Enhanced Quantitative Imaging of Living Cells and Dissected Tissues UtilizingMulti-Spectral Digital Holographic Microscopy

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Abstract: We explored, if coherence properties of partial coherent light sources can be generated synthetically utilizing spectrally tunable lasers. The performance of the method is demonstrated by label-free quantitative phase imaging of living pancreatic tumor cells.
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1. Introduction

Main restrictions of using laser light in digital holographic microscopy (DHM) are coherence induced noise and parasitic reflections in the experimental setup as these disturbances affect the reconstructed amplitude and phase images and thus limit the measurement accuracy. Partially coherent light reduces such effects. However, the application of light sources with a very low coherence length requires special experimental arrangements or precise alignment of the optical equipment. We explored if coherence properties of partial coherent light sources can be generated synthetically utilizing spectrally tunable laser systems. Therefore, amplitude and phase distributions are numerically superimposed that result from the reconstruction of digital holograms, which are recorded separately at different laser wavelengths. Thus, a robust alignment of a laser-based experimental setup is combined with the noise reduction advantages of partial coherent light. The application of fiber coupled tunable lasers simplifies the usage of the multi-wavelength approach with existing DHM setups. We demonstrate the capabilities of multi-spectral DHM for label-free quantitative phase imaging of living cells and tissues with an experimental configuration that utilizes a commercial microscope and a tunable super continuum laser light source that covers a spectral range of > 230 nm.

2. Experimental setup and numerical reconstruction of digital holograms

Fig. 1 shows the concept for multi-wavelength DHM setup than can be modular integrated into a common inverted microscope (AE30, Motic, Hong Kong, China). Light from a tunable super continuum laser light source (SuperK EXTREME EXW-12 with Super Select 4xVIS/IR acousto-optic tunable filter (AOTF), NKT Photonics A/S, Birkerød, Denmark) is coupled into a Michelson interferometer-based DHM configuration in which one of the mirrors is slightly tilted to generate digital off-axis holograms [1]. The sample is illuminated via the condenser lens of the inverted microscope. The Michelson interferometer configuration allows a robust and simplified alignment that is insensitive to changes of the object illumination and vibrations. Moreover, it can be operated even with light with low coherences properties (l_c < 50 µm). Digital off-axis Holograms were recorded at different wavelengths λ from 470 nm to 700 nm utilizing an industrial camera (The Imaging Source, Bremen, Germany). The reconstruction of quantitative phase images from the acquired off-axis holograms was carried out by spatial phase shifting [2]. If the sample was not imaged sharply during hologram recording at different wavelengths, numerical refocusing was applied [3]. In order to reduce coherence disturbances, short coherence properties are generated numerically by superposition of optical path length (OPL) distributions, which are calculated from quantitative phase images that are captures at different wavelengths as reported earlier [4].

3. Quantitative phase imaging of living pancreatic tumor cells

The performance of multi-spectral DHM is illustrated by quantitative phase imaging of living pancreatic tumor cells (PaTu 8988 T E-Cad). Cells were cultured subconfluent in Petri dishes (µ-dish with glass lid, ibidi GmbH, Munich, Germany) and observed in cell culture medium with an experimental setup as shown in Fig. 1. Fig. 2 shows representative OPL distributions. N holograms of the cells were recorded at 470 nm (N = 1) and stepwise in different wavelength ranges (470 nm to 480 nm, N = 5, $\Delta\lambda$ = 10 nm; 470 nm to 510 nm, N = 20, $\Delta\lambda$ = 20 nm; 470 nm to 550 nm, N = 40, $\Delta\lambda$ = 80 nm; 470 nm to 700 nm, N = 65, $\Delta\lambda$ = 230 nm). For the quantitative phase images reconstructed

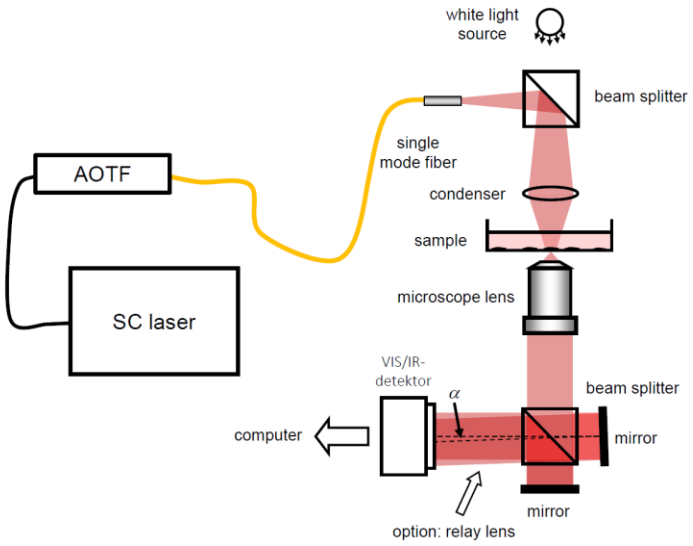

Figure 1. Michelson interferometer-based concept for multi-spectral digital holographic microscopy. α: off-axis angle between object and reference wave, AOTF: Fiber coupled acousto-optic tunable filter, SC laser: fiber-coupled super continuum laser source.

from a single hologram (N = 1) the thin cell borders are difficult to recognize. For the OPL distributions that were retrieved from N = 5, 10, 20 and 40 and 65 averaged quantitative phase images that were recorded at different wavelengths, with increasing spectral width, these areas appear with reduced background noise and significantly improved contrast. Moreover, N = 20, 40 and 65 holograms also subcellular structures like the nucleoli are clearly resolved in the OPL distributions.

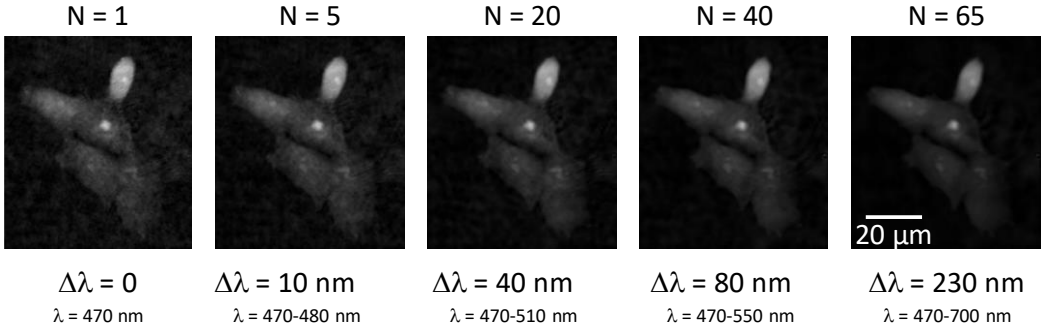

Figure 2. Averaged OPL distributions of pancreatic tumor cells (PaTu 8988 T E-Cad cells) retrieved from a single hologram (N = 1, 470 nm, Δλ = 0), N = 5 holograms (470 nm to 480 nm, Δλ = 10 nm), N = 20 holograms (470 nm to 510 nm, Δλ = 40 nm), N = 40 holograms (470 nm to 550 nm, Δλ = 80 nm) and N = 65 holograms (470 nm to 400 nm, Δλ = 230 nm).

5. Conclusions

Our approach significantly decreases coherent induced image disturbances in DHM by numerically superimposing monochromatic wave fields that were reconstructed from holograms acquired with a wavelength tunable super continuum laser. The image quality was significantly improved and the contrast of the thin boundary areas of cells as well as the visibility of subcellular structures highly benefitted from the reduced noise level. In conclusion, multi-spectral DHM is a promising flexible tool for high-resolution quantitative phase imaging of living cell cultures.

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