doi:10.1017/S1431927621009168
Microsc. Microanal. 27 (Suppl 1), 2021

Multi-Scale Imaging of Connectomes With Photons and Electrons

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A fundamental gap in our knowledge is understanding how brain function and behavior arises from the structure of neuronal circuits. This gap has arisen because anatomical connectivity and neuronal dynamics are typically studied separately and at vastly different spatial and temporal scales. We recently established methods to scale electron (Phelps et al., 2021) and X-ray microscopy (Kuan et al., 2020) and to combine these high-resolution structural imaging approaches with cellular resolution *in vivo* two-photon calcium imaging (Bock et al., 2011; Lee et al., 2016). This combination of imaging modalities at multiple scales enables us to directly bridge behavior, neuronal computation, and circuit structure; however, linking them remains a challenge. I will present our efforts and open-sourced solutions for light, X-ray, and electron microscopy (EM) and their coordinated analysis through automated segmentation and synaptic prediction using deep neural networks.

We use *in vivo* multi-photon microscopy to image neuronal dynamics while measuring animal behavior. These data have 1 μm spatial resolution allowing us to identify and track the same neuronal cell bodies over time. Using genetically encoded calcium indicators, we monitor neuronal population activity at 5.3 Hz while animals are performing perceptually-guided, decision- making tasks in virtual reality (Driscoll et al., 2017; Harvey et al., 2012).

We use synchrotron-based X-ray holographic nano-tomography (XNH) to image millimeter-scale volumes with sub-100 nm resolution, enabling reconstruction of dense wiring in nervous system tissue (Kuan et al., 2020). This allows comprehensive imaging of cells and their morphology across large distances. Because X-ray imaging is non-destructive, it can act as a bridge between light and EM. In the future, however, we expect to achieve synapse-resolution over large fields of view with further development of high-resolution X-ray microscopy.

We use serial-section transmission EM (TEM) to acquire structural data at 4 nm resolution to analyze the synaptic connectivity between neurons. We recently developed GridTape, a technology that combines automated serial-section collection with automated high-throughput TEM (Phelps et al., 2021). The data quality afforded by GridTape-based, automated TEM allows dense, automated segmentation and synapse prediction using convolutional neural networks. We provide open access to our datasets, reconstructions, instrumentation designs, software to make functional connectomics more accessible to the community and fuel further discovery.

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