Jul 21, 2025

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

DOI
https://dx.doi.org/10.17504/protocols.io.kqdg3wj7ev25/v1

Song Pang[1], C. Shan Xu[2]

[1]FIB-SEM Collaboration Core, Yale School of Medicine, New Haven, CT, USA;
[2]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

Song Pang
Yale School of Medicine

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg3wj7ev25/v1

Protocol Citation: Song Pang, C. Shan Xu 2025. 3D Isotropic large-volume high-resolution enhanced FIB-SEM pipeline. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3wj7ev25/v1

Protocol status: Working
We use this protocol and it's working

Created: June 07, 2025

Last Modified: July 21, 2025

Protocol Integer ID: 219744

Keywords: enhanced focused ion beam scanning electron microscopy, focused ion beam scanning electron microscopy, embedded cultured cell, sem pipeline, correlative fluorescence microscopy, sem pipeline this protocol, cultured cell, efib, data registration of epon, sem, pipeline, cell

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.

Attachments

PDF

eFIB-SEM pipeline.pd...

127KB

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

1 Enhanced FIB-SEM Sample Preparation

1.1 One Epon-embedded cultured cell sample of RPE-1 cells, was mounted onto the top ofa 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

2.1 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 × 6 × 12 µm³ was acquired over 8 days at a rate of 1 minute per frame.

3 Image Registration and Alignment

3.1 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 × 4 × 4 nm³ voxels, which can be viewed in any arbitrary orientations.

Protocol references

S. Pang, C. S. Xu, “Chapter 11 - Methods of enhanced FIB-SEM sample preparation and image acquisition” K. Narayan, L. Collinson, P. Verkade, Eds. (Academic Press, 2023; https://www.sciencedirect.com/science/article/pii/S0091679X23000420) vol. 177 of *Volume Electron Microscopy*.

C. S. Xu, K. J. Hayworth, Z. Lu, P. Grob, A. M. Hassan, J. G. García-Cerdán, K. K. Niyogi, E. Nogales, R. J. Weinberg, H. F. Hess, Enhanced FIB-SEM systems for large-volume 3D imaging. *eLife* **6**, e25916 (2017).

C. S. Xu, S. Pang, G. Shtengel, A. Müller, A. T. Ritter, H. K. Hoffman, S. Takemura, Z. Lu, H. A. Pasolli, N. Iyer, J. Chung, D. Bennett, A. V. Weigel, M. Freeman, S. B. van Engelenburg, T. C. Walther, R. V. Farese, J. Lippincott-Schwartz, I. Mellman, M. Solimena, H. F. Hess, An open-access volume electron microscopy atlas of whole cells and tissues. *Nature* **599**, 147–151 (2021).