Manipulation on human red blood cells with femtosecond optical tweezers

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Received July 17, 2008

Different types of femtosecond optical tweezers have become a powerful tool in the modern biological field. However, how to control the irregular targets, including biological cells, using femtosecond optical tweezers remains to be explored. In this study, human red blood cells (hRBCs) are manipulated with femtosecond optical tweezers, and their states under different laser powers are investigated. The results indicate that optical potential traps only can capture the edge of hRBCs under the laser power from 1.4 to 2.8 mW, while it can make hRBCs turn over with the laser power more than 2.8 mW. It is suggested that femtosecond optical tweezers could not only manipulate biological cells, but also subtly control its states by adjusting the laser power.

OCIS codes: 350.4855, 120.4820, 170.4520.
doi: 10.3788/COL20080612.0919.

Manipulating single cell is of paramount importance in areas of biomedical research such as *in vitro* fertilization, cell-cell interaction, cell adhesion, embryology, microbiology, stem cell, tissue engineering and regenerative medicine, and single cell transfection. Since the development of optical tweezers by Ashkin *et al.*[1] in 1986, it has emerged as an essential tool for manipulating single biological cell and performing sophisticated biophysical/biomechanical characterizations. Distinct advantages of optical tweezers for these characterizations include non-contact force for cell manipulation, force resolution as accurate as 100 aN, and amiability to liquid medium environments. Ashkin *et al.* captured neutral atom and living bacterium using optical tweezers, which greatly impelled the development of optical tweezers technique[1−5].

Recently, the femtosecond laser has become a powerful tool for many applications[6−10]. In contrast to nanosecond or continuous-wave (CW) laser, femtosecond pulses with a tiny single pulse energy and a high peak power hardly causes any damage in the vicinity of the targeted tissue while achieving an ultra-high spatial resolution. Therefore, many researches using femtosecond optical tweezers have been carried out. Agate *et al.* compared the capturing efficiency of femtosecond laser with that of CW laser through the single-beam gradient force optical trap[11]. Their group also fulfilled similar experiments with Bessel optical trap and observed second harmonic of the KTP crystallites induced by femtosecond laser[12]. In 2006, they captured array micro-particles using femtosecond laser double-beam fiber optical trap and achieved the real-time two-photon fluorescence imaging[13]. Morrish *et al.* reported the relations between two-photon exciting effects and the appearance of particles using femtosecond laser single-beam gradient force optical trap[14]. Im *et al.* used femtosecond laser and CW laser single-beam gradient force optical traps to capture and manipulate red blood cell (RBC)[15,16]. Meantime, they studied the damage threshold of RBC captured by femtosecond laser. Xing *et al.* calculated the gradient forces and achieved stable capturing of RBC using tightly focused femtosecond laser[17]. However, how to control the irregular targets using femtosecond optical tweezers remains to be explored.

In this work, we present an optical manipulation system of femtosecond optical tweezers. Stable capturing of biological cells such as human red blood cells (hRBCs) are observed and the threshold powers of different states of hRBCs captured using femtosecond laser tweezers are measured.

Figure 1 shows an optical manipulation system for femtosecond optical tweezers. Femtosecond Ti:sapphire laser (TFS-1, Institute of Physics, Chinese Academy of Sciences, Beijing) of 800-nm wavelength, 82-MHz repetition frequency, and 30-fs pulse duration was coupled to an optical microscope (Olympus IX-71, Japan), and focused by a microscope objective lens (100×, numerical aperture NA=1.35) into the sample. The laser power was adjusted by an attenuation system composed of a rotatable polarizer and a half-wave plate. A shutter was used to control the irradiation of laser on sample. The cell specimen was mounted on a three-dimensional (3D) stage controlled by a three linear actuators. Real-time observation of the sample was achieved using a charge-coupled device (CCD) camera connected to the computer. The hRBCs used in our experiment was derived from the human blood. The blood added with anticoagulant agent was preserved in icebox at 4 °C. In the experiment, a droplet of blood added to physiological saline solution

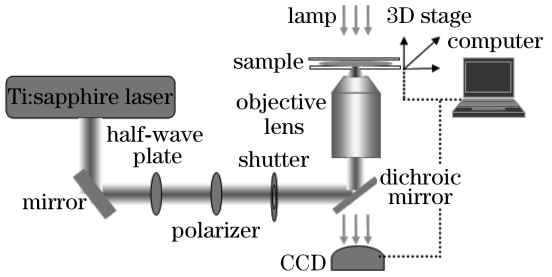

Fig. 1. Optical manipulation system for femtosecond optical tweezers.

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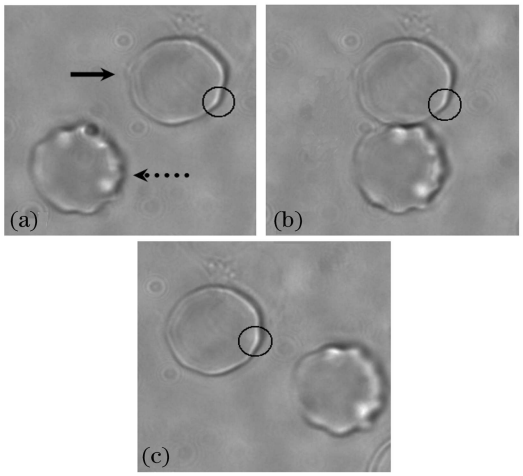

Fig. 2. Capturing in the edge of hRBC induced by femtosecond optical tweezers with 2.0-mW laser power. The center of circle represents the position of laser focus. The target hRBC, and neighboring cell are pointed by real line arrow, and dashed arrow, respectively.

was dropped on the cover glass. The hRBCs look like a disk with about 8-μm diameter and 1.7-μm thickness[18].

Steady capturing in the edge of hRBC was achieved using femtosecond optical tweezers with the laser power of 2.0 mW (Fig. 2). The target hRBC and neighboring cell were pointed by real line arrow and dashed arrow, respectively, in Fig. 2(a). After the target cell was placed on the focus plane, its edge was moved to the laser focus with the velocity of 2 μm/s and captured steadily. Meanwhile, the neighboring cell moved from the left of the target cell to below it firstly (Fig. 2(b)), and then to the right (Fig. 2(c)). It was concluded that the capturing depends on the dimension of laser focus and shape of hRBC. If the target is a regular sphere, the lateral gradient force of femtosecond optical tweezers would make the center of target move to the laser focus according to conventional single-beam gradient force model. Based on the theory of paraxial approximation, the dimension of Gaussian beam paraxial focus can be deduced as

$$\omega_0 = \frac{2\lambda}{\pi \cdot \mathrm{NA}}, \tag{1}$$

where λ is the wavelength, NA is the numerical aperture of objective. The diameter of laser focus is 754 nm, which is much smaller than the dimension of hRBC. Therefore, the laser focus located in different positions of hRBC (Fig. 3) will affect its capturing effect. When the laser focus is located in the point "a" or "c" (Fig. 3), the lateral force acting on the hRBC is zero because of its symmetry. When in "b" or "d", the lateral force will drive the laser focus to move to point "c". It is concluded that the probability of capturing in "c" is the greatest

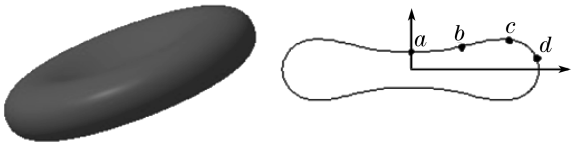

Fig. 3. Section of hRBC.

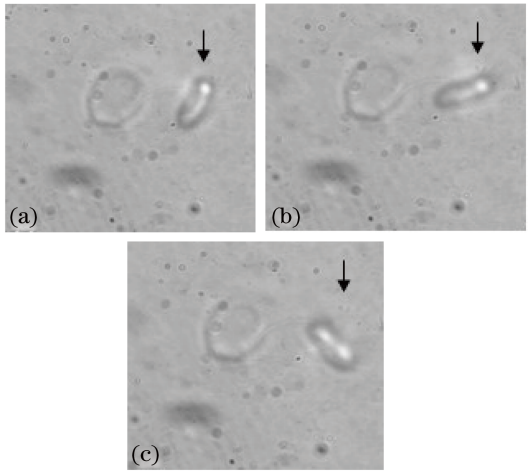

Fig. 4. Turnover and rotation of hRBC induced by femtosecond optical tweezers with 4.0-mW laser power. The target cell is labelled by black arrow.

because of the smallest potential energy of hRBC.

Figure 4 shows the turnover of hRBC induced by femtosecond optical tweezers with 4.0-mW laser power. Firstly, the hRBC was captured and turned over rapidly when the laser focus was moved to the target cell (Fig. 4(a)). Then the cell rotated immediately (Fig. 4(b)) and reached to the steady state (Fig. 4(c)). It is inferred that these results are attributed to the different force gradients in lateral and axis directions. Adopting the polar coordinates, the intensity distribution of the Gaussian beam in laser focus is[19]

$$I(r,\theta,t) = I_0 \mathrm{e}^{-2(\frac{r}{\omega_0})^2} \mathrm{e}^{-4\ln 2(\frac{t}{\tau})^2}, \tag{2}$$

where τ is full-width at half-maximum (FWHM) of femtosecond laser, and I_0 is the optical intensity of peak value. Furthermore, the ratio of axis-lateral dimension can be concluded as[20]

$$k = \frac{l}{d} = \frac{1.64n}{\mathrm{NA}}, \tag{3}$$

where l is the axis dimension, d is the lateral dimension, and n is the refractive index of the sample. We can calculate that k is 1.82 here, which means that the gradient of lateral force is larger than that of axis force. When the laser power is higher, the hRBC turns over under torsional moment induced by lateral force and axis force. The cell has the smallest potential energy when its plane is parallel to the direction of optical axis (Fig. 4). After the turnover of hRBC, femtosecond laser-induced strong electric field in focus makes the electricity neutral biomolecule have a relative displacement, which forms an electric dipole and an electric moment along the direction outward the electric field. This is a possible reason for the rotation of the hRBC.

Now we investigate the dependence of capturing in the edge and turnover of hRBCs on the laser power of femtosecond optical tweezers. When the femtosecond laser was focused on the hRBC moving at the velocity of 2 μm/s, the states of hRBC at different laser powers were shown in Fig. 5. To obtain the statistical results, 20 hRBCs were chosen for each laser power. It was concluded that the hRBCs cannot be captured when the laser power is smaller than 1.2 mW. Ten hRBCs were

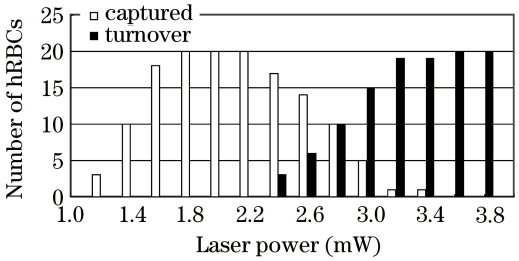

Fig. 5. Dependence of capturing and turnover of hRBCs on the laser power of femtosecond optical tweezers.

captured when the laser power was 1.4 mW. All cells can be captured when the laser power was between 1.8 and 2.2 mW. Therefore, the 1.4-mW laser power was regarded as the capturing threshold. As the laser power increases, the number of turnover cells increases and that of captured cell in the edge decreases. When the laser power was 2.8 mW, the number of captured hRBCs in the edge was equal to that of turnover cells. When the laser power was higher than 3.6 mW, the hRBCs can be turned over entirely. With the laser power increasing, the RBCs will be damaged due to high peak power of femtosecond laser[8,9]. Therefore, we can easily control the state of hRBCs through adjusting the laser power accurately.

Based on the femtosecond optical tweezers system, the states of hRBCs, including capturing in the edge and turnover of hRBCs, were observed by adjusting the laser power. The capturing in the edge of hRBC was achieved when the laser power was 2.0 mW, which depended on the dimensions of laser focus and hRBC. The hRBC turned over when the laser power was 4.0 mW because of enough torsional moment induced by lateral force and axis force. The threshold powers of capturing in the edge and turnover of hRBCs were measured by adjusting the laser power, respectively. The states of hRBCs can be easily controlled by selecting laser power of femtosecond optical tweezers.

This work was supported by the National "863" Program of China (No. 2006AA04Z307), the Foundation for the Author of National Excellent Doctoral Dissertation of China (No. 2006039), the National Natural Science Foundation of China (No. 50775140), the Natural Science Foundation of Jiangsu Province (No. BK2006507), and the Jiangsu Province Research Innovation Program of College Graduate (No. CX07B_086z). M. Zhou is the author to whom the correspondence should be addressed, his e-mail address is zm_laser@126.com.

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