doi:10.1017/S1431927622005979
Microsc. Microanal. 28 (Suppl 1), 2022
© Microscopy Society of America 2022

Building a Volume EM Atlas of Whole Cells and Tissues with Enhanced FIB-SEM

C. Shan Xu[1,2*]

[1.] Yale School of Medicine, Department of Cellular & Molecular Physiology, New Haven, CT, United States.
[2.] Janelia Research Campus, HHMI, Ashburn, VA, United States.
* Corresponding author: xuc@janelia.hhmi.org

Isotropic resolution and volume are probably the two most important criteria that define the applications of a volume electron microscopy (vEM) technique. Resolution in x, y, z dimensions all below a few nanometers is imperative to reveal those biological structures smaller than 20 nm in size. In addition, the ability to image an entire cell is crucial to obtain structural information related to quality, distribution, interactions, and perturbations in the inhomogeneous cellular environment and increases the probability of capturing rare events in their native context. The development of enhanced FIB-SEM technology transformed a conventional FIB-SEM lacking long-term reliability into a robust platform capable of months to years of continuous imaging at 8-nm isotropic voxels without defects in the final image stack [1, 2], enabling the largest and most detailed connectome to date [3].

The unique potentials of FIB-SEM imaging with 4-nm voxels to resolve intracellular structures were demonstrated in a pioneering study [4]. However, it was challenging to obtain volumes of ~500 μm^3 at such resolution due to the combined challenges from slow SEM scan using backscattered electron detection via a sample biasing scheme and FIB milling instability induced by higher electron radiation energy density [1]. Thus, higher-resolution isotropic 3D whole cell data remained mostly out of reach.

Here, I will present FIB-SEM datasets greater than 100,000 μm^3 volume with 4-nm voxels. The two orders of magnitude improvement in imageable volume primarily comes from: 1) higher precision and stability of FIB milling control to extend reliable long-term acquisition to 4-nm voxels, and 2) enhanced SEM signal detection using secondary electrons to achieve faster imaging, hence reduce electron radiation energy density that further improves FIB milling control. As a result, we have generated more than a dozen diverse datasets, from cancer cells to immune cells, from mouse pancreatic islets to *Drosophila* neural tissues. Together, they represent the beginning of an open-access vEM atlas (https://openorganelle.janelia.org/) of whole cells and tissues [5].

References:

[1] CS Xu et al., eLife **6** (2017), e25916. doi: 10.7554/eLife.25916
[2] CS Xu et al., in "Volume Microscopy", ed. I Wacker et al., (Humana, New York) p. 221. doi: 10.1007/978-1-0716-0691-9_12
[3] LK Scheffer et al., eLife **9** (2020), e57443. doi: 10.7554/eLife.57443
[4] Y Wu et al., PNAS **114** (2017), e4859. doi: 10.1073/pnas.1701078114
[5] CS Xu et al., Nature **599** (2021), p. 147. doi: 10.1038/s41586-021-03992-4

https://doi.org/10.1017/S1431927622005979 Published online by Cambridge University Press