



Microscopy

How to Use FIB-SEM Data for 3-D Reconstruction

The ability to acquire, display and interrogate three-dimensional volumes of image data has been well-established through various scientific disciplines. The medical field, in particular, has exposed the public to tomographic methods through now common medical procedures such as computed axial tomography (CAT), magnetic resonance imaging (MRI) and positron emission tomography (PET). In an analogous fashion, the focused ion beam (FIB) and scanning electron microscopy (SEM) can combine to generate tomographic data.

While less common, the FIB-SEM tomographic method has demonstrated the ability to complete 3-D volumetric reconstruction at a resolution of 10 nm or better in all three dimensions. Thus, the method holds tremendous potential for materials and life science investigations.

With the advent of simultaneous high-resolution SE imaging during the FIB sectioning process, it is possible to acquire several hundred tomographic SEM image slices a few nanometers (nm) “thick” in the span of less than one hour. This ability to rapidly acquire high density and high-resolution tomographic SEM slices at the nanoscale contributes to practical implementation of the FIB-SEM tomographic technique.

Step by step

The process begins by defining a volume that will be FIB sectioned. Volumes are typically 200 to 500 μm^3 , which translates into typical dimensions of 10- μm wide, 5- μm deep and 10 μm in height. Suitable FIB milling currents range from 10 to 100 pA. Once milling of the volume is started the entire process is recorded continuously in real-time via AVI capture of the SEM image.

Frames are captured every 10 to 20 sec with scan conditions that result in 10,000 to 60,000 electrons per pixel per frame, yielding a very good signal-to-noise ratio. Two frames recorded from one such capture are shown in Fig. 1. The sample is a precipitate phase from the heat affected zone of a stainless steel weld. The dark contrast

is the precipitate phase and there is a platinum protective layer on the top. The inherent resolution of the technique is based primarily upon the interaction volume of the electron beam with the sample. The resolution of the technique is therefore in the range of 5 to 20 nm depending upon the SEM voltage and the material. Best results are obtained at lower voltage, 0.75 kV to 1.0 kV to maximize depth resolution.

Following acquisition of the successive image slices the

image data matrix is processed to perform the 3-D volume reconstruction. Process options include the ability to apply selective transparency to specific features based upon image or feature contrast, as shown in Fig. 2 where the precipitate phase is separated from the matrix. The arrows in Figs. 1 and 2 highlight a common structure in the 2-D frame and 3-D volume. All three axes are quantified in the process, enabling feature and volumetric analysis.

Latest updates

The latest exciting evolution in FIB-SEM tomography is the use of an in-lens backscattered electron (BSE^I) detector for the SEM signal, available on the Carl Zeiss SMT EsB XB. This in-lens BSE^I detector produces a low voltage, short working distance, high-resolution compositionally weighted image signal with minimal topographic contrast. In addition to being possibly the ideal signal for 3-D FIB-SEM tomography, it opens new opportunities for biological and life science samples through the ability to distinguish contrast associated with various forms of organic materials while providing maximum charge control.

Stay tuned!

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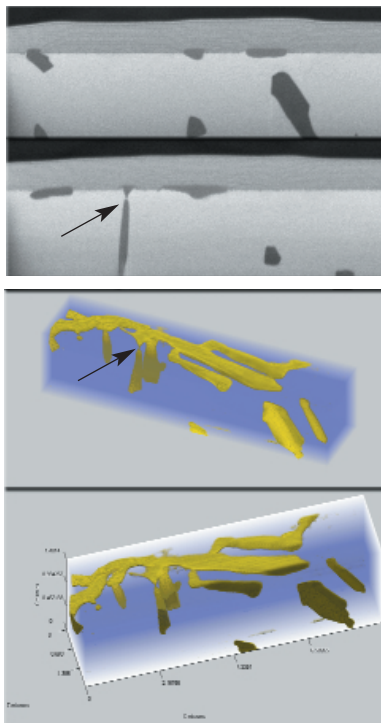


Fig. 1: Two raw images extracted from the original movie used to produce the 3-D volume reconstruction. Fig. 2: 3-D FIB-SEM reconstruction with transparency applied to highlight phases formed in a heat-affected zone of a stainless steel weld. Sample courtesy of Mahesh Chaturvedi, Univ. of Manitoba.

Web Resources for SEM:

- www.smt.zeiss.com
- www.mos.org/sln/SEM
- www.mse.iastate.edu/microscopy/home.html