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ABSTRACT

In vivo optical trapping is a novel prospective area of application for cell optical manipulation, which enables one to measure mechanical properties of cells and tissues in living organisms noninvasively. However, actual application of the approach is limited because of strong light scattering in most of biological tissues. Optical clearing enables to decrease the scattering and therefore increases depth of light penetration, reduces distortion of light beam, and improves image contrast and resolution. A novel trend in the study of optical clearing mechanisms and impact is its cellular level. In this paper, we present a novel method of evaluation of optical clearing agent concentration that is based on the measurement of optical trap stiffness. We studied optical trap stiffness on concentration of optical clearing agent (glucose) for 1.5 μm polystyrene beads and red blood cells (RBCs).

1. INTRODUCTION

In vivo optical trapping of a RBC demonstrated a possibility of noninvasive manipulations of cells and force measurements of cellular interactions in living animals.[1,2] Optical trapping was applied for manipulation of injected nanoparticles and bacteria; and for analysis of adhesion properties and membrane deformation of endothelium and macrophages in living animals.[3] Basics of optical trapping were developed by Arthur Ashkin[4,5] and this technique has been called optical tweezers.[6] Optical tweezers are widely used in microbiology: for example, study of molecular motors at the single-molecule level and mechanical properties of biopolymers,[7,8] study of RBC aggregation,[9] etc. Basic limitations of in vivo optical trapping are caused by strong scattering of many biological tissues[10,11] and the use of a microscope objective (MO) with a high numerical aperture (NA). Therefore, optical trapping is possible only within superficial blood capillaries since a MO with a high NA has a relatively short working distance. Optical trapping with a small NA can be still possible when a cell is pressed against a vessel wall, however this trapping is not informative for estimation of cell mechanical properties.

The strong scattering of biological tissues can be significantly reduced by application of optical clearing agents (OCAs).[12] This is a so called immersion optical clearing when tissue or cell impregnation by an agent is used for matching of refractive indices of tissue fibers and cell organelles against interstitial fluid and cytoplasm. Some other mechanisms, such as reversible dehydration and destruction of hydrated shell of collagenous molecules, are also important. The major measurement techniques of degree of optical clearing are used at macroscopic level:[13] integrating sphere technique; collimated transmittance, backscattering spectroscopy, fluorescence spectroscopy, optical coherence tomography (OCT); confocal microscopy; nonlinear microscopy, etc.[12,13] Recently, optical clearing had been studied at cellular level by direct measurement of the scattering phase function of a single cell.[14] Contrast-enhanced imaging of cellular structures using digital holographic microscopy[15] and hyperspectral dark-field microscopy at nanoparticle labeling[16] was also demonstrated by applying of optical clearing technology. In this paper, we are presenting a further

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development of a single-cell concept in optical clearing, in particular, we are studying optical trap stiffness for 1.5 μm polystyrene beads and red blood cells in dependence of OCA concentration. Potentially, these studies could be applicable for in vivo measurements of concentration of different agents, such as metabolic substances, drugs, contrast agents, etc.

There are two basic categories of stiffness measurement of single beam traps:[17] 1) by analyzing thermal fluctuations of a trapped particle and 2) by application of an external force (for example, viscous drag force). The first category is not applicable for particles in flows, especially in blood flow. The second category is divided into two basic subgroups: 1) a particle moves relative to the optical trap (OT) and 2) the OT with a trapped particle moves relative to an environment. In this paper, we are using a technique from the second basic subgroup for estimation of OT stiffness dependence on OCA concentration.

2. MATERIALS AND METHODS

2.1. Experimental setup

A scheme of experimental setup is presented in Fig. 1. A part of this instrument is presented in a simplified linear scheme in Fig. 2. A light source is a CW diode laser (650 nm, 65 mW). The OT is provided by a laser beam focused by a microscope objective (MO; 70x, NA 1.23, water immersion, achroplan, a focal length is 2.52 mm, LOMO, Russia).

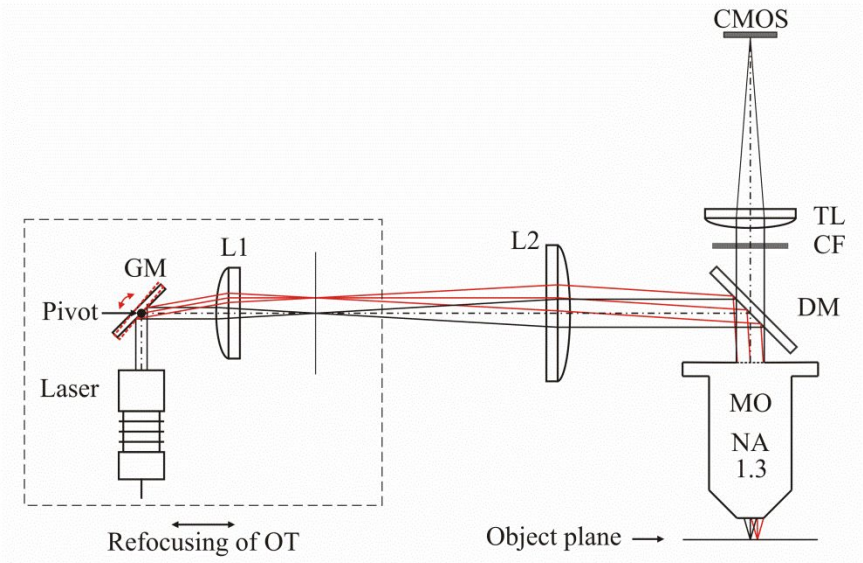


Figure 1. The scheme of our experimental setup. GM – galvanometer scanning mirror; L1, L2 – lenses; DM – dichroic mirror; MO – microscope objective; CF – coloured filter; TL – tube lens.

The galvanometer scanning mirror (GM; 6210H, Cambridge Technology), lenses (L1, L2) are used simultaneously as a beam expander and a position controller of the OT. The beam expander is used to fill by light exit aperture (A_{MO}) of the microscope objective (Fig. 2). A magnification of the beam expander is determined by a ratio of focal lengths of lenses L1 and L2. The position controller of the OT is a quasi 4F-optical system. An afocal system (lenses L1, L2) images pivot of GM into a center of A_{MO} (Fig. 2). Therefore, light beam equally fills exit aperture A_{MO}, thus light intensity and correspondingly stiffness of the OT are constant for various angles of the GM. An angle of the GM is controlled by a driver (The MicroMax 677XX, Cambridge Technology) and a multifunction data acquisition device (PCIe-636, NI). The angle of GM determines lateral position of the OT. Parallel axial displacement of the laser, the GM, the L1 relative to the

L2 causes axial displacement of the OT. A relative ratio of these displacements is determined by ratio of focal length of the L2 and the MO. Images are registered by the tube lens (a focal length is 250 mm; AC254, Thorlabs) and the CMOS sensor (CMOS camera; DCC1545M, Thorlabs).

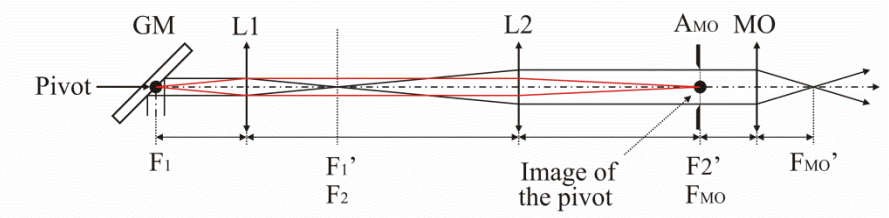


Figure 2. The simplified linear scheme of the part of the experimental setup (Fig.1). L1, L2 – lenses; MO – microscope objective; A_{MO} – aperture of the microscope objective; F_1, F_1', F_2, F_2', F_{MO}, F_{MO}'– focal points and focal length of lens L1, L2 and MO respectively.

2.2 Optical trap stiffness calibration

A particle experiences the viscous drag force when it moves in a viscous medium. The drag force can be derived from Stokes' law[18] for slowly moving small spherical particles (i.e. at low Reynolds number)

$$F_{drag} = 6\pi\eta r V \ , \tag{5}$$

where η is the dynamic viscosity of a fluid, r is the radius of a particle, V is the velocity of a particle. A trapped particle is in the OT until the trap force is greater than the drag force. Then,

$$F_{trap} = F_{drag} = 6\pi\eta r V_{critical}, \tag{6}$$

where $V_{critical}$ is the velocity by which a trapped particle leaves the OT.

2.3 Dynamic position control

In this work, the measurement technique of trap stiffness is based on use of an externally applied force. This force is a viscous drag force produced by a motion of a trapped particle relative to environment. A mobile trap enables one to regulate simpler and precisely velocity of a trapped particle and to move this particle with the accelerated velocity. The accelerated velocity enables one to reduce considerably amount of measurements.

A lateral optical trap position is linearly associated with an angle of the GM. Angle of GM is controlled by the driver and the mDAQ device. Angle of GM is linearly dependent on the signal of the mDAQ device (linearity is 99.9%) which is used as a digital-to-analog converter (DAC) (resolution of 16 bits and time response of 10 ns).

A calibration of a lateral OT position with mDAQ signal is realized by microscope image with the working MO. A lateral OT position is defined as a lateral position of OT image achieved by focusing of the microscope on any plane surface. Rotating of the GM moves OT along x-axis. The calibration is realized by two various mDAQ signals. Then a lateral position of the OT is determined as

$$P_x = A_x \cdot U + B_x \ , \tag{1}$$

where

$$A_x = (P_{x2} - P_{x1})/(U_2 - U_1)\,; \tag{2}$$

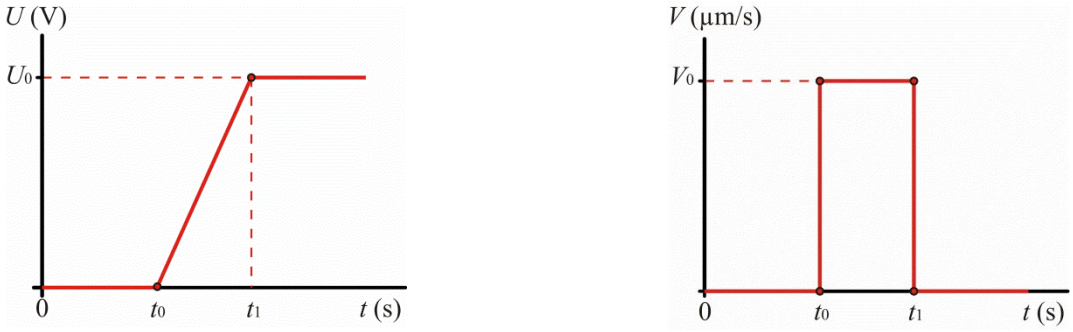
$$B_x = P_{x1} - A_x \cdot U_1\,; \tag{3}$$

U is the voltage of mDAQ; P_1, P_2 are the lateral positions of OT corresponding to various voltage of mDAQ (U_1, U_2), respectively.

Stiffness of the OT is determined by a critical velocity $V_{critical}$ when the trapped particle leaves the OT. Velocity of the OT and a trapping particle is

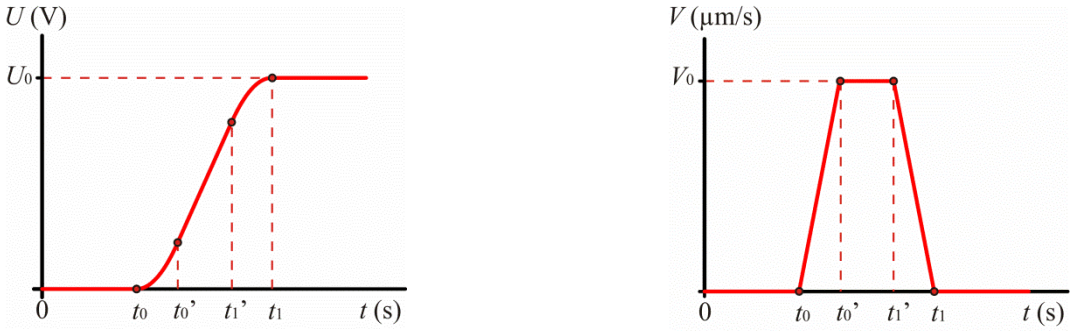
$$V_x = d(P_x)/dt\,. \tag{4}$$

If an OT position is linearly changed (Fig. 3) acceleration of particles is quasi infinite at the moments t_0 and t_1. Thus, the particle may leave at initial or final moments of the movement. Therefore we are proposing to change the OT position as it is shown in Fig. 4, where OT position is parabolically changed in the first (t_0 to t_0') and the third (t_1 to t_1') stages of the movement. In this case, the trapped particle is accelerated linearly.



(a) (b)

Figure 3. The temporal dependences of mDAQ device signal (a) and trap velocity (b) by linear increase of the signal.



(a) (b)

Figure 4. The temporal dependences of mDAQ device signal (a) and trap velocity (b) by linear increase of the velocity.

3. RESULTS

3.1 The optical clearing for 1.5 µm beads

The calibrating graph of OT force is presented in Fig. 5 for 1.5 µm polystyrene beads (n_p = 1.57) in distilled water (n = 1.3305) at 20ºC.

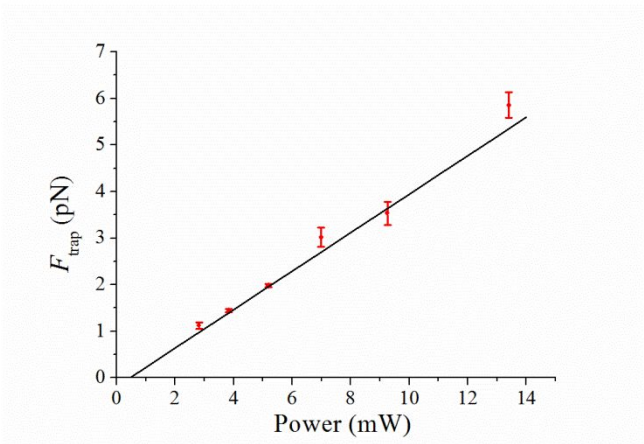


Figure 5. The dependence of OT force on light power incident on MO aperture.

The dependence of $V_{critical}$ on refractive index of the medium (n_m) is presented in Fig. 6 for 1.5 µm polystyrene beads for distilled water (n = 1.3305); physiological solution (saline) (n = 1.3318); isotonic solution with 5% concentration of glucose (n = 1.3382). $V_{critical}$ is used instead of OT force presentation since at refractive index change there are two processes involved, such as optical clearing and change of medium viscosity, which are not separated. $V_{critical}$ is increased around 18% for isotonic solution of 5%-glucose instead of saline.

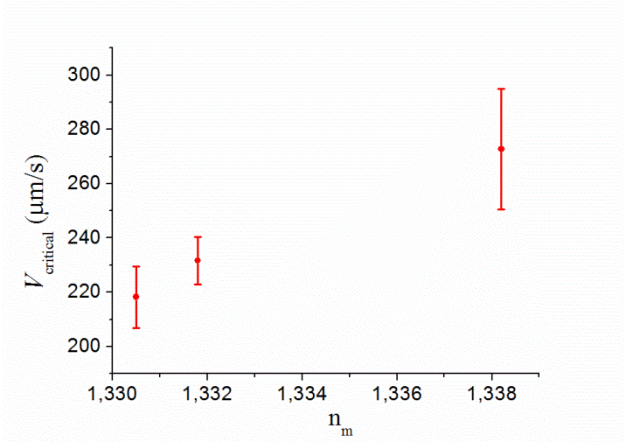


Figure 6. The dependence of $V_{critical}$ on refractive index n_m for 1.5 µm polystyrene beads in distilled water (n = 1.3305); saline (n = 1.3318) and isotonic solution of 5%-glucose (n = 1.3382).

3.2 The optical clearing of red blood cells

The $V_{critical}$ for two media - saline (n = 1.3318) and isotonic solution of 5%-glucose (n = 1.3382) is presented in Fig. 7. For RBCs, refractive index n_{RBC} is around 1.4.[19] The images of trapped RBCs are presented for these two solutions in Fig. 8. The isotonic solution of 5%-glucose is used for RBCs since it does not make any visible changes of RBC morphology (Fig. 8) and has a quite different refractive index and viscosity. The $V_{critical}$ is increased around 6.5% for isotonic solution of 5%-glucose in comparison to saline.

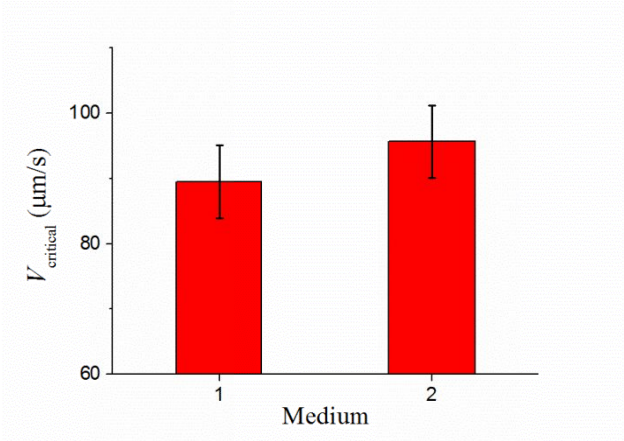


Figure 7. The $V_{critical}$ for two media - saline (n = 1.3318) and isotonic solution of 5%-glucose (n = 1.3382)

(a) (b)

Figure 8. Images of trapped RBCs in saline (n = 1.3318) and isotonic solution of 5%-glucose (n = 1.3382).

4. CONCLUSION

In this paper, we present the study of influence OCA concentration on optical trap force for 1.5 μm polystyrene beads and RBCs. It is shown that optical clearing enables one not only to decrease scattering and increase light penetration depth but also increase the optical trap force (Figs. 6 and 7). This finding is important for *in vivo* cell trapping since allows for reduction of power density of a laser. A solution of inverse problem enables one to measure OCA concentration *in vivo* and *in situ*. Since OCA application modifies relative refractive index, viscosity of surrounding medium and morphology of cells, optical trap force is very sensitive to OCA concentration. This fact requires providing a high precision study for accurate estimation of OCA concentration.

ACKNOWLEDGEMENTS

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