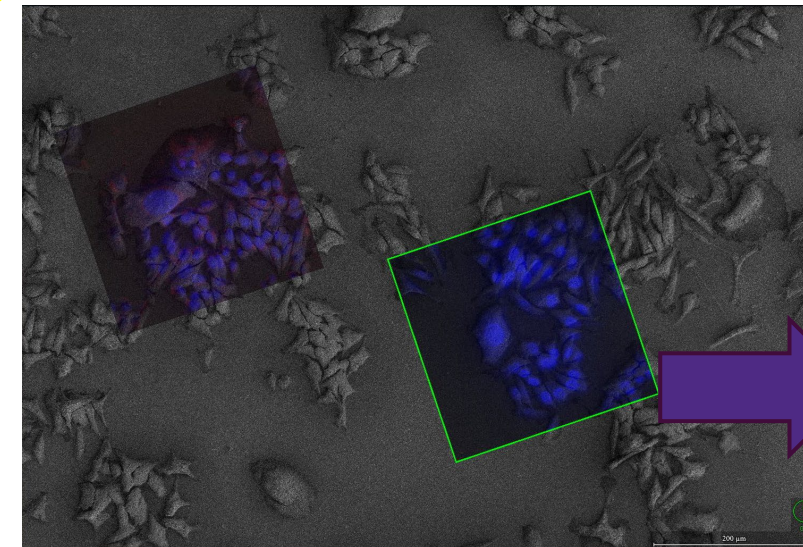
- Other techniques besides (P)FIB-SEM
 - Example: Fluorescence imaging / FIB-SEM tomography
- Within the (P)FIB-SEM
 - Examples: 3D EDS of solid oxide fuel cell and superalloy

Correlative Workflows – Fluorescence / FIB-SEM Tomo

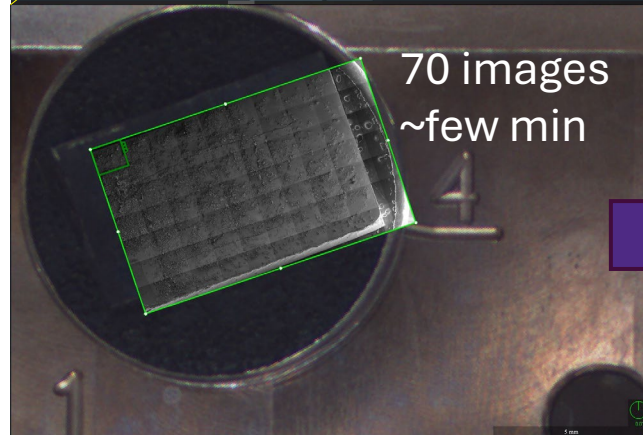
MAPS Correlative Software



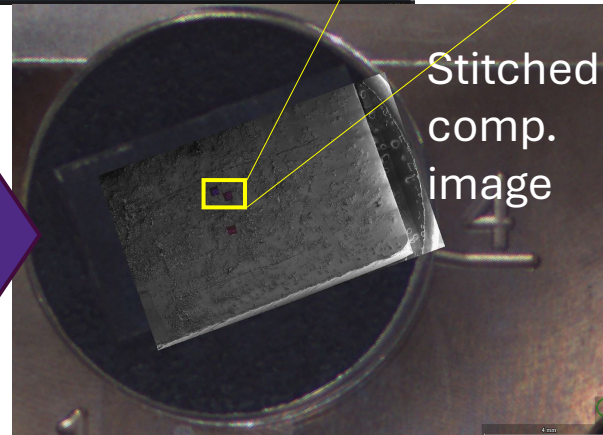
No overlaid
fluorescence
data



Overlaid
fluorescence
data (DAPI)



70 images
~few min

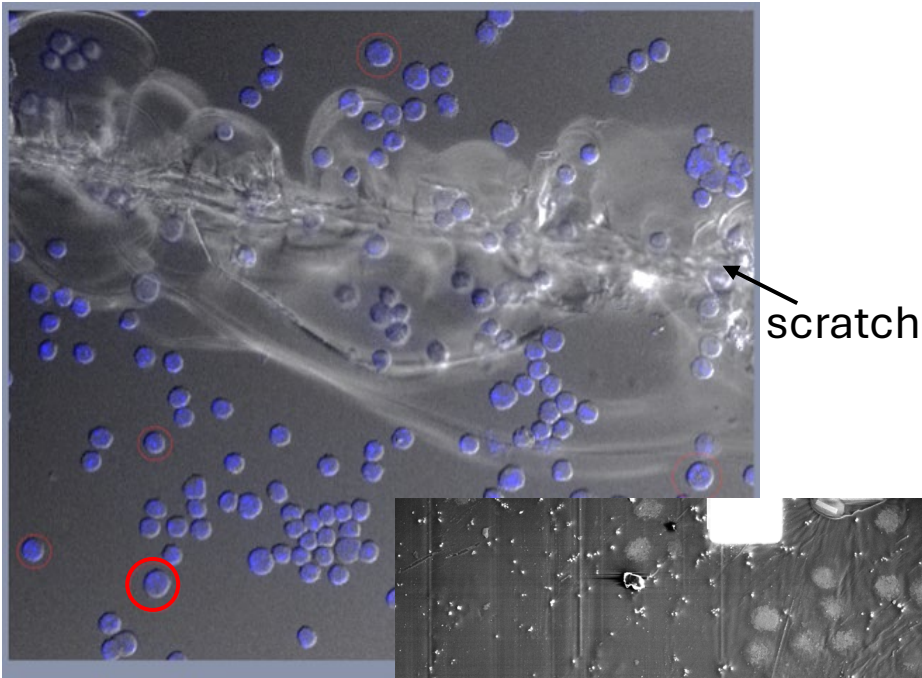


Stitched
comp.
image

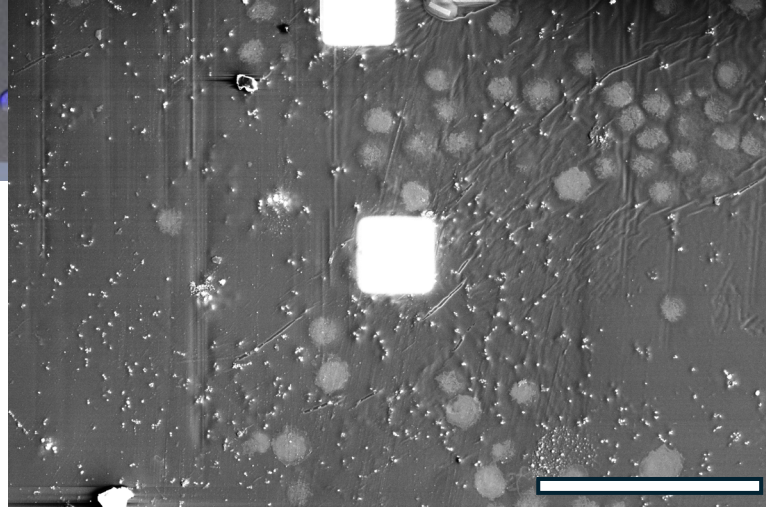
Correlative Workflows – Fluorescence / FIB-SEM Tomo

Bone-marrow derived cells from Mouse

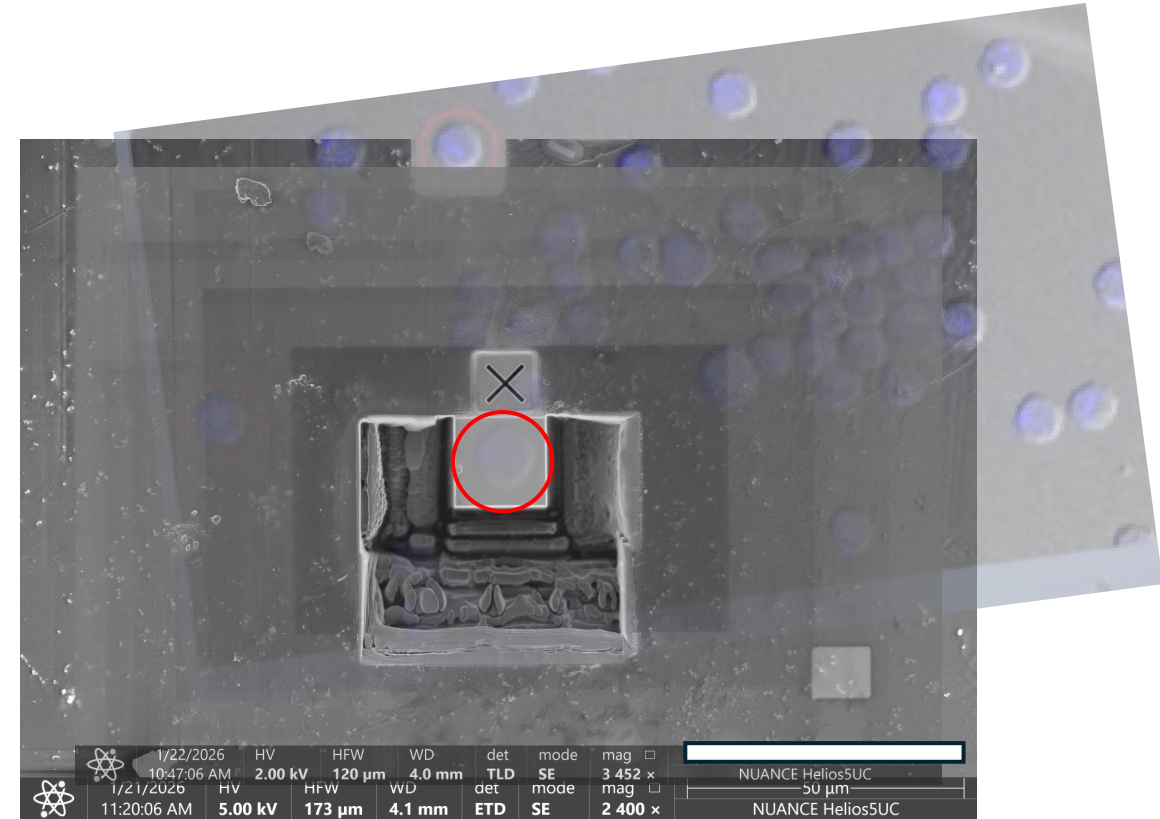
Fluorescence Image



SEM Image



Composite Fluorescence/SEM Image

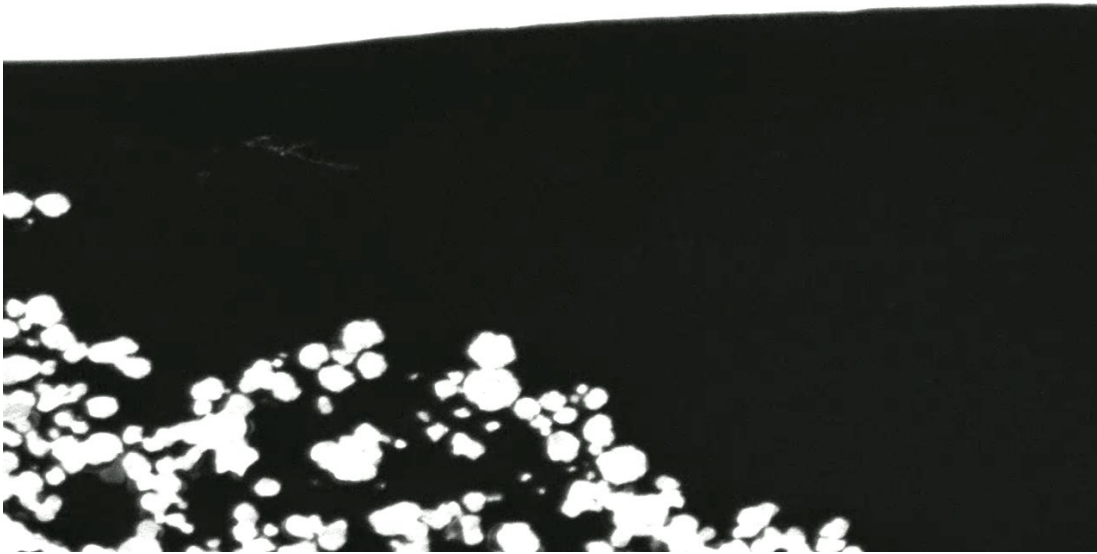


ROI before FIB-tomo acquisition

Sample Preparation credit: FSM collaborators,
Chris Sharpe & Reiner Bleher

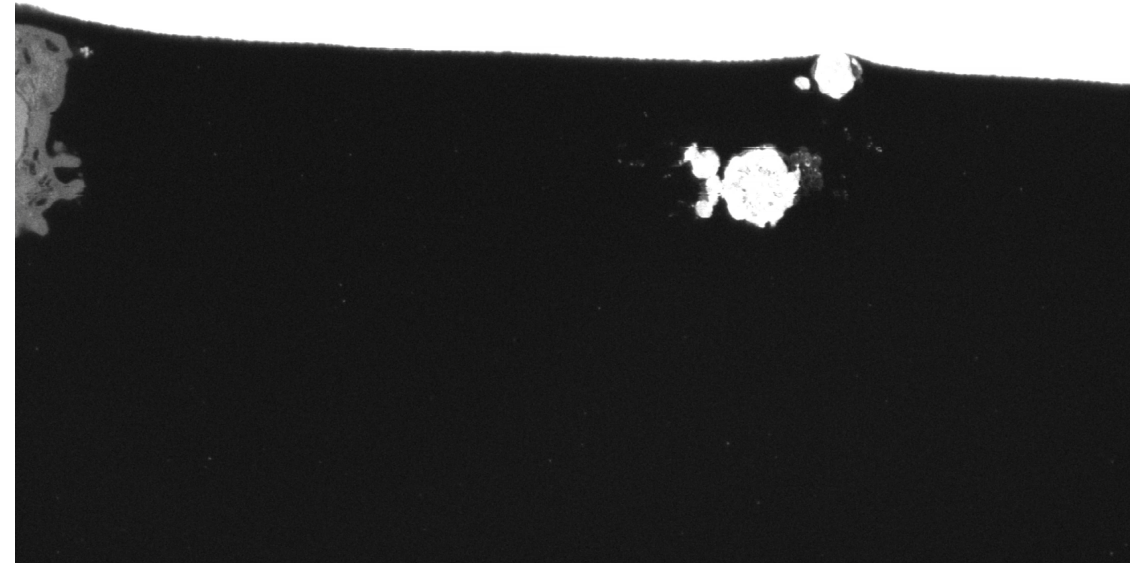
Correlative Workflows – Fluorescence / FIB-SEM Tomo

Bone-marrow derived cell



Slicing: 5nm thickness; **1974** slices total (~**120 fps**)
Raw images: BSE 5nm pixel size; 2598 * 1295 pixels

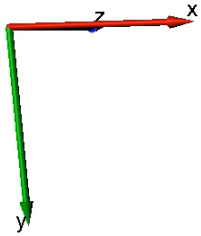
Spleen-derived cell



Slicing: 5nm thickness; **1322** slices total (~**60 fps**)
Raw images: BSE 5nm pixel size; 2649 * 2300 pixels

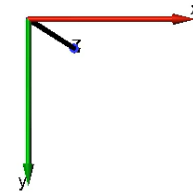
Visualization of Targeted Cells

Bone-marrow derived cell



1500 nm

Spleen-derived cell



1000 nm

Segmentation/visualization credit: Yu Wen

Ga-FIB Tomography of Targeted Cells - Quantification

Cell	Volume (nm ³)	Ratio	
Euchromatin	1.97E10	38.6%	43.1%
Heterochromatin	3.13E10	61.4%	
Cell shell	6.73E10		56.9%

Spleen-derived cell

Cell	Volume (nm ³)	Ratio	
Euchromatin	1.17E10	43.1%	34.8%
Heterochromatin	1.55E10	56.9%	
Cell shell	5.1E10		65.2%

Bone-marrow derived cell

Submitted - Collaboration with 36 authors

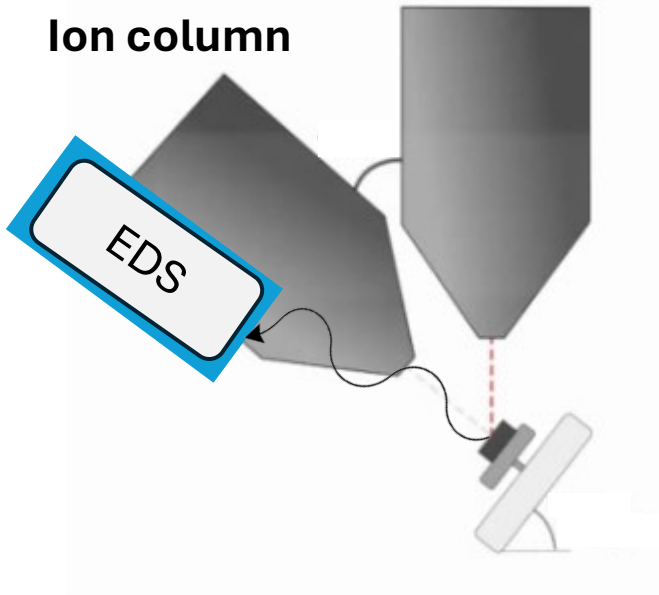
Correlative Workflows – 3D EDS

- Acquire EDS data from cross-section

EDS / SEM Imaging

Electron column

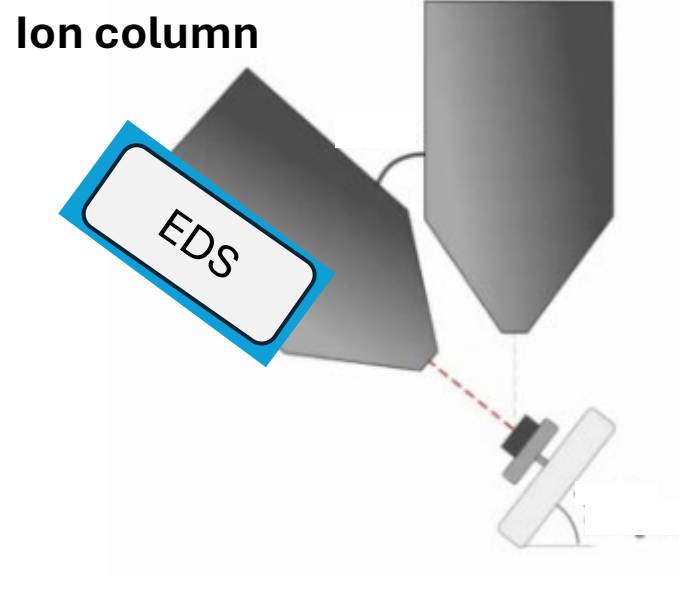
Ion column



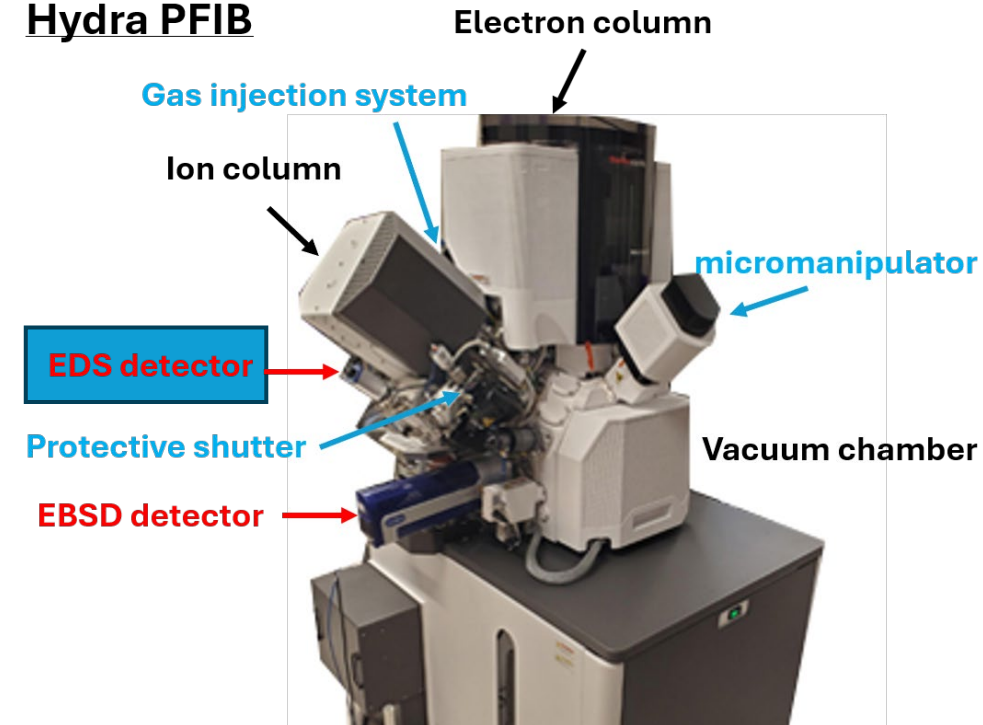
(P)FIB Milling

Electron column

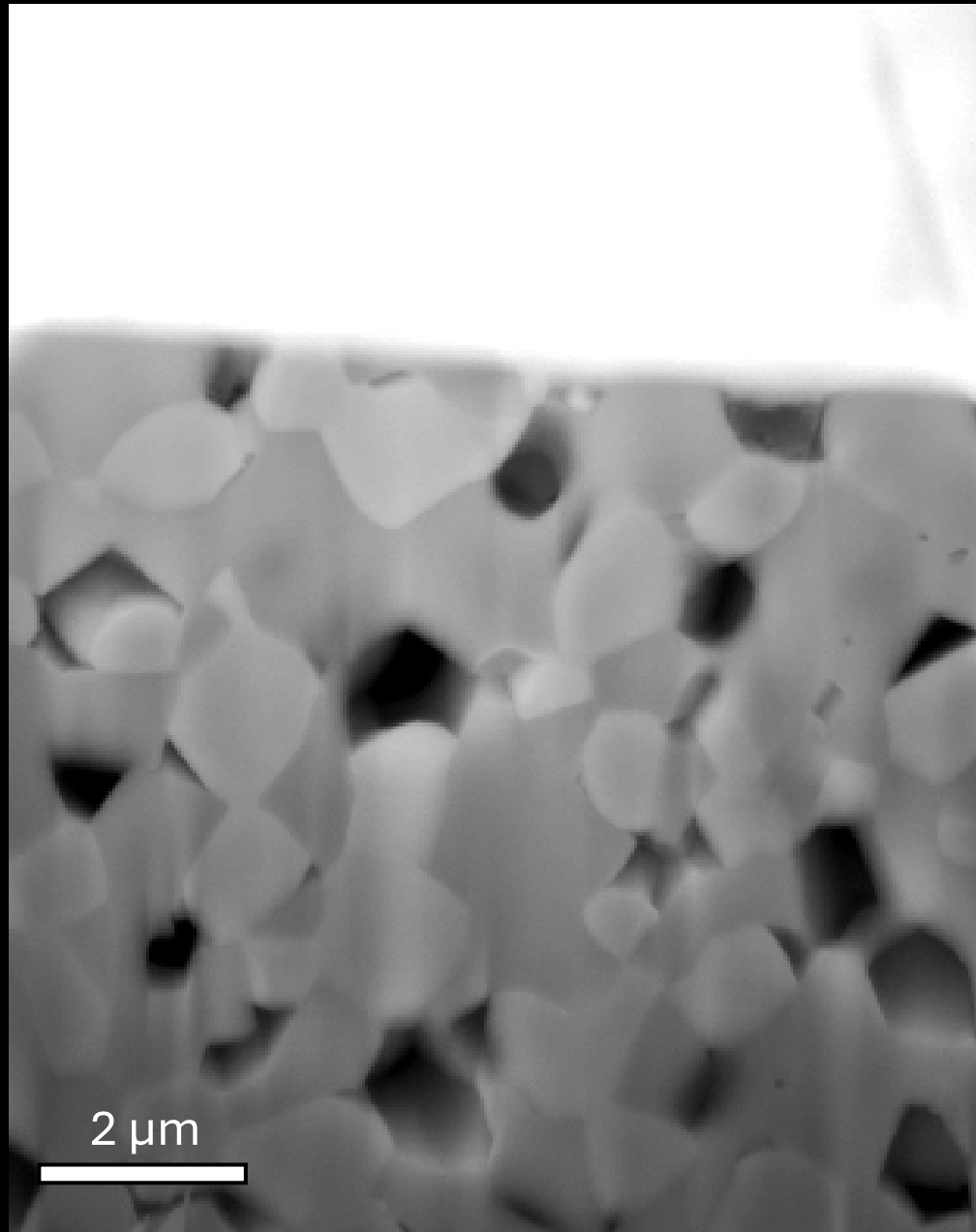
Ion column



Hydra PFIB



3D EDS of Ni-YSZ anode in SOFC

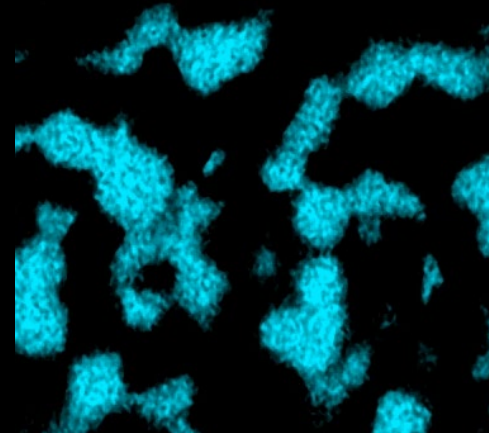


Slicing/Imaging:

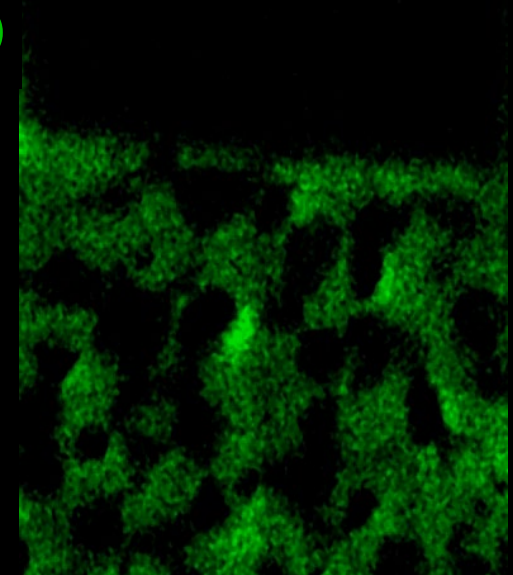
15 kV, 50 pA
2560 × 1920 px
50 nm

Processing: Vertical destriping
Stack alignment

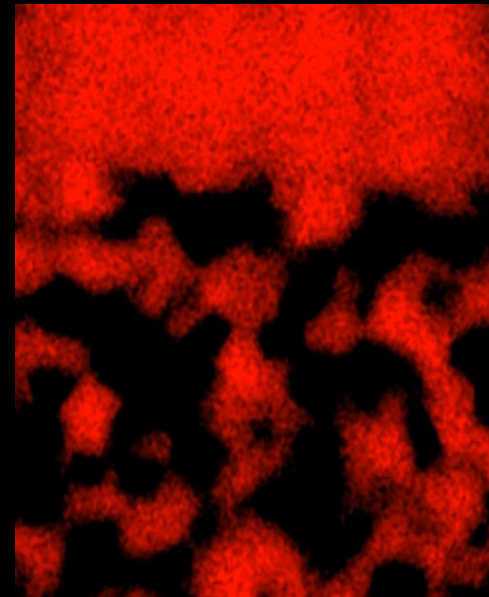
Ni



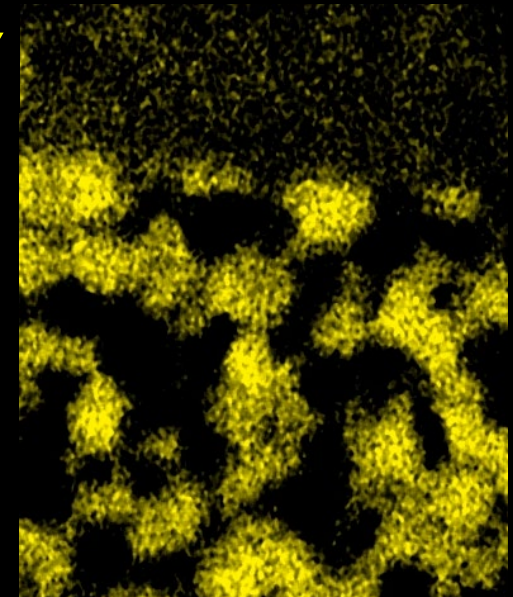
O



Zr



Y

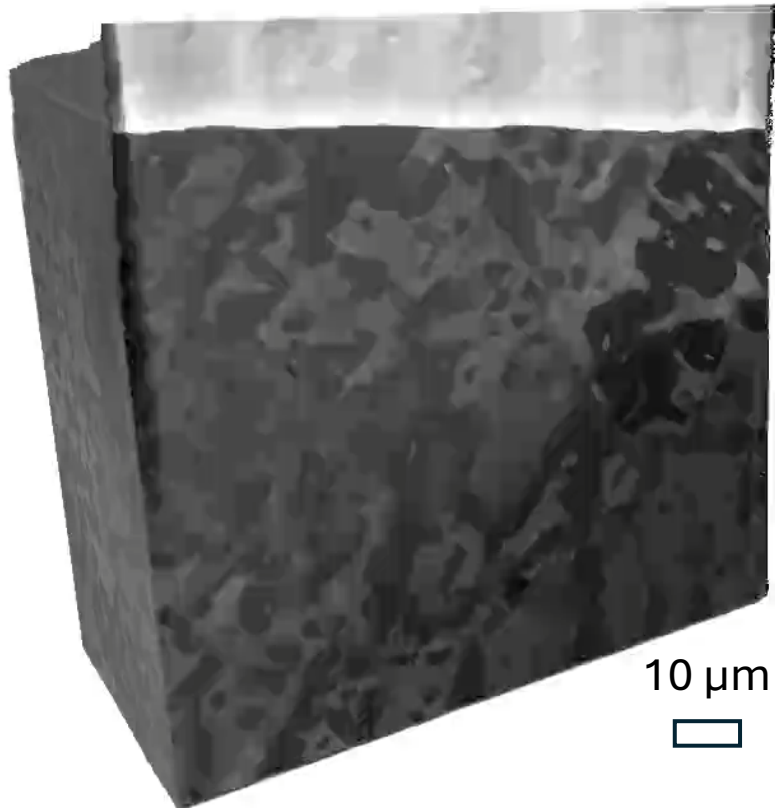


EDS mapping: 5 μs dwell time
7 passes
1048 × 765 px

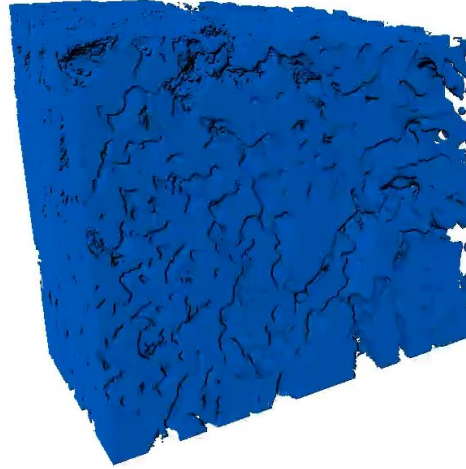
Processing: Gaussian filter
Median filter

3D EDS of Ni-Cr-Nb Superalloy

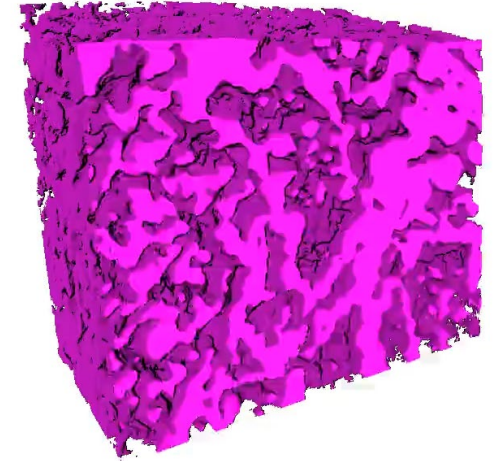
BSE



Cr



Ni



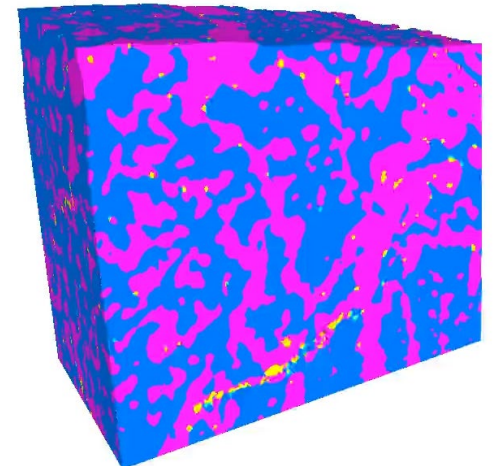
Nb



Ni

Cr

Nb



Segmentation/visualization credit: Yu Wen

Thank you for your Attention

Paul Smeets, PhD

NUANCE TEM/FIB Manager / Research Assistant
Professor

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Email: paul.smeets@northwestern.edu



NUANCE
Learning Exchange

Seminars
Workshops
Demos

Optimized 3D FIB-SEM Workflows for
Multimodal Materials Characterization and
VolumeEM: Spin Mill Method

DATE / TIME / LOCATION

Seminar:

August 18, 2026
Ford Hive, Room #2350
9:00 - 11:00 AM

In-Person Demos:

August 18-20, 2026
Tech AG98



[VIEW SCHEDULE
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curr	det	mode	mag	tilt	WD	HFW
kV 0.80 nA	TLD BSE	4 144 ×	52.0 °	4.1 mm	50.0 μm	

20 μm

NUANCE Helios 5 Hydra CX PFIB