

Hyperspectral digital holographic microscopy approach for reduction of coherence induced disturbances in quantitative phase imaging of biological specimens

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ABSTRACT

Coherence induced noise and parasitic reflections in the experimental setup are main restrictions that limit the resolution and measurement accuracy in laser light-based digital holographic microscopy (DHM). We explored, if coherence properties of partial coherent light sources can be mimicked by utilizing spectrally tunable lasers. Moreover, the performance for label-free quantitative phase imaging of biological specimens is illustrated utilizing an experimental configuration including a commercial microscope and tunable super continuum laser sources with a wavelength range of up to 230 nm.

Keywords: digital holographic microscopy, quantitative phase imaging, multi-wavelength, hyperspectral imaging, coherence induced image disturbances, laser speckle.

1. INTRODUCTION

During the past decade quantitative phase microscopy (QPM) was continuously improved for high resolution label-free quantitative live cell imaging [1-17]. Digital holographic microscopy (DHM) [1], a variant of QPM, is an established tool for industrial non-destructive testing and a promising method for minimally invasive label-free analysis of biological specimens like living cells or dissected tissues. 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 scattering, speckle, and parasitic reflections in the experimental setup. These disturbances affect the reconstructed amplitude and phase images and thus limit the measurement accuracy. Application of partially coherent light reduces such effects [26,27]. However, utilization of light sources with very low coherence lengths, like for example light emitting diodes (LEDs) [26,27] requires special experimental arrangements or highly precise alignment of the optical equipment.

Based on previous work [28-31], we explored if coherence properties of partial coherent light sources can be generated synthetically utilizing spectrally tunable laser systems. Therefore, we superpose numerically several amplitude and phase distributions 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 pancreatic tumor cells and dissected mouse retina by an experimental con-

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figuration that utilizes a commercial microscope and tunable super continuum laser light sources that cover a spectral range up to 230 nm.

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 integrated into a common inverted microscope (AE30, Motic, Hong Kong, China). Light from a tunable laser light source (SuperK EXTREME EXR-15 or EXR-9, NKT Photonics A/S, Birkerød, Denmark) combined with an acousto-optic tunable filter (AOTF) (Super Select 4xVIS/IR or nIR1, NKT Photonics A/S, Birkerød, Denmark) was coupled into a Michelson interferometer-based DHM configuration in which one of the mirrors was slightly tilted to generate digital off-axis holograms [31]. The sample was 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 coherence properties ($l_c \approx 50 \mu\text{m}$). 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, or from 800 nm to 850 nm utilizing a standard industrial camera (DKM 23UP1300, The Imaging Source, Bremen, Germany).

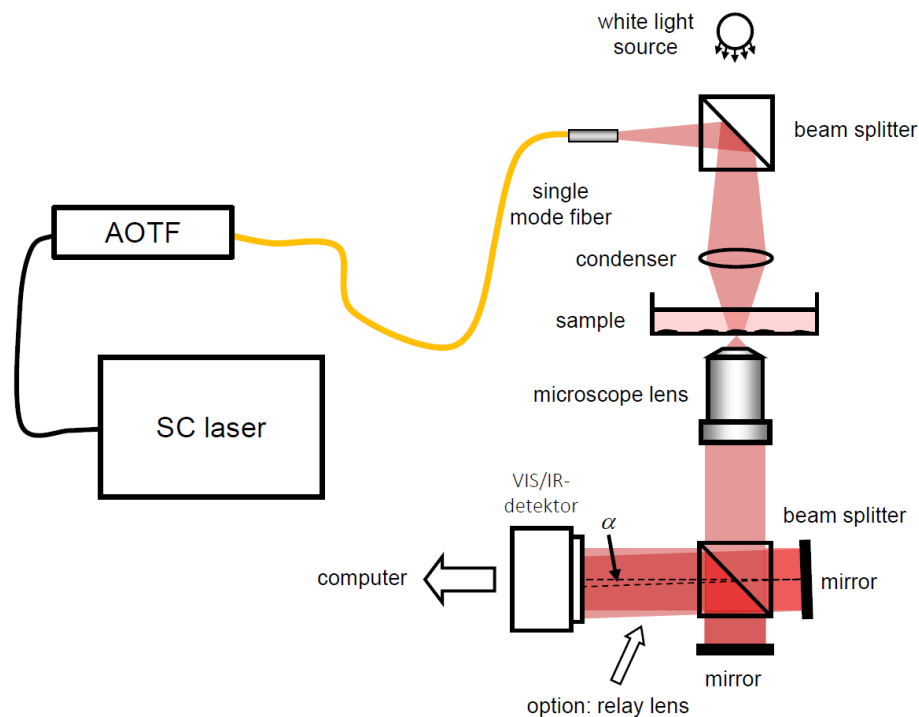


Figure 1. Concept for multi-spectral digital holographic microscopy. α : off-axis angle between object and reference wave, AOTF: Fiber coupled acousto-optic tunable filter, SC laser: fiber-coupled super continuum laser source.

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 re-focusing 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]

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

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

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

3. QUANTITATIVE PHASE IMAGING OF LIVING CELLS AND DISSECTED TISSUES

The performance of multi-wavelength DHM is firstly 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. Figure 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 with steps of $\Delta\lambda = 2$ nm (470 nm to 480 nm, $N=5$; 470 nm to 510 nm, $N=20$; 470 nm to 550 nm, $N=40$; 470 nm to 700 nm, $N=65$).

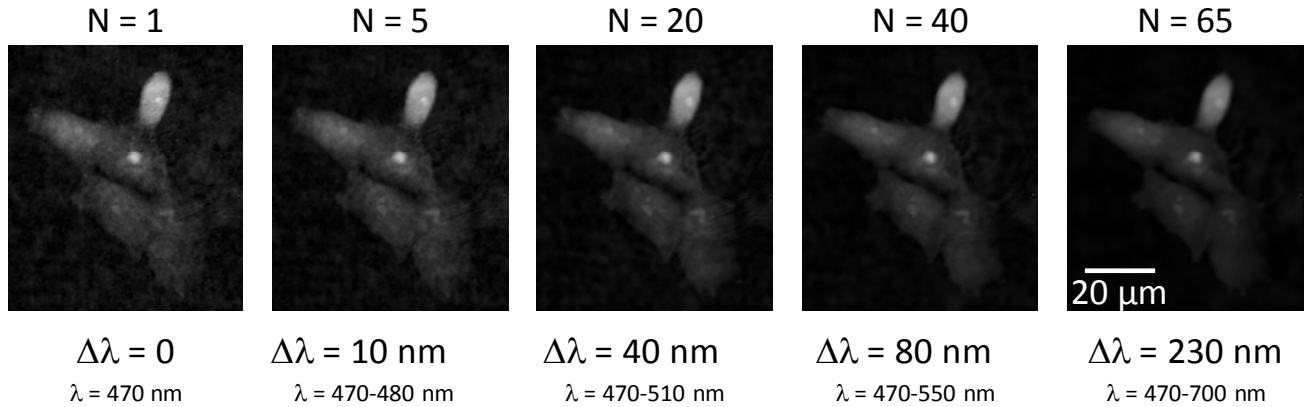


Figure 2. Averaged OPL distributions from PaTu 8988 T E-Cad cells retrieved from a single hologram $N=1$ ($\lambda=470$ nm), $N=5$ holograms ($\lambda=470$ nm-480 nm), $N=20$ holograms ($\lambda=470$ nm-510 nm), $N=40$ holograms ($\lambda=470$ nm-550 nm), and $N=65$ holograms ($\lambda=470$ nm-700 nm). Adapted from [33].

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.

In a second demonstration, and to show the capability of the system to image tissue sections, multi-wavelength DHM was employed for quantitative phase imaging of dissected mouse retina. The tissue sections were prepared with a specif-

ic thickness and fixated on conventional glass carriers. For DHM measurements, the samples were embedded in phosphate buffered saline (PBS) and covered with a cover slip. To minimize possible unwrapping errors resulting from the thickness of the sample tissue, longer wavelengths were employed in this case. In particular, several digital holograms were recorded at different wavelength from $\lambda = 800 \text{ nm}$ to $\lambda = 850 \text{ nm}$ in steps of $\Delta\lambda = 10 \text{ nm}$, which are relevant in optical coherence tomography(OCT)-based ophthalmological imaging. Fig. 3 shows representative quantitative phase map distributions of a mouse retina (field of view $170\mu\text{m} \times 170\mu\text{m}$). The detected phase distribution reflects the layer structure of the retina and its refractive index properties. As it can be observed in Fig. 3, coherent disturbances that are present in quantitative phase images calculated from a single hologram (white arrow in Fig.3a) are reduced by reconstructing several quantitative phase images that were recorded at different wavelengths (Fig.3b), and by averaging them to produce a noise-reduced quantitative phase image (Fig.3c). A pseudo 3D representation of the quantitative phase map of Fig.3c is depicted in Fig.3d.

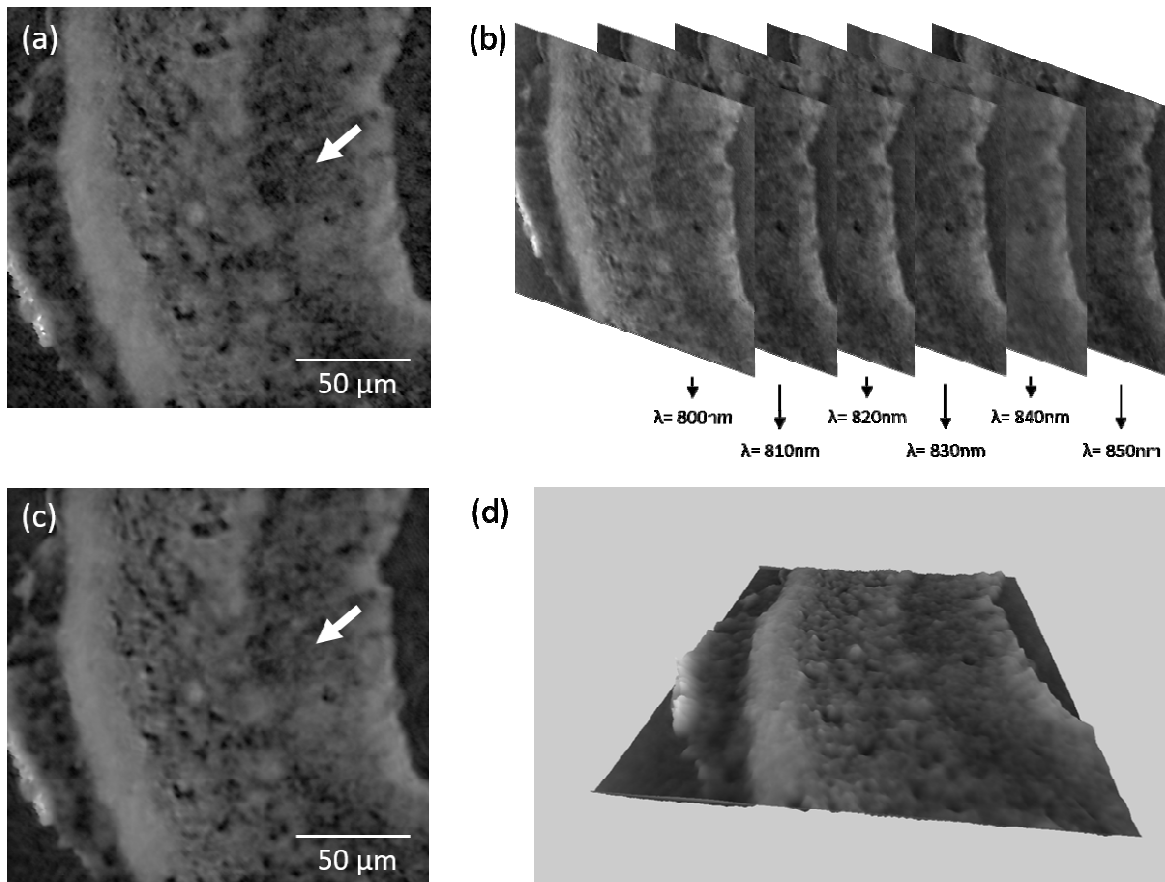


Figure 3. Reduction of coherence-induced disturbances in quantitative phase images (QPIs) of dissected mouse retina using the presented hyperspectral digital holographic microscopy approach. (a) QPI of a region of interest (ROI) of the retina that was obtained from a single hologram acquired at $\lambda=800 \text{ nm}$. (b) Stack of QPIs of the same ROI that were acquired at different wavelengths (from $\lambda=800 \text{ nm}$ to $\lambda=850 \text{ nm}$ in steps of $\Delta\lambda = 10 \text{ nm}$). (c) Averaged QPI resulting from numerically superimposing the stack of QPIs in (b). (d): pseudo 3D plot of the QPI in (c). The white arrows in (a) and (c) serve to compare the reduction of coherence induced disturbances.

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 living pancreatic tumor cells and of dissected mouse retina. 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 and dissected tissues.

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