3D X-ray Vision
Non-destructive Imaging of Internal Structures
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Visualizing buried features and microstructures at high resolution traditionally has required destructively sectioning a sample to prepare it for 2D optical or electron microscope imaging. While newer serial sectioning techniques (FIB-SEM and microtomy) have emerged as pathways to 3D imaging, the damage due to sectioning leads to consumption of the sample during measurement. ZEISS has the first 3D X-ray microscopes (XRM) truly capable of non-destructive analysis for quantification of internal structural parameters at submicron to nanometer scale. This also preserves the integrity of samples for further investigation.

Challenges Inherent in 2D Slicing Methods
Historically, microscopy studies aimed at understanding the interior of a sample began with 2D methods, like optical or electron microscopy. However, these imaging methods faced major challenges due to requiring mechanical sectioning of the sample which can damage structures and alter or destroy sensitive features, produce mechanical cutting artifacts and face damage, whether due to physical cutting tool, FIB-SEM, or laser damage, and expose internal structures to the atmosphere.

Reliance on 2D images for 3D conclusions
While some systems have proven quantifiable using stereo-graphy under ideal conditions, research shows that extrapolating 2D images to 3D metrics can be highly inaccurate, especially for real-world materials that are often heterogeneous or anisotropic.1, 2, 3

3D Imaging via Serial Sectioning
In response to these limitations, new techniques were developed for 3D characterization, including extending optical and electron microscopy to 2D-based serial sectioning microanalysis with associated drawbacks. In these techniques, samples are repeatedly sliced while 2D surface images are taken. The resulting images are used for 3D reconstruction. While this moved closer to 3D characterization, reliance on slicing caused limited depth resolution dependent on slice thickness, spacing, and accuracy of cut placement, causing highly non-cubic voxels, and retains the damage issues resulting from 2D interior sectioning. This approach ultimately consumes the sample during the imaging process, which prevents re-imaging the sample for 4D (3D imaging over time) studies or multi-length scale analysis through use of another imaging modality.

The 3D X-ray Solution
High-resolution 3D X-ray microscopes (XRM) resolve these issues and create the possibility of non-destructively imaging in 3D at comparable length scales. The deep penetration of X-rays can remove or minimize the need for extensive sample preparation. Full X-ray tomography also does not alter the sample and hence does not suffer from mechanical sectioning artifacts and noncubic voxels. The result is superior visualization and quantification of 3D microstructures.

ZEISS offers Xradia Versa and Xradia Ultra families to extend X-ray microCT abilities to provide: (1) submicron down to nanometer scale resolution, (2) high contrast to accurately quantify internal structural information, and (3) high resolution at large working distance, which are critical for a wide range of studies including the following.
Semiconductor failure analysis: Imaging of submicron defects within intact packages or nanometer voids in advanced TSV

Porous materials and virtual core analysis: Large data set acquisition to provide maximum statistics on porous networks for fluid flow models and analysis

Ex-vivo preclinical investigations: ZEISS systems are uniquely high contrast for unstained soft materials (see tech note “High Contrast”)

In-situ and microstructural evolution: Repeated imaging of samples under load and other conditions for quantification of structural variation

Fragile materials: Non-destructive imaging of fragile polymer foams or fossils

**Why ZEISS?**

Unlike traditional flat-panel microCT systems, ZEISS’s unique 3D X-ray microscope detector architecture provides multi-length scale imaging with resolution down to 50 nm, phase contrast imaging technology for low contrast features, and high resolution maintained for large sample sizes and working distances.

*ZEISS’s X-ray microscopes allow the best resolution X-ray vision into large samples.*

![Figure 1: Application examples for 3D X-ray imaging](image)

(a) Geology – Segmented sandstone that is composed of grain matrix (yellow), clay (blue), pore (green), and high Z materials (red).

(b) Materials Science – Wood tomography visualization of fiber microstructure and can be used to determine hygroscopic properties.

(c) Semiconductor industry – Tomography of Through Silicon Vias (TSVs) detects voids formed.

(d) Life Sciences – Freshwater bryozoans Cristatellamucedo stained with PTA (Image provided by Brian Metscher and Gerd Müller of the Department of Theoretical Biology, University of Vienna).

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**Suggested Reading**
