Spatial and temporal resolution of optical tweezers

Introduction

Since their invention more than 20 years ago [1], optical tweezers have been used extensively not only to manipulate biomolecules and cells, but also to directly and accurately measure the minute forces (on the order of fractions of picoNewtons) involved. Most often, the biomolecules of interest are not trapped themselves directly, but manipulated through functionalized microspheres. Those microspheres are usually very small, with diameters ranging between a few tens of nanometers and several micrometers. Because of its small size and the low stiffness of the optical trap, the probe will always perform Brownian motion of considerable extent due to interactions with molecules of the solvent. Since this movement within the trap volume can be as large as 50–100 nm, one needs to average the position signals if smaller displacements have to be measured accurately. An improvement of the resolution is possible because the Brownian motion is normally distributed. The resolution can be enhanced by averaging, reducing the standard deviation, when averaging over N samples, by $1/\sqrt{N}$.

This resolution enhancement may be intrinsically limited by the stability of the setup. For setups designed for optimal stability—as for JPK's NanoTracker™ optical tweezers platform—the instrinsic limit is more likely set by the timescale of the process one is looking at. On the other hand, more measurements per time unit can trivially be made by increasing the sampling rate of the position detection. Depending on the scheme used for position detection, this can be done down to the timescale where the particle undergoes diffusive motion effectively undisturbed by the optical trap, a timescale characterized by the so-called trap auto-correlation time τ. An intuitive interpretation of this quantity is the average relaxation time on which the particle returns to the trap center after a displacement by an external force [2].

Now, if small displacements or forces are to be measured, some considerations should be made before doing the experiment. The same holds when performing force-feedback experiments, since the feedback loop should never be faster than the time needed to average to the resolution one wants to achieve.

This technical note serves to illustrate the need for a fast detection scheme in force-sensing optical tweezers experiments in view of these intrinsic physical effects.

Theoretical background

The mean square displacement σ of a particle in an optical trap with stiffness κ is:

$$\sigma = \sqrt{\langle x^2 \rangle} = \sqrt{\frac{k_B T}{\kappa}} , \quad (1)$$

with x the time-dependent particle position and $k_B T$ the available thermal energy. For times longer than the particle's characteristic time $t_{char} = m/\gamma$, effects caused by inertia do not play a role and the particle's movement can be described by its mean square displacement [2]. Here, m is the mass of the particle and γ the Stokes drag $\gamma = 6\pi\eta r$ with the particle radius r and the viscosity of the surrounding medium η. Figure 1 shows t_{char} for polystyrene and silica particles in the size range used in optical-tweezers experiments. For typically used particles with a diameter of around 1 µm, the characteristic time is in the microsecond

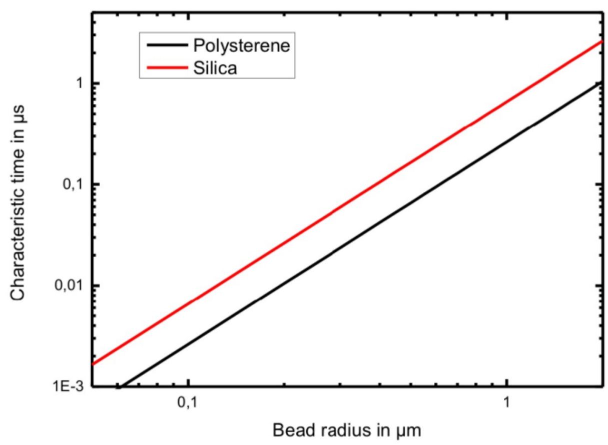

Figure 1 Characteristic time for polystyrene and silica particles with diameters between 10 nm and 4 µm as typically used in experiments with optical tweezers.

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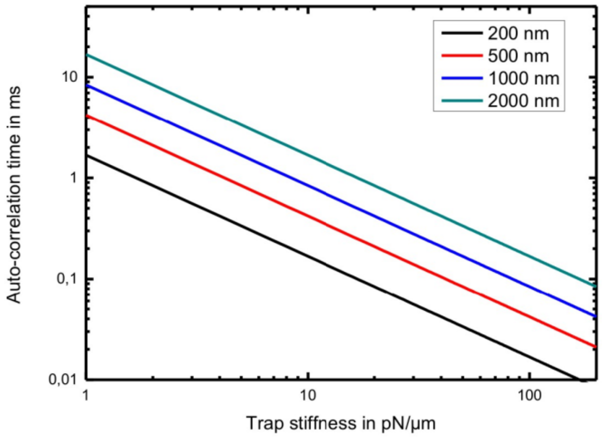

Figure 2 Auto-correlation time for trap stiffness values ranging from 1 pN/µm to 200 pN/µm calculated for four different bead diameters.

range.

If the particle is moving in an optical trap, the auto-correlation time τ can be calculated as $\tau = \gamma / \kappa$ with the trap stiffness κ. Since the auto-correlation time is independent of the mass of the particle, it is determined only by the particle's size and not by its material properties. As shown in Figure 2, large particles in weak traps have the largest auto-correlation time, i.e., they need longer to explore the trap volume. From the auto-correlation time also the corner frequency f_c is given as $\tau = 1/(2\pi f_c)$. Looking at the Brownian motion of a particle in the frequency domain, the motion is governed by interaction with the harmonic trap potential at frequencies below f_c. For frequencies above f_c, the motion is essentially that of a free particle.

Measuring small distances and forces in optical traps

If one wants to measure a displacement Δs of a particle from the center of an optical trap, it should be larger than the mean square displacement σ of the Brownian motion. However, that only gives a statistical significance of 68.3%, so one has to use even more stringent criteria of for example 2σ for 95.5% or 3σ for 99.7% significance. Let us call this additional factor W, so we get a minimum measurable distance of:

$$\Delta s_{\min} \geq W \sqrt{\frac{k_B T}{\kappa}} \qquad (2)$$

The harmonic potential formed by an optical trap follows Hooke's law and the force acting to obtain a displacement Δs can be calculated as $F = -\kappa \Delta s$. Therefore, the minimal distinguishable force accordingly amounts to:

$$F_{\min} \geq W \sqrt{\kappa k_B T} \ . \qquad (3)$$

Thus, if one wants to measure distances or forces with a single measurement, the limitations caused by the intrinsic Brownian motion of the particle have to be considered. For force measurements, the optimal resolution is obtained by using a relatively weak trap; if distance measurements are key, relatively strong traps are best. The best accessible distance and force resolution intrinsic to the physics of

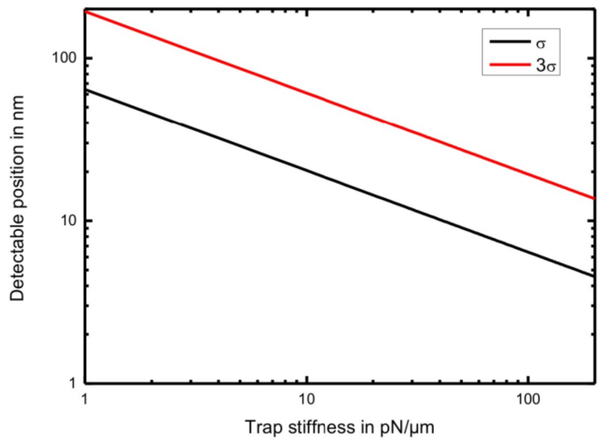

Figure 3 Spatial accuracy (up to 68.3% and 99.7% accuracy, respectively) for measurements with a particle undergoing Brownian movement within an optical trap as a function of trap stiffness.

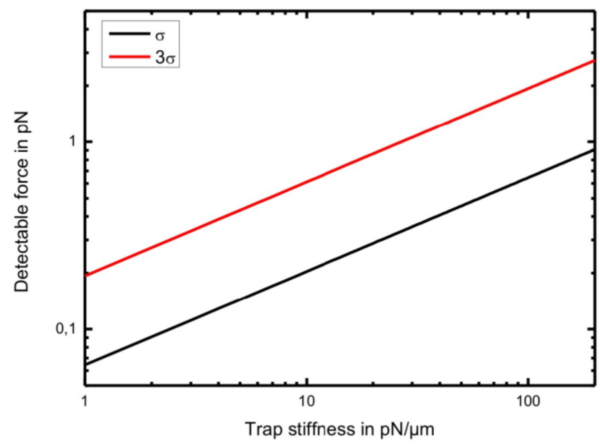

Figure 4 Achievable force resolution (up to 68.3% and 99.7% accuracy, respectively) as a function of trap stiffness for a single measurement.

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optical tweezers are shown in Figures 3 and 4 for the two cases of 68.3% and 99.7% significance.
In practice, the Brownian motion of the particle in the trap causes the best obtainable spatial accuracy to be around 10 nm, *assuming a single position measurement is made*. On the other hand, forces below 100 fN can already be accessed using a weak trap, again for a single measurement.

Fortunately, since the Brownian motion is normally distributed, one can average measurements of either position or force over time and thus arrive at a significantly higher precision. Over a time $t_{average}$, the number of samples N is set by the sampling frequency f_s: $N = t_{average} f_s$.
When averaging over N samples, the smallest detectable force becomes:

$$F_{min} \geq \frac{W}{\sqrt{N}} \sqrt{\kappa k_B T} \ . \qquad (4)$$

However, sampling faster than the inverse of the auto-correlation time will not lead to a further enhancement of the resolution, thus limiting the effective sample number to:

$$N_{eff} \leq 2\pi f_c t_{average} \qquad (5)$$

Taking this into account, the smallest distance that can be resolved is:

$$\Delta s_{min} \geq W \sqrt{\frac{k_B T}{N_{eff} \kappa}} = \frac{W}{\kappa} \sqrt{\frac{k_B T 6\pi\eta r}{t_{average}}} \qquad (6)$$

With W=1, this is referred to as the thermal resolution limit [5,6]. In addition to the trap stiffness it now depends on the particle radius and the measurement time, making it attractive to use small particles in strong traps to measure small displacements. An example for measuring distances with stiff traps is the famous 8-nm stepsize of the motor protein kinesin [3]. Here the optical trap is supported by the compliance of the tether that links the protein to the microsphere, resulting in a higher effective stiffness and thus better displacement resolution if the particle is already pulled out of the trap [4]. However, since also the trap stiffness depends nonlinearly on the particle diameter [7, 8], in practice one needs to find the right trade-off for such experiments.
Figure 5 shows the smallest displacement that can be measured as a function of the averaging time t for a particle radius of 1000 nm and various trap stiffnesses. Here W=3 has been used to achieve a significance of 99.7%. To determine a difference in position for a particle, twice the time for averaging has to be taken, since both the position before the displacement and the one after have to be determined with the desired precision. Here, the sampling frequency always is taken to be the 'optimum' frequency $f_s \geq \frac{\kappa}{6\pi\eta r}$. To achieve a spatial resolution below 1 nm, averaging times of 10 ms and more are necessary for moderately stiff traps. As mentioned before, a high stiffness obviously is the better choice for measurements if spatial resolution is a concern.
The smallest force then is:

$$F_{min} \geq W \sqrt{\frac{k_B T 6\pi\eta r}{t_{average}}} \qquad (7)$$

This equation demonstrates the interesting fact that the

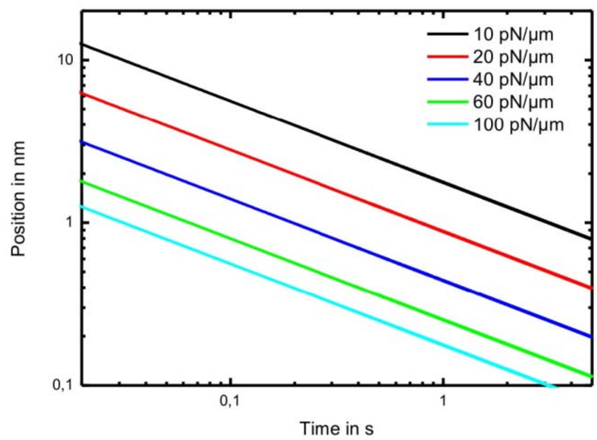

Figure 5 Smallest displacement that can be measured for a trapped particle with a diameter of 1000 nm at various trap stiffnesses.

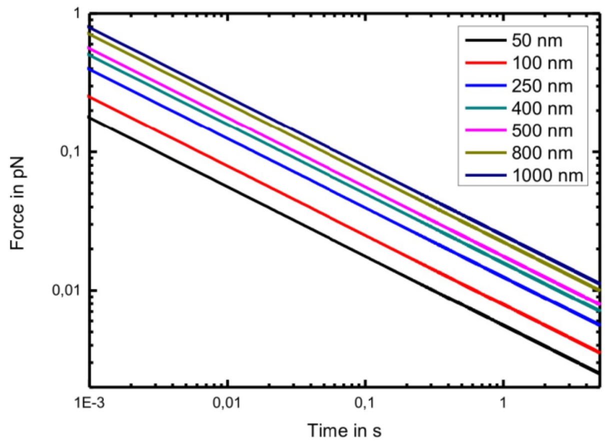

Figure 6 Smallest force that can be measured in a time t with particles of different diameters.

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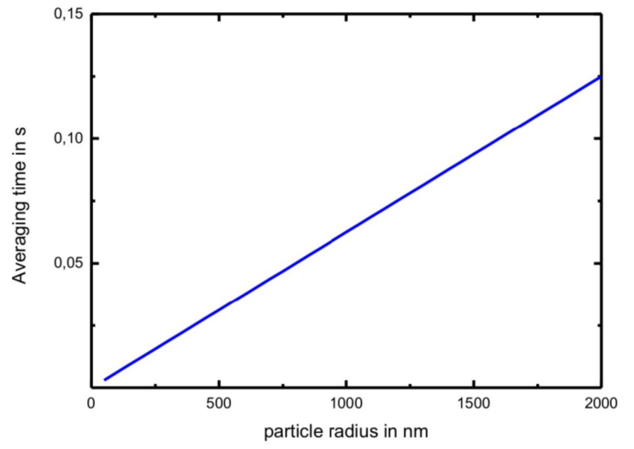

Figure 7 Time necessary to significantly measure a force of 100 fN acting on a particle.

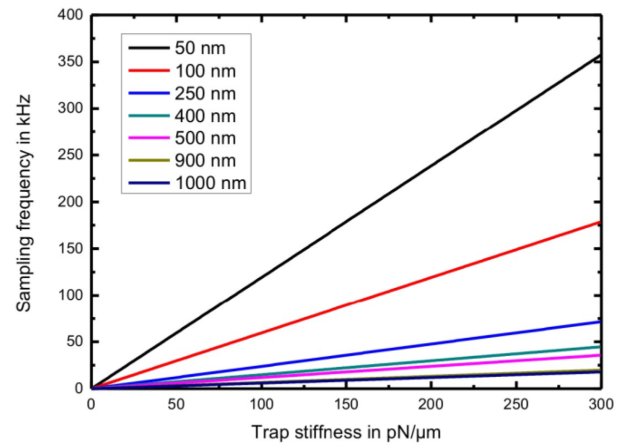

Figure 8 Highest useful sampling frequency if only events slower than the auto-correlation time are to be measured (for different particle diameters).

resolution of force measurements with optical traps is independent of the trap stiffness. The forces that can be resolved after averaging the Brownian motion for a time *t* are shown in Figure 6 for different particle diameters and also *W*=3. For all particle diameters shown, 1 ms is sufficient averaging time to measure a force of 1 pN. However, forces below 100 fN require significantly longer times.

Time constraints for measurements and feedback

As explained above, the intrinsic Brownian motion of a particle in an optical trap sets a lower limit on the time necessary to average for a result and (therefore) also for the integration time used in closed-loop feedback. Even for displacements larger than 3σ, one has to average the Brownian motion away, since otherwise the error will never be smaller than those 3σ.

With a sampling rate of the detection system that is always high enough ($f_s \geq \frac{\kappa}{6\pi\eta r}$), the time needed for averaging to determine a displacement Δs is:

$$t_{average} \geq \frac{W^2}{\Delta s^2 \kappa^2} k_B T 6\pi\eta r \qquad (8)$$

For a force ΔF this leads to:

$$t_{average} \geq \frac{W^2}{\Delta F^2} k_B T 6\pi\eta r \qquad (9)$$

As an example, the time needed to determine an external force of 100 fN on a trapped particle with a certainty of 99.7% is shown in Figure 7. If a small force has to be measured in short times, small particles will be the best choice. The sampling rate necessary to always get as many data points as rendered useful by the Brownian motion is shown in Figure 8 for some particle diameters over the trap stiffness. A sampling rate of 50 kHz will typically suffice, however to exclude aliasing, at least twice the sampling rate should be used.

Practical considerations

When it comes to actual experiments, most physical or biological processes have their own intrinsic timescales. This mostly limits the time available for averaging, rendering high sampling rates always beneficial. **Note that, in view of this, detection based on video tracking is usually not sufficient because the sample rate (~25 Hz) is far too low.**

A change in the effective trap stiffness, such as caused by the series compliance of the trap stiffness with a tether molecule as mentioned above [4,9], might be exploited to enhance the spatial resolution. This should be taken into account by calculating the auto-correlation time and choosing the appropriate sampling frequency.

When choosing particle size and trap stiffness for an experiment, also the dimensions of the harmonic trapping potential as well as range of the detection system used have to be taken into account. If a particle is displaced significantly more than its radius from the center of the trap, the trapping potential may no longer be harmonic [7],

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leading to an overestimation of the applied force. If using Back-Focal-Plane (BFP) interferometry [10, 11] to detect the particle position, such as in JPK's NanoTracker™ optical tweezers platform, care must be taken that the particle does not leave the linear range of the detector. Fortunately, since the detector response is linear up to displacements of about a particle radius, this does not impose a further limitation. The combination of trap stiffness and particle diameter should always be chosen in a way that the particle is not displaced more than its radius by external forces during the course of an experiment.

Obviously, the resolution cannot be better than the intrinsic resolution of the detection system. The best choice in view of that is a BFP interferometry based detection system, for which this limit is far better than a nanometer.

The mechanical stability of the optical setup might hamper measurements that require long averaging times. To assess the mechanical stability of a setup, one can use the Allan-variance [6], yielding the resolution limit over a large range of timescales. For properly designed, drift-compensated setups such as JPK's NanoTracker™, one can minimize mechanical drift to a level that allows measurements with a resolution limited by the thermal limit over several seconds.

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