Propagation based phase contrast x-ray imaging of Caenorhabditis elegans

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Introduction: Imaging of multicellular organisms with nano-scale resolution is one of the challenges of modern life sciences. To solve biological problems, often the combined information of several microscopy techniques is necessary. While confocal fluorescence microscopy and optical super-resolution fluorescence can detect the distribution of markers in live cells, electron microscopy (EM) offers highest resolution in fixed specimen. This, at the price of invasive sample preparation involving fixation, cutting and staining. Yet another important contrast is provided by x-ray microscopy which can yield quantitative density distribution in bacterial cells[1]. At the P10 nanofocus holography endstation at PETRAIII we performed a full-field phase contrast x-ray tomography on the multicellular nematodes Caenorhabditis elegans which serve as model organism for biological research fields including neuroscience, cell biology and signaling[2, 3]. This correlative imaging experiment uses protocols from EM[4] and molecular neurogenetics [5].

Experiments: A sample is placed at a variable distance \( z_1 \) upstream of the KB-focused x-ray beam, while the detector, a scintillator based microscope, is set at a fixed distance \( z_2 = 5.51 \) m downstream of the KB-focus. The magnification \( M \) of the setup can then be tuned by changing the sample position respectively to the focus (sketched in figure A). This results in effective pixel sizes ranging from \( 1 \) \( \mu \)m down to 100 nm. Here, tomograms of epon-embedded worms as well as projection images of living worms were recorded.

Figure 1: (A) Sketch of the possible high and low magnifying P10 detector and sample setups. (B) Light microscopic image of the sample, a free-standing, epon-infiltrated worm on a needle. The flatfield corrected phase-contrast projection image in the C) high- and D) low-magnification setup. The projections are devoid of vertical intensity fluctuations or KB-mirror artifacts. (E) A volume-texture rendering with an orthoslice of the unreconstructed dateset. The orthoslice depicts internal organ structures. Final segmentation is ongoing. (F) A diffraction pattern of a living nematode reveals internal organ structures. Scale bars = 50 \( \mu \)m.
Results: For micro-resolution imaging the sample (presented in figure B) was placed 5.32 m upstream of the focus about 18 mm in front of the detector. The detector system consists of a scintillator-based microscope using a 20 µm LuAG:Ce scintillator on 1 mm glass substrate, a PCO-2000 CCD and a 10-fold magnifying objective. The resulting effective pixel size of this setup with a small magnification of 1.03-fold is 0.72 µm. A series of 450 projection recordings, equally spaced around 180 degree and 450 flatfield images of 5 s exposures were used to create a flatfield-corrected holographic dataset that can be used for tomographic reconstruction. For nano-resolution the sample can be placed closer to the focus to exploit the magnifying properties of this divergent (cone) beam illumination. Here, the sample was placed 0.19 m upstream of the focus about 5.3 m in front of the detector. When using a 4-fold magnifying objective the effective pixel size of the setup with a large magnification of 29.1-fold is 0.13 µm. Here, a series of 300 projection recordings and immediately recorded flatfield images of 5 s exposure was used to create the flatfield-corrected holographic tomography dataset. Exemplary projections for both the high- and the low-magnification tomograms are shown in figure C and D respectively (empty beam corrected).

Current algorithmic limitations: For final tomographic reconstructions the previously developed algorithms for pure phase contrast imaging in this setup[1] have to be adapted for mixed phase and absorption contrast samples. A simple filtered backprojection (Ram-Lak filtered) of the raw data (after empty beam division), however, gives a first impression of the 3D structure. The volumetexture rendering with an orthoslice of the unreconstructed dataset (compare figure E) illustrates that the contrast intensity in this dataset is mainly given by the surface of the nematode. However, in the unreconstructed dataset the internal organs are already vaguely identifiable (see orthoslice).

Live-Worm-Imaging: In order to demonstrate the speed and radiation-dose-efficiency of the setup, we have furthermore imaged a living nematode in the micro-resolution setup. Phase wrapping issues were avoided by matching the density of the embedding media with the approximated worm density. We constructed an air-tight, framed polypropylene holder that contained agarose pad (2%) with previously anestesized (30 mM Na3) worms. An 450 s image of the nematode, presented in figure F, reveals great details of the anatomy of the worm and proves that this experimental scheme well suited for tomographic studies with live nematodes or other living samples.

Conclusion: We have demonstrated the potential of synchotron based x-ray microscopy for multicellular organisms in tomography experiments. The presented methods have proven potential for the observation of various biologicl specimen with x-ray phase contrast. The imaging techniques can be used for the investigations off single cells, tissues or even complete organisms both in fixed as well as living conditions. Phase reconstruction is in progress on all recorded datasets (high- and low magnification). With one future beamtime extension the completion of live-tomographic experiments seems possible based on dose fractionation.

References