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Oscillatory disturbance in force calibration of optical tweezers*

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In the calibration of the optical trap stiffness, it is found that there appears an attenuating oscillation as an oscillatory disturbance added to the trapped bead movement, when the scanner is driven by a triangular wave input. An equivalent oscillator model is put forward to explain the mechanism of the oscillatory disturbance. Both the measurements and calculations show that the attenuating oscillation comes from the oscillation of the scanner and the triangular wave drive causes this additional oscillation of the scanner. Furthermore, the analysis indicates that the oscillatory disturbance will become stronger, when the stiffness of the trap increases or the natural frequency of the scanner decreases. We adopt another driving way, i.e. a sinusoidal wave input is used instead of the triangular wave input. Our experiment has verified that in this case the oscillatory disturbance is eliminated completely.

Keywords: optical tweezers, force calibration, oscillatory disturbance
PACC: 3280P, 4260, 3310G

1. Introduction

In recent years, optical tweezers have become an important means for biological researches. Living organism can be manipulated in the non-contact and non-intrusive way. Meanwhile, the optical tweezers have been extensively used to measure accurately the interaction force between two objects and the displacement of biological object of sub-micro size.[1−6] In order to know the trapping force accurately, the force calibration must be carried out. There have existed several methods of force calibration, such as fluid dynamics method based on Stokes' law,[7−9] Brownian motion analysis,[7,8] and the method by measuring the response time of trapped bead to the rapid displacement of optical trap.[7,9] Fluid dynamics method is widely used owing to its high precision. A viscous force is applied to the trapped bead when a piezoelectric transducers (PZTs) -controlled stage (i.e. a scanner) is moved, on which a sample cell is located. The force can be calculated according to the Stokes' law and then the relationship between trapping force and displacement of trapped bead can be established. Usually the scanner is driven by a triangular wave input. However, in our experiments we find that there appears an attenuating oscillation superposed on the regular square-wave periodic motion of the trapped bead. This oscillation degrades the stability of the trapped bead and shrinks the force calibration range. The cause of the oscillatory disturbance may be understood as follows. When the scanner, driven by a triangular wave input, moves to its turning point, the remaining kinetic energy of the scanner can be converted into the oscillation energy of the scanner, which further induces an additional oscillation of the trapped bead due to the viscosity of solution. It is well known that when the scanning frequency of the scanner is close to its natural resonant frequency, the scanner will oscillate. If it is far from the resonant frequency, the induced oscillation is negligible. However, our observation shows that as long as the scanner's speed is not zero at its turning point, the release of

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its kinetic energy may still result in an additional oscillatory disturbance. In this paper, we report our experimental and theoretical studies on the cause of the oscillatory disturbance. Moreover, a substitution method is adopted, i.e. the scanner is driven by a sinusoidal wave input. In this case the additional disturbance is completely avoided.

2. The equivalent oscillator model of the scanner

The schematic diagram of our optical tweezers and the detection system have been described in Refs.[10] and [11], respectively. In our experiment, we adopt fluid dynamics method for the trap stiffness calibration. When the scanner is driven by a triangular wave, it is found that there appears an attenuating oscillation superposed on the periodic motion of the trapped bead, as shown in Fig.1. The scanner is a three-dimensional piezoelectric scanning stage (NIS-70, Nanonics, Israel). Its scanning ranges in x, y and z directions are all up to 70μm and the displacement resolution is at nanometer level. The structure of the scanner is described in Ref.[12] in detail. Based on its structure, an equivalent oscillator model is assumed to analyse the source of the oscillation. We regard the scanner, which consists of a sample frame, 4 flexure connectors and the piezotubes, and the sample cell, as an equivalent oscillator (EO). The EO is put in a periodic motion under the triangular wave input. At the turning point its kinetic energy is converted into the oscillatory energy, and the EO starts to oscillate. Because of the viscosity of solution the trapped bead oscillates too. To prove whether the scanner can be treated as an equivalent oscillator, an experiment is designed and carried out as follows. An object with a mass m' is added to the scanner, thus the total mass of the EO is changed. Then the responses of the trapped bead are measured for different additional mass. The results are shown in Fig.1. It can be seen that the frequency of the superposed oscillation decreases with the increase of total mass. Based on the EO model we have $m' = K/(4\pi^2 f^2) - m_0$, where K and m_0 are the stiffness and the mass of the scanner respectively, f is the oscillatory frequency. By using the experimental data in Fig.1, we obtain m_0=5.4g and K=1.63×10^{7}pN/nm. Therefore, the natural frequency of the scanner is obtained to be

$$f_0 = (K/4\pi^2 m_0)^{1/2} = 276\text{Hz}. \quad (1)$$

This result coincides with its nominal value of 280Hz (provided by Nanonics imaging Ltd). The agreement indicates that the scanner may be treated as an EO and the additional oscillation is related to the movement of the scanner, rather than other sources, such as the electrical circuit.

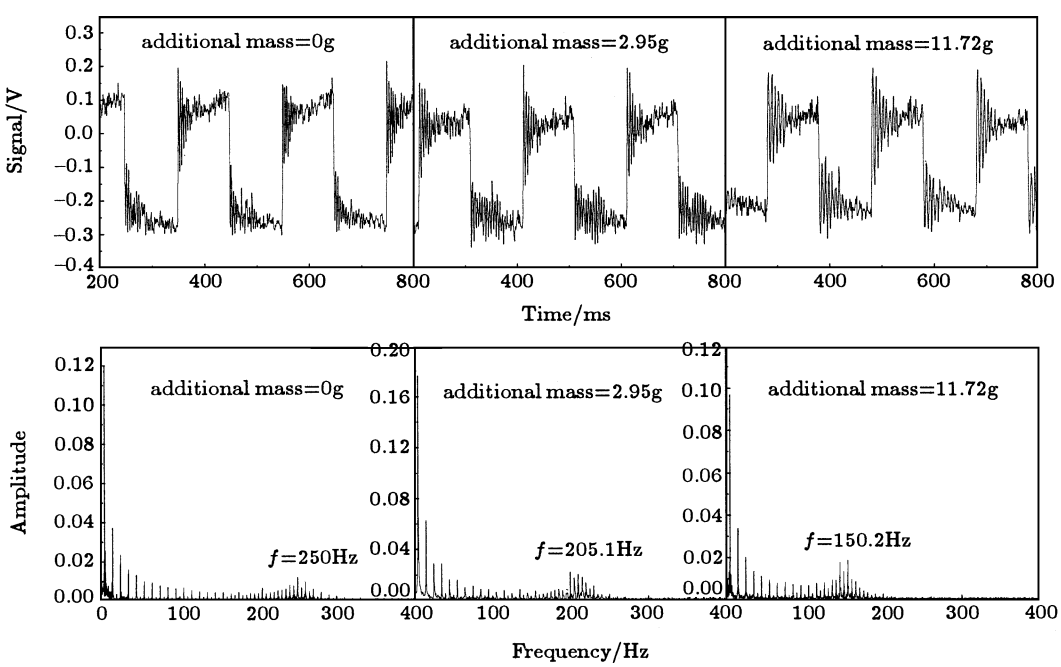


Fig.1. Responses of a trapped bead for different total mass of the scanner and the corresponding frequency spectra.

3. Analysis of the oscillatory disturbance

In order to understand deeply the source of observed oscillatory disturbance, the attenuating oscillations of both the EO and the trapped bead are analysed in detail.

3.1. Oscillation of the EO

At the turning point, the EO begins to oscillate due to the release of its kinetic energy. The corresponding kinetic equation is

$$M\ddot{X} + R\dot{X} + KX = 0, \tag{2}$$

where M is the mass of the EO and R is the damping factor. At the turning point, due to the response time limitation of the scanner the duration of the speed of the scanner from $+V$ turning to $-V$ is not infinitesimal but 2ms (provided by Nanonics Imaging Ltd). However, the oscillatory period measured in our experiment is about 4ms. Because the establishing process of the disturbed oscillation is very complex, it is difficult to estimate accurately the extent of the momentum conversion to the exciting of the oscillation. Therefore, we consider approximately that the maximum momentum conversion into oscillation is MV which corresponds to a quarter-period of oscillation, so the initial speed of the scanner is V. Thus, with the initial condition: t=0, X=0 and $\dot{X} = V$, the solution of Eq.(2) is

$$X = A_0 \mathrm{e}^{-Bt} \sin(Wt), \tag{3}$$

where $A_0 = V/W$, V is the scanning speed of the scanner, and $B = R/2M$ is the attenuating constant of oscillation ($B \approx$68s^{-1} is our measured value). $W = \sqrt{W_0^2 - B^2}$=1578s^{-1} and $W_0 = \sqrt{K/M}$=1579s^{-1} are the oscillatory angular frequency and the natural angular frequency of the EO respectively.

3.2. Oscillation of trapped bead

The kinetic equation of the trapped bead driven by the attenuating oscillation of the scanner can be expressed by

$$\begin{aligned} &m\ddot{x} + b\dot{x} + kx \\ &= b\dot{X} = bA_0 \mathrm{e}^{-Bt}[W\cos(Wt) - B\sin(Wt)], \end{aligned} \tag{4}$$

where m is the mass of polystyrene bead, for 1μm-diameter bead m=5.55×10^{-13}g, $b = 6\pi\eta r$ is the viscosity of the solution, and k is the trapping stiffness, in our experiment k=0.04pN/nm. For $W \gg B$, the second term in the force on the right-hand side can be neglected. By solving Eq.(4), the attenuating oscillation of the trapped bead is

$$x = a\mathrm{e}^{-Bt}\cos(Wt + \phi) \tag{5}$$

with

$$\begin{aligned} a &= \frac{H/m}{\sqrt{(\omega_0^2 - W^2 + B^2 - 2\beta B)^2 + [2W(\beta - B)]^2}}, \\ \phi &= -\mathrm{arctg}\frac{2W(\beta - B)}{\omega_0^2 - W^2 + B^2 - 2\beta B}, \end{aligned} \tag{6}$$

where $\omega_0 = \sqrt{k/m}$=2.7×10^{5}s^{-1} and $\beta = b/2m$ = 8.5×10^{6}s^{-1} are the natural angular frequency and the attenuating constant of the trapped bead oscillation, respectively. For $\beta \gg B$ and $\omega_0 \gg W$, the initial amplitude a and the phase angle ϕ of the disturbance oscillation of trapped bead can be respectively simplified as

$$a = \frac{V}{\sqrt{(k/b)^2 + W_0^2}} \quad \text{and} \quad \phi = -\mathrm{arctg}\frac{W_0 b}{k}. \tag{7}$$

In our experiment, the speed of the scanning stage V is set to be 0.053cm/s. The initial amplitude a calculated from Eq.(7) is 117nm, and the corresponding experimental result is about 113nm. The experimental result is in good agreement with the theoretical one. This further proves that the oscillatory disturbance comes from the attenuating oscillations of the scanner. Based on the expression (7) and relationship $F_{\mathrm{trap}} = kl = bV$, the ratio of the initial amplitude a to the bead displacement l, which represents the degree of the disturbance, can be obtained as follows:

$$\frac{a}{l} = \frac{1}{\sqrt{1 + (bW_0/k)^2}}. \tag{8}$$

The values of a/l under different trapping stiffness k and natural frequencies f_0 of the scanner are shown in the following table 1.

Table 1. The values of a/l under different trapping stiffness k and natural frequencies f.

k/(PN/nm)	0.002	0.005	0.01	0.02	0.04
f_0/Hz					
280	12%	29%	51.6%	77%	92%
350	9.6%	23.5%	43.5%	69.4%	88.8%
500	6.7%	16.6%	32%	56%	80%

Obviously, the oscillatory disturbance becomes stronger, when the trapping stiffness k increases or the natural frequency f_0 of the scanner decreases.

3.3. Analysis of frequency spectrum of the trapped bead motion

To further test the accuracy of our analysis about the origin of the oscillatory disturbance to the trapped bead motion, we compare the frequency spectrum obtained by the theoretical calculation with the experimental result. In Figs.2(a) and (b) the measured and calculated frequency spectra of trapped bead motion are respectively plotted. They are consistent with each other. This means that the equivalent oscillator model is suitable to analysing the disturbance occurring in trapping force calibration.

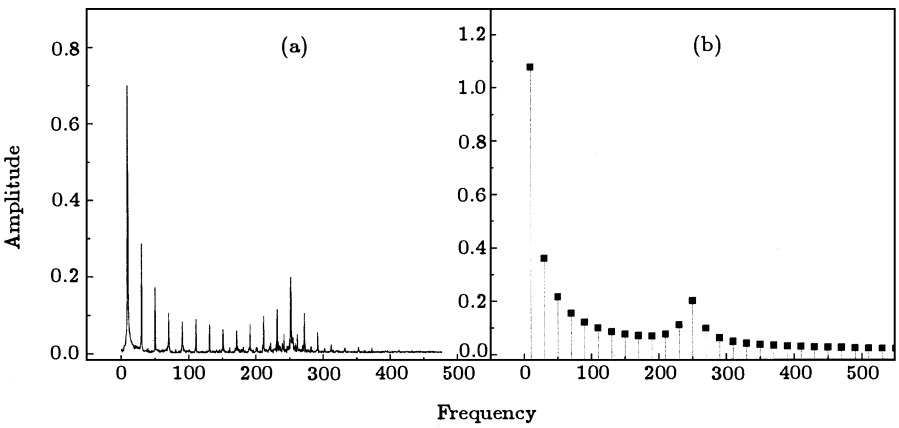


Fig.2. Frequency spectra of trapped bead movement (a) experimental, (b) calculated.

4. Elimination of oscillatory disturbance by sinusoidal wave drive

In order to avoid the oscillation in force calibration, a sinusoidal wave input is substituted for a triangular wave one to drive the scanner. When the scanner moves to its turning point, its velocity decreases down to zero, i.e. the kinetic energy of the scanner diminishes greatly at the turning point. Therefore, the oscillatory disturbance can be eliminated completely.

In Figs.3 (a) and (b), the solid curves denote the responses of the trapped bead to a sinusoidal wave input and a triangular wave input, respectively. It is obvious that there appears no oscillation in the bead movement and then the trapping stability is improved largely, when a sinusoidal wave input is used. The dotted curve in Fig.3(a) represents the corresponding fit curve with the expression $y = c_0 + c\sin(\pi(z - z_0)/d)$, where c_0 is the mean value of bead displacement, c,

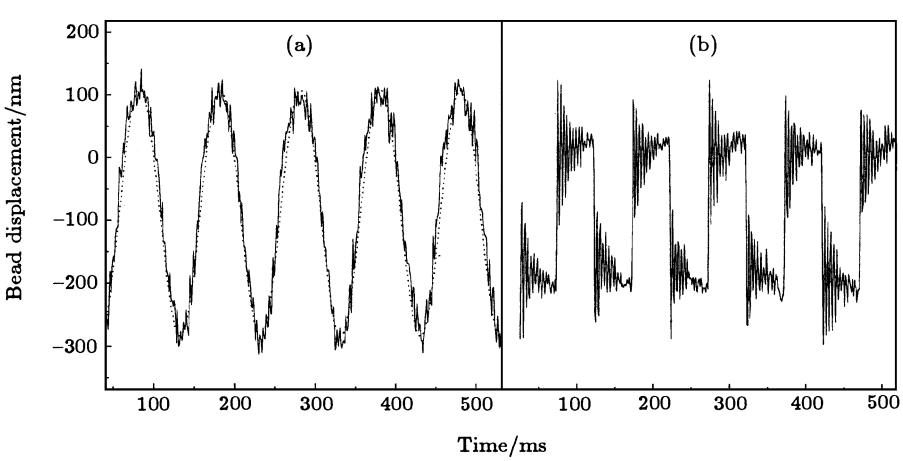


Fig.3. Response of a trapped bead and the corresponding fit curve with sinusoidal wave input (a) and triangular wave input (b).

d and z_0 are amplitude, half-frequency and the starting time of a period of bead movement, respectively. Using this curve fitting, the processing of the experimental data is easy and timesaving. Figures 4(a) and (b) show force calibrations obtained by applying the triangular wave input and the sinusoidal wave input to the scanner, respectively, under the same experimental conditions. It can be seen that the values of the trapping stiffness k are equal, however, the maximum displacement of the bead that can be trapped stably increases from ±180nm to±220nm when the triangular wave input is replaced by the sinusoidal wave input. This result is of significance, especially, if a large range of force calibration is necessary, for example, in the measurement of membrane tension.

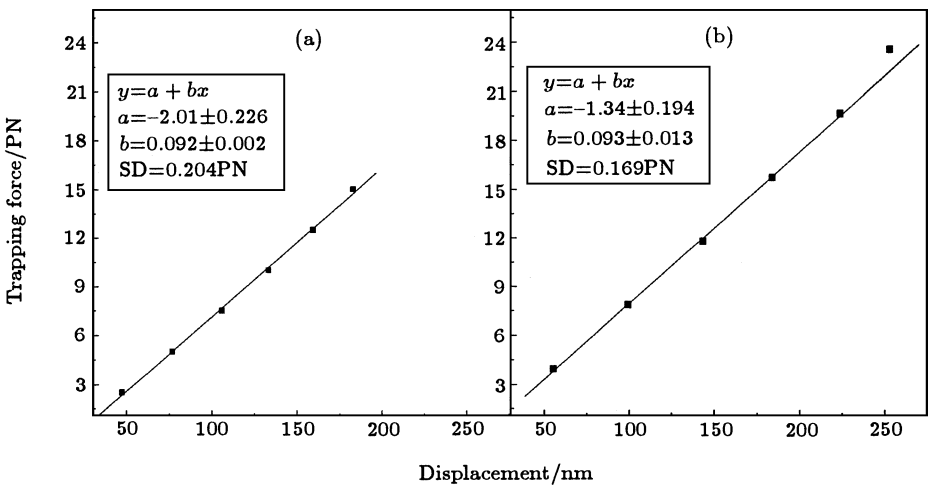


Fig.4. Trap stiffness calibration with triangular wave input (a) and sinusoidal wave input (b).

5. Conclusions

In the calibration of the optical trap stiffness, it is found that there appears an attenuating oscillation as an oscillatory disturbance added to the trapped bead movement, when the scanner is driven by a triangular wave input. An equivalent oscillator model is put forward to explain the mechanism of the oscillatory disturbance. Both the measurements and calculations show that the attenuating oscillation comes from the oscillation of the scanner and the triangular wave drive causes the additional oscillation of the scanner. Furthermore, the analysis indicates that the oscillatory disturbance will become stronger when the stiffness k increases or the natural frequency f_0 of the scanner decreases. This means that the scanner should not be driven by the triangular wave input when the stiffness k is relatively large. We adopt another driving way, i.e. we use a sinusoidal wave input instead of the triangular wave. Our experiment has verified that in this case the oscillatory disturbance is eliminated completely. Therefore, the range of force calibration has been extended greatly. It is believed that our experimental and calculated results may be valuable as a reference to accurately measuring the interaction force by optical tweezers.

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