Microscopy in 3D

Identify, access, prepare and analyzes Samples within your Samples

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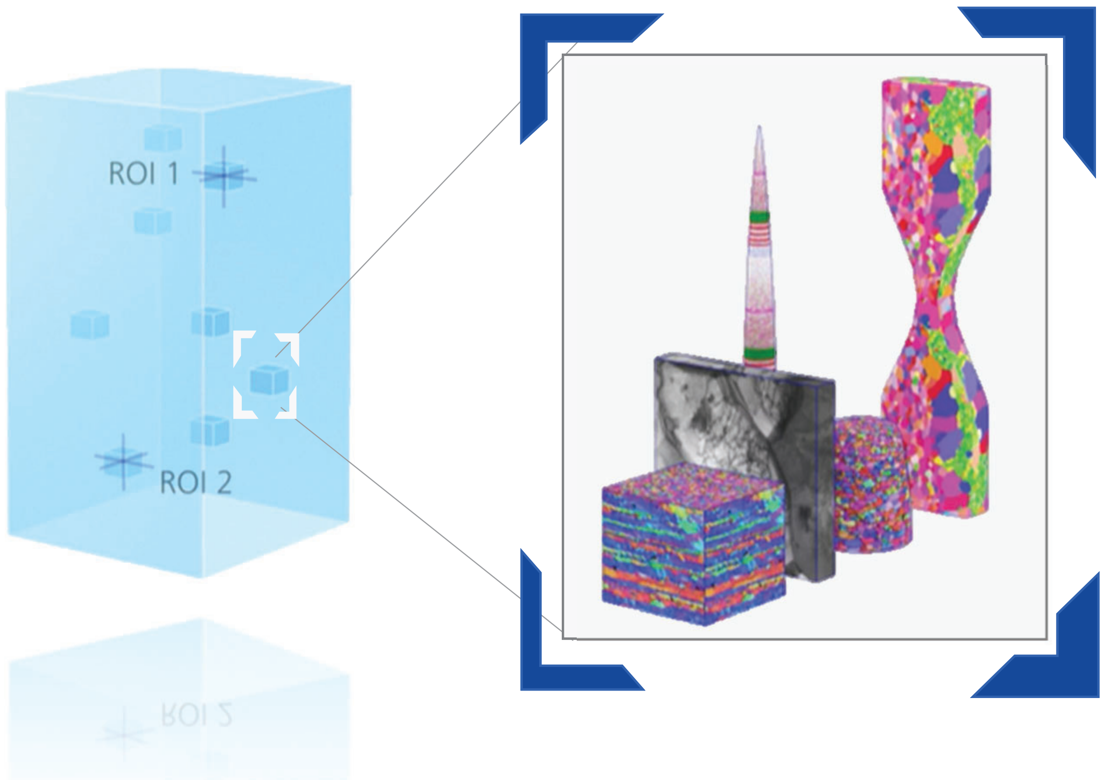
ZEISS Microscopy

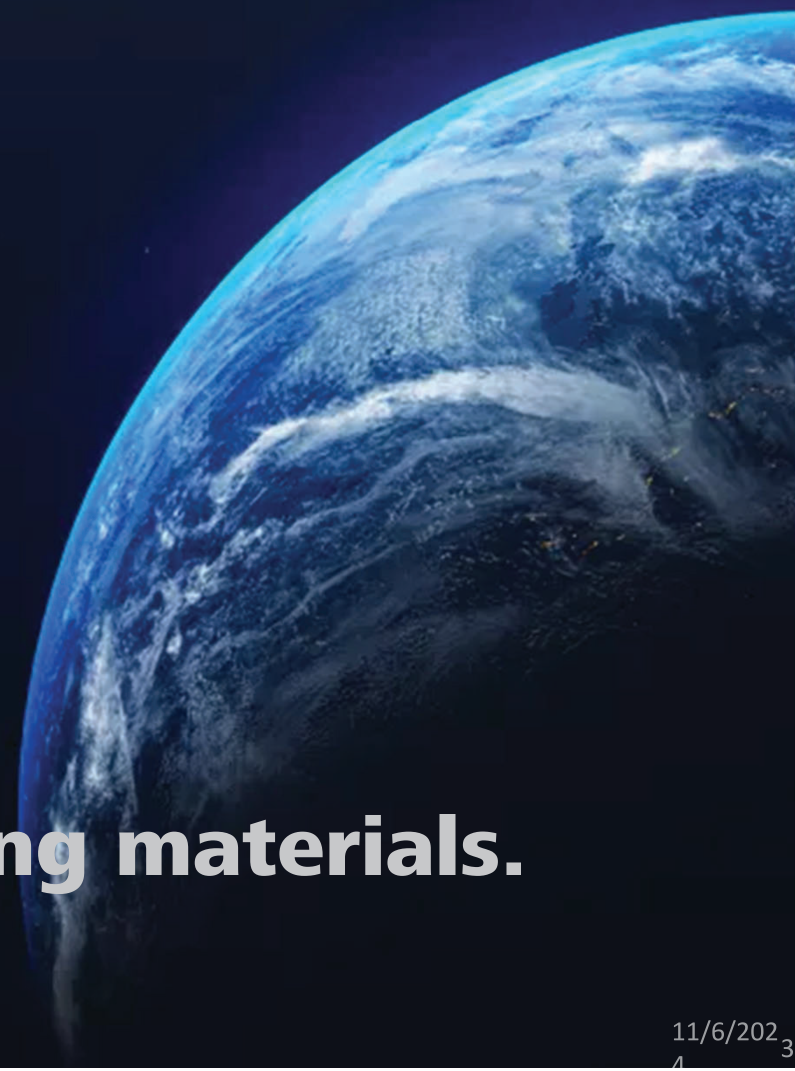
6 November 2024



ZEISS Sample in Volume Analysis workflow
Agenda

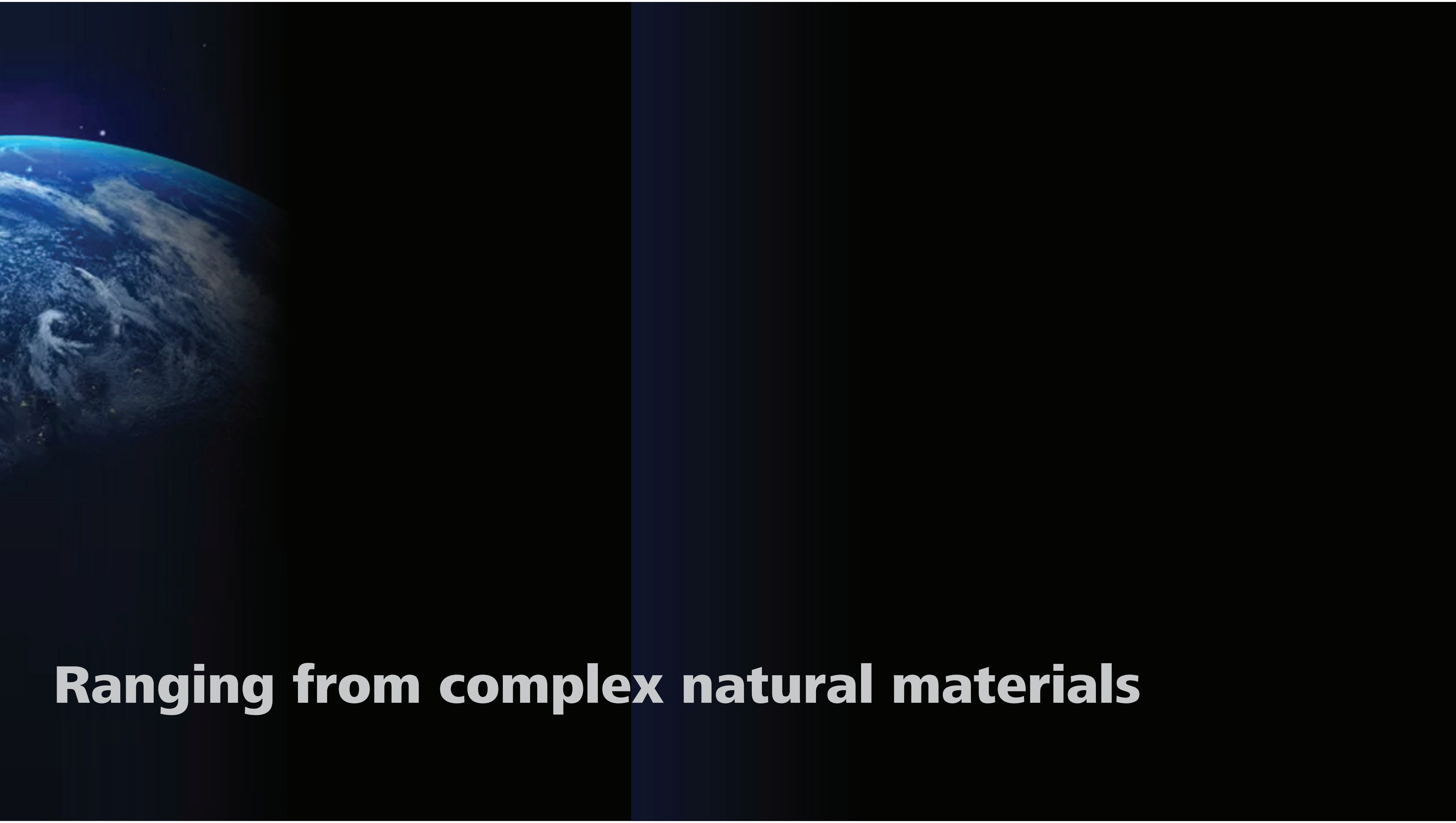
- ❑ **Motivation**
- ❑ Microscopy Challenge of Multiscale Materia
- ❑ Introduction to ZEISS Sample in Volume Ana
  workflow; What is it?
- ❑ Applications Use Cases

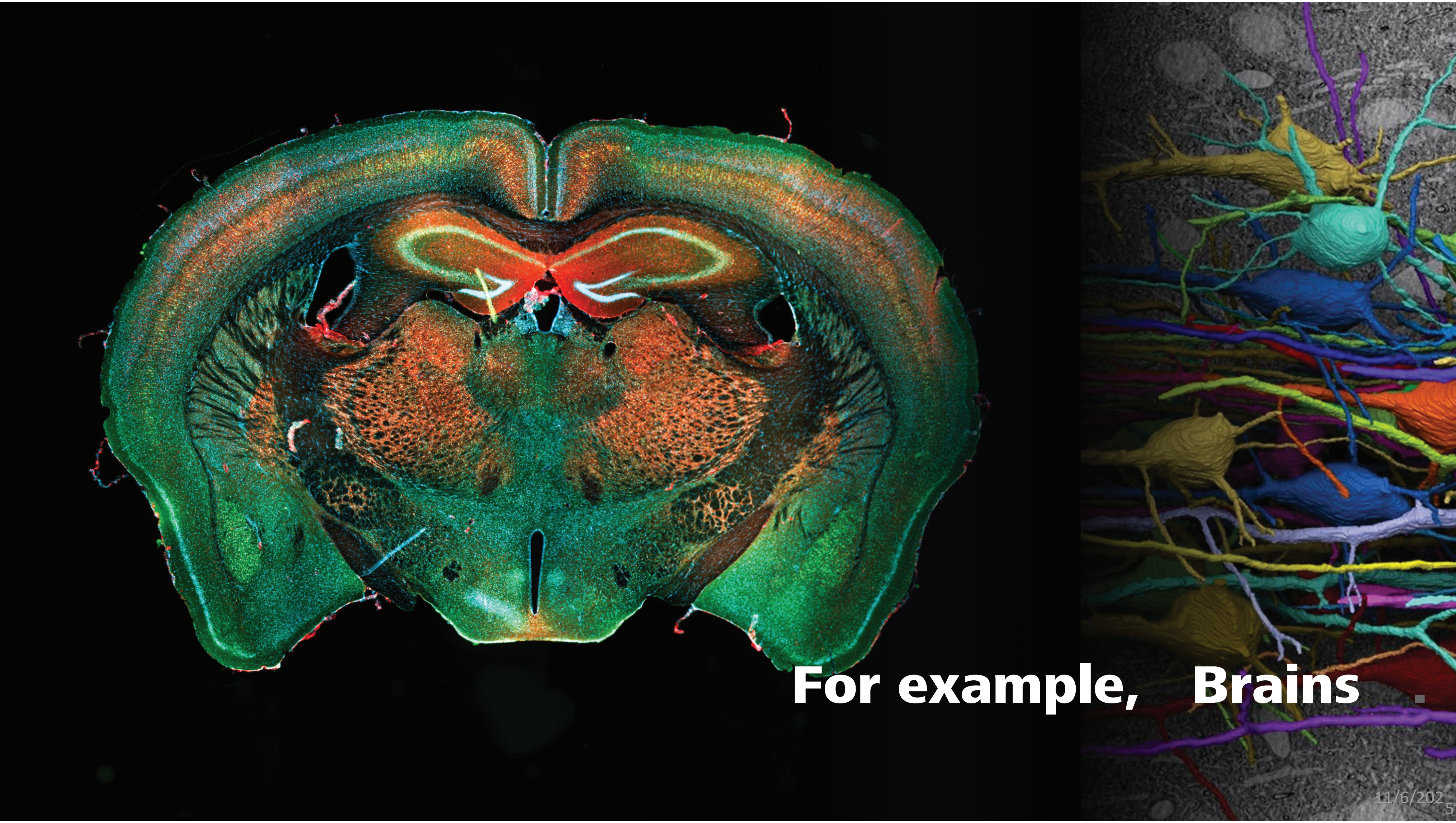
Motivation

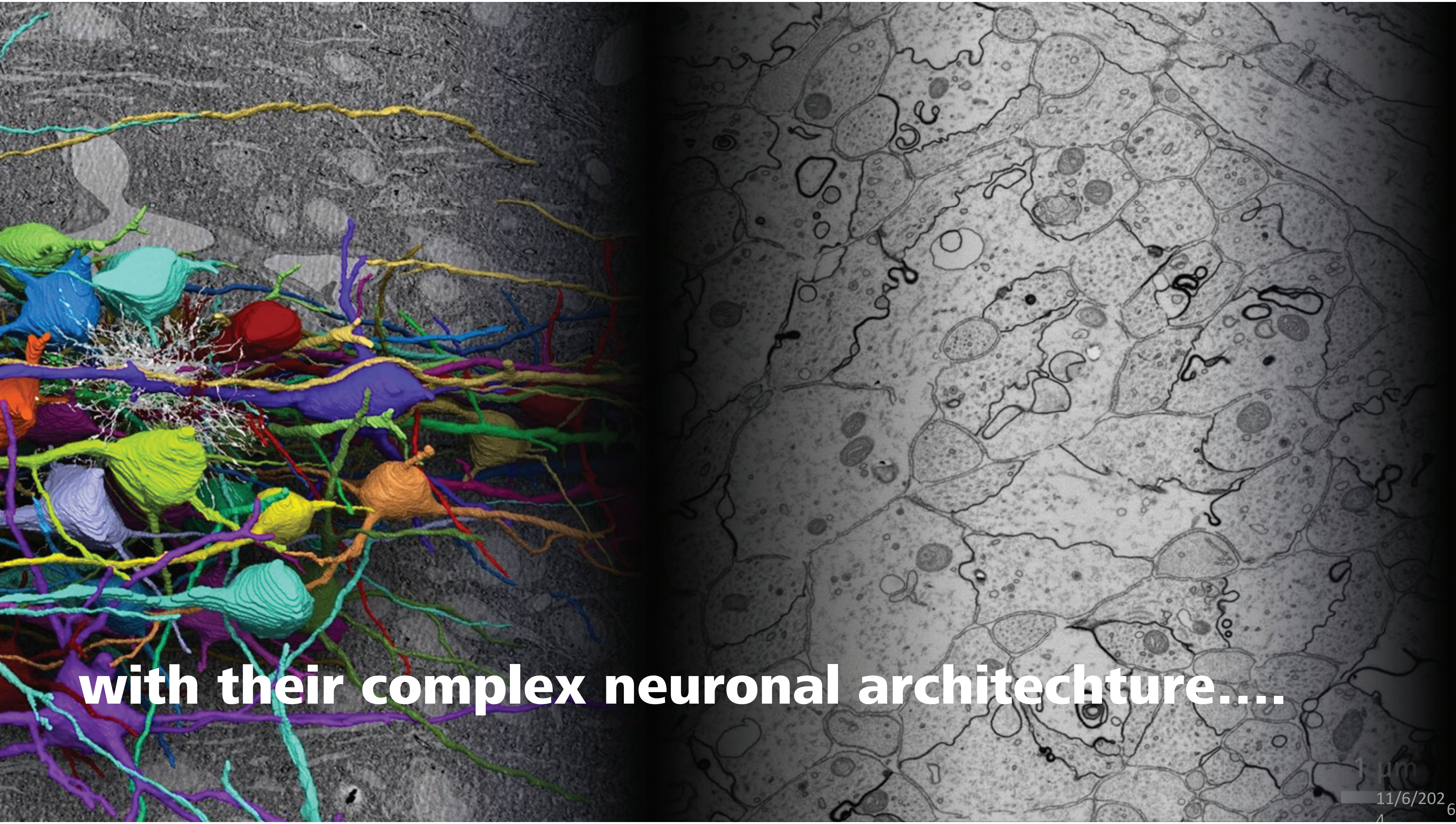


Let’s take a short visual journey.

Our world is filled with fascinating materials.


Ranging from complex natural materials


For example, Brains .

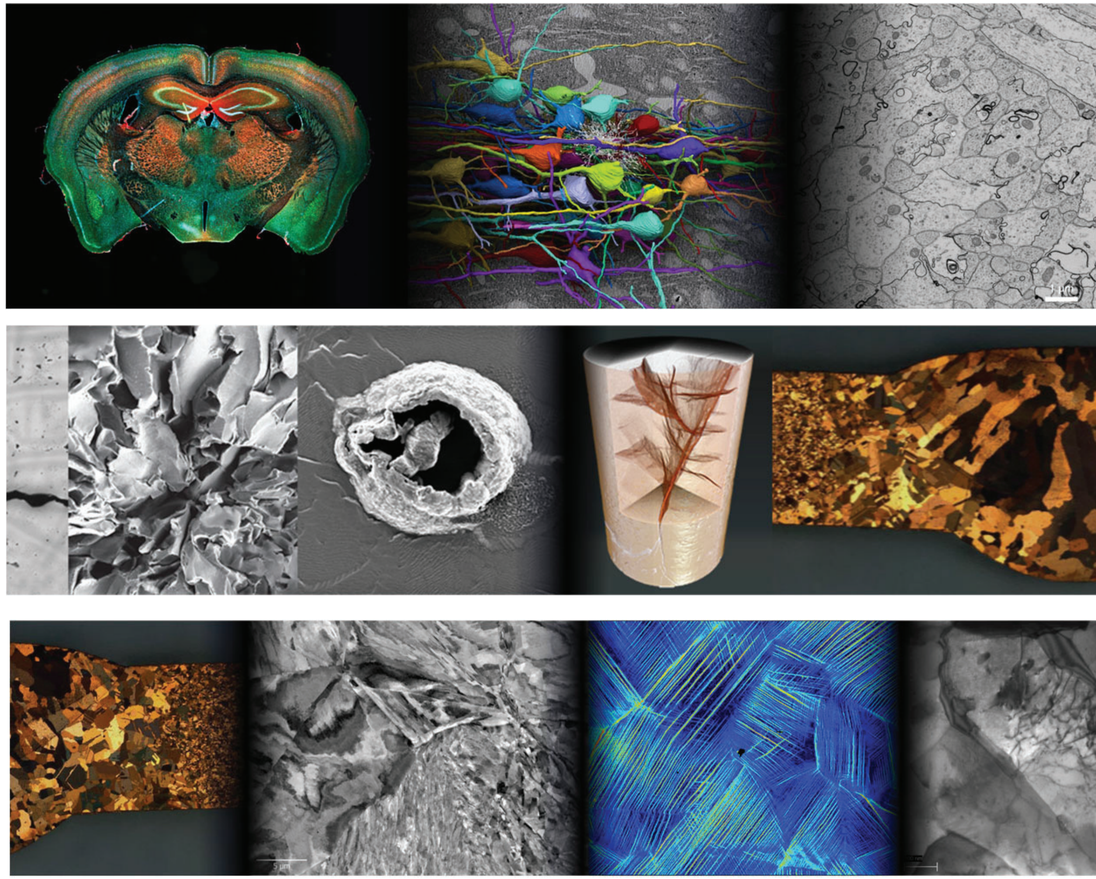


with their complex neuronal architechture….


To some of the strongest man-made materials
10µm

Common ground: a complex challenge to solve

- Research involves complex microstructures that are three dimensions (3D, or more)
- Each length scale macro-to-nano presents a different perspective, a new insight.
- Microstructures are Multiscale
- Length scales are connected, i.e. the frame of reference is extremely important.



Breaking it down to the basics

Unraveling the mysteries of complex samples

What challenges do researchers commonly face?

I need observations on a large representative sample volume. Inside that sample, I want to find a specific site to do more experiments, at high resolution.

- I need to first reliably identify the regions of interest
- How do I now access those volumes for further analysis?

From my macro-sample, I need to prepare specific samples for my next analysis that is either in the TEM, atom probe or synchrotron.

- I need to ensure for these high-value experiments I prepare a sample from the right region of interest.
- I need to understand where that region of interest is, access and prepare a sample(s).

Workflow backbone

1. Identify
2. Access
3. Prepare
4. Analyze

ZEISS Sample in Volume Analysis

Complete Multiscale Solution for Precision Site Specific Sample Preparation and Analysis

Solving the fundamental challenges in connected microscopy

I need observations on a large representative sample volume. Inside that sample, I want to find a specific site to do more experiments, at high resolution.

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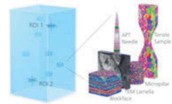
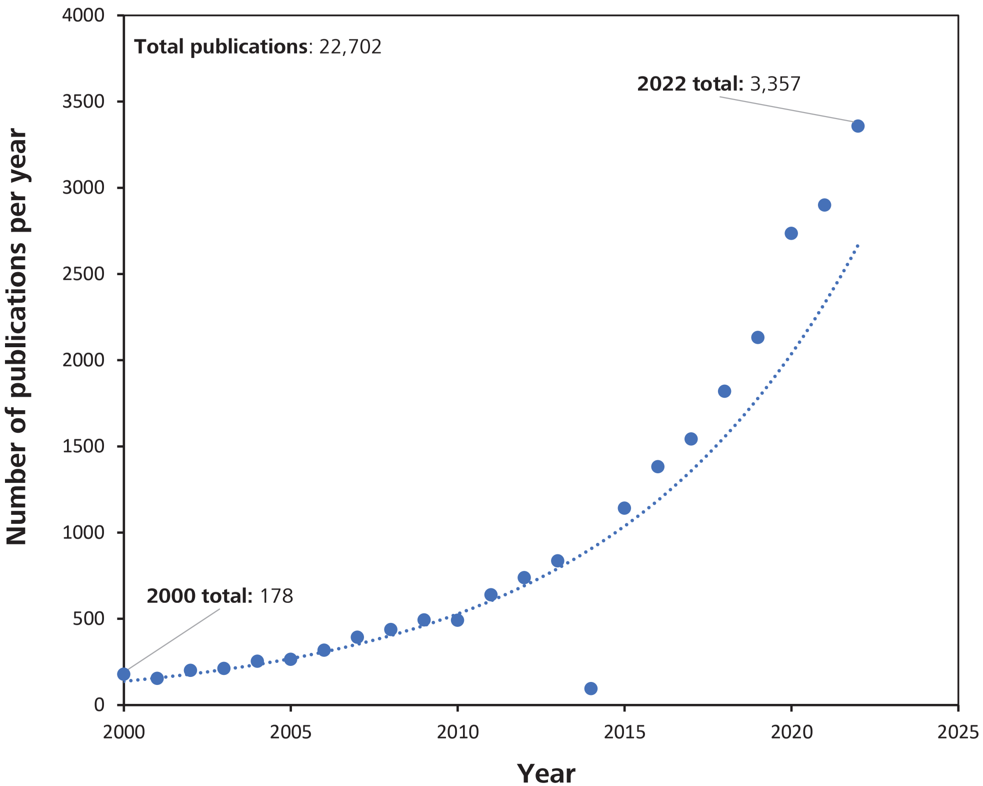
The growing importance of "correlative microscopy" for multi-scale research in material sciences





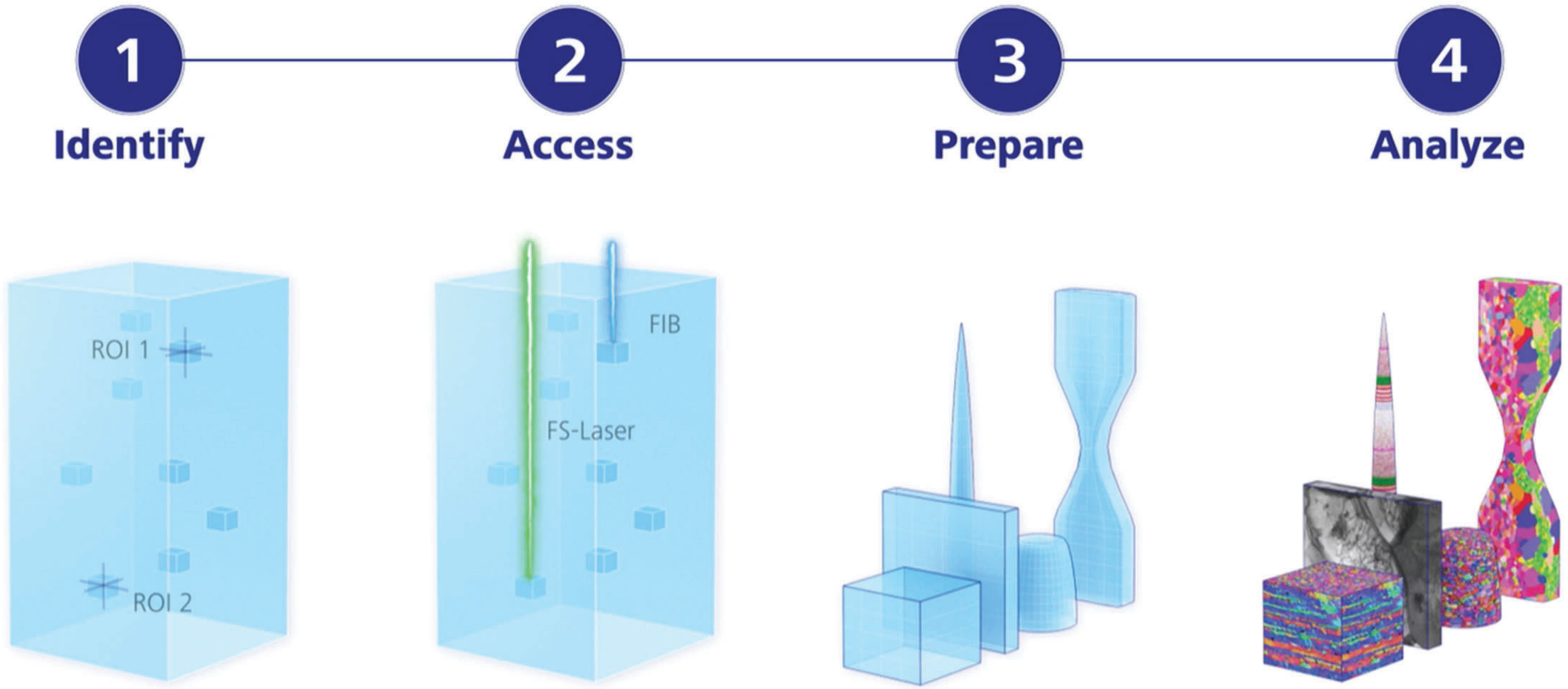
Data collection from dimensions.ai online database. "Correlative microscopy" is used as the search term. Total data is filtered by physical sciences with "Engineering", "Chemical Sciences", "Materials Engineering", "Physical Science" and "Earth Science" selected.

„Correlative microscopy" is now a megatrends in material science

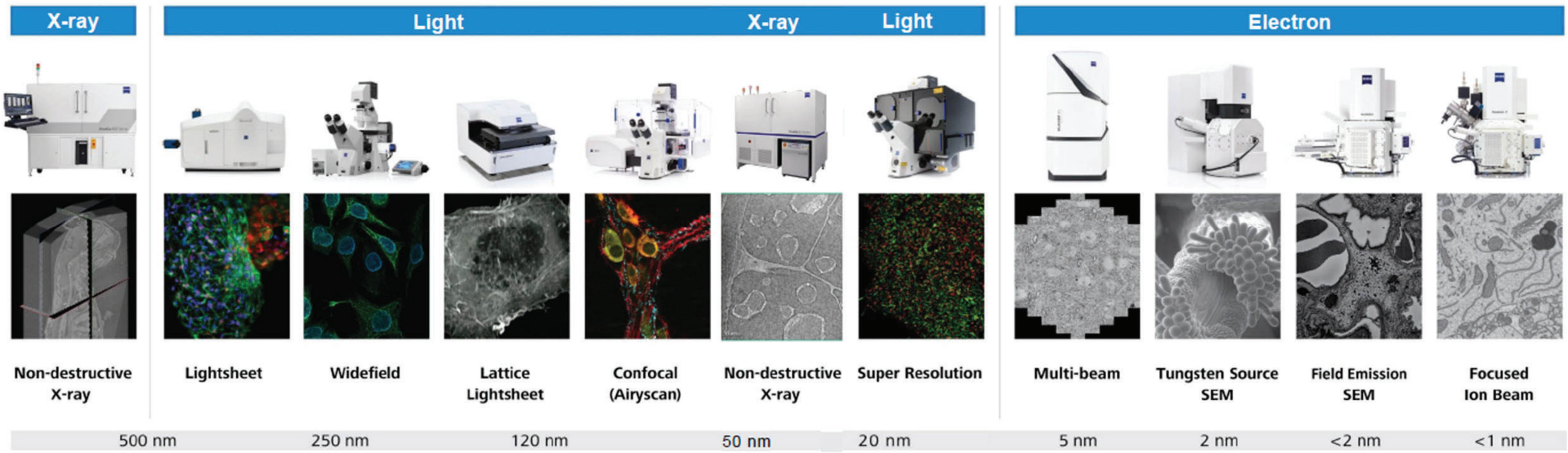
- Publications in material science that are using „correlative microscopy" have grown from 178 in 2000 to 3,357 in 2022.
  - This is 1,786% increase in 22 years
  - This is a 15% compound annual growth rate (CAGR)
- **Microscope technology** is one **driver** of this megatrend.
- However the **direction** of **R&D** in material science is also **accelerating** the need for **macro- to sub-nm workflows**.
  - Battery, semiconductor, additive manufacturing, biomaterials (etc.), are all well-funded mega-trends and hot areas of research with multi-scale workflow resquirements spanning from macro- to sub-nm lengthscales

The Concept

Sample in Volume Analysis.. Identify, access, prepare and analyze samples in volumes..



Multimodal and Multiscale Microscopy



APPLICATIONS

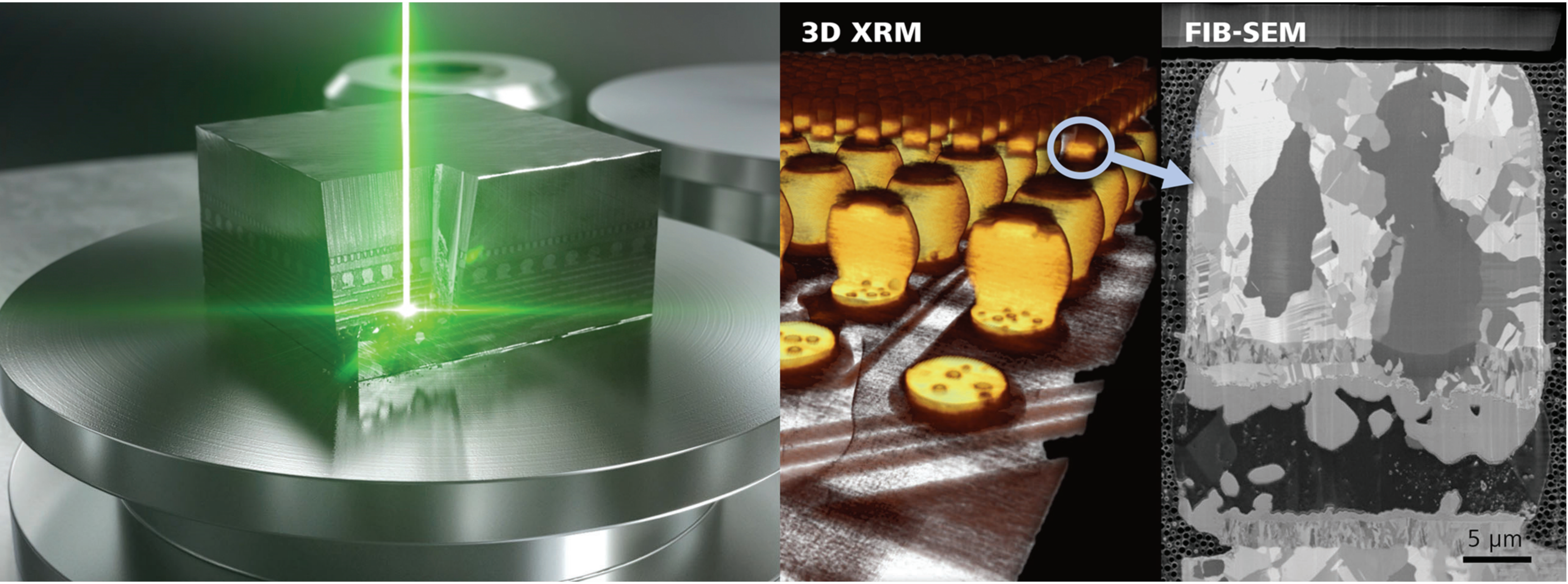
Correlative microscopy in Electronics & Semiconductors

Collection of case studies from Semi FA

Back to Overview

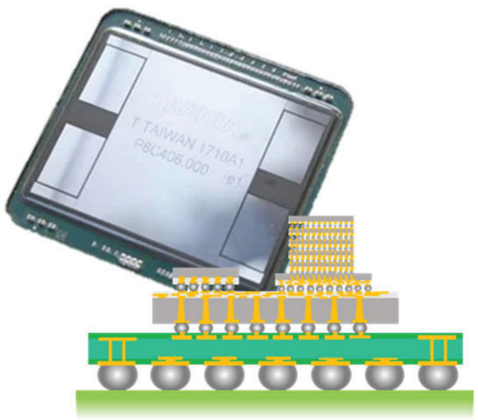
APPLICATIONS

Emerging Technologies for Advanced 3D Package Characterization to Enable the More-Than-Moore Era

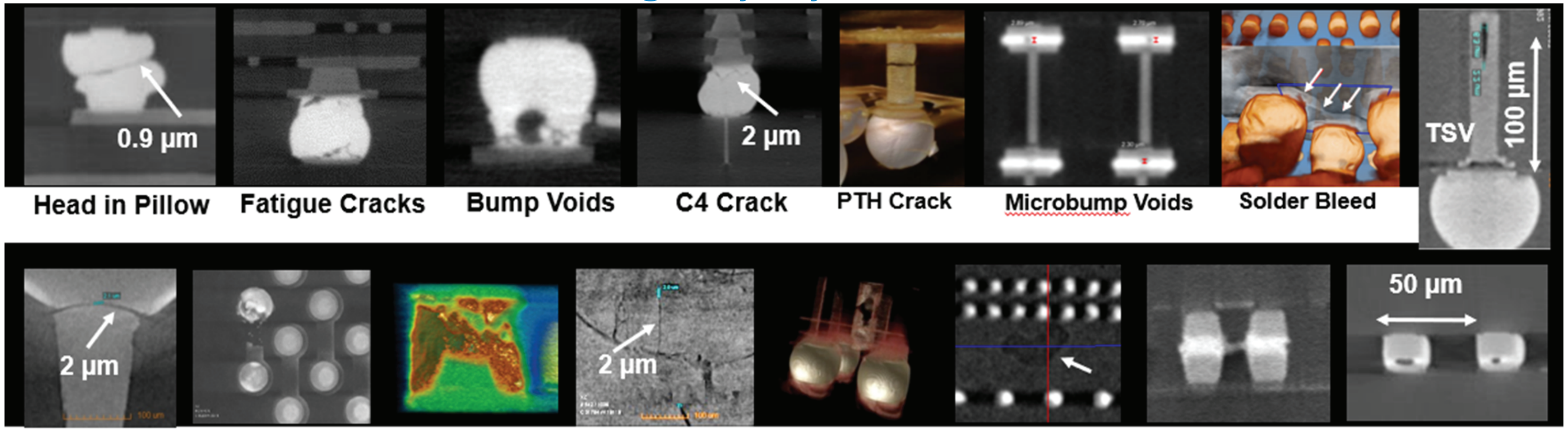


Package Inspection And Physical Analysis

How to target and image small buried features with high resolution and speed?



3D XRM submicron-resolution images of defects



Head in Pillow, Fatigue Cracks, Bump Voids, C4 Crack, PTH Crack, Microbump Voids, Solder Bleed, TSV

Trace Cracks, Breaks, Electromigration, Si Cracks, Broken Vias, Underfill Void, Microbump Shorts and Voids

Fault Isolation
- Optical inspection
- Electrical test
- TDR

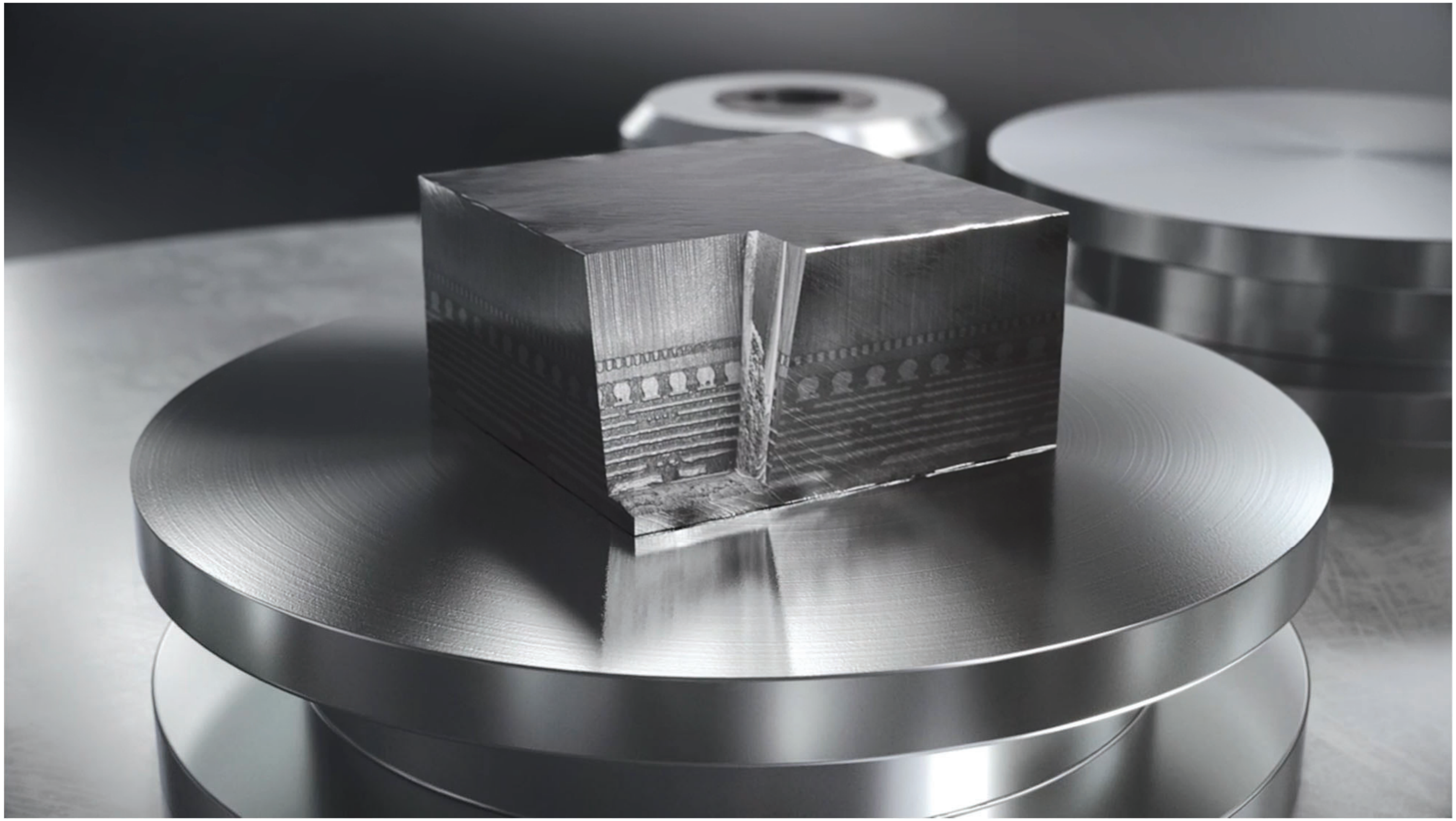
Non-destructive Imaging
- C-SAM
- 2D X-ray

Destructive Failure Analysis
- Parallel polish → Microprobe
- TEM

- **High success rate**
- **Artifact-free**
- **Submicron resolution**
- **High throughput**

Results. Fast.

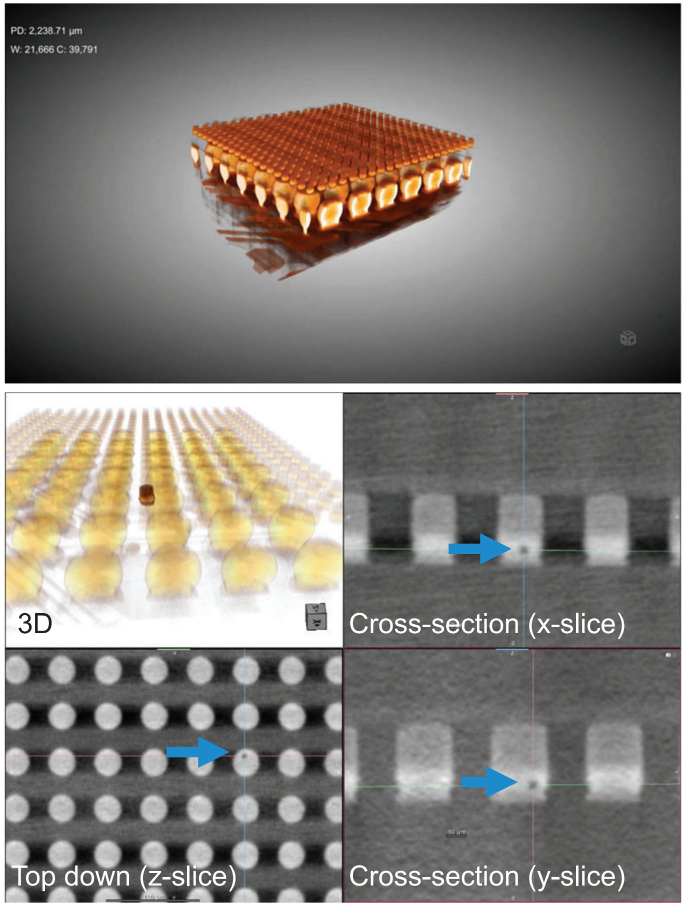
Correlated analysis – Fast sample transfer – Clean SEM chamber



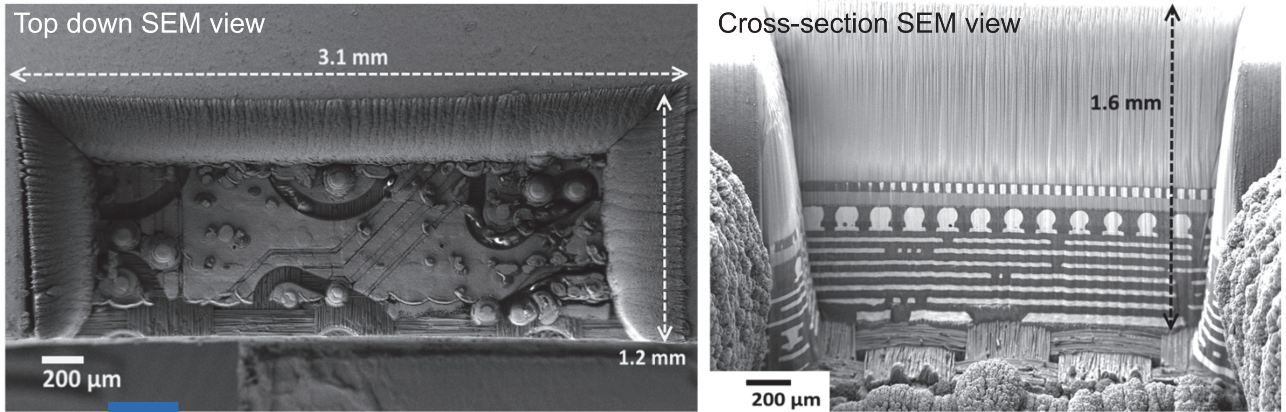
Example of LaserFIB Cross-Section Accuracy

3D package test vehicle for 14 nm Si node & 5-um void

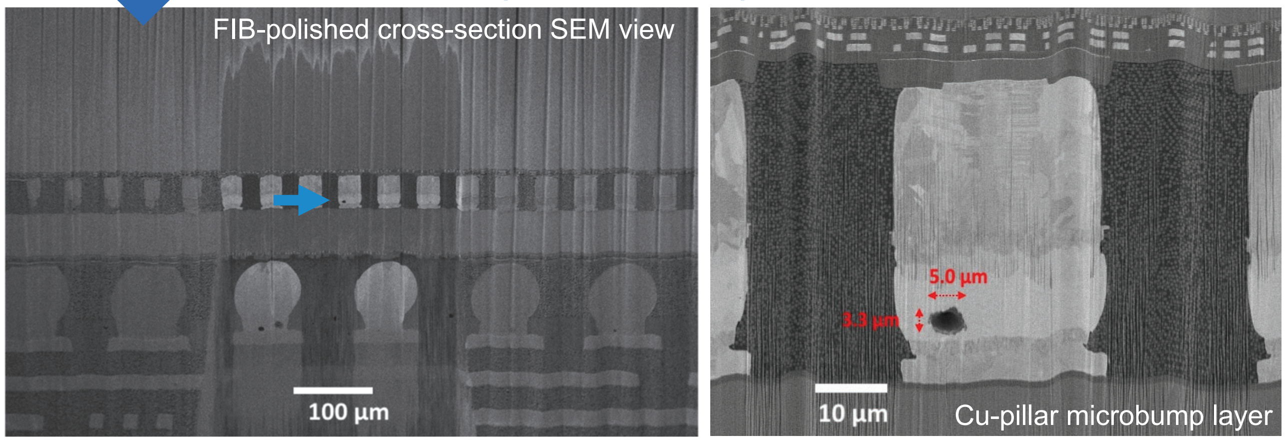
❶ Identify ROI (5 µm void) in XRM scan



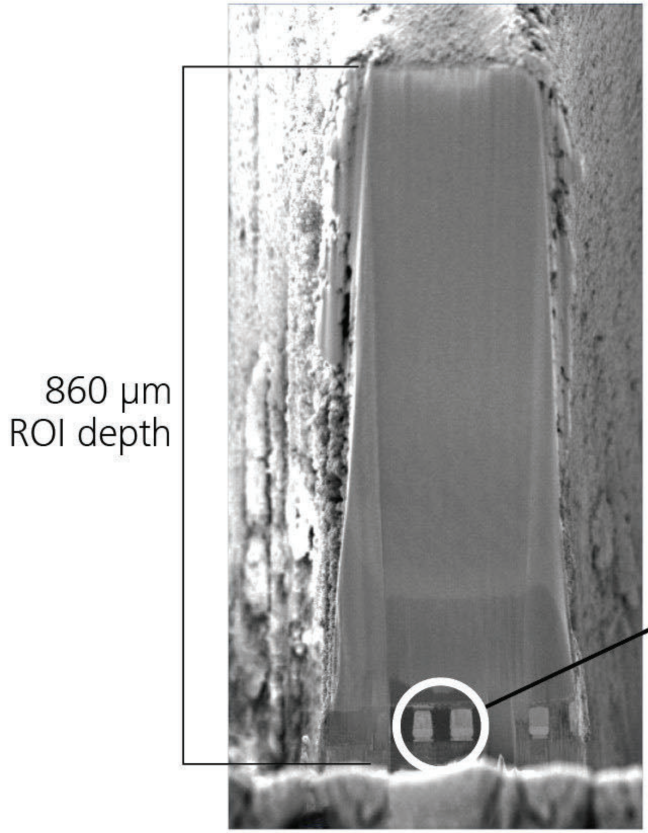
❷ Large-scale opening towards ROI by laser and fine polish

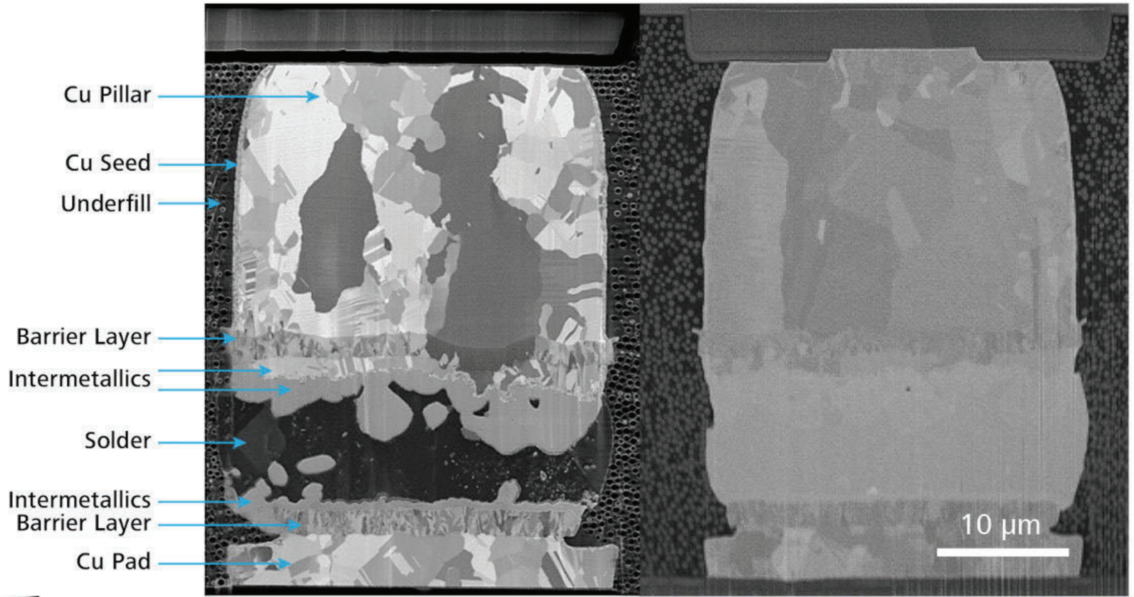


❸ Final FIB polish into 5 µm void



3D Stacked Die Interconnect (14 nm Si Node)



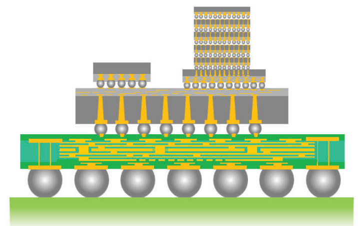


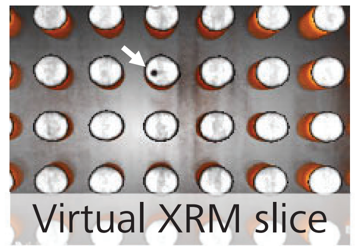
Time Budget

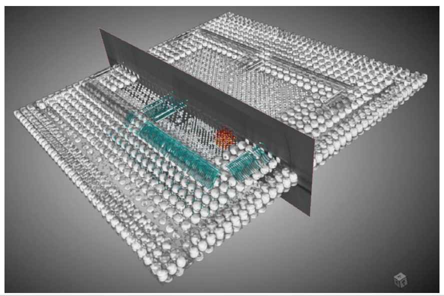
■ fs-laser preparation time	29 minutes
■ FIB polish time	22 minutes
Total time-to-results	**51 minutes**

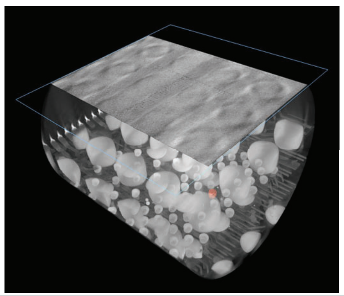
Summary

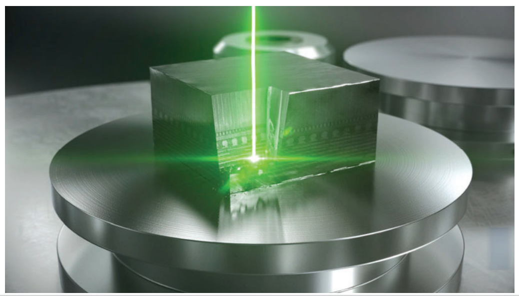
1. Advanced package architectures with higher I/O densities, finer pitch and buried 3D interconnects present new challenges in development and FA
2. New solutions extend 3D XRM and FIB-SEM capabilities by orders of magnitude, opening an exciting new characterization era
3. Advanced deep learning reconstruction engines enable high-resolution 3D X-ray images $\geq$4X faster while retaining image quality
4. A new LaserFIB gives rapid access to site-specific buried structures: millimeter volumes of material are removed with minimal artifacts & micron scale site-specificity
5. Correlated microscopy improves efficiency and insights from macro to nano levels
6. Continued efforts in AI to improve speed and resolution, as well as automated correlated workflows, will complement advancements in sources and detectors for these instruments

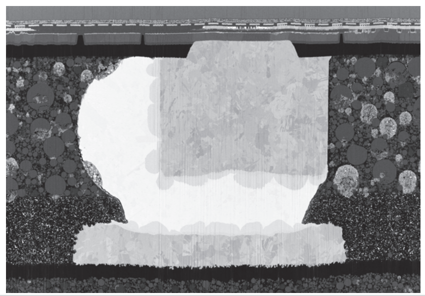


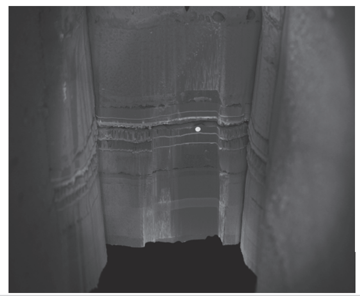








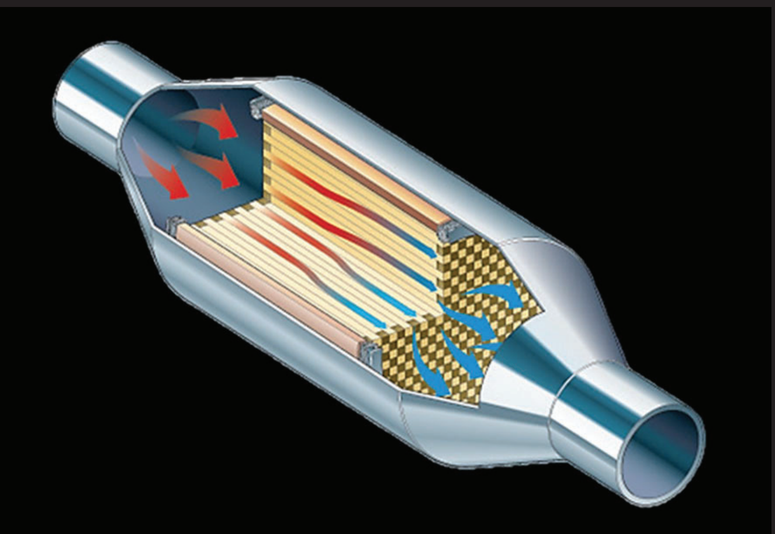


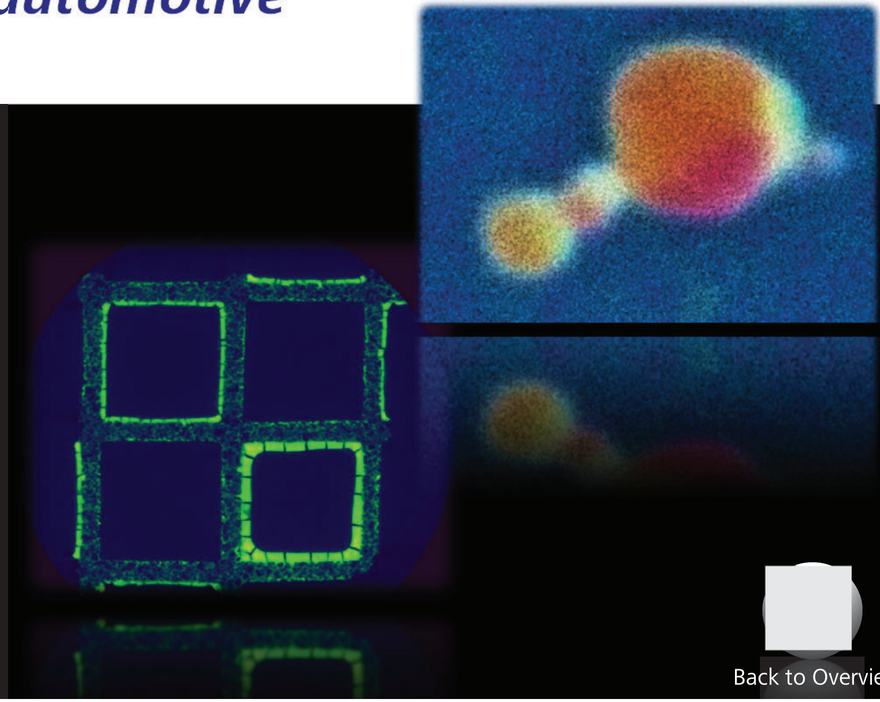


APPLICATIONS

Correlative microscopy in Industrial Research

A case study on multiscale characterization of automotive catalytic converter





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Advanced FIB-SEM Tomography
Generating 3D stacks of SEM images

Johnson Matthey — Inspiring science, enhancing life

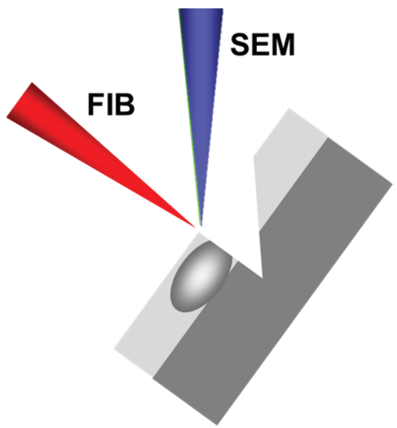
FIB-SEM Tomography

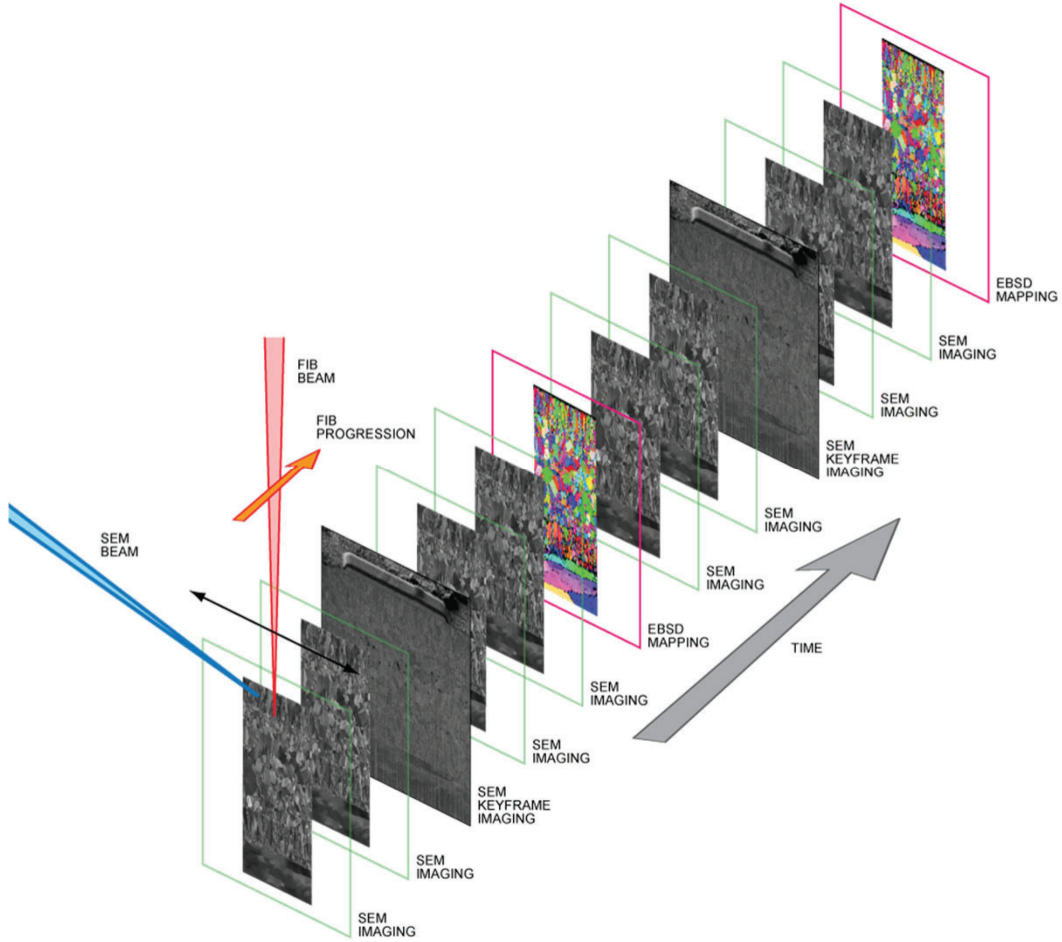
- Use FIB to mill a volume of interest slice by slice
- Image cross-section face with the SEM
- Optional: EDS/EBSD maps after defined distance

Challenges

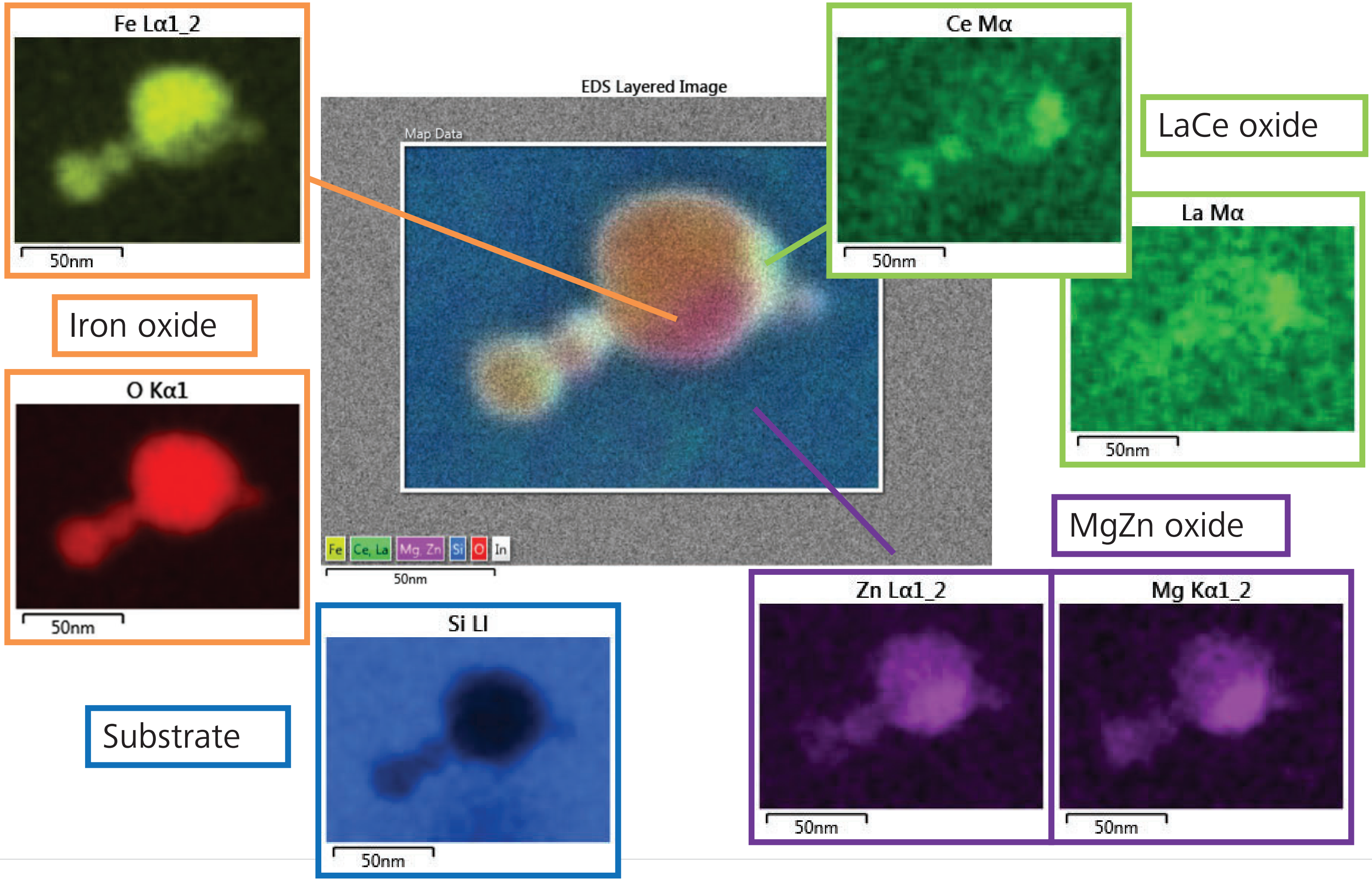
- Speed, Accuracy, Resolution, Analytics

ZEISS Crossbeam

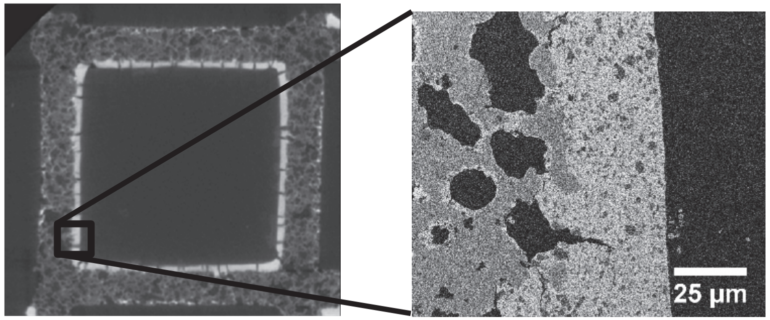




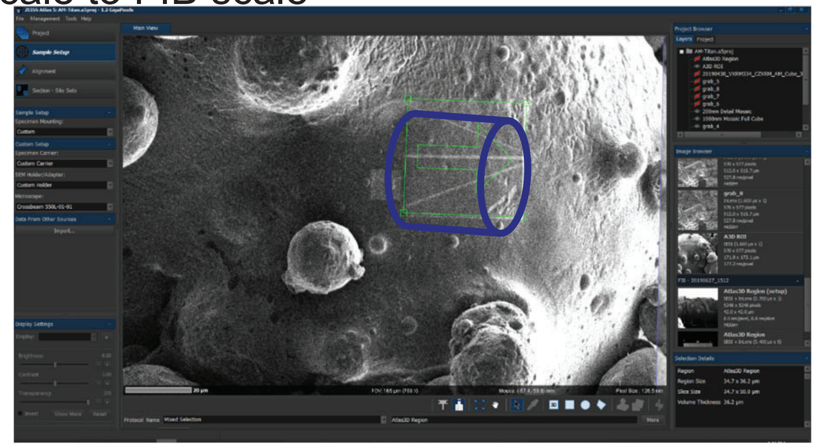
EDS analysis of the Oxide Nano-particles



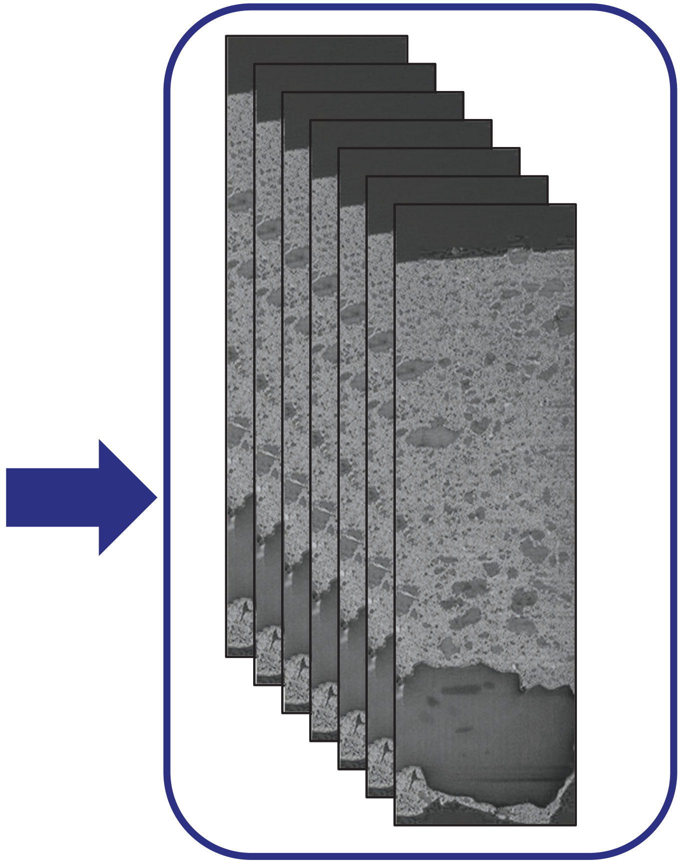
Extended Resolution: Correlative Microscopy XRM-FIB
composition of the washcoat layer slice by slice



Gaining more resolution by moving from XRM scale to FIB scale



Correlating positions of XRM volume (Versa) FIB tomography



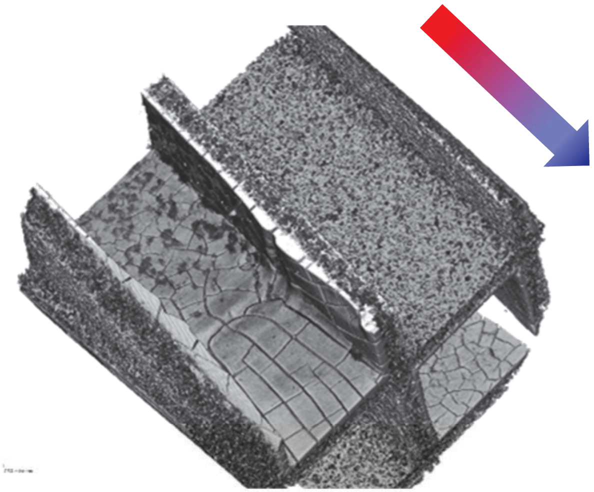
FIB milling automatic slice by slice

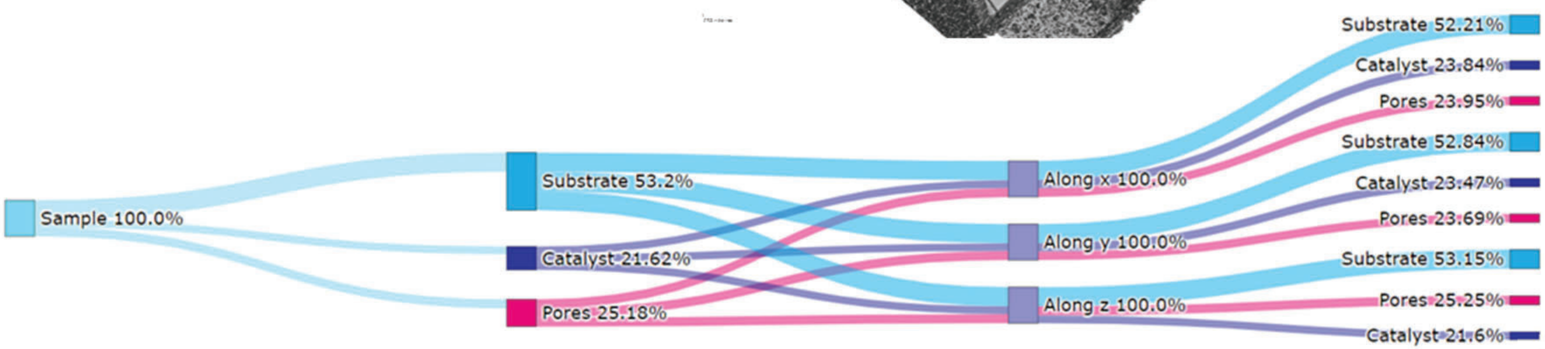
20nm resolution achievable

Result
- Correlative 3D volume by FIB sectioning
- Visibility of the internal structure

Summary

- The mean sample decomposition plot (Sankey plot) quantitatively breaks down the sample into components such as catalyst, pores and substrate.
- The mean sample decomposition along the various axial directions shows consistent coverage
- With deep learning a complete understanding of the GFP's macroscopic properties can be achieved. The data from multiple scales and modalities can be used to improve the performance of a gasoline particulate filter





Correlative Microscopy FIB-TEM

FIB-TEM sample prep

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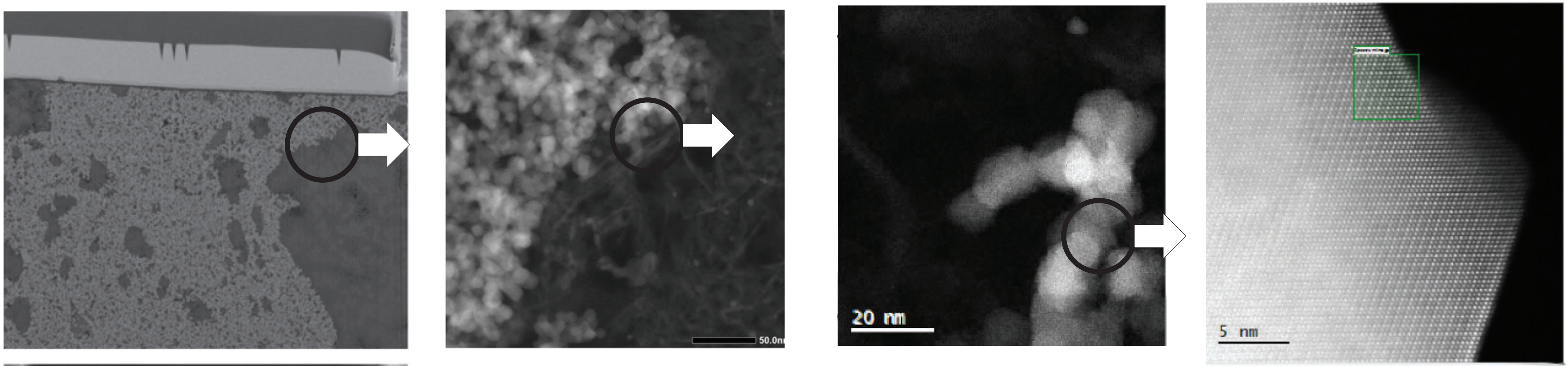
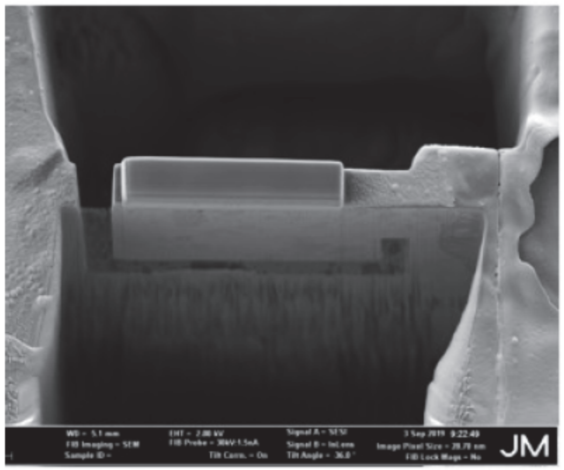
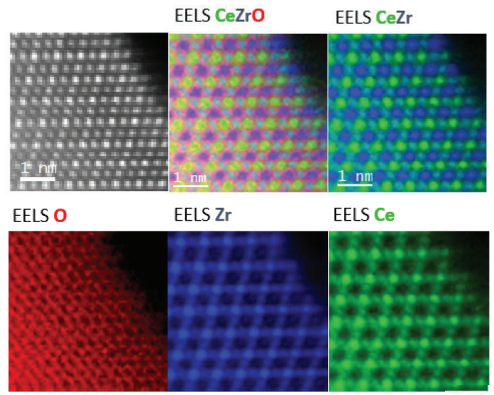




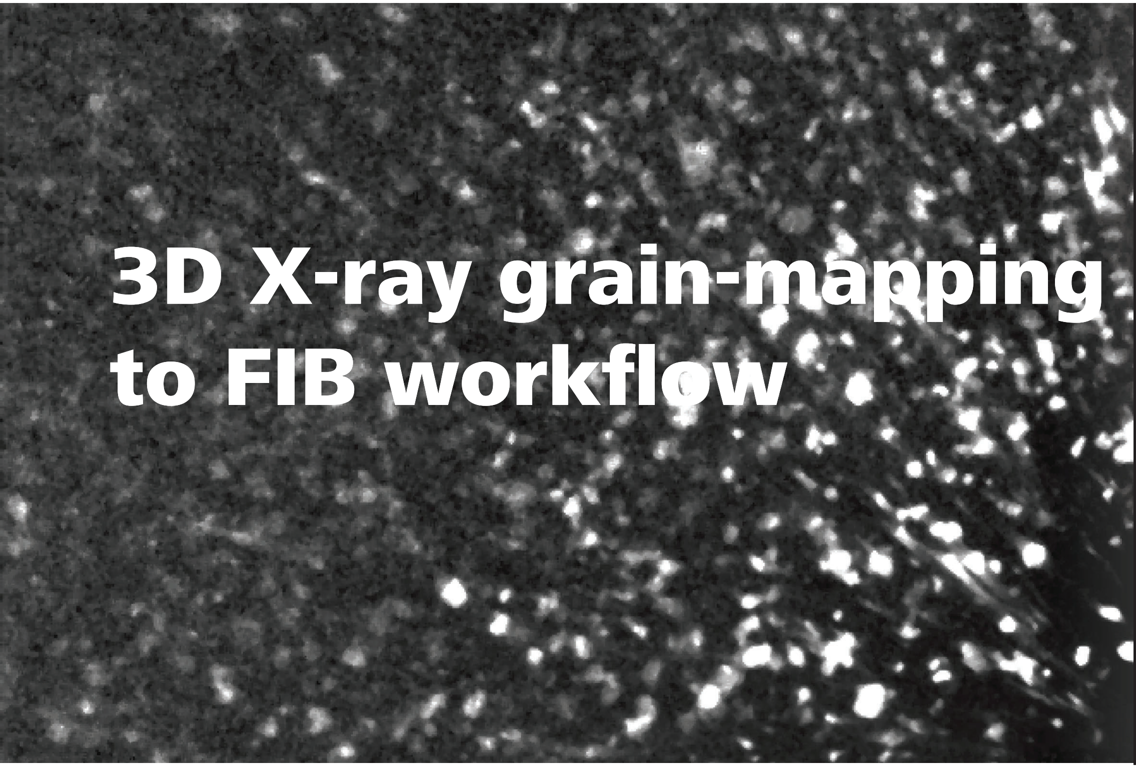
Extending to higher resolution
- Highest quality TEM sample preparation of coating layer for HR-STEM

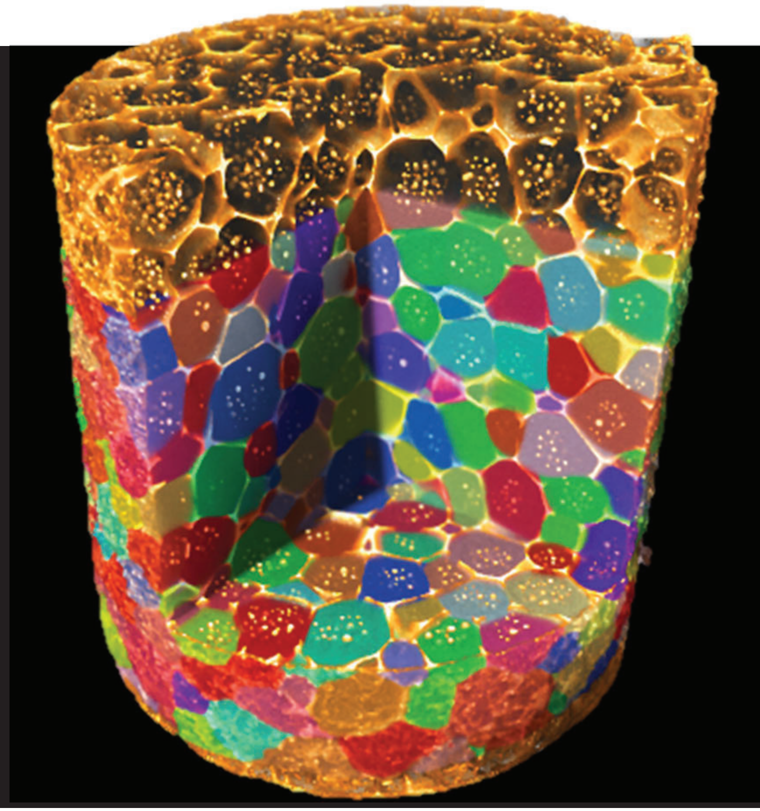
Extending the modalities
- Further EELS analytics

Result: Composition and crystallography at atomic scale



3D X-ray grain-mapping to FIB workflow





Overview of Crystallographic Grain Mapping

Large-Volume 3D X-ray Grain-mapping to FIB-SEM

Laser → 3D X-ray → FIB-SEM

Industry-standard Approaches To Grain Structure Analysis

Surface analysis

2D — **Surface Preparation** → **EBSD Mapping of Surface Grains**

Limited to surface

Sub-surface analysis

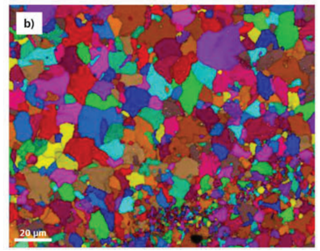
2D — **FIB-SEM Preparation** → **EBSD Mapping of Exposed Grains**

"Blind" selection to ≤100μm depth

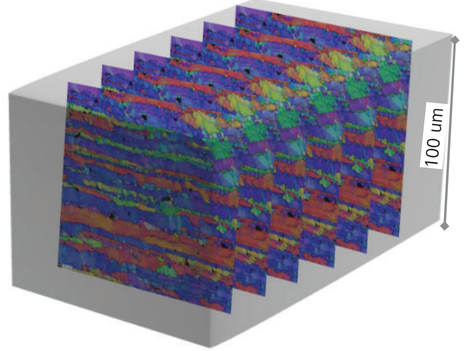
3D — **FIB-SEM Preparation** ⇄ **EBSD Mapping of Exposed Grains**

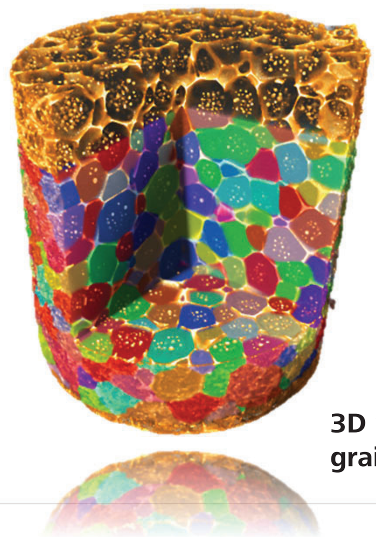
Limited to 100μm depth, high-resolution

EBSD-Mapping
individual grains colored



3D EBSD
Combining serial sectioning and EBSD mapping





3D non-destructive DCT grain mapping

Sample Representivity

Enabling massive volume investigation

Current DCT capabilities

Sample ROI needs to be fully illuminated in the field of view (FOV) for all rotational angles.

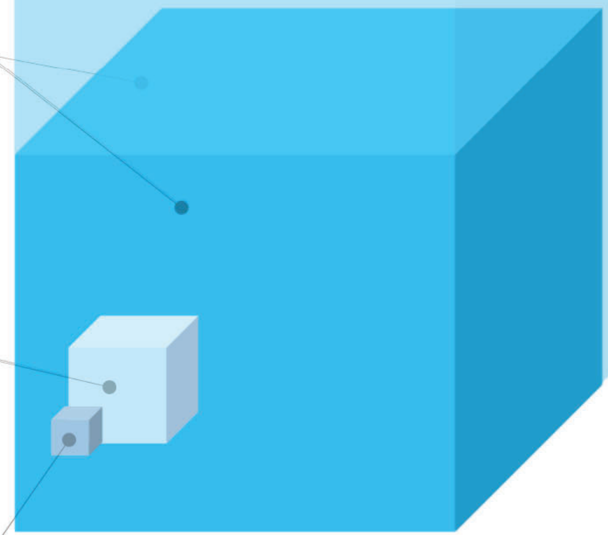
Next generation lab based DCT

- Enable larger sample volumes, less sample prep, and handling of irregular/realistic sample shapes
- Increased speed
- Provide sample representivity
- Address sample specificity

New generation DCT

Non-destructive DCT
Volume $(1000)^3$ µm^3
Isotropic voxels up to 2 µm*
Voxel aspect ratio = 1

Volume >> $(1000)^3$ µm^3 and beyond
Isotropic voxels up to 2 µm*
Voxel aspect ratio = 1



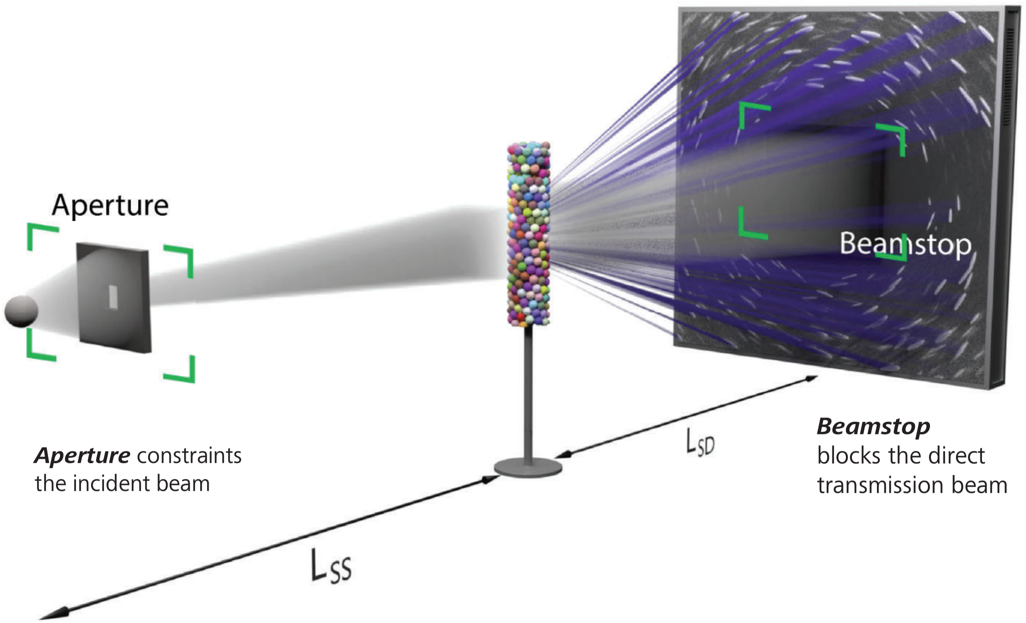
PFIB + EBSD
Volume $(250)^3$ µm^3
Slice thickness 0.2 - 5um
Voxel aspect ratio ≥ 50

Ga-FIB + EBSD
Volume $(100)^3$ µm^3
Slice thickness 10 nm
Voxel aspect ratio ≥ 1

Laboratory Diffraction Contrast Tomography (LabDCT)

Non-destructive 3D crystallographic for accurate ROI pin-pointing

Non-destructive 3D grain mapping using X-ray diffraction contrast tomography



Laboratory diffraction contrast tomography

3D Grain Mapping offers:

- ✓ Grain Centroid Position
- ✓ Grain Size
- ✓ Grain Orientation
- ✓ Grain Shape
- ✓ Grain Boundary Information

Left-right:
Faces of a selected grain color coded in random color, by IPF color, misorientation to neighboring grains, grain boundary curvature and grain boundary normal direction in crystal reference system.

3D grain map of an Armco iron sample.
Sample courtesy of Prof. Burton R. Patterson, Univ. of Florida

Correlative crystallographic imaging

Workflow 1 | Non-destructive 3D crystallographic for accurate ROI pin-pointing

Non-destructive 3D grain mapping using DCT

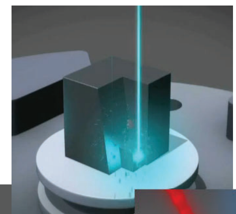
3D grain analytics to identify specific ROI site based on

- **Grain boundary characteristics**
- **Features in grain vicinity**
  - Inclusion
  - Voids
  - Precipitates
  - Cracks

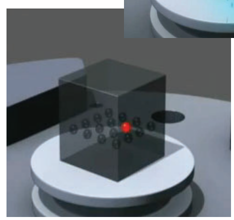
Data guided navigation Site preparation / deep ROI excavation

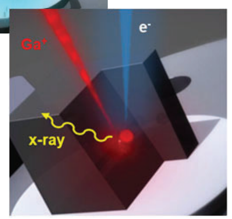
- Use fs-laser to expose deep ROI's
- FIB to polish or precise site preparation

Fs-laser preparation



XRM/LabDCT

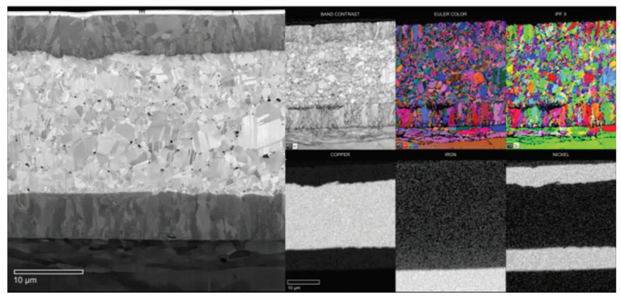




Ga-FIB processing

Full Context high resolution analytics

- High resolution 3D-EBSD on specific grain boundary site.
- 3D-EDS
- Image fine grain features in greater detail



Correlative crystallographic imaging for

Workflow 2 | Non-destructive 3D crystallographic for in-situ guidance

Laser → **3D X-ray** → **FIB-SEM**

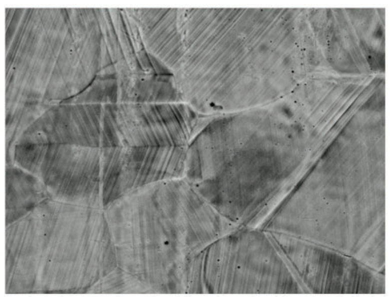
Non-destructive 3D grain mapping using LabDCT

3D grain analytics to identify

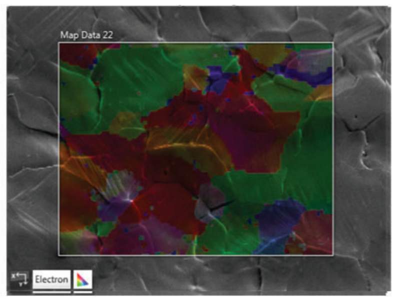
- **Grain boundary characteristics**
- **Other non-crystallographic features**
  - Inclusion
  - Voids
  - Precipitates
  - Cracks

High-res 2D in-situ experiments

- 2D surface in-situ measurements combining
  - ECI, BSE, EBSD



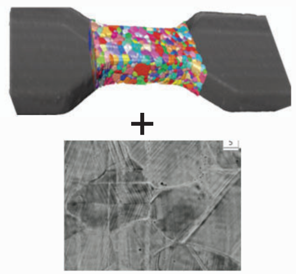
Electron Channeling Contrast Imaging



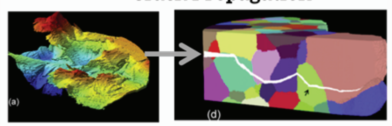
Electron Back Scattered Diffraction

Gain new insights

Combine 3D non-destructive and 2D destructive in-situ data to connect the dots



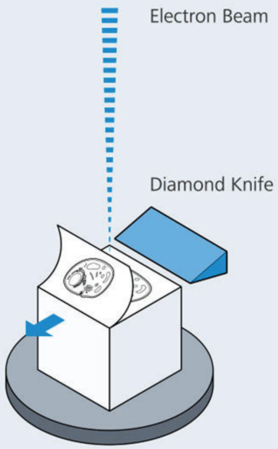
Crack Propagation



M. Herbig, A. King, P. Reischig, H. Proudhon, E.M. Lauridsen, J. Marrow, J-Y Buffiere, W. Ludwig, Acta Materialia 59 (2011) 590–601

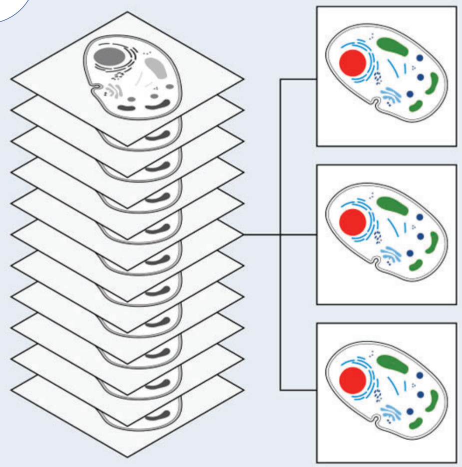
Volume EM at Room Temperature
Serial Block-face 3D Results with ZEISS Volutome

1



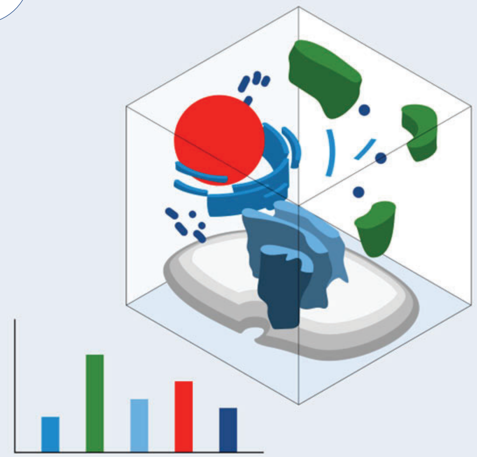
- Cutting
- Imaging
- Cutting & imaging cycle is repeated thousands of times

2



- Image Processing
- 3D Reconstruction
- Segmentation

3

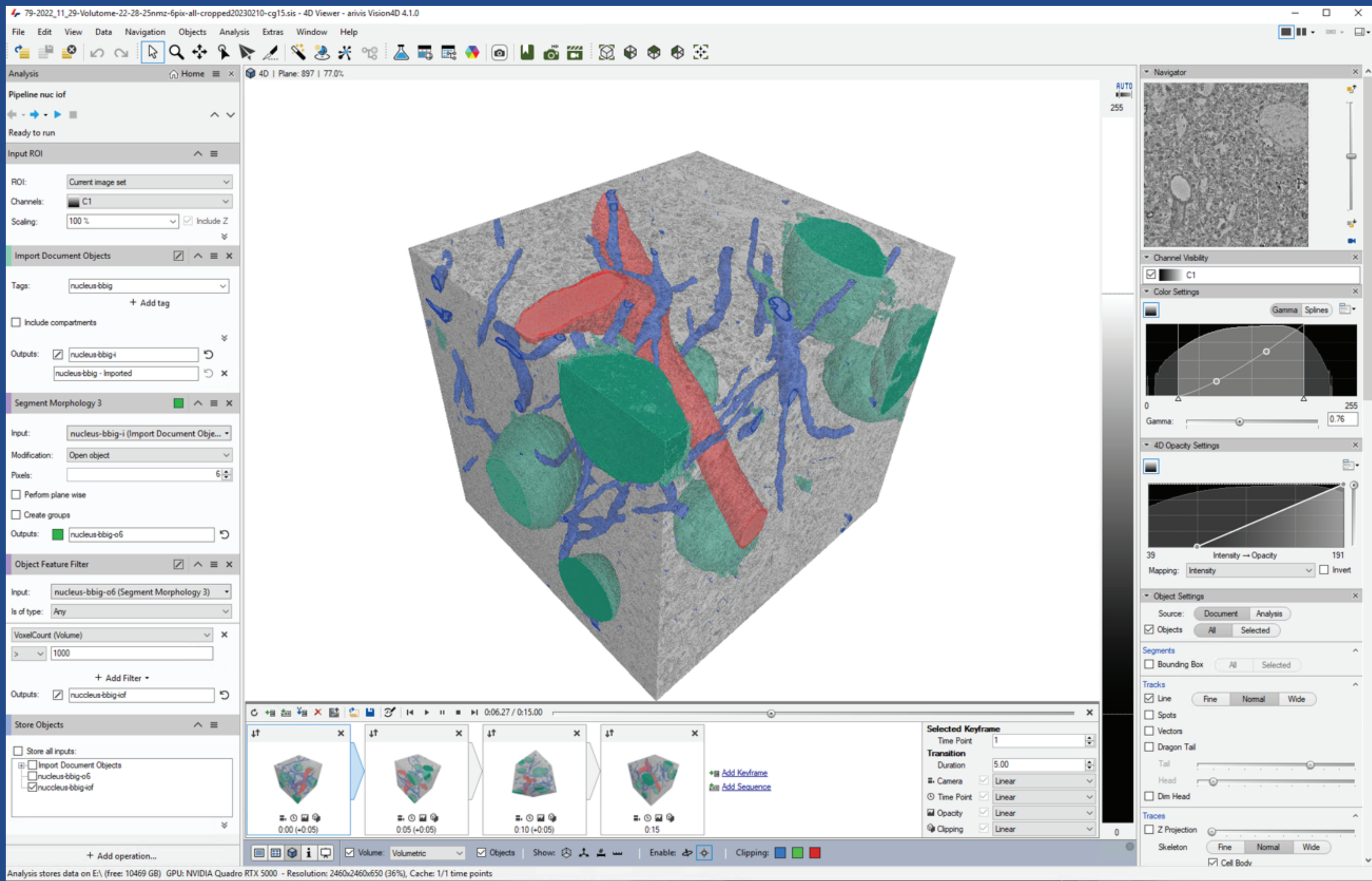


- Visualization
- Investigation
- Analysis


Voluto

ZEISS arivis for processing, segmentation and visualization

Turn your large volume sample data into a 3D reconstruction



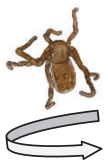
ZEISS

Seeing beyond

X-ray Imaging

How are the 3D images generated?

Introducing X-ray Tomography



Capture multiple 2D images at different sample rotations

Calculate the 3D render of internal structure

Slice your sample in any orientation

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

X-ray Imaging

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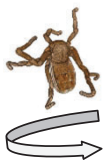
Introducing X-ray Tomography



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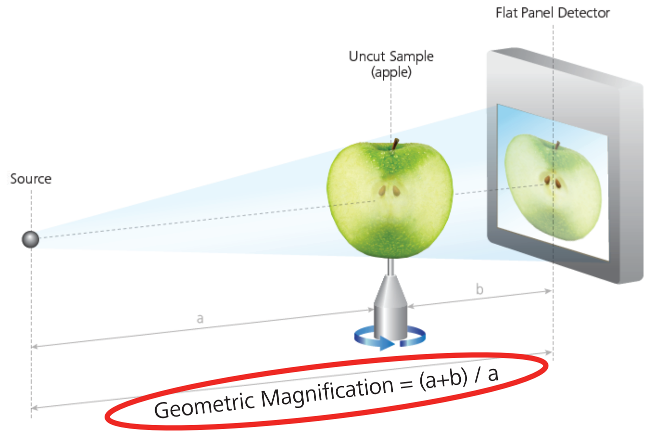
How does micro CT (µCT) work?



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Moving source and sample closer together uses geometric magnification to increase resolution but **gains are limited by sample size**

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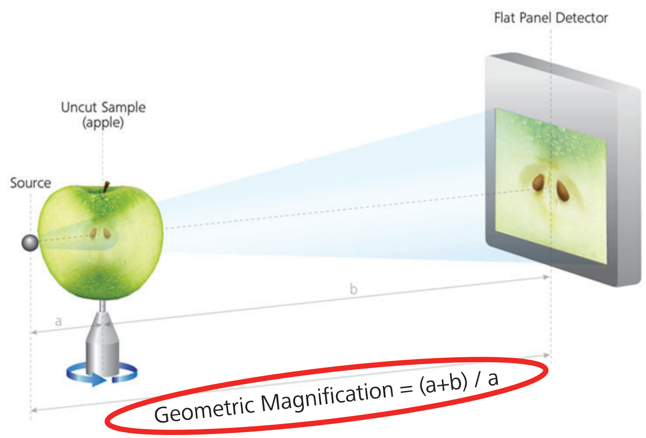
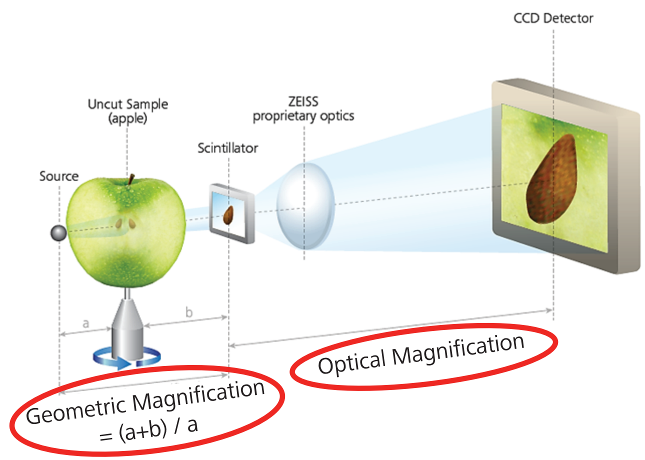


Moving source and sample closer together uses geometric magnification to increase resolution but **gains are limited by sample size**

How does X-Ray Microscopy (XRM) work?



X-Ray Microscopy uses **objective lenses** in addition to geometric magnification to enable high resolution even in larger samples