Digital Holographic Phase Microscopy

Cell Refractive Index Determination Using Microspheres

We explored a method to determine the refractive index of the cytoplasm by using microspheres that have been incorporated by living cells as reference in quantitative digital holographic phase contrast microscopy images. As many cells show a phagocytic behavior the method may be used with a variety of different cell types.

Introduction

Full-field quantitative phase microscopy (QPM) techniques enable label-free minimally-invasive live cell analysis [1-4]. In QPM-based live cell imaging the intra-cellular refractive index distribution represents an important parameter. On one hand decoupling of the cellular refractive index and the cell thickness is required, e. g., for reliable investigations on the cell thickness and shape. On the other hand the information about the refractive index is related to the concentration of the intracellular content. For these reasons, up to the present several QPM-based concepts for cell refractive index determination have been developed (see for example reference collections in [5, 6]). However, in particular the reliable determination of the refractive index of adherent cells still represents a challenge. We explored a method to determine the mean refractive index of the cytoplasm with quantitative digital holographic phase contrast microscopy by using silica microspheres as reference that have been incorporated by living cells. As many cells show a phagocytic behavior the method may be used with a variety of different cell types. Furthermore, as no modification of the experimental setup is required, the method prospects to be used with several existing quantitative phase contrast imaging techniques.

Principle of Refractive Index Determination and Results

Multi-focus quantitative phase imaging was performed in transmission with an inverted modular digital holographic microscopy (DHM) setup (fig. 1, light source: frequency doubled Nd:YAG laser, λ = 532 nm) based on a principle described in detail previously [1,4,7] which allows live cell imaging in a temperature stabilized environment. Adherent cells were incubated with SiO2 microspheres (diameter
about 3.44 µm) and observed in conventional Petri dishes.

Figure 2 illustrates the internalization of two silica microspheres by Chinese hamster ovary cells (CHO cells) by representative quantitative DHM phase images that were obtained from time lapse investigations.

Figure 3 demonstrates the principle for the measurement of the intracellular refractive index with microspheres as reference in quantitative phase images [8]. Figure 3a shows an exemplary bright field image of a SiO$_2$ particle in cell culture medium. In figure 3b the corresponding quantitative DHM Phase contrast image is depicted. Figure 3c shows a cross-section through the DHM phase contrast image of the microsphere in figure 3b along the dashed white line. The maximum phase contrast in figure 3c corresponds to the value that is expected by the diameter of the microsphere and the refractive index data given by the manufacturer of the microspheres. Figure 3d shows a bright field image of an adherent pancreatic tumor cell (PaTu 8988 T) with an incorporated microsphere. In figure 3e the corresponding quantitative DHM phase contrast image is depicted. In figure 3f cross-sections through the phase distribution near and through the microsphere are shown that are marked with dashed lines in figure 3e. The maximum phase contrast caused by the micro particle to the surrounding cytoplasm is lower than the value that is obtained in cell culture medium (fig. 3c). This indicates that the refractive index of the cytoplasm is higher than the refractive index of the cell culture medium. Using a fitting procedure based on the least square method [9,10] that takes into account the spherical shape of the microparticle with n(SiO2) = 1.435±0.006 (previously obtained for λ = 532 nm with the same method from the isolated particles) the intracellular refractive index is quantified to n(cell) = 1.38 ± 0.01.

Figure 4 shows a representative result from an experiment with an adherent PaTu 8988 T cell in which the osmolality of the cell culture medium was stepwise decreased by dilution with deionized water. A linear dependency of the refractive index from the osmolality is obtained. The experimental data are found in agreement with previously published values that were obtained by other quantitative phase imaging-based procedures to obtain the intracellular refractive index (see for example [11]).

**Summary and Prospects**

The results demonstrate that internalized microspheres can be used as reference in quantitative phase images for the determination of the refractive index of the
cytoplasm. The experimental data in figure 4 are found in good agreement with previously published data for the cellular refractive index. As no modification of the experimental setup is required, the measurement principle prospects to be used with several existing quantitative phase contrast imaging techniques. As many cells show a phagocytic behavior the method may be applicable with a variety of different cell types. In conclusion the method prospects to be a helpful tool in quantitative phase microscopy-based live cell imaging of adherent cells to enhance the precision for morphology measurements and for the label-free quantification of intracellular solute concentration changes.

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References
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