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Transforming FIB-SEM for Large Volume Imaging: A Powerful Discovery Platform for Biological Sciences

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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 unique benefits such as high resolution (< 10 nm in x, y, and z) and robust image alignment. However, its prevailing deficiencies in imaging speed and duration cap the maximum possible image volume. I will present a detailed description of 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 months to years of continuous imaging without defects in the final image stack [1]. The two most important technological advances are imaging speed improvement, and system reliability improvement including error detection at all known failure modes and seamless recovery from any interruptions. 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. By trading off imaging speed, the system can readily be operated at even higher resolutions achieving voxel sizes of 4 x 4 x 4 nm^3. Primarily limited by time, the maximum volume can be greatly extended.

This powerful discovery platform enables a vast new regime in scientific learning, where nano-scale resolution coupled with meso and even macro scale volumes is critical. The largest and most detailed connectome to date (Figure 1) has been generated through this enhanced FIB-SEM platform, where the superior z resolution empowers automated tracing of neuronal processes and reduces the time-consuming human proofreading effort [2]. Likewise, many discoveries have also been unveiled in cell biology studies at even higher resolution on smaller volumes [3], where entire mammalian cells can be imaged at 4 x 4 x 4 nm^3 voxel resolution [4]. Nearly all organelles can be resolved and classified with whole cell imaging at 4 nm voxel resolution. Higher resolution further improves the interpretation of otherwise ambiguous details (Figure 2), thereby generating ground truth for machine learning in connectomics and cellular organelle segmentation. 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 [5].

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. Transformative images in these datasets have revealed fine details of various brain circuitries and ultra-fine cellular structures. 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 [6-10].

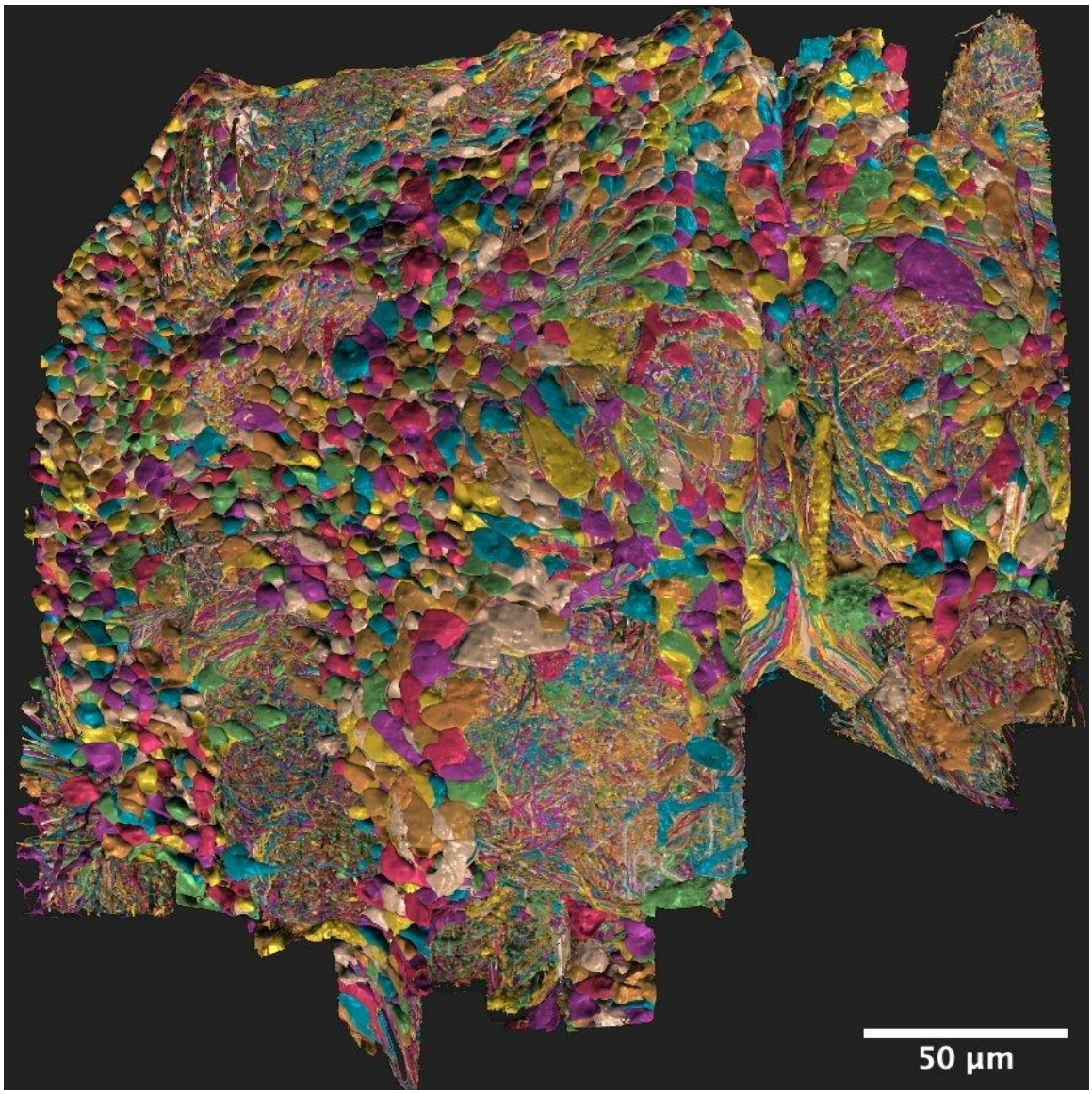

Figure 1. A 3D rendering of densely packed neurons in the adult Drosophila central brain connectome generated by enhanced FIB-SEM systems. It is the largest and most detailed connectome to date, containing around 25 thousand neurons and 20 million synapses.

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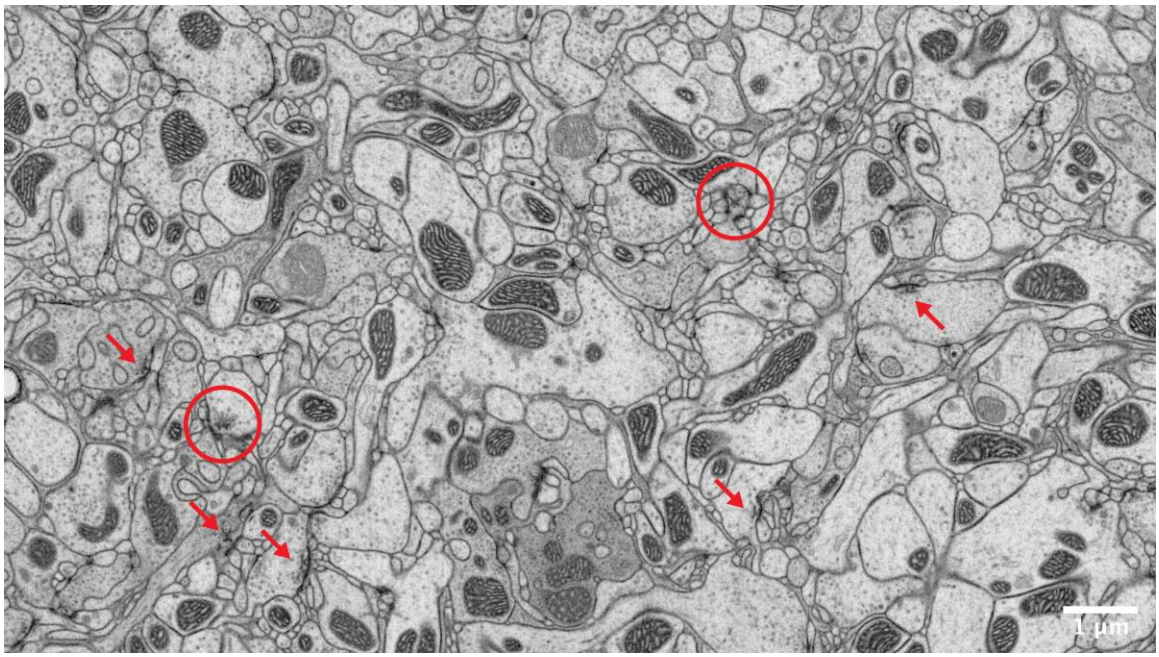

Figure 2. A FIB-SEM image of 4 nm resolution reveals detailed structure of synapses in Drosophila optic lobe: cross-section view of T-bars indicated by red arrows and surface view of T-bars indicated by red circles.

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