Structure of myelinated nerves by x-ray scanning diffraction and phase-contrast tomography

M. Bartels, M. Krenkel, and T. Salditt
Institut für Röntgenphysik, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

To understand cellular networks in the nervous system and to map connectivity, 3D structural information of multi-cellular tissue over hundreds of micrometers is required with resolution and contrast high enough to resolve sub-cellular features. The goal of the present research, in collaboration with Dr. W. Moebius (MPI Exp.Medizin, Göttingen), is to develop an x-ray phase-contrast imaging method capable of delivering such data for entire uncut myelinated mouse nerves, extending our previous cone-beam phase contrast tomography work [1]. The myelin sheath is a concentric multilamellar membrane stack which is wrapped around axons of neurons. The segmental structure of the insulating myelin sheath along the axon, with segments separated by so-called nodes of Ranvier (where myelin is lacking), enables the saltatory conduction of nerve impulses, needed for fast signal transduction. Three dimensional data of thousands of axons running parallel in the nerve along with their respective myelin sheath segments would allow to study if and how positions of the nodes of Ranvier in neighboring axons are correlated.

Figure 1: (a) A hard x-ray beam is first focused by two KB-mirrors and subsequently filtered by a 2D waveguide system, yielding a highly coherent and divergent illumination. The nerve samples are positioned at multiple source-sample distances to facilitate phase reconstruction. (b,d) Virtual orthogonal slices through the reconstructed 3D electron density of a mouse nerve. (d,e) Virtual slices through the nerve of a mouse mutant (recorded at GINIX/P10). (f) Rendering of 20 axons (turquoise) based on a previous test experiment (recorded at ID22NI) illustrates the high degree of structural information of such 3D datasets. Nodes of Ranvier are rendered red, Schmidt-Lantermann incisures green. Scale bars, (b-e) 50 µm, (f) 100 µm, (f inset) 20 µm.
**Results:** At beamline P10, a 13.8 keV x-ray beam was focused by two KB-mirrors down to 300 nm (FWHM), using the Göttingen Instrument for Nano-Imaging with X-rays (GINIX). A two-dimensional waveguide systems was placed in the focal plane of the mirrors, acting as a spatial and coherence filter (Fig. 1a). The waveguide system consists of two crossed planar waveguides, each with a sputtered thin film sequence Ge/Mo/C/Mo/Ge optimized for high transmission [2]. With a C guiding layer thickness of 59 nm, a maximum photon flux of $5 \times 10^8$ photons/s exiting the 0.764 mm long waveguide system was measured with a pixel detector (Pilatus). Entire uncut high-pressure frozen sciatic nerves were placed on a tomographic sample stage at a distance $z_1$ behind the waveguide and magnified Fresnel diffraction patterns (in-line holograms) were recorded with a fibre-coupled scintillator-based sCMOS camera (Photonic Science) with 6.54 $\mu$m pixel size placed at the waveguide-detector distance $z_1 + z_2 = 5.07$ m. The tomography stage allows waveguide-sample distances between $z_1 \approx 1$ mm up to $z_1 = 200$ mm and thus a powerful zooming capability, based on the geometric magnification supplied by the spherical wavefront of the waveguide illumination. At $z_1 = 190$ mm, tomographic overview scans were performed with an (effective) pixel size of $p_e \approx 250$ nm and a large field of view of $470 \mu$m $\times$ 265 $\mu$m (1920 $\times$ 1080 pixels). The overview scan allowed to easily identify a suitable region of interest (ROI). A subsequent ROI scan was performed with a pixel size of $p_e \approx 50$ nm. The sample was placed at three different distances $z_1 = [40, 42, 45]$ mm to facilitate phase reconstruction based on the inversion of the phase contrast transfer function [3]. At each sample position a tomographic scan with 720 projections covering an angular range of 180 degrees was recorded with 3.5 seconds dwell time per projection. Phase retrieval was performed individually for every angle and filtered backprojection was applied to the reconstructed phase maps, yielding a 3D representation of the electron density of the investigated sample.

Figure 1 (b-d) shows virtual 2D slices (orthogonal to each other) through the reconstructed 3D electron density of the investigated mouse nerves. Individual axons with surrounding myelin are clearly visible. Previous test experiments at beamline ID22 (ESRF) have revealed that the approach is capable of identifying myelin sub-structure, such as nodes of Ranvier and Schmidt-Lantermann incisures (see Fig. 1f). The present experiment allowed higher spatial resolution, and data for both wildtype and mutant mice (with altered myelin properties) were recorded. While qualitative difference can already be seen in the 2D slices a full 3D analysis. It may reveal differences in myelin and axon thickness as well as distribution of and correlation between nodes of Ranvier and Schmidt-Lantermann incisures within control and mutant mice.

**Conclusion:** We have implemented 3D phase-contrast imaging at the GINIX setup of beamline P10, capable of resolving thousands of axons running parallel within entire uncut mouse nerves as well as sub-cellular structures. The zooming capabilities and the high degree of coherence provided by waveguide illumination have proven particularly important for overview and subsequent high resolution ROI scans. By moving the waveguide out and moving the sample into the focus, scanning x-ray diffraction can be performed on the same sample on the same setup to deliver complementary data on the sub-10 nm scale. While this imaging mode was successfully implemented in 2D [4] at GINIX, application to thick nerve samples requires extension to 3D scanning x-ray diffraction, which will be the focus of future research.

**References**