Digital Holographic Microscope for the Analysis of Living Cells

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The analysis of life processes with cellular and even sub cellular resolution is one of the particular interests in life sciences. Life processes in (semi) transparent probes which produce refractive index changes and variations of the shape can be analyzed by the interferometric determination of optical path length changes. Digital holography allows a fast, non destructive and quantitative high resolution full field measurement of the optical amplitude and phase. The application of a microscope lens to the system improves the lateral resolution that is normally restricted by the pixel pitch of the utilized CCD sensor. At the Laboratory of Biophysics of the University of Münster a system for digital holographic microscopy has been realized. The lateral and axial resolution of the system is characterized and the applicability on investigations on living cells is demonstrated.

1 Introduction

Digital holography provides spatial and temporal high resolution three dimensional shape and deformation measurement of technical and biological samples down to the cellular and sub cellular level. The axial component is evaluated from the simultaneously reconstructed object wave’s phase. The lateral resolution that is normally restricted by the image recording device can be improved by the application of a microscope lens to the experimental setup. Results from investigations on test charts for system characterization and on living cells are presented and discussed.

2 Experimental Setup

Fig. 1 shows the experimental setup for digital holographic microscopy. Here the incident light arrangement is used for metrology measurement of technical probes while the inverse transmitting light arrangement is used for the detection of optical path length changes of transparent probes, such as living cells in suspension.

The emitted light of a frequency doubled Nd:YAG laser (λ = 532 nm) is divided into object wave and reference wave which are spatially filtered. The object wave is focused for object illumination while the reference wave is collimated due to reconstruction with a plane wave model. To enhance the system’s lateral resolution that is restricted by the pixel pitch of the CCD array the incident/transmitting object wave is magnified by a microscope lens. Holographic off-axis geometry is realized by superposing a reference wave slightly tilted to the object wave. The digital hologram is captured with an 8 bit CCD array with IEEE1394 interface.

3 Numerical Reconstruction

Numerical reconstruction of the object wave is performed by the application of a non diffractive reconstruction method (NDRM) for off-axis digital holography. The complex object wave (amplitude and phase) within the hologram plane (CCD array) is calculated by solving the interferogram equation. As a result the reconstructed image does not contain the disturbing terms: twin image and zero order [1].

In a second step multiple image planes can be calculated. Therefore, the complex object wave is propagated by numerical evaluation of the Fresnel-Kirchhoff diffraction integral, e. g. by the discrete Fresnel transform (DFT) [1, 2].

The advantages of combining the NDRM with the DFT reconstruction method are:
• full pixel resolution and contrast enhancement of the reconstructed image caused by the elimination of the twin image and zero order with the NDRM,
• reconstruction of multiple object planes by propagation with the DFT, e.g. for subsequent focusing or zooming.

4 Results and Discussion
To demonstrate the lateral resolution of the measurement technique an USAF 1951 resolution chart was captured in transmitting light arrangement with a 20x microscope lens (NA = 0.4) and its amplitude was reconstructed numerically (Fig 2 a)). The elements of group 9.2 which correspond to a line width of 0.85 µm are well separated. This result is in good accordance with the Abbe criterion and means that the lateral resolution is only restricted by the diffraction limit.

Fig. 2: a) USAF 1951 resolution chart: group 7,8 and 9; b) axial test chart (chrome on chrome), step height = 30 nm.

Fig. 2 b) shows the reconstructed phase (subpart as colored 3D plot) of a chrome on chrome test chart with axial 30 nm steps that appears as reflective phase object. It was captured in incident light arrangement with a 5x microscope lens (NA = 0.1).

The axial resolution of the reconstructed object morphology was quantified by the analysis of noise and amounts to 8 nm.

Fig. 3: Three dimensional quantitative analysis of the migration process of living human pancreas cells (PaTu8988T).

For cancer research the migration process of living tumorous human pancreas carcinoma cells (PaTu8988T) was recorded with high temporal resolution (video-frequency). Fig. 3 shows the reconstructed phase image at t=0, 5 min, 10 min and 15 min exemplarily.

Two reconstructed phase images of a single PaTu8988S cell are presented in Fig. 4. At t=0 5 µl Latranculin-B were added to the culture medium to destroy the actin filaments of the cell's cytoskeleton. The deformation of the cell is quantified by the horizontally cross sections.

Fig. 4: Reconstructed phase images of a single PaTu8988S cell and corresponding horizontally cross sections through the quantitative phase contrast images at t=0 and t=140 s.

The presented results show that digital holographic microscopy allows spatial and temporal high resolution quantitative three dimensional measurements. The technique has particular advantages on biological samples: marker free, non destructive and full-field recording. Therefore it can be applied, e.g. for the analysis of dynamic processes like cell migration or effects of drug delivery. In conclusion the technique has the potential to form a versatile tool for microscopy applications in the life sciences and biophotonics.

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Literature