Computational Diffraction Microtomography with Sample Rotation and with Illumination Direction Variation

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We report first results about techniques for expanding the Optical Transfer Function (OTF) when considering observation of weakly scattering specimens with a tomographic microscope, and combining the sample rotation method with the varying illumination direction method. In particular, we present simulations of the effect of the remaining missing frequencies on the image reconstructions.

1 Introduction

We aim at the development of a new approach to observe transparent samples having a low refractive index (RI) contrast, that makes it difficult to identify their intra-cellular structures. Tomographic diffractive microscopy in coherent light is one of the techniques proposed to address this issue. For weakly diffractive samples, the 3-D distribution of the complex RI can be reconstructed from the knowledge of the measured scattered fields sampled under various viewing and illumination angles according to the diffraction tomography theorem.

2 Tomographic Diffractive Microscopy (TDM)

The weakly scatterer is illuminated by a monochromatic plane wave. Within the framework of the first-order Born approximation, and using the diffraction tomography theorem, the Fourier transform values of the RI distribution in the diffractive case are distributed along a spherical cap of the Ewald sphere, as shown in fig. 1 [1, 2].

The 3-D observation of weakly diffractive samples have been so far realized by varying the sample illumination with a fixed sample [3,4,5] or by rotating the sample using a fixed illumination [6,7,8]. In case of the varying illumination direction method, the sample is illuminated under different angles (different vector $\boldsymbol{k_i}$), and the diffracted wave is recorded with a microscope objective. The lateral resolution is doubled with respect to holographic microscopy, but a strong anisotropy along the optical axis is observed, due to the missing cone, represented in fig. 2 [3,4].

For the sample rotation method, the sample is rotating around the *x*-axis with a fixed illumination (constant vector $\boldsymbol{k_i}$). The extended support of frequencies mapped by recording spatial frequencies for a large number of rotations is not a complete sphere, as illustrated in fig. 3, but the isotropy of the obtained spatial resolution is improved [8].

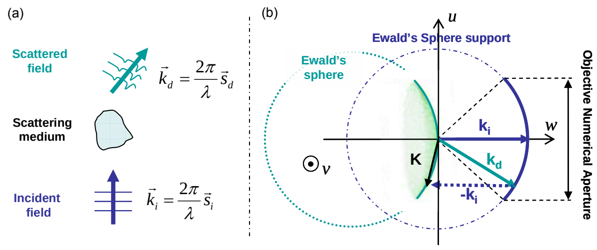

Figure 1: (a) Definition of the propagation vector of the scattered light from a scattering medium. (b) The Fourier transform of the object scattering potential is distributed over the surface of a spherical cap, the top of which coincides with the frequency origin.

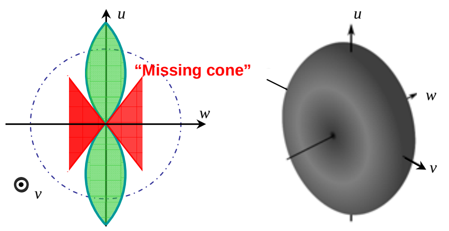

Figure 2 : Representation of the OTF obtained by illuminating the object under different angles.

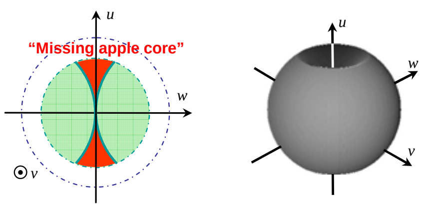

Figure 3 : Representation of the OTF obtained by object rotation along the x-axis.

3 Tomographic Diffractive Microscopy: possible configurations and simulations

In order to both enhance the resolution, and improve its isotropy, we propose different possible configurations combining the two previous methods, described in fig. 4.

• Performing successively the varying illumination direction method and the sample rotation method in order to fill missing frequencies areas existing in the two corresponding OTF, which are distributed along different axes. (b+c→d)

• Rotating the sample of few steps and performing the varying illumination direction method after each angular rotation. (Equivalent to "rotate the donut" in order to "increase the isotropy".) (c→e-h)

• Rotating the sample by 90° successively along the *x*- and *y*-axes. (c→i).

The effect of these different frequency supports on the reconstruction is investigated by filtering the spatial frequencies of a cell-like object by each frequency support and by calculating the inverse Fourier Transform (simulations performed using Matlab). Parameters: illumination wavelength 633 nm, water immersion objective with NA=1.2. The (mimicking a bacteria) cylindrical membrane-like object closed by two semi-spheres on its top and containing two solids spheres (diameter 1.16 µm and 925 nm), has a length of 6.5 µm, a width of 2.77 µm, and a membrane thickness of 92 nm.

4 Conclusion

For weakly scattering specimens, tomographic diffractive microscopy with sample rotation along one axis or with illumination direction variation does not result in isotropic resolution. Combining both methods permits to fill the missing cone characteristic of transmission microscopes, but the resolution along the optical axis is still inferior to the lateral one. The varying illumination direction method combined with a few specimen rotation steps results in the best isotropy and resolution. Note that performing a precise sample rotation, compatible with interferometric measurements, remains a crucial point [9].

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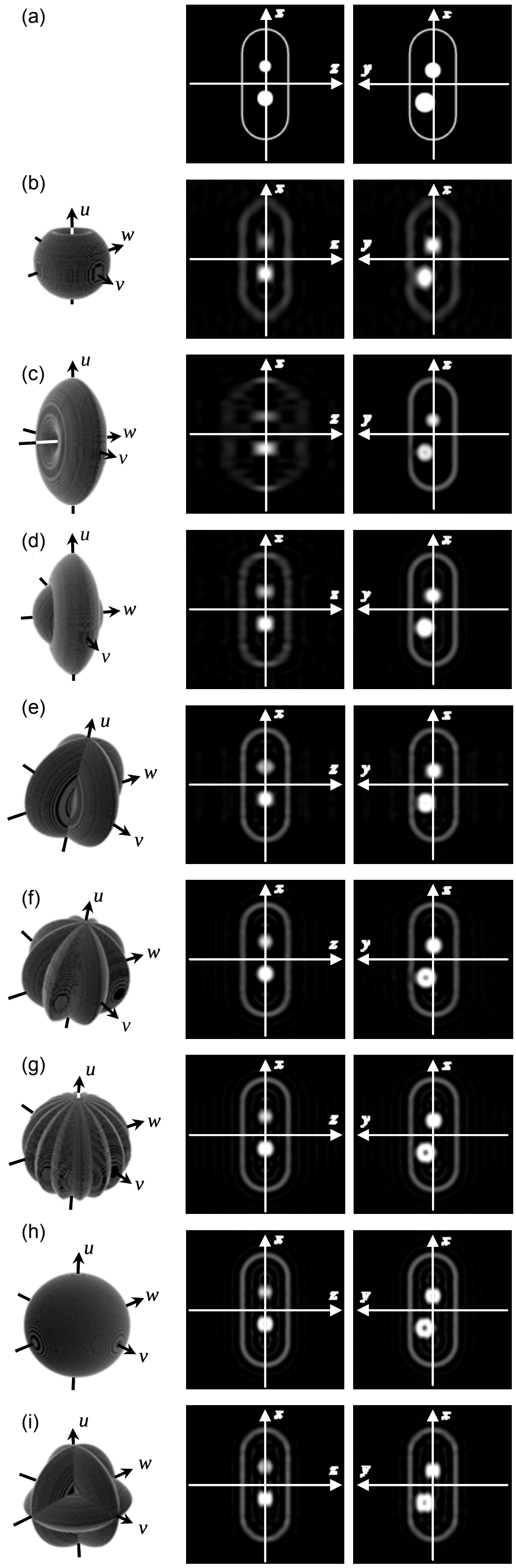

Figure 4 : (a) Simulated object. (b-h) OTF extension for different proposed tomographic methods and their respective object reconstruction simulations.