Optical trapping and orientation of Escherichia coli cells using two tapered fiber probes

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We report on the optical trapping and orientation of Escherichia coli (E. coli) cells using two tapered fiber probes. With a laser beam at 980 nm wavelength launched into probe I, an E. coli chain consisting of three cells was formed at the tip of probe I. After launching a beam at 980 nm into probe II, the E. coli at the end of the chain was trapped and oriented via the optical torques yielded by two probes. The orientation of the E. coli was controlled by adjusting the laser power of probe II. Experimental results were interpreted by theoretical analysis and numerical simulations. © 2015 Chinese Laser Press

1. INTRODUCTION

Manipulation of single cells is of great importance in biomedical research, microbiology, cell–cell interaction, and microbiology. Trapping and orientation of individual nonspherical bacteria can promote the study of the interaction between specific regions of biological objects, which provides an efficient method to observe and describe the behavior of single cells and, further, to study the dynamics of bacteria populations [1,2]. Thus, it is of great importance in understanding the mechanisms of cell function [3]. In addition, scanning the angle between the long cell axis and the optical axis of the optical tweezer may allow for reconstruction of the three-dimensional structures using standard computerized tomography methods [4]. Moreover, when the cell was illuminated in different orientations, by studding the scattering light of different orientation, more information on the morphology of the cell can be obtained which can be used to discriminate between different cell types or, more importantly, between different cell states [5]. One of the most widely investigated nonspherical bacteria is Escherichia coli (E. coli) cells due to their critical role in biological engineering and industrial microbiology [6,7]. Some challenges, however, exist in realizing stable trapping and controllable orientation of E. coli cells. First, the E. coli cells in liquids swim in two patterns, “runs” and “tumbles” [8,9], which are related to the rotation direction of the flagella [10]. Second, the E. coli cells are strongly affected by Brownian motion in liquids due to their nanometer size [11]. To solve these challenges, optical methods based on resonant optical antennas [11] and optical tweezers [4,12] have been proposed to trap and orientate E. coli cells. However, the operations were limited to a fixed substrate or a specific depth of cell solutions, and the bulk structure of the optical system and focusing objective make it difficult to move and focus. Moreover, the difficulty in penetrating thick samples by the focus generated by the objective make it difficult to apply the system to thick samples. Fortunately, optical fibers provide an alternative approach to manipulate E. coli cells [13–15]. Among them, the fiber probe is a miniaturized and highly flexible tool for particle manipulation because light beams can be simply focused by the tip of the probe without the use of complicated optical components such as high-numerical-aperture objective. However, with only one laser beam applied for the cell manipulation, the orientation will only be determined once the cells are trapped [12,13], and therefore the accessible rotational axes or feasible angles are restrained. In this work, we report on the optical trapping and orientation of E. coli cells using two tapered fiber probes. With the 980 nm laser beams injected into the probes, an E. coli cell chain was formed and trapped at the tip of one probe while the cell at the end of chain can be controllably orientated by adjusting the power injected into the fiber probes.

2. RESULTS

Figure 1 shows the experimental scheme. Two probes with different divergence angles were placed in an E. coli solution [Fig. 1(a)], with an angle of 150° between the probe axes. After launching a laser beam into probe I, an E. coli chain consisting of three cells was formed at the tip of probe I [Fig. 1(b)]. Another beam was then injected into probe II. Due to the superposition of the two beams, the energy density at the intersection point of two probes, i.e., point O in Fig. 1(a), was higher than those around it, resulting in a much stronger gradient force than the scattering force. Therefore, an E. coli cell can be trapped by the gradient force at point O and the orientation of the cell can be realized by adjusting the powers in the two probes. At \( P_2 > P_1 \), the E. coli at point O will be oriented with its axis along probe II due to the restoring torque [Fig. 1(c)], while at \( P_1 > P_2 \), the cell will be orientated along probe I [Fig. 1(d)]. The experimental setup is schematically
shown in Fig. 1(e). Two laser beams at 980 nm wavelength (with a very weak absorption by *E. coli* cells) were launched into the two probes. The probes were fabricated by drawing single-mode fibers (connector type, FC/PC; core diameter, 9 μm; cladding diameter, 125 μm) through a flame-heating technique. Fibers I and II were fixed by two tunable microstages I and II, respectively. The lengths of the tapered part and the fiber stick out of the holding stage were about 75 μm and 9 cm, respectively. To decrease the oscillations in our experiment due to the environmental fluctuations, each fiber probe was sheathed by a glass capillary (inner diameter, 0.9 mm; wall thickness, 0.1 mm; length, 12 cm). One side of the glass capillary was fixed on the stage while the other side was stuck out. The length of the probe outside the capillary was about 2 mm. The probes were immersed in the *E. coli* solution. An optical microscope with a CCD camera was used for real-time monitoring and image capturing.

At the beginning of the experiment (*t* = 0), laser I was turned on to launch a beam of 25 mW into probe I while no laser was launched into probe II, i.e., *P*_1 = 25 mW and *P*_2 = 0 [Fig. 2(a)]. *E. coli* cells were trapped one after another by the optical forces generated by probe I and gradually formed into a cell chain. At *t* = 2 s, a chain consisting of three *E. coli* cells was formed [Fig. 2(b)]. At *t* = 3 s, laser II was also turned on to launch a beam of 20 mW into probe II, i.e., *P*_1 = 25 mW and *P*_2 = 20 mW. The *E. coli* at the end of the chain was pulled away from the chain by the optical force from probe II [Fig. 2(c)], and then began to rotate anticlockwise. At *t* = 3.3 s, the azimutal angle of the *E. coli* θ, which is defined as the angle between the axes of the *E. coli* and probe II, was −19° [Fig. 2(d)] and the orientation became stable. At *t* = 4.2 s, with *P*_2 increased from 20 to 35 mW, the cell was finally oriented along the axis of probe II, i.e., θ = 0 [Fig. 2(e)].

At *t* = 6 s, with the power of probe II decreased from *P*_2 = 35 to 15 mW, the *E. coli* was rotated clockwise [Fig. 2(f)] and kept oriented along the axis of probe I [Fig. 2(g)], i.e., θ = −30° again.

3. ANALYSIS AND DISCUSSION

To interpret the phenomena just described, simulations were performed based on a finite element method (COMSOL Multiphysics 4.3b). In the simulations, the diameter of probe I was set as 4 μm at the input port and then linearly decreased to 2 μm within a length of 15.7 μm, with a parabolic extremity. The diameter of probe II was set as 8 μm at the input port and then linearly decreased to 4 μm within 10.3 μm. The axis of probe II was along the X direction and the angle between the axes of the two probes was 150°. The *E. coli* cell was treated as a rod (diameter, 500 nm; length, 2 μm) with two hemispherical caps. The refractive indices are 1.33, 1.39,
and 1.44 for the water, *E. coli*, and probes at the laser wavelength of 980 nm. Distributions of energy density for the two probes were obtained by the simulations (Fig. 3). When only laser I was launched into probe I, the energy was concentrated near the tip of probe I [Fig. 3(a)] with a focal plane at \( X = 5.5 \mu m \) [Fig. 3(b)], which results in a strong gradient force exerted on the *E. coli*. When only laser II was launched into probe II, the energy was concentrated at a position further away from the tip of probe II [Fig. 3(c)], with a focal plane at \( X = 0.1 \mu m \) [Fig. 3(d)]. The light output from probe I was focused nearly at the tip while the light from probe II was focused 3.6 \( \mu m \) away from the tip, which is attributed to the different profiles and sizes of the two probes. As a result, probe I was used to form and trap a cell chain of *E. coli* while probe II was used to manipulate the single cell at the end of the chain.

The optical force \( \mathbf{F}_o \) exerted on the *E. coli* can be calculated by integrating the time-dependent Maxwell stress tensor \( \mathbf{T}_o \) around the *E. coli* with a closed surface \( S \) [16,17]:

\[
\mathbf{F}_o = \oint_S (\mathbf{T}_o \cdot \mathbf{n}) dS,
\]

where \( \mathbf{n} \) is the surface normal vector. The optical torque \( \mathbf{T} \), which determines the orientation of the *E. coli* cell, can be calculated by [18]

\[
\mathbf{T} = \int r_i \times d\mathbf{F}_o i.
\]

where \( d\mathbf{F}_o i \) is the optical force element at an arbitrary point \( i \) within the *E. coli* cell that interacts with light, and \( r_i \) is the position vector from the center point of the *E. coli* cell where \( d\mathbf{F}_o i \) is generated. Figure 4(a) shows the calculated optical torque on the *E. coli* cell as a function of the azimuthal angle \( \theta \) with respect to the +\( X \) direction. The inset of Fig. 4(a) describes the calculation model. For probe I, \( \mathbf{T} \) is 0 when \( \theta = -120^\circ, -30^\circ, \) and \( 60^\circ \). In the regions of \(-120^\circ < \theta < -30^\circ \) and \( 60^\circ > \theta > 30^\circ \), the values of \( \mathbf{T} \) are positive and negative so that the *E. coli* is rotated anticlockwise and clockwise, respectively. For probe II, \( \mathbf{T} \) is 0 when \( \theta = 0^\circ, -90^\circ, \) and \( 90^\circ \). In the regions of \(-90^\circ < \theta < 0^\circ \) and \( 90^\circ > \theta > 0^\circ \), the values of \( \mathbf{T} \) are positive and negative so that the *E. coli* is rotated anticlockwise and clockwise, respectively. Therefore, the *E. coli* trapped by probes I and II tends to be rotated toward \( \theta = -30^\circ \) and \( \theta = 0 \), respectively. The rotational potential energy \( U \) of the two probes were evaluated by integrating \( \mathbf{T} \) with respect to \( \theta \). For stable trapping, \( U \) must overcome the thermal energy \( k_b T \) [19], i.e., \( |U| > k_b T \), where \( k_b \) was the Boltzmann constant and \( T \) was the absolute temperature of the solution. In this work, \( U \) has to satisfy that \( |U| \geq 100k_b T = 4.14 \times 10^{-10} \) J. For probes I and II, \( U \) reaches minimum at \( \theta = -30^\circ \) and \( \theta = 0 \), respectively, confirming that \( \theta = -30^\circ \) and \( \theta = 0 \) are the most stable orientations for the *E. coli* trapped by probes I and II, respectively. *E. coli* cells will be trapped at the axis of either probe by the optical force and rotated by the optical torque until the axis of the *E. coli* coincides with the probe axis. With an input power of 25 mW, four *E. coli* cells can be trapped by the probe [13]. Figure 4(b) shows the simulated energy density distributions of *E. coli* cell chains consisting of 1–4 cells at an input power at 25 mw. Light was confined in the *E. coli* cells and propagated along the chain, indicating that the *E. coli* cells trapped by probe I can be seen as the extension of the fiber tip, resulting in more *E. coli* cells being trapped. Figure 4(c) shows the energy density distribution along the axis of probe I with cell numbers of \( N = 0, 1, 2, \) and 3. When the number of the *E. coli* chain is increased from 0 to 3, more energy will be concentrated along
For probe I with a cell chain formed at the tip, the optical intensity at point $O$ is stronger than that without a chain. Figure 4(d) shows the calculated resultant optical force ($F$) exerted on the last $E. coli$ of the chain, consisting of 1–4 cells. The optical forces exerted on the last $E. coli$ were directed to the probe, which means that this force was the trapping force.

To analyze the orientation process, simulations were then performed by increasing the input power of probe II to 20 mW while keeping the input power of probe I at 25 mW. In the simulation, two $E. coli$ cells were connected with probe I. Figure 5(a) shows the simulated distributions of energy density emitted from the two probes. The distances between the probe tip and the intersection point of probes ($X = 0.6 \, \mu m$, $Y = 0$) were $D_1 = 5.9 \, \mu m$ and $D_2 = 3.4 \, \mu m$ for probes I and II, respectively. At the intersection point, energy density was higher than those around it because of the superposition of two laser beams. The $E. coli$ near the intersection point will be trapped by the gradient force. Figure 5(b) shows the energy density distributions along the axes of the probes projected onto the $X$ coordinate. The strongest optical intensity was at $X = 1.1 \, \mu m$ rather than the focus point of probe II ($X = 0.1 \, \mu m$), indicating that the trapping area of the $E. coli$ can be adjusted by changing the positions of the probes.

Figure 5(c) shows the calculated optical torques $T$ as a function of input power. For $P_1 = 25 \, mW$ and $P_2 = 20 \, mW$, the torque $T$ is 0 when $\theta = -16^\circ$. The torque $T$ becomes positive and negative when $\theta < -16^\circ$ and $\theta > -16^\circ$, respectively, indicating that the orientation is stable at $\theta = -16^\circ$. Similarly, for $P_1 = 25 \, mW$ and $P_2 = 35 \, mW$ and for $P_1 = 25 \, mW$ and $P_2 = 15 \, mW$, the orientation is stable at $\theta = -1.55^\circ$ and $\theta = -25.8^\circ$, respectively. The rotational potential energy $U$ of two probes reached the corresponding minimum at $\theta = -16^\circ$, $-1.55^\circ$, and $-25.8^\circ$ [Fig. 5(d)], which also indicates the angles for the stable orientations. Note that the experimental results presented in Figs. 2(d) and 2(g) are a bit different from those obtained by calculations. This is because in our models for calculations, the $E. coli$ was simplified as a rod with two hemispherical caps, while actually the $E. coli$ was not always so symmetrical and straight, as shown by the scanning electron micrograph image in the inset II of Fig. 1(e). The slight difference in shape and size also exists in the fiber probe models and the fabricated ones.

To further analyze the optical torques on the $E. coli$ with different distance between the probe and $E. coli$, a series of simulations were performed by changing the position of probe II while fixing probe I at $D_1 = 5.9 \, \mu m$. Figure 6, as an example, shows calculated optical torque $T$ and corresponding rotational potential $U$ as a function of the azimuthal angle $\theta$. At input power $P_1 = 25 \, mW$, $P_2 = 35 \, mW$, the variation of the values of $T$ for $D_2 = 2.4, 2.9, and 3.9 \, \mu m$ is very small compared with those for $D_2 = 3.4 \, \mu m$ [Fig. 6(a)], which indicates that the cell can be rotated toward $\theta = 0$. The corresponding rotational potential $U$ reaches a minimum of less than $-1.1 \times 10^{-18} \, J$ around $\theta = 0$ [Fig. 6(b)]. The $E. coli$ will be oriented along the axis of probe II so that the orientation of the cell can be controlled, with $D_2$ ranging from 2.4 to 3.9 $\mu m$. Similarly, at input power $P_1 = 25 \, mW$, $P_2 = 15 \, mW$, the variation of values of $T$ for $D_2 = 2.9 and 3.9 \, \mu m$ is also very small compared with those for $D_2 = 3.4 \, \mu m$ [Fig. 6(c)]. The corresponding minima of $U$ were $-5.22 \times 10^{-19}$ and $-6.1 \times 10^{-19} \, J$ [Fig. 6(d)], which indicates that the $E. coli$ can also be oriented by probe I. For $D_2 = 2.4 \, \mu m$, the minimum of $U$ was $-1.7 \times 10^{-19} \, J$, which was more than $-4.14 \times 10^{-19} \, J$. Thus, in this situation, the cell orientation cannot be realized by probe I. This is because the focus of probe II was 1.2 $\mu m$ away from the intersection point of the axes of the probes in this case. The input power of probe I was decreased and thereby the energy density at the point where the $E. coli$ was trapped was not strong enough to control the orientation of the $E. coli$.

4. CONCLUSIONS

An optical method for trapping and orientation of $E. coli$ cells has been experimentally demonstrated using two probes. By launching a laser beam at 980 nm into probe I, an $E. coli$ chain was formed at the probe tip, followed by a trapping and orientation of the $E. coli$ at the end of the chain by launching...
another beam into probe II. The orientation of the *E. coli* was then controlled by adjusting the laser power of probe II. The performance of the method in terms of resultant optical torque and the corresponding rotational potential energy has been numerically analyzed with simulations and calculations. These results are expected to find application in single-cell studies in which optical manipulation of nonspherical bacteria is desired.

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