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Effect of Low-Pass Filtering in Force Calibration of Magnetic Tweezers *

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In typical experiments where magnetic tweezers are involved, precise measurement of the magnetic forces is of crucial importance. To achieve this, a widely applied method is to track the bead's Brownian motion trajectory and to calculate the force from its mean-squared-displacement. However, this method does not take into account the fact that the bead-tracking device always has a finite bandwidth, acting effectively as a low-pass filter. The result could be subjected to significant system errors, which overestimates the magnetic force. We analyze the power spectrum of the bead's Brownian motion, and provide a corrected formula to calculate the magnetic force, which is free of system errors induced by limited detection bandwidth. A dsDNA force-extension curve is experimentally measured. The curve is consistent with the WLC model, exhibiting correctness of the new formula. On the other hand, the force given by the traditional method shows significant deviation from the WLC model, which is 3 times larger at most.

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In the recent decades, experimental techniques based on single molecule manipulation have improved enormously, and have created a novel branch of research in molecular biology. Among them there are, to name a few, optical traps, atomic force microscopy (AFM), magnetic tweezers, etc. Traditionally, researchers often focus on the ensemble average of various properties, while with the help of single molecule techniques they can now directly observe the reaction between individual molecules. In this way, researchers can achieve much more information which in the past is hidden behind the ensemble average and inaccessible, such as statistical distributions of variables, reaction pathways and other details of certain reactions.[1,2]

In a typical experiment where magnetic tweezers are involved, a super-paramagnetic micro bead is attached to one end of a DNA molecule (either single strand or double strand) through biotin-streptavidin interaction. The other end is tethered to the container wall via the interaction between digoxingenin and anti-digoxingenin. When an uneven magnetic field is applied to the bead, a force along the direction of ∇B will exert on the bead (Fig. 1). Under such circumstance, its motion consists of two parts: the confined Brownian motion and possible changes of the DNA end-to-end length. By carefully tracking the bead's position, some details about the reaction might be revealed.

The tethering force of the DNA molecule is of crucial importance in many DNA-related reactions. For example, forces of different magnitudes may cause significantly different energy profiles, which will cause different reaction rates, or even different pathways.[3,4] In magnetic tweezers, to accurately measure the exerted force, one can track the Brownian motion of the bead. From the equal-partition theorem, the force can be calculated from the mean squared displacement (MSD) of the bead's trajectory:[5]

$$F = \frac{k_{\mathrm{B}} T l}{\langle \delta x^2 \rangle}, \tag{1}$$

where l is the DNA's end-to-end length, k_{B} is the Boltzmann constant, T is the temperature of the experimental environment and δx^2 is the mean squared displacement.

There are many ways to track the bead's position, e.g. video-based software tracking via CCD or CMOS, quadrant photodiodes (QPD), laser-based polarization interferometry.[6,7] These methods have different detection bandwidths. For example, CCD is relatively slow with a typical bandwidth of 5–500 Hz. CMOS and QPD are much faster, which can easily achieve a frequency above 1 kHz.[8–11]

However, this method does not take into account the bandwidth limit of the detection device. Calculating MSD statistically is prone to system errors if the bandwidth is not large enough compared with the signal.[12] The bandwidth varies across different experimental setups, which mainly depends on the contour length and the persistence length of the DNA molecule involved, the temperature, and the size of the magnetic bead. Typically, the upper limit of the bandwidth, described by cut-off frequency, lies in 10–500 Hz. On the other hand, the device's bandwidth is determined by the detection method. If a CCD is involved, under many circumstances the sample rate is less than 100 Hz. The limited bandwidth behaves like a low-pass filter, which results in smaller MSD and overestimates forces. To solve the problem, a modified

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method is needed.

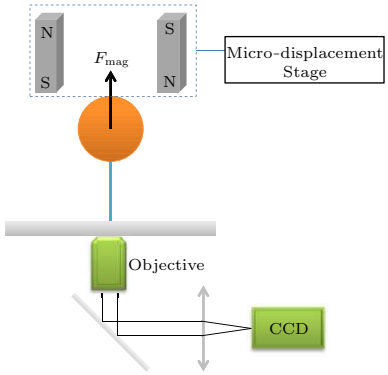


Fig. 1. A typical configuration of magnetic tweezers. The bead's image is captured by a CCD.

According to Einstein–Ornstein–Uhlenbeck's theory about Brownian motion, the micro bead's Langevin equation is

$$m\ddot{x}(t) + \beta\dot{x}(t) + \kappa x(t) = (2k_{\rm B}T\beta)^{1/2}\eta(t), \quad (2)$$

where $\eta(t)$ describes the stochastic force, while κ is the stiffness of the potential well, m is the micro bead's mass, β is the liquid's viscosity, $k_{\rm B}$ is the Boltzmann constant and T is the temperature of the environment.

In magnetic tweezers, the bead's motion is strictly confined to a range that is not longer than the DNA molecule's contour length, Within such a short range, the magnetic force is essentially constant.[13] As long as x, which is the displacement of the bead from the center of the potential well, is orders of magnitude less than the DNA end-to-end length l, the potential well can be considered to be approximately harmonic, and the magnetic force $F_{\rm magnetic}$ equals to the ensemble average of the tethering force $F_{\rm DNA}$. Therefore, $\kappa = \langle F_{\rm DNA}\rangle/(l+a)$, where a is the radius of the micro bead.

In the system described above, the relaxation time $t_{\rm inert} = m/\beta$ is many orders of magnitude less than the device's temporal resolution, so we can ignore the inertial term and obtain

$$\dot{x}(t) + 2\pi f_c x(t) = (2D)^{1/2}\eta(t), \quad (3)$$

where $D = k_{\rm B}T/\beta$, $f_c = \kappa/2\pi\beta$.

From the Wiener–Khinchin theorem, we can obtain the Brownian motion's one-side power spectrum:[14]

$$S_{\rm one-side}(f) = \frac{k_{\rm B}T}{\pi^2\beta(f^2+f_c^2)}. \quad (4)$$

The power spectrum is Lorentzian, with a cut-off frequency depending on both the potential well's stiffness and the friction the micro bead is subjected to. For small micro beads which are tethered via short DNA molecules, the cut-off frequency of the system f_c might be comparable or even larger than the tracking device's bandwidth. According to Nyquist–Shannon sampling theorem, such low-frequency sampling cannot accurately record the original signal. Effectively this effect can be considered as a low-pass filter applied to the original signal (Fig. 2).

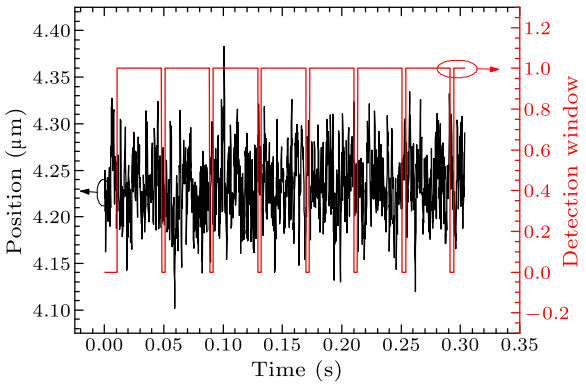


Fig. 2. Black: the original Brownian motion signal. Red: a serial of rectangle window functions related to the CCD's exposure process. The video-based tracking algorithm will analyze each frame and calculate the position series, which is essentially the time average of the original signal within the exposure period.

Let $x(t)$ be the original Brownian motion trajectory. The observed samples can be written as

$$x_{\rm sample}(n) = \int_{-\infty}^{\infty} x(\tau)g_w(n\Delta - \tau)d\tau, \quad (5)$$

where Δ is the exposure time and g_w is an origin-centered rectangle window function with the width of Δ, which is essentially the CCD's exposure time. The observation is essentially the convolution of the original signal and the window function. The sample's filtered power spectrum is[15,16]

$$S_{\rm sample}(f) = \frac{k_{\rm B}T}{\pi^2\beta(f^2+f_c^2)}\left[\frac{\sin(\pi f\Delta)}{\pi f\Delta}\right]^2, \quad (6)$$

where f is the frequency of the signal's power spectrum, and f_c is the cut-off frequency.

According to Parseval's theorem, the sample MSD is

$$\begin{aligned}\langle\delta x^2_{\rm sample}\rangle &= \int_0^{\infty} S_{\rm sample}(f)df \\ &= \frac{k_{\rm B}T(2\pi f_c\Delta + e^{-2\pi f_c\Delta} - 1)}{4\pi^3\beta f_c^3\Delta^2}. \end{aligned} \quad (7)$$

If we define r as the ratio between the cut-off frequency and the Nyquist frequency $f_{\rm bandwidth}$, which is $(2\Delta)^{-1}$, we have

$$r = \frac{f_c}{f_{\rm bandwidth}} = \frac{F}{2\pi\beta(l+a)f_{\rm bandwidth}}, \quad (8)$$

where l is the end-to-end length of the DNA molecule, and a is the radius of the magnetic bead. The correction factor between the original MSD and the sample MSD can be written as

$$g = \frac{\langle\delta x^2_{\rm sample}\rangle}{\langle\delta x^2(t)\rangle} = \frac{2(\pi r + e^{-\pi r} - 1)}{\pi^2 r^2}. \quad (9)$$

The corrected equation to calculate magnetic forces is

$$F = \frac{k_{\rm B}T(l+a)}{\langle \delta x^2(t)\rangle} = \frac{k_{\rm B}T(l+a)}{\langle \delta x^2_{\rm sample}\rangle}\cdot\frac{2(\pi r + e^{-\pi r} - 1)}{\pi^2 r^2}. \quad (10)$$

When the bandwidth is much larger than the cut-off frequency, g is approximately 1 and Eq. (1) is applicable. However, g decreases rapidly with the increase of r. When $r = 1$, which means that the device's bandwidth equals the cut-off frequency, g drops to about 0.44, which means that Eq. (1) will overestimate the force approximately twice.

We have designed an experiment to prove the corrected force-calculation equation. The configuration is illustrated in Fig. 1.

The magnet pair consists of two anti-parallel NdFeB magnets. Its position can be adjusted by a micro-displacement stage (MP285, Sutter Instrument), and exerts various magnetic forces on a 2.8 μm micro bead (M280, Invitrogen), which is attached to a tethered 1 μm dsDNA. The micro bead-DNA complex is in biological buffer (135 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM NaH_2PO_4). Through the objective (Olympus APON 60XOTIRF) and the microscope (IX71, Olympus), the image of the bead is captured by a CCD (Andor EMCCD DV885). Those images are analyzed by a homemade software.[17] Besides tethered micro beads, we also introduce some micro beads that are attached to the wall through non-specific adsorption.

In this experiment, there are two independent ways to measure the magnetic force. One is to use the WLC model, which is valid when the force is less than 50 pN:[5,18–20]

$$\frac{FP}{k_{\rm B}T} = \frac{1}{4}\left[\left(1-\frac{l}{L}\right)^{-2} - 1\right] + \frac{l}{L}, \quad (11)$$

where P is the DNA's persistence length, and L is its contour length. The DNA end-to-end length l can be measured in the following way. The adsorbed beads can serve as indicators of the container wall position. By measuring the displacement between tethered beads and absorbed beads, we can obtain the end-to-end length, and subsequently the magnetic force.

On the other hand, the force can also be measured by tracking the micro bead. We can examine the MSD and calculate the force by Eq. (10).

By adjusting the magnets' position, we can exert different forces on the tethered micro bead. The tracking results are shown in Fig. 3.

With the magnets at several various positions, we measured the magnetic forces and then compared them with the WLC model.

In Fig. 4, when the exposure time is 30 ms, the forces measured from both the traditional uncorrected Eq. (1) and the corrected Eq. (10) are compared side by side, together with a standard WLC model force-extension curve. For small forces which are less than 0.5 pN, both equations give similar results. This is because the potential well stiffness is relatively small and the cut-off frequency is much lower than the device's bandwidth under such condition. The majority of the Brownian motion energy is distributed in the low-frequency regime, and can be accurately recorded even by slow devices such as CCD. Therefore the correction factor g is very close to 1. However, when the force gets larger, g decreases. In such a regime, when the corrected equation still remains consistent with the WLC model, forces measured by Eq. (1) quickly deviate. When the force is 4.8 pN, the traditional equation overestimates it by more than twice (11.7 pN), indicating that the traditional method is no longer suitable to calculate magnetic forces.

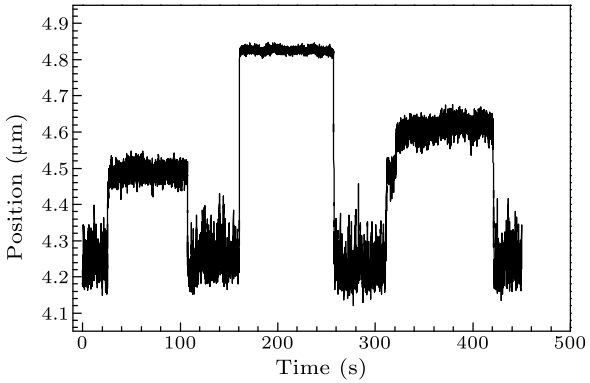


Fig. 3. Brownian motion of a bead tethered by a 1 μm dsDNA with various magnetic forces applied to it. The y-axis denotes the bead's relative position change, which reflects the change of the DNA molecule's end-to-end length.

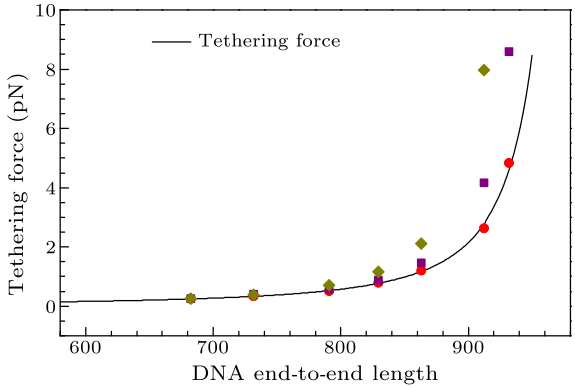


Fig. 4. Red and purple: the exposure time is 30 ms. Red: forces measured from Eq. (10); purple: forces measured from Eq. (1). Brown: forces measured from Eq. (1) with the exposure time of 100 ms.

When the exposure time is set to 100 ms, the device's bandwidth is even smaller (5 Hz). From Fig. 4, forces calculated by the traditional method deviate from the WLC model much faster, indicating a much larger system error while calculating the MSD from the low-pass filtered signal.

In experiments where magnetic tweezers are involved, the applied magnetic force is of crucial importance. We can measure the force by tracking the micro bead's Brownian motion and calculate the force from the mean square displacement by Eq. (1). However, this method does not take into account the impact of limited bandwidth of the tracking device. Insufficient bandwidth might induce large system error in the MSD estimation, and the force will be significantly overestimated.

By analyzing the Brownian motion's power spectrum, we have introduced a corrected equation which gives more accurate results when the tracking device's bandwidth is small compared with the cut-off frequency of the bead's motion. This new force-measuring method will help shed light on future studies where magnetic tweezers are involved.

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