

1 X-ray phase-contrast in vivo tomography

Four-dimensional imaging techniques are essential tools in biology to understand the behaviour of cells during embryonic development. Here, we apply X-ray phase-contrast microtomography to capture the early development of the optically opaque African clawed frog (*Xenopus laevis*) over the course of time and in 3D. *Xenopus* embryos lack conventional X-ray absorption contrast and act as pure-phase objects for hard X-rays. The wave front exiting the sample is thus characterised by a 2D phase map representing the projection of the object along the X-ray beam. Employing quasi-monochromatic and sufficiently spatially coherent X-rays, we make use of propagation-based phase-contrast.

In Fresnel theory, we study the formation of 2D intensity contrast upon free-space propagation from a given phase map and how phase retrieval based on linear contrast transfer breaks down at strong phase variations. Using a single-distance intensity measurement only, we devise a phase-retrieval method for moderately strong phase variations which, due to large propagation distances, exhibits high spatial resolution and contrast at low photon statistics.

We discuss constraints imposed by in vivo imaging and present results from experiments on living *Xenopus* embryos.

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We study how a 2D phase map, representing the absorption-free projection of an object along a hard, quasi-monochromatic and sufficiently spatially coherent X-ray beam, is converted into a 2D map of intensity variations upon free-space propagation over a distance z .

In Fresnel theory, the according Fourier-space expansion in the strength of phase variations starts with a linear and local term which is modified by an infinite series of non-local corrections. We show simulationally and by an actual experiment that two important properties of linear contrast transfer are conserved for a wide range of nonlinear phase variations and propagation distances and how this can be understood in terms of the breaking pattern of a phase-scaling symmetry which is exact in the limit of vanishing phase variations. An according phase-retrieval algorithm is devised which, thanks to large values of z , exhibits high spatial resolution and contrast at low photon statistics. Finally, we compare our results of 3D reconstructed, time-lapsed electron density in living vertebrate model embryos with those obtained by a conventional phase-retrieval algorithm.