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Data Processing Challenges for Proper Interpretation of FIB-SEM Nanotomography Imaging Applications

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FIB-SEM serial sectioning nanotomography has become a widely adopted technique for inspection of three-dimensional (3D) microstructure in life, materials, and geosciences. Despite the rapid adoption of the technology, there remain data processing and interpretation challenges that are unique to this method. Among those challenges are computationally aligning the sections and estimating the thickness of each section. Ignoring these challenges will corrupt the image segmentation and proper quantitative 3D interpretation of the data stack.

In this study, we illustrate these challenges and consider the consequences of failing to address them. We use FIB-SEM serial section image stacks of matrix material from the Tagish Lake 5b meteorite described in [1]. Approximately 1000 serial sections were imaged in backscatter electron (BSE) mode on a Helios NanoLab 650 (FEI) with in-plane pixel size of 5.6 nm x 7.1 nm, with a nominal 10nm milled slice thickness. We applied various image processing routines from software packages including ImageJ (National Institutes of Health, public domain) and Avizo Fire (FEI Visualization Sciences Group).

Serial section alignment is an obvious challenge. FIB milling is routinely performed with the sample''s top surface tilted normal to the ion beam. Because of instrument design, the electron beam (e-beam) is angled 52° with respect to the ion beam. Consequently, if the serial section imaging is performed without further stage tilting, the milled surface is imaged at an oblique angle to the e-beam. Under these geometrical constraints the milled surface image is foreshortened on the y-axis, unless the scan raster is altered to compensate. Furthermore, each successive image of the nascent milled surface appears to be displaced upwards in y. The systematic y-displacement is routinely predicted and corrected in modern instruments by applying a compensating beam shift. Further beam shift can be applied on-the-fly to compensate for specimen drift which may be monitored in preview images. Even with compensations, the resulting stack does not have a high accuracy alignment, so users must perform an additional computational alignment with image processing software.

Many alignment routines aggregate pairwise alignments into one global solution. Pairwise alignments proceed by aligning the each slice with its subsequent or preceding slice. Those approaches can cause errors to propagate. A better option is to align all of the slices to one extrinsic fiducial mark that is unvarying and observed in every section; all sections are aligned against one section held as a fixed reference so that errors don't propagate. Here we show the initial stack and compare it to a pairwise-aligned solution and a fixed-reference solution.

Poor alignments can confound the image segmentation. The pore-back effect, sometimes called shine-through, causes material from a deeper slice to appear prematurely in an early slice. The intensity is usually depressed by local shadowing, but that signal is not representative of the composition of the

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early slice so it should not appear at all. Routines to identify pore-back effects have been described, but they depend on a highly accurate alignment [2-3]. Failure to properly label pore-backs results in missegmentation and erroneous findings. We show with the meteorite sample how segmentation is aided with the proper alignment.

Measuring section thickness is a second challenge. A proposed solution has the user mill a pattern in the surface normal to the ion beam. By monitoring how that pattern intersects the milled surface, the ablated slice thickness can be computed [4]. In instances where that approach is not practical, ion images can be captured, and the distance from some fiducial to the milled surface can be measured. The difference between that measured distance observed on successive images gives the ablated section thickness. For many image processing tools, the image stack needs to have a uniform section thickness; for those cases, the image stack must be computationally resampled to a uniform section thickness.

Despite these challenges, visual inspection of serial section experiments is usually very informative. The brain compensates for minor displacement errors and gives the user a good qualitative view of the sectioned material. In many cases, the user can easily discriminate pore-back effects from real in-plane observations. Because most experimentalists seek quantitative descriptions, however, the aforementioned challenges must be addressed or the interpretations are suspect.

Disclaimer: Certain commercial equipment and software are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the software or equipment identified are necessarily the best available for the purpose.

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