Application of Color Digital Holographic Microscopy

Xiaoli Mo[1,2], Björn Kemper[1], Patrik Langehanenberg[1], Angelika Vollmer[1],Jinghui Xie[2], Gert von Bally[1]

[1]*Center for Biomedical Optics and Photonics, University of Muenster, Germany,*

[2]*School of Information Science and Technology, Beijing Institute of Technology, China*

moxiaoli@gmail.com

Color digital holographic microscopy offers subsequent multi-focus true color imaging with simultaneous quantitative phase contrast analysis. Investigations on the numerical reconstruction of color digital holographic images have been performed by applying an off-axis transmission microscope experimental setup to stained tissue sections.

1 Introduction

The analysis of pathological sections represents an important tool in medical diagnostics. Pathological tissue is enclosed in a supporting material and treated with a reagent or dye that visualizes structures before sectioning for microscopic examination. Digital holographic microscopy offers subsequent multi-focus imaging with simultaneous quantitative phase contrast analysis [1-4]. Investigations on digital recording and numerical reconstruction of color digital holographic images have been performed by applying a transmission microscope experimental setup in holographic off-axis geometry. Three monochromatic digital holograms are recorded a different wavelengths in red, green and blue spectral range [5-6]. After digital holographic refocusing and compensation for aberrations of the microscope imaging system by digital image correlation the numerically reconstructed amplitude distributions are combined into color images. The applicability of the method is demonstrated by results obtained from a stained intestine tissue section.

2 Setup for Color Digital Holographic Microscopy

Fig. 1 illustrates the concept of a color digital holographic microscopic system. The light of three lasers with different wavelengths (red: λ=632.8 nm, green: λ=532 nm, blue: λ=460 nm) is divided respectively into an object illumination wave (object wave) and a reference wave which are each arranged to propagate in the same optical path. For simplification of the experimental setup polarization maintaining single mode optical fibers are applied. Holographic off-axis geometry is achieved by a beam splitter that generates a slight tilt between the reference wave and the object wave. The magnification of the sample is performed by a microscope lens (Zeiss Acroplan LD 5×). The holograms that are formed by the superposition of object wave and reference wave are recorded with a CCD camera (Sony XCD-X700, 8 bit, 1024×768 pixels, pixel pitch 6.25µm×6.25µm) and are transferred via an IEEE1394 (“FireWire”) interface to an image processing system for digital holographic reconstruction. Color images can be generated by combination of images that are recorded by illumination of the sample with three different light wavelengths (e.g. red, green, blue). Thus, the reconstructed amplitudes obtained from digital holograms that are recorded separately by application of three monochromatic lasers contain the color information of the investigated object.

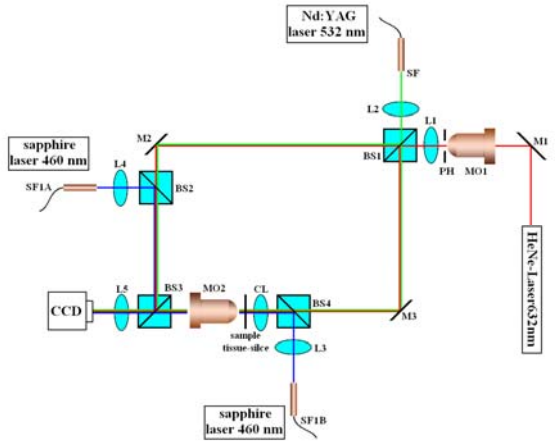

Fig. 1 *Experimental setup for color digital holographic microscopy. M1 – M3: mirrors; BS2 – BS4: beam-splitter cubes;L1 – L5: lenses, MO1 – MO2: microscope lenses; PH: pinhole; SF, SF1A, SF1B: single mode optical fibers.*

3 Digital holographic reconstruction

Diffraction based digital holographic reconstruction methods like the Fresnel-transformation generate not only the information about the object wave but also the intensity of the reference wave (“zero-order”) and the “twin-image” [7]. In addition, the size of the reconstructed holographic image depends on the propagation distance. Thus, for microscopy applications non diffractive reconstruction methods (NDRM) that use spatial phase shifting

for the retrieval of the complex object wave from digitally captured off-axis holograms [2, 8] in combination with subsequent convolution based numerical propagation [9] are of particular advantage. As a consequence of the applied reconstruction algorithms the reconstructed complex object wave $O(x, y, z_{IP})$ in the image plane at $z=z_{IP}$ does not include the disturbing terms "twin-image" and "zero-order". From $O(x, y, z_{IP})$, in addition to the amplitude $|O(x, y, z_{IP})|$ that represents the image of the sample, the phase information $\Delta\varphi_S(x, y, z_{IP})$ of the sample (quantitative phase contrast image) is reconstructed simultaneously:

$$\Delta\varphi_S(x, y, z_{IP}) = \arctan \frac{\mathrm{Im}\{O(x, y, z_{IP})\}}{\mathrm{Re}\{O(x, y, z_{IP})\}} (\mathrm{mod}\, 2\pi) \qquad (1)$$

For color digital holographic microscopy three holograms of the sample are recorded at different wavelengths. After digital holographic refocusing and compensation for aberrations of the microscope imaging system by digital image correlation the numerically reconstructed amplitude distributions are combined into color images.

4 Experiment results

Fig. 2 illustrates the color fusion process of the numerical reconstruction by a result obtained from of a stained intestine tissue section. Figures 2(a)-(c) show the amplitude distributions reconstructed from the holograms recorded at λ=632.8 nm, 532 nm and 460 nm. By defining a three-layer RGB matrix, and adding the reconstructed amplitudes for red, green and blue, the color image in Fig. 2(d) is obtained. Fig. 2(d) shows that the color holographic image appears with clear boundaries and details. Fig. 2(e) shows a corresponding quantitative phase contrast image of the sample (mod 2π).

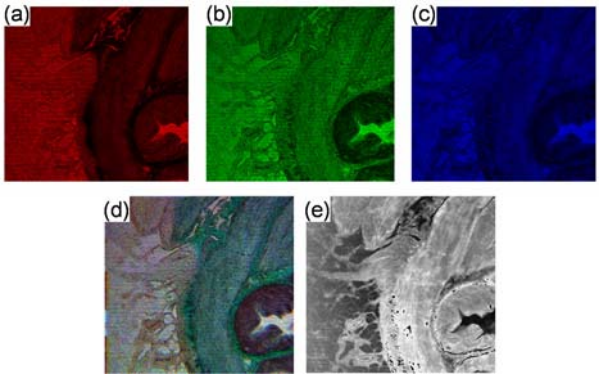

Fig. 2 *Results obtained from of a stained intestine tissue section by using color digital holographic microscopy. Reconstructed amplitude distributions at (a): λ=632.8 nm; (b): λ=532 nm; (c): λ=460 nm. (d): RGB image resulting from fusion of (a), (b) and (c). (e): corresponding quantitative phase contrast image of the sample.*

5 Conclusions

The results presented in section 4 demonstrate that color DHM can be used for the analysis of stained tissue sections and prospect subsequent refocusing of microscopic color images. Furthermore, the quantitative phase contrast offered by DHM can be utilized for quantitative analysis, e.g. of the refractive index distribution, of such samples. Thus, additional quantitative and impartial information and parameters in digital histopathology are provided.

6 Acknowledgements

Financial support by the German Federal Ministry for Education and Research as part of the "Biophotonics" funding program and the China Scholarship Council (CSC) is gratefully acknowledged.

References

[1] E. Cuche, P. Marquet, C. Depeursinge, "Simultaneous amplitude-contrast and quantitative phase-contrast microscopy by numerical reconstruction of Fresnel off-axis holograms," Appl. Opt. 38, 6994-7001 (1999).

[2] D. Carl, B. Kemper, G. Wernicke, G. von Bally, "Parameter-optimized digital holographic microscope for high-resolution living-cell analysis," Appl. Opt. 43, 6536-6544 (2004).

[3] P. Marquet, B. Rappaz, P. J. Magistretti, E. Cuche, Y. Emery, T. Colomb, C. Depeursinge, "Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength," Opt. Lett. 30, 468-470 (2005).

[4] C. Mann, L. Yu, C. Lo, M. Kim, "High-resolution quantitative phase-contrast microscopy by digital holography," Opt. Express 13, 8693-8698 (2005).

[5] H. Toge, H. Fujiwara, K, Sato, "One-shot digital holography for recording color 3-D images," Proc. SPIE 6912, 69120U (2008).

[6] I. Yamaguchi, T. Matsumura, J. Kato, "Phase-shifting color digital holography," Opt. Lett. 27, 1108-1110 (2002).

[7] U. Schnars, W. Jüptner, "Direct recording of holograms by a CCD target and numerical reconstruction," Appl. Opt. 33, 179-181 (1994).

[8] M. Liebling, T. Blu, M. Unser, "Complex-wave retrieval from a single off-axis hologram," J. Opt. Soc. Am. A 21, 367-377 (2004).

[9] B. Kemper, D. Carl, J. Schnekenburger, I. Bredebusch, M. Schäfer, W. Domschke, G. von Bally, "Investigation of living pancreas tumor cells by digital holographic microscopy," J. Biomed. Opt. 11, 034005 (2006).