Optical tweezers induced photodamage in living cells quantified with digital holographic phase microscopy

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ABSTRACT

Optical tweezers are a versatile technique to manipulate living biological specimen in a contact-less way. The interaction with living cells can be performed, for example, through direct manipulation of cell organelles or by movement of an internalized particle within the cytoplasm. However, the risk of damage that the trapping beam may induce in the biological sample due to the energy deposition has to be considered. This optically induced damage or photodamage depends mainly on the wavelength of the trapping beam, the exposure time and the biological specimen that is investigated. In this work, we explore a method to analyse the photo damage in living cells in a multimodal biophotonic workstation that is based on combining a holographic optical tweezers (HOT) microscope with a self-interference digital holographic microscopy (DHM) module. A time-dependent investigation shows that no observable changes in the cell morphology are induced at room conditions with the used laser power of the trapping beam during periods of time < 20 min of laser application. In addition, results from investigations of the photodamage increasing the working temperature to 37°C demonstrate that the optical tweezers beam can provoke severe but reversible morphology changes in the cell.

Keywords: quantitative phase imaging, holographic optical tweezers, digital holographic microscopy, photodamage

1. INTRODUCTION

Laser light-based techniques are unique tools for the manipulation of biological specimen in a contact-less way. A well-established technique are optical tweezers which enable to trap and control objects with higher refractive index than its surrounding medium in the focus point of a tightly focused beam [1]. The forces in the range of piconewtons that are achievable with this technique [2] and sophisticated approaches as holographic optical tweezers (HOT) [3] enable the manipulation of single and multiple living cells [4, 5], the study of molecular motors [6] or the manipulation of intracellular organelles or internalized particles [7-9]. However, the damage induced in the sample by interaction of the trapping beam light with the biological specimen, the so-called photodamage, must be considered [10-12]. A main factor to consider is the damage due to light absorption of the specimen. Thus, it is recommended to work at wavelengths in the near infrared where the absorption of light by water and biological material is minimal [2, 13]. Other factors, like the power of the laser at the specimen plane and the duration of the manipulation are also important to secure the viability of the cell [6, 12 and 14]. Nevertheless, the mechanisms of the cell response to the photo damage are still not fully understood. Thus, new techniques to analyse the photodamage, sometimes termed "opticution" [2], induced by the optical tweezers is of particular interest. We report the use of a multimodal biophotonic workstation to study the photodamage caused by an optical tweezers beam. The workstation combines HOT based on light fields tailored by a phase-only spatial light modulator [15], and self-interference off-axis digital holographic microscopy (DHM) [16]. DHM provides label-free quantitative phase imaging of the cellular samples with optional subsequent autofocusing [17]. We show that our biophotonic workstation enables the quantitative evaluation of the HOT-induced changes in the morphology of living pancreatic tumor cells as a model system. In a first experiment, we present a time-dependent investigation of the photodamage while trapping internalized polystyrene microspheres in the cells. The particles serve to localize the position of the trapping beam focus. Then, in a second experiment, the photodamage in the cells at a working temperature of 37°C is investigated.

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2. MULTIMODAL BIOPHOTONIC WORKSTATION

Figure 1 depicts a schematic of the multimodal biophotonic workstation that is used to perform the experiments. In order to combine HOT and DHM, a self-interference DHM module is mounted to one of the camera side ports of the inverted microscope (Eclipse Ti, Nikon) used for observation and optical trapping. A high numerical aperture microscope objective (Nikon Apo TIRF, 100x/1.49 oil-immersion) is used for both imaging and optical manipulation. The principles of the HOT and DHM systems are described in detail in [3, 15] and [16], respectively. In order to control and increase the temperature of the sample a heating chamber (ibidi HT 200, ibidi GmbH, Munich, Germany) was used.

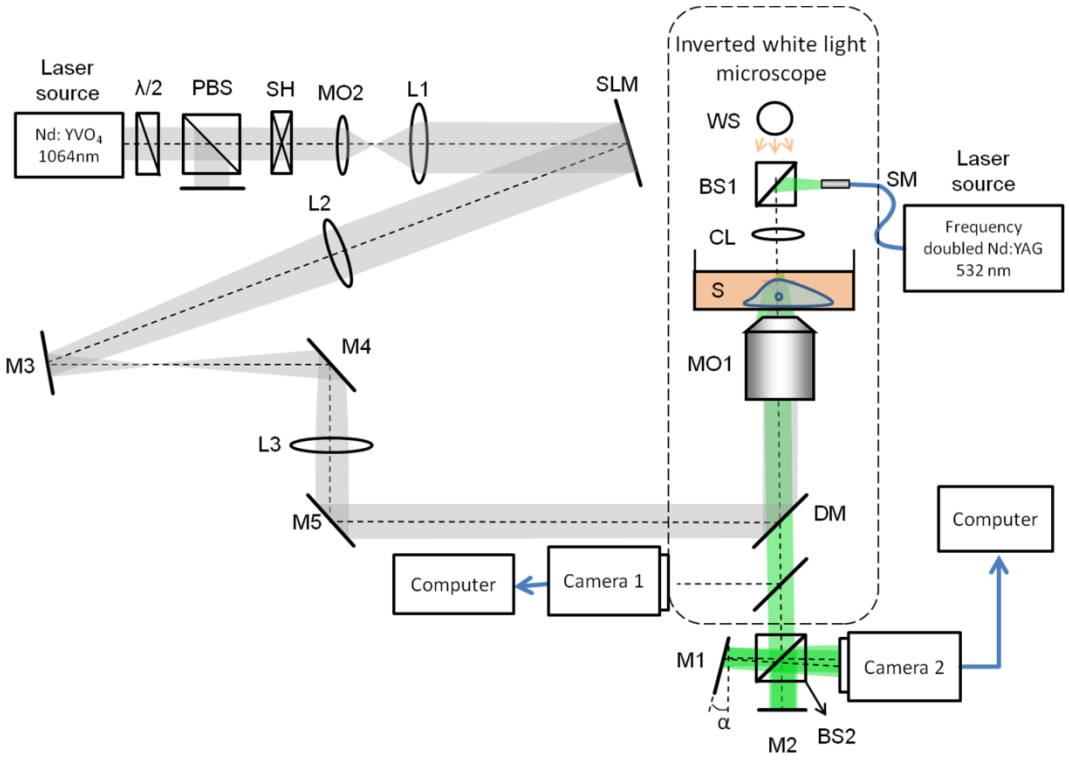

Figure 1. Schematic of the combined workstation. HOT elements: λ: rotatable half wave plate, PBS: polarizing beam splitter, SH: shutter, MO microscope objective, L: lens, SLM: spatial light modulator, M: mirror, DM: dichroic mirror, WS: white light source; DHM elements: SM: single mode optical fiber, BS: non-polarizing beam splitter, CL: condenser lens.

The light source used for the optical tweezers is a Nd:YVO4 laser operating at λ = 1064 nm (max. output power 2.5W) in order to have a good compromise between high power available and best wavelength to minimize the photodamage due to light absorption. The light of the laser is expanded via a telescope in order to illuminate adequately the surface of a spatial light modulator (SLM) (Pluto phase-only, 1920 x 1080 pixels, Holoeye Berlin, Germany). The position of the optical trap in the specimen plane is controlled by means of the appropriate digital hologram displayed in the SLM. To investigate the optical induced damage due to the laser trapping beam the laser power was set to a relatively high level of 440 mW at the entrance of the inverted microscope. The transmission of the microscope objective according to the manufacturer is approximately 60% at 1064 nm. Camera 1 (Pixely qe, PCO, Kelheim, Germany) is used together with the SLM and custom-made software [18] to configure and control in three dimensions the focus of the trapping beam.

The biological sample is observed by using white light illumination of the inverted microscope for conventional bright field imaging while the coherent light of a frequency-doubled Nd:YAG laser (λ = 532 nm; max. output power 100 mW) is used for recording of the digital holograms. A non-polarizing 50:50 beam splitter and a microscope condenser lens are

adjusted to obtain suitable Koehler-like illumination of the sample with the laser. The microscope objective MO1 is used for magnification of the sample (S). Then, the object wave enters into a Michelson-interferometer formed by two mirrors (M1 and M2) and a second beam splitter (BS2). In order to produce a suitable spatial carrier fringe pattern for off-axis holography, one of the mirrors (M1) is tilted by a small angle α in such a way that an area of the sample that contains no object is superposed with the image of the specimen. Thus, both wave fronts interfere with this angle on the sensor of a charge coupled device sensor (Camera 2) (CCD, The Imaging Source DMK 41BU02, Bremen, Germany) where the hologram is created and recorded. The digital recorded holograms are transferred to a computer for posterior reconstruction.

The numerical reconstruction of the digitally recorded holograms is performed by a spatial phase shifting method as described in detail in [19, 20]. In case the sample is out-of-focus numerical auto-focusing was used as reported previously in [21].

3. SAMPLE PREPARATION

Experiments were performed using as a model system adherent pancreatic tumor cells (PaTu 8988T) with internalized polystyrene (PS) spheres (diameter ≈ 1 μm, n = 1.59). The cells were cultured at 37°C in 10 % CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) with 3.7 g/l $NaHCO_3$, 580 mg/l L-glutamine, 110 mg/l Na-pyruvate, 4.5 g/l D-glucose (DMEM FG0455 Biochrom, Berlin, Germany) and supplemented with 5% fetal calf serum (FCS of PAA, Pasching, Austria) and 5% horse serum (HS of Biochrom, Berlin, Germany).

For the internalization of the PS spheres, the adherent cells were cultivated with the PS spheres in Petri dishes with a physically treated bottom (μ-Dish ibiTreat, ibidi GmbH, Munich, Germany). After one night of incubation the beads were incorporated by the cells by means of phagocytosis. Prior to the experiment, the cell culture medium was replaced with DMEM buffered with Hepes to prevent intracellular acidosis at room conditions. In addition, the cells were washed twice to remove the non-incorporated beads since they may be undesirably trapped by the optical tweezers and would disturb the phase contrast measurements.

4. INVESTIGATION OF PHOTODAMAGE IN TUMOR CELLS

In order to investigate the photodamage induced during the typical exposure time for manipulation of cells with optical tweezers, an internalized polystyrene microsphere in a cell was trapped with the optical tweezers for 20 minutes while a sequence of digital holograms was recorded. For this purpose, only a single optical trap was generated, having nearly the full power of 440 mW resulting in an energy deposition of roughly 300 J at the specimen plane. By trapping a particle, the position of the optical tweezers beam could be easily localized. The experiment was performed at a room temperature (24°C) and repeated for 40 cells.

Figure 2.a illustrates the bright field image of an investigated cell with various internalized polystyrene microspheres. The reconstructed quantitative DHM phase images were evaluated to study the induced optical damage on the cell caused by the optical tweezers beam. Figures 2.b-2.c show the DHM phase images during the exposition of the trapping laser at the initial time and after t = 20 min, respectively. In Figure 2.e the corresponding cross-sections along the dashed lines that are drawn in the phase map are plotted. Figure 2.e shows that after 20 minutes of exposure neither significant change in the phase distribution $\Delta\varphi$ around the place where the optical tweezers are applied nor in the rest of the cell is detected.

Then, in a proof-of-principle experiment, the optically induced damage by the trapping beam at a larger exposure time (1 hour, corresponding to an energy deposition of approx. 900 J at the specimen plane) was investigated for only this single cell. Figures 2.d and 2.f show the DHM phase image after t = 1 h of the laser exposition and the corresponding cross-sections along the dashed lines in the phase map. In the phase image, no obvious changes are observed at different times of exposure. In contrast, in Figure 2.f, the plots of the cross-sections through the phase images show an increase of the

phase change Δφ of 1 rad from the initial time to after one hour of laser exposure. This indicates cell rounding which indicates that the cell viability decreases.

In conclusion, no significant cell morphology changes were observed in the quantitative DHM phase images during interval of time $t < 20$ minutes. Nevertheless, the result of the proof-of-principle experiment demonstrates that it is advantageous to minimize the time of exposure to the laser light.

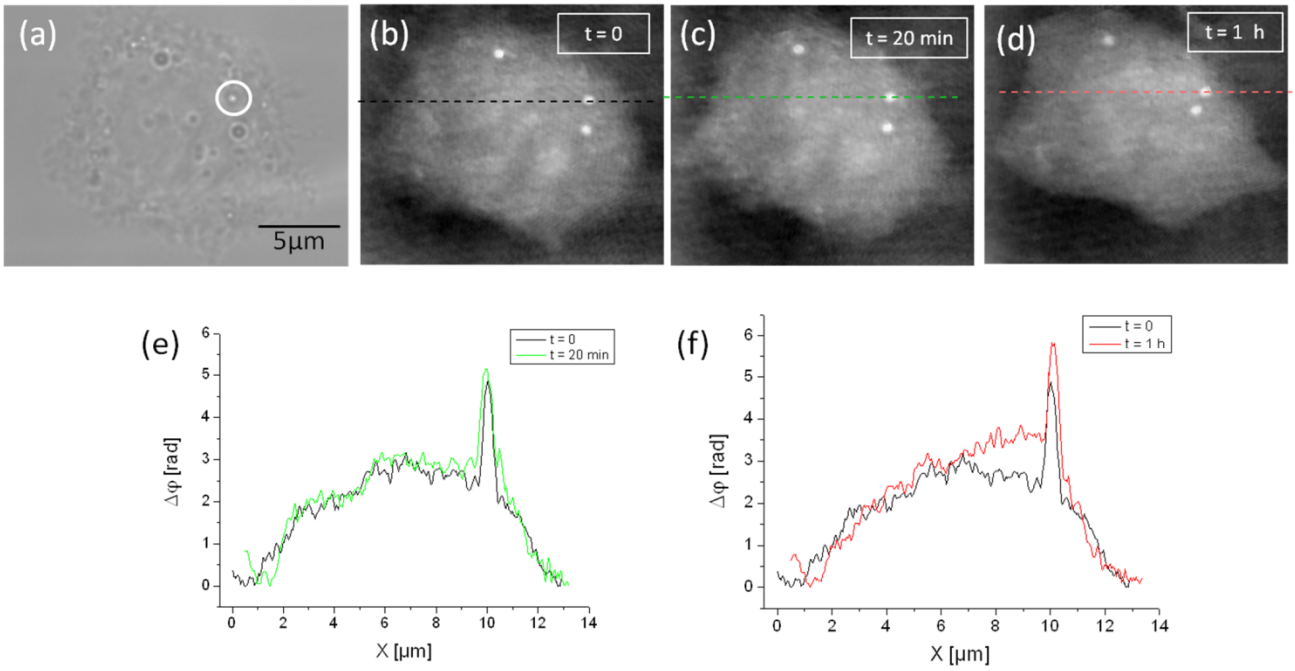

Figure 2. Analysis of the photo damage caused by optical tweezers at room conditions. (a) Bright field image of the investigated adherent PaTu 8988T cell with incorporated 1 µm polystyrene spheres. The white circle marks the position of the optical tweezers and the trapped particle. (b)-(d) Corresponding quantitative DHM phase images at $t = 0$, $t = 20$ minutes and $t = 1$ hour of exposure time, respectively. (e)-(f) Comparison of the cross-sections through the cell along the dashed lines in the DHM phase images between the initial time (black) and after $t = 20$ min (green) and $t = 1$ hour (red), respectively.

In a second experiment, the photodamage induced in cells at 37°C was investigated. In this case, also cells with non incorporated particles were studied with the optical tweezers while digital holograms were recorded. We observed drastic changes in the cell morphology only in two cells among 30 investigated cells. Figures 3.a-3.c show a white light image sequence of the morphology change process in one of the adherent PaTu 8988T cells. The optical tweezers beam (indicated by a white circle in Figure 3.a) produced changes in the cell appearance after few seconds of exposure (Figure 3.b). After blocking the laser beam of the optical tweezers, the cell recovered its initial appearance (Figure 3.c).

Figures 3.d-3.f show DHM phase images that are obtained from the recorded digital holograms before using the optical tweezers ($t = 0$ s), during the exposure of the optical tweezers beam ($t = 10$ s) and after turning off the laser ($t = 40$ s). Note that these images were taken during manipulation of the cell in different area of the cell and thereby do not correspond with the white light images of Figures 3.a-3.c. Figures 3.g-3.i show corresponding pseudo 3D phase images of the quantitative phase images in Figures 3.d-3.f. The horizontal and vertical cross sections through the dashed line in the 2D phase images are plotted in Figures 3.j-3.k.

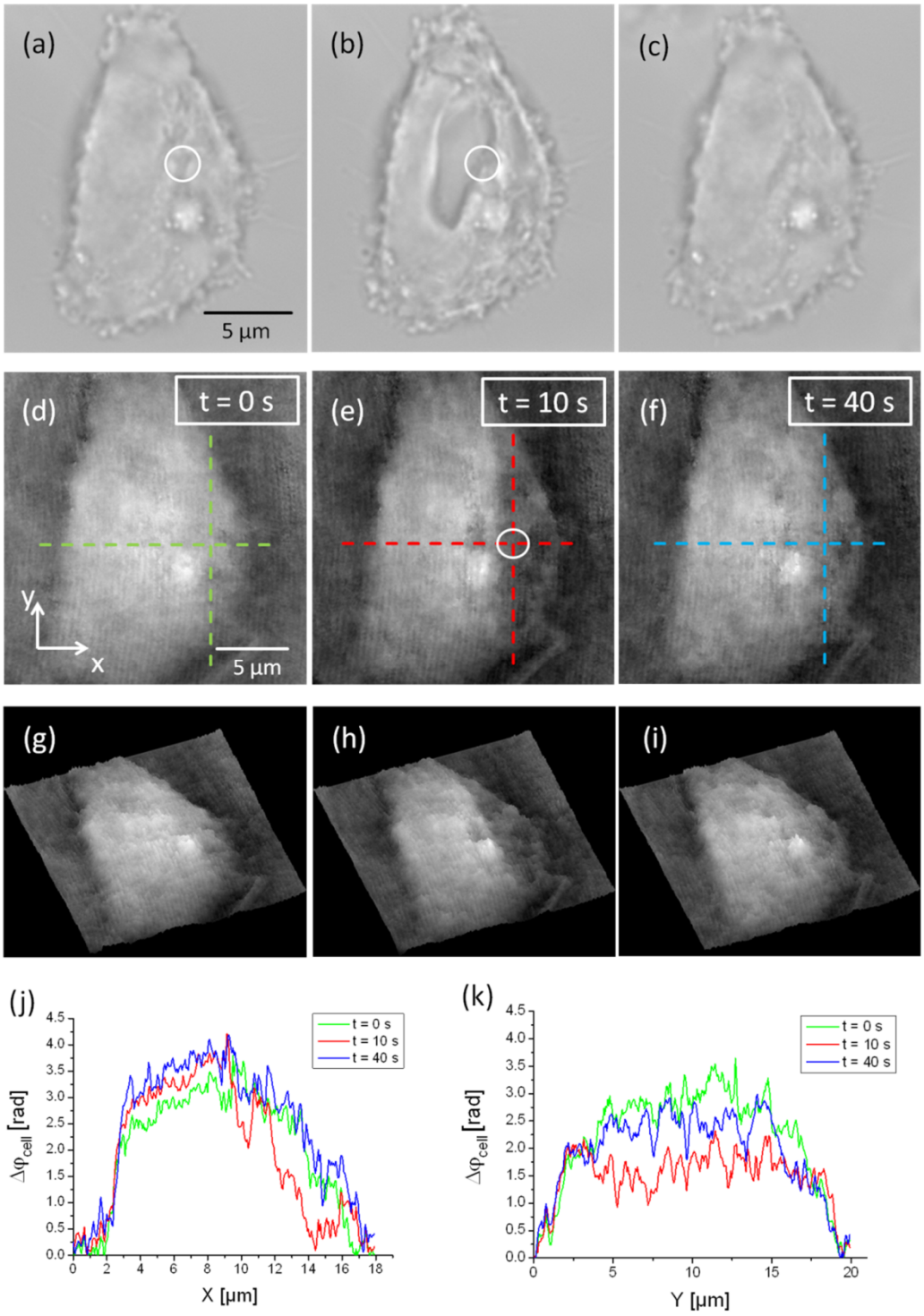

Figure 3. Analysis of the photodamage by optical tweezers at 37°C. (a)-(c) White light image sequence during optical manipulation of the cellular morphology in adherent PaTu 8988T cells at 37°C ((a): before, (b) during, (c) after blocking the optical tweezers beam). The white circle marks the position of the optical tweezers. (d)-(f) 2D and (g)-(i) pseudo 3D DHM phase image sequence during application of the optical tweezers in another region of the cell. (j)-(k) Cross-sections in *x*-direction and *y*-direction through the color dashed lines of the DHM phase images.

The morphology changes of the cells become also visible in the quantitative DHM phase images (Figures 3.d-3.f). The pseudo three-dimensional representation of the phase image sequence (Figures 3.g-3.i) gives a better perspective to analyse the morphology changes. As it is observed, the optical density on the right side of the cell is reduced. The decreasing of the phase contrast $\Delta\varphi_{cell}$ is clearly visible in the plots of the phase change and thickness profile of the cell, in Figures 3.j-3.k, in which the difference between the green line (before turning on the trapping laser beam) and the red line (during deformation due to the optical tweezers) shows the deformation of the cell. Then, when the laser of the optical tweezers was blocked, the cell recovered in a few seconds almost completely its shape. Thus, the changes of the cell morphology induced by the optical tweezers are quite drastic but the observed effect seems to be also reversible, since the cell reorganized its structure and density rapidly to the initial form.

This behaviour of the cell may be explained by the optoporation of cells by which the cell membrane becomes fluid and thereby permeable to the surrounding medium [22]. However, it also could be that a localized highly absorbing area of the cell caused the release of a gas bubble. In any case, after the treatment of the laser, the cell membrane recovers and become again semipermeable to the medium.

5. CONCLUSIONS

In this work, we reported a novel method to study the optically induced damage in living cells using a combination of holographic optical tweezers and quantitative phase digital holographic microscopy. Results from a time-dependent investigation demonstrate the feasibility of the method to analyse dynamic changes in the cell morphology that are provoked by the optical tweezers. Moreover, investigations of cell manipulation at 37°C showed that drastic changes in the cell may occur. Thus, as these are the typical working conditions to investigate living cells, increased damage due to the laser should be considered in experiments that are performed at these conditions. We conclude that the proposed method prospects to be a helpful tool to detect and evaluate the photodamage induced by an optical tweezers system used for biological specimen manipulation.

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