

Photonic force microscope calibration by thermal noise analysis

E.-L. Florin, A. Pralle, E.H.K. Stelzer, J.K.H. Hörber

Cell Biophysics Program, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, Postfach 10.2209, D-69117 Heidelberg, Germany
(Fax: +49-6221/387-306, +49-6221/387-242, E-mail: *lastname@embl-heidelberg.de*)

Received: 25 July 1997/Accepted: 1 October 1997

Abstract. The forces of a photonic force microscope based on optical tweezers are determined using the Boltzmann distribution of thermally driven position fluctuations. As a fundamental method, only a minimum of information about the system is required, i.e. the solution's temperature. This calibration method provides detailed information about the trapping potential with nanometer resolution. In contrast to other calibration methods, no numerical hydrodynamic corrections are necessary. The lateral force constant of the optical trap was measured along the optical axis and found to decrease linearly by $\approx 8\%/ \mu\text{m}$ for a latex bead 600 nm in diameter. Oscillations in the lateral force constant were found along the optical axis close to the surface.

Small forces are encountered in physical sciences as well as in life sciences. They determine properties such as colloidal stability, cell adhesion, cell motility, stability of specific bonds and conformational changes in proteins. Various methods for measuring small forces have been developed in the past (optical tweezers, AFM, magnetic beads, hydrodynamic flow). Optical tweezers [1] turn out to be especially suitable for biological applications [2] because they are easily combined with optical microscopes, applicable to transparent objects and cover a biologically relevant force range.

Recently, we introduced a three-dimensional scanning probe microscope, the photonic force microscope (PFM) [3]. This microscope is based on optical tweezers and is especially suitable for biological applications. The three-dimensional trapping potential of optical tweezers is only harmonic for small displacements and may also vary due to local changes in refractive index or absorption. Therefore, a fast three-dimensional in situ force calibration method for optical tweezers is required that is not restricted to harmonic potentials and is independent of other parameters such as the bead diameter or the local viscosity.

The methods of calibrating the force field of optical tweezers can be divided into two categories: first, calibration

against known forces (e.g. Stokes friction and gravitation) [4] and second, the analysis of thermal fluctuations. Calculation from first principles of the forces acting on a trapped particle have been performed [5, 6], but they rely on parameters that are not exactly known and neglect effects such as spherical aberration. Conventional calibration methods based on thermal noise analysis have to assume a harmonic potential and require parameters such as the viscosity of the medium [7].

Boltzmann statistics has been used by Prieve et al. [8] to measure the potential caused by colloidal forces between a small latex sphere and a glass surface along the z -axis with 1 ms temporal and several nm positional resolution. More recently, Rädler et al. [9] extended this method to measure the interaction between artificial lipid membranes on solid support and lipid vesicles.

Both techniques rely on the sedimentation of the particles (diameter $\approx 10 \mu\text{m}$) due to gravitation. The accessible parts of the potentials are given by the equilibrium position, determined by parameters such as the salt concentration. Because of the lack of active three-dimensional positional control over the bead, the methods are limited to large spheres, preventing fast diffusion in solution.

We report a calibration method relying on the Boltzmann statistics of the thermally activated position fluctuations of the trapped particle and compare it with other established methods. Our method overcomes the restriction of harmonic potentials and requires only the medium's temperature. The diameter of the particles and therefore the spatial resolution is only restricted by the ability of the optical trap to generate a potential well with a depth of about 10 times the thermal energy $k_B T$ and by the sensitivity of the position detectors.

Furthermore, our method allows the measurement of energy landscapes even below the thermal energy with high spatial and temporal resolution. Interestingly, since this calibration method depends only on the temperature, it allows us to measure other parameters, such as the local viscosity, by combining it with other calibration methods.

1 Experimental setup

A schematic of our optical tweezers setup is outlined in Fig. 1. The optical tweezers are built around an inverted microscope (Axiovert 35, Zeiss, Jena, Germany) with standard DIC equipment. The expanded beam emitted by an Nd:YVO₄ laser (T20-B10-106Q, Spectra Physics) is coupled into the back aperture of a piezo-driven (P721.00, Physics Instruments, Germany) oil-immersion objective lens (Plan Neofluar 100×, Zeiss, Jena, Germany) with a high numerical aperture (NA = 1.3). The back aperture is overfilled to ensure high trapping efficiency. The objective lens generates a diffraction-limited spot in the medium-filled sample chamber and traps particles with a refractive index slightly higher than that of the surrounding medium in all three dimensions. The forward scattered light is collected by the condenser lens and focused on a quadrant photodiode (S5981, Hamamatsu) to control the position of the trapped particle with respect to the position of the geometric laser focus. To separate the detection from the regular DIC illumination path a dichroic mirror, designed to reflect only the wavelength of the laser (1064 nm), directs the light through an absorption filter (NG, Schott, Germany) to the detector.

The original solution, containing latex beads (FluoSpheres, CML, Molecular Probes) with a nominal diameter of 200 nm (actual diameter 216 nm ± 3.8%) was diluted 10000× in distilled water. Similarly, latex beads in PBS (phosphate-buffered saline) with 600 nm ± 6.3% (FluoSpheres, Molecular Probes) were used in measurements along the optical axis.

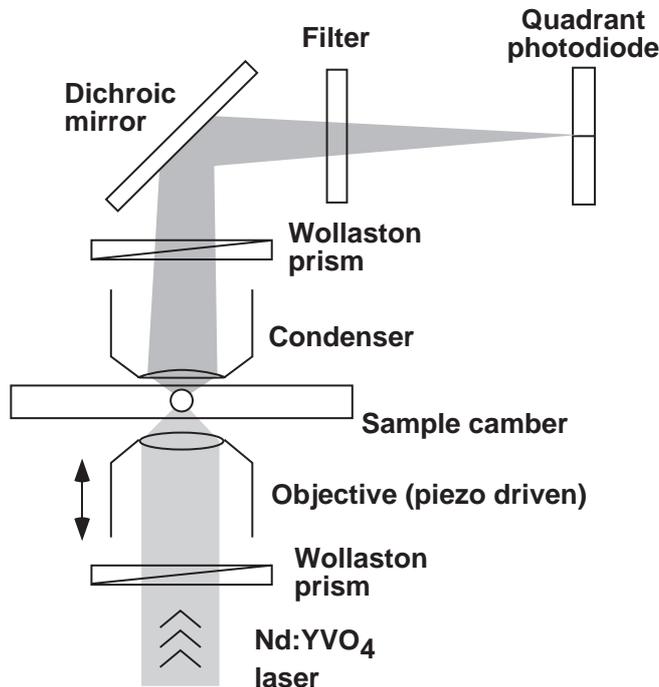


Fig. 1. Experimental setup: an expanded parallel Nd:YVO₄ laser beam is directed through a Wollaston prism into the back aperture of an oil-immersion and piezo-driven objective lens. A latex bead is trapped close to the focal point in the object plane. The forward-scattered light is collimated by a condenser lens and directed by a dichroic mirror onto a four-quadrant photodiode after passing another Wollaston prism and before an absorption filter

The detection signal for a lateral displacement of the bead was calibrated after all measurements had been performed on the same bead. To ensure reliable calibration, strong adhesion between the bead and the glass surface was forced by pressing the bead against the glass surface for several seconds. The laser focus was then scanned across the bead and the detector response as a function of the x -position was recorded on a storage oscilloscope (9304 AM, LeCroy) and later analyzed (IgorPro, Wavemetrics, OR, USA). The thermal noise data were either recorded on the same storage oscilloscope or via a data acquisition board (DAS-1600, Keithly Instruments or AD-Win 5F, Jäger, Lorsch, Germany). The analysis of the data was performed using IgorPro. To avoid digitization effects, we corrected the data for slow drift. For z -position-dependent measurements, the objective lens was moved by the piezo translator (without feedback) controlled synchronously with data acquisition.

2 Results and discussion

Boltzmann statistics

$$p(x) dx = C e^{-\frac{E(x)}{k_B T}} \quad (1)$$

describe the probability $p(x) dx$ of finding a molecule or a small particle in a potential $E(x)$ in a volume dx in thermal equilibrium. C normalizes the probability distribution ($\int p(x) dx = 1$). The coupling between the particle and the external field is not important as long as the force field is conservative. By measuring the probability density $p(x)$, the potential experienced by the particle can be calculated:

$$E(x) = -k_B T \ln p(x) + k_B T \ln C. \quad (2)$$

The last term determines the potential offset and is neglected.

Figure 2a shows a 100 ms period of the position fluctuations along the x -axis of a latex bead 200 nm in diameter trapped 5.5 μm above the coverslip in solution sampled at a frequency of 50 kHz. Amplitude fluctuations of up to 60 nm were recorded. The histogram in Fig. 2b shows the frequency at which bead positions were recorded. The bin width in this histogram is 1 nm. To extract the actual potential $E(x)$ from this histogram we calculate the logarithm of the frequency in Fig. 2b and multiply it by $k_B T$ (Fig. 2c). This is fitted numerically by a harmonic potential with a force constant $k = 1.45 \times 10^{-5} \text{ N/m}$. The bin width reflects how well one measures the position of the bead along the x -axis, but not the resolution with which we probe the focal spot's intensity profile. This is of the order of the bead diameter. The temporal resolution is the time required to record uncorrelated data for a histogram. In these experiments the time resolution was $\approx 75 \text{ ms}$ for an error tolerance below 10%. For comparison we performed established calibration methods on exact the same bead at the same height above the coverslip.

Calibrating the trapping force against the Stokes friction force is one of the most common methods. The bead is moved in a medium at a certain height above the coverslip. The force acting on the bead is according to Stokes's formula $F_{\text{Stokes}} = 3\pi d\eta v$, where d denotes the diameter of the bead, η the dynamic viscosity of the medium and v the velocity of the

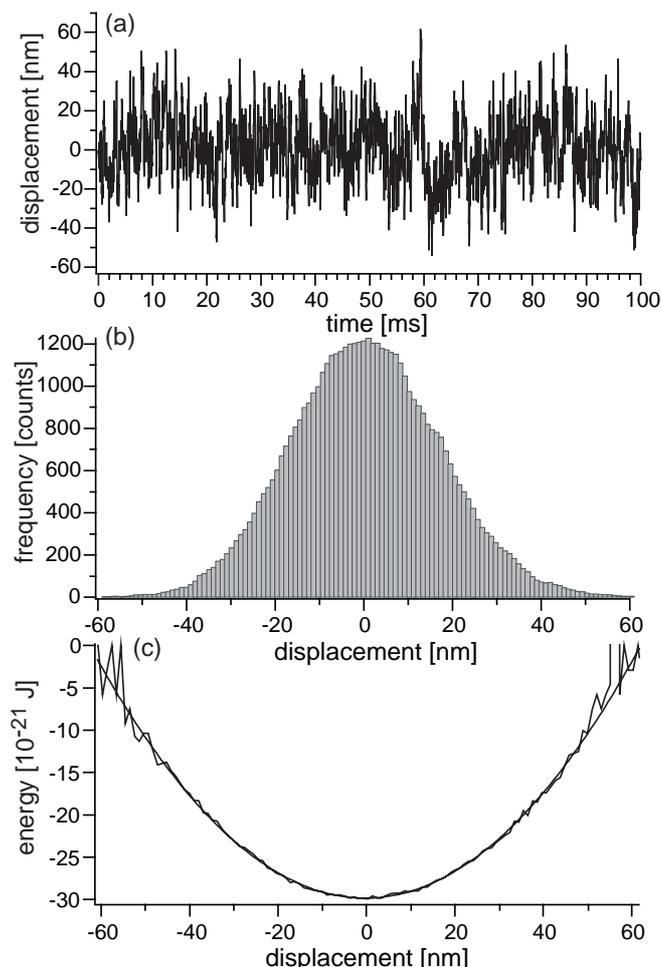


Fig. 2a–c. Typical trace of thermal position fluctuations of a 200 nm bead trapped 5.5 μm above the glass surface (a) (recording time 5 s). **b** Histogram of the position fluctuation in a (bin width of 1 nm). Energy profile for the trapped bead along the x -position calculated from the histogram in b using Boltzmann statistics. The solid line shows a parabolic fit assuming a harmonically bound Brownian particle

bead. Although apparently simple, this calibration method is complicated by the dependence of the viscosity on the temperature and the hydrodynamic corrections which become necessary when the distance to the coverslip is of the order of the bead diameter [10] to calculate the force constant of the trap, one must plot the displacement in the trap versus the applied force, and therefore the calibration of the position detector must be known. Taking into account errors due to bead diameter, temperature, detector calibration and statistics the absolute error of this method was 21% (Table 1). The large

Table 1. Comparison of different calibration methods (same bead in all cases)

Method	k (N/m)	Δk (N/m)
Boltzmann statistics	1.45×10^{-5}	0.10×10^{-5} (7%)
Corner frequency	1.36×10^{-5}	0.15×10^{-5} (11%)
Mean square displacement	1.50×10^{-5}	0.10×10^{-5} (7%)
Stokes friction	1.39×10^{-5}	0.29×10^{-5} (21%)

error, the restriction to lateral dimensions and the restriction to move the bead are disadvantages.

One of the simplest and fastest methods of calibrating the force constant is the measurement of the mean square displacement ($\langle x^2 \rangle$) of the position fluctuations due to thermal excitations, since for a harmonic potential the equipartition theorem predicts $\frac{1}{2}k_B T$ per degree of freedom.

However, this method always assumes a harmonic potential and is easily disturbed by external noise, leading to an apparently softer force constant for the trap. A total error of about 7% has been calculated for this method under the given experimental conditions, taking detector calibration and temperature errors into account.

Another method relying on the equipartition theorem is the measurement of the corner frequency [7]. A Brownian particle in a harmonic potential has a Lorentzian power spectral density with a corner frequency ω_C . The corner frequency itself ($\omega_C = \frac{k}{\gamma}$) depends on the force constant and the viscous drag γ of the particle. The viscous drag can be calculated using Stokes's formula ($\gamma = 3\pi\eta d$). Errors arise from fitting the uncertainties of the corner frequency, the bead diameter and the temperature-dependent viscosity. We calculated a total error of $\approx 11\%$. The advantage of this method is that the detector calibration need not be known. Therefore, the force constant can be measured in two independent ways. A further advantage of calculating the power spectral density is the discrimination against mechanical and electronic noise.

Besides providing additional control of the experiment, the combination of noise analysis by Boltzmann statistics and the corner frequency allows the measurement of an independent parameter such as the local viscosity of the medium, which was not accessible before. This is especially useful because the viscosity is the most uncertain parameter, depending on both temperature and hydrodynamic corrections.

Figure 3 shows the force constant as a function of the axial position of the bead. For this measurement the thermal noise has been continuously recorded as a function of the axial position in the range 0–9 μm . In this range most of the single-beam gradient trap experiments are performed. The force constant increases constantly by 8%/ μm , normalized to

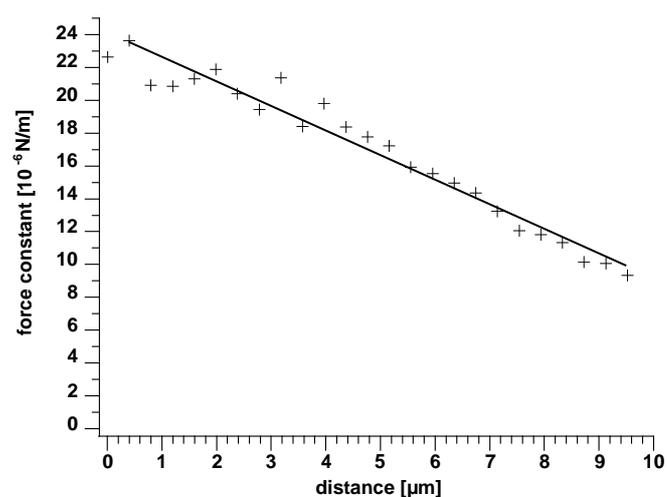


Fig. 3. Force constant of the trapping potential of a 600 nm bead versus distance of the bead from the glass surface ($T = 29^\circ\text{C}$)

its value at 5 μm . The oscillations close to the glass surface, with amplitudes up to 15%, are caused by interference effects between the bead and the glass surface (unpublished result). Preliminary results show that these interference effects really change the force constant. We attribute the linear decrease of the force constant along the z -axis to spherical aberration, which could affect the force constant twofold. First, the focal spot broadens in the solution and therefore the force constant decreases. Second, in addition to this effect, the resting point of the bead in the trapping potential may be shifted further out from the geometrical focus with increasing distance to the glass surface. This view is supported by the observation of additional stable trapping minima far into the solution by Felgner et al. [4].

3 Conclusions

Analysis of the thermal noise by Boltzmann statistics enables us to measure potential profiles below the thermal energy level with high spatial and temporal resolution. The potential profiles are allowed to be anharmonic and asymmetric.

Compared with other calibration methods, Boltzmann statistics provide a very accurate unbiased method, depending only on the temperature and applicable in all three dimensions. For an uncalibrated energy profile even knowledge of the temperature is not required. The combination with other

calibration methods allows independent measurements of additional parameters such as the local viscosity or the particle diameter. The latter is especially important for particle diameters far below a light microscope's resolution. Energy landscape profiling as well as viscosity mapping are in situ measurements and we expect them to become a major tool for photonic force microscopy [3].

Acknowledgements. We thank Steffen Lindek for helpful discussions and presenting some parts of this work at a DFG meeting (Rostock, Germany, March 1997). This work was supported by the DFG.

References

1. A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu: *Opt. Lett.* **11**, 288 (1986)
2. K. Svoboda, S.M. Block: *Ann. Rev. Biophys. Biomol. Struct.* **23**, 247 (1994)
3. E.-L. Florin, A. Pralle, J.K.H. Hörber, E.H.K. Stelzer: *J. Struct. Biol.* **119**, 202 (1997)
4. H. Felgner, O. Muller, M. Schliwa: *Appl. Opt.* **34**, 977 (1995)
5. A. Ashkin: *Biophys. J.* **61**, 569 (1992)
6. T. Wohland, A. Rosin, E.H.K. Stelzer: *Optik* **102**, 181 (1996)
7. L.P. Ghislain, W.W. Webb: *Opt. Lett.* **18**, 1678 (1993)
8. D.C. Prieve, S.G. Bike, N.A. Frej: *Faraday Discuss. Chem. Soc.* **90**, 209 (1990)
9. J. Rädler, E. Sackmann: *Langmuir* **8**, 848 (1992)
10. J. Happel, H. Brenner: *Low Reynolds Number Hydrodynamic* (Prentice Hall, Englewood Cliffs, NJ 1965)