Investigations on Label-Free Identification of Subcellular Tumor Cell Structures by Digital Holographic Phase Contrast Microscopy

Sabine Przibilla[*], Björn Kemper[*], Christina Rommel[**], Steffi Ketelhut[*], Angelika Vollmer[*], Patrik Langehanenberg[*], Jürgen Schnekenburger[**], Gert von Bally[*]

** Center for Biomedical Optics and Photonics, University of Muenster, Robert-Koch-Str. 45, D-48149 Muenster, Germany*

*** Department of Medicine B, University of Muenster, Albert-Schweitzer-Straße 33, D-48129 Muenster, Germany*

mailto: bkemper@uni-muenster.de

In order to interpret quantitative digital holographic phase contrast images for the analysis of subcellular structures, comparative investigations were performed using fluorescence microscopy with DAPI-staining. Results from statistical studies on adherent pancreas tumor cells with fluorescence microscopy and DHM demonstrate that nucleus components can be identified label-free by digital holographic microscopy.

1 Motivation

Digital holographic microscopy (DHM) offers a label-free quantitative phase contrast for minimally invasive analysis of living single cells and cell cultures with low demands on the sample preparation [1-4]. In order to identify subcellular structures in DHM images comparative investigations using fluorescence microscopy with DAPI (4',6-Diamidino-2-phenylindol) were performed since DAPI-staining visualizes cell components containing DNA. Results from statistical studies on fixed adherent pancreas tumor cells with fluorescence microscopy and DHM show that nucleus components can be identified label-free in digital holographic phase contrast images.

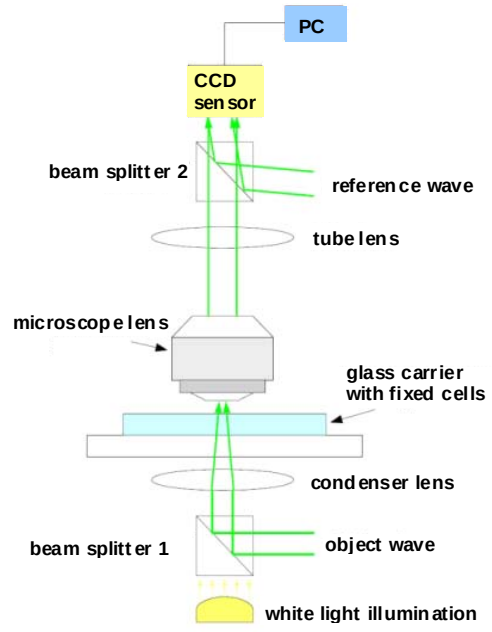

Fig. 1 *Sketch of the setup for digital holographic microscopy.*

2 Digitalholographic Microscopy Setup

The experiments are performed with an upright fluorescence microscope which has been modified for DHM [3]. Fig. 1 shows the setup for DHM. The light of a frequency-doubled Nd:YAG laser (λ = 532 nm) is divided into object illumination and reference wave. The light transmitted by the sample is superposed with the reference wave to generate an off-axis hologram which is digitally captured by a CCD sensor. The subsequent numerical reconstruction of the digital hologram yields quantitative phase contrast images [1-2] that can be refocused automatically [4] (see green and red arrows in Fig. 2).

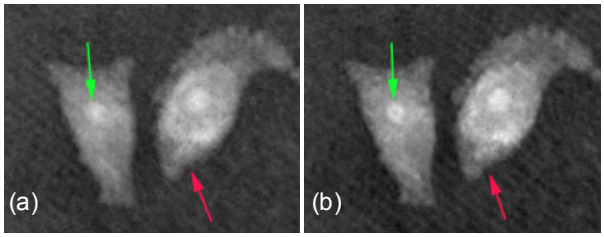

Fig. 2 *Quantitative DHM phase contrast images of fixed human pancreas tumor cells (PaTu 8988T). (a): slightly defocused, (b): numerically refocused.*

The measured phase distribution $\Delta\varphi_{cell}$, effected by a cellular sample in a surrounding medium, depends on the cell thickness d_{cell}, the wavelength λ of the applied laser light, the integral cellular refractive index n_{cell} and the refractive index of the medium n_{medium} [2]:

$$\Delta\varphi_{cell} = \frac{2\pi\left(n_{cell} - n_{medium}\right)d_{cell}}{\lambda} \quad . \qquad (1)$$

3 Results and Discussion

3.1 Phase Information and Cell Thickness

Formalin fixed pancreas tumor cells (PaTu 8988T) in physiological dilution (PBS/PBS++) result in a cell thickness d_{cell} of 8 to 11 µm, which is in agreement with previously published data for living cells [2]. Neither DAPI (fluorescence stain) nor Triton (used to enhance the cell membrane permeability) affect the phase contrast and cell thickness data. In contrast, embedding of the cells in Glycerol yields a reduced DHM phase contrast and thus results in a cell thickness of 2 to 4 µm. This can be explained by diffusion of Glycerol into the cell and the nucleus which induces a change of n_{cell} that differs from that in PBP/PBS++.

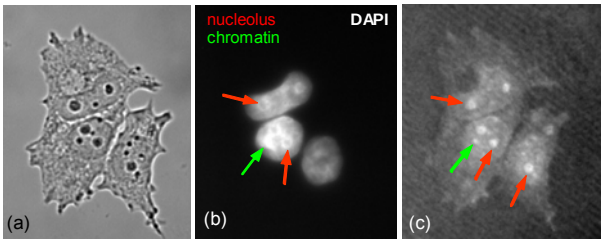

Fig. 3 *Fixed pancreas tumor cells (PaTu 8988T). (a): bright field image, (b): fluorescence image (DAPI staining), (c): quantitative DHM phase contrast image.*

3.2 Visibility of Cell Components

Cell nucleus components (nucleolus, chromatin) are detected at identical positions in bright field images, fluorescence images and digital holographic phase contrast images. The nucleus and different subcellular components of investigated PaTu 8988T cells are visible and appear bright and dark in fluorescence images as well as in the digital holographic phase contrast images (see exemplarily result Fig. 3). Bright areas in fluorescence images indicate a high DNA concentration while bright areas in digital holographic phase contrast images correspond to cell nucleus structures with high optical density. Dark areas in nucleoli correspond to a phase difference as illustrated in Fig. 4.

Due to the visibility of dark areas in the nucleoli 38 % of the nucleoli are identified only with a digital holographic phase contrast image. For 39 % of the investigated cell structures the identification of chromatin and nucleoli is possible by comparison with the fluorescence image. Fig. 5 shows that Triton induces an enhanced contrast in bright field, fluorescence and digital holographic phase contrast images of different pancreas tumor cell lines (PaTu 8988T, PaTu 8988S). Furthermore, it is demonstrated that, due to the numerical autofocusing feature of DHM, for thin PaTu 8988T cells the best visibility of the cell nucleus components is obtained for the digital holographic phase contrast images. In comparison, for thick PaTu 8988S cells with $d_{cell} > 20$ µm nucleus components are better visible in fluorescence images.

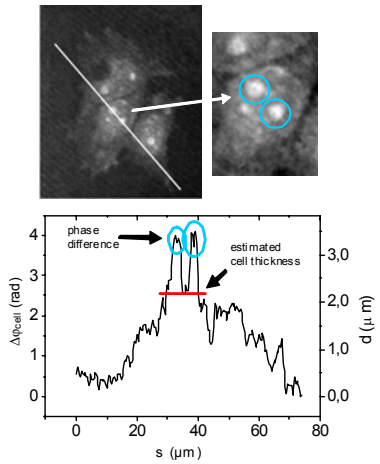

Fig. 4 *Cross-section through the phase contrast image of two nucleoli of a fixed PaTu 8988T cell in Glycerol.*

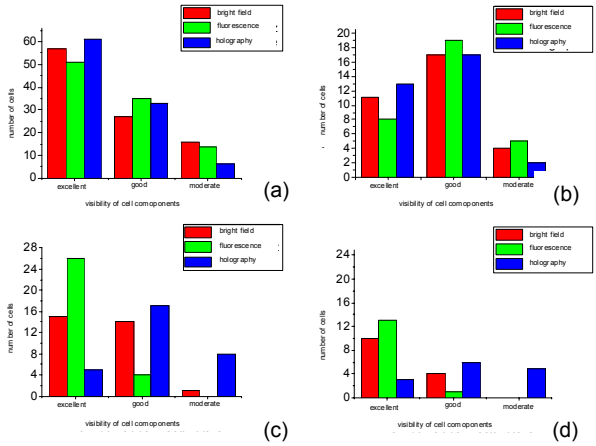

Fig. 5 *(a): fixed PaTu 8988T cells with Triton, (b): fixed PaTu 8988T cells without Triton, (c): fixed PaTu 8988S cells with Triton, (d): fixed PaTu 8988S cells without Triton.*

References

[1] D. Carl, et al., "Parameter-optimized digital holographic microscope for high-resolution living-cell analysis,"*Appl. Opt.* **43**, 6536-6544 (2004).

[2] 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).

[3] B. Kemper, D. Carl, A. Höink, G. von Bally, I. Bredebusch, J. Schnekenburger, „Modular digital holographic microscopy system for marker free quantitative phase contrast imaging of living cells", *Proc. SPIE* **6191**, 61910T (2006).

[4] P. Langehanenberg, B. Kemper, D. Dirksen, and G. von Bally, "Autofocusing in digital holographic phase contrast microscopy on purephase objects for live cell imaging," *Appl. Opt.* **47**, D176-D182 (2008).

Acknowledgements

Financial support by the German Federal Ministry for Education and Research is gratefully acknowledged.