DEALING WITH RECONSTRUCTED COMPLEX FIELDS IN MICROSCOPY: GOING TO THE RESOLUTION LIMIT AND BEYOND?

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1. INTRODUCTION

Dealing with the complex electromagnetic wavefield scattered by the specimen, such as it can be obtained by reconstruction of digital holograms or any other method like TIE (transport of intensity equation) [1], DIC, multi-wave lateral shearing interferometry, shearography and more generally, any so-called quantitative phase microscopy (QPM) method, is a growing modality in microscopy, which will find its own path among intensity based imaging methods like fluorescence and other light scattering or emitting methods. By itself, quantitative phase is acknowledged to provide a wealth of data on the sizes and composition of the specimen by the analysis of the optical path length and the refractive index with its dispersion law. Significance of these data has been improving recently in biology and medicine.

2. THE PROBLEM OF RESOLUTION LIMIT

Another appealing feature of reconstructed complex wavefields is to allow synthetizing directly the aperture of a virtual microscope: the distribution of the complex field reconstructed on the pupil of the microscope objective permits the easy computation of the angular spectrum of the scattered light. A synthetic aperture up to full 2- or 4π is handy provided that a suitably engineered scanning concept is found. We illustrate the concept development by considering different practical approaches that allow to match the 2- or 4π concepts most completely. Before being in position to synthetize fully the aperture, 3D deconvolution of the complex field reconstructed from the measured hologram must be achieved [2]. A method to derive experimentally and to model the coherent transfer function (CTF) has been already described in some papers [2, 3]. The approach of complex deconvolution offers the advantage of directly correcting for phase aberrations within the CTF spatial frequency domain.

Stitching the filtered pupils allows fillingup most completely the wavevector space inside the Ewald sphere. As a consequence of this synthesis procedure, the spatial resolution can be significantly improved in such coherent microscopy systems.

As an illustration of the resolution improvement achieved with the synthetized aperture approach, Fig. 1 shows the resolution improvement obtained on a phase image. In summary, synthetizing the spatial frequencies spectrum in the reciprocal space leads to resolution improvement.

3. REFERENCES Figure 1: Resolution scale of a neuronal axon in phase.

[1] Kou et al., *Quantitative phase restoration by direct inversion using the OTF*, Opt. Lett. **36** (2011). [2] Cotte et al., *Realistic 3D CTF inverse filtering of complex fields*, Biomed. Opt. Express **2** (2011). [3] Cotte et al., *Microscopy image resolution improvement by deconvolution of complex fields*, Opt. Express **18**, (2010).