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Multi-spectral digital holographic microscopy for enhanced quantitative phase imaging of living cells

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ABSTRACT

Main restrictions of using laser light in digital holographic microscopy (DHM) are coherence induced noise and parasitic reflections in the experimental setup which limit resolution and measurement accuracy. We explored, if coherence properties of partial coherent light sources can be generated synthetically utilizing spectrally tunable lasers. The concept of the method is demonstrated by label-free quantitative phase imaging of living pancreatic tumor cells and utilizing an experimental configuration including a commercial microscope and a laser source with a broad tunable spectral range of more than 200 nm.

Keywords: digital holographic microscopy, quantitative phase imaging, multi-wavelength, hyperspectral imaging.

1. INTRODUCTION

During the past decade quantitative phase microscopy (QPM) was continuously further improved for high resolution label-free quantitative live cell imaging [1-17]. Digital holographic microscopy (DHM) [1], an interferometry-based variant of QPM, allows the reconstruction of quantitative phase images by numerical evaluation of digitally captured holograms and (subsequent) numerical refocusing without mechanical focus realignment. Moreover, DHM can be integrated modular into common research microscopes [18] for multimodal label-free imaging [19, 20] and utilized for quantification of migration [21] and motility [22] as well as for analysis of living cell cultures in three-dimensional environments [23, 24].

Main restrictions of using laser light in 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 [25-27]. However, the application of light sources with a very low coherence length requires special experimental arrangements or precise alignment of the optical equipment [26,27].

Based on previous work [28,29,30], 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 superposed that result from the reconstruction of digital holograms which are recorded separately at different laser wavelengths. Thus, the robust alignment of a laser-based experimental setup is combined with the noise reduction advantages of partial coherent light. The application of single-mode fiber coupled tunable lasers, simplifies the usage of the multi-wavelength approach with existing DHM setups.

Here, we demonstrate the capabilities of multi-spectral DHM for label-free quantitative phase imaging of living cancer cells by an experimental configuration that utilizes a commercial microscope and a tunable super continuum laser light source that covers a spectral range of more than 230 nm.

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2. MULTI-WAVELENGTH DIGITAL HOLOGRAPHIC MICROSCOPY

2.1 Experimental setup and reconstruction of digital holograms

Fig. 1 shows the concept of an experimental setup for multi-wavelength modular DHM that was be integrated into a common inverted microscope (AE30, Motic, Hong Kong, China). Light from a tunable laser light source (SuperK EXTREME EXW-12 combined with a 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 [31]. 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 with light with low coherences properties ($l_c \approx 50$ µm). For imaging of the sample, a 20x microscope lens (Zeiss LD Acroplan 20x/0.4 Korr) was used. Holograms were recorded at different wavelengths λ from 470 nm to 700 nm utilizing a standard industrial camera (DKM 23UP1300, The Imaging Source, Bremen, Germany).

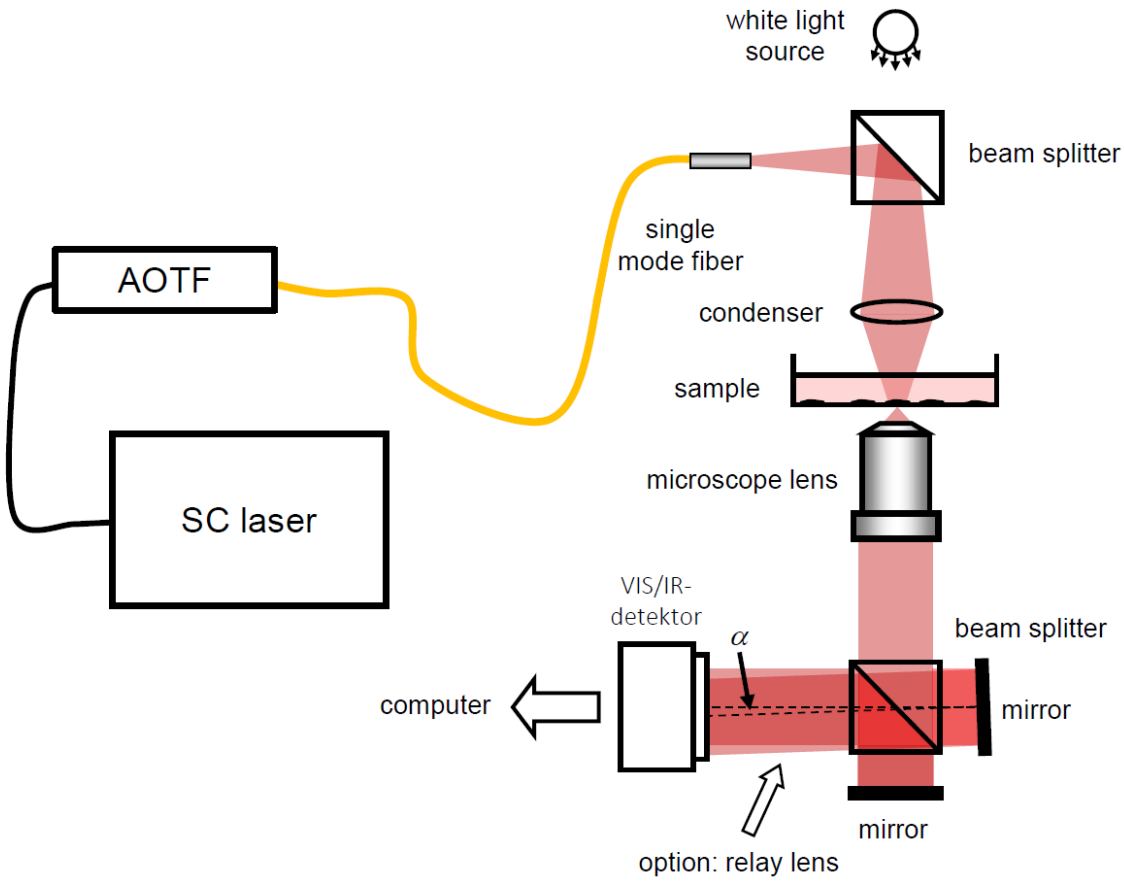

Figure. 1. Concept for multi-spectral digital holographic microscopy. α: off-axis angle between object and reference wave, AOTF: Fiber coupled acousto-optic tunable filter, SC laser: fiber-coupled super continuum laser source.

The reconstruction of quantitative phase images from the acquired off-axis holograms was carried out by spatial phase shifting [2,7]. If the sample was not imaged sharply during hologram recording at different wavelength, numerical refocusing was applied [32].

2.2 Evaluation of quantitative phase images for numerical reduction of coherence induces disturbances

The relation between the measured quantitative phase delay $\varphi_{O,\lambda}(m,n)$ of the object and the corresponding optical path length changes OPL(m,n) [29,30]

$$\mathrm{OPL}(m,n) = \frac{\lambda}{2\pi}\varphi_{O,\lambda}(m,n) \tag{1}$$

depends on the applied laser wavelength λ. The parameters m,n denote the pixel coordinates of the hologram recording device. In order to reduce coherence disturbances short coherence properties are generated numerically by superposition of N OPL distributions (Eq. 1) [29,30]:

$$\mathrm{OPL}_{\mathrm{sum}}(m,n) = \frac{1}{N}\sum_{i=1}^{N}\frac{\lambda_i}{2\pi}\varphi_{O,\lambda_i}(m,n)\ . \tag{2}$$

3. QUANTITATIVE PHASE IMAGING OF LIVING PANCREATIC TUMOR CELLS

The performance of multi-wavelength DHM is illustrated by quantitative phase imaging of living pancreatic tumor cells with different morphological properties (PaTu 8988 T, PaTu 8988 S, PaTu 8988 T PLXIN, 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 of thin PaTu 8988 T E-Cad cells. 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, Δλ = 10 nm; 470 nm to 510 nm, N = 20, Δλ = 20 nm; 470 nm to 550 nm, N = 40, Δλ = 80 nm; 470 nm to 700 nm, N = 65, Δλ = 230 nm).

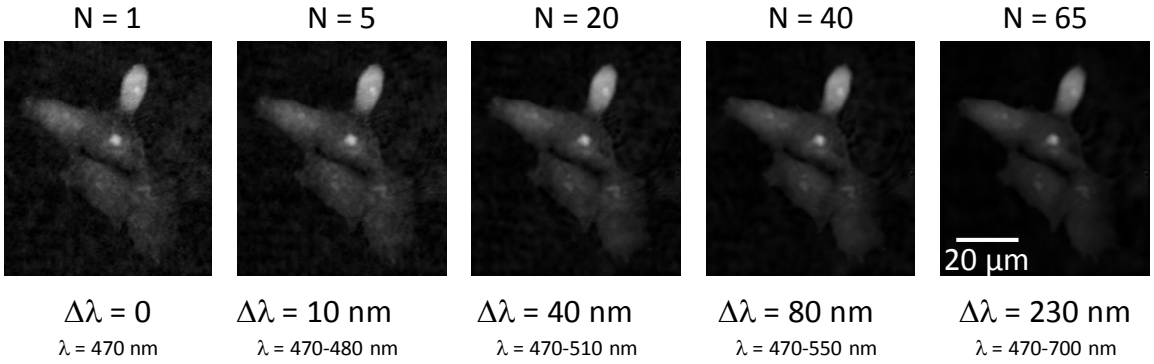

Figure 2. Averaged OPL distributions from PaTu 8988 T E-Cad cells retrieved from a single hologram (N = 1, 470 nm, Δλ = 0), N = 5 holograms (470 nm to 480 nm, Δλ = 10 nm), N = 20 holograms (470 nm to 510 nm, Δλ = 40 nm), N = 40 holograms (470 nm to 550 nm, Δλ = 80 nm) and N = 65 holograms (470 nm to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

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

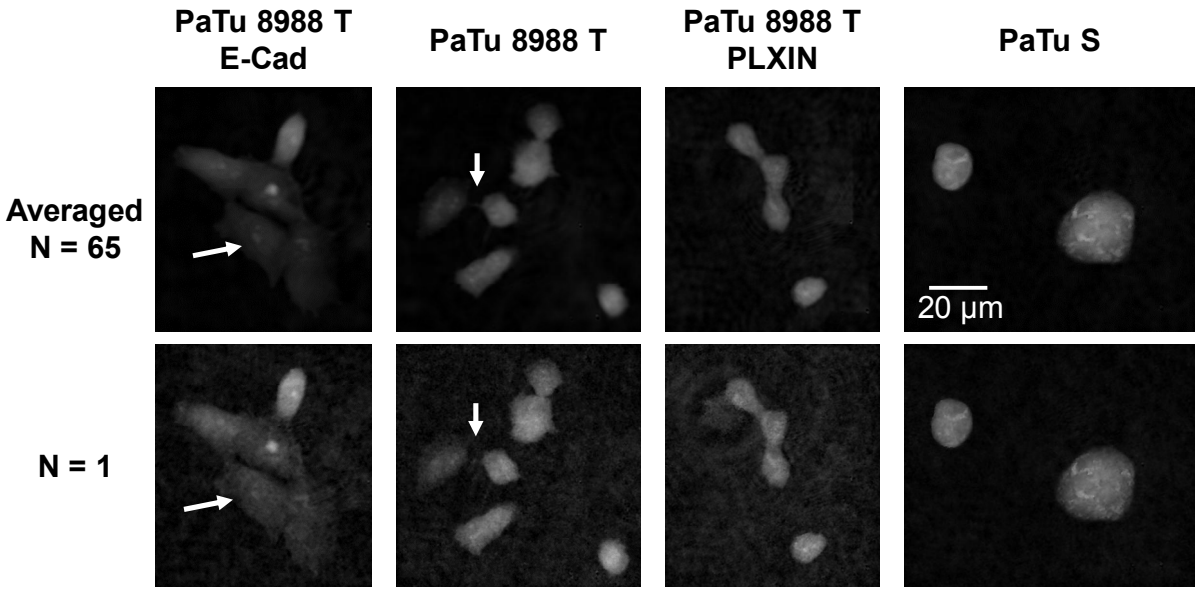

Figure 3. Upper row: Averaged OPL distributions from of living pancreatic tumor cells with different morphological properties (PaTu 8988 T E-Cad, PaTu 8988 T, PaTu 8988 T PLXIN, PaTu 8988 S) that were retrieved from and $N = 65$ holograms recorded in a wavelength range 470 nm to 700 nm ($\Delta\lambda = 230$ nm) second row: Corresponding OPL distributions retrieved from single holograms ($N = 1$) at 470 nm.

Fig. 3 shows in the upper row averaged OPL distributions retrieved by multi-spectral DHM from different pancreatic tumor cells (PaTu 8988 T, PaTu 8988 S, PaTu 8988 T PLXIN, PaTu 8988 T E-Cad) that were retrieved from and $N = 65$ holograms recorded in a wavelength range 470 nm to 400 nm ($\Delta\lambda = 230$ nm) in comparison to corresponding OPL distributions retrieved from single holograms ($N = 1$) at 470 nm. In all images the cells, cell borders, subcellular structures and tiny cell-cell contacts are visible with significantly improved contrast while the background noise appears reduced.

4. CONCLUSIONS

In summary, our approach significantly decreases coherent noise in DHM by numerically superimposing monochromatic wave fields that were reconstructed from holograms acquired at different wavelengths of a tunable laser. The applicability of the method was illustrated by quantitative DHM phase contrast imaging of a living pancreatic tumor cells. The image quality was significantly improved and the contrast of the thin boundary areas of the cells as well as the visibility of subcellular structures benefitted from the reduced noise level. In conclusion, multi-spectral DHM is a promising tool for high-resolution quantitative phase imaging of living cell cultures.

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