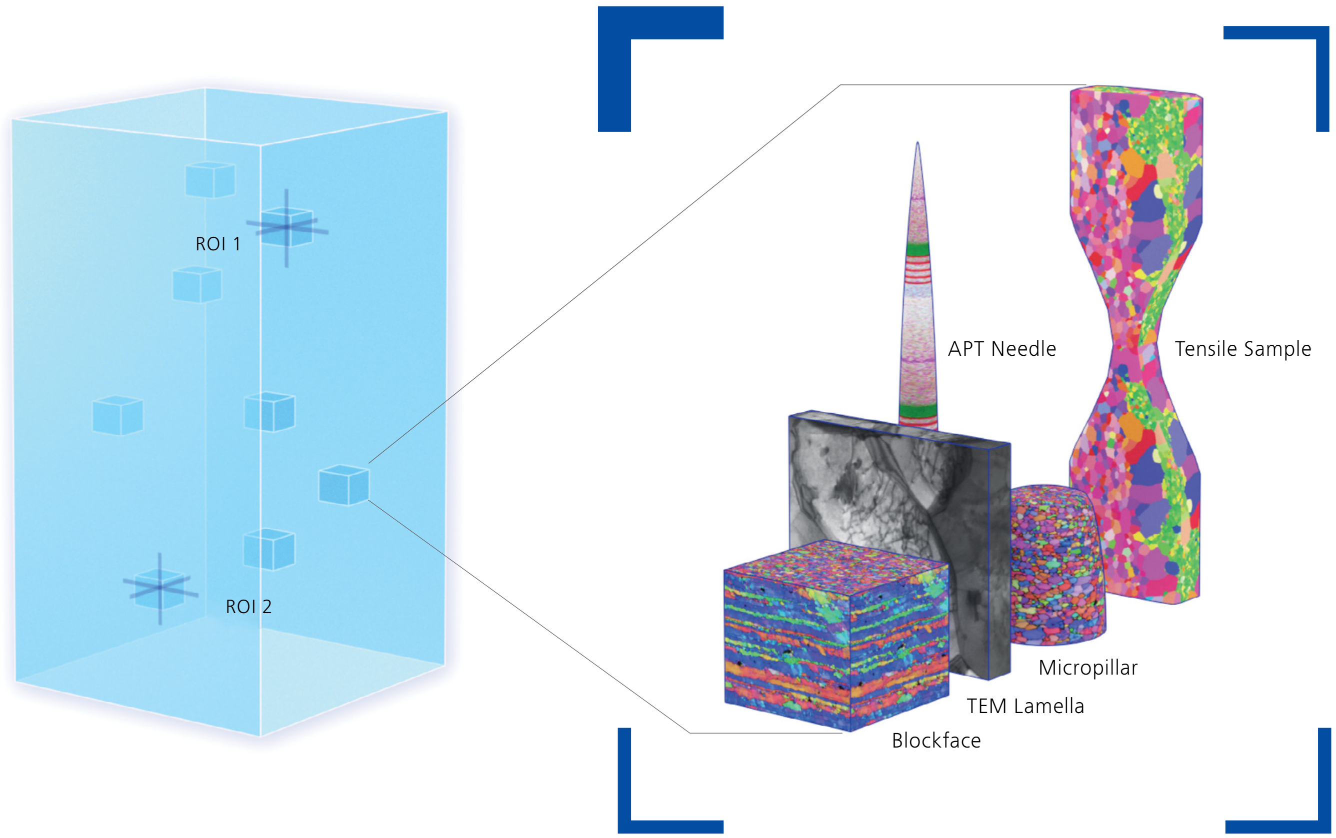
# Identify, Access, Prepare and Analyze With Precise Navigational Guidance




**Sample-In-Volume Analysis Workflow from ZEISS**

ZEISS
Seeing beyond

www.zeiss.com.sg/sample-in-volume

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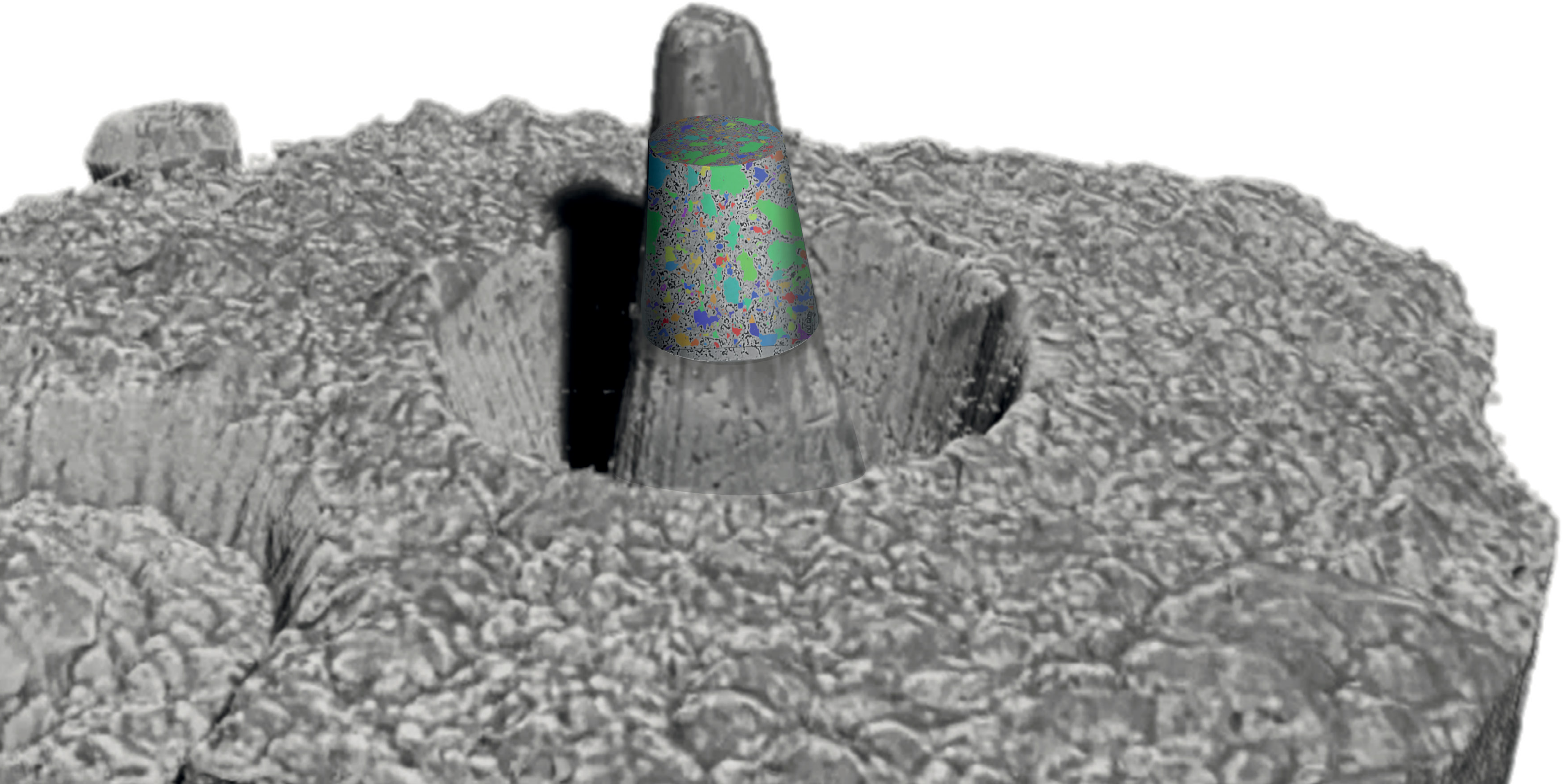


# Understanding the Multi-scale Microscopy Challenge in Materials Science

How do I identify and precisely investigate a region of interest in my multiscale sample?

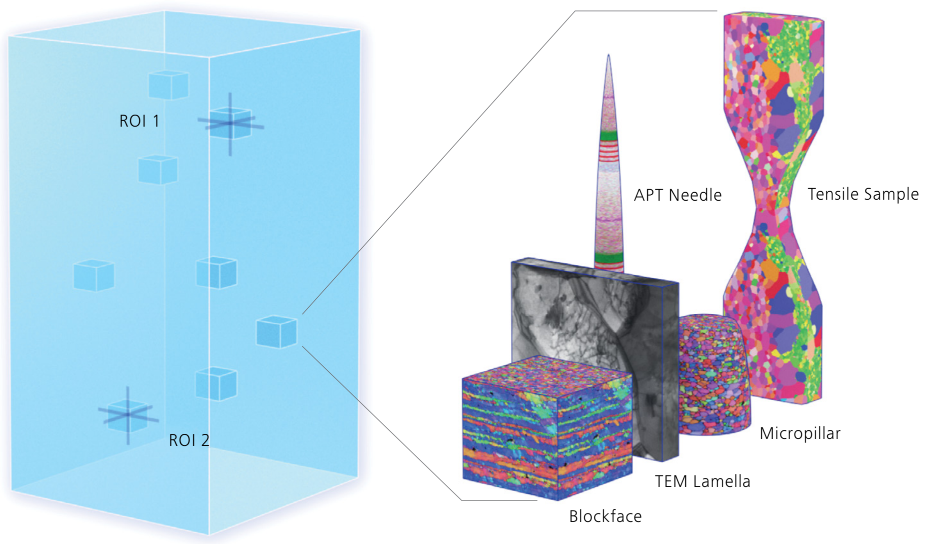
Advanced materials research is challenging. The challenge is based on our requirement as materials scientists to understand structures, properties and processes across different length scales within a material. This requires a range of imaging and analysis technologies that enable us to understand our materials from macro-to-sub-nanometer scales.

As we move from the macro-scale towards sub-nanometer, the materials scientist requires a workflow that enables the selection of the best decisions possible for the best experimental outcomes.

**The workflow must:**

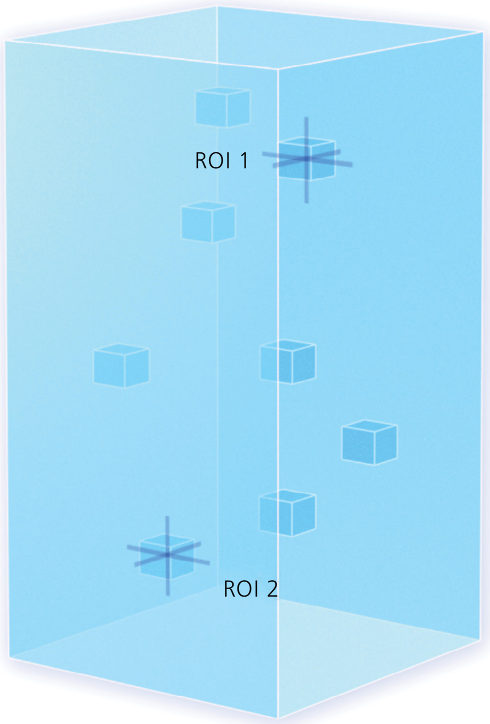
- Help one to identify regions of interest in large samples
- Provide a method to precisely access regions buried within our samples that contain the nm-scaled information we require
- Provide a preparation step that allows the creation of high-quality surfaces and structures for further analysis

ZEISS has developed a unique correlative workflow to address these multiscale challenges. This workflow is known as the Sample-in-Volume Analysis workflow and synergizes the latest innovations in multi-scale microscopy.




*Materials science is a multi-scale discipline. This poses challenges for identifying and accessing regions of interest or "samples" within larger volumes, and the preparation of surfaces and structures for downstream analysis*

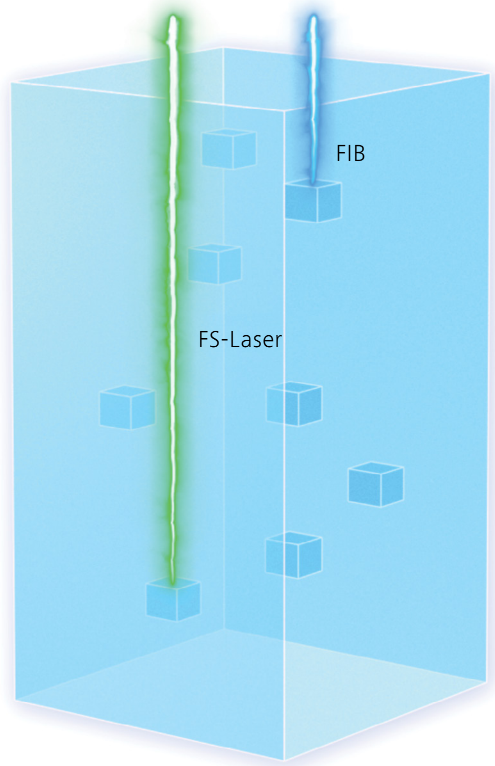
**My sample is hidden in a large volume, how do I identify this region of interest?**




## 1 Identify

Use Resolution at a Distance (RaaD) and Scout-and-Zoom capabilities of the ZEISS Xradia Versa to perform non-destructive imaging of multiple regions of interest.

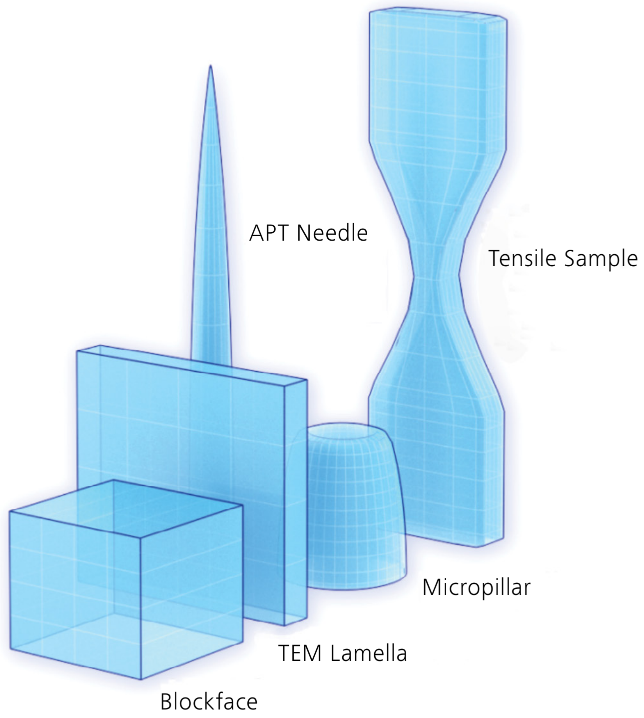
**How do I access and expose this deeply buried region of interest? How do I precisely access this ROI?**




## 2 Access

Use the integrated fs-laser on the Crossbeam FIB-SEM platform to perform massive material ablation and to precisely access the buried ROI

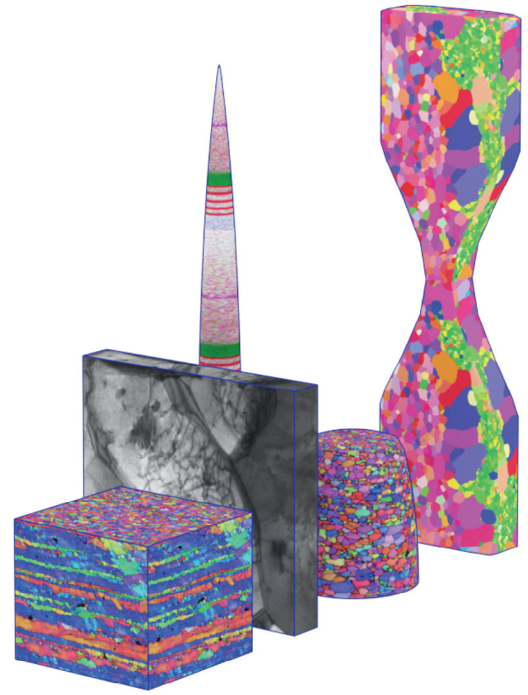
**I need a high-quality and site-specific sample for downstream analysis. How do I prepare a surface or structure at my identified ROI?**




## 3 Prepare

Use the Ion-sculptor $Ga^{+}$ ion FIB column on the Crossbeam platform for high quality preparation of surfaces and structures.

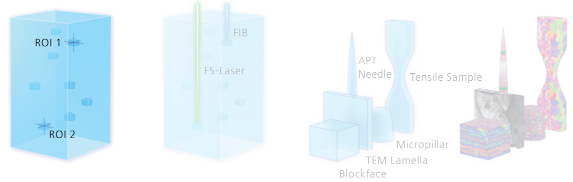
**What analysis can I perform using my prepared sample?**



## 4 Analyze

Use the prepared surfaces for a wide range of imaging, microanalysis or 3D-FIB tomography. Use prepared structures for APT, TEM, nanoCT and synchrotron experiments

# Identify Your Sample-In-Volume With a 3D X-ray Microscope



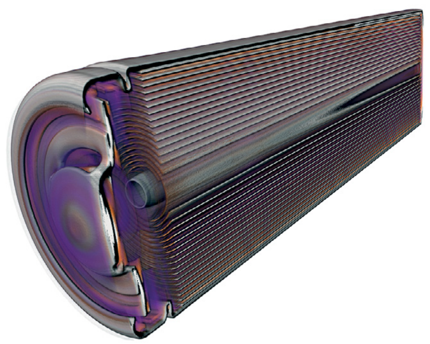

**Challenge**

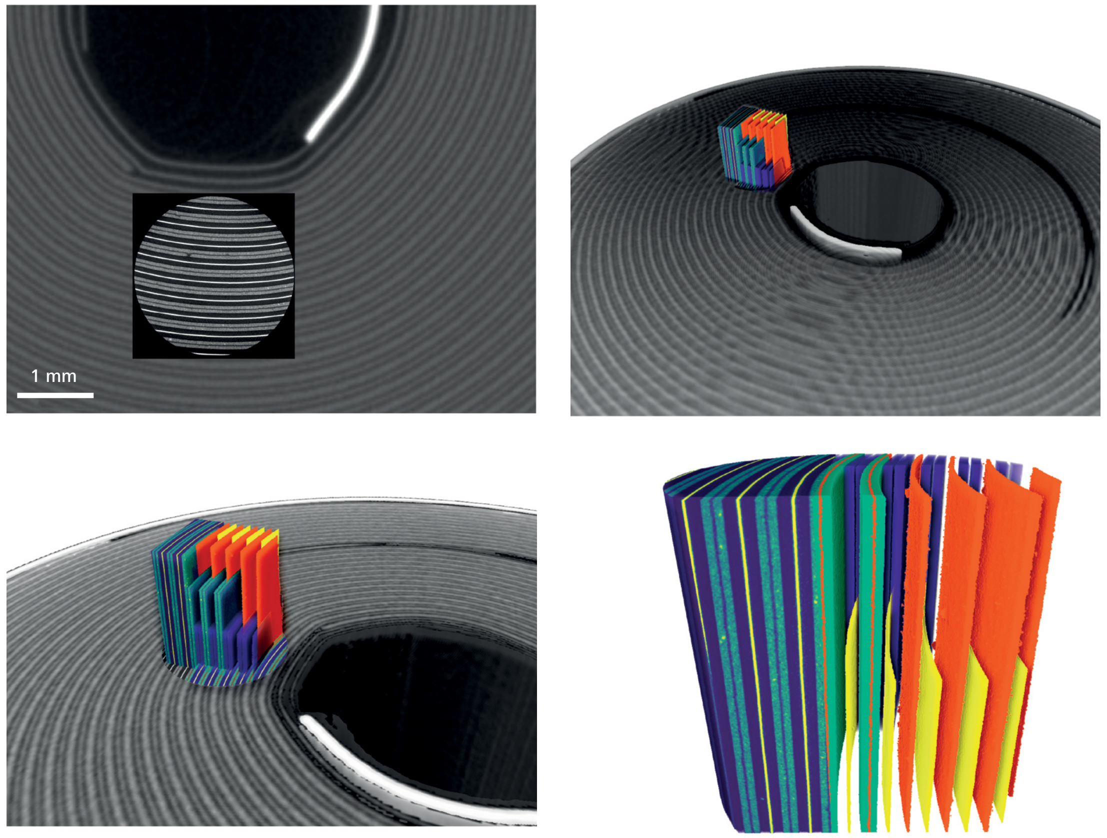
Material science samples present a multi-scale problem. These samples can be large and contain features that span from macro- to sub-nanometer length scales. The challenge is to identify regions of interest for further analysis.

**How can the ZEISS Xradia Versa help to identify regions of interest in larger sample volumes?**

The ZEISS Xradia Versa enables non-destructive 3D X-ray microscopy (XRM) for imaging of large samples. The synchrotron-like optics provide two distinctive advantages in addressing the multi-scale microscopy challenge:

- Use Resolution at a Distance (RaaD) technology to maintain a high spatial resolution while imaging large sample volumes
- Benefit from the Scout-and-Zoom capability to locate multiple regions of interest and perform higher resolution non-destructive “interior tomography” investigations for region of interest identification.






*Intact 18650 Li ion battery imaged using RaaD & Scout and Zoom. Internal tomography reveals remarkable detail of the electrode, including aging effects, foreign particles and cracks.*

*Full field of view: 30 µm voxel size, high res scan 1 µm voxels.*



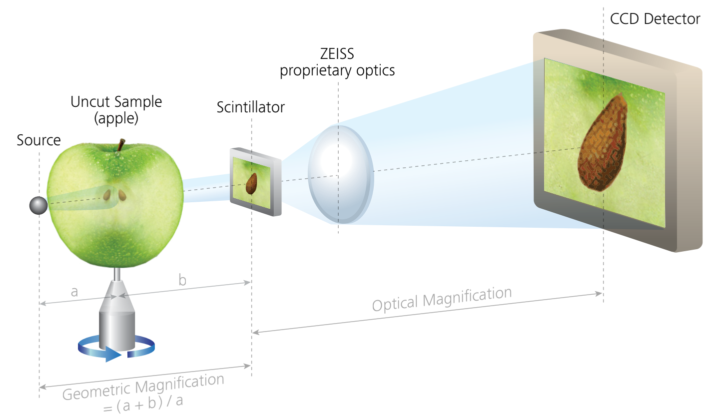
# Technology Highlight: ZEISS Xradia Versa X-ray Microscope

## 3D Non-destructive XRM Imaging at Sub-micron Resolution

Begin your sample-in-volume workflow with non-destructive 3D X-ray imaging at sub-micrometer resolutions using an XRM of the  ZEISS Xradia Versa family. Obtain data for a wide range of samples and working environments. Xradia Versa XRMs feature dual-stage magnification based on synchrotron-caliber optics, a distinctive advantage compared to traditional micro-computed tomography.

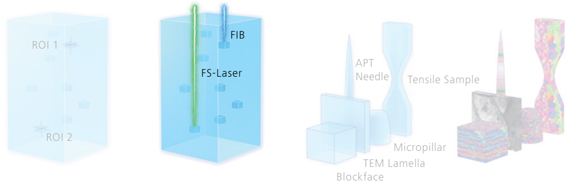

**Benefit from:**

- Resolution at a Distance (RaaD) that enables high resolution 3D imaging of large sample volumes.
- Scout-and-Zoom, a unique capability that allows exploratory scout scans across a large field of view to identify interior ROIs for higher resolution zoom scans.
- *In situ* kits that enable 3D and 4D studies letting you understand how materials perform in different environments or under different stimuli like time, tension or temperature.
- Laboratory-based Diffraction Contrast Tomography (LabDCT) that enables access to 3D crystallographic information, the acquisition of non-destructive mapping of grain orientation and the investigation of microstructures in 3D.
- The high flux X-ray source and acquire tomography scans faster without sacrificing resolution and contrast.
- AI-driven advanced reconstruction technologies with the Advanced Reconstruction Toolbox (ART)




*ZEISS XRM two-stage magnification architecture (RaaD). Sample is imaged independent of distance to source, enabling interiors of larger samples to be imaged non-destructively at higher resolution.*

# Access Your Sample With the LaserFIB and Find Your ROI

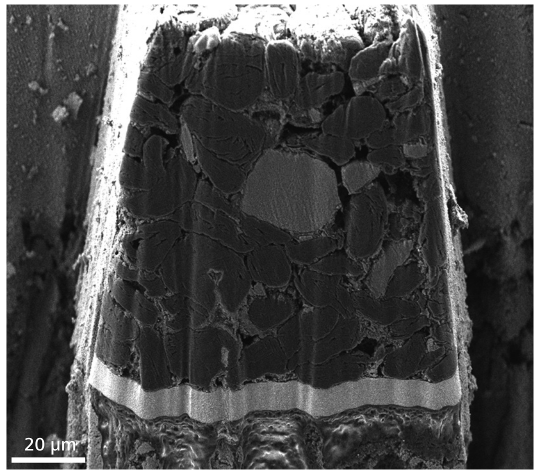


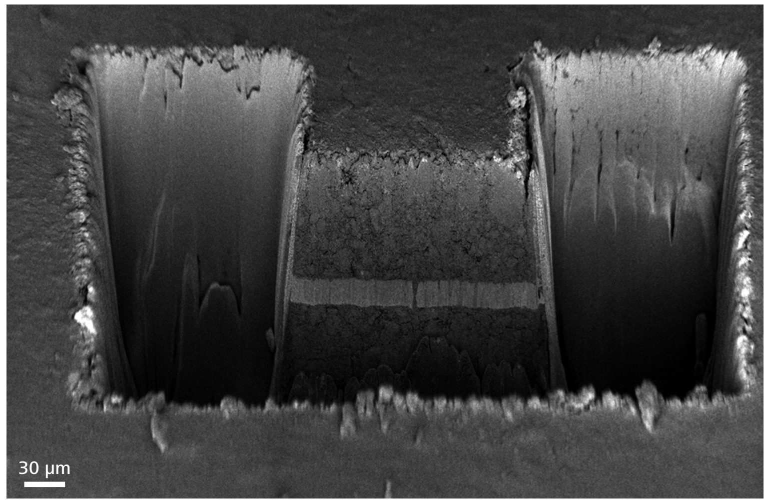

**Challenge**

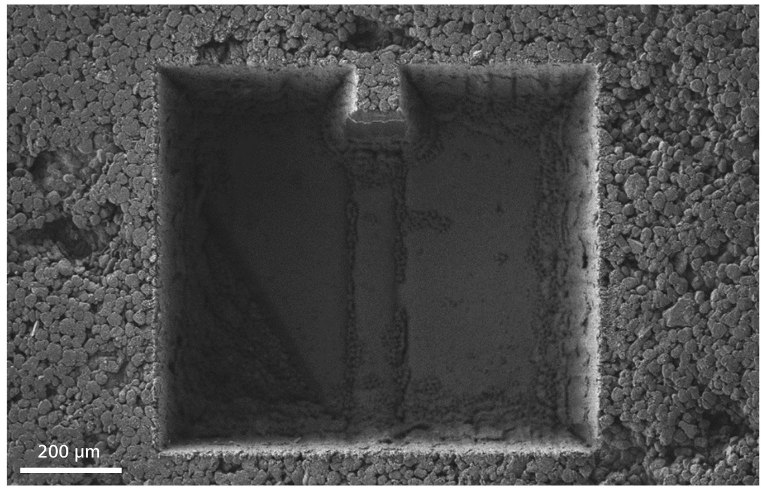
Accessing the identified region of interest is a two-fold problem: Firstly, the region of interest is buried within a large sample volume, requiring massive material ablation to access the target region. Secondly, precise access of the region of interest requires software correlation of the 3D X-ray tomogram with 2D FIB-SEM stage coordinates to ablate precisely to the buried location.

**How does the ZEISS Crossbeam laser combined with the ZEISS Atlas 5 software provide a solution for targeting deeply buried regions of interest?**

Use the LaserFIB from ZEISS which combines an integrated femto-second laser on the Crossbeam platform to perform massive material removal. Furthermore, the Atlas 5 software allows correlation of the XRM 3D tomogram and stage coordinates of the FIB. This enables the accurate location of (i.e., depth) regions of interest within the volume and targeted fs-laser sample preparation to access this region. The dedicated fs-laser chamber means the main instrument chamber is protected from contamination and the cross-jet technology ensures laser power is constant throughout the ablation process.




*Left: Fs-laser milling to expose a lithium ion battery (LIB) cathode foil. Aluminium collector foil can be observed between two layers of active material which has been sprayed and calendered onto the aluminium foil. The cut quality is sufficient to do a quick assessment of the manufacturing parameters. Right: 600 µm wide square milled into battery anode material to a depth of more than 400 µm in just 1 minute; Inset image shows the surface quality immediately after laser milling. The cross-section surface is clean and is an ideal starting condition for final sample preparation and polishing by FIB.*



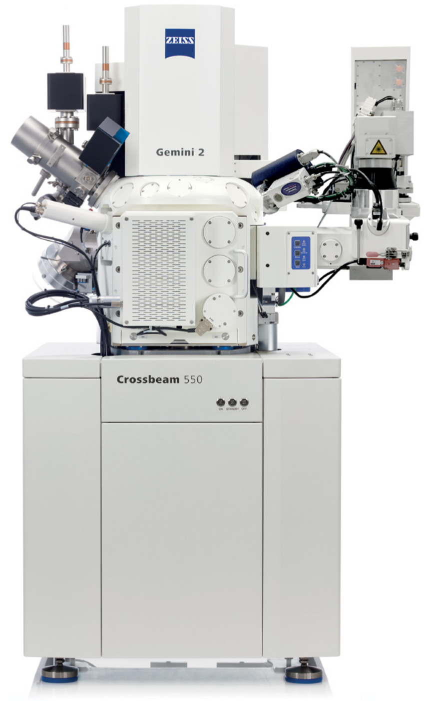
# Technology Highlight: ZEISS Crossbeam laser (LaserFIB)

## Rapidly Access ROIs Buried Deeply within the Sample Volume

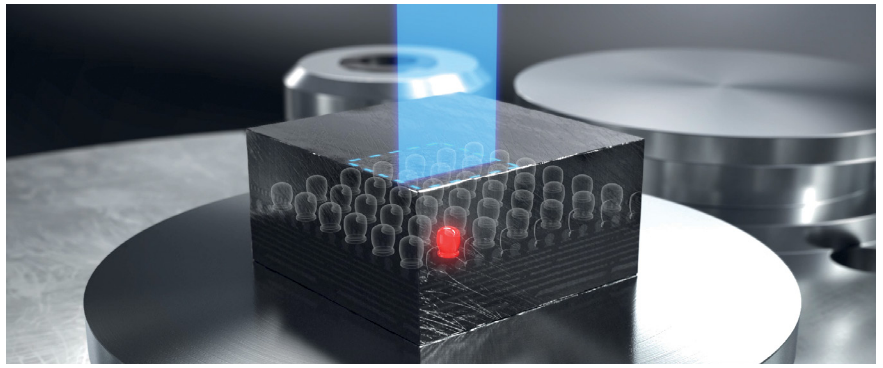
Move to step two in the workflow: Access your ROI precisely and rapidly with ZEISS Crossbeam laser. Its fs laser enables massive and rapid material removal. Take advantage of a ZEISS FIB-SEM that lets you combine the imaging and analytical performance of a high-resolution field emission SEM with the processing ability of a next-generation FIB. Finally, expand the capacity of Crossbeam laser with ZEISS Atlas 5 and enjoy seamless navigation with contextual image viewing in a sample-centric workspace, a GUI tailored to high-end correlative microscopy.

**Benefit from:**

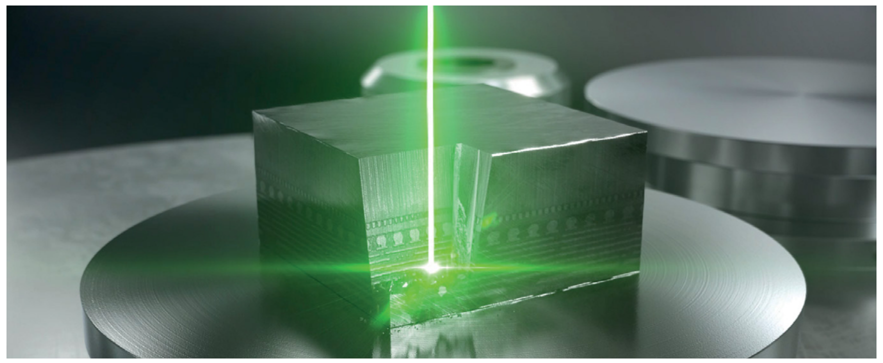
- the 515 nm green laser with burst pulsing that enable massive material ablation with rates up to 15 Mio. µm3/s (for Si) and resolution <2 µm on local areas
- preparation of extremely large cross-sections up to millimeters in width and depth within minutes.
- avoiding contamination of your FIB-SEM main chamber by performing laser work in a dedicated integrated load lock chamber with isolation to maintain cleanliness
- minimal damage to your sample from the quasi-athermal ablation provided by the ultrashort laser pulses
- a dedicated solution for multi-scale experiments, ZEISS Atlas 5, which lets you correlate to your ROI from the XRM dataset and enables the LaserFIB to access the site deeply buried in your sample




*Example of the ZEISS LaserFIB. The LaserFIB has an integrated femtosecond laser unit (right side of the chamber) for massive material ablation with a dedicated chamber and cross-jet debris handling system to protect the main FIB chamber*

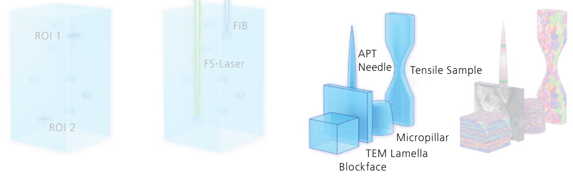


*Schematic showing how the LaserFIB is being used to access the ROI identified in an XRM dataset (red) in a large volume. Sample: electronic device, a TSV multichip package with defects in the microbumps with SEM beam in blue.*



*Schematic of the laser beam (green) during material removal in the separated chamber.*

# Prepare Your Sample for Further Analysis with a FIB-SEM




**Challenge**

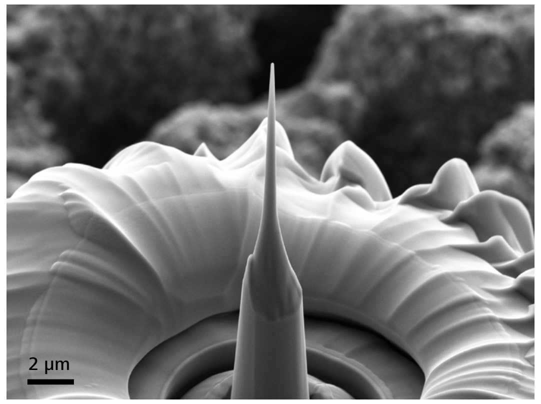
For reliable experiments, a high-quality surface or structure needs to be prepared on the regions that have been exposed through massive material removal using the fs-laser. Sample regions that are prepared for analytical characterization need to be of the highest quality. Milling precision needs to ensure the region of interest is not damaged by milling or polishing which can introduce artefacts in the analysis.

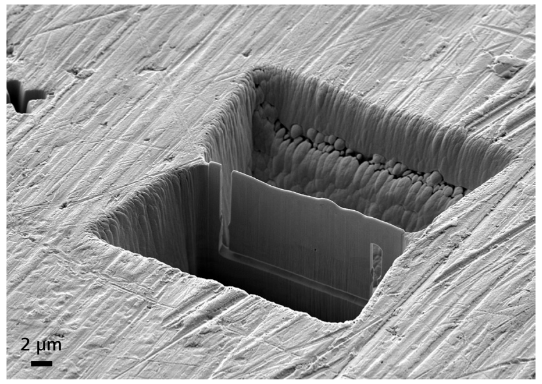
**How does the Ion-sculptor (Ga⁺ ion column) provide you with the best-in-class ion column for precise and reliable preparation?**

The Ion-sculptor Ga⁺ ion FIB column on the ZEISS Crossbeam platform enables you to precisely arrive at the region of interest and prepare high quality surfaces and structures. Take advantage of Ion-sculptor to minimize sample damage, maximize sample quality and prepare for experiments faster

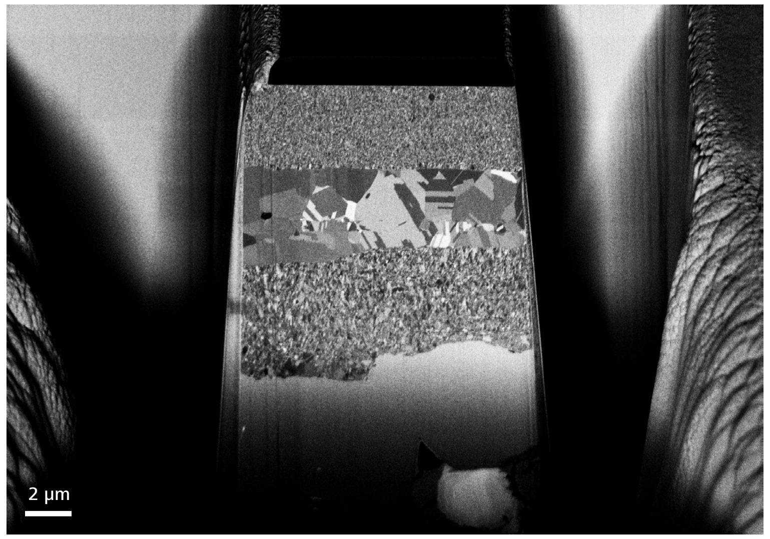
- Benefit from the 100 nA current to prepare surfaces and structures of your region of interest with speed and precision
- Use the low voltage capabilities of the Ion-sculptor column to prepare high quality (i.e., thin, minimum amorphization) surfaces and structures
- Observe live imaging and ion milling and watch your sample preparation taking place in real time

These capabilities ensure that once you arrive at the region of interest, you are able to prepare the highest quality sample possible.




*Prepare sample structures for further downstream analysis.*
*Top: A LaserFIB pre-prepared pillar is isolated from a sample volume for FIB milling and shaping of an atom probe needle.*
*Bottom: TEM lamella of an AgNiCu layered system ready for lift out.*




*Sample surface preparation through a non-metallic polymer protective layer on a NdFeB magnet, ready for EBSD analysis*



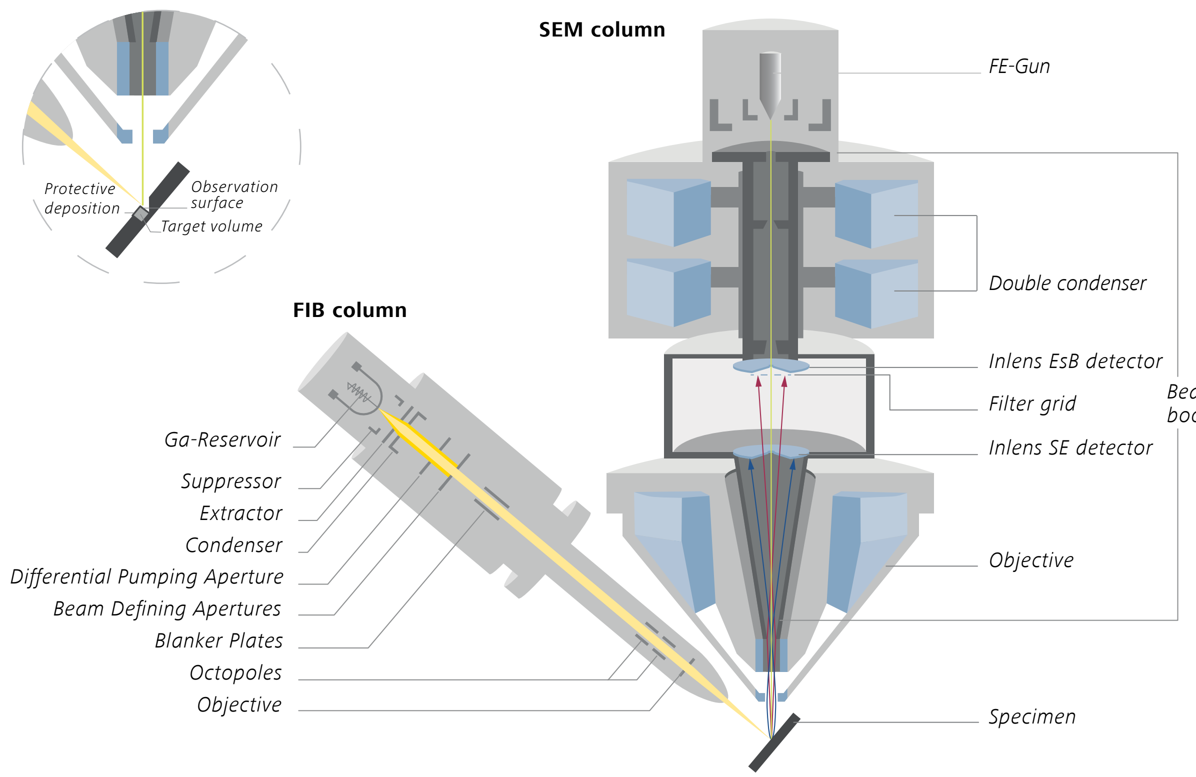
# Technology Highlight: Crossbeam With Ion-sculptor Column (FIB-SEM)

## Combine Precise Sample Preparation with Informative Imaging


Once the ROI has been identified with XRM and accessed with the LaserFIB, dive into the details: the preparation of delicate structures. ZEISS Crossbeam laser allows you to prepare samples precisely and efficiently. Exceptional surface finishing can be achieved with the high resolution of the FIB and its unique capabilities when being operated at low kV. Perform live imaging and milling. For further analysis note that 3D EDS and 3D EBSD can be obtained while milling, letting you increase the FIB-SEM's efficiency even further.

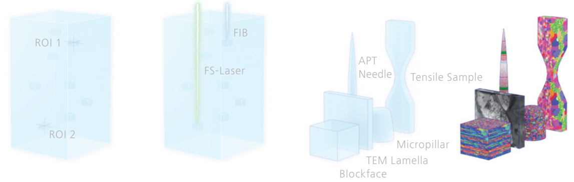
**Benefit from:**

- the LaserFIB to prepare tensile, compression or cantilever sample geometries for *in situ* nanomechanical testing, arrays of nanopillars, TEM lamellae, or atom probe tomography samples
- combining the laser with the Gallium FIB column, Ion-sculptor, for fine polishing if applicable
- Ion-sculptor's low voltage capabilities to get ultra-thin samples like TEM lamellae while keeping amorphization damage to a minimum
- the Ion-sculptor's 100 nA probe current enabling fast and precise preparation without compromising on FIB resolution
- Crossbeam's capability to perform live imaging while milling, a feature that simultaneously ensures precision in arriving at identified regions of interest
- the 6-axis stage allowing you to access and prepare complex specimens easily
- ZEISS Gemini optics, enabling you to achieve distortion-free, high-resolution imaging and best-in-class low kV performance without the need for stage biasing.




*The focused ion beam column, the Ion-sculptor of the Crossbeam product line.*

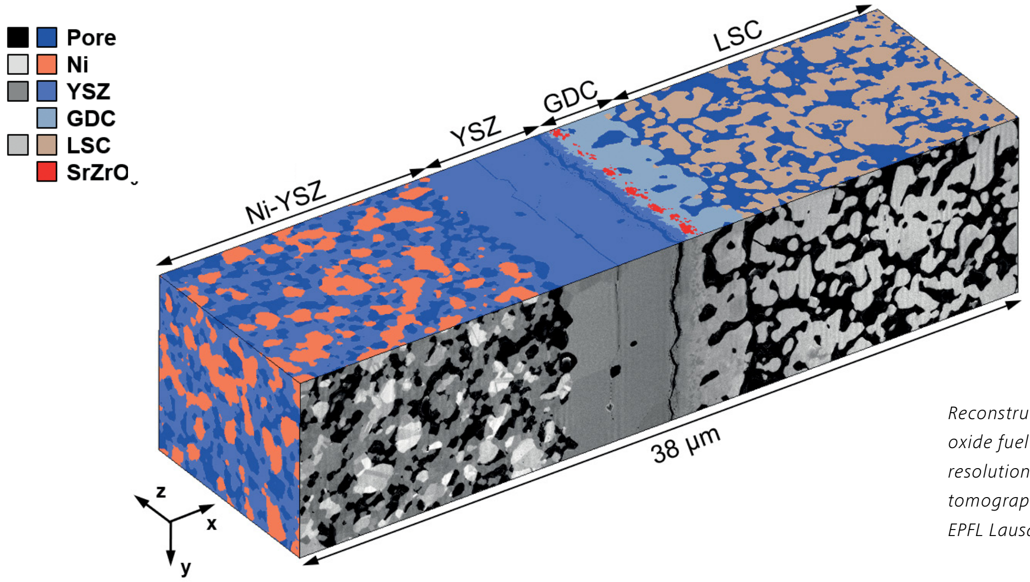
# Analyze Your Prepared Sample in Detail using Multiple Modalities



What experiments can you conduct on the surfaces or structures prepared in the FIB-SEM and with which microscopic or analytical modalities can you continue to work?

**Challenge**

- How to correlate to one or multiple ROIs you have identified in the large volume in the earlier steps of the workflow?
- How to ensure high resolution imaging and analysis, maybe even in 3D?
- Which structures or properties can be analyzed to characterize the sample comprehensively or design materials to perform better?




*Reconstructed 3D FIB tomography of a solid oxide fuel cell showing the advantages of high-resolution imaging and analysis with 3D FIB tomography. Sample courtesy of: M. Cantoni, EPFL Lausanne, Switzerland.*

**Solution**

- Correlate to your ROIs such as readily prepared cross-sections, with the FIB-SEM Crossbeam. Keep the context to your XRM dataset with the help of Atlas 5.
- Perform high resolution imaging with multiple detection modes directly with Crossbeam. Benefit from unique material contrast, transmission imaging and ECCI (electron channeling contrast imaging).
- When needing 3D visualization and quantitative analysis of your 3D XRM dataset before your FIB-SEM experiment, use the powerful ORS Dragonfly Pro software.
- Perform 3D tomography with Crossbeam and benefit from the best 3D voxel resolution and from its live milling and imaging capability.
- For highest demands in large area imaging, 3D tomography, 3D EDS and 3D EBSD analysis or nanopatterning use Atlas 5.
- Analyze trace elements with a ToF-SIMS spectrometer attached to Crossbeam in a completely air free workflow.
- Switch to other modalities like:
  - TEM analysis or STEM in SEM
  - *in situ* tests on a ZEISS FE-SEM
  - nanoCT on a ZEISS XRM Xradia Ultra or ZEISS Xradia Synchrotron end station
  - LabDCT on a ZEISS Versa XRM
  - Atom probe tomography



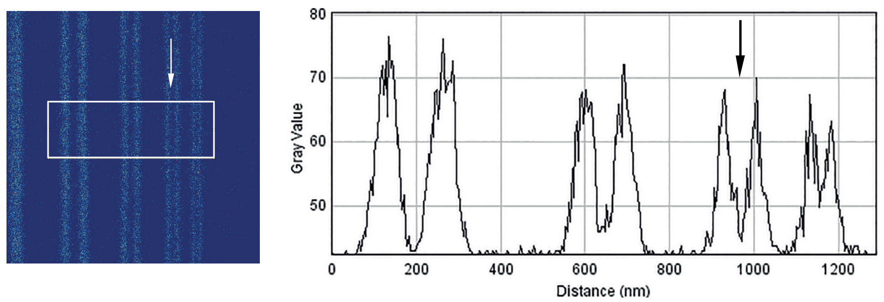
# Technology Highlight: Software for Correlation and Analysis

## Bridge the Gap over Multiple Scales and Modalities

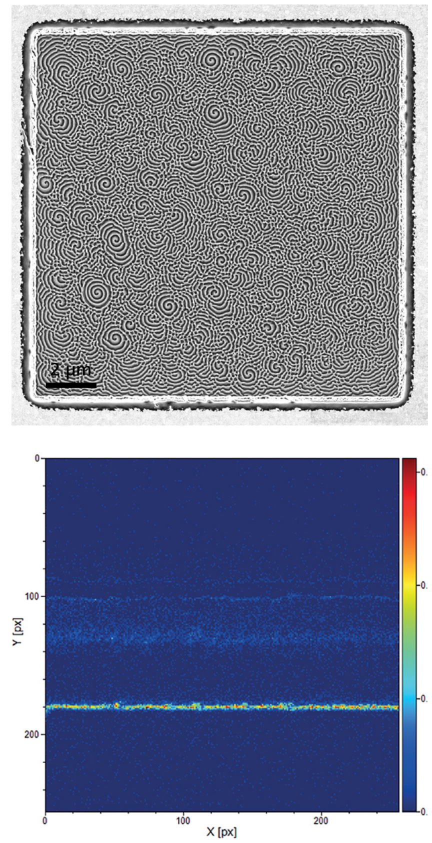
Analyze your specimen in depth and connect all scales and modalities with ZEISS Atlas 5 or ORS Dragonfly Pro software. For 3D visualization and quantitative analysis of X-ray and EM multi-scale and multi-modal data, use Dragonfly Pro. Atlas 5 efficiently correlates data in 2D & 3D workflows from any source. It lets you maximize the results of your 3D nanotomographies and 3D analytics. Lastly, from the prepared samples you can perform various analyses using ZEISS Microscopy Solutions, or switch to other modalities.

**Benefit from:**

- Multi-scale 3D visualization and analysis with the advanced analysis software ORS Dragonfly Pro and perform 3D image processing and segmentation.
- The automated acquisition of large 2D & 3D datasets without operator supervision using Atlas 5.
- Correlating images from multiple sources to build the seamless multi-modal, multi-scale dataset of your sample with Atlas 5.
- Simultaneous imaging and analysis while milling in the Crossbeam. This allows you to assess the process and quality of milling or 3D analytics with different imaging modes like secondary or backscatter electron detection or even use Inlens detection.
- Use of the integrated 3D analysis for EDS and EBSD of Atlas 5 and take advantage of the "True Z" slice thickness control, even below 5 nm slice thickness. Achieve best 3D resolution to produce isotropic voxels in 3D FIB-SEM tomography for the most reliable and high-resolution 3D FIB analysis.
- Switching to other modalities with ZEISS solutions, e.g. use a range of micro- and nanoscale imaging and analysis techniques such as the Xradia Ultra or ToF-SIMS on Crossbeam.

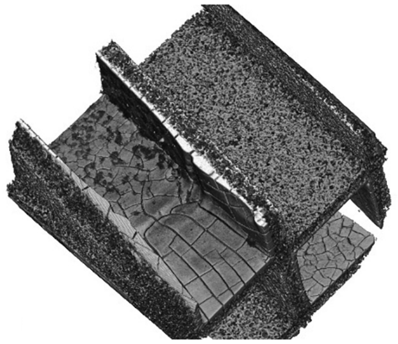



*Left: Al (27 amu) map of a calibrated BAM L200 sample. The FOV is 2 µm. Right: Line profile for the area within the green frame. Lines with a width and separation of 33.75 nm can be resolved clearly (arrows).*



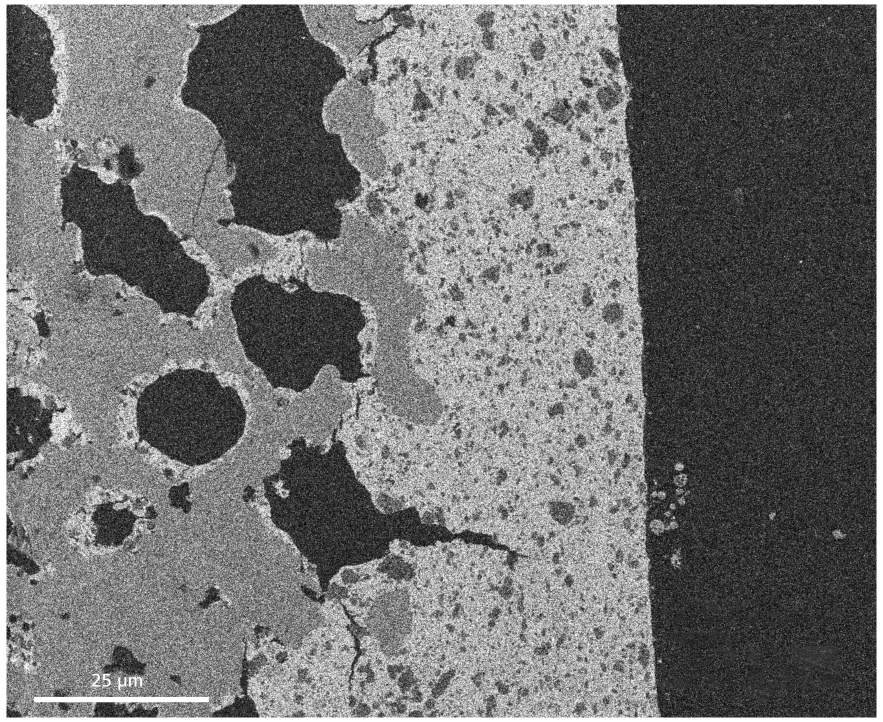

*SEM image (top) of a perovskite solar cell sample after a top-down SIMS measurement, acquired with Crossbeam with a ToF-SIMS, Na SIMS map (bottom).*

# Applications
## Catalytic Systems and Nanoparticles



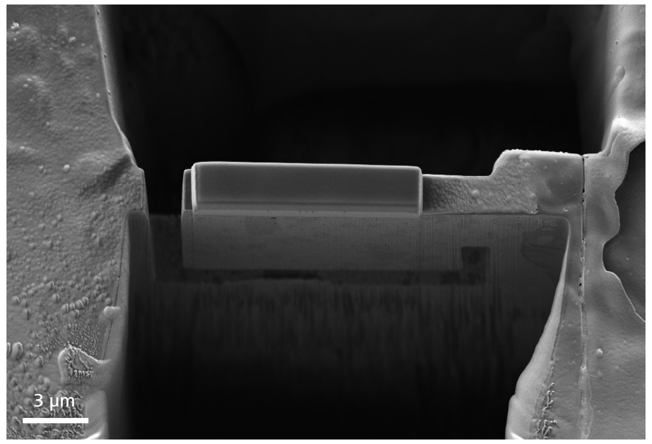
**XRM is used to identify a region of interest within the catalyst structure**
**Scale:** macro– to micrometer structures

This region of interest will be investigated to understand the catalyst active sites and whether the morphology of the nanoparticles provide enough active sites.



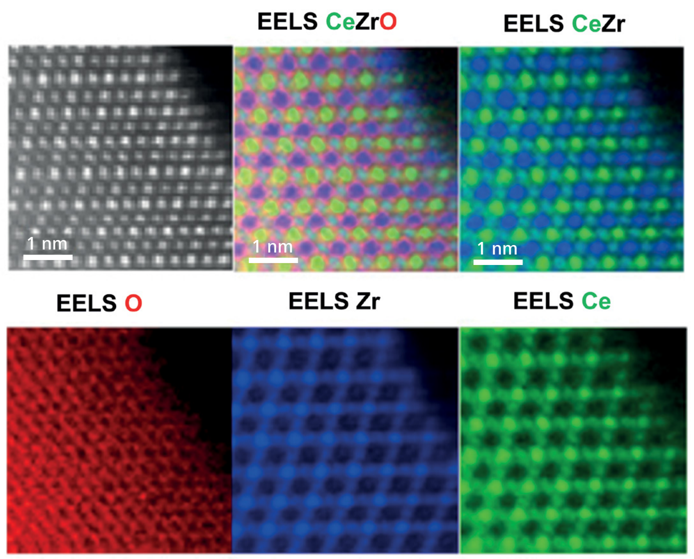

**Access using the LaserFIB or Crossbeam**
**Scale:** macro- to nanometer structures

Correlation of the XRM data volume with the FIB-SEM stage coordinates using Atlas 5 enables targeted milling to access the region of interest.




**Prepare using the Crossbeam and Ion-sculptor $Ga^+$ ion column**
**Scale:** sub-micrometer

The $Ga^+$ ion column is used to prepare a TEM lamella at the targeted site. The low kV performance of the $Ga^+$ ion column is utilized to produce the highest quality TEM sample possible.

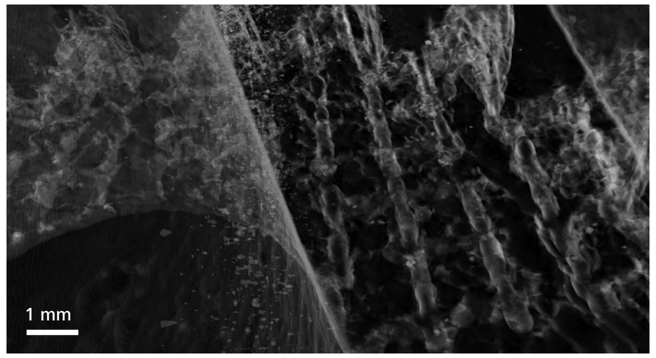



**Analyze the prepared surface or structure**
**Scale:** sub-nanometer

Prepared TEM lamellae are taken to a TEM for HR-STEM and further EELS analytics

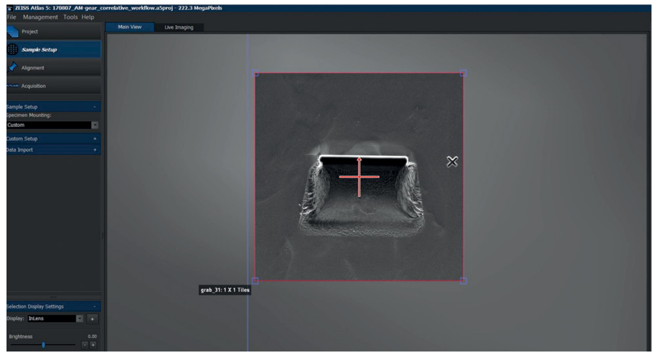


# Applications
## Metals



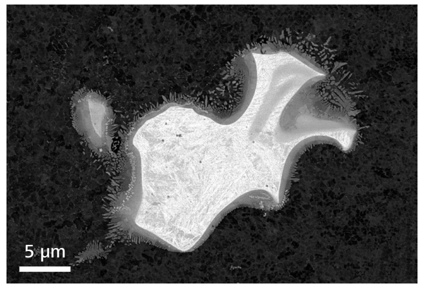

**XRM is used to identify an unknown inclusion in a Laser Powder Bed Fusion (L-PBF) additively manufactured gear**
**Scale:** macro– to micrometer structures

A low resolution scan identifies an unknown and "bright" inclusion within the gear part. Using the Scout and Zoom workflow, a higher resolution scan confirms the feature of interest for further studies.



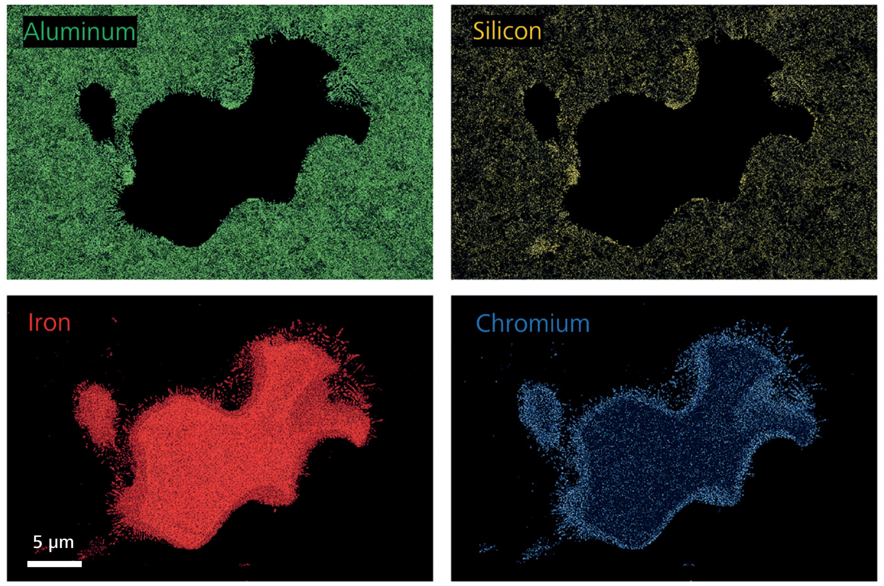
**Access using the LaserFIB or Crossbeam**
**Scale:** macro- to nanometer structures

The feature of interest was buried at a depth out of reach for FIB milling. The femtosecond laser was used to ablate into the sample and provide precise access to the feature.




**Prepare using the Crossbeam and Ion-sculptor $Ga^+$ ion column**
**Scale:** sub-micrometer

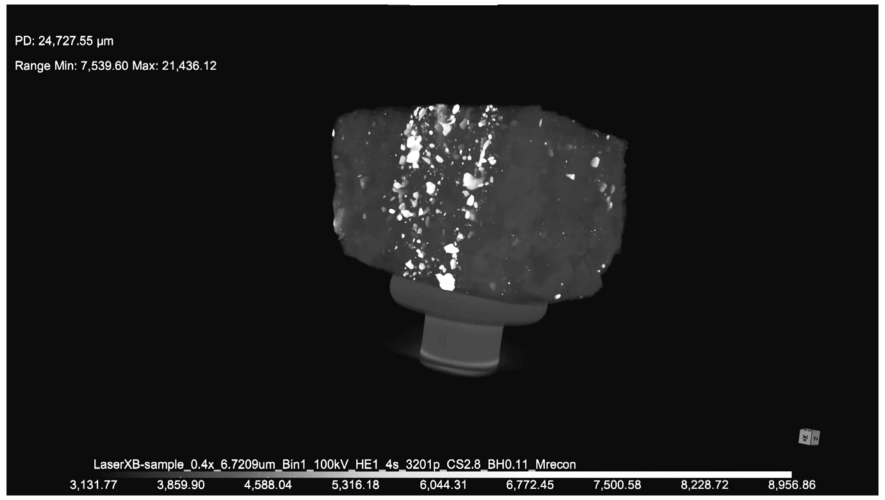
A high-quality surface is prepared across the feature of interest. The location of the feature was confirmed with the XRM dataset to guide milling depth.




**Analyse the prepared surface or structure**
**Scale:** sub-micrometer

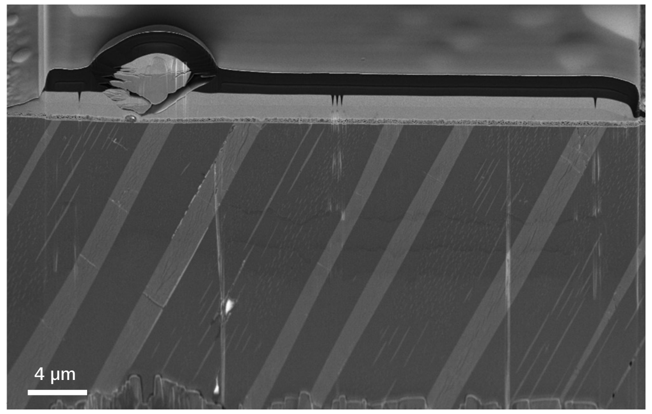
The FIB cross section and backscattered electron contrast provide an early indication of chemical differences across the feature. An Energy Dispersive X-ray spectroscopy (EDS) element map outlines the distribution of Fe and Cr within the inclusion

# Applications
## Geoscience



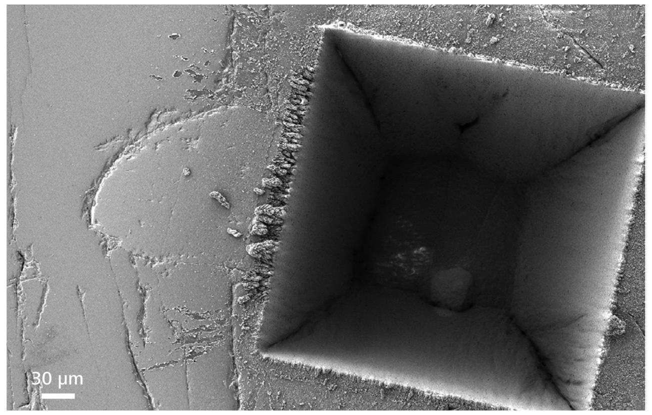

**XRM is used to identify an unusual mineral texture buried deeply within a rock sample.**
**Scale:** macro– to micrometer structures

An overview XRM analysis helps to identify regions of interest within the sample which are then further imaged using RaaD for high resolution, non destructive interior tomography.




**Access using the LaserFIB or Crossbeam**
**Scale:** macro- to nanometer structures

Within 32 seconds a 300 µm × 300 µm × 300 µm trench is cut into the sample to access the identified phase of interest buried in the sample.

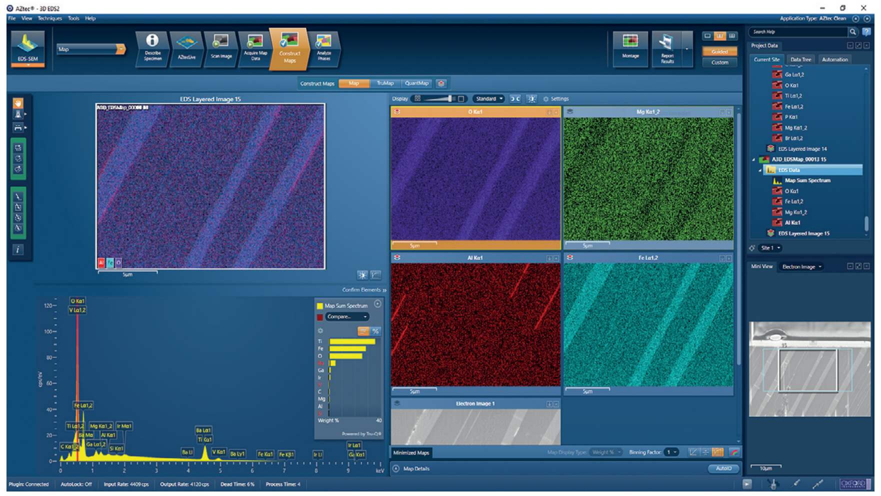



**Prepare using the Crossbeam and Ion-sculptor Ga⁺ ion column**
**Scale:** sub-micrometer

The sample surface is prepared with FIB milling to allow for high-quality further analysis using EDX.

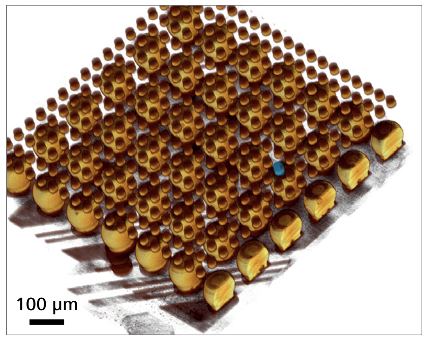
**Analyse the prepared surface or structure**
**Scale:** sub-nanometer

EDS measurements and phase mapping outlines chemical variations across the minerals present.



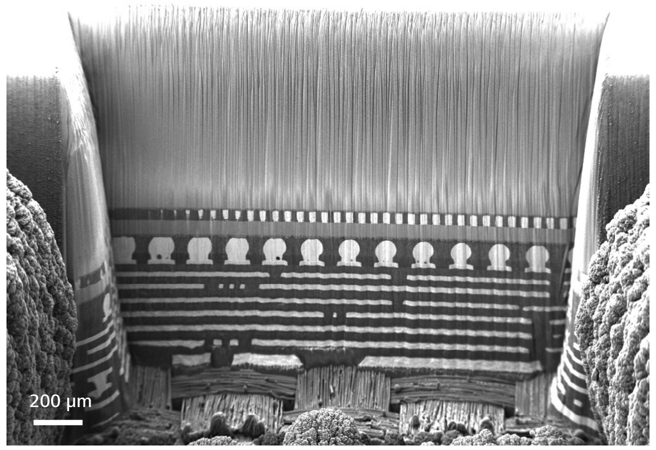


# Applications
## Electronics



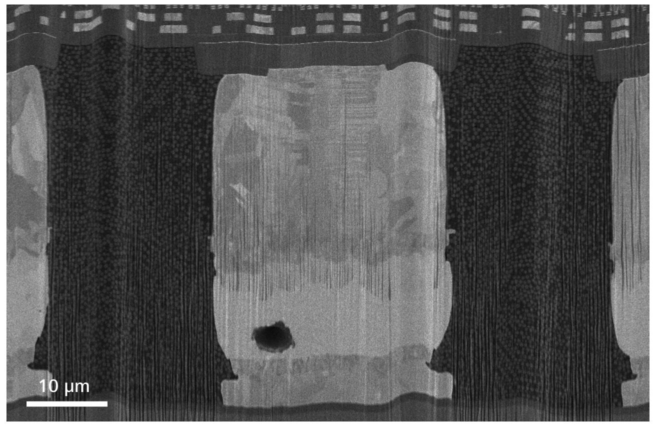

**XRM is used to identify a failure point in a packaged electronic device**
**Scale:** macro– to micrometer structures

An XRM scan is used to identify a ~5 µm void within a Cu-pillar microbump. The void is further imaged using RaaD to provide higher resolution without cutting the sample.

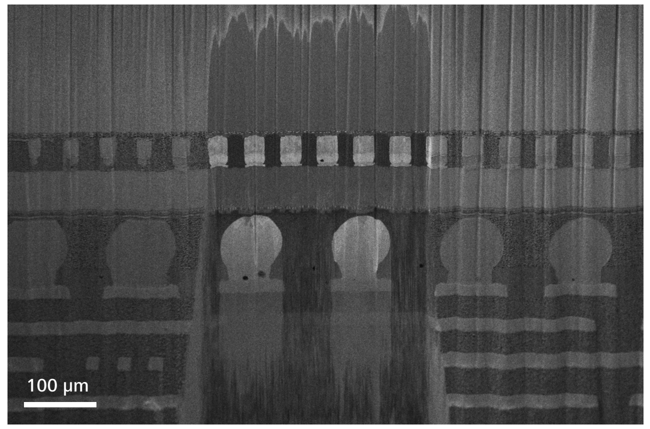

**Access using the LaserFIB or Crossbeam**
**Scale:** macro- to nanometer structures

A 3.1 mm × 1.2 mm × 1.6 mm trench is milled to precisely access the region of interest for further analysis. The cross-jet is used to maintain laser power throughout the laser milling process.




**Prepare using the Crossbeam and Ion-sculptor Ga⁺ ion column**
**Scale:** sub-micrometer

A final FIB-SEM polish is used to prepare a high-quality sample surface at the region of interest, exposing the void identified by XRM.




**Analyse the prepared surface or structure**
**Scale:** sub-nanometer

Imaging is performed to understand the void size, shape and location.

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