Optical tomography with digital holographic microscopy

I. Bergoënd, N. Pavillon, F. Charrière, C. Depeursinge
EPFL, Advanced Photonics Laboratory, 1015 Lausanne, Switzerland
e-mail: isabelle.bergoend@epfl.ch

Summary

Three-dimensional optical imaging by tomographic techniques is presented, especially with object rotation in a transmission configuration. In digital holographic microscopy, the complex field is measured, which permits either to study transparent objects with varying index of refraction or to compute diffraction tomography in which the phase is needed.

Introduction

Digital holographic microscopy is an imaging technique which consists in recording on a CCD camera the pattern (hologram) produced by the interference between an object beam and a reference beam. An off-axis Mach-Zehnder interferometer mounted in a microscope is used here. After a digital demodulation equivalent to illuminating the hologram with the reference wave, propagation is computed until the plane in which the object is focused. Corrections are applied to the hologram to compensate for tilt aberrations, phase offset and wavefront curvature [1] [2]. In the case of large phase variations, an unwrapping procedure is applied.

Digital holographic microscopy, as usual microscopy, does not permit a three-dimensional reconstruction of the object unless a scan is introduced. We consider here the rotation of the object under fixed illumination and acquisition device. From a large number of views taken at different rotation angles, we reconstruct the three-dimensional variations of index of refraction in the object. Under the assumption of very low index variation, the filtered backprojection algorithm gives satisfying results; however diffraction must be taken into account for a certain class of objects.

Optical tomography using filtered backprojection

Some of the biological objects are transparent and can be mainly characterized by the variations of their inner refractive index. In this case, digital holographic microscopy has the great advantage of providing the phase of the scattered field, which is related to the optical path encountered by the wave. Moreover, a single hologram is needed foreach angle, therefore high acquisition rates can be reached.

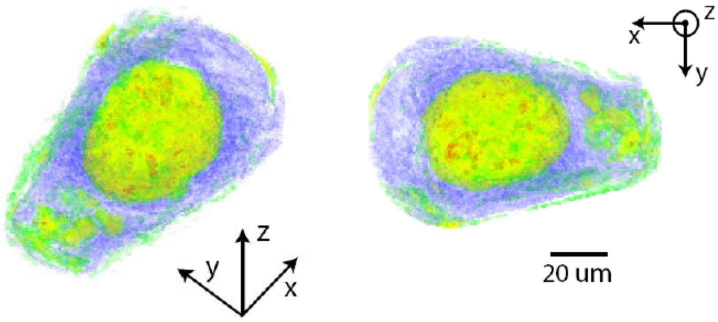

Fig 1: Transparency view of a tomographic measurement of a tek amoeba, in (a) angular view and (b) x-y projection. Colors correspond to refractive index value range: Blue: 1.448-1.467, Green: 1.474-1.478, Yellow: 1.478-1.493, Red: 1.493-1.513.White: refractive index of the immersion medium.

When studying objects of order of some millimeters with weakly-varying refractive index, diffraction effects can be neglected, owing to the optical wavelength which is

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less than one micron. Thus, straight rays are considered here and the Radon transform is used for the 3D index reconstruction.

Experimental results have been obtained with a tek amoeba (*Hyalosphenia papilio*). The rotation is achieved by clamping the observed specimen with a micropipette fixed on a motorized stage, so that it could be rotated within a chamber containing the immersion medium (glycerol, n=1.473). The total angle of rotation is 180° with steps of 1°, the axis being perpendicular to the illumination direction. The sample is illuminated with a laser diode (wavelength: 635 nm), and imaged with a 0.4 NA 20X microscope objective. Figure 1 presents a visualization of the 3D reconstructed amoeba, whose shell is approximately 130 μm long, 70 μm wide and 35 μm deep, where the internal structure can be readily identified. Some internal elements in the amoeba are a few microns in size, showing that the tomographic reconstruction is close to the optical resolution of the setup. The resolution in refractive index is approximately 0.005.

Diffraction tomography

In order to account for diffracting objects, we have implemented diffraction tomography according to Wolf's theory [3], using the technique of Fourier interpolation [4]. As in this case knowledge of the complex scattered field is needed, digital holography is especially well suited.

The spatial frequencies obtained from each angle of view lie on part of a sphere called the Ewald's sphere if diffraction is taken into account, and reduces to a simple plane when diffraction is neglected. Each new angle permits to reach another sphere of same radius, with a different center. Using only one rotation axis, a cone is missing in the frequency space [5]. By rotating the object according to two Euler angles, we can fill the 3D frequencies space with Ewald's spheres, using the 2D Fourier transforms of each view. Then, an inverse 3D Fourier transform gives the 3D scattering potential from which the quantitative variations of refractive index can be deduced.

Conclusion

Digital holographic microscopy has a great potential in optical tomography because of its ability to measure the complex scattered field from a single hologram, especially for 3D imaging of biological objects that possibly induce diffraction of optical waves.

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