

3D isotropic large-volume high-resolution enhanced FIB-SEM pipeline

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Abstract: This protocol outlines the 3D isotropic, large-volume, high-resolution enhanced Focused Ion Beam Scanning Electron Microscopy (eFIB-SEM) pipeline, including sample preparation, image acquisition, and data registration of Epon-embedded cultured cells, with precise targeting guided by correlative fluorescence microscopy and X-ray tomography.

1. Enhanced FIB-SEM Sample Preparation

1.1. One Epon-embedded cultured cell sample of RPE-1 cells, was mounted onto the top of a 1 mm copper stud using Durcupan, ensuring optimal charge dissipation by maintaining contact between the heavy metal-stained sample and the copper stud.

1.2 The vertical sample post was then trimmed to a small block containing the Region of Interest (ROI), with dimensions of 80 μm in width (perpendicular to the ion beam) and 75 μm in depth (in the ion beam direction). A 20 μm -thick of Durcupan layer was preserved at the sample front to serve as a “self-healing” layer, mitigating the milling artifacts. Precise ROI targeting and trimming were achieved using overlay images from light fluorescence microscopy and X-ray tomography data acquired with a Zeiss Versa XRM-620. The detailed approach was previously described by Pang & Xu 2023.

1.3 To enhance conductivity, thin layers of conductive material—10 nm of gold followed by 40 nm of carbon—were deposited onto the trimmed sample using a Leica EM ACE600 coater.

2. Enhanced FIB-SEM Imaging Acquisition

The FIB-SEM prepared sample was imaged using a customized enhanced FIB-SEM microscope (Xu et al., 2017 and Xu et al., 2021). The images were acquired using a 500 pA current SEM probe at 0.7 keV. The scan rate was 400 kHz, with a 2-nm pixel along x and y axes. A 2-nm z-step was achieved by ~5 seconds of milling with a 15-nA Ga⁺ beam at 30 kV. A total volume of 12 \times 6 \times 12 μm^3 was acquired over 8 days at a rate of 1 minute per frame.

3. Image Registration and Alignment

The raw image stack was aligned using a SIFT based MATLAB script (https://github.com/cshanxu/Enhanced_FIB-SEM/tree/main/Matlab) and binned 2-to-1 along x, y, and z axes to create a final dataset with 4 \times 4 \times 4 nm^3 voxels, which can be viewed in any arbitrary orientations.