

TOMOGRAPHIC MICROSCOPY OF TRANSPARENT SAMPLES

B. Simon, M. Debailleul, V. Georges, O. Haeberlé and V. Lauer
Laboratory MIPS, University of Haute-Alsace
IUT Mulhouse, 61 rue Albert Camus, 68093 Mulhouse Cedex, France
Lauer Optique et Traitement du Signal
1, Villa de Beauté, 94130 Nogent/Marne, France

Keywords: Diffraction, Holographic microscopy, Tomographic microscopy

In 1969, Wolf [1] proposed the principle of 3D semi-transparent object imaging. The wave resulting from the diffraction of a plane wave by a semi-transparent object is decomposed as a sum of plane waves. Within the first Born approximation [2], it is possible from the scattered and illumination waves to reconstruct a Fourier representation of the specimen index of refraction distribution. Under the elasticity condition, this representation depicts the surface of the Ewald sphere in the spatial frequency domain.

Classically, in holographic microscopy, off-axis holography or phase-shifting holography is generally used to record the amplitude and phase of both the illumination and scattered waves, from one illumination direction only. Because of the limited numerical aperture of the objective, one collects a portion only of the scattered wave. As a consequence, the reconstructed object frequency support corresponds to a cap only of the Ewald sphere, and therefore the main limit is the low resolution, especially along the z-axis.

Lauer has proposed [3] a method to construct an extended frequency support by recording series of hologram for different incidence angles of the illumination wave. We have now built such a set-up on an Olympus IX71 inverted fluorescence microscope.

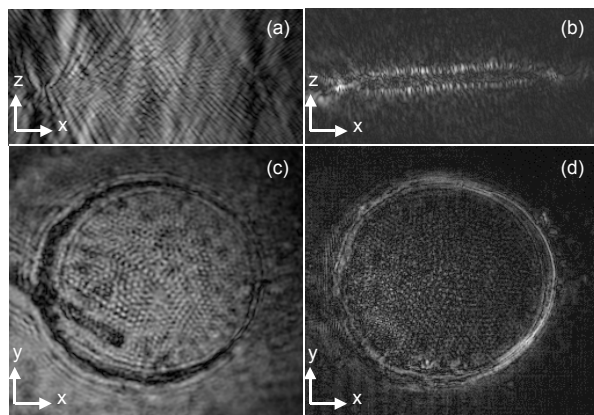


Figure 1: Diatom cell wall imaged through holographic microscopy (a,c) and optical diffraction tomography (b,d). (a,b): longitudinal views and (c,d): transversal views (arrows represent 5 μ m).

Figure 1 compares holographic microscopy for one illumination incidence and optical diffraction tomography for 372 incidences of illumination. On Fig. 1(a) characteristic longitudinal diffraction fringes from punctual objects are visible: there is no discrimination along the z-axis. In Fig. 1(b) these fringes disappear and the horizontal walls of the diatom cell are now visible. Note also that the other elongated diatom, which appears superimposed on the round diatom in Fig. 1(c) is not visible on Fig. 1(d). In fact, it belongs to another plane of the 3-D image, which is discriminated in Fig. 1(d), whereas it is not in Fig. 1(c).

This demonstrates the better 3-D imaging capabilities of diffraction tomographic microscopy, compared to classical holographic microscopy. We now intend to couple tomographic microscopy with fluorescence microscopy, in view of estimating fluorescence image inhomogeneities induced by specimen index of refraction variations [4].

1. E. Wolf, "Three-dimensional structure determination of semi-transparent object from holographic data". *Opt. Comm.* **1**, 153-156 (1969)
2. M. Born and E. Wolf, *Principles of Optics*, Chapter 8 (Pergamond Press, Exeter, 1991)
3. V. Lauer, "New approach to optical diffraction tomography yielding a vector equation of diffraction tomography and novel tomographic microscope." *J. Microscopy*. **205**, 165-176 (2002)
4. J. B. Pawley, "Limitations on Optical Sectioning in Live-Cell Confocal Microscopy", *Scanning* **24**, 241-246 (2002)