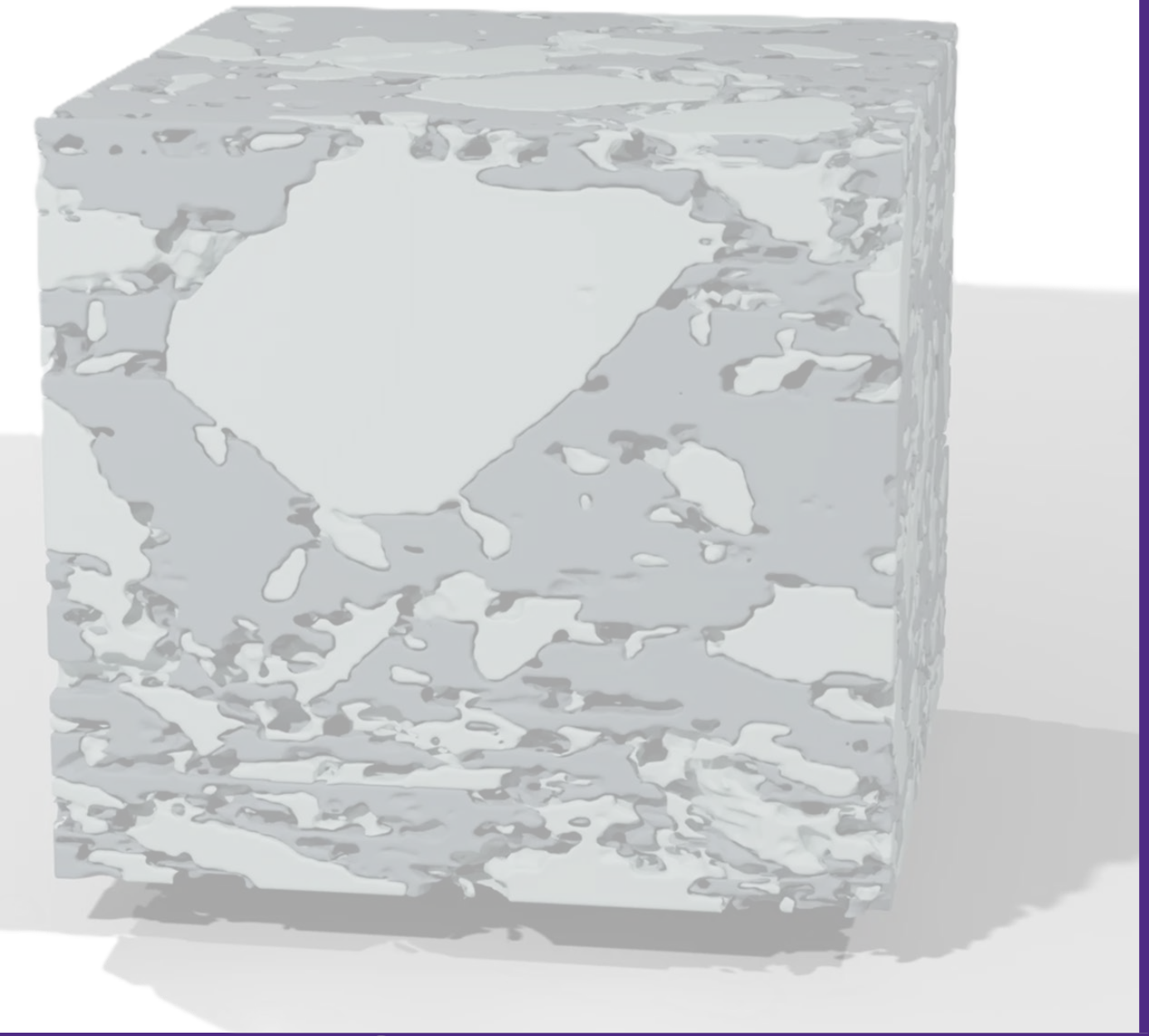

Below the Surface

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

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1. Use a knife to cut away a piece
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Instead of a

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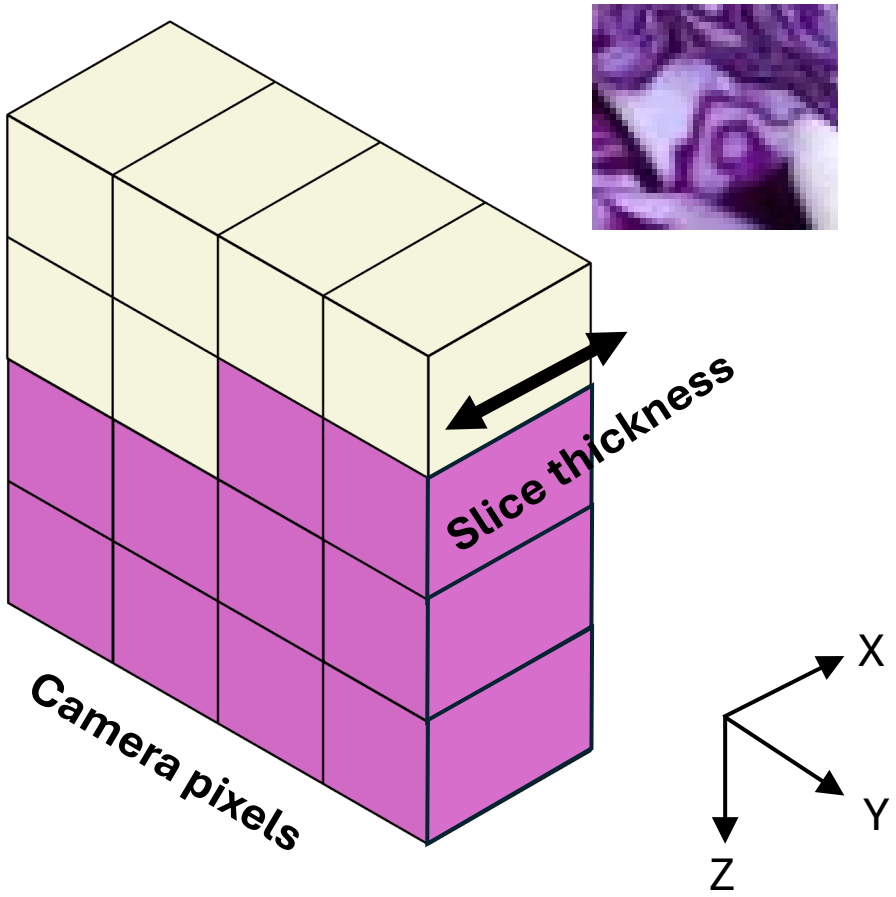
From Pixels (2D) to Voxels (3D)



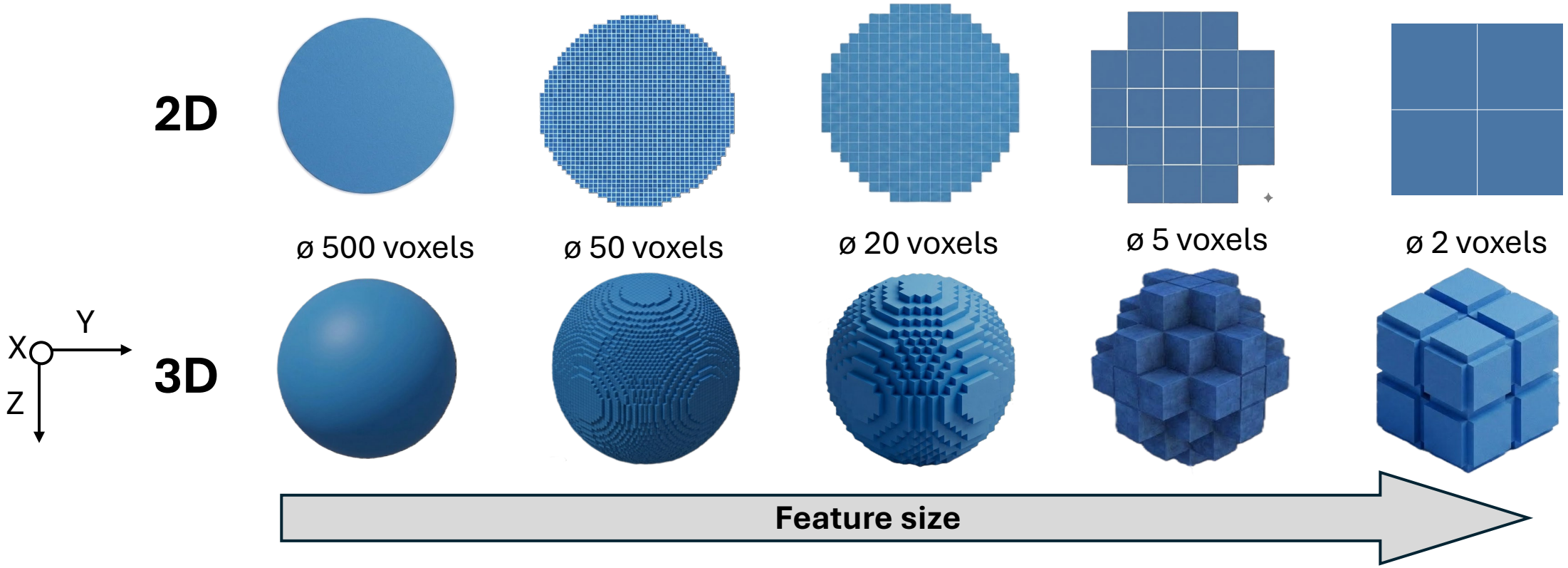
Important considerations for 3D acquisition

- **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↑**

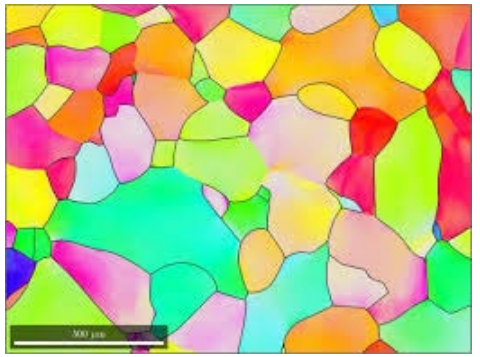


High measurement precision / high fidelity

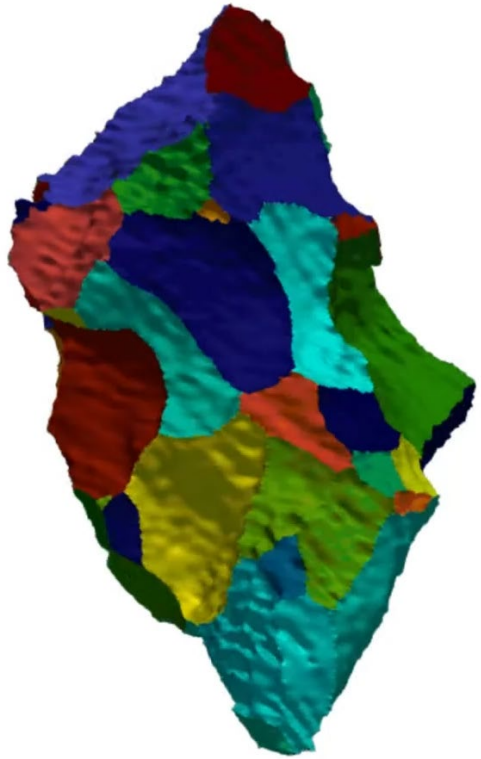
Low measurement precision / low fidelity

Why do we need 3D Materials Characterization?

- Failure analysis of buried features

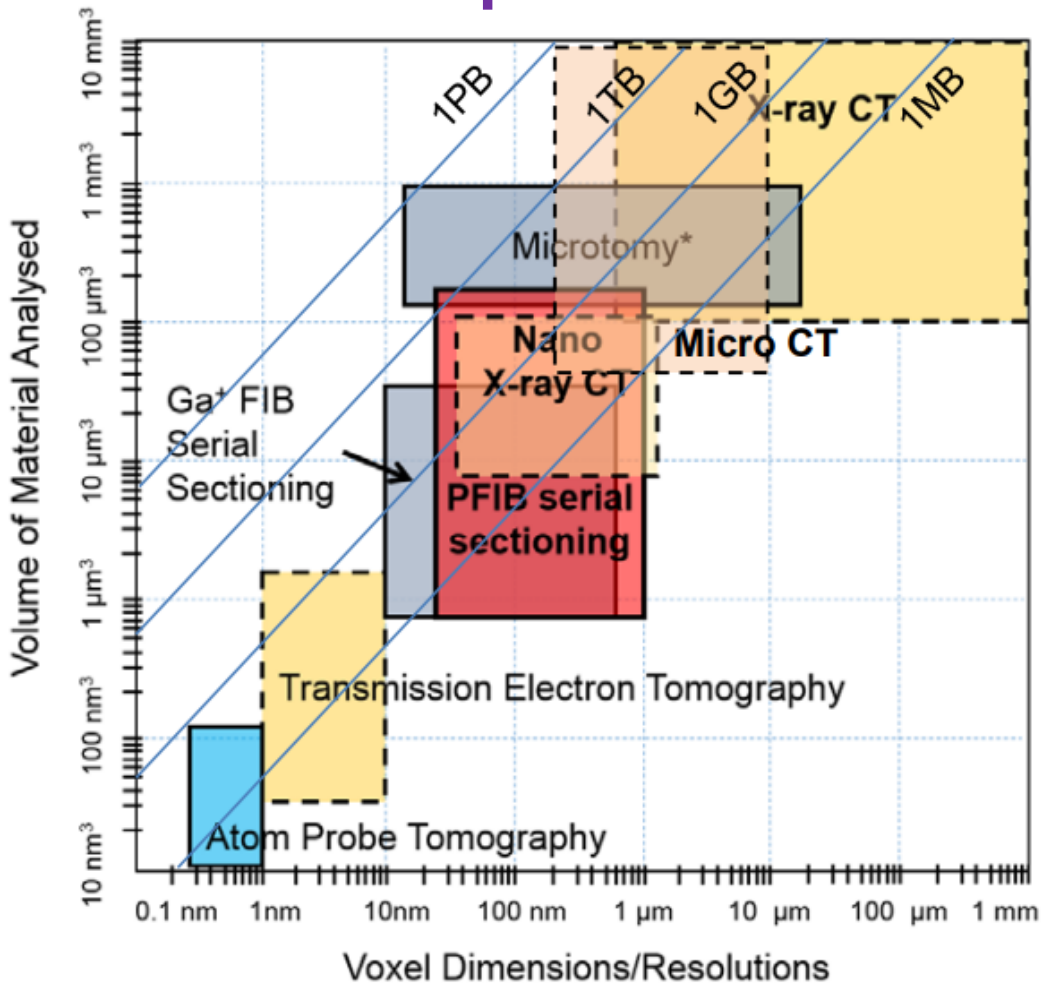


- Quantification of features
  - **Materials Science:** Grain size analysis, thickness deviations in layered films, porosity fractions, localizing subsurface defects…
  - **Life Sciences:** Revealing cellular ultrastructure, mapping neural circuits…



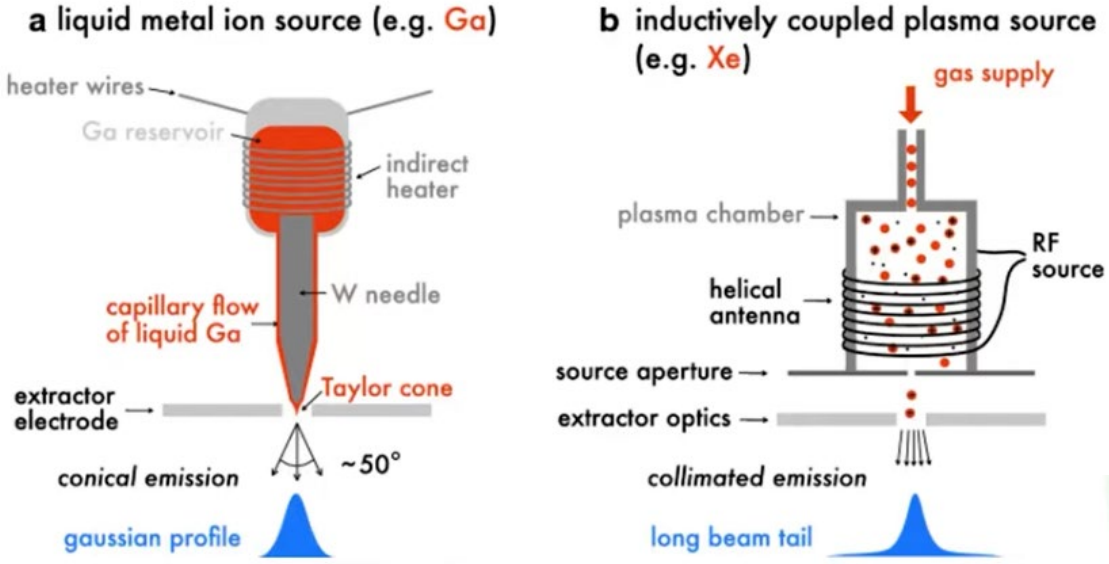
3D (P)FIB-SEM vs other 3D Techniques

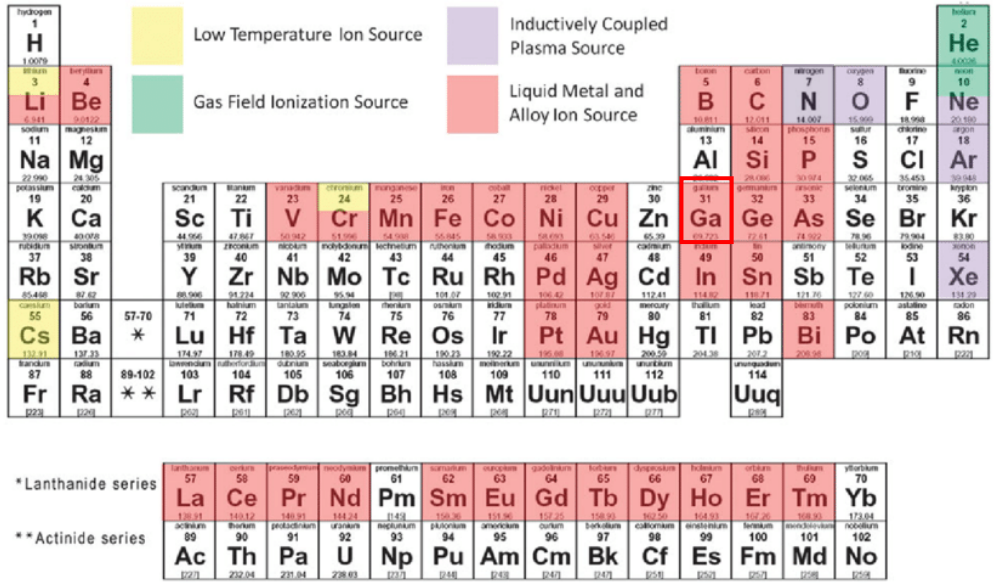
- Generally, when the amount of material analyzed increases, the spatial resolution drops
- (P)FIB-SEM tomography provides a good balance between bulk volume and spatial resolution
- However: destructive technique



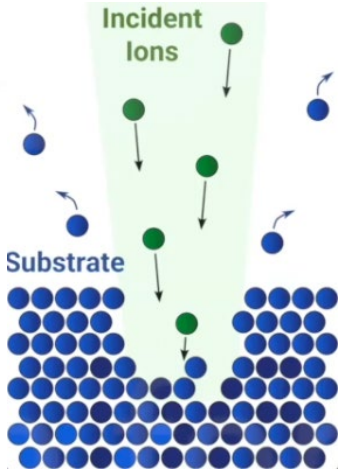
How is Matter Removed in (P)FIB-SEM?

- Ion sources: LMIS vs inductively coupled plasma source



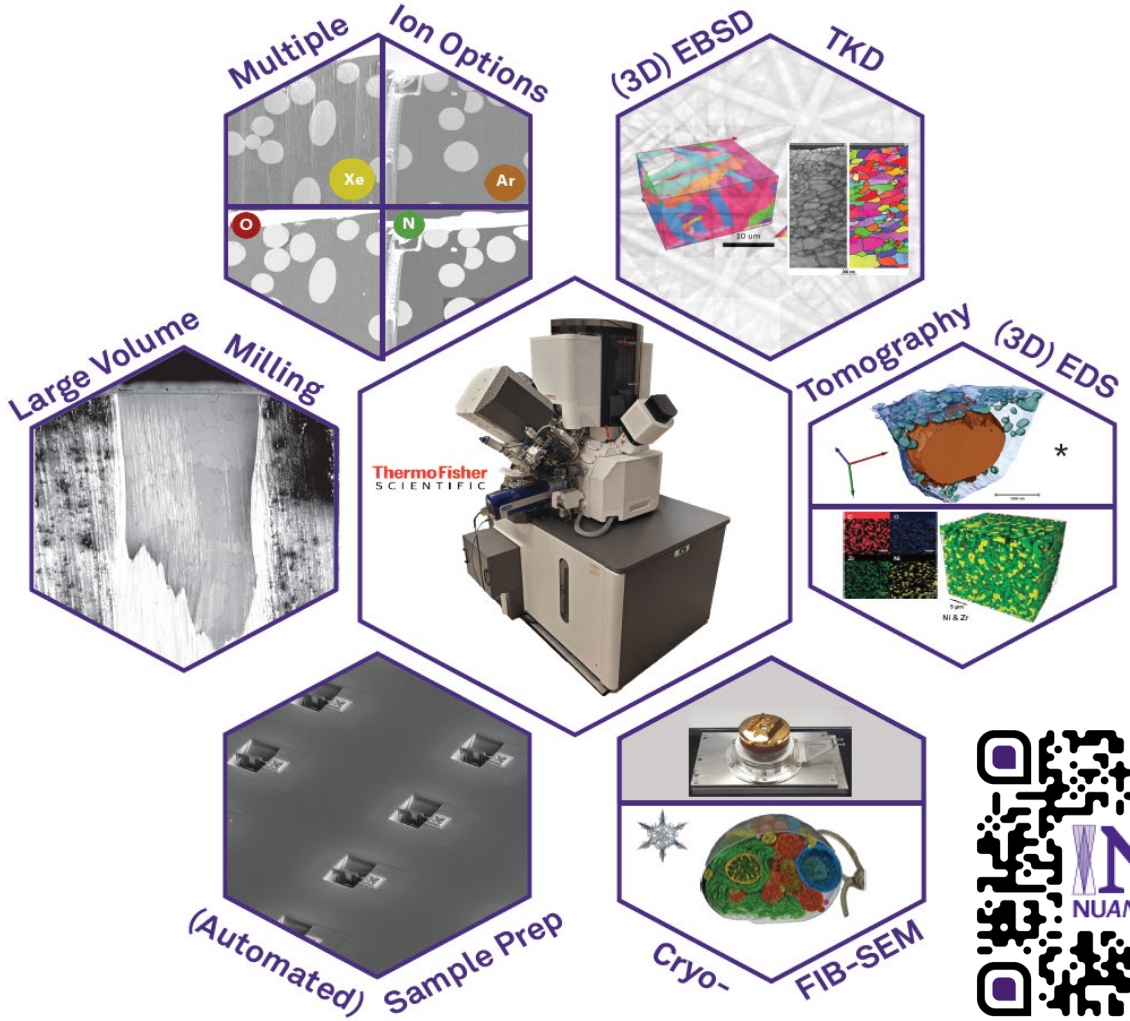


- Milling (sputtering)



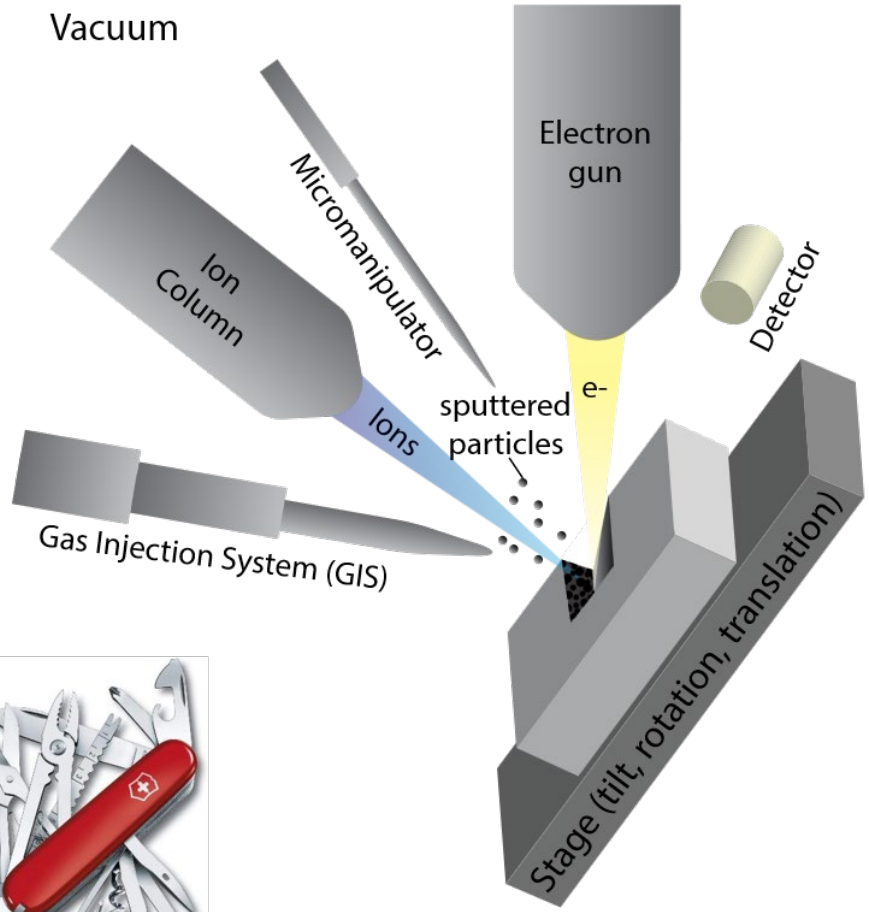
- 5 ion sources
  Ga, Xe, Ar, O, N

Helios 5 Hydra CX Plasma DualBeam



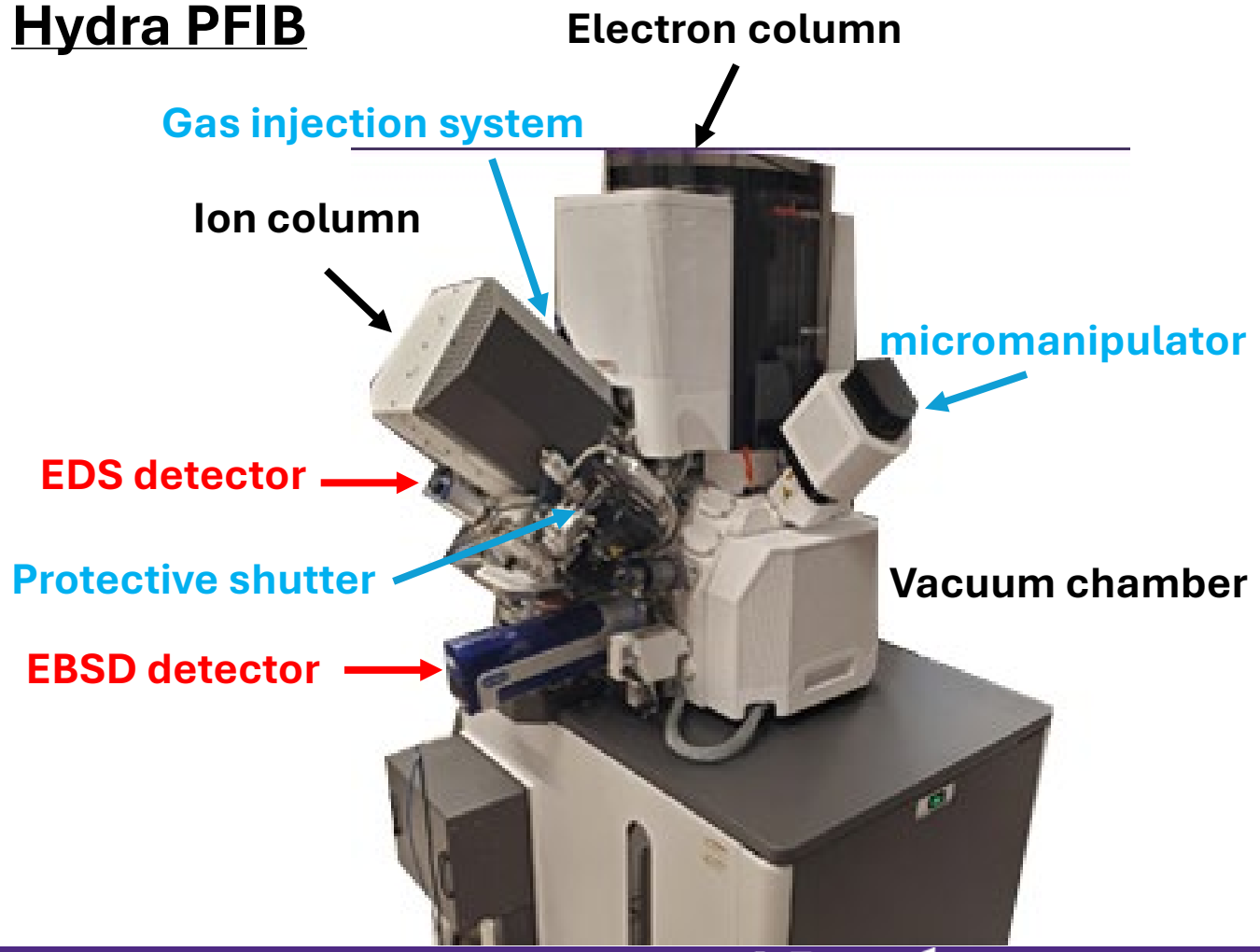
Helios 5 UC Ga DualBeam

Configuration of the (P)FIB Instrument



"Swiss Army Knife"

Hydra PFIB



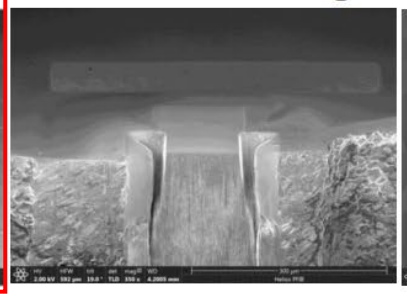
FIB-SEM Tomography Configurations

1. Default – In Bulk

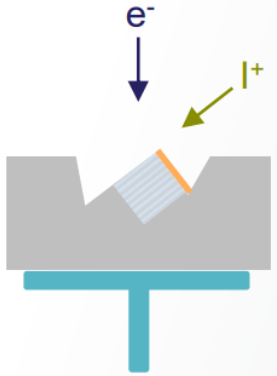
Stage tilt: 52°

2. Default – At Edge

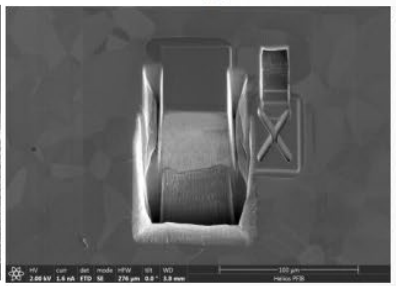
Stage tilt: 52°



3. Zero Degree Tilt



Stage tilt: 0°

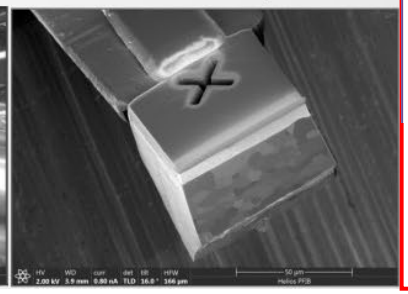


4. Pre-Tilted – At Edge

Stage tilt: 16°
Pre-tilt: 36°

5. Chunk Liftout (Pre-Tilted)

Stage tilt: 16°
Pre-tilt: 36°



6. Spin Mill (planar milling)

NUANCE
Northwestern University Atomic and Nanoscale Characterization Experimental Center

NUANCE Learning Exchange
Seminars
Workshops
Demos

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

DATE / TIME / LOCATION

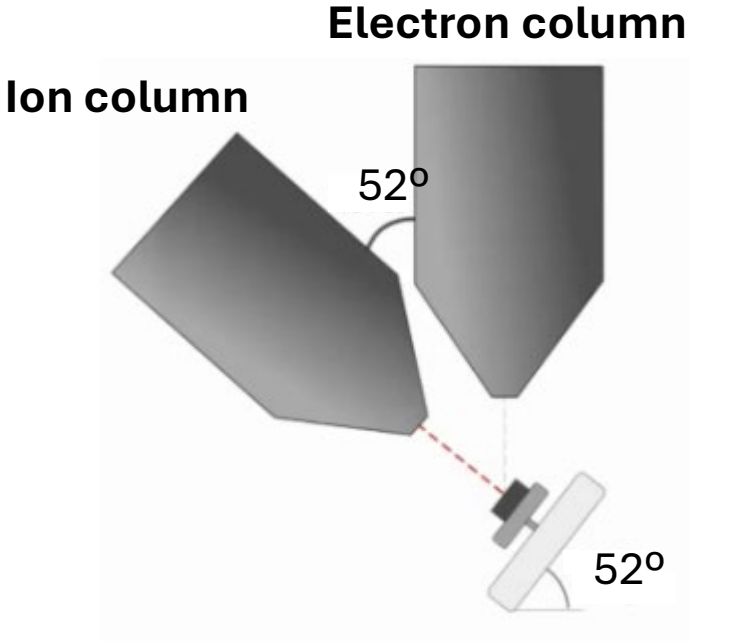
Seminar:
August 18, 2026
Ford Hive, Room #2350
9:00 - 11:00 AM

In-Person Demos:
August 18-20, 2026
Tech AG98

VIEW SCHEDULE REGISTER

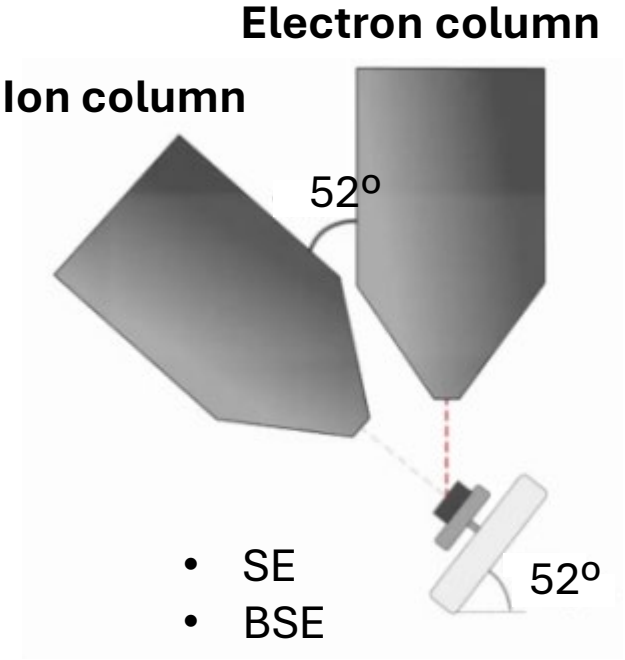
In-Bulk Tomography Configuration

(P)FIB Milling



- Choice of 5 ion sources

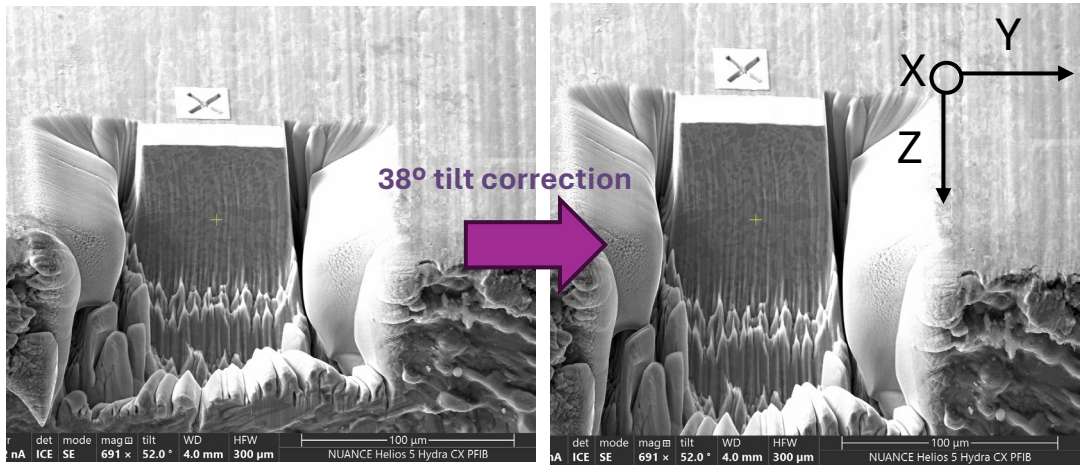
SEM Imaging



- SE
- BSE
- Hydra: 5 detectors
- Helios: 6 detectors
- Mix and match

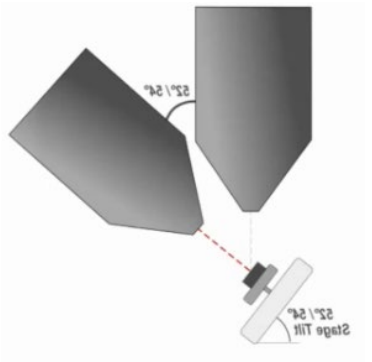
Sample does not move (or minor movements) between imaging and milling positions (faster)

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



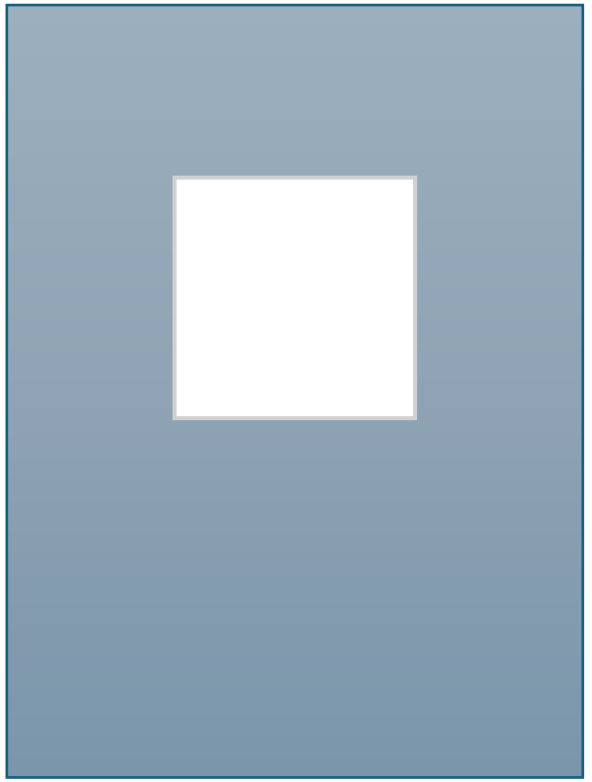
ROI Preparation for In-Bulk Tomography

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

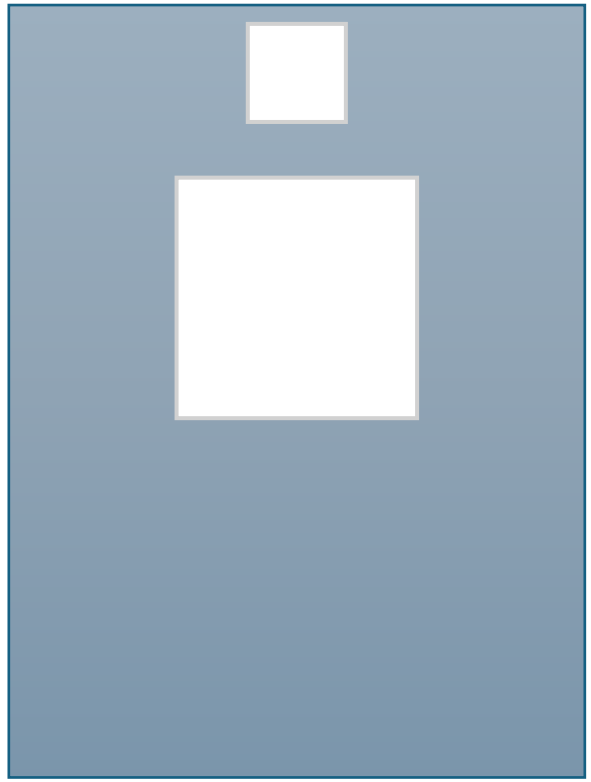
As viewed by FIB:



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 um height, ~ 4-5 um away from first protective cap
   - Tracking marker to position for slice milling

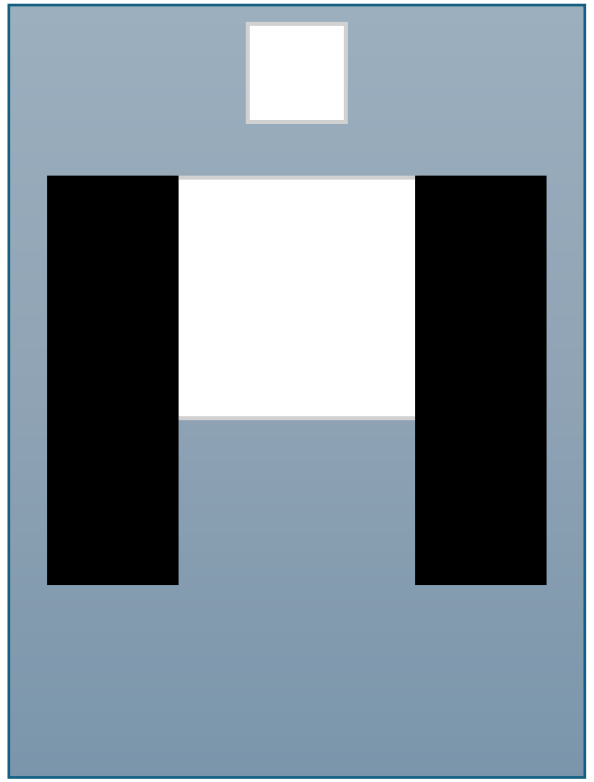
As viewed by FIB:



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

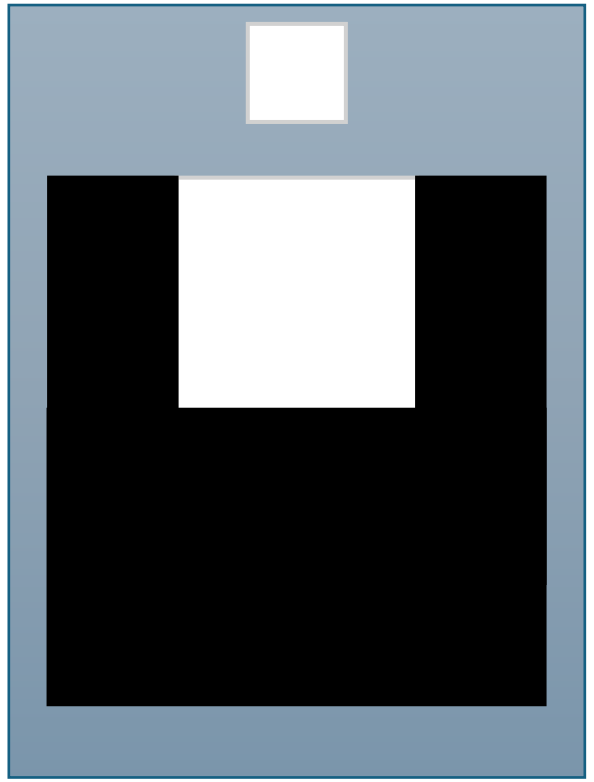
As viewed by FIB:



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)

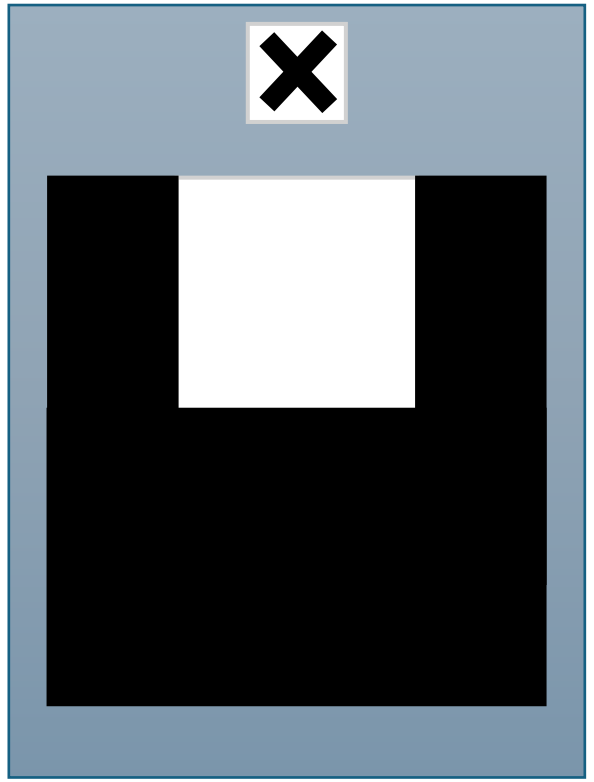
As viewed by FIB:



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

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 µA

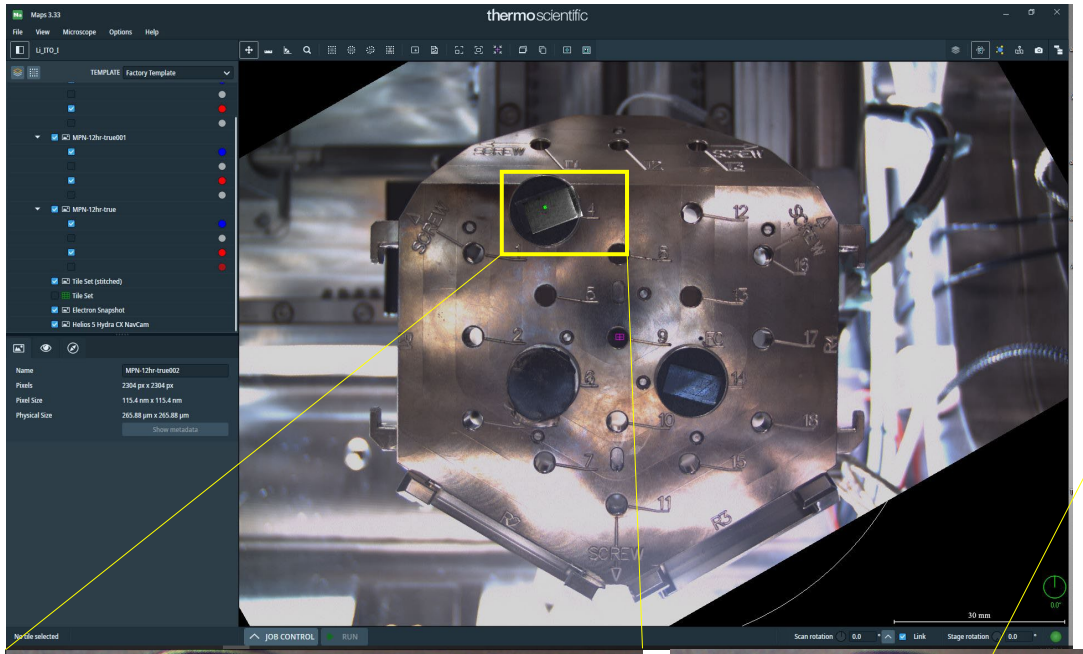
8 – 24+ hrs

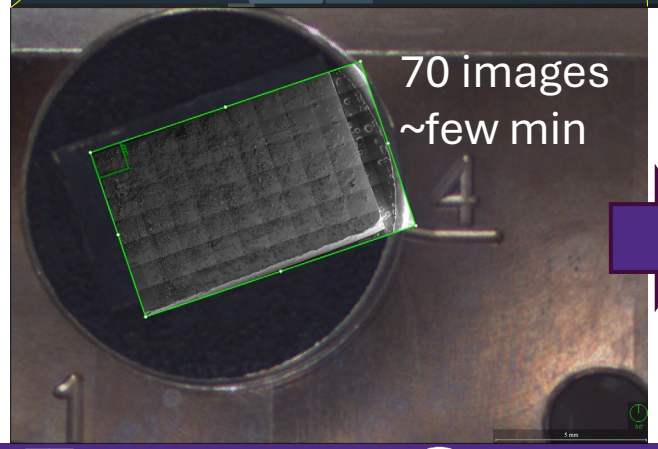
Correlative (P)FIB Tomography Workflows

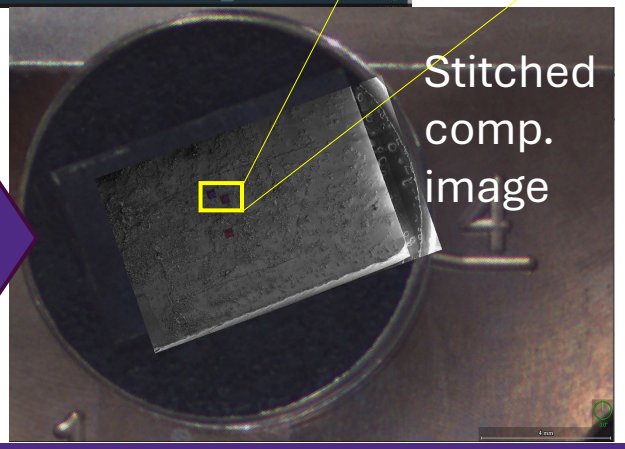
- Other techniques besides (P)FIB-SEM
  - Example: Fluorescence imaging / FIB-SEM tomography
- Within the (P)FIB-SEM
  - Examples: 3D EDS of solid oxide fuel cell and superalloy

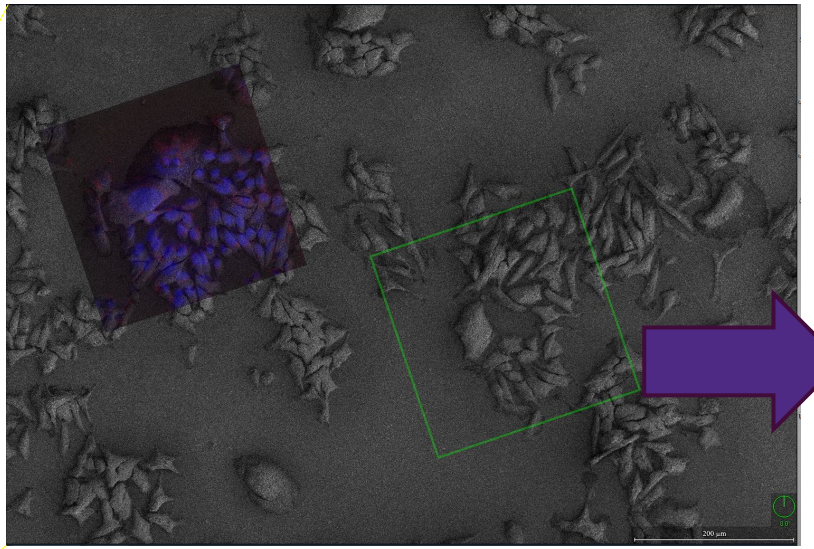
Correlative Workflows – Fluorescence / FIB-SEM Tomo

MAPS Correlative Software

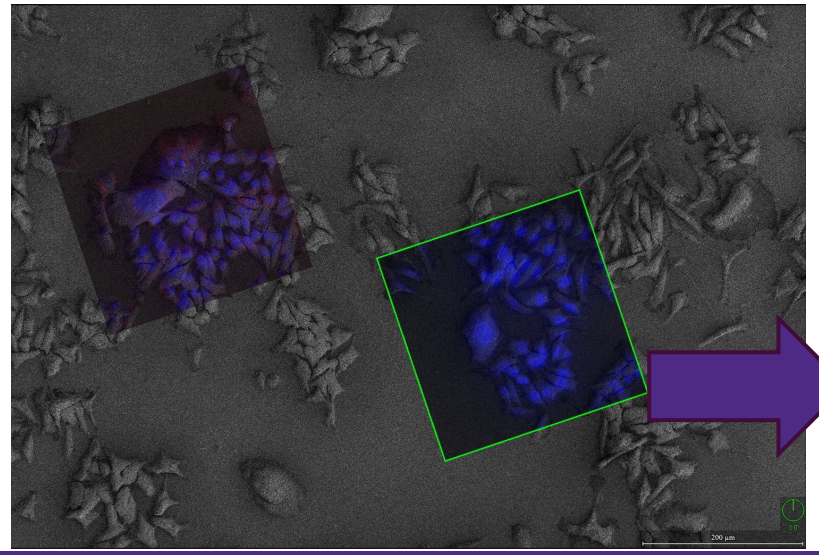








No overlaid fluorescence data

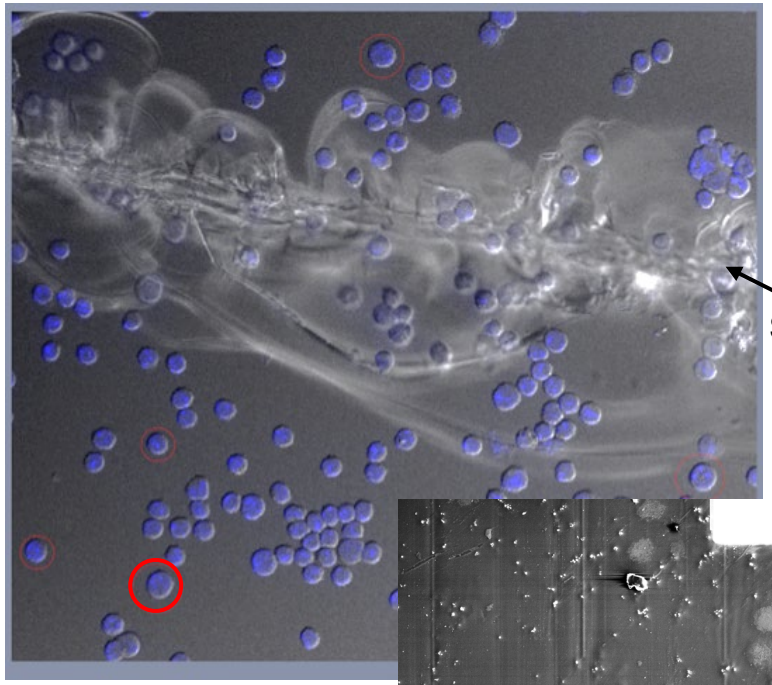


Overlaid fluorescence data (**DAPI**)

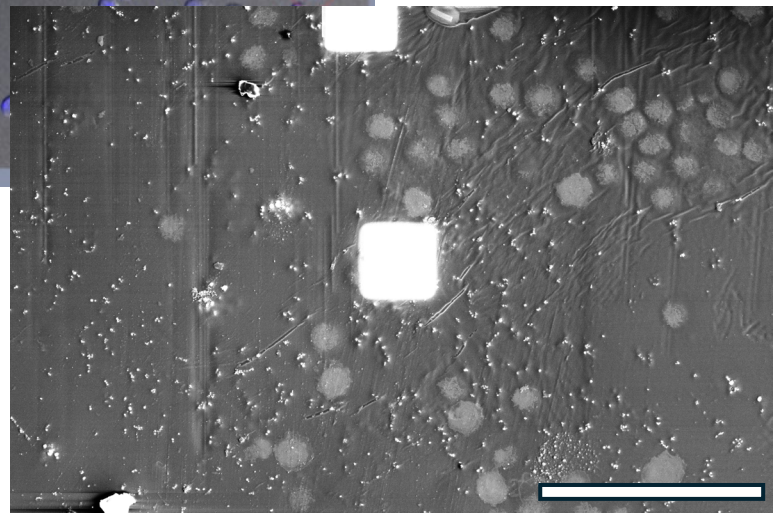
Correlative Workflows – Fluorescence / FIB-SEM Tomo

Bone-marrow derived cells from Mouse

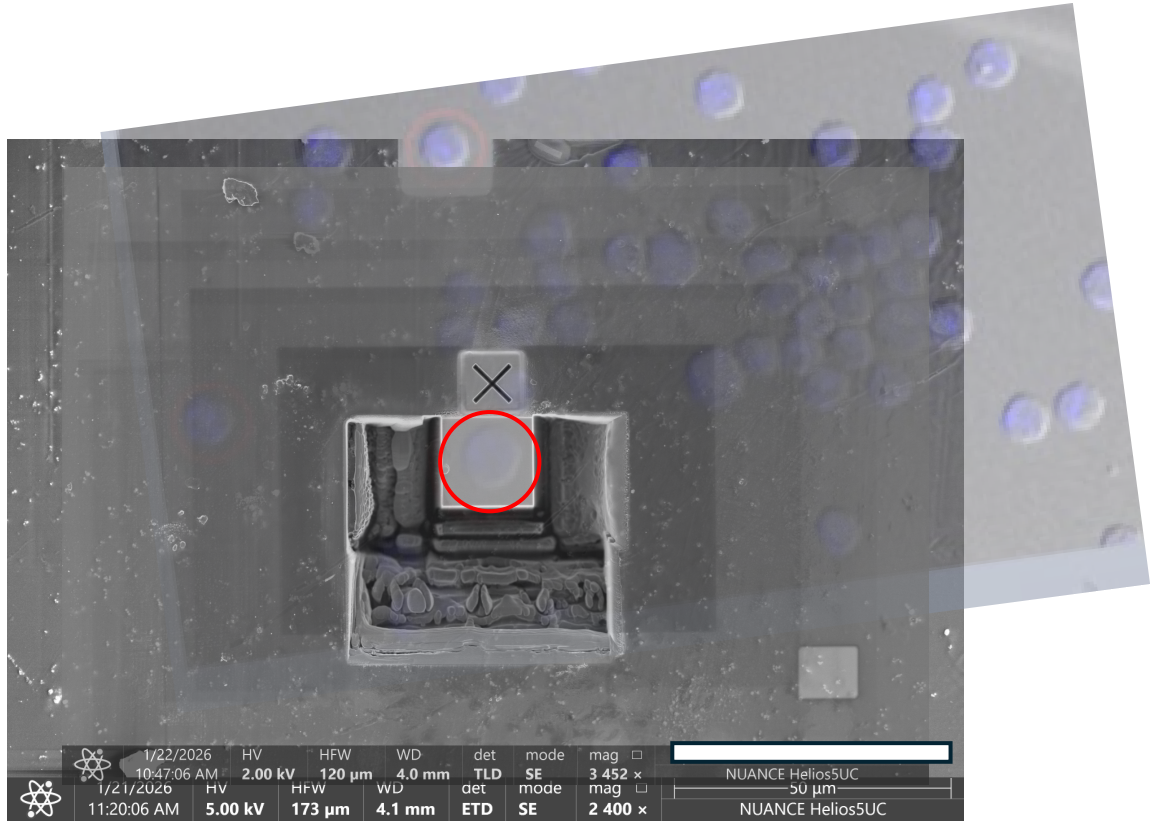
Fluorescence Image



SEM Image



Composite Fluorescence/SEM Image

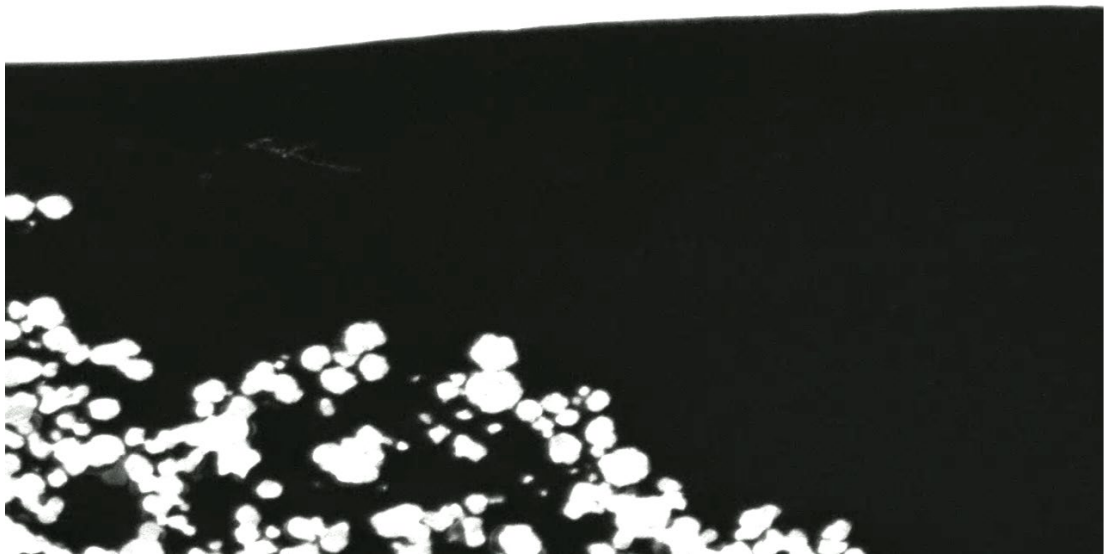


ROI before FIB-tomo acquisition

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

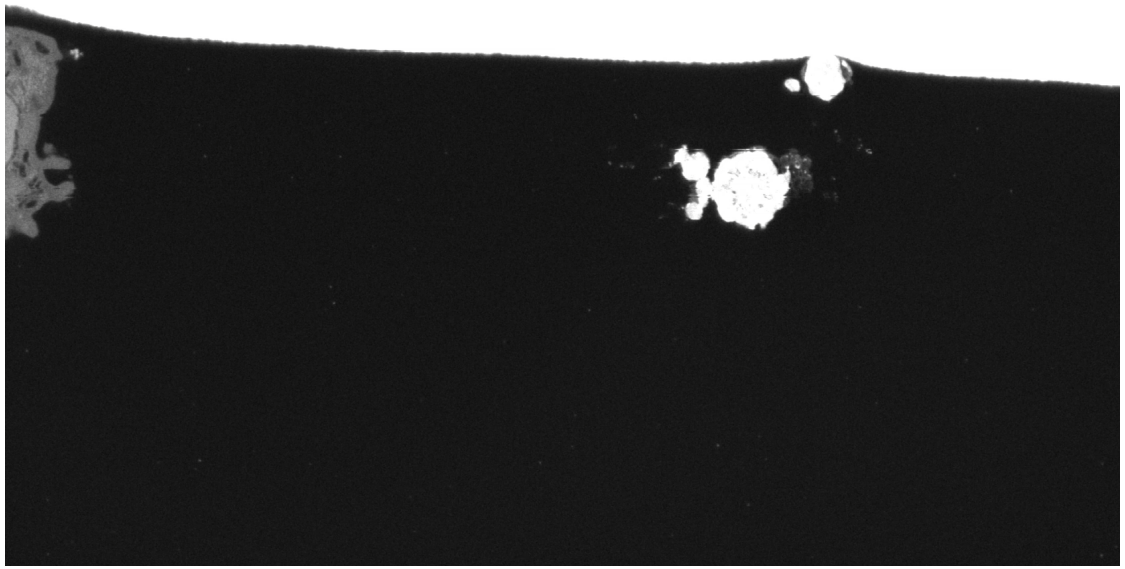
Correlative Workflows – Fluorescence / FIB-SEM Tomo

Bone-marrow derived cell



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

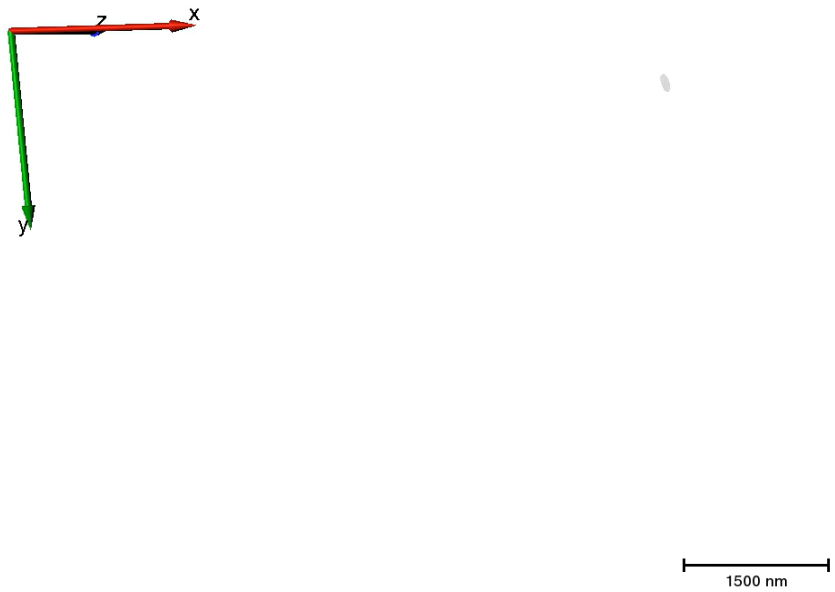
Spleen-derived cell



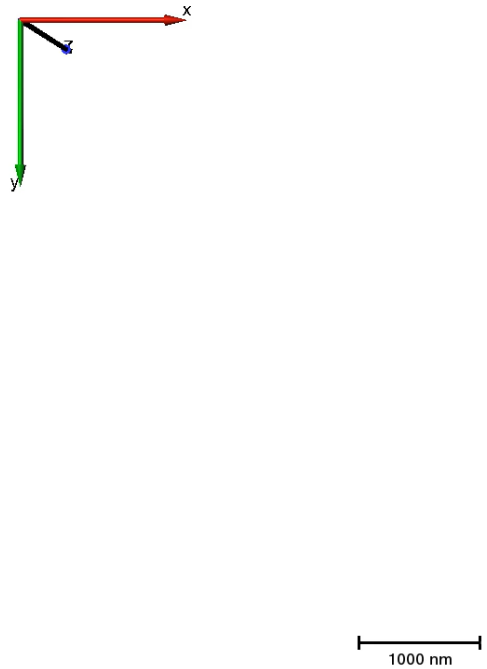
Slicing: 5nm thickness; **1322** slices total (~**60 fps**)
Raw images: BSE 5nm pixel size; 2649 * 2300 pixels

Visualization of Targeted Cells

Bone-marrow derived cell



Spleen-derived cell



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%

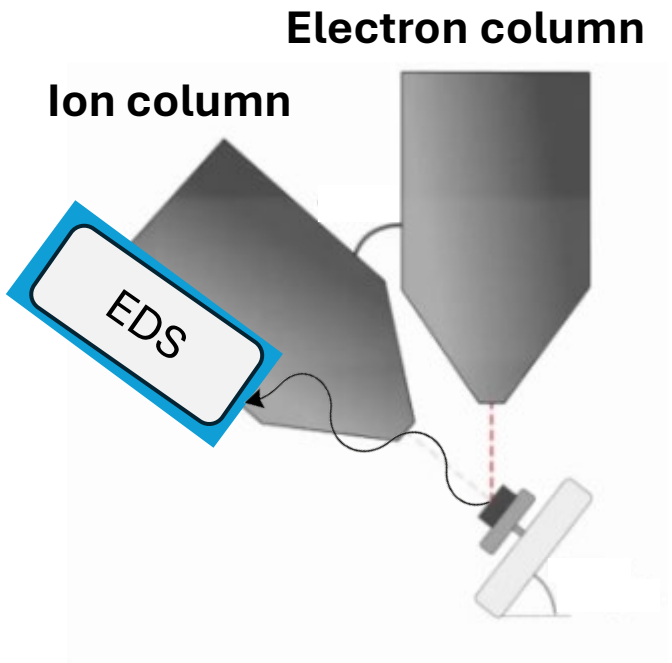
Bone-marrow derived cell

Submitted *- Collaboration with 36 authors*

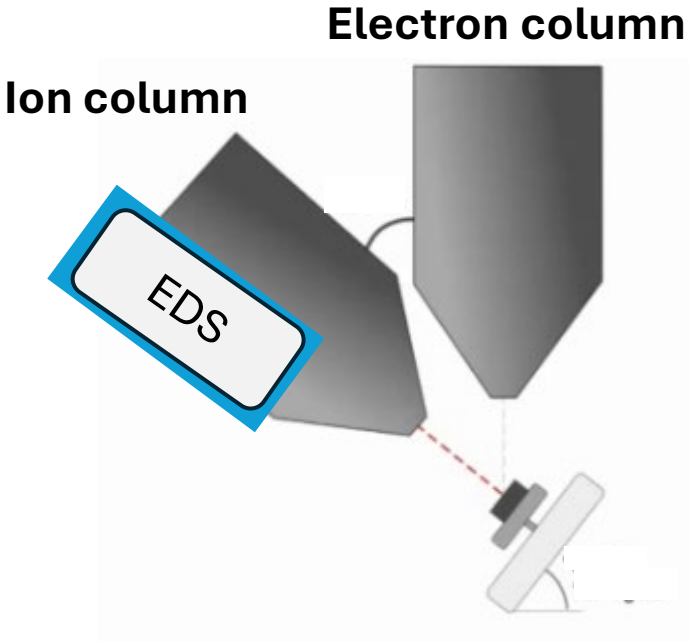
Correlative Workflows – 3D EDS

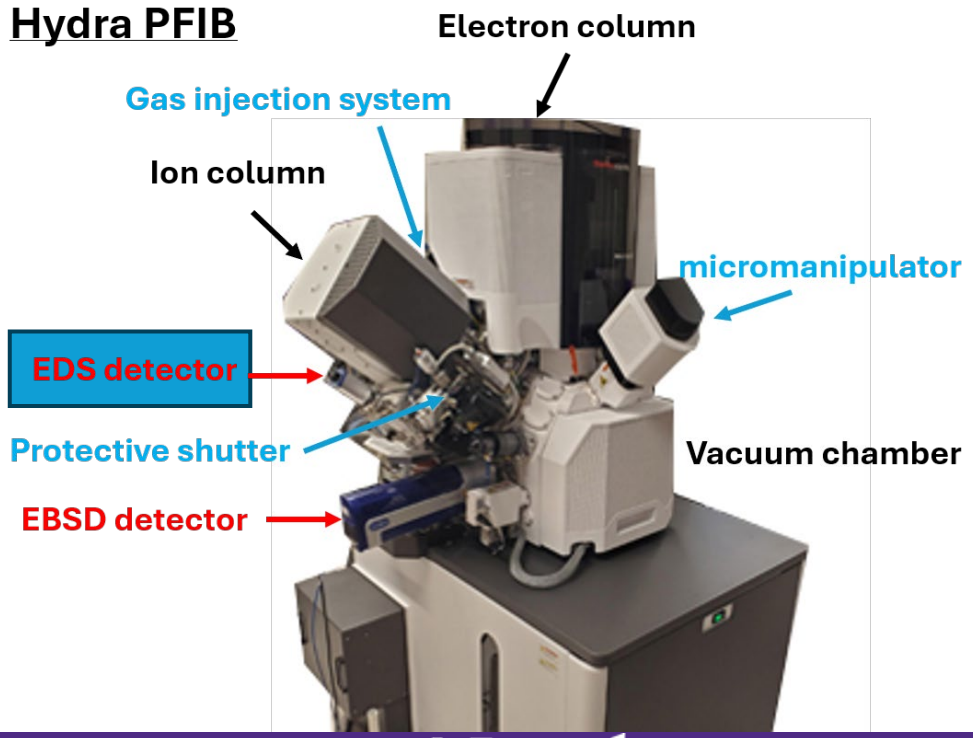
- Acquire EDS data from cross-section

EDS / SEM Imaging

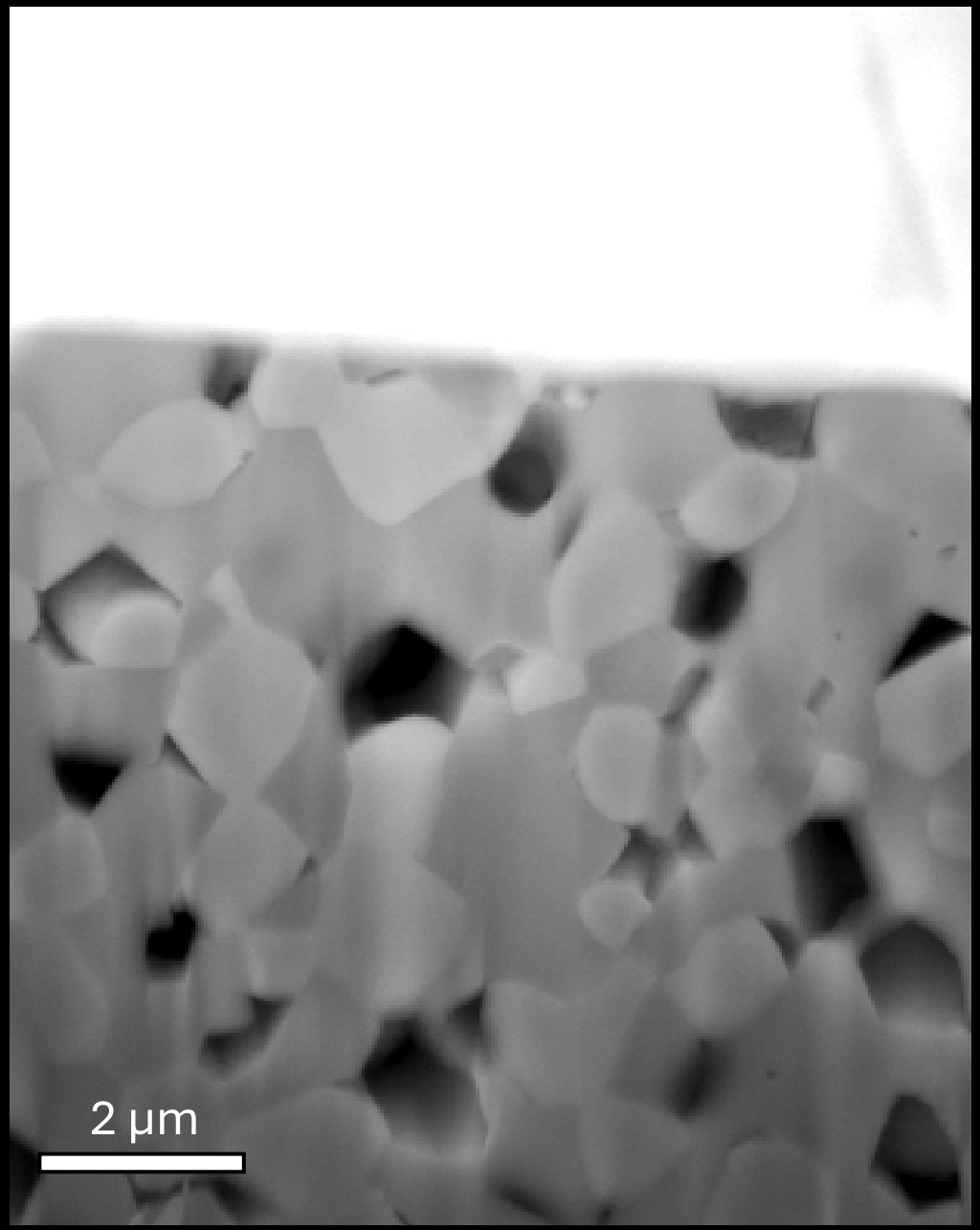


(P)FIB Milling

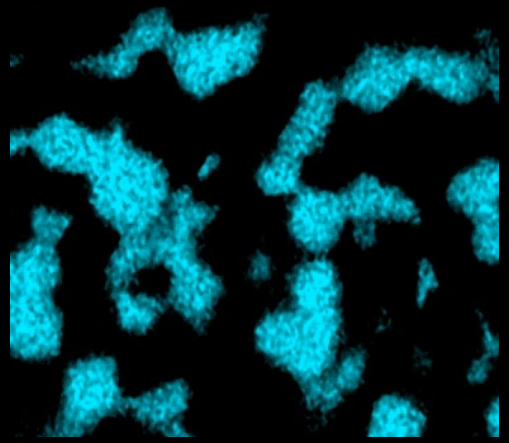




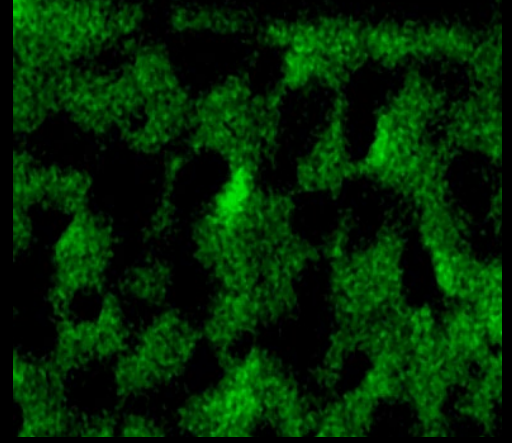
3D EDS of Ni-YSZ anode in SOFC



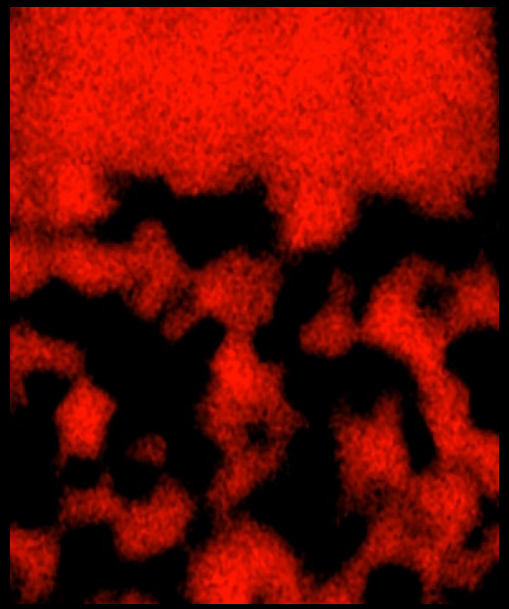
Ni



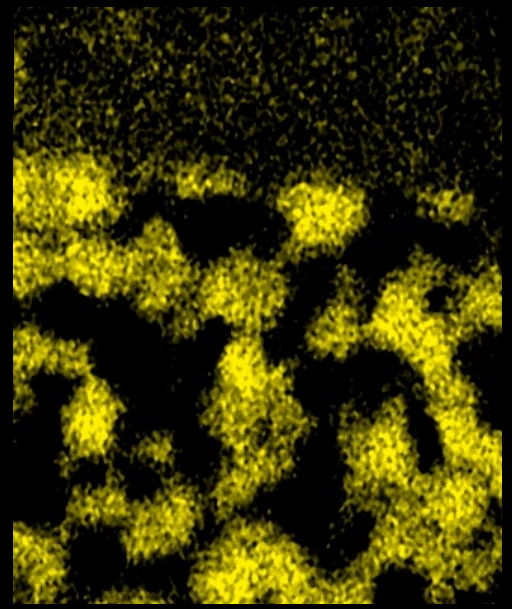
O



Zr



Y



Slicing/Imaging: 15 kV, 50 pA
2560 × 1920 px
50 nm

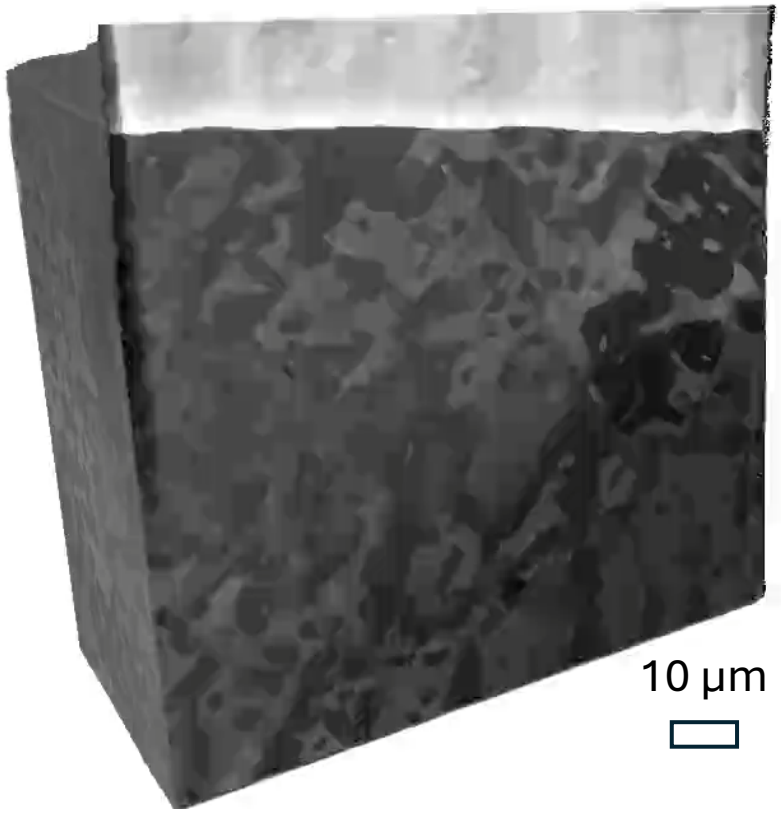
Processing: Vertical destriping
Stack alignment

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

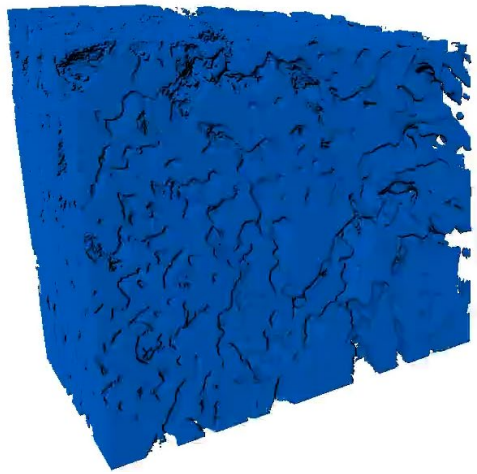
Processing: Gaussian filter
Median filter

3D EDS of Ni-Cr-Nb Superalloy

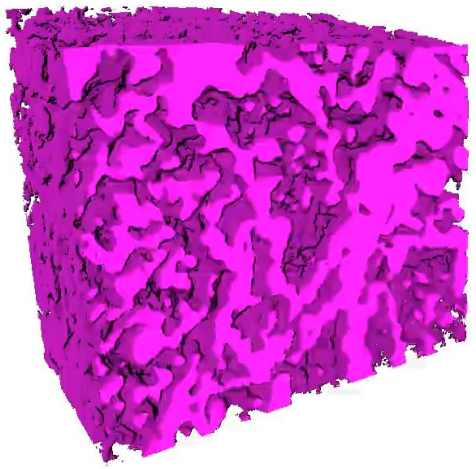
BSE



Cr

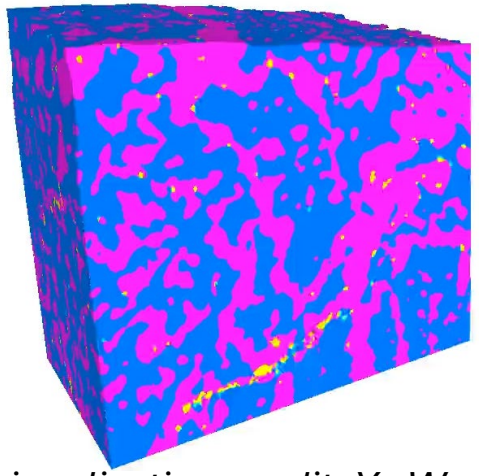


Ni



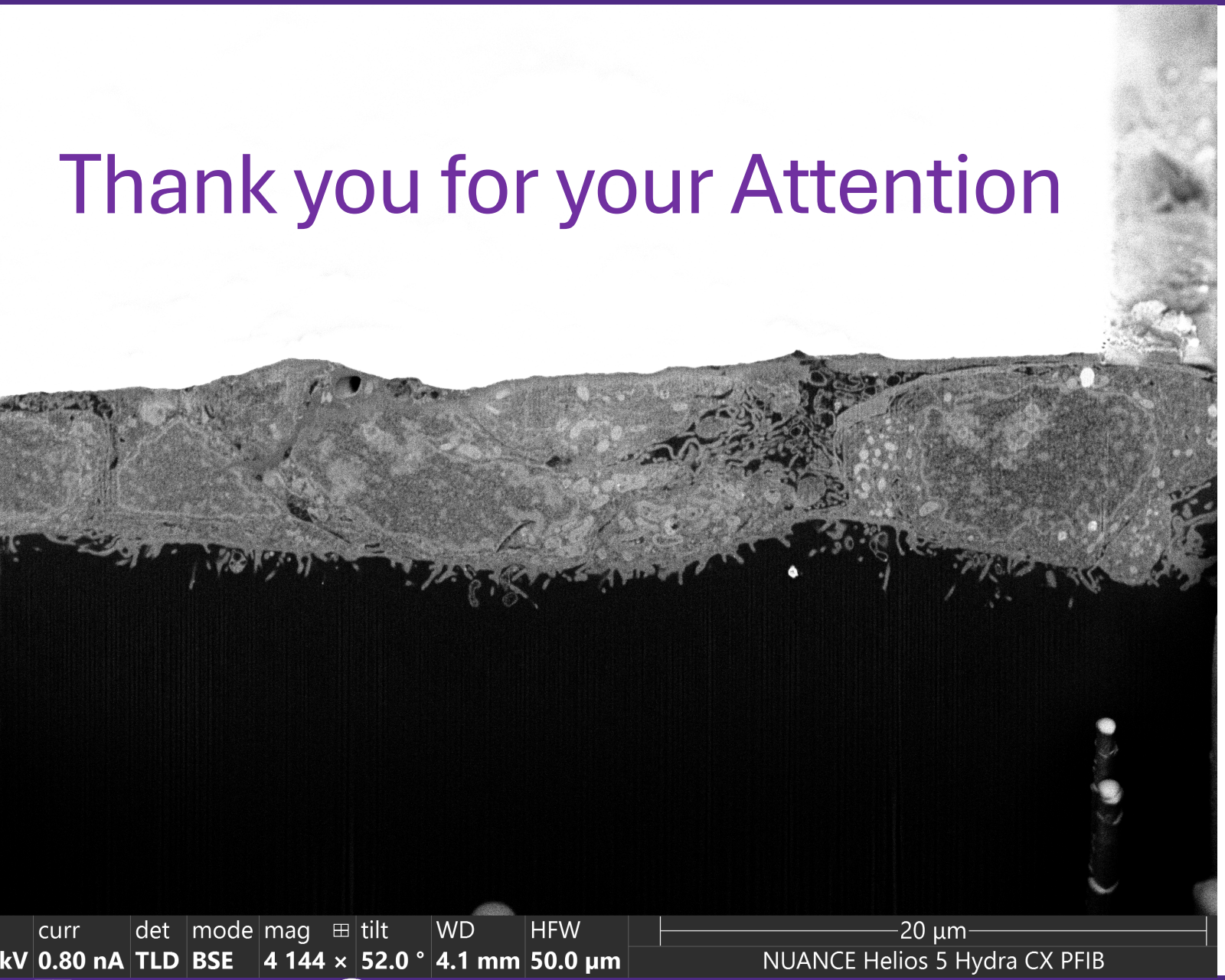
Nb

Ni
Cr
Nb



Segmentation/visualization credit: Yu Wen

Thank you for your Attention



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NUANCE
Northwestern University Atomic and Nanoscale Characterization Experimental Center

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