# Appendix

## Expanded description of the phase-contrast x-ray tomography setups

### **Laboratory setup equipment and reconstruction pipeline**

The laboratory scans were carried out at two home-built phase-contrast μCT setups. One is based on a liquid-metal jet x-ray source (Excillum) with a Galinstan liquid metal jet anode (characteristic photon energy of 9.25 keV), operated at an acceleration voltage of 40 kV, electron power of 57.1 W and a projected focus size of 10 x 10 µm [1, 2]. The other setup is equipped with a micro-focus rotating anode x-ray tube (Rigaku MM007 HF) with a Cu target (peak energy of 8 keV), and was operated at 40 kV acceleration voltage, 1200 W electron power and a circular 70 x 70 µm x-ray spot size [3]. For both setups, projection images were recorded using a scintillator-based lens-coupled CCD detector with a pixel size of 0.54 µm (2504 × 3326 pixels; Rigaku). Following Bartels et al. [2], the source-to-sample distance z01 and sample-to-detector distance z12 were chosen in view of sufficient partial coherence and optimized system resolution (reduced source blurring due to high resolution of the detector and geometric setting with magnification M ~1), resulting in z01 = 159 mm and z12 = 27.91 mm for the liquid metal jet setup, and z01 = 500 mm and z12 = 5 mm for the rotating anode setup.

In both setups, the fully motorized sample stage allowed to adjust the position of the sample based on automated alignment scripts and to rotate it during the CT scan, which was performed with an angular range of 180°, uniformly covered by a set of 1000 projections, each acquired with an exposure time of 50 s. Additional images of the detector thermal noise (10 dark images in total) and of the empty beam profile (2x25 images liquid metal jet setup, 1x25 images rotating anode setup) were acquired with the same exposure time, for correction purposes. The total scan time, including read-out and motor movement overhead, was ~ 15 h.

As a first step of the image processing pipeline [4], the obtained images were 2 x 2 binned in order to increase the signal-to-noise ratio. Phase retrieval was then performed with the Bronnikov-aided correction algorithm [1,2,5] on the empty-beam corrected projections. A preliminary ring removal was carried out by wavelet filtering [6]. Lastly, the tomographic reconstruction was achieved with the FDK algorithm [7] implementation in the ASTRA toolbox [8].

The resulting virtual volumes contain the whole 1 mm wide samples with an effective voxel size of ~ 1 µm (0.92 µm for the liquid metal jet setup, 1.07 µm for the rotating anode setup). The image contrast is very similar between the two setups, and enables a clear identification of the neural tissue structure. The healthy colon sample was scanned in the liquid metal jet setup, while the ileum sample from a patient was scanned in the rotating anode setup. The only reason for this distinction was time optimization, as the two systems could run in parallel.

### **Synchrotron setup equipment and reconstruction pipeline**

CT scans with synchrotron radiation were recorded using the GINIX endstation [9] at the P10 undulator beamline of the PETRA III storage ring (DESY, Hamburg).

Synchrotron radiation was monochromatized to a photon energy of 7.5 keV by a Si (111) channel-cut monochromator and focused by a pair of Kirkpatrick-Baez (KB) mirrors to a spot size of around 300 nm onto an x-ray waveguide channel, fabricated in silicon by e-beam lithography and wafer bonding. The waveguide channel exit served as secondary source of a size in the range 15-50 nm (bidirectional, depending on the channel used) with corresponding cone-beam emission by diffraction, homogeneous illumination and increased spatial coherence.

As in the laboratory setup, the samples were placed downstream on a fully motorized stage, allowing for sample alignment and for its rotation during the tomographic scan. The samples were the ones used for the laboratory scans, without any further preparation, mounted on the same holders; a mark on the latter was crucial to position the samples with the same orientation in both imaging stations (laboratory and synchrotron), in order to more easily correlate the images.

A scintillator-based fiber-coupled sCMOS detector with a pixel size of 6.5μm (2048 × 2048 pixels; Photonic Science) was placed behind the samples (detector distance z02=5100 mm), resulting in geometrical magnification in the range of M~35-40.

In this setup, image formation is in the in-line holographic regime with high sensitivity to small phase differences [10]; for this reason, several projections at different source-sample distances are acquired for each tomographic angle and their different information is merged together in the subsequent data analysis. Three full tomographic scans were performed for each imaged region of interest (ROI) in each sample, with source-to-sample distances of 138 mm, 143 mm and 168 mm. The smallest effective pixel size was therefore 176 nm.

Every tomographic scan was performed with an angular range of 180°, 1000 projections, 2 s exposure time per projection. Additional images of the detector thermal noise (25 images in total) and of the empty beam profile (50 images) were also acquired with the same exposure time. The total scan time per ROI (all three distances) was ~ 2.5-3 h.

For (linearized) phase retrieval, the contrast transfer function (CTF) approach was used [11]. In order to account for the different effective pixel sizes in the images due to the changing source-to-sample distances, all the images were scaled to the one with the smallest effective pixel size, aligned, and cropped to the same field-of-view. A ring removal algorithm was then applied [6]. Lastly, the tomographic reconstruction was performed with the iradon function in Matlab® (Mathworks) environment. The resulting digital volumes correspond to 320x320x320 µm3 regions with an effective voxel size of 176 nm.

The methodology used in phase image recording and phase retrieval, both for the laboratory and the synchrotron setups, is described in more detail in the work from Töpperwien et al. [12].

## **References**

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