Imaging of synapses in 3D with non-destructive synchrotron X-ray ptychography

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Wiring diagrams of neural circuits are of central importance in delineating mechanisms of computation in the brain (*1*). Hereby, the individual parts of neurons - axons, dendrites and synapses - need to be densely identified in 3-dimensional volumes of neuronal tissue. This is typically achieved by volume electron microscopy (*2*), which requires ultrathin physical sectioning or ablation, using high precision slicing techniques or ion beams, either before or during the image acquisition process (*3-6*). Here, we demonstrate that cryogenic X-ray ptychographic tomography (*7-9*), a coherent diffractive X-ray imaging technique, can acquire 3-dimensional images of metal-stained mouse neuronal tissue with sufficient resolution to densely resolve axon bundles, boutons, dendrites and synapses without physical sectioning. We show that the tissue volume can be subsequently imaged in 3D using high-resolution, focussed ion beam-scanning electron microscopy (FIB-SEM). This suggests that metal-stained neuronal tissue can be highly radiation-stable. Using FIB-SEM as ground truth, we could show that X-ray ptychography reliably resolves 60% of the synaptic contacts in the mouse olfactory bulb external plexiform layer with an 80% precision. Ongoing improvements in synchrotron, X-ray and detector technologies (*8, 10, 11*) as well as further optimization of sample preparation and staining procedures (*12, 13*) could lead to substantial improvements in acquisition speed. Combined with laminography (*14*) and nano-holotomography (*15, 16*) it could allow for non-destructive x-ray imaging of synapses and neural circuits in increasingly larger volumes.

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