A calibration method for optical trap force by use of electrokinetic phenomena

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An experimental method for calibration of optical trap force upon cells by use of electrokinetic phenomena is demonstrated. An electronkinetic sample chamber system (ESCS) is designed instead of a common sample chamber and a costly automatism stage, thus the experimental setup is simpler and cheaper. Experiments indicate that the range of the trap force measured by this method is piconewton and sub-piconewton, which makes it fit for study on non-damage interaction between light and biological particles with optical tweezers especially. Since this method is relevant to particle electric charge, by applying an alternating electric field, the new method may overcome the problem of correcting drag force and allow us to measure simultaneously optical trap stiffness and particle electric charge.

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Optical tweezers have become a versatile tool in cellular and molecular biologies. Presently the optical trap force (OTF) must be determined by empirical force calibration. Thus, different methods have been developed for OTF calibration[1−6]. One group of methods is based on the observation of the Brownian motion of a trapped particle. For micron-sized cells, the motional amplitudes are very small and thus require very precise position measurements with resolutions on a subwavelength scale[7]. The other methods (called Stokes friction method) compare the OTF with a viscous drag, generated by a relative motion of the particle with respect to its liquid environment. In order to produce this relative motion, a way is that the chamber containing fluid is moved past a stationary trapped sphere, and the velocity of the stage at which the bead escapes is measured[1,4,8]. In this case, a costly motorized or piezo-driven stage is needed. Another way is that using a flow chamber, fluid is pumped past a stationary trapped sphere at increasing velocity until the object just escapes[9,10]. This procedure requires a flow chamber connected to a variable pump.

Our approach is similar in principle to Stokes friction method. However, the relative motion of the cell with respect to its liquid environment is produced by electrokinetic phenomena, and the cell OTF is determined by comparing it with the electrophoretic and viscous drag forces in its solution.

Most of bio-cells used in experiments suspended or dissolved in an aqueous electrolyte solution often carry electric charges. If an external electric field E is applied to the solution, the cells will produce electrophoresis, the solution will produce electroosmosis, both of these are called electrokinetic phenomena. In this case, the cells acquire uniform drift velocity. This drift velocity is proportional to the applied field. So the cell drift velocity can be accommodated by changing the applied field. We applied this technique to cell optical tweezers experiments, and realized the measurement of a cell's OTF.

A method used to measure the force on trapped particles or beads in biological experiments is to estimate the "escape force". For a fixed system, experiments have indicated that the escape force increases linearly with laser beam power. It is assumed that the solution around the cell has electroosmosis velocity v_o. At the beginning, $E = 0$, the cell is trapped by optical tweezers. When applying the electric field, the trapping cell is all the same in the state of mechanical equilibrium, the force analysis is illustrated in Fig. 1. The OTF f_l is determined by the condition

$$f_l = f_r + f_{\mathrm{E}}, \tag{1}$$

where f_{E} is electric field force, and f_r is viscous drag force generated by a relative motion of the cell with respect to its liquid environment, due to the viscosity η of the liquid, we consider the cell as a spherical object of radius r, f_r is given by Stokes law as

$$f_r = 6\pi r\eta v_o. \tag{2}$$

Increasing the applied voltage gradually until the cell is carried away from the trap, at this point, the OTF rises to a maximum that the trap can exert, which is called escape force $f_{l\,\max}$. Holding the voltage, the velocity of the escaping cell released with zero initial velocity will have increased to a point where the drag force has caught up with the electric field force, the cell has reached terminal velocity v'. This terminal velocity is given by the condition

$$f_{\mathrm{E}} = f'_r, \qquad f'_r = 6\pi r\eta(v' - v_o). \tag{3}$$

Inserting Eqs. (2) and (3) into Eq. (1), we find

$$f_{l\,\max} = f_l = 6\pi r\eta v'. \tag{4}$$

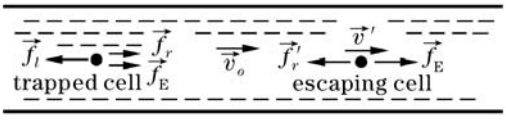

Fig. 1. Forces at the trapped and escaping cells.

Equation (4) indicates that the escape force $f_{l\max}$ can be obtained by measuring a cell's terminal velocity v' and the viscosity η of the liquid.

In order to realize the method above to measure a cell's OTF, we designed an electrokinetic sample chamber system (ESCS), whose structure diagram is shown in Fig. 2. It is a homebuilt sandwich construction of two glass coverslips separated by two pieces of organic glass, where the flow channel is formed. The size of the cross section is 75×25 (mm). There is a look-in hole for the inverted microscope objective in underside, called look-in chamber, whose wall is 0.17 mm thick. The width/height ratio is 10, and the height is 1 mm, this ensures constant electroosmosis velocity in microscope visual field. Salt bridge chambers are designed symmetrically at each end of the flow channel, which are used to place salt bridge (the gel constitutive of agar-agar and NaCl solution) and airproof the sample solution. The electrodes are inserted into the salt bridges and joined with a direct current (DC) power.

Figure 3 shows a schematic diagram of the experimental setup, which is built on the basis of a normal optical tweezers system, but has an ESCS instead of a common sample chamber and a costly automatism stage. A 780-nm diode laser (60 mW adjusted) is used as the light source of the optical tweezers. The laser beam is introduced into an inversed microscope, reflected by a dichromatic mirror which is inserted in the microscope, and incident upon a high numerical-aperture (100×, NA 1.25) oil-immersion microscope objective, which focuses the strongly convergent beam into the sample chamber to form the optical tweezers.

The observation of the motion of cells and the measurement of their velocities were finished by the charge-coupled device (CCD) camera and the image analysis software (30 frame/s). Firstly, we recorded the motion of cells in a period of time and got the position image sequence versus time; then, by choosing two frames of the images, the velocity was calculated by reading the cell's displacement and the time interval between the two images.

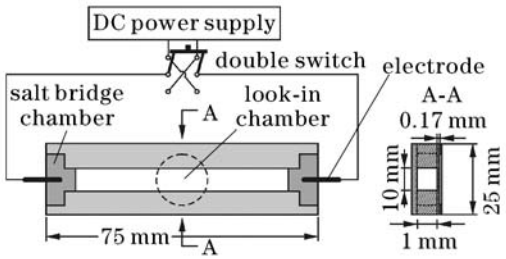

Fig. 2. Schematic diagram of the electrokinetic sample chamber system.

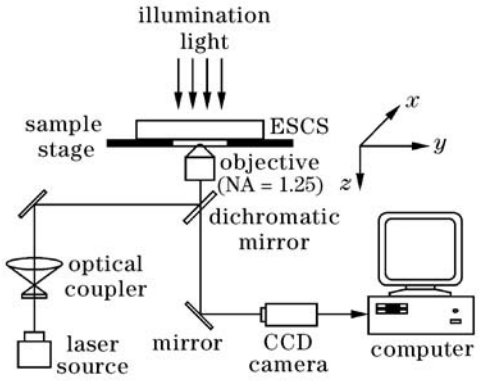

Fig. 3. Schematic diagram of the experimental setup.

In order to verify the feasibility of the method, we measured the average velocity of yeast cells in 0.9% NaCl solution as a function of voltage gradient at room temperature (25 °C). The results are shown in Fig. 4. Each velocity is an average of 20 yeast cells. The yeast cells' velocity was found to increase linearly with gradient voltage, as expected from literature reported.

By the ESCS coupled with optical trap, we measured the escape force upon yeast cells at different laser powers according to Eq. (4). The drag force was calculated via Stokes friction law, where $r = 2$ μm, $\eta = 10^{-3}$ Pa·s. The results are shown in Fig. 5, where each point is an average of 20 data sweeps. The results are similar with human red cells reported[10].

Electrophoresis is the phenomenum of migration of small charged particles suspended or dissolved in an aqueous electrolyte solution under the influence of an applied electric field. These "small particles" may be bacterial cells, viruses, globular protein molecules, or synthetic small particles. It turns out that most of these objects naturally carry electric charges. So the method can be used to them.

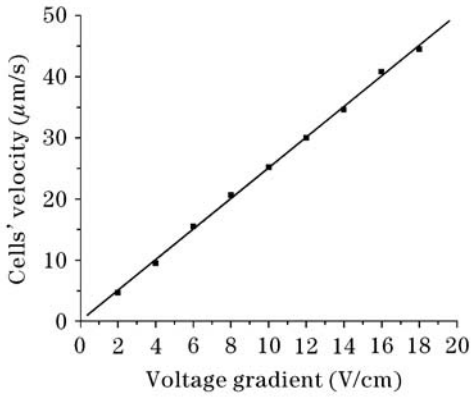

Fig. 4. Relationship between the voltage gradient and the cells' velocity. There is a linear relation between them over the range of 2—18 V/cm (correlation coefficient> 0.99).

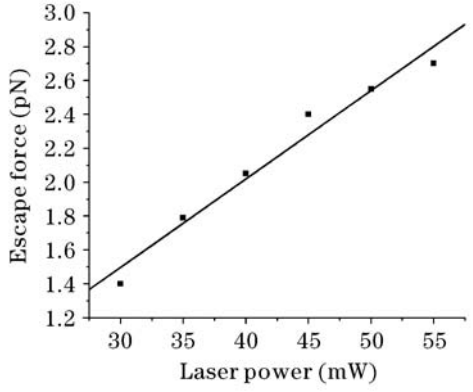

Fig. 5. Relationship between the laser power and the optical trap force on yeast cells (correlation coefficient> 0.98).

Notice that in order to avoid ohmic heating of the solution, the applied electric field must be kept small, a few volts per centimeter. Since the electrokinetic velocity is proportional to the applied field, a few dozen microns per second velocity can be measured with this method. For micron-size particles, the maximum escape trap force given by Stokes law is in the order of piconewton. This indicates that the range of the trap force measured by this method is piconewton and sub-piconewton. The maximum escape trap force of the yeast cells in this paper is about 3.6 pN, as would be expected. For biological particles, low power laser optical tweezers are often used without causing over damage. In this case, the optical trap force upon particles sometimes small, which makes new method be especially fit for non-invasive manipulation of biological particle with optical tweezers.

Since trapping is often done near a wall of the sample chamber, the shear field in the flow tends to push the trapped particle out of the focal plane towards the wall, the main drawback to Stokes friction method is that the drag must be corrected for the proximity to the wall, the effect of which can be large when the distance from the surface is comparable to the particle radius. The new method may overcome the problem, it is possible to apply an alternating electric field with such a frequency whose electroosmosis is suppressed to lead the shear field absent, whereas the particles are still able to follow the electric field according to their DC mobility[11]. For the first time, Galneder *et al.* combined microelectrophoresis and laser trap technologies to measure the electrophoretic force, thereby monitoring the activity of enzymes by using optical trap as a force transducer[12]. On the contrary, the new method may use alternating electric field to simultaneously calibrate trap force and obtain electrophoretic force, then use similar procedure in Ref. [12] to get the cell surface charge. Those should be the object of future studies.

In summary, we have put forward and demonstrated a method of measuring OTF by use of electrokinetic phenomena to produce velocity field. An ESCS adaptive for the measurement of OTF upon cells in an optical tweezer was designed and realized. The ESCS is used to manipulate the relative velocity of a cell with respect to its liquid environment, so a costly automatism stage is avoidable, and thus the system is simpler and cheaper. The principle experiments show that the method is feasible, and the measurement of OTF is in piconewton and sub-piconewton range. By applying an alternating electric field, the new method may overcome the problem of correcting drag force and allow us to measure simultaneously optical trap stiffness and particle electric charge. We believe that the method will find a variety of important applications in colloid science and cell biology.

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