Transforming FIB-SEM for Large Volume Imaging to Enable Discoveries in Bioscience

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Isotropic high-resolution imaging of large volumes provides unprecedented opportunities to advance connectomics and cell biology research. Conventional Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) offers high resolution and robust image alignment. However, its prevailing deficiencies in imaging speed and duration cap the maximum possible image volume. I will present the instrumentation technologies that have transformed the conventional FIB-SEM from a lab tool that is unreliable for more than a few days to a robust volume EM imaging platform with 100% effective reliability: capable of years of continuous imaging without defects in the final image stack [1]. As a result, we have expanded the imageable volume by more than four orders of magnitude from 10^3 μm^3 to 3 x 10^7 μm^3 while maintaining an isotropic resolution of 8 x 8 x 8 nm^3 voxels. The largest and most detailed connectome in the world has been generated through this enhanced FIB-SEM platform, where the superior z resolution and fewer artifacts empower automated tracing of neuronal processes and reduces the time-consuming human proofreading effort [2]. By trading off imaging speed, the enhanced system can readily be operated at even higher resolutions achieving voxel sizes of 4 x 4 x 4 nm^3. Higher resolution further improves the interpretation of otherwise ambiguous details [3]. Nearly all organelles can be resolved and classified with whole cell imaging at 4 nm voxel resolution. Primarily limited by time, the maximum volume can be greatly extended. The expanded volumes enable a vast new regime in scientific learning, where nanoscale resolution coupled with meso and even macro scale volumes is critical. Moreover, combining with super-resolution fluorescence imaging, CLEM applications at the whole cell level unleash the full potential of intracellular organelle identification with labelling insights [4].

In this presentation, a variety of examples including *Drosophila* CNS (adult and larvae), mouse liver, and mammalian cells will be described to illustrate the power of fine isotropic resolution coupled with large imaging volume. The resulting neuronal wiring diagram and whole-cell segmentation have already had significant impact on the research community and will ultimately lead us to the understanding of how the brain works and how cells function [5,6].

[1] C.S. Xu, K.J. Hayworth, Z. Lu, et al., "Enhanced FIB-SEM systems for large-volume 3D imaging," *eLife*, **6**: e25916 (2017).
[2] C.S. Xu, M. Januszewski, Z. Lu, et al., "A connectome of the adult *Drosophila* central brain," *bioRxiv* 2020.01.21.911859 (2020).
[3] Y. Wu, C. Whiteus, C.S. Xu, et al., "Contacts between the endoplasmic reticulum and other membranes in neurons," *PNAS* **114**, E4859 (2017).
[4] D.P. Hoffman, G. Shtengel, C.S. Xu, et al., "Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells," *Science* **367**, eaaz5357 (2020).
[5] J. Nixon-Abell, C.J. Obara, A.V. Weigel, et al., "Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER," *Science* **354**, 433 (2016).
[6] R. Gao, S.M. Asano, S. Upadhyayula, et al., "Cortical column and whole-brain imaging with molecular contrast and nanoscale resolution," *Science* **363**, eaau8302 (2019).