Methods of calibration to optical trapping force upon non-spherical cells

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The dynamical equation of a trapping cell is solved to find calibration methods for the trapping force, and two methods are compared by synthetic experiment data. Results indicate that: Boltzmann distribution method (BDM) is available for the force calibration of non-spherical or anisotropic cells in arbitrary trap potential; the mean square displacement method (MSDM) is available only for a symmetric harmonic optical trap. The spatial resolution requirement of the calibration system is about a nanometer. The results agree with the reported experiments.

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Optical tweezers (OTs) have become a popular manipulation and force measurement tool in cellular and molecular biology[1−3]. Presently, there is no theory that can be used to directly calculate the trapping force for cells. The force must be determined empirically, and the force calibration is necessary. There are a number of ways to calibrate optical trapping force[4−7]. These ways are available for spherical isotropic particles and the harmonic potential profiles. Biological cells, organisms, and biological structures in a trap often have complex shapes and typically have unknown optical properties, hence, the potential profiles are sometimes inharmonic and asymmetric. Therefore, it is desired to demonstrate an available optical trap force calibration method for non-spherical and anisotropic cells with a general potential profiles.

In this paper, we assume biological cells as Brownian particles in OTs, and report on solving the dynamical equation of a cell in an optical trap. According to the results obtained, we analyze available force calibration methods directly for non-spherical cells in a common potential. The results are applied to the case of a harmonic approximate trapping potential. The two methods, namely, Boltzmann distribution method (BDM) and mean square displacement method (MSDM), are compared by synthetic experiment data.

As the cells size is in micrometer range, they can be considered as Brownian particles. The trap in OT is ordinary divided into the lateral and axial directions to be discussed. For simplicity, we consider in the following only the projection onto the lateral x axis and treat the problem as one-dimensional motion. We suppose that the cell is at $x = 0$ at initial time. The motion equation of a cell with mass m laying out on a horizontal plane is

$$-\alpha\frac{\mathrm{d}x}{\mathrm{d}t} + F_x(t) - \frac{\mathrm{d}U(x)}{\mathrm{d}x} = m\frac{\mathrm{d}^2x}{\mathrm{d}t^2}, \tag{1}$$

where α is the frictional coefficient; $-\frac{\mathrm{d}U(x)}{\mathrm{d}x} = T_x$ is the force acting on the cell by OTs, which is called optical trapping force; $U(x)$ is the cell's potential; $F_x(t)$, which represents the Langevin force by thermal fluctuation activating, obeys

$$\begin{aligned}&\langle F_x(t)\rangle = 0\\ &\langle F_x(t)F_x(t')\rangle = 2\alpha k_{\mathrm{B}}T\delta(t-t')\end{aligned}, \tag{2}$$

where k_{B} is the Boltzmann constant and T is the sample temperature. For large frictional coefficient α, the cell has low Reynolds number, we may neglect the second derivative with respect to time in Eq. (1). Thus, Eq. (1) can be rewritten as

$$-\alpha\frac{\mathrm{d}x}{\mathrm{d}t} + F_x(t) - \frac{\mathrm{d}U(x)}{\mathrm{d}x} = 0. \tag{3}$$

Corresponding to Eq. (3), the Fokker-Planck equation for the probability distribution $P(x,t)$ of the cell takes the form

$$\frac{\partial P}{\partial t} = \frac{1}{\alpha}\frac{\partial}{\partial x}\left[\frac{\mathrm{d}U(x)}{\mathrm{d}x}P + k_{\mathrm{B}}T\frac{\partial}{\partial x}P\right]. \tag{4}$$

For steady-station solution, we have

$$\frac{\partial}{\partial x}\left[\frac{\mathrm{d}U(x)}{\mathrm{d}x}P + k_{\mathrm{B}}T\frac{\partial}{\partial x}P\right] = 0. \tag{5}$$

It is easy to test that the particular solution of Eq. (5) is

$$P(x) = C\exp\left(-\frac{U(x)}{k_{\mathrm{B}}T}\right), \tag{6}$$

where C is the normalized constant ($\int P(x)\mathrm{d}x = 1$). Equation (6) illustrates that the trapped cell obeys Boltzmann distribution.

The different interaction models between an OT and different cells implies different potential functions $U(x)$. By measuring potential $U(x)$, we can calculate the force acting on the cell by $-\frac{\mathrm{d}U(x)}{\mathrm{d}x} = T_x$. This procedure is called OTs trapping force calibration.

According to Eq. (6), a random shape cell potential can be measured and the corresponding trapping force can be

calibrated. The method is that by measuring the probability density $P(x)$, the potential experienced by the cell can be calculated by

$$U(x) = -k_{\rm B}T \ln P(x) + k_{\rm B}T \ln C. \tag{7}$$

The last term determines the potential offset and is neglected by choosing zero potential value. The corresponding potential $U(x)$ may be fitted numerically. This procedure is called BDM. In principles, BDM is suitable for all cell sizes and shapes, and for arbitrary trapping fields. There have been some experiments in agreement with the conclusion for non-spherical particles[8,9].

For the harmonic potential, $U(x) = \frac{1}{2}kx^2$, k is the trap stiffness coefficient, the potential can be obtained by measuring k through the procedure called trap stiffness calibration (TSC). In the case, the calibration of the trap force translates into TSC, we have

$$P(x) = C \exp\left(-\frac{x^2}{2k_{\rm B}T/k}\right). \tag{8}$$

Equation (8) is applicable for anisotropic cells[10,11]. Two approaches of TSC can be obtained, one is BDM, the other is MSDM. By measuring the mean square displacement $\langle x^2 \rangle$, k can be obtained as $k = k_{\rm B}T/\langle x^2 \rangle$[12].

We adopt $\sqrt{\langle x^2 \rangle} = \sqrt{\frac{k_{\rm B}T}{k}}$ to calculate the spatial resolution for the calibration system. For bio-cell experiments, we use $T \sim 3 \times 10^2$ K, presently there is no experiment data reported for cell's trap stiffness coefficient. For the polystyrene micro-spheres of 0.5-μm radius (refractive index 1.58 in Ref. [13]), the trap stiffness coefficient is about 10^{-5} N/m. By numerical simulation, Ref. [9] gave that the trap stiffness coefficient of 50-nm-in-radius polystyrene beads (refractive index 1.56) are about 4.5 times of those of artificial CHO (Chinese hamster ovary, refractive index 1.38) cell, and considered that the difference in the trap stiffness coefficient mainly results from the difference in the refractive indices between them. According to these data, we may calculate the trap stiffness coefficient of microns-in-radius cell in the order of 10^{-5}–10^{-6} N/m. So the scope of displacement from the cell to the center of optical trap is about 10^{-8} m, we use one tenth of this value (1 nm) as the spatial resolution for the calibration system. The resolution is nanometer, suitable for inharmonic and asymmetric potential cases also.

In order to compare MSDM and BDM, we generated samples of synthetic data $p^{\rm tr}(x_i)$ with a prefixed set of parameters $k^{\rm tr} = 10^{-5}$ N/m, $T = 300$ K, the superscript "tr" means true data or equivalently error-free data. Therefore, discrete values of $p^{\rm tr}(x_i)$ in a given range of $x_i = -7, -6, \cdots, 0, \cdots, 6, 7$ were obtained by the relation: $p^{\rm tr}(x_i) = \exp(-k^{\rm tr}x_i^2/2k_{\rm B}T)) = \exp(-x_i^2/8.28)$, here the unit of x_i is 10^{-8} m.

We use $p^{\rm ex}(x_i)$ to express the experimental data which involve errors, different levels of the pseudo experimental error δ are introduced in the probability. Therefore, discrete values of $p^{\rm ex}(x_i)$ in the range of $x_i = -7, -6, \cdots, 0, \cdots, 6, 7$ were obtained as

$$p^{\rm ex}(x_i) = p^{\rm tr}(x_i - x_0)(1 + \delta G), \tag{9}$$

where G is the Gaussian random number with zero mean and unit variance which is included to simulate the experimental noise, x_0 is the displacement offset between the center of the trap and the origin of position coordinate. Therefore, according to Eq. (9), experiment data with different levels of error ($\delta = 0.02, 0.1, 0.5$; $x_0 = 0, 10, 20$ nm) were produced. The two calibrating procedures were applied to the data to obtain the potential energy. The representative results are shown in Fig. 1, where the potential energy as a function of displacement for different displacement offsets x_0 ($x_0 = 0, 10, 20$ nm) at the same experiment error δ ($\delta = 0.5$) are plotted. By using a harmonic potential model, $k^{\rm ex}$ can be fitted. By comparing $k^{\rm ex}$ obtained above with $k^{\rm tr} = 10^{-5}$ N/m, the error is measured as it is defined to express the fit goodness as

$$\text{err} = \frac{|k^{\rm ex} - k^{\rm tr}|}{k^{\rm tr}} \times 100\%. \tag{10}$$

The stiffness coefficient errors are listed in Table 1. It is shown that BDM can be used to measure potential

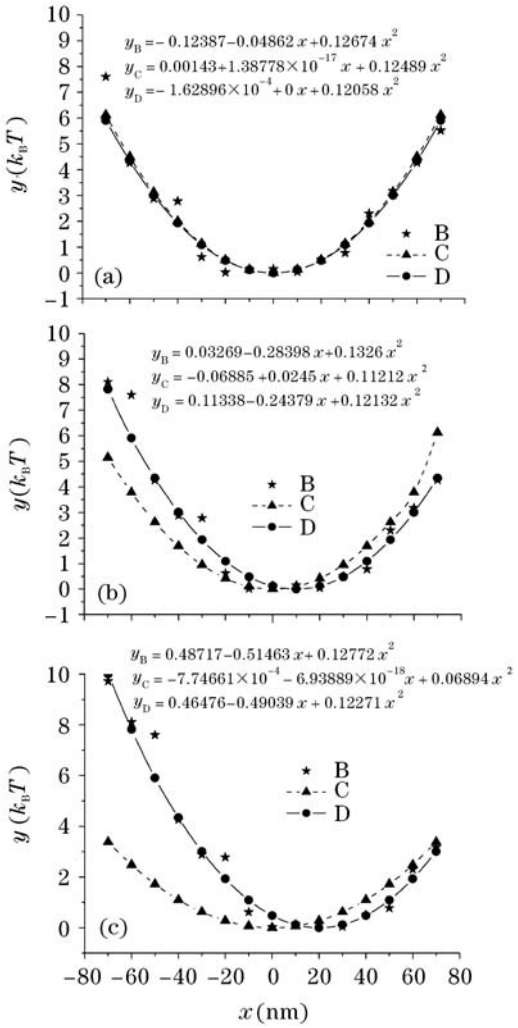

Fig. 1. Potential energy y as a function of displacement x obtained from synthetic data for different potential offsets x_0 of 0 (a), 10 (b), and 20 nm (c). "D" represents the predictions of the true data, "B" refers to the BDM, and "C" to MSDM. The binomial equations fitted by the data accordingly are included for comparison.

Table 1. Stiffness Coefficient Errors from the Two Calibration Methods in Various Cases (Unit: %)

Methods	$\delta = 0.02$ $x_0 = 0$	$\delta = 0.1$ $x_0 = 0$	$\delta = 0.5$ $x_0 = 0$	$\delta = 0.5$ $x_0 = 10$ nm	$\delta = 0.5$ $x_0 = 20$ nm
BDM	1	2	5	6	7
MSDM	2	2	4	13	42

profiles which are allowed to be inharmonic and asymmetric, but MSDM is only applicable for a harmonic potential.

In summary, the interaction between cells and OTs are expressed with potential function. Two calibration methods can be used for cellular optical trap forces calibration. BDM is a all-purpose method, MSDM is only applicable for a harmonic potential. The spatial resolution for the calibration system is about a nanometer. The results can be applied to analyze the optical force calibration methods for a trapped biological cell, and give references for studying cells mechanics and light interaction with biological cells by OT technology.

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