Complex field imaging for diffraction tomography

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Abstract: We present a technique to reconstruct 3D refractive index distribution of cells using Digital Holographic Microscopy. Diffraction tomography is performed by two-axes rotation of the sample and aberrations corrected imaging with high numerical aperture.

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Diffraction tomography is a novel technique to reconstruct 3D refractive index (RI) distributions for biological samples taking into account diffraction of a weakly scattering object. Recently, true 3D conformations of cells have been published by different groups. In our approach, the complex scattered field is obtained from a single hologram acquisition based on DHM. True 3d information is assessed through direct sample rotation on two axes. Thus, the object 3D Fourier space can be regularly sampled according to tomographic reconstruction theory [1].

The DHM experimental setup is used in transmission configuration and equipped with a high-NA long-working distance microscope objective (MO). Additionally, the two axes rotation device is directly built in the setup and the biological sample may be placed on a conventional coverslip. However, the rotation of the coverslip induce strong aberrations, typically coma like, that add to the intrinsic MO's aberration and to the amplitude point-spread function (APSF). At high tilt-angles, the measured complex fields of a test sample [an immersed glass sphere whose complex image is illustrated in cf. Fig. 1(a)] are prone of asymmetrically deformed diffraction patterns.

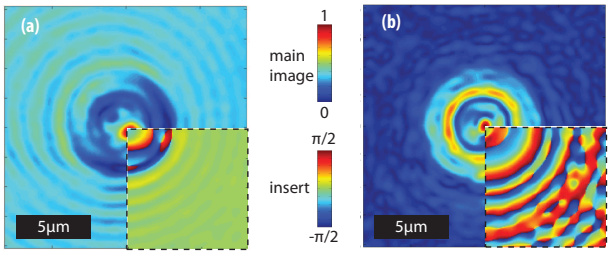

Fig. 1: Complex field (main: amplitude part, insert: phase part) of RI-unmatched glass sphere at 15° of sample tilt. Image (a) shows the distorted measured complex field, while the aberration compensated complex field is depicted in image (b). Background and diffraction patterns are successfully removed and the expected symmetric object is only visible in image (b).

Contrary to low-NA systems, the MO's intrinsic diffraction plays an important role and the two mentioned kinds of aberrations prevent from recovering the object's complex scattered field. Consequently, we explore methods to recover the 'true' complex scattered field required by diffraction tomography. A physical method consists in the use of special optics, while a numerical method is based on a 3D-modified version of deconvolution of complex fields [2], using experimentally measured APSF for all tilt-angle configurations. As exemplarily seen in the result of correction, depicted in Fig. 1(b), aberration and APSF deformation can be effectively corrected. Consecutively, the method can be applied to each complex 2d image at all tilt measurements. Eventually, the aberration compensated complex fields can be used for diffraction tomographic reconstruction. Based upon this principle, we present full 3d reconstruction of biological samples.

References

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