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High-sensitivity phase-contrast tomography of rat brain in phosphate buffered saline

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Abstract. We report advances and complementary results concerning a recently developed method for high-sensitivity grating-based x-ray phase-contrast tomography. In particular we demonstrate how the soft tissue sensitivity of the technique can be used to obtain in-vitro tomographic images of rat brain specimens. Contrary to our previous experiments with fixated specimen (chemically modified or formalin fixed), the present results on the rat's brain are closer to the in-vivo situation. The findings are particularly important from a clinical point of view, since a similar approach using three gratings can be implemented with more readily available x-ray sources, such as standard x-ray tubes.

Phase-sensitive x-ray imaging, which uses the phase shift rather than the absorption as the imaging signal has the potential of substantially increased contrast in soft biological tissue [1, 2]. While various phase-sensitive x-ray imaging methods were developed in the past years, we have particularly focused on the development of grating-based differential phase-contrast imaging [3, 4]. In previous work we have demonstrated how phase-contrast tomography with a grating interferometer setup can yield images of biomedical specimens with unprecedented soft tissue sensitivity at synchrotron x-ray sources [4, 5, 6]. In particular we have shown that this x-ray method can be used to discern between subtle details in the tissue structure of animal brains [4, 6] and other organs [5], an application field which until now has almost exclusively been reserved for other, *i.e.*, MRI techniques.

Contrary to the above mentioned previous studies, where the samples were fixated using formalin, the results presented in this contribution were obtained on rat brain specimens prepared in phosphate buffered saline (PBS). This is particularly important to evaluate the real potential of the method for future pre-clinical or clinical in-vivo applications. In the latter context, experiments based on formalin-fixed specimens could be misleading, since formalin-fixation (like most other fixation methods) significantly alters the chemical conditions of the sample, thus prohibiting an assessment of the performance of phase-contrast tomography under real in-vivo conditions.[1]

[1] To prepare the brain samples prior to extraction from the animal, we used microwave irradiation to the head of anesthetized rats (4kW at 2450 MHz for 2s). This procedure preserves the neurochemical environment of the entire brain in situ.

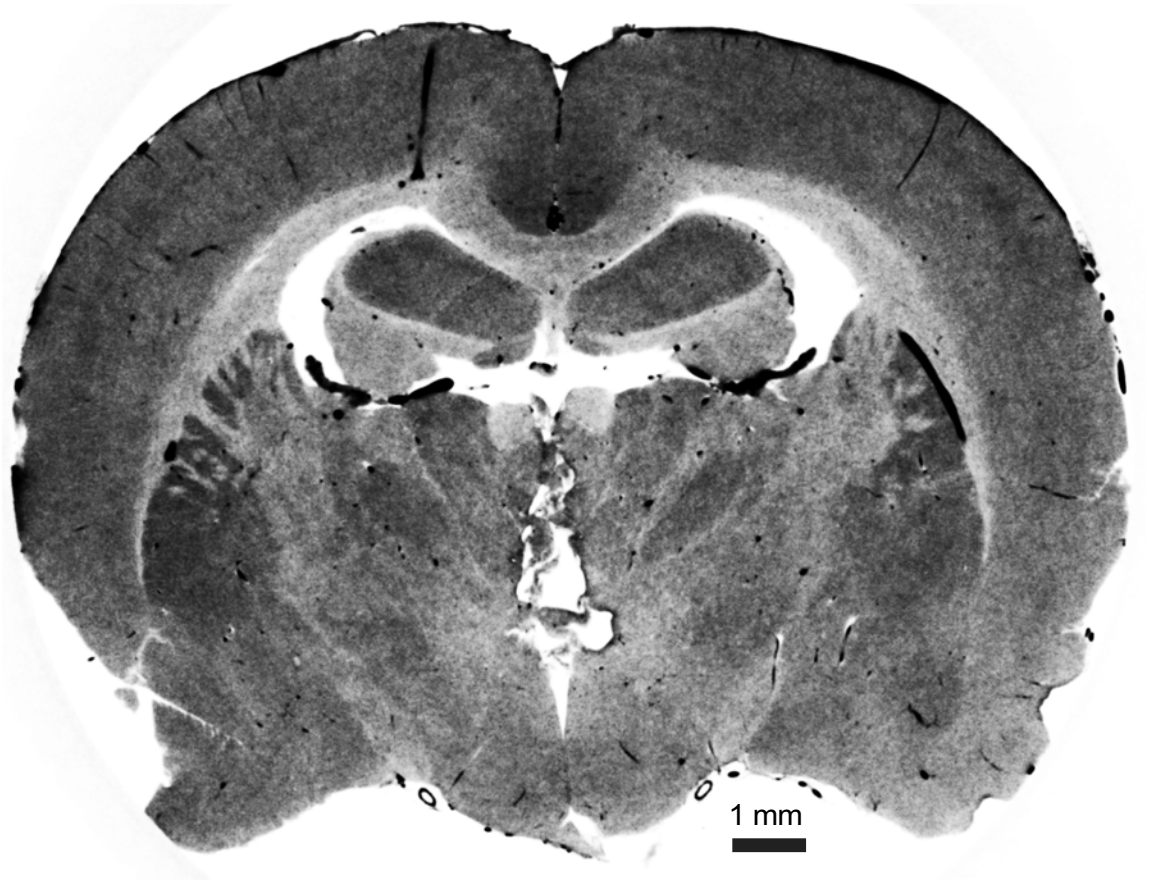

Figure 1. One out of several hundred reconstructed tomographic slices of a rat brain specimen measured in phosphate buffered saline (PBS). The image is displayed on a linear gray scale.

The principles of differential phase-contrast imaging using a grating interferometer have been described in detail in [3, 4], and are only briefly reviewed here. The experimental arrangement consists of the specimen on a high-precision rotation stage, a phase grating G1, and an analyzer absorption grating G2. The first grating (G1) acts as phase mask, and imprints periodic phase modulations onto the x-ray wave front. The second grating (G2) with absorbing lines and the same periodicity and orientation as the fringes created by G1 is placed in the detection plane, immediately in front of the detector. When one of the gratings is scanned along the transverse direction ('phase stepping'), the intensity signal in each pixel in the detector plane oscillates as a function of the grating position. The fundamental idea of the method is to analyze, for each pixel, the changes of these oscillations, when an object is placed into the x-ray beam. Three dimensional (3D) information of the specimen using computed tomography (CT) can be obtained, when the specimen is rotated around the rotation axis and a phase stepping series is recorded for each angle. The differential phase-contrast projection tomography data set can be reconstructed by using a modified filter kernel (Hilbert transform) in combination with standard filtered back-projection algorithms [9, 10].

The imaging experiments were carried out at the beamline ID19 of the European Synchrotron Radiation Facility (ESRF, Grenoble). A monochromatic x-ray beam of 26 keV was used for the measurements. The interferometer was placed at a distance of 145 m from the wiggler source. The gratings were fabricated by a process involving photolithography, deep etching into silicon and electroplating of gold. The first grating (Si phase grating, G1) had a period of: $p_1 = 3.99\ \mu$m

and a height of $h_1 = 31.7$ μm. The corresponding values for the second grating (gold absorber grating, G2) were $p_1 = 2.00$ μm and $h_2 = 30$ μm. To achieve a very high angular, and thus phase sensitivity, the distance between G1 and G2 was chosen to be as large as 376 mm (ninth fractional Talbot distance). The images were recorded using a 15 μm micron thick polycrystalline gadolinium oxysulphide scintillation screen with a magnifying optical lens system and a cooled charge coupled device (CCD). We used the FReLoN 2000 (a Fast-Readout, Low-Noise CCD developed at the ESRF) with 2048×2048 pixels, with a final effective pixel size of 15.0 × 15.0 μm^2.

Figure 1 shows one out of several hundred reconstructed virtual tomographic slices of the rat brain specimen measured in phosphate buffered solution (PBS). 1001 projections, each consisting of four images, within a total exposure time of approx. 33 minutes (0.5 seconds exposure time per individual image) were used for this reconstruction. As for formalin fixation [4], one clearly observes numerous anatomical features well above the noise level. Although such differentiation is usually hardly possible based on x-ray CT scans, our method clearly resolves these small density differences in the brain tissue structure. Based on the standard deviation of the gray values in background (PBS) regions of the reconstructed tomographic slices we deduce a measurement sensitivity for the real part of the refractive index of 0.7×10^{-10}. This corresponds to an electron density sensitivity of 0.04 e/nm^3 and a mass density sensitivity of approximately 0.1 mg/cm^3 for aqueous specimens.

In summary we have demonstrated how an improved grating based phase-contrast tomography setup yields images of biomedical specimens with unprecedented soft tissue sensitivity at synchrotron x-ray sources. In particular we have shown that this x-ray method can be used to discern between subtle details of the tissue structure of animal brains, an application field which until now has almost exclusively been reserved for other, *e.g.*, MRI techniques. While the results can be applied immediately for biomedical studies at synchrotron x-ray sources, they are potentially interesting from a clinical point of view, since a similar approach can be implemented with more readily available x-ray sources, such as standard x-ray tubes [11, 12].

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