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Quantitative optical trapping and optical manipulation of micro-sized objects

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Abstract. An optical tweezers technique is used for ultraprecise micromanipulation to measure positions of micrometer scale objects with a precision down to the nanometer scale. It consists of a high performance research microscope with motorized scanning stage and sensitive position detection system. Up to 10 traps can be used quasi-simultaneously. Non photodamage optical trapping of *Escherichia coli* (*E. coli*) bacteria cells of 2 μm in length, as an example of motile bacteria, has been shown in this paper. Also, efficient optical trapping and rotation of polystyrene latex particles of 3 μm in diameter have been studied, as an optical handle for the pick and place of other tiny objects. A fast galvoscanner is used to produce multiple optical traps for manipulation of micro-sized objects and optical forces of these trapped objects quantified and measured according to explanation of ray optics regime. The diameter of trapped particle is bigger than the wavelength of the trapping laser light. The force constant (k) has been determined in real time from the positional time series recorded from the trapped object that is monitored by a CCD camera through a personal computer.

Keywords: optical tweezers, multiple traps, force calibration, nanometer displacement, *E. coli* bacteria.

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1. Introduction

Optical tweezers are commonly used for trapping and manipulation with micro- and nanoparticles [1-4]. Since their invention in 1986, the field of optical trapping has become a hot-spot topic for research, and its applications have extended from microfabrication [5] to drug delivery [6]. Furthermore, optical tweezers are considered as a quantitative tool to analyze forces applied to trapped objects and precisely determine these forces in the range of pico-newton scale, with resolution at the femto-newton, and nanometer scaled displacements. Many experiments have been done by using these analytical optical tweezers in many fields of research, namely: physics, biology, nanotechnology, and materials science. In recent time, manipulation, rotation and assembly of different kinds of nanostructures [7, 8], such as carbon nanotubes [9], nanowires [10, 11], and polymer nanofibers [12], have been also done by using the optical tweezers technique. Also, they have used to manipulate living cells [13-16] and to investigate the motility and flagellar rotation of single bacterial cells [17-19].

In general, optical trap is performed by using a highly focused laser beam into a transparent particle with a higher refractive index than that of its surroundings, to generate a large electric field gradient helps to attract the particle towards the focal spot without any mechanical contact [20]. The infrared

wavelength is commonly used for optical trapping because of its low absorptivity in biological material and low degree of optical photodamage on biological samples. The precision of using the optical tweezers instruments is crucial for accuracy of the calibration method aimed at a trapped transparent object. A well-known calibration procedure is recording the stochastic movement of the trapped transparent particle and comparing its statistical behaviour with the theory of the Brownian motion in a harmonic potential [21].

2. Theory and overview

The kinetic force induced by light on matter relies on the size of trapped transparent particle. In case of trapping micro-sized particles, the ray optics approach is used to describe the origin of this force as presented by Ashkin [22]. The obtained results from Ashkin's calculation are valid only for particles with sizes larger than the wavelength of the laser light. In this regime, the light beam consists of individual rays. Each ray reflects and refracts on the surface of the transparent particle. The optical trapping force produced by each ray is simply calculated by the exchange of momentum between light and matter. This kinetic force is presented by the momentum change per unit time. The total force is the sum of the forces calculated for each ray.

The total force from a single ray is divided into two components, F_1 and F_2 for vertical and horizontal directions, respectively, and is presented by the following simple formulae [23]:

$$F_1 = \frac{nP}{c}\left(1 + R\cos 2\theta - \frac{T^2\left[\cos(2\theta - 2r) + R\cos 2\theta\right]}{1 + R^2 + 2R\cos 2r}\right) \quad (1)$$

$$F_2 = \frac{nP}{c}\left(R\sin 2\theta - \frac{T^2\left[\sin(2\theta - 2r) + R\sin 2\theta\right]}{1 + R^2 + 2R\cos 2r}\right), \quad (2)$$

where n, r, P and c are the refractive index of surrounding medium, angle of refraction, power of a single ray and the speed of light, respectively. R and T are the Fresnel reflection and refraction coefficients of the incident angle θ. The total force on the trapped particle is the sum of these two components expressed by Eqs (1) and (2) over individual rays of the laser beam. The force is estimated from Stokes' law based on the drag force induced by moving surrounding medium.

An optical tweezers is generally achieved by tightly focusing a laser beam in order to generate a high intensity hotspot towards which the particles are attracted. The resulting optical forces are typically distinguished into scattering forces, due to the forward radiation pressure, and gradient forces, generated by the gradient of the light intensity. Successful trapping can be obtained, when the gradient forces exceed the scattering ones. In general, there are several requirements for achieving stable optical trap such as trapping power, particle size, particle shape, refractive index, surface roughness, absorption, numerical aperture of the objective and transverse intensity distribution of the laser light [23]. The trapping beam has Gaussian focal intensity distribution, so that the gradient force can be expressed as a harmonic restoring force acting on the particle:

$$F = k\left(x - x_0\right), \quad (3)$$

where k is the spring constant describing the stiffness of the potential, x – position of the particle, and x_0 denotes its equilibrium position.

Trap stiffness calibration (Drag force method). Calibration of trapped object is essential for quantitative force and displacement measurements, and it can be done in several ways. In this experiment, Stokes drag calibration method presented to measure the displacement of a trapped micro-sphere and *E. coli* bacterium cell from its equilibrium position. This method depends on the response to viscous forces produced by the medium, and it is given by

$$k\,x = 6\pi\eta r\upsilon, \quad (4)$$

where η is the viscosity of the liquid, r – diameter of the trapped particle, and υ – velocity of the fluid.

Since nanoscale measurements of both force and displacement require a well-calibrated system for determining the position, high sensitive position detection system is required to track microscopic objects.

3. Experimental set-up

The schematic of optical trapping system is shown in Fig. 1. The optical trap used for quantitative measurements of the optical forces is implemented in a Nikon inverted microscope to visualize the sample. As it can be seen, the experimental setup consists of the continuous wave laser beam (1064 nm, 8 W) with a symmetrical intensity distribution around the beam axis. It is strongly focused by a high numerical aperture objective of a microscope (Plan Fluor 100x NA 1.3). Before entering the microscope objective, a beam expander (two convex lenses) is used to expand the diameter of the laser beam for achieving a tiny beam spot size on the trapping region. An extremely sensitive position detector, quadrant photodiode, is used to measure 3D position of micrometer scale objects with a precision down to the nanometer scale. The position of the trapped micro-sized object is traced by back focal plan interferometry. The sample chamber mounted on a motorized scanning stage to properly navigate and provide positional control of the trap inside the chamber with nanometer resolution. For achieving the optimal

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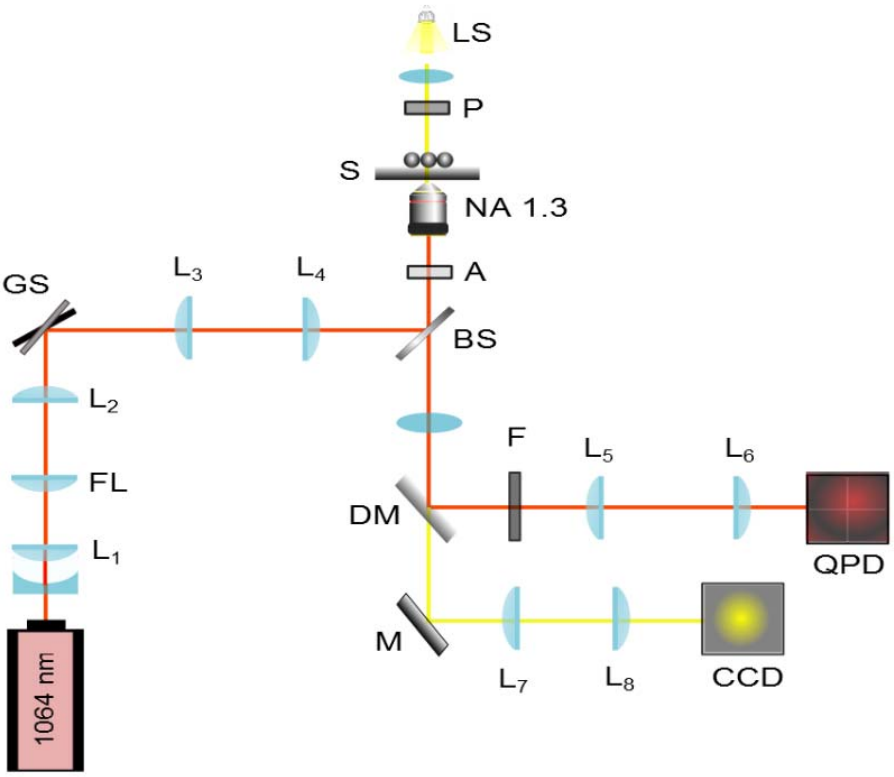

Fig. 1. Layout of the experimental setup. L – lenses, FL – focus lens, GS – galvoscanner, BS – beam splitter, A – analyser, S – sample, P – polarizer, LS – light source, DM – dichroic mirror, F – infrared fillter, QPD – quadrant photodiode, M – mirror, CCD – charge couple device camera to image the sample.

conditions of trapping, immersion oil with a refractive index of 1.54 is used with the large numerical objective to minimize spherical aberrations. A very rapid galvanic scanner system is used in the path of the laser beam to create multiple quasi-simultaneous traps, up to 10 traps, and to move them in the field of view. Groups of trapped objects can be rotated around their center or scaled up and down. In this experiment, multiple trapping is easily achieved by scanning the laser beam rapidly to prevent escaping the particle from the trapping region before the next beam scan take places. White light source illuminates the sample that is imaged in a CCD camera for videomicroscopy. Finally, the optical tweezers instrument is mounted on an anti-vibration table to eliminate the mechanical vibrations.

4. Results and discussion

Quantifying optical trapping forces for micro-sized objects is done by using the high-tech optical tweezers instrument for ultra-precise micromanipulation. This study has been performed in a liquid environment using polystyrene micro-spheres of 3 μm (Sigma, UK, LB30) and *E. coli* bacteria of 2 μm in length (Strain No. ATCC25922). All measurements were made at room temperature (22 °C). In this experiment, the single microscopic object was trapped in three dimensions with efficient detection of position. The calibration procedure was performed by converting the detected voltage signal to displacement units, and the forces exerted on the trapped object displaced from its equilibrium position was defined by tracking and analysing its dynamics.

Trapping of *E. coli* bacteria. In general, *E. coli* bacteria cells in the aqueous environment can swim, run, tumble, and move anywhere inside the sample chamber. It is considered as an example of motile bacteria. Bacterial dynamics are directly related to bacterial motility, and it can be observed in the trapping process [19, 24]. The single cell level of *E. coli* can be damage-free trapped in the focus without any mechanical contact and tends to align itself along the optical axis [25]. This type of bacteria has two kinds of movements: Brownian motion and flagella-mediated propulsion. The positional signal recorder from trapped bacterium cell contains of both movements, so it has unreasonable information about force constant. Also, trapping more than one *E. coli* bacterium cell in the trap shows a complicating noise in to the recorded positional signal. In this work, very diluted sample is prepared to avoid bacterial accumulation at the trap during measurements. Fig. 2 shows the calibration of optical tweezers by measuring

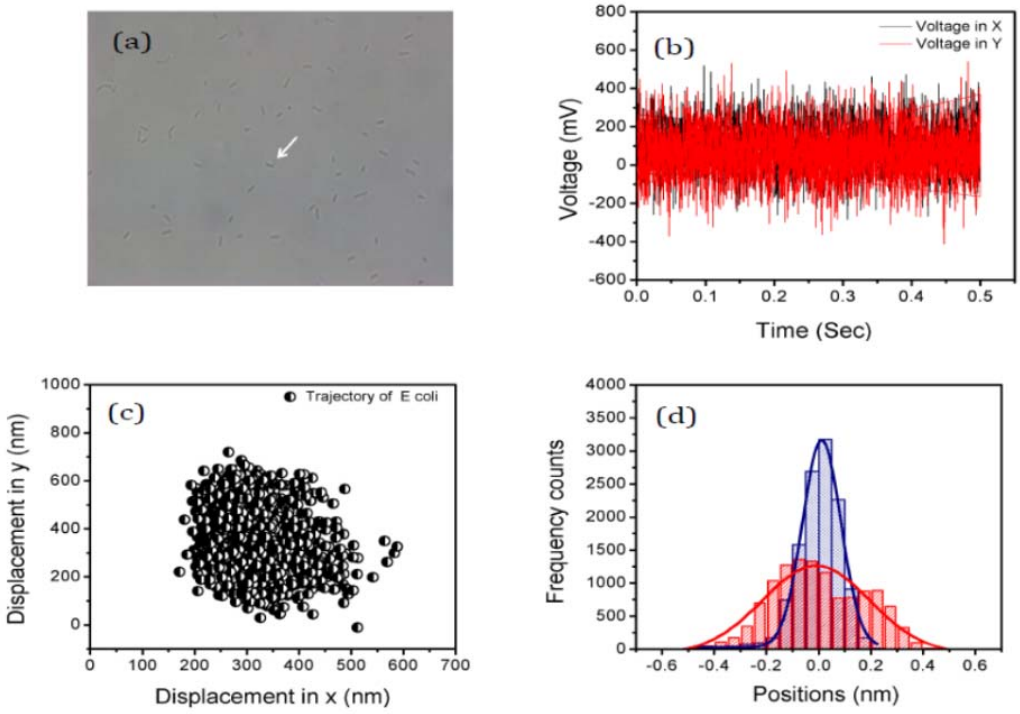

Fig. 2. Calibration of optical forces on an *E. coli* bacterium cell of 2 μm in length. (a) Image of *E. coli* bacterium (white arrow pointed to the trapped cell). (b) Voltage signal of trapped cell in *x* and *y* as recorded from a quadrant photodiode. (c) Trajectory of trapped cell. (d) Two position histograms of trapped single *E. coli* bacterium cell (blue one) and trapped two *E. coli* bacteria cells (red one) for the trapping power 0.86 W.

the Brownian motion of the trapped *E. coli* bacterium cell. Fig. 2a shows the image of *E. coli* bacteria. The voltage signal from the quadrant photodiode is detected as shown in Fig. 2b, and the trajectory has shown in Fig. 2c. As the optical trap is exerting a harmonic potential on the trapped particle, the position distribution is well described by a Gaussian distribution as shown in Fig. 2d. During the experiment, the trapped single bacterium cell showed different dynamics. It can be trapped, arrested for a while, and then escaped away from trapping region. This behaviour is happened because of the energy stored in the trapped cell released and converted into kinetic energy leading to escape away from the trap [19].

Trapping of polystyrene spheres (The optical handles). Optical tweezers are commonly used to perform force measurements in the range of femto- to pico-newton scale and to detect nanometer scaled displacements. The stiffness constant, *k*, can be evaluated from the Stokes drag calibration method by monitoring the Brownian motion of an optically trapped polystyrene latex sphere. This kind of micro-spheres can be used as an optical handle to grab and manipulate other tiny objects, such as DNA [26, 27], viruses [28] and molecules [29] by attaching them to the surface of spherical bead. This could be useful for biological studies and for optically assisted self-assembly processes. The stiffness constants of trapped bead perpendicular to the propagation direction of the trapping laser (k_x and k_y) measured directly against trapping power as shown in Fig. 3a, and they are nearly identical. A laser trap is dragged across 3-μm polystyrene latex particles exerting optical force to trap objects in a potential well formed by light as shown in Fig. 3b. In this experiment, multiple trapping is created by rapid scanning of laser beam with galvoscanner to prevent the trapped particle from escaping from the trap before the next beam scan occurs. It is fairly easy to trap and manipulate two or more particles of almost the same size independently at the same time by using the same number of laser beams as particles. Fig. 4 shows three trapped particles placed in a triangle shape and rotated around their center without affecting on each others by reflected or scattered light.

5. Conclusions

To conclude, optical tweezers are very attractive techniques for trapping and manipulation of microscopic objects without any mechanical contact. Here, damage free trapping of *E. coli* bacterium cell and trap characterization and calibration of polystyrene spheres are described. These micro-spheres can be used alone or as optical handles attached to extremely small objects, such as virus and DNA, to apply the calibrated force.

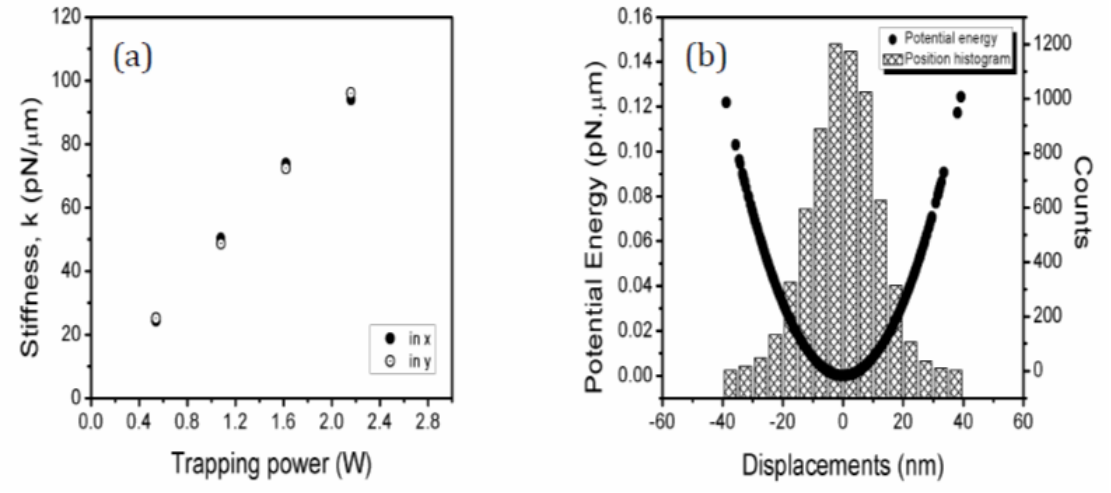

Fig. 3. Calibration of optical forces on a polystyrene sphere. (a) Estimated optical force constant on 3-μm polystyrene bead as a function of the trapping power in *x* and *y* directions. (b) Potential energy and position histogram of 3-μm polystyrene bead at trapping power 1.2 W.

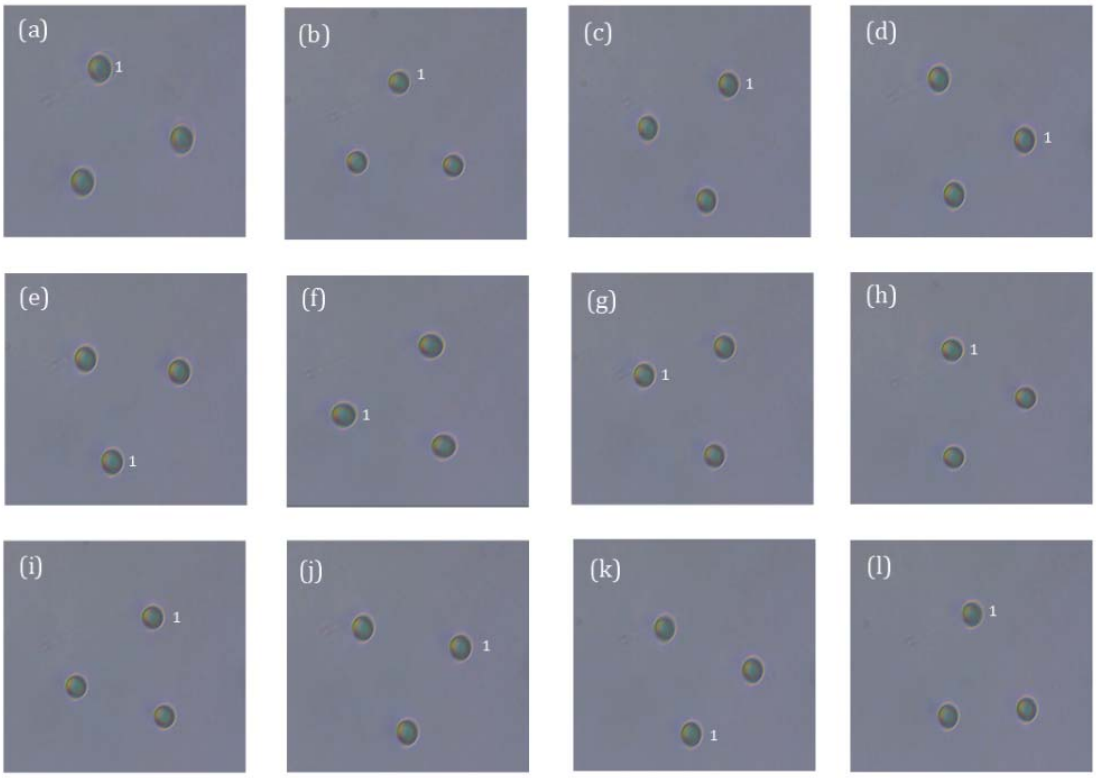

Fig. 4. Rotation of the optical handles (Polystyrene particles of 3-μm diameter) around their centres.

This experiment may be extended and developed for studying bacterium drug interactions and cell-cell interactions.

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