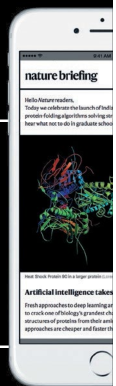


NEUROSCIENCE

X-ray connectomics

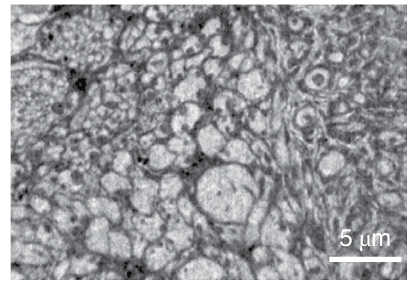
X-ray holographic nano-tomography is a promising complement to electron microscopy for connectomics studies.

Detailed mapping of neuronal circuitry has traditionally been the domain of electron microscopy. However, this approach is time-consuming and technically challenging for large samples. "We were thinking of other potential imaging modalities," says Wei-Chung Allen Lee from Harvard Medical School in Boston. "X-ray imaging … is powerful because we think that we can apply such approaches to large circuits; and it is non-destructive, able to image comprehensively, and there's a simplicity to the data alignment handling and speed of the technique," explains Lee.

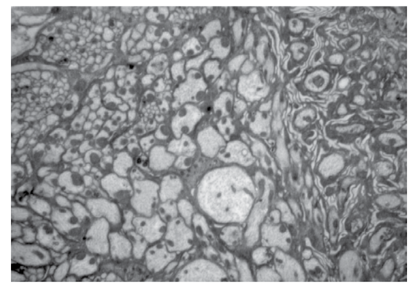
Hence, Aaron Kuan and Jasper Phelps in Lee's lab teamed up with Alexandra Pacureanu at the European Synchrotron in Grenoble to use X-ray holographic nano-tomography (XNH) for the reconstruction of neurons. In XNH, the sample is placed on a rotating stage and subjected to a coherent, high-energy X-ray beam. The resulting self-interference patterns, or holograms, are then recorded and processed before a 3D image is reconstructed.

While XNH is an established technique, the researchers adapted the approach for improved resolution. The team developed some tricks for sample preparation, used a cryogenic system, and optimized the reconstruction algorithms. These tweaks allowed Lee and his colleagues to image the *Drosophila* central nervous system or the innervation in the leg with a resolution that approaches that of electron microscopy. Although they could not resolve the finest details, they could resolve thicker neurites without problem, with the added benefits that the sample can be imaged label-free, relatively quickly and in a non-destructive manner. This last feature makes it possible to image the sample multiple times at different resolutions or to follow up with electron microscopy if desired.

Another advantage is that post-processing of the data is straightforward because, in contrast to electron microscopic datasets, "the tomographic reconstruction essentially gives you the alignment for free, which is key to automated segmentation," says Lee. Such segmentation remains challenging in large-scale electron microscopy. At the same time, the researchers were able to trace neurons using the same approaches used for electron microscopy datasets. "We could use essentially deep-learning-based methods that were developed for large-scale EM connectomics on our X-ray data," says Lee.



X-ray holographic nano-tomography



Transmission electron microscopy

Comparison of XNH and transmission electron microscopy images of the *Drosophila* leg. Reprinted with permission from *Nat. Neurosci.* https://doi.org/10.1038/s41593-020-0704-9, Springer Nature.

Lee acknowledges current limitations of XNH. "The resolution is not as good as is electron microscopy currently, but we're actually far away from the theoretical limits of the resolution of this technique." Lee and his colleagues are trying to push the approach to achieve higher resolution while maintaining large fields of view. "I think there's still ways for us to go, technically, but I think these are engineering challenges that are solvable," says Lee.

Nina Vogt

Published online: 29 October 2020
https://doi.org/10.1038/s41592-020-00994-4

Research paper

Kuan, A. T. et al. Dense neuronal reconstruction through X-ray holographic nano-tomography. *Nat. Neurosci.* https://doi.org/10.1038/s41593-020-0704-9 (2020).