Photothermal quantitative phase imaging of living cells with nanoparticles utilizing a cost-efficient setup

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ABSTRACT

We explored photothermal quantitative phase imaging (PTQPI) of living cells with functionalized nanoparticles (NPs) utilizing a cost-efficient setup based on a cell culture microscope. The excitation light was modulated by a mechanical chopper wheel with low frequencies. Quantitative phase imaging (QPI) was performed with Michelson interferometer-based off-axis digital holographic microscopy and a standard industrial camera. We present results from PTQPI observations on breast cancer cells that were incubated with functionalized gold NPs binding to the epidermal growth factor receptor. Moreover, QPI was used to quantify the impact of the NPs and the low frequency light excitation on cell morphology and viability.

Keywords: Photothermal imaging, live cell imaging, nanoparticles, quantitative phase imaging, digital holographic microscopy, tumor cell biology, molecular specificity

1. INTRODUCTION

During the past decade, various quantitative phase imaging (QPI) techniques were developed and continuously further improved for high resolution label-free quantitative live cell imaging [1-17]. In photothermal quantitative phase imaging (PTQPI) cells are incubated with functionalized nanoparticles (NPs) that bind to specific target structures to achieve molecular specificity [18, 19]. Excitation of the NPs with laser light induces a photothermal signal. The resulting optical path length changes can be detected by quantitative phase imaging. Compared to other techniques, e.g., fluorescence imaging, the method allows simultaneous acquisition of global cell parameters, like thickness, refractive index or dry mass, and molecule specific information about the target structures with the same experimental setup during a single measurement. Typically, PTQPI is performed with high excitation frequencies. This requires specific equipment like fast image recording devices that can be expensive. QPI can be integrated modular into common research microscopes [20] for multimodal imaging [21, 22, 23]. Thus, we explored PTQPI with a cost-efficient setup on the basis of a modified cell culture microscope that is suitable for usage in a biomedical environment. The excitation light was modulated by a mechanical chopper wheel with low frequencies, while quantitative phase imaging was performed with Michelson interferometer-based digital holographic microscopy. For off-axis digital holographic microscopy acquisition a standard industrial camera was used. We present results from PTQPI observations on breast cancer cells, which were incubated with functionalized gold nanoparticles that bind to the epidermal growth factor receptor. Moreover, quantitative phase imaging was used to quantify the impact of NPs and the low frequency light excitation on cell morphology and viability.

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Quantitative Phase Imaging III, edited by Gabriel Popescu, YongKeun Park, Proc. of SPIE Vol. 10074, 100740Q · © 2017 SPIE · CCC code: 1605-7422/17/$18 · doi: 10.1117/12.2252873

2. METHODS AND MATERIALS

2.1 Setup multimodal quantitative phase imaging

For PT imaging of living cells an inverted microscope (Motic E31, China) with an attached self-interference DHM module (Fig. 1) based on a principle described previously [24] was utilized. The coherent light source for the recording of digital holograms was a diode laser (Quantum Analysis, Muenster, Germany, λ=635nm). For the experiments described in section 3.3 an electrically focus tunable lens was applied for enhanced quantitative phase imaging [25]. An incubator (HT 200, ibidi GmbH, Munich, Germany) allowed temperature stabilization at 37°C. The light source for photothermal imaging, a frequency-doubled neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (Compass 315M-100, Coherent, Lübeck, Germany, λ=532nm, P_{max} = 100 mW) was coupled into the microscope's illumination path by a dichroitic mirror. The light intensity was temporally modulated by a mechanical chopper wheel (SciTech Model 300CD).

For the experiments, MDA-MB-468 breast cancer cells were cultured subconfluently in Petri dishes (ibidi µ-dish, ibidi GmbH, Munich, Germany) and incubated with functionalized gold nanoparticles (diameter: 55 nm) that bind to the epidermal growth factor receptor (EGFR) as reported previously [18,19]. For PT imaging and observation with quantitative DHM phase contrast the initial cell culture medium was replaced by 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffered cell culture medium and the conventional cap of the Petri dish was replaced by a glass lid (ibidi GmbH, Munich, Germany). Digital off-axis holograms of the cells were recorded continuously with a charge-coupled device camera (DMK 41BF02, The Imaging Source, Bremen, Germany) with a maximum image acquisition rate of 15 Hz. The numerical reconstruction of the digitally captured holograms was performed as reported previously by spatial phase shifting reconstruction in combination with holographic autofocusing as reported with details previously [2, 7, 25, 26].

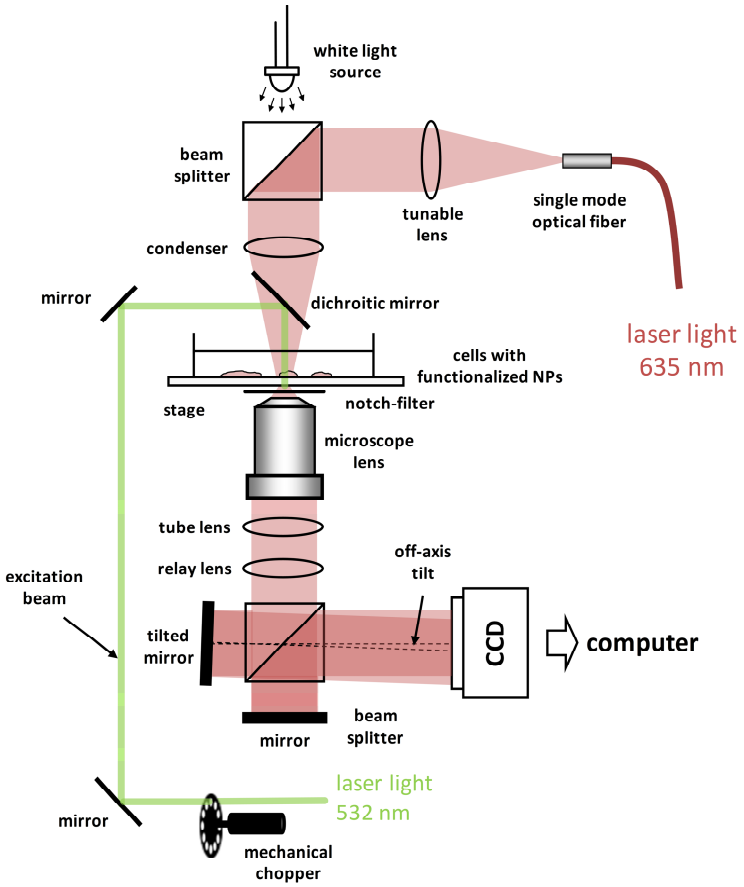

Figure 1. Integrated self-interference DHM setup for multi-modal photothermal quantitative phase imaging.

2.2 Evaluation of quantitative phase contrast images

The retrieved series of quantitative phase images during photothermal excitation was evaluated as described in [18, 19]. In order to quantify cellular growth and the response of the cells to incubation with functionalized nanoparticles, the cell occupied area and the cellular dry mass were obtained from quantitative phase images by image segmentation as described in [21].

3. RESULTS

3.1 Characterization of the influence of functionalized nanoparticles on cell morphology and growth

First, we analyzed the influence of the functionalized gold NPs on MDA-MB-468 cells in time-lapse experiments in in which the growth of nanoparticle incubated adherent cells was observed vs. untreated control cells for at least 16 h (Δt = 3 min) with a separate Mach-Zehnder interferometer-based DHM setup described in [27] (10x magnification, NA=0.3). From the obtained series of quantitative phase images, the cell occupied area and the relative dry mass change was retrieved. Figure 2 shows representative quantitative phase images at t = 0, t = 8 h and t = 16 h as well as the corresponding temporal developments of the cell occupied area and dry mass. The results in Fig. 2 indicate that the NPs do not significantly influence cell morphology and cell division but cause a slight decrease in cellular growth.

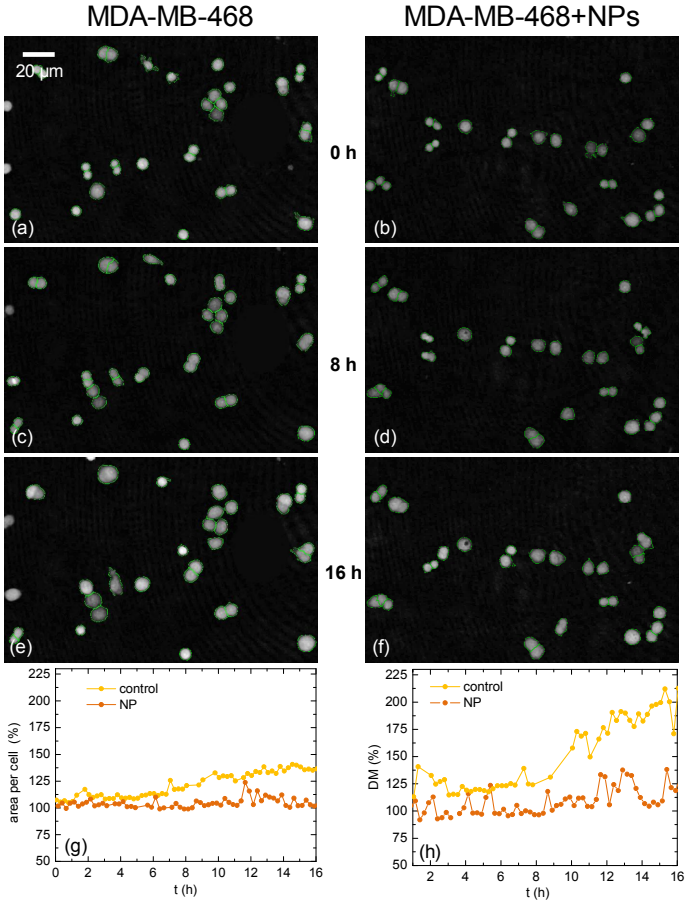

Figure 2. Cell growth of MDA MB-468 breast cancer cells after incubation with functionalized gold nanoparticles. (a), (c), (e): representative segmented quantitative phase images of control cells at indicated times; (b), (f): representative quantitative phase images of cells incubated with functionalized gold nanoparticles. (g): average cell occupied area for control cells and NP incubated cells; (h): relative temporal development of the cellular dry mass (DM).

Moreover, we observed the impact of the functionalized gold NPs on single cell volume, refractive index and dry mass. Therefore, control cells and nanoparticle incubated cells were detached and holograms of at least 120 cells with spherical

appearance where recorded for each fraction with the setup in Fig. 1. From the reconstructed quantitative phase images (Fig. 3a) cell volume V (Fig. 3e), refractive index n_{cell} (Fig. 3f), and dry mass (Fig. 3g) were calculated by fitting of the sphere function (Figures 2b-2d) as described in [28]. For all parameters, no statistically significant differences between the control cells and the cells incubated with functionalized nanoparticles are found. This indicates that the treatment of the cells with functionalized nanoparticles does not significantly influence the cell morphology.

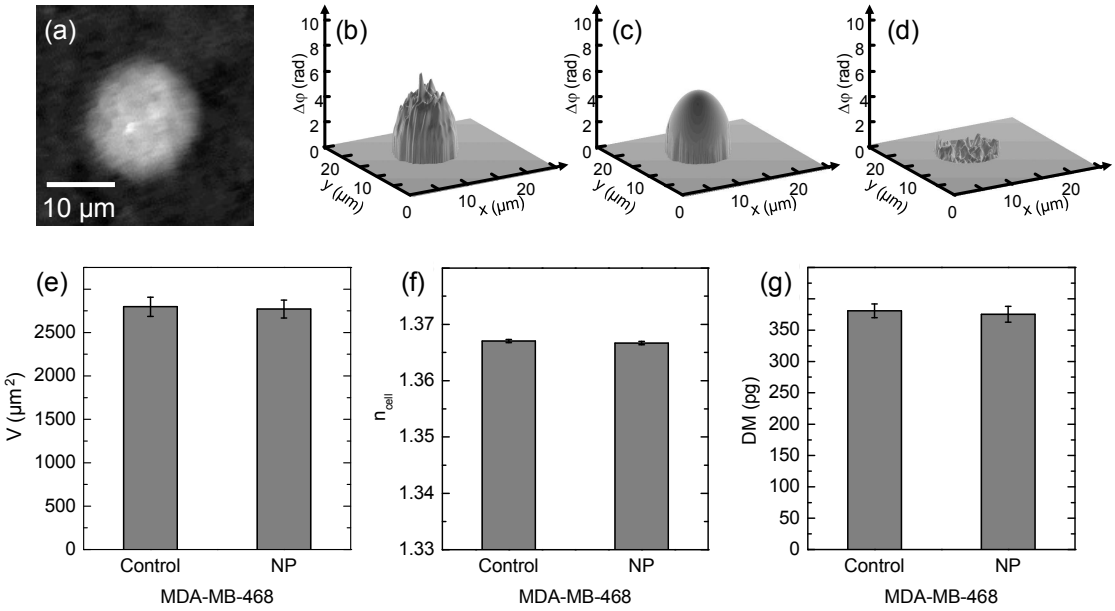

Figure 3. Quantification of the cell response of MDA-MB-468 breast cancer cells to incubation with functionalized gold nanoparticles. (a): quantitative phase image of a suspended MDA-MB-468 cell. (b): pseudo 3D plot of the phase data in (a); (c): fit of the sphere function to the phase data in (b); difference of the data in (b) and (c). (e), (f), (g): volume V, cellular refractive index n_{cell}, and cellular dry mass DM of control cells (Control) and nanoparticle incubated cells (NP) Data are mean±standard error.

3.2 Photothermal imaging

Fig. 4 presents representative images from photo thermal imaging of MDA-MB-468 cells that are labeled with functionalized gold nanoparticles that where retrieved with the experimental setup in Figure 1. Therefore, quantitative phase images were acquired with 15 Hz at a PT excitation frequency of 5 Hz. Fig. 4a shows a representative photothermal signal while Fig. 4b depicts the corresponding quantitative phase image of the observed area. The results in Fig. 4 demonstrates photo thermal imaging at low excitation frequencies that can be resolved by standard industrial cameras with low maximum image acquisition rates of 15 Hz.

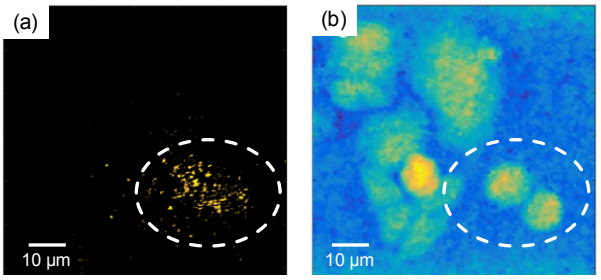

Figure 4. Photothermal imaging of MDA-MB-468 cells labeled with functionalized gold nanoparticles which were excited at 532 nm with a frequency of 5 Hz. (a): Photothermal signal; (b): corresponding quantitative phase image of the observed area. The area marked with a dotted line indicates cells that are labeled with functionalized gold particles.

3.3 Impact of PTI on cell viability

Finally, we observed the cell morphology during photothermal imaging with low excitation frequencies. Therefore, time-lapse series of digital holograms from adherent MDA-MB-468 cells were recorded during photothermal excitation with a frequency of 5 Hz. Holograms were recorded with the setup in Fig. 1 every 5 minutes with enhanced quantitative DHM phase imaging utilizing the electrically focus tunable lens for object illumination. The resulting series of quantitative phase images were evaluated for the temporal dependency of the average cell occupied area and the average single cell dry mass. Fig. 5 presents representative results. Figures 5a, 5b and 5c show representative segmented quantitative phase images at indicated time while in Figures 5d, 5e and 5f corresponding pseudo 3D representations are depicted. In Figures 5g and 5h the temporal development of the average cell occupied area and the corresponding average cellular dry mass per single cell are plotted. Up to one hour cells showed no significant changes of cell morphology. Then, the cell occupied area increased while simultaneously a dry mass decrease was observed which indicates a burst of the cell membrane. However, in summary, the results in Fig. 5 demonstrate that with the proposed cost efficient setup photothermal imaging can be performed continuously up to one hour without significant cell morphology changes.

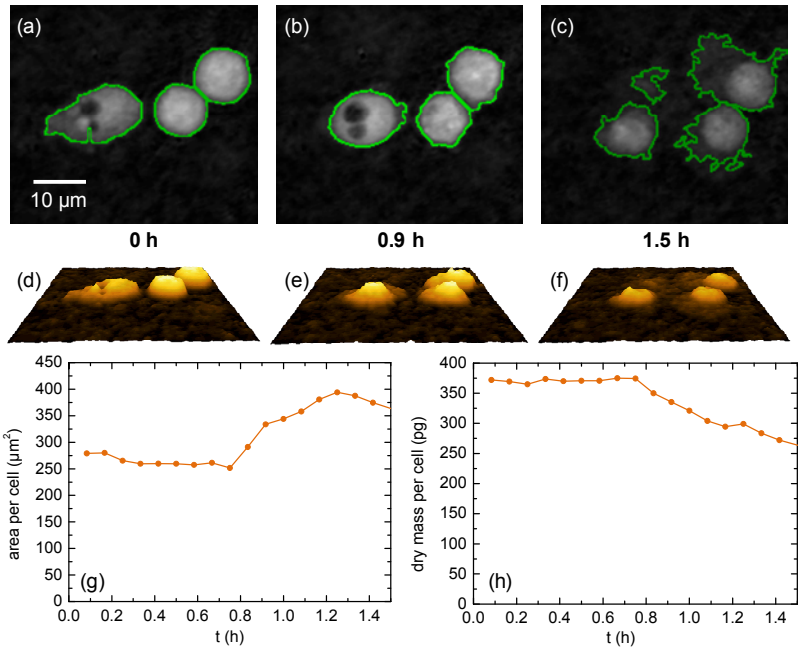

Figure 5. Quantification of cell morphology of MDA-MB-468 breast cancer cells labelled with functionalized gold nanoparticles during photothermal excitation with 5 Hz. (a), (b), (b): representative segmented quantitative phase images at indicated time; (d), (e), (f): pseudo 3D representation of the data in (a), (b) and (c). (g): temporal development of the average cell occupied area corresponding to the segmented areas in (a), (b), (c). (h): temporal development of the average cellular dry mass per single cells corresponding to the segmented areas in (a), (b) and (c).

4. CONCLUSIONS

We have presented a cost-efficient setup for multi-modal quantitative phase microscopy including photothermal imaging of cells which are labeled with functionalized gold nanoparticles that bind to the epidermal growth factor receptor. Moreover the setup is capable for time-lapse observation for analysis of cellular growth as well as biophysical cell parameters like volume, refractive index and dry mass. Our results show that labeling of breast cancer cells with functionalized gold nanoparticles does not cause significant cell morphology changes. Moreover, we demonstrate that breast cancer cells can be observed by photothermal quantitative phase imaging for up to one hour at low excitation frequencies in the range of 5 Hz. Although further systematic investigations are required to validate that nanoparticles have not significant influence on physiological processes of the cells, our results demonstrate that PT imaging can be integrated cost-efficiently into multi-modal quantitative phase imaging systems for introduction of molecular specificity, e.g., for cell identification, without additional equipment for fluorescence imaging.

ACKNOWLEDGEMENTS

This work was supported by The German-Israeli Foundation for Scientific Research and Development (GIF), Grant number I-1260-401.10/2014. The authors further thank Annette van Dülmen for excellent technical assistance the non-linear photonics group of the department of applied physics of the University of Muenster with the chopper wheel system.

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