Optimizing active and passive calibration of optical tweezers

This article has been downloaded from IOPscience. Please scroll down to see the full text article.
2011 J. Opt. 13 044020

View the table of contents for this issue, or go to the journal homepage for more

Download details:
IP Address: 130.225.212.4
The article was downloaded on 07/03/2011 at 08:17

Please note that terms and conditions apply.
Optimizing active and passive calibration of optical tweezers

M Andersson¹,², F Czerwinski¹ and L B Oddershede

The Niels Bohr Institute, University of Copenhagen, Blegdamsvej 17, 2100 Copenhagen, Denmark

E-mail: magnusan@nbi.dk, czerwinski@nbi.dk and oddershede@nbi.dk

Received 29 April 2010, accepted for publication 12 October 2010
Published 4 March 2011
Online at stacks.iop.org/JOpt/13/044020

Abstract

To obtain quantitative information from optical trapping experiments it is essential to perform a precise force calibration. Therefore, sources of noise should be pinpointed and eliminated. Fourier analysis is routinely used to calibrate optical trapping assays because it is excellent for pinpointing high frequency noise. In addition, Allan variance analysis is particularly useful for quantifying low frequency noise and for predicting the optimal measurement time. We show how to use Allan variance in combination with Fourier analysis for optimal calibration and noise reduction in optical trapping assays. The methods are applied to passive assays, utilizing the thermal motion of a trapped particle, and to active assays where the bead is harmonically driven. The active method must be applied in assays where, for example, the viscoelastic properties of the medium or the size or shape of the trapped object are unknown. For measurement times shorter than the optimal calibration time the noise is larger in active than in the passive assays. For times equal to or longer than the optimal measurement time, though, the noise on passive and active assays is identical. As an example, we show how to quantify the influence on measurement noise of bead size and chamber geometry in active and passive assays.

Keywords: optical tweezers, active calibration, passive calibration, Allan variance, power spectrum, noise, drift, laser

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The possibility of performing precise micro-manipulation and measuring interactions and dynamic properties of single molecules has resulted in optical tweezers being a valuable technique in the field of biophysics [1]. To use optical tweezers in quantitative force studies, a bead, typically a polystyrene bead in the micrometer regime, is used as force transducer. To calculate the exact forces exerted on the bead it is crucial to perform force calibrations as precisely as possible. Calibration protocols for precise force and position determination typically involve either passive calibration, where the Brownian motion of the trapped particle is monitored, or active calibration where the measurement chamber is oscillated with respect to the trapped bead [2, 3]. Active calibration has the advantage of reducing the number of variables, for example neither the viscosity nor the size of the trapped object nor its distance to nearby surfaces need to be known [2, 4, 5]. The number of assumptions in the final calibration step can thereby be reduced. In addition, since the forces measured are in the sub-pN to hundreds of pN range, optical trapping assays are extremely sensitive to noise picked up by the instrumentation from the surrounding environment and to drift originating from laser instabilities and temperature gradients, for example. Clever modifications to the classical single beam optical trapping assay have been invoked to minimize the effect of drift and noise [6–10]. However, the impact of drift on active calibration protocols has not yet been systematically addressed and quantified.

Most high precision optical trapping assays routinely perform Fourier analysis of the trapped particle positions, which yield a power spectrum that can be used to deduce
the spring constant, characterize the quality of the trap, and quantify high frequency noise [11]. However, Fourier analysis is not a very efficient method for pinpointing low frequency noise and determining the optimal measurement time for calibration, for example for single molecule assays. For this purpose, Allan variance has proven to be a better choice [12–14]. In previous studies, the effect of very low frequency noise sources present in typical passive assays was investigated, these include the noise dependence of detection frequency, the type of photodiode, presence of a passive piezo stage with capacitive feedback loop, and presence of acoustic noise [12]. Here, we focus on quantification of noise in active calibration assays. Also, we show how to routinely implement Allan variance analysis alongside power spectral analysis and provide an optimized protocol that will allow the user to improve the signal to noise ratio in both passive and active optical trapping assays. As an example of how to use this optimized method, we performed both active and passive Allan variance analysis of optically trapped beads of different sizes in different chamber geometries with the goal of quantifying the corresponding noise contribution and determining the optimal measurement time for both passive and active calibration assays.

2. Materials and methods

2.1. Sample preparation

Measurement chambers with different heights and width-to-length ratios were made from two 100 μm thick glass slides, with either a single layer of Parafilm 110 ± 5 μm or a double layer of Parafilm 220 ± 10 μm that were cut into different geometries and placed between the cover slides as spacers. Cover slides were mended by heating the flow cell to 80 °C. Air bubbles in the film were removed by gently applying pressure to the top slide. Open chambers were made in a similar manner to that described in [15]. Phosphate buffered saline (PBS, 1×) was filtered with a 50 nm pore sized Millipore filter and degassed in ultrasound for 5 min. The buffer was used to prepare dilutions of polystyrene microspheres (1:50 000 from stock) of nominal diameter 792 ± 45 nm that were cut into different lengths with either a single layer of Parafilm 110 ± 5 μm or a double layer of Parafilm 220 ± 10 μm that were cut into different geometries and placed between the cover slides as spacers. The bead positions were measured for 12 min with the position sensitive photodiode. The acquisition frequency was 22 kHz. Furthermore, acquisition card settings were chosen to ensure the best possible data reliability. A schematic illustration of the experimental setting is shown in figure 1.

We performed both passive and active calibrations along the optimized protocol outlined in section 2.4. For the passive calibration we simply recorded the positions of the bead performing thermal motion within the trap. For the active measurements the piezo stage was driven in a sinusoidal motion at 8, 32, or 128 Hz with an amplitude of 300 nm. Piezo and bead positions were recorded for all measurements. The data were streamed to the computer utilizing a LabVIEW program optimized for reliable streaming of large time series [16]. After each measurement, both active and passive, the data were scrutinized to find the trapping stiffness by the power spectrum calibration procedure [11, 17] and the noise in the experiment was quantified using the Allan variance approach [12, 18, 19]. The Allan variance of each data set was calculated by a custom-written Matlab program available for download [20].

2.3. Allan variance

Allan variance was designed to extrapolate the drift of a system at infinity from finite measurements [18]. In contrast to the normal variance, Allan variance converges to a finite value for a broad range of typical noise sources found in biophysical experiments, such as purely stochastic white noise, shot noise, damped oscillations etc. Therefore, Allan variance has proven itself a valuable tool to quantify the stability and noise of optical tweezers [12, 19, 21]. It gives an exact quantification of the variance for neighboring measurement intervals of length τ. This allows for extrapolating the timescale at which the system is affected by high frequency noise, for example that originating from electronic components, as well as low frequency noise such as vibrations, temperature gradients, etc.

For a time series with N elements and a total measurement time \( t_{\text{acq}} = N/f_{\text{acq}} \) the Allan variance is defined as

\[
\sigma_\tau^2 = \frac{1}{2} \langle (x_{i+1} - x_i)^2 \rangle_{\tau},
\]

where \( x_i \) is the mean over a time interval \( \tau = m/f_{\text{acq}}, m \) being the number of elements in the interval. \( \langle \cdot \cdot \cdot \rangle \) denotes the average over all combinations of neighboring intervals. The convergence of the Allan variance results in a significant gain in resolution, especially for \( \tau > \tau_c \), where \( \tau_c \) is the typical correlation time of a particle undergoing Brownian motion in a harmonic trap. For measurement times significantly longer than \( \tau_c \), drift that is intrinsically present in every experimental setup becomes dominant over the fluctuations. At this transition a minimum of the Allan variance occurs, the corresponding time being the optimal measurement time that originating from electronic components, as well as low frequency noise such as vibrations, temperature gradients, etc.
Figure 1. A schematic of the optical tweezers setup. A continuous Nd:YVO<sub>4</sub> laser operated at 800 mW was used to trap polystyrene beads 50 μm above the lower surface of the measurement chamber. The scattered light from the bead was imaged via the condenser onto a photodiode and digitized with custom-made LabVIEW software. The sample was mounted on a piezo stage, and during active calibration driven sinusoidally at 8, 32, and 128 Hz. Different measurement chamber geometries were tested.

For calibrations [12], Allan variance is a direct measure of the accuracy and it is assumption free. It makes it possible to extrapolate the impact of typical low-frequency 1/f-noise without the need to define bandwidth or filters. Since Fourier analysis lacks this quality, Allan variance and Fourier analysis probe complementary regimes of the frequency spectrum.

The thermal limit is given by the inherent uncertainty in determining the position of a bead with drag coefficient γ in a harmonic potential with spring constant k during the measurement time τ [12]:

\[
SE_{(x)} = \sqrt{\frac{2k_{B}T\gamma}{k^2\tau}}.
\]  

(2)

If not oversampled, the Allan deviation cannot overcome this standard error. The closer the Allan deviation is to the thermal limit, the less noisy the trapping facility. For τ > 2\(\pi^2\)\(\tau_c\), the Allan deviation follows the \(-1/2\) scaling of the thermal limit with respect to τ.

2.4. Optimized protocol for calibration of optical tweezers setups

To quantitatively address and possibly minimize the noise present in an optical tweezers assay, we suggest an optimized protocol for optical trapping calibration that combines power spectral and Allan variance analysis:

(i) Sample position data in a passive calibration experiment for long time series with a sufficiently high acquisition frequency.

(ii) Calculate Allan variance of adjacent time series for a set of measurement times τ.

(iii) Determine optimal measurement time \(\tau(\sigma_x, \text{min})\) where the Allan deviation is minimal. Use this optimal measurement time for passive and active calibrations.

(iv) Calibrate over statistically independent intervals of length \(\tau(\sigma_x, \text{min})\) using the power spectral density method [11].

(v) If desirable, perform (i)–(iv) with active driving and fit power spectral density accordingly to [2]. This is particularly useful if the drag coefficient of the trapped object or the viscoelastic properties of the surrounding media are unknown [2, 3, 22].

(vi) Average the fitting parameters (corner frequency \(f_c\), conversion factors relating output voltages to distance) of consecutive, statistically independent intervals.

(vii) Convert the Allan deviation from voltage units to meters, compare to the thermal limit.

(viii) Compare internal (e.g. active driving) and external noise (e.g. drift) levels. Quantify and possibly minimize noise sources.

This optimal protocol is not limited to optically trapped spherical particles, but is applicable to any kind of commonly used optical tweezers handles. For example, its use on gold nanorods and quantum dots has been demonstrated [13, 14].

3. Results

3.1. PSD in combination with Allan variance

Allan variance analysis can easily be performed together with the more conventional power spectral analysis, typically performed to find the force characteristics of the optical trap. To show how both power spectral density (PSD) and Allan variance can even be presented in the same plot, we used the data from a trapped 792 nm bead and sampled its position for 12 min. In figure 2, the corresponding power spectrum, averaged 4, 32, and 8192 times, respectively, is plotted together with the Allan variance of the same time sequence. To get the same physical values plotted on the ordinate axis for both the Allan variance and the PSD, the Allan variance was plotted as \(2\sigma_x^2\) as a function of \(\pi \tau\), where \(\pi \tau = \omega^{-1}\). The reason for the emergence of the factors π and 2 is due to the particular form of Fourier transformation used [11]. Interestingly, the...
Allan variance (solid black line) is able to reconstruct the power spectrum and yields a high resolution analysis of the data, particularly at low frequencies. Considerable averaging is needed to make the PSD equally precise as the Allan variance of the same dataset. The power spectrum yields noisier information for the low frequencies, hence details regarding noise and drift are lost. For high frequencies, the product of Allan variance and measurement time is slightly smaller than the power spectral density, due to the fact that Allan variance only considers neighboring intervals.

### 3.2. Active versus passive calibration

Active calibration has the advantage of needing less pre-knowledge about the system for which the force constant, for example, is sought. However, introducing an oscillatory motion could potentially increase the overall noise and influence the optimal measurement time. To investigate these issues we performed an experiment where a 792 nm bead was held by a stationary trap and the chamber was moved in a sinusoidal motion at 32 Hz. Also, we performed a passive calibration of the same bead. The resulting Allan deviation plots of the positions of the bead as well as the output from the piezo stage are shown in figure 3. The solid lines show the Allan deviation of the bead’s positions and dashed lines denote the output from the piezo stage, which was turned on during both the active and passive experiments but kept stationary for the passive experiments. Green lines denote active calibration, black lines passive calibration. The dashed–dotted line denotes the thermal limit given by equation (2). It represents the lower limit for position detection. The trace of the passively calibrated particle (solid black line) shows that for low frequencies, noise levels are very close to the thermal limit, a benchmark of a stable setup. For high frequencies, the product of Allan variance and measurement time is slightly smaller than the power spectral density, due to the fact that Allan variance only considers neighboring intervals.

3.3. Variations in bead size and chamber geometry

As an example of how to go through the experimental settings with the aim of minimizing noise, we performed active and passive calibrations to pinpoint the effect of changing bead size and chamber geometry. Two different beads, with diameters of 792 and 2035 nm, were trapped with the same laser power and the frequency of the active calibration was 32 Hz. Instead of calibration, figure 3 can be divided into two distinct regions, one where the driven oscillation still dominates the motion of the bead, \( \tau < 6 \text{ s} \), and another for longer measurement times, \( \tau > 6 \text{ s} \), where the Allan deviations originating from passive and active calibrations, respectively, overlap completely. In this region drift dominates the measurement. Hence, our results show that active methods have a position accuracy that is equally as good as passive calibrations provided that the measurement time is long enough. The inset of figure 3 shows the maximum and minimum values of the positions of the bead during active calibration, when the piezo is run at 32 Hz (green symbols and lines). The maxima and minima converge and become indistinguishable for \( \tau > 3 \text{ s} \). Also shown are the thermal limit (dashed–dotted line) and a guide-to-the-eye of slope \(-1\) (dashed–double-dotted line).

To zoom in on the optimal time measurement period for active calibration, we considered the divergence of the Allan deviation from the thermal limit for various driving frequencies (8, 32, and 128 Hz). Figure 4 shows the Allan deviations of a bead undergoing active calibration subtracted from the thermal limit. Fits with \(\tau^{-1}\) scaling to the maximum peak values for each data set show that the deviation from the thermal limit rapidly decreases and they converge, with a deviation less than one 0.1 nm, already at approximately 1 s. The inset is a zoom in on the interesting region where the influence of the oscillations falls below the noise present from other external sources.

4
sensitivity is plotted versus $\tau$. Some results are shown in figure 5(b), where the force ratio in the lateral direction with respect to the trapping laser for a quadratic chamber with $2 \times 2 \text{ mm}^2$ (solid line), a quadratic chamber $2 \times 2 \text{ mm}^2$ (dashed–double-dotted line), and open chamber (dashed line). (C) Comparison of the force sensitivity for chambers with a height of 100 $\mu$m (solid lines) and 200 $\mu$m (dashed–double-dotted lines) during active calibration (32 Hz, gray) and passive calibration (black). All dashed–dotted lines denote the thermal limit.

The effect of varying chamber height, doubling the volume, was minor, with regard to both the optimal measurement time and the absolute force sensitivity, though with a tendency for the shallower chamber to pick up more noise.

4. Discussion and conclusion

As Fourier analysis and Allan variance analysis have different strengths, the optimal strategy encompasses the use of both methods. It is convenient to have both present in the same representation, as shown in figure 2. Fourier analysis is especially efficient for pinpointing high frequency noise contributions, whereas Allan variance analysis elegantly quantifies low frequency contributions and provides the optimal measurement time, balancing the gain of drawing additional data points from a given distribution with the fact that inevitably for long enough timescales drift kicks in.

We systematically investigated the noise contribution from both passive and active calibrations, the latter involving a sinusoidal motion of the chamber with respect to the trapped bead. Active calibration procedures are often attractive, especially if, for example, the friction coefficient of the trapped particle or the viscosity of the medium are unknown. If active methods are used in combination with passive methods, one can even deduce trapping properties in an unknown viscoelastic environment like the cytoplasm of a living cell [3, 22]. Due to the active driving, it is not surprising that at measurement times shorter than the optimal measurement time the noise on active assays is considerably larger than on passive assays. It was previously showed that the mere presence of a turned on but not actively moving piezo stage introduces significant noise to an optical
trapping assay [12]. Interestingly, our results showed that on timescales equal to or longer than the optimal measurement time, active calibration does not introduce additional noise in comparison to passive calibration. This is true for all driving frequencies tested (8–128 Hz). Therefore, the contribution of low frequency noise from the piezo stage might be linked to the feedback of the controller itself. However, care should be taken, since piezo stages have different inherent properties, for example resonance frequencies. A comparison of the maxima of the bead response in active calibration to the thermal limit in an Allan deviation graph allows for a good estimate of suitable driving frequencies and optimal measurement times.

The optimized active and passive calibration routine can be used to pinpoint noise in specific experimental settings. As an example, we investigated if the level of noise would be dependent on the bead size or chamber geometry. For both short and long times the smaller beads picked up less noise than the larger beads, but the noise levels for large and small beads were identical in the optimal measurement time interval. These conclusions are valid for both active and passive calibration. The noise picked up by a bead in chambers where we systematically varied the lateral aspect ratio and the height of the chamber did not depend on the chamber geometry. However, a bead in an open chamber picked up significantly more noise than in a closed chamber. Hence, to minimize noise we would recommend using closed chambers.

Every experimental setup and its environmental conditions are slightly different. By employing a combination of power spectral analysis and Allan variance analysis and reporting the outcomes to systematic changes, noise sources can be found, quantified and sometimes eliminated. Implementing these routines on a daily basis holds the potential to compare various setups and measurements across different laboratories with the goal of improving optical trapping standards.

Acknowledgments

We acknowledge useful discussions with Joanne L. Cornall, Liselotte Jauffred, Ulrich F. Keyser, and Oliver Otto and thank Nicola Girotti for help with illustrations. MA acknowledges financial support from the Swedish Research Council and LBO acknowledges support from the KU excellence program.

References