BioMedical Engineering OnLine

BioMed Central

Research

Open Access

Quantitative phase imaging with scanning holographic microscopy: an experimental assesment

Guy Indebetouw*, Yoshitaka Tada and John Leacock

Address: Virginia Tech, Physics Department, Blacksburg, VA 24061/0435, USA

Email: Guy Indebetouw* - gindebet@vt.edu; Yoshitaka Tada - ytd@vt.edu; John Leacock - leacock@vt.edu

* Corresponding author

Published: 28 November 2006

BioMedical Engineering OnLine 2006, **5**:63 doi:10.1186/1475-925X-5-63

Received: 19 September 2006

Accepted: 28 November 2006

This article is available from: http://www.biomedical-engineering-online.com/content/5/1/63

Abstract

This paper demonstrates experimentally how quantitative phase information can be obtained in scanning holographic microscopy. Scanning holography can operate in both coherent and incoherent modes, simultaneously if desired, with different detector geometries. A spatially integrating detector provides an incoherent hologram of the object's intensity distribution (absorption and/or fluorescence, for example), while a point detector in a conjugate plane of the pupil provides a coherent hologram of the object's complex amplitude, from which a quantitative measure of its phase distribution can be extracted. The possibility of capturing simultaneously holograms of three-dimensional specimens, leading to three-dimensional reconstructions with absorption contrast, reflectance contrast, fluorescence contrast, as was previously demonstrated, and quantitative phase contrast, as shown here for the first time, opens up new avenues for multimodal imaging in biological studies.

1- Background

Microscopy is an essential tool in biological research, micromechanical testing, the integrated circuit industry, etc. The demand for higher resolution and contrast, shorter acquisition time, and multimodal imaging, among other desirable properties, has resulted in the recent invention, demonstration, and often rapid commercialization of a number of new technologies. In biological studies, two modalities appear to be of primary importance. They are fluorescence imaging for the specific identification of biomolecules in a labeled sample, and phase imaging for the determination of internal structures in unstained specimens. The conventional phase imaging methods (i.e. Zernike phase contrast, and Nomarski differential interference contrast) usually provide only the visualization of the phase of biological structures in a qualitative way, although it is possible to extract quantitative phase information with the differential interference contrast method [1]. Recently, quantitative phase imaging, as provided by digital holographic microscopy [2] gave a new dimension to phase imaging by allowing the quantitative measurement of, for example, biomasses and fluid concentrations in cells. Quantitative phase imaging is also essential in measuring non-destructively the surface topography of biological samples, as well as of micromechanical systems, and integrated electrical circuits.

To our knowledge, no single instrument or imaging method can capture both fluorescence and quantitative phase information of 3D specimens simultaneously in holographic form. In this paper, we demonstrate experimentally that scanning holographic microscopy [3,4], which was developed to obtain holograms of incoherent objects [5,6], and has recently been shown to provide