The connectivity of neural networks within the brain is an important problem in neuroscience. Phase contrast x-ray imaging and tomography with contrast formation by free propagation may provide a tool of unique potential to unravel morphologies and ultimately also connectivity information without slicing, given significant further research in optimizing imaging parameters, algorithms and sample preparation. Towards this goal of virtual histology on tissue of the central nervous system (CNS), we have investigated basic imaging modalities and parameters in view of different samples and preparation procedures. The investigated samples ranged from the organ level (mouse cochlea) to a single cell layer of primary neurons, but not the initially envisioned spiral ganglion neuronal tissue in cochlear, since these samples gave already excellent tomography results at our in-house liquid jet micro-focus source [1]. At the same time, in view of higher resolution, they proved to be very challenging samples for the cone-beam high resolution tomography setup of GINIX, not only in view of the problems associated with region-of-interest tomography, but also since they led to extremely high contrast of strongly holographic nature, which has so far precluded successful reconstruction. Therefore, we concentrate on the samples of ‘smaller contrast’ such as single neurons and brain tissue with thickness of 1 mm. We used waveguide based x-ray holography for 2D as well as tomographic measurements on the samples. The experiment was performed at the P10 beamline (GINIX setup) where a crossed multilayer waveguide [2] was inserted in the KB mirror focal plane to reach the highest possible resolution. The waveguide consists of two 59 nm thick crossed multilayers resulting in a 0.764 mm long waveguiding channel creating an effective source of 16 nm FWHM according to finite difference simulations. Due to the waveguide, the illuminating beam shows a higher coherence as well as a resolution down to below 30 nm for 2D test structures [3].

As a first step to phase-contrast imaging of neural connectivity in the brain, we tested the ability to resolve unstained neurons in 2D. Fig. 1(a) shows a brightfield microscope image (40× magnification) of the sample with the examined region marked with a black rectangle. The reconstructed image is shown in 1(b). The nucleus of the neuron as well as the shape of the cell itself can be recognized. However, contrast is low and finer structures as dendrites are not resolved. The next challenge is to image the neurons in brain tissue. To this end, two samples with differently prepared parts of mouse brains were examined. The first sample was a chemically fixed 1 mm thick brain slice mounted between two polypropylene foils on aluminium rings (see Fig. 2(a)). For a small volume in the middle of the slice, a tomogram was recorded which is shown in Fig. 2(b). The nearly homogeneous part in the upper left edge is a water-filled volume within the brain. The dark spots in the tissue can be associated with the nuclei of the neuronal cells. However, without staining, the axons and dendrites of the neurons are not resolved.

For the second sample, the cerebellum of a mouse brain was prepared by removing all water and protein contents. For a better overview of the sample and comparison, an in-house tomogram (liquid-jet anode, BAC phase retrieval) is shown in Fig. 2(c). The green region marks the volume for which a high resolution tomogram was recorded at the synchrotron, shown in Fig. 2(d). In both tomograms, the same blood vessel is labeled. In the synchrotron data, smaller branches of the vessel can be resolved. However, the quality of phase retrieval (3-distance CTF) is compromised by the fact that the regime was strongly holographic as well as complications associated with the region-of-interest tomography. A similar finding has previously been reported in [4]. Further analysis based on alternative phase retrieval strategies is ongoing.
Figure 1: Measurements of fixated primary neurons between two silicon nitride membranes. (a) Brightfield microscope image of the sample with a 40× magnification. The measured region is marked with a black rectangle. (b) Reconstructed phase for the region marked in (a). The nucleus as well as the shape of the cell are visible. Scale bar, 10 µm.

Figure 2: (a) Examined 1 mm thick brain slice which was mounted between two polypropylene foils. (b) Reconstructed density distribution for a volume in the middle of the slice. Scale bar, 10 µm. (c) In-house micro-focus source measurement of the cerebellum which was prepared by removing all water and protein contents. The green region was measured with a higher resolution at the P10 beamline. Within this region a blood vessel is labeled. (d) Reconstructed density distribution for the synchrotron measurement of the region marked in (c). The blood vessel is labeled as well showing smaller branches that are resolved.

References