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

C. Bosch¹, A. Diaz², A. Pacureanu³, M. Holler⁴, E. Müller⁵, A. Schaefer¹

¹The Francis Crick Institute, London, UK, ²cSAXS beamline, Paul Scherrer Institut, Villigen, Switzerland, ³ID16A beamline, ESRF, Grenoble, France, ⁴Electron Microscopy Facility, Paul Scherrer Institut, Villigen, Switzerland

Email of communicating carles.bosch@crick.ac.uk

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, focused 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.

4. K. J. Hayworth et al., Gas cluster ion beam SEM for imaging of large tissue samples with 10 nm isotropic resolution. Nature methods 17, 68-71 (2020).

Keywords: synapse; ptychography; neuroscience

We acknowledge the Paul Scherrer Institut, Villigen, Switzerland for provision of synchrotron radiation beamtime at the cSAXS beamline X12SA of the SLS under the proposals 20190654 and 20200783.