Product Data

Extended Slice and View

Routine 3D tissue imaging

Benefits

- Improved isotropic resolution
- Extends the resolution of the 3D image
- Extends the volume of the area to be imaged
- High accuracy of slice placement using image recognition algorithms
- Large field of view imaging through the automated acquisition of tiled images

Extended Slice and View enables you to create 3D tissue images in high resolution and within a fully automated workflow. Based on the popular Slice and View, Extended Slice and View has been enhanced based on the feedback and requirements of the research community. FEI has developed an automated image acquisition method that improves the isotropy of the resolution of the cross-sectional face by moving the sample perpendicular to the SEM column and positioning the sample close to the end lens. Dramatic improvements in resolution can be archived which reveal greater insight into biological processes.

Since FEI first introduced the DualBeam™ instrument in 1993, a series of applications have been introduced which take advantage of the DualBeam's unique capabilities. Extended Slice and View represents the latest application to utilize the sample manipulation and imaging capabilities of the DualBeam and adds extensive automation functionality which makes routine volume imaging of biological samples a reality.

Imaging Biological Samples with a DualBeam

DualBeam instruments combine the imaging capability of a scanning electron microscope (SEM) with the cutting and deposition capability of a focused ion beam (FIB). This combination provides biologists with a powerful tool for investigating three-dimensional structure with nanoscale resolution.

The high-energy ions (Ga+) of the FIB remove material by sputtering atoms and molecules from the sample surface. Their confinement in a tightly-focused beam provides precise spatial control of the milling process.

Volume interaction graphs

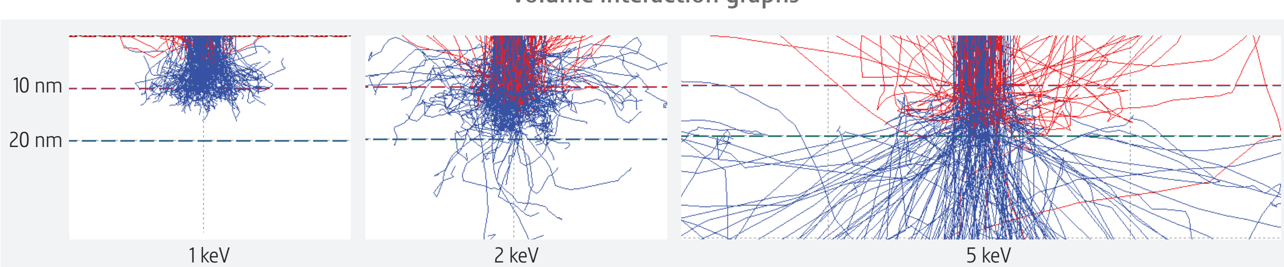

Figure 1: Volume interaction graphs generated using Monte Carlo simulations illustrate the beam penetration at 1 keV, 2 keV, and 5 keV.

Other techniques, such as Transmission Electron Microscopy (TEM), can provide sub-Angstrom resolution in some materials, though not generally in biological applications. Also, TEM-based electron tomography is playing an increasingly important role in visualizing the 3D architecture of cells and the imaging of macromolecular machines.

DualBeam instruments offer an attractive alternative for nanoscale imaging and 3D investigations of structures as large as a whole cell or a even across multiple cells. The DualBeam slice and view technique consists of acquiring a sequence of cross sectional images spaced evenly through a region of a bulk specimen, and reconstructing those two-dimensional images into a three-dimensional representation of the sampled volume. It has been used successfully on tissues and whole cells, and is particularly well-suited for composite specimens that include both hard and soft components. It also provides an effective method for preparing thin sections for TEM imaging and analysis.

FIB cross sectioning has several advantages over mechanical sectioning techniques commonly employed, such as ultramicrotomy:

- You are able to reproducibly remove slices with thicknesses even less than 10 nm; this provides more isotropic resolution than mechanical sectioning techniques.
- Knife marks and other cutting artifacts are largely eliminated.
- FIB milling performs particularly well on samples that include both hard and soft materials that would tend to tear or smear when cut by mechanical means.

Resolution Improvement

As resolution on an SEM is optimal at shorter working distances, we make use of a high-precision piezo stage to move the sample to an optimal imaging position while acquiring the images and move it back to the milling position for creating the next FIB slice. By using a position perpendicular to the SEM, the acquired pixels are not tilted like they are in a conventional beams-coincidence position. This results in a resolution improvement in the Y direction of greater than 25 percent.

Additionally, by optimizing the working distance the achievable resolution can be improved significantly.

Beam geometry

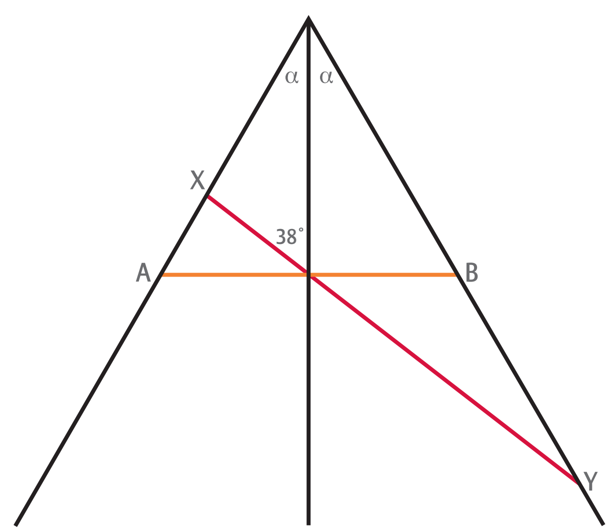

Figure 2: Beam geometry while imaging at the traditional DualBeam angle of 38° (XY) and the resolution enhancement when imaging perpendicular to the sample (AB).

Volume Improvement

Extended Slice and View makes use of FEI's advanced digital 16-bit scan generator, enabling the acquisition of 8k x 8k pixel images on the Helios platform. Compared to FEI's standard 4k x 4k scan engines, this enables acquiring 4 times more data in a single scan using the same pixel resolution.

In addition, the high-precision piezo stage technology is used for automated acquisition and subsequent tiling of images of the sample surface. This provides a large field of view for the 2D image and volume for the 3D image.

Brain tissue

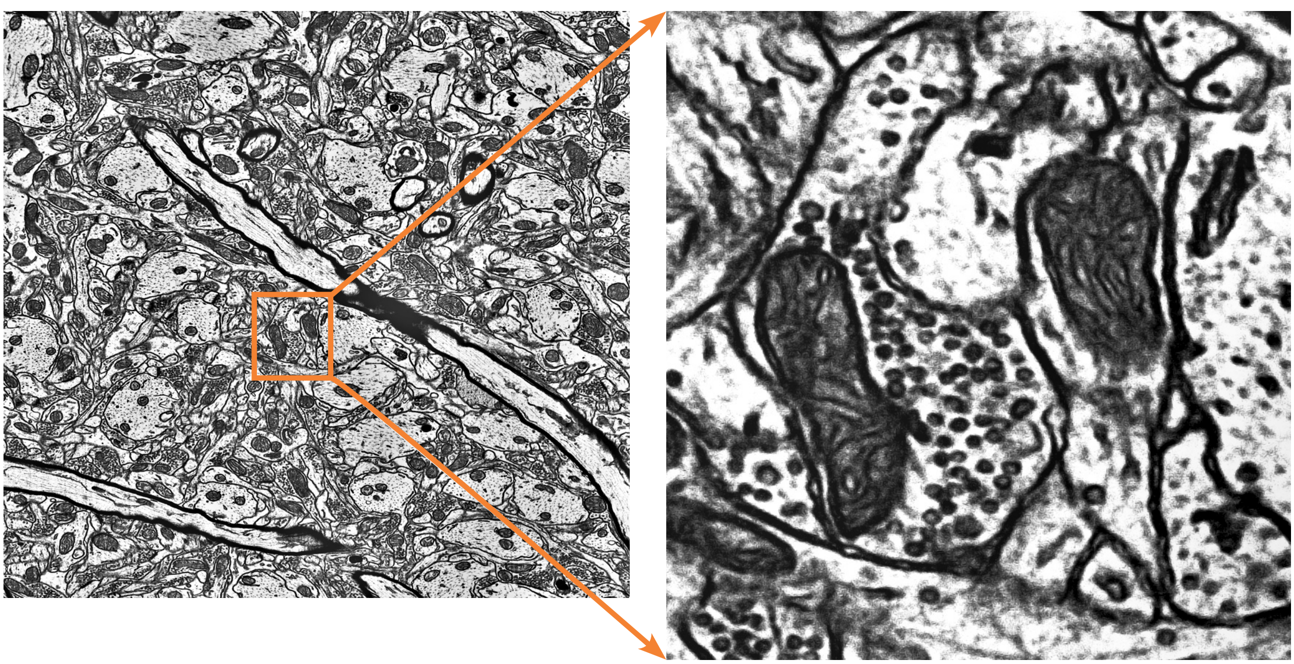

Figure 3: Brain tissue (from the region of the hippocampus).
Sample courtesy Dr. C. Genoud, Facility for Analysis of Images and Microscopy, Friedrich Miescher Institute, Basel.

Zooming

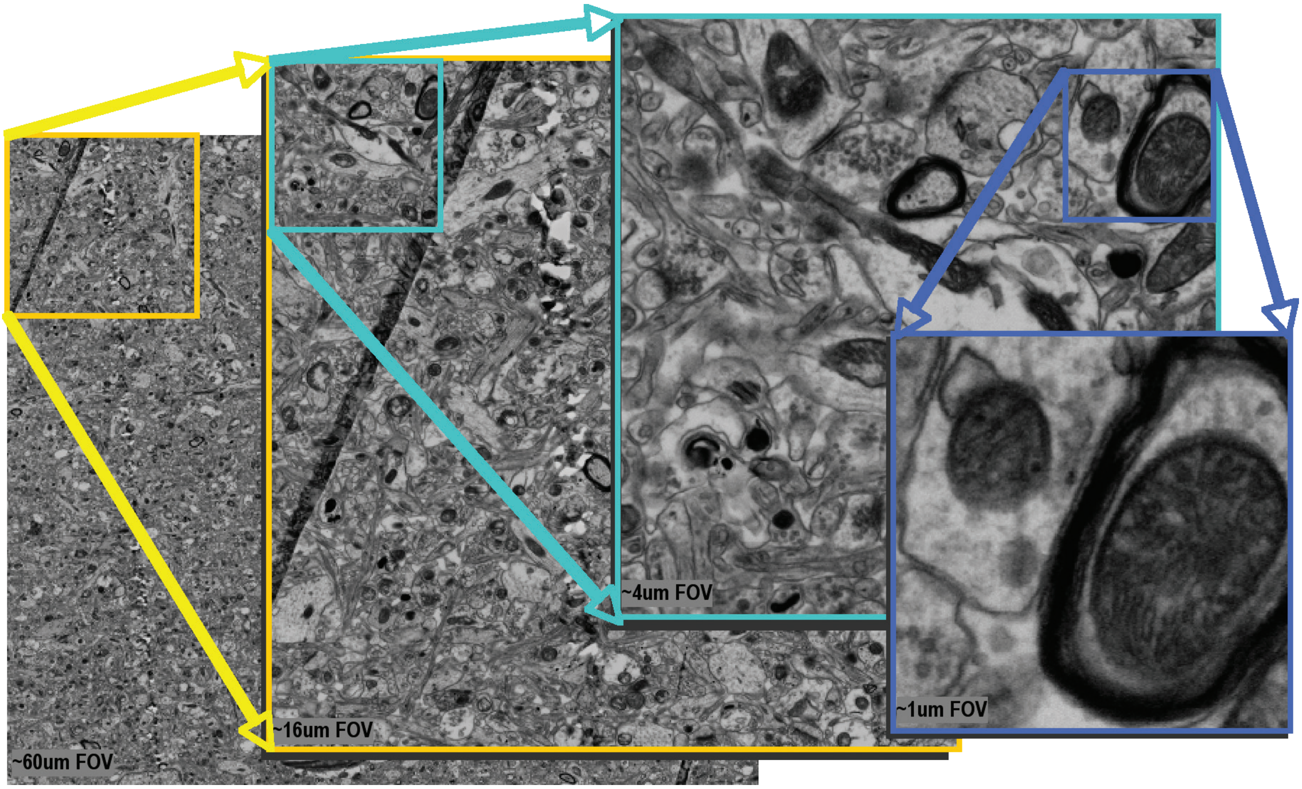

Figure 4: The 60 um FOV image consists of 4x4 tiles of 4 k images using a pixel size of 4 nm.

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