Super-resolution Phase Tomography

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Abstract: Digital Holographic Microscopy (DHM) yields reconstructed complex wavefields. It allows synthesizing the aperture of a virtual microscope up to 2π , offering super-resolution phase images. Live images of micro-organisms and neurons with resolution less than 100 nm are presented.

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1. Introduction

Dealing in microscopy with the complex electromagnetic wavefield scattered by the specimen, such as it can be obtained by reconstruction of digital holograms or any other methods so-called quantitative phase microscopy, is a growing modality in microscopy, which will find its own path among intensity based imaging methods like fluorescence. 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 pathlength and the refractive index with its dispersion law. Significance of these data has been improving recently in biology and medicine. The exploitation of phase data also permits the improvement of the image resolution [1].

Fig. 1: illustration of the angular scanning of the illuminating beam

2. The problem of resolution limit

Another appealing feature of reconstructed complex wavefields is to allow synthesizing directly the aperture of a virtual microscope: the distribution of the complex field reconstructed on the pupil of the MO allows the easy computation of the angular spectrum of the scattered light. Synthetic aperture up to a full 2π is handy provided that a suitably engineered scanning concept is found. We have illustrated the concept development by considering different practical approaches allowing to full match to the full 2π or 4π concepts most completely. Before being in position to synthesize fully the aperture, 3D deconvolution of the complex field reconstructed from the measured hologram must be achieved. A method to derive experimentally and to model the coherent transfer function (CTF) has been developed and described in [2] and [4].

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 to fill-up 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 by Digital Holographic Microscopy (DHM) is a method capable of providing the exact reconstruction from an hologram of the complex wavefront, both in amplitude and phase. Accordingly, its propagation can be computed exactly for any scattered wavefield.

3. Tomography.

The knowledge of the exact complex wavefield scattered for different illuminating beams allows computing the 3D spatial distribution of the specimen refractive index by means of the diffraction tomography theorem. The exact determination of the complex wavefront also provides a mean of synthesizing an enlarged numerical aperture (NA) and therefore providing super-resolved phase images. Resolutions better than 100 nm have been achieved recently

by pushing the synthetic NA to its maximum value. This development corresponds nearly to a full 2π DHM [4],[5].

4. Applications to biological cell imaging

Applications of this tomographic imaging technique to super-resolved phase imaging are described in the field of neurobiology. Dynamic observations of fluid circulation at the cellular scale (neuron body, dendrites) open a promising field of investigation.

Fig.2: illustration of the 3D images resulting from phase tomography of a neuron body. Refractive index appears as color coded 3D shells. Two different sections are shown: a) horizontal section, b) oblique section showing the nucleus (blue) and some details of the cytoplasm, most probably Golgi app. , mitochondria...

As an illustration of the resolution improvement achieved with the synthesized aperture approach, Fig. 2 shows the internal structure of the body of a living neuron by sectioning the cell with a movable plane. The observation of details of the nucleus and peri-nucleus illustrates the resolution improvement with synthetic aperture.

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6. References

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