Calibration of optical tweezers based on acousto-optic deflector and field programmable gate array

Qingbao Song (宋清宝), Cheng Wen (闻 丞), Yan Zhang (张 岩), Guangfu Wang (王广福), and Anpei Ye (叶安培)

School of Electronics Engineering and Computer Science, Peking University, Beijing 100871

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Accurate calibrations of stiffness and position are crucial to the quantitative measurement with optical tweezers. In this paper, we present a new calibration scheme for optical tweezers including stiffness and position calibrations. In our system, acousto-optic deflectors (AODs) are used as laser beam manipulating component. The AODs are controlled by a field programmable gate array (FPGA) connected to a computer using universal serial bus (USB) communication mode. Our results agree well with the present theory and other experimental results.

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The optical tweezers[1] have become an important tool in both physics and biology[2,3]. They have an ability to apply piconewton-level force to micron-sized particles, which are frequently used to study molecular motors at single-molecule level[4] and the physics of mesoscopic systems etc.[5] Among the techniques applied to measure extremely weak forces, optical tweezers are of particular interest since they allow both force measuring and manipulation without any mechanical contact and hence will not cause overt damage. In quantitative measurement using optical tweezers, it is very important to calibrate optical tweezers[6−14] accurately including position calibration and stiffness determination.

At present, there have been several kinds of methods to calibrate the tweezers[15], and here we give an alternative method. In brief, we steer optical tweezers in a sinusoidal pattern with a given amplitude. Then we measure the amplitude of the motion of the bead trapped by the tweezers with quadrant detector (QD). Thereafter, we can theoretically calculate the stiffness of the tweezers. It is necessary to notice that our method is different from Nemet's[13], which calibrates the stiffness of tweezers by measuring the phase difference between the motion of tweezers and that of the bead. The demonstrated method is also different from the approach introduced by Neuman *et al.*[15], in which the stage moves in a sinusoidal pattern but tweezers keep stable.

Our work is based on the following theory. In a single Gaussian-beam optical trap, a particle experiences a potential which can be approximated by a harmonic one. If the displacement of the particle from the center of the trap is not too large, the motion of a particle in such a harmonic potential is described by the Langevin equation. Considering the motion of the laser beam in Fig. 1, the Langevin equation for one dimension is

$$m_{\mathrm{b}}\frac{\mathrm{d}^2x_{\mathrm{b}}(t)}{\mathrm{d}t^2}=-\gamma\frac{\mathrm{d}x_{\mathrm{b}}(t)}{\mathrm{d}t}-k[x_{\mathrm{b}}(t)-x_{\mathrm{l}}(t)]+F(t), \quad (1)$$

where $x_{\mathrm{b}}(t)$ is the displacement of the bead, m_{b} is the mass of the bead, $x_{\mathrm{l}}(t)$ is the displacement of the laser beam, γ is the friction coefficient, k is the stiffness of the optical tweezers, $F(t)$ is the random force with zero mean. $\gamma=6\pi\eta a$, η is the viscosity of the liquid (for water at 20 °C, $\eta=1.009\times10^{-3}$ Pa·s), a is the radius of the bead.

The random force $F(t)$ is much smaller than the force exerted by the tweezers, $k[x_{\mathrm{b}}(t)-x_{\mathrm{l}}(t)]$, so it can be omitted. In the case of low Reynolds number, the motion of a trapped bead is approximate to an over damping vibrator and the inertia force can be ignored compared with the viscous force[16]. Therefore, the inertial term in Eq. (1) is negligible. Since the laser beam moves in a sinusoidal trajectory in our experiment, namely $x_{\mathrm{l}}(t)=A_0\sin 2\pi ft$, where A_0 and f are the amplitude and the frequency of tweezer motion, respectively, the solution of Eq. (1) is

$$x_{\mathrm{b}}(t)=c\mathrm{e}^{-2\pi f_0 t}+\frac{A_0}{\sqrt{(f/f_0)^2+1}}\sin(2\pi ft+\varphi), \quad (2)$$

where $f_0=k/(2\pi\gamma)$ is the characteristic roll frequency, c is a constant which can be determined by initial conditions, and $\varphi=-\arctan(f/f_0)$.

If we measure the amplitude of the bead after a time which is much longer than $1/f_0$, the first term on the right side of Eq. (2) can be omitted. Therefore, the amplitude of the bead motion A and the amplitude of the

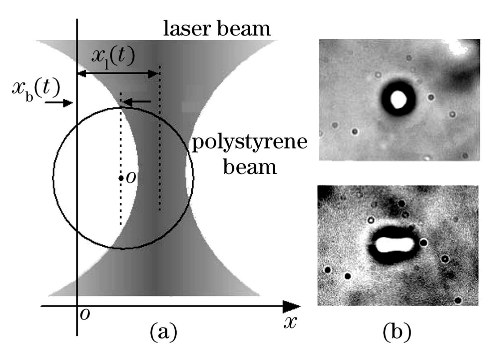

Fig. 1. (a) Schematic sketch of our calibration method; (b) the bead held by the tweezers when the tweezers are stable and scanning, respectively.

tweezer motion A_0 have the following relation,

$$A = \frac{A_0}{\sqrt{(f/f_0)^2 + 1}}. \quad (3)$$

The motion control is achieved by an acousto-optic deflector (AOD), which is modulated with a field programmable gate array (FPGA). Thus, A_0 and f can be given by a computer. The amplitude of the bead motion A can be measured with the QD during our experiment. Therefore f_0 can be calculated from Eq. (3), and then we can calculate the stiffness from

$$k = 2\pi\gamma f_0 = 2\pi\gamma f/\sqrt{(A_0/A)^2 - 1}. \quad (4)$$

Our experiments were performed in a custom-built inverted microscope, as depicted in Fig. 2. Nd:YAG laser (1064 nm) was directed through a 1:10 telescope system, and then the laser went through the AOD, which was used to steer the direction of the laser beam. In front of the AOD, there was a polarizer to change the polarization of the laser beam to satisfy the requirement of the AOD. The first-order deflected beam was coupled into the objective (100×, oil-immersion, numerical aperture (NA) = 1.25) to form optical tweezers. A halogen lamp illuminated the sample. The image of beads was collected with a charge-coupled device (CCD) camera. The QD was used to detect the displacement of the particle with the illuminating light going through the sample. The diameter of the polystyrene bead (refractive index $n = 1.59$, made by Sigma-Aldrich Company) was 5 μm with an error of ± 0.1 μm.

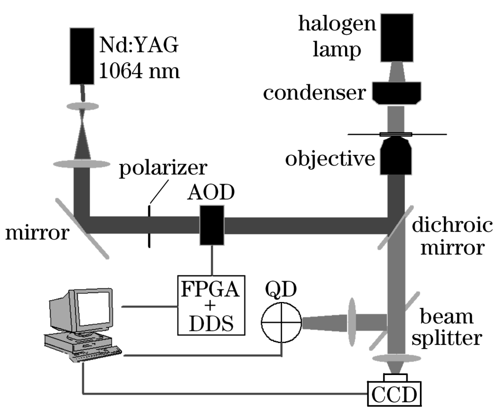

Fig. 2. Experimental setup. DDS: direct digital synthesizer.

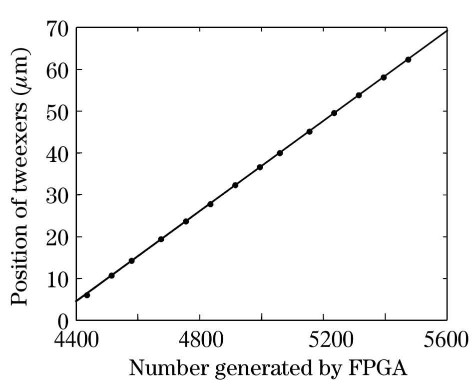

Fig. 3. Dependence of position of the tweezers on numbers generated by FPGA. Experimental results are labelled with dots.

Firstly, we calibrated the AOD with a grating. The distance between the grooves in the grating was known, and the precision was pretty high. We let the tweezers move from one groove to another by changing the FPGA number, as shown in Fig. 3. We could know that the displacement of the tweezers was 0.0541 μm if the FPGA number was increased by one.

Secondly, we determined the relation between the voltage signal from QD and the real value of the amplitude of the bead motion. We let the tweezers scan very slowly with the frequency of 0.24 Hz and amplitude of 0.812 μm (actually amplitude from 0.8 to 1.2 μm all can satisfy the requirement of harmonic approximation, and can ensure the good linearity and high signal-to-noise ratio of the QD's output), respectively. The laser power held a large value, and this made f_0 a large value, about 400 Hz. According to the Eq. (3), we could get a good approximation that $A \approx A_0$. Here, A_0 was known. Under the above conditions, the corresponding voltage signal from QD was 2.4734 V. Therefore the relation between the voltage signal from QD v and the real value of the amplitude of the bead motion A can be determined as $A/v = 0.328$ μm/V.

Thirdly, the tweezers do sinusoidal scanning at a fixed amplitude of 0.812 μm. The power of the laser was fixed at 155 mW. The frequency of tweezers motion was changed, and the corresponding amplitude of bead movement could be measured. The results are shown in Fig. 4. Fitting the experimental result with Eq. (3), the characteristic roll frequency f_0 was obtained, and then we can calculated the stiffness with Eq. (4).

At last, we changed the power of the tweezers from 23 to 153 mW and repeated the process described above. Thus the stiffness of the tweezers at different laser powers could be obtained, as shown in Fig. 5. The figure reveals that the stiffness has good linearity versus laser power, and accords with the following relation: k (pN/μm) = 0.415 × P (mW). So the slope is 0.415 pN·μm^{-1}·mW^{-1} under our experimental conditions. This value agrees well with 0.42 pN·μm^{-1}·mW^{-1} from Simmons[7]. Their conditions (NA = 1.25, $\lambda = 1064$ nm, $d = 3$ μm) are similar to ours. We note that, for large particles ($d \gg \lambda$), where the ray optic regime holds, particles intercept all the converging rays at the laser focus, so the trapping force is the same, irrespective of diameter[7]. Therefore the above two results are accurately comparable with each other.

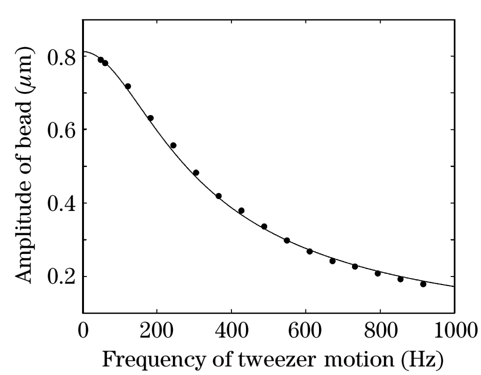

Fig. 4. Dependence of amplitude of the bead on frequency of the tweezer motion. Experimental results (dots) are fitted with Eq. (3).

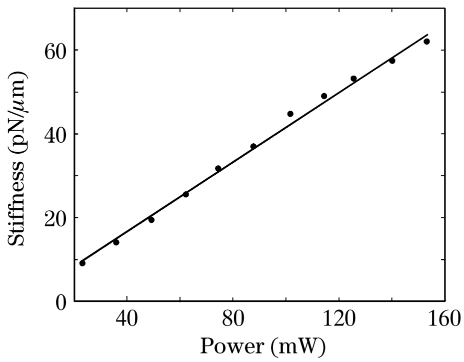

Fig. 5. Dependence of tweezer stiffness on laser power. The dots are experimental results.

The precision of our method depends on the error of the temperature, the diameter of the bead, the viscosity of the liquid, and the precision of the QD. Compared with other methods such as moving the stage or power spectrum, our method does not involve other factors that can reduce the precision, e.g. the turbulent flow for the method of moving the stage and the poor signal-to-noise ratio when laser power is high for the method of power spectrum. Our method has several advantages. Firstly, the bead used for the calibration is just the one at the same position in the following experiment, but the method of moving the stage cannot do this. Secondly, determining the stiffness of tweezers by the power spectrum method requires a detector system with sufficient bandwidth to record faithfully the power spectrum well beyond the rolloff frequency[15], and the power of tweezers should not be too large. But our method can overcome the above two disadvantages. Thirdly, because the state for calibration can hold on for a long time, and AOD can scan very fast, our method is convenient and fast relatively.

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