Influence of Sample Preparation in Digital Holographic Phase Contrast Microscopy

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The interpretation of quantitative digital holographic microscopy (DHM) phase contrast images that are obtained from fixed adherent cells requires the consideration of the sample preparation. To quantify the influence of different cell preparation methods empirical models that quantify the maximum cell effected phase contrast and the visibility of nucleus components were developed and applied to fixed tumor cells.

1 Introduction

Quantitative phase contrast imaging with digital holographic microscopy (DHM) enables a label-free analysis of living and fixed cellular specimens [1-3]. For DHM measurements on fixed adherent cells the sample preparation (e. g. the embedding medium) has to be considered. Thus, investigations were performed to analyze the influence of the sample preparation on the DHM phase contrast and the resulting calculated cell thickness. A second aim of the study was to quantify the visibility of subcellular components like the nucleoli. The maximum cell effected phase contrast and the visibility of the nucleoli were determined numerically by empirical models which were fitted to the measured phase data.

2 Methods

By using a modified iMIC-system (TILL Photonics, Gräfelfing, Germany) (Fig. 1) as digital holographic microscope, quantitative phase contrast images of living and fixed tumor cells (PaTu 8988T, PaTu 8988S, HT-1080) in cell culture medium (DMEM), phosphate buffered saline (PBS) and glycerol were recorded. For the determination of the maximum

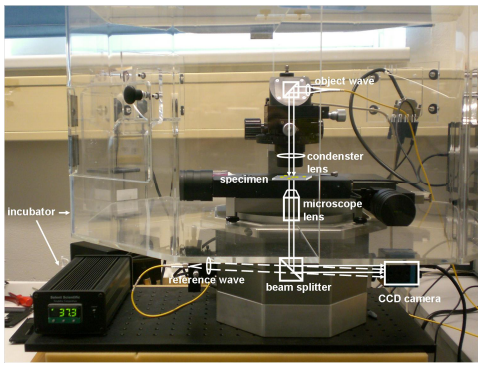

Fig. 1 *iMIC-microscope (TILL Photonics, Gräfelfing, Germany) with schematic of the modification for DHM.*

phase contrast $\Delta\Phi_{max}$ the empirical function

$$\Delta\Phi(s) = \Delta\Phi_{max} \cdot e^{\frac{(s-a)^4}{b}} + c + d \cdot s\,, \qquad (1)$$

with the variable parameters *a, b, c* and *d* was fitted to the measured phase distribution $\Delta\Phi_{cell}$. caused by the cells (Fig. 2).

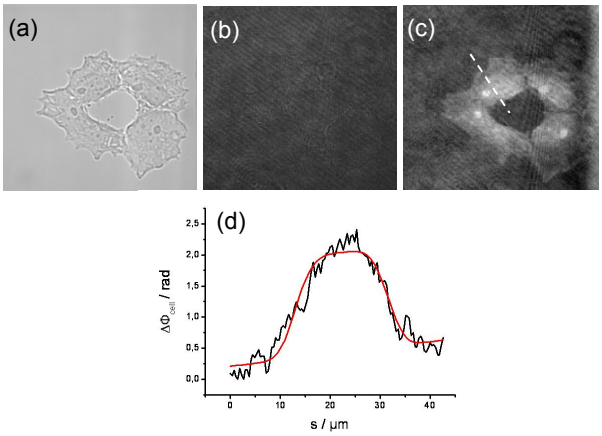

Fig. 2 *Exemplary results obtained from pancreas tumor cells (PaTu 8988T) in PBS. (a): Bright field image, (b): digital off-axis hologram, (c): DHM phase contrast image with cross section (white line) through a cell, (d): phase contrast $\Delta\Phi_{cell}$ along the cross section s in (c) (black line) and fit corresponding to Eq. (1) (red line).*

$\Delta\Phi_{max}$ depends on the wavelength λ of the applied laser light, the integral cellular refractive index n_{cell} the refractive index of the surrounding medium n_{medium} as well as on the maximum cell thickness d_{cell}, [2]

$$\Delta\Phi_{max} = \frac{2\pi}{\lambda}(n_{cell} - n_{medium})d_{cell}\,. \qquad (2)$$

The visibility of the nucleoli was quantified by the difference Δn between the refractive index of the nucleolus n_{nucleo} and the refractive index n_{cell} of the surrounding area of the cell. Estimating a spherical shape for the nucleoli the function

$$\Delta\Phi_{\text{nucleo}} = \frac{4\pi}{\lambda} \cdot \sqrt{R^2 - (s - s_0)^2} \cdot \Delta n + \Delta\Phi_0 \,, \quad \text{with} \quad \Delta n = n_{\text{nucleo}} - n_{\text{cell}} \,, \tag{3}$$

and the variable parameters R, s_0, $\Delta\Phi_0$ and Δn were fitted to the phase contrast $\Delta\Phi_{\text{nucleo}}$ of the nucleolus to determine the parameters Δn the radius R of the nucleoli [3].

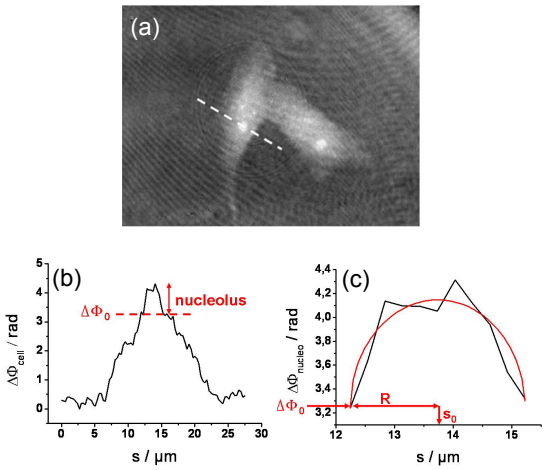

Fig. 3 *(a): Exemplary DHM phase contrast image of fixed pancreas tumor cells in PBS with cross section through a cell and the nucleolus, (b): phase contrast $\Delta\Phi_{\text{cell}}$ along the cross section s in (a) with marked peak effected by the nucleolus, (c): selected phase contrast data of the nucleolus $\Delta\Phi_{\text{nucleo}}$ (black line) from (b) and fitted data from Eq. (3) (red line) with the fit parameters $\Delta\Phi_0$, R and s_0.*

3 Results and Discussion

The glycerol embedding decreases the maximal phase contrast of the cells (Fig. 4a) and results in an underestimation of the cell thickness of about 71 % (Fig. 4b). For the fixed cells in PBS similar results as for the living cells in DMEM are obtained.

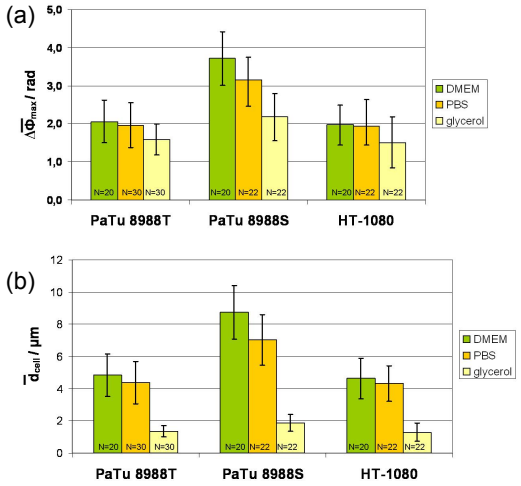

Fig. 4 *Results for (a): the average maximum phase contrast and (b): the average cell thickness of tumor cells in different embedding media. The fixed cells were embedded in PBS and glycerol. Living cells were investigated in cell culture medium (DMEM).*

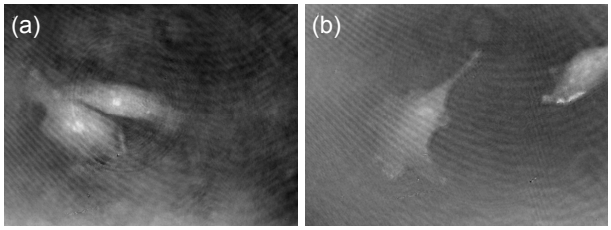

Fig. 5 *Comparison of fixed pancreas tumor cells in PBS: (a): cells with a high value Δn=0.016, (b) cells with a lower value Δn=0.010.*

The results in Figures 5 and 6 show that the parameter Δn represents a figure of merit for the visibility of subcellular components. Furthermore, the results of the nucleoli analysis in Fig. 6 indicate that the cell preparation (embedding medium and substrate) does not affect the cell radius and the DHM phase contrast of the nucleoli within the measurement accuracy.

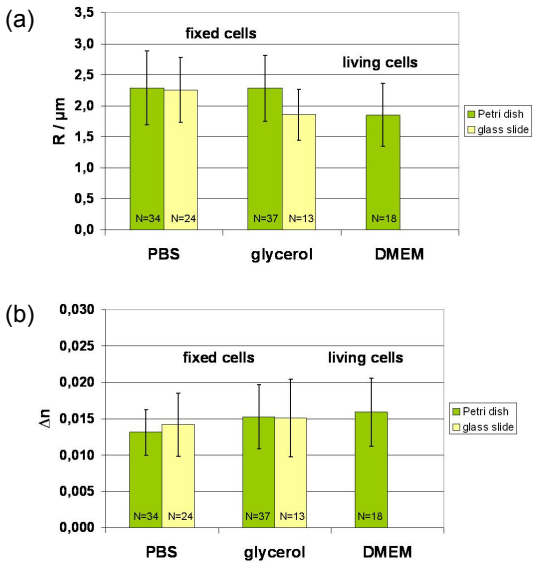

Fig. 6 *(a): Radius R of the nucleoli and (b): difference Δn between the refractive index of the nucleoli and the surrounding area of the cell. The shown results were achieved for the pancreas tumor cell line PaTu 8988T in different embedding media (PBS, glycerol and DMEM) in comparison for different substrates (Petri dishes and glass slides).*

References

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Acknowledgements

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