FIB-SEM Nanoscale 3D Volume Reconstructions

Application Note

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. In contrast to established medical procedures, FIB-based tomographic methods are in an early phase of implementation. While less common, the FIB-SEM tomographic method has demonstrated the ability to complete 3D volumetric reconstruction at a resolution of 10nm 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 "thick" in the span of less than one hour in an automated fashion. 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.

The process begins by defining a volume that will be FIB sectioned. Volumes are typically 200-500 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 10-100 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 10-20 seconds 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 figure 1.

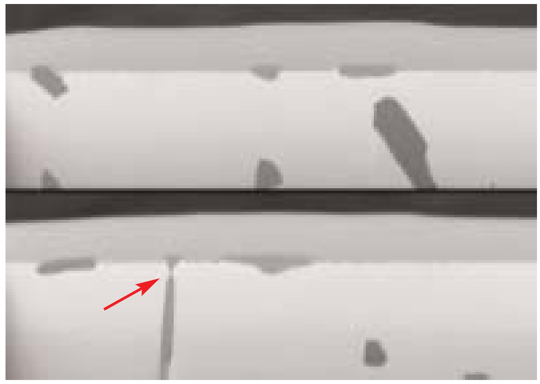

Fig. 1a: Two raw images extracted from the original movie used to produce the 3D volume reconstruction.

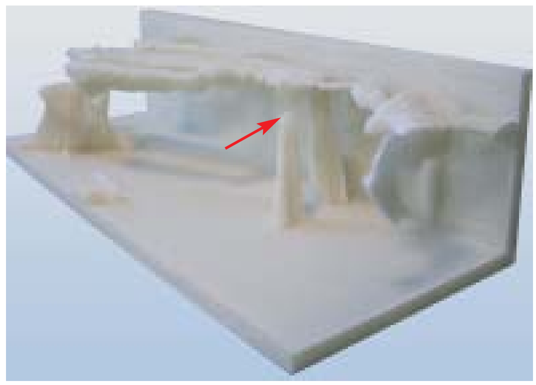

Fig. 1b: From nanometers to centimetres: Stereolithographic reconstruction of the of the recorded 3-D data in PMMA. The size of the solid plastic model is about 0.3 m x 0.1 m x 0.1 m. Note the excellent resolution on the small interconnect (arrow)

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 5-20 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 3D volume reconstruction. Process options include the ability to apply selective transparency to specific features based upon image or feature contrast, as shown in figure 2 where the precipitate phase is separated from the matrix. The arrows in figures 1 and 2 highlight a common structure in the 2D frame and 3D volume. All three axes are quantified in the process, enabling feature and volumetric analysis.

The latest exciting evolution in FIB-SEM tomography is the use of an in-lens back-scattered electron (BSEI) detector for the SEM signal, available on the Zeiss EsB XB. This in-lens BSEI 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 3D 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.

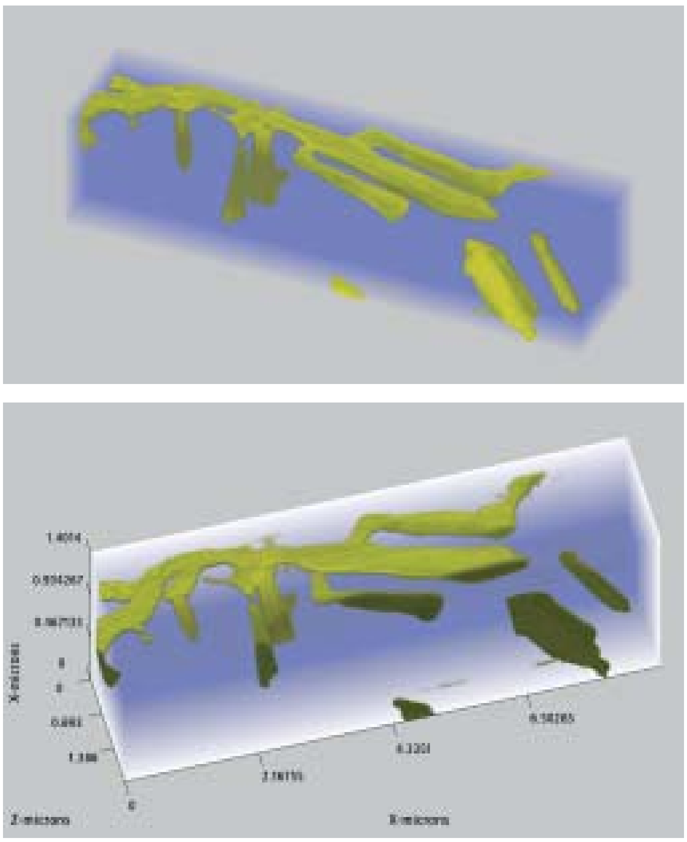

Fig. 2: 3D FIB-SEM reconstruction with transparency applied to highlight phases formed in heat affected zone of a stainless steel weld. A common feature detail is identified by the dashed lines. Axes units are microns. Sample courtesy of Mahesh Chaturvedi, University of Manitoba.

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