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Serial block face imaging using a Xe-plasma FIB-SEM system

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Serial block face scanning electron microscopy comprises a group of techniques for three-dimensional characterization of tissue and cells. Typically, biological structures are contrasted by heavy metal stains followed by embedding in plastic resin. Thin sections of the resin block are then continuously removed by either an integrated ultra-microtome inside the SEM (SBF-SEM) or focused ion beam (FIB-SEM) and subsequently imaged with a back-scattered electron detector. The regions of interest are segmented within the image stack and visualized in 3D.

SBF-SEM has been routinely used for reconstructions of large volumes of tissue with excellent resolution. However, slicing tissue of uneven hardness (e.g. dental tissue) can cause damage to a diamond knife or create artifacts to the sample. A gallium FIB-SEM system has been shown to provide a very precise, high resolution reconstructions with isotropic voxel resolution <10 nm. However, the maximum milling currents are limited, so reconstructing bigger volumes become time consuming.

FIB-SEM with a Xe plasma source has been shown as a high speed milling alternative to gallium FIB-SEM. It provides up to 50x higher milling rates, so large amounts of material can be removed in the same time.

In this talk, we demonstrate serial block face imaging of resin embedded dental tissue using Xe-plasma FIB-SEM [1]. First, we removed a large volume of resin and localized boundaries of interest in the tissue. We performed the serial slicing and reconstructed the regions of interest, such as veins and dentin structure in 3D.

The presented approach of using Xe-plasma FIB-SEM for serial block face imaging is a very effective method for site-specific visualization of structures embedded in large resin blocks. It could also be a method of choice for 3D imaging of large, nonhomogeneous samples.

Reference:
[1] Khatibi Shahidi, M., et al., J. Dent. Res. (2015), in press.