Influence of Illumination Conditions on Temperature in Sample Cell and the Output of a Quadrant Detector in an Optical Tweezers System

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Introduction

In an optical tweezers system, the output signal of a photo-diode quadrant detector and the temperature in a sample cell are two key factors for the quantitative measurements of mechanical properties of living biological objects such as cells, organelles and macro-molecules. In order to enhance the output of a quadrant detector and effectively control the temperature in a sample cell, the dependence of the temperature in the sample cell and the output of the quadrant detector for different illumination conditions are studied. The results show that appropriate illumination conditions can ensure both nearly constant temperatures in the cell and the desired output signal, which provides for the possibility of high-precision and damage-free analysis of living biological objects.

In an optical tweezers system, a CCD camera and a photodi-ode quadrant detector (QD) are employed to make displacement measurements. The precision of a CCD measurement is superior, so CCD measurements are routinely used in experiments of tiny displacement measurements, such as the measurement of the 8-nm step displacement of a kinesin motor.[1] However, the time response of a CCD is very limited, so for measurements of rapid motion, such

Fig.2 Schematic diagram of path illumination path for the optical tweezers. Infrared light is blocked by the heat filter. Symmetrical illumination light is obtained with a ground-glass diffuser. Kohler illumination is formed with the Field diaphragm, aperture diaphragm, and condenser (C1, C2). Piezo-electric-transducers (PZT) are used for micro-operation of the sample cell and force calibration; The sample in the object plane of the objective is imaged on the surface of the QD.

as the measurement of the step of myosin motor,[2] a QD is usually used instead of a CCD.

The sensitive wavelength range of most QDs is in the near infrared, which happens to be the region of strong heat absorption of water. In order to avoid water absorption-induced temperature increases in the sample cell, the infrared wavelength components of the microscope standard illumination are blocked. This has little effect on displacement measurements with a CCD. However, this will result in a decreased output signal from a QD, which, at best, is only in the range of tens of millivolts, while the noise signal from a QD is in the level of millivolts–making the signal-to-noise ratio (SNR) of the measurement system problematical. To increase the SNR, the standard illumination conditions of the microscope should be changed to guarantee the required output signal of the QD, but the consequence of this change is to maximize the near infrared light. This will bring about a temperature rise in sample cell because of water absorption, which is usually fatal for the study of living objects.

The volume of the sample cell in an optical tweezers is usually very small–only about 100 μl–so the temperature in a sample cell is easily affected by its environment. The effect of an optical laser on the temperature in a sample cell has been studied in the references[3-5] and laser-induced cell damage with different wavelengths has been reported.[6-9] Using an infrared trap laser as the input of a QD can greatly increase the output of the QD while having little influence on the temperature inside the sample cell.[5,10,11] In this way, however, the relationship of the motion of a bead image on the QD plane and the actual motion of a bead in the objective plane is nonlinear, and this will make it very difficult to exactly calibrate the output of a QD and the trap stiffness. Certainly it can be considered as linear within a certain range of deviation from the trap center, but the scope is very limited when a trap laser is

Fig.1 Thermocouple Calibration. The thermocouple is put into a water bath at different temperatures. The temperature of the water is measured by a calibrated thermometer to ± 0.1°C. The thermocouple output microvoltmeter data is fitted with a quadratic function. The standard deviation of this fit is 0.133°C, yielding a thermocouple temperature measurement accuracy of 0.5°C.

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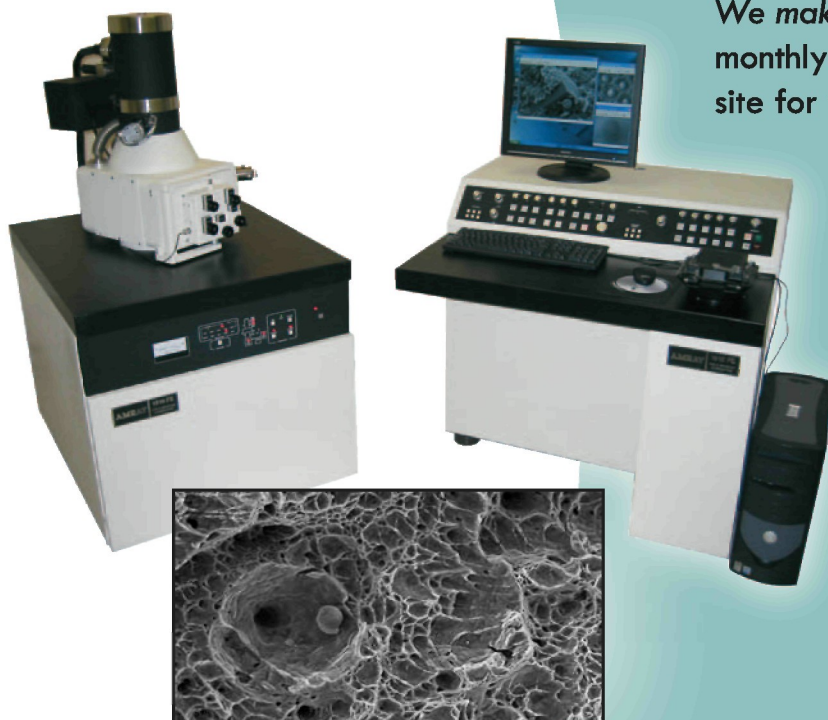We specialize in pre-owned, rebuilt and upgraded products, including:

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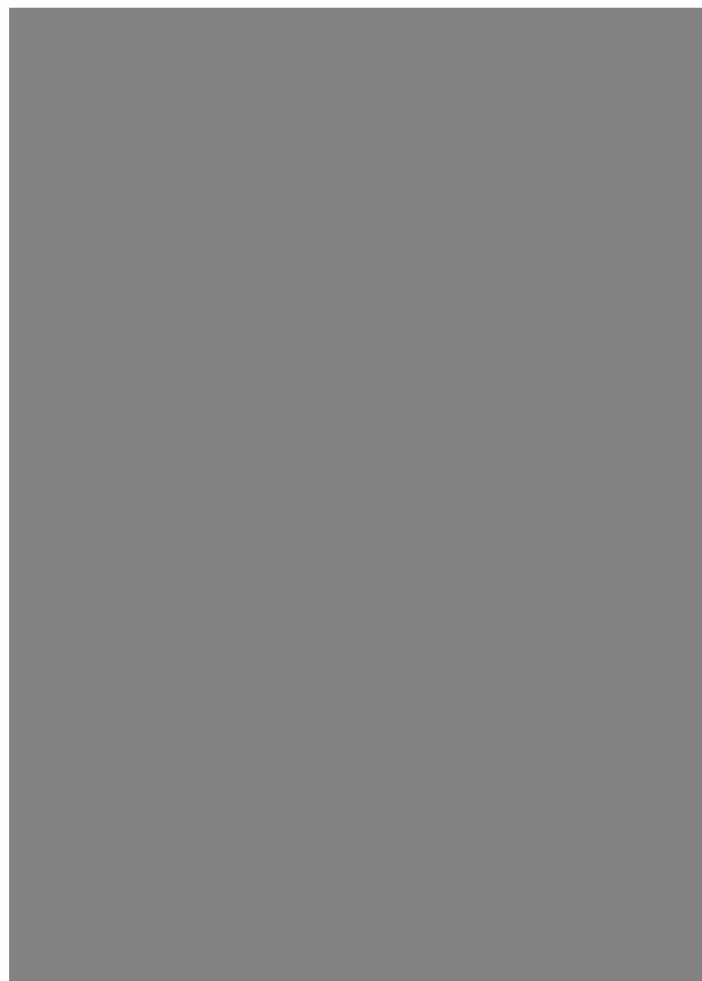North Billerica, MA 01862
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Fig.5 Dependence of the temperature in the sample cell and the output of the QD on the voltage of light lamp when the aperture of the field diaphragm is set to correspond to the field-of-view.

Comparatively, using the illumination light of the microscope as the input to a QD is a convenient method if it is feasible to compensate for the heating effects of the infrared component of the illumination light. Few reports, however, concern the influence of illumination light on the temperature and output signal of QD.

In order to find the desired illumination conditions for both constant temperature and a larger output signal from the QD, we designed a special sample cell, which is equipped with a thermocouple and a cooling sub-system. The influence of different illumination conditions on the temperature in the sample cell and output signal of the experimental QD is studied systematically.

Materials and Methods

The optical tweezers system consists mainly of an inverted optical microscope ((Leica DMIRB, Germany) with a high numerical aperture objective (HCXAPO 100× / NA 1.30) and a photodiode quadrant detector (Hamamtsu1557-03). The sample cell with a built-in temperature sensor is designed by ourselves and its volume is about 150 μl ($\phi10\times2$ mm). The sensor is a thermocouple made of copper and constantan, and its position in the sample cell is adjustable so that the temperature in different locations in the sample cell can be measured. The temperature calibration curve is shown as Fig.1, the accuracy of the temperature measurement is ±0.5°C. The schematic diagram of the optical tweezers system can be seen in reference [12], the only difference is that the CCD camera is replaced by the QD. The detailed illumination light path is shown in Fig.2.

Fig.3 Dependence of the temperature in the sample cell and the output of the QD on the voltage of illumination lamp. (a) With the heat filter in the illumination light path. (b) Without the heat filter in the illumination light path. The voltage of the lamp is changed from 2.5V to 12V. The temperature in the sample cell is monitored with a thermocouple system. At each point, when the temperature is stable (illumination time duration is about 2 mins), the value of temperature in the sample cell and the output of the QD are recorded.

used as an input of a QD.

An ideal arrangement would be to use an infrared laser as an input to the QD instead of the standard illumination light of the microscope in the illumination path. But, this needs a larger laser and considerable changes to the microscope structure.
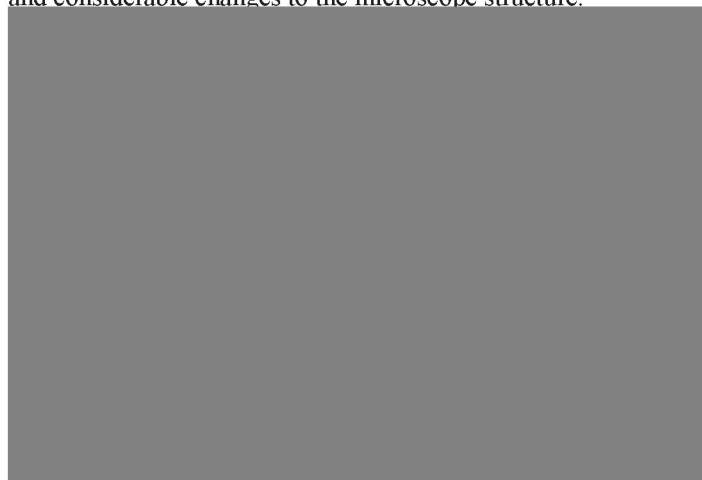

Fig.4 Dependence of the temperature in the sample cell and the output of the QD on the field diaphragm aperture size.

Fig.6 Dependence of the temperature in the sample cell and the output of the QD on the value of the aperture diaphragm.

Firstly, we shall study the influence of the heat filter in the illumination path and the voltage of the illuminating lamp on the temperature inside the sample cell and the output signal of the QD. Under standard illumination conditions of the microscope, i.e. with the heat filter in place, the dependence of the temperature and output signal on the voltage of the illumination lamp is measured under the condition of maximum field and aperture diaphragms openings. The output signal of the QD is determined as follows: first, a 1-μm-diameter polystyrene bead is trapped by the laser tweezers, then we adjust the position of the QD to make sure that the bead image is in the center of the QD. At this time, the output of the QD should be zero, we then adjust the QD to make the QD center deviate from the trap center until the output of QD reaches its maximum. In our experiment, the QD is moved 0.3mm from its center position, which is corresponding to 500nm displacement in the object plane of objective. By comparing the maximum outputs, the influence of illumination conditions on the output of the QD can be determined. Fig. 3a shows the dependence of temperature and output signal on the voltage of the lamp at room temperature (20°C). It can be seen that the temperature in the sample cell changes from 26°C to 33°C with an increase of voltage from 2.5V to 12V. Despite the fact that the temperature change is not very large, the output signal of QD is small. When the voltage reaches its maximum of 12V, the output of the QD is only 55mV. Allowing for its linear range, the output signal that can be best used in practice is about 30mV. Under this condition, the nanometer steps of some biological motor proteins corresponds to just a few millivolts output signal from the QD. Such a small signal is obviously not enough for the quantitative measurements of small displacements.

In order to enhance the SNR of the QD, an improved design for the illumination light path is put forward. We take away the heat filter from the light path. Then the light sensitivity of the QD is compensated for. Fig.3b is the dependence of temperature and output signal on the voltage of the lamp; the illumination conditions are similar to that in Fig.3a except that the heat filter is removed in this experiment. From Fig.3b we can see that the output of the QD increases almost linearly from 28 mV to 450 mV with the increase of lamp voltage. This results show that the output of the QD is an order of amplitude larger than that with the heat filter in the illumination path, increasing the SNR of the QD greatly. Taking this advantage into consideration, it can be concluded that the heat filter should be taken away. However, its unfavorable aspect is that the temperature in the sample cell increases greatly without the heat filter in place. When the lamp voltage is set to 12V, the temperature in the sample cell can reach 80°C, which is fatal for living biological samples.

To guarantee both a large output signal from the QD and a minimal temperature increase in the sample cell, the effect of different illumination conditions, such as varying the apertures of the field diaphragm and on the output of the QD, while monitoring the temperature in the sample cell are studied after removing the heat filter from the light path. The changes of the output of the QD and the temperature in the sample cell with the aperture of the field diaphragm are shown in Fig.4, where the lamp voltage is set to be 12V and the aperture diaphragm opened to its maximum. Similar results are obtained with different lamp voltage and the aperture diaphragm. So we take the Fig. 4 case as an example

to analyze. It can be seen from Fig.4 that as long as the aperture of the field diaphragm is not less than the field of view (1.4mm diameter of diaphragm or 200μm in the object plane of the objective), there is no obvious effect on the output of the QD, whereas the temperature in the sample cell is affected strongly by opening or closing the field diaphragm. The temperature varies from 80°C to 23°C as the field diaphragm closes from its maximum (φ30mm) to minimum(φ0.5mm), which indicates that when the aperture of field diaphragm is adjusted to a suitable size, both the large output of the QD and the small temperature increase in the sample cell can be assured. Fig. 5 is the dependence of the QD output and the temperature in the sample cell while varying the lamp voltage under the condition that the field diaphragm is set to correspond to the field of view and the aperture diaphragm opened to its maximum. It shows that there is no obvious temperature change with increase in lamp voltage while the output of the QD increases greatly.

Next, the effect of varying the aperture diaphragm on the temperature and the output signal is studied. In this experiment, the field diaphragm is fixed to correspond to the field of view and the lamp voltage is set to 12V. Fig.6 shows the change of temperature in the sample cell and the output of the QD with different aperture diaphragm sizes. It can be seen that the output signal of the QD changes greatly with adjusting the aperture diaphragm while the effect on the temperature is small. With opening of the aperture diaphragm, the output of the QD increases at first, then reaches a maximum when the aperture of the diaphragm is 8, and then decreases slowly.

Discussion

For optical tweezers combined with a QD as a means to measure the displacement and force of moving micro objects, the ideal illumination condition requires both a high SNR and a constant temperature in the sample cell.

The SNR of QD can be described as follow:

$$\qquad\qquad\qquad\qquad\qquad\qquad (1)$$

Where V_N is noise voltage of the QD. It can be determined by measuring the output of a QD when a fixed bead in the object plane of the objective is imaged on the center of the QD. For a stable system, V_N can be considered to be a constant. V_s is the signal output of the QD corresponding to the trapped bead motion. This is related directly to the displacement of the bead and the intensity of the effective illumination light. V_s can be expressed as:

$$\qquad\qquad\qquad\qquad\qquad\qquad (2)$$

Where S is the sensitivity of the photodiode, and k is a constant that is related to the magnification factor of the electric circuit of the QD. I_m is the light intensity of the bead image; I_n is the light intensity of the background, Δx is the displacement of the bead image on the QD, and r is the radius of the bead image on the QD.

For biological experiments, such as myosin and kinesin, the displacement step is usually very small. So, to acquire a desired high V_s, the intensity of the illumination light that is sensitive to the QD has to be enhanced. However, the sensitive wavelength

range of most QDs is within the near infrared, which will inevitably raise the temperature in the sample cell.

Taking all these situations into account, the conclusion can be drawn from our experimental results that in order to obtain the larger output signal and minimal temperature change, the heat filter should be removed from the light path and the field diaphragm and aperture diaphragm should be adjusted to appropriate sizes.

For the situations shown in Fig.5 and Fig.6, when the heat filter is removed, the lamp voltage is set to 12V, the field diaphragm corresponds to the view field and the aperture diaphragm is in position 8, the influence of the illumination conditions on the SNR compared with standard illumination shown as Fig.3 can be estimated. After removing heat filter from the illumination path, the output of the QD increases from 55mV to 450mV while the temperature changes no more than 3°C. The change of V_N can be neglected, but the SNR can be improved:
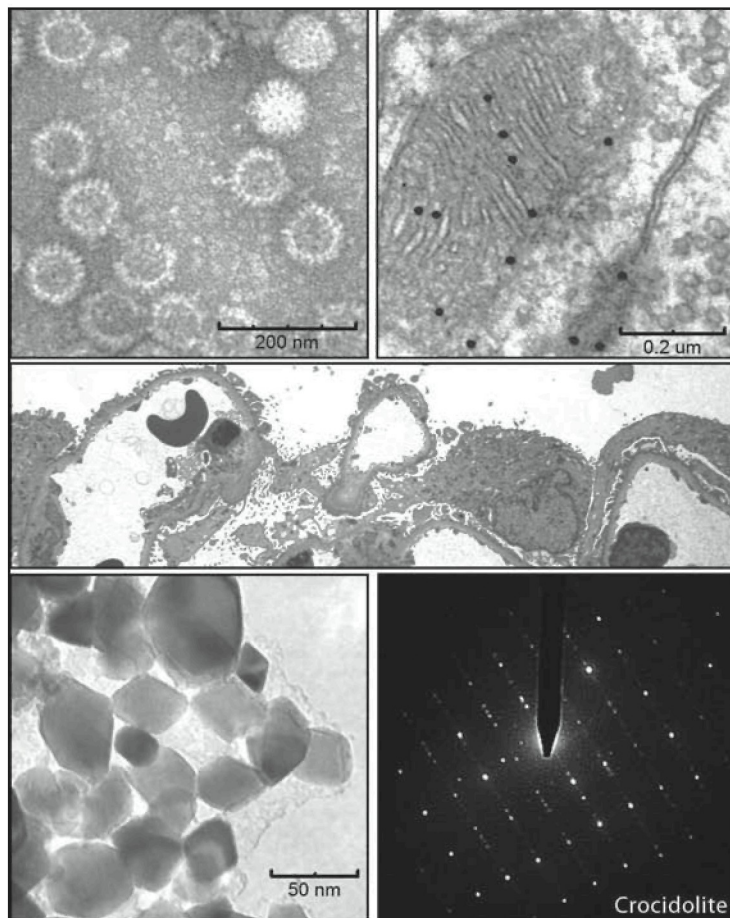
$$\Delta_{SNR} = \Delta V_S / V_N = (450 - 55)/V_N$$

$$\delta_{SNR} = \Delta_{SNR}/SNR \cong 800$$

This is a very favorable result for both the signal strength from the QD and for maintaining a constant temperature in the sample cell, providing for the possibility of quantitative measurements of nanometer displacement and piconewton forces. ■

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