Displacement and Force Measurements with Quadrant Photodetector in Optical Tweezers *

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A technique of displacement and force measurements with a photodiode quadrant detector in an optical tweezers system is presented. The stiffness of optical trap is calibrated and the leukemia cell membrane tension is measured. The results show that the optical tweezers combined with the quadrant detector is a very useful tool for detecting the displacement and force with a millisecond-order response.

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In recent years, applications of optical tweezers in biology have attracted significant attention. The potential utility is opened in biological field since Ashkin found that the optical tweezers could be used to trap and manipulate viruses, bacteria and cells.[1,2] In the study of the trapping force, it is found that the force in the range from piconewtons to tens of piconewtons is well suitable for the study of mechanical properties of cells, sub-cells and macromolecules. In addition, due to characteristics of non-contact and non-intrusive manipulation of living objects, the optical tweezers combined with the high-precision displacement and the force measurement system become one of the most powerful tools for study of the mechanical properties of biological objects.[3−10] In the measurements of displacement and force, the standard weighted centroid calculation for the image of an object is widely used due to its simplicity and high precision.[11,12] However, the range of the force measurements is limited by its response time and the standard weighted centroid calculation cannot be used to measure the fast change of displacement and force. Moreover, the data processing is so time-consuming that the results cannot be obtained in time. In contrast, the photodiode quadrant detector (QD), owing to its fast time response and simple data processing, becomes an alternative device used for displacement and force measurements. In this Letter, we theoretically and experimentally describe the displacement and force measurements with QD in optical tweezers system and exhibit the typical results of force calibration and leukemia cell membrane tension measurements in details.

In the optical tweezers system, dielectric particle such as polystyrene bead is usually used as a *handle* by binding to the biological objects so that the mutual interaction and mechanical properties of the biological objects can be learned by measuring the displacement of the trapped bead and the force on it. The trapping force on bead is similar to that of an object attached to a spring and it is commonly calibrated against the viscous drag exerted by fluid flow. The relative velocity between the trapped bead and fluid is produced by a piezo-electronic driven stage (Nanonics NIS-70). In our experiments, the scientific microscope (Leica DMIRB) and oil-immersion objective (100× NA 1.3) are used for observing the micro-objects and focusing the laser beam. The bead is magnified 100× and 5× by the objective and the auxiliary lens, respectively, then projected onto the front surface of the QD (Hamamtsu1557-03), so the displacement of the bead can be detected with QD. The schematic diagram can be seen in Ref. [13], except that the CCD camera is replaced by the QD.

The data acquisition process is as follows. Firstly, the photocurrent from the bead image on the surface of four independent photodiodes is converted to the corresponding voltage by the first-stage $I-V$ converters, then the second and third stages of the electronics, respectively, add and subtract the quadrant signals to generate the x- and y-positions with the output voltages. Finally, these analogue signals are digitized by 12-bit A/D converters (HY 8201), then acquired and saved in a computer. The quadrant detector circuit can be seen in Ref. [14].

Because the intensity differences between the bead image and background are very small, an appropriately designed illumination path and suitable optical magnification of the bead are important to acquire the desired output signal. In order to know the relationship between the bead magnification and output signal of QD, we carried out the numerical simulation. In the calculation, the diameters of the QD and bead

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are 1 mm and 1 μm, respectively. Under the assumption that the distribution of optical intensity is homogeneous and incidence aperture angle is constant, the dependence of output signal on the optical magnification is shown in Fig. 1. The seven curves represent the output signal change with the displacement of the bead from the QD centre to out of the QD under the different bead magnifications which vary from $A = 400$ to $A = 1000$. It can be seen that the output signal increases with the displacement of the bead initially. When the bead moves to some extent, the output signal reaches its maximum, then gradually decreases to zero with the bead moving out of the QD. In addition, the maximal output signal decreases with increase of the magnification and the linear ranges of the output signal become shorter when the magnification is larger than 500. In practice, the displacement range of the bead that can be well trapped by optical tweezers is within a radius of about 0.3 μm. Taking all these factors into consideration, we found that it is appropriate to choose the magnification from 400 to 600 for an ideal output signal.

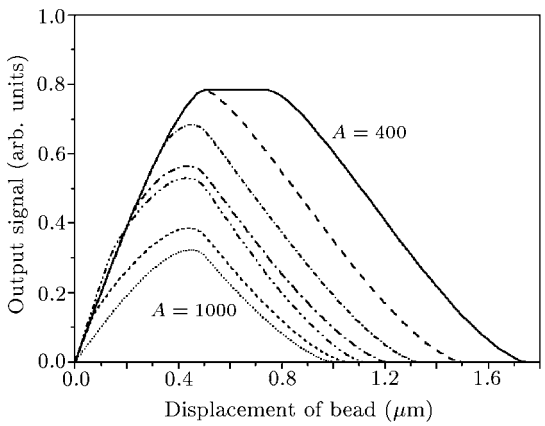

Fig. 1. Dependence of output signal on the bead displacement under different magnifications from $A = 400$ to $A = 1000$.

In order to know the real displacement of bead, the dependence of the QD output signal on the bead displacement must be calibrated experimentally. In our experiments, by scanning the QD in the x direction, the relative displacement of the image of a trapped bead to the QD is indicated by the output voltage of QD, as shown in Fig. 2. In this figure, the horizontal coordinates represent the readings on micrometre in the x direction of the QD. It can be seen that the output voltage changes linearly with the displacement of the bead image in a certain region. The slope of the linear fit for this region gives the ratio of output voltage to the displacement of bead image. The ratio of the voltage to the real displacement of the bead can be obtained by multiplying the magnification of the bead in the experiment. In our experiments, the magnification is about 505, so their ratio is 4.8 mV/nm. Moreover, according to Fig. 2, we can also learn the deviation of the bead from the QD centre. Consequently, we can adjust the position of the QD to ensure that the centre of the bead is coincident with that of the QD.

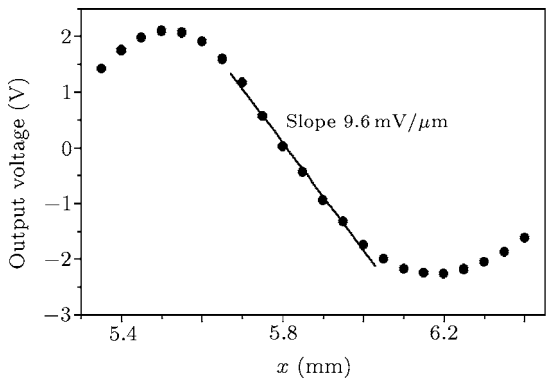

Fig. 2. Output signal versus displacement x of bead image with respect to QD. The dots represent the output voltages acquired by moving the QD with a step of 50 μm in the x direction. A linear fit of the chosen range gives the ratio of output voltage to displacement of the bead image.

In the force calibration, a fluid dynamics method is adopted to calibrate the force. The fluid flows through the trapped bead and the bead is displaced away from the centre of the trap due to the viscous force exerted by the fluid flow. When the viscous force equals to the trapping force, the bead stays in a stable position. Thus, the force can be calculated from Stokes' law:[15] $F = 6\pi\eta r v$, where v is the velocity of fluid, η is the viscosity of fluid, and r is the radius of bead. In our experiments, the 1-μm polystyrene bead is taken as a standard object for the force calibration and the trapping stiffness is 0.1 pN/nm at the laser power of 305 mW.

As an application, we measured the dynamic and static forces on membrane tethers of leukemia cells. Two representative results are shown in Figs. 3 and 4. In details, we take the logarithm prophase leucocytes and lectin-coated polystyrene beads to the poly l-lysine coated sample cell with the amount ratio of 2:1. Then we mix them and put a cover slip onto the sample cell and seal it with resin. We place the sample cell on the sub-stage of a microscope. Because of the adhesion of poly l-lysine, most leukemia cells are attached to the bottom of the sample cell. We make a bead trapped and then make a chosen leukemia cell approach the trapped bead. Taking a few seconds to ensure a stable combination, we drag the leukemia cell away from the bead at a constant velocity by moving the stage. When the cell membrane is displaced from the trapped bead, a tether is formed between the bead and membrane and the trapped bead is displaced from the trap centre. The displacement of the trapped bead is detected by the QD. Thus, the tether force can be

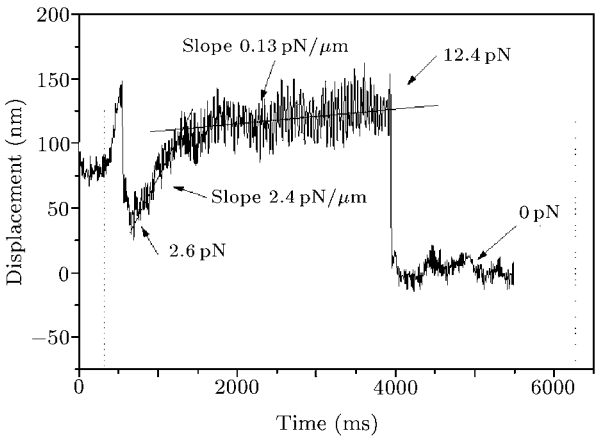

Fig. 3. Displacement of trapped bead during the membrane tether formation and break.

calculated by the displacement of bead. Figure 3 shows the displacement of the trapped bead under the drag of the cell which is combined with the bead at the velocity of 4.4 μm/s. The duration of the movement of the stage is 6 s. The two vertical lines in Fig. 3 represent the beginning and finish points of stage movement, respectively. The output signal is acquired once every millisecond. Before the movement of the stage, the bead stays at the centre of the trap and the displacement of the bead should be zero. However, due to the influence of leukemia cells on imaging of the bead, an apparent displacement of the bead comes into existence and increases with the drag of the leukemia cell. Then an abrupt decrease of the measured displacement occurs as the image of the leukemia cell is completely out of the QD. At this time, the displacement of the bead can correctly reflect the membrane tether force. It can be seen from Fig. 3 that the displacement of the bead is no longer zero, which is because the tether has been formed between the cell membrane and the bead. The corresponding tether force is 2.6 pN as shown in Fig. 3. With the elongation of the tether, the force on the tether increases by the slope of 2.4 pN/μm. When the tether elongates to some extent, increasing the force becomes slower and the elongation of 1 μm corresponds to the force increase of 0.13 pN. Then the tether breaks at the force of 12.4 pN, the bead returns to the trap centre, and the data acquiring terminates before stopping the movement of the stage. The process shown in Fig. 4 is similar to Fig. 3, but the tether always exists during the movement of the stage. After stopping the movement, the displacement of the bead decreases and the corresponding force of 8.4 pN marked in Fig. 4 represents the static force on the tether. From these two figures, some key parameters can be obtained. Firstly, it can be seen that the response time of QD is at a millisecond scale. Secondly, the influence of the leukemia cell on the displacement of the bead is clearly reflected. Thirdly, the dynamic and static tether forces can be learned and accordingly the membrane tension and viscosity can be calculated. Furthermore, the two different change rates of the force during the tether elongation imply that the cell membrane deformation may be based on two different kinds of membrane materials. All these results indicate that our system can be well used to study the membrane mechanical property.

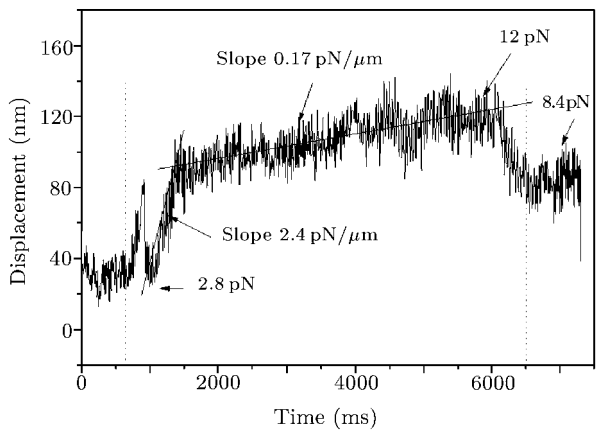

Fig. 4. Displacement of a trapped bead before and after stopping movement of the stage. The dynamic and static tether forces are calculated by the displacement of the bead.

In conclusion, we have provided the theoretical calculation and experimental measurements of both displacement of a bead and the force of optical tweezers on the bead with a QD. The theoretical calculation shows that the bead magnification chosen to be about 500 may be advantageous for a desired output signal of the QD. The experimental results show that the response time of the QD is in a millisecond scale, which means that the real-time measurement of the displacement and the force can be carried out based on this system. This special characteristic just meets the need of study of the mechanical property of biological objects.

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