**Introduction:** The aim of the proposed experiment was to image the structure of entire myelinated nerves of several hundreds of micrometer in diameter with sub-cellular resolution. Coherent x-ray imaging offers a new route to image the ultra-structure of unsliced and unstained biological cells and tissues without invasive sample preparation at high resolution [1,2]. The setup for propagation imaging installed at the coherence beamline P10 [3] provides well controlled and curved illumination fields with lateral source dimensions down to 200 nm with high flux of about $2 \cdot 10^{11}$ cps suitable for propagation based phase-contrast tomography.

Figure 1: (a) A 7.9 keV x-ray beam focused by two KB-mirrors is used to record propagation based phase-contrast tomograms of entire myelinated nerves exploiting the magnifying capabilities of cone-beam illumination. (b) Reconstructed slice of the data recorded in sample plane 1, showing an optical nerve within a glass capillary. (c) Three-dimensional visualisation of the reconstructed volume revealing characteristic dense regions which can be attributed to oligodendrocytes, 720 nm voxel size. (d) Volume rendering of the (preliminary) reconstruction of the data recorded in sample plane 2, voxel size 64 nm.

**Results:** A 7.9 keV x-ray beam was focused by two KB-mirrors down to 200 nm (FWHM) with a photon flux of about $2 \cdot 10^{11}$ cps, as measured by a pixel detector (Pilatus). A pinhole with 1.4 μm diameter was positioned close to the focal plane acting as a low pass filter to remove spurious high frequency oscillations in the illumination wave field (see Fig.1 (a)). A high-resolution x-ray detector system (Optique Peter) using a 20μm LuAG:Ce scintillator screen (Crytur) and an optical CCD with 2048 times 2048 pixels (PCO.2000) in combination with 4- or 10-fold magnifying objectives (Olympus) was placed at a distance $z_2 = 5.51$ m from the focal plane to record the magnified
in-line hologram (Fresnel diffraction pattern). Entire unstained sciatic and optical nerves of wild
type mice were fixated in liquid (4% PFA in phosphate buffer, 2.5% glutaraldehyde) and kept in
thin glass capillaries of $300 - 700 \mu m$ diameter. To advantageously utilize the intrinsic geometric
magnification of cone-beam illumination the samples were placed at different distances $z_1$ from the
focus (see Fig.1 (a)). The geometric magnification $M = (z_1 + z_2)/z_1$ results in correspondingly
demagnified (effective) detector pixels and the experiment can be described in a well-known equi-
valent parallel-beam geometry with an effective sample-detector distance $z_{eff} = z_1 z_2/(z_1 + z_2)$. At
$z_1 = 5.33 m$ (sample plane 1) the magnification of $M=1.03$ in combination with a 10-fold mag-
nifying objective of the detector system resulted in an effective pixel size of 720 nm. The large
field of view of more than $1 mm^2$ was used to image the entire capillary. In total 900 angles were
recorded with 10 seconds illumination time each. A selected region of interest (ROI) of the sam-
ple was then imaged with high resolution at $z_1 = 195 mm$ (sample plane 2). The magnification
of $M=28$ in combination with a 4-fold magnifying objective resulted in an effective pixel size of
64 nm. A total of 900 angles were recorded, each with 5 second accumulation time. Depending
on the effective propagation distance $z_{eff}$ the phase reconstruction scheme varies. As a first step the
recorded in-line holograms were corrected by the intensity distribution of the illuminating wave
field without the sample in the beam, recorded during the respective illumination time. In sample
plane 1 a single-step method based on the Transport-of-Intensity Equation was used to reconstruct
the phase distribution. In sample plane 2 a holographic one-step reconstruction was used. To sup-
press the spoiling twin-image of holographic reconstruction, iterative GS-like (Gerchberg-Saxton)
reconstruction schemes will be adapted, which is currently in progress.

Figure 1 (b) shows a reconstructed slice of the data recorded in sample plane 1. The optical nerve
within the capillary is clearly visible. Figure (c) shows two orthogonal slices through the recon-
structed three-dimensional volume inside the capillary along with a volume rendering of a subset of
the data. Oligodendrocytes, which form the myelin, typically have a higher density of the cellular
nucleus and the cytoplasm compared to astrocytes [4]. Therefore the characteristic dense regions
can be tentatively attributed to oligodendrocytes allowing to determine the distribution and three-
dimensional arrangement of oligodendrocytes within the nerve. Figure (c) shows a volume rendering
of the (preliminary) reconstruction of the data recorded in sample plane 2 which can be regarded
as a zoom into the area indicated by the dashed rectangle in (a). Again, characteristic dense regions
corresponding to oligodendrocytes are visible. Due to the one-step holographic reconstruction spu-
rious artifcats spoil this preliminary data and an improvement of quality is expected for the final
reconstruction. After final analysis, structures will be interpreted in collaboration with Prof. Dr.
K.D. Nave and Dr. W. Moebius, MPI Experimentelle Medizin, Göttingen.

Conclusion: The structure of an entire unstained optical nerve of a wild type mouse was imaged
successfully using propagation based phase-contrast tomography in cone-beam geometry. Reso-
lution and contrast was high enough to visualize characteristic dense regions corresponding to
oligodendrocytes. The three-dimensional arragement of oligodendrocytes within the nerve in com-
bination with quantitative structural information of myelinated axons could be highly relevant to
understand myelin related deseases [4]. The setup at P10 could be advantageously used to obtain
this information by scanning diffraction tomography in combination with phase-contrast tomogra-
phy demonstrated here.

Literatur

[1] D. Shapiro et al., PNAS 102, 15343 (2005); P. Cloetens et al., PNAS 103, 14626 (2006)