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Optical Tweezers: Probing Biological Surfaces

LENE ODDERSHEDE[a*], SONIA GREGO[a†], SIMON F. NØRRELYKKE[a] and KIRSTINE BERG-SØRENSEN[b]

[a]*The Niels Bohr Institute and* [b]*NORDITA, Blegdamsvej 17, 2100 Copenhagen Ø, Denmark*

A pair of optical tweezers is an eminent tool for manipulating biological specimens at the macromolecular level. Optical tweezers measure corresponding forces and distances typically in the pico Newton and nanometer regim0es. Compared to the Atomic Force Microscope (AFM) optical tweezers are capable of accurately measuring forces which are orders of magnitude lower than those obtainable with AFM. We have built an experimental setup based on optical tweezers and in this paper describe its technical properties, design, abilities, and use in probing biological surfaces.

Keywords: Optical tweezers, laser traps, membrane proteins, diffusion

INTRODUCTION

The use and refinement of optical tweezers is a rapidly evolving field and our setup is constructed according to the most recent developments. In its simplest implementation, the trap acts as a manipulator for optically confined, micrometer-sized objects. Often, such a micrometer-sized object is bound to the system of interest, e.g. a protein which can be much smaller than the size of the trapped object. The motion of the trapped object can be monitored with a spatial resolution of nanometers. When the displacements of the trapped object are used for calibration purposes, the optical trap becomes a force transducer (force scope) in the range of pico Newton and nanometers. Digital data analyses allow for a real-time measurement of the corresponding forces and distances. The spatial resolution of optical tweezers is typically limited by thermal fluctuations of the probe particle. This prevents the optical tweezers from reaching the same spatial resolution as the AFM (Stout and Webb, 1998). As the stiffness of a typical AFM cantilever is around 10^2 to 10^{-3} Newtons/meter and the stiffness of the optical trap is generally 1 to 2 orders of magnitude lower than that of the softest AFM cantilever, the two surface probe methods complement each other nicely. Therefore, the AFM is better suited for exploring strong interactions and the optical tweezers for studying weaker interactions.

Since their early development, optical tweezers have been used to study biological samples, especially optical tweezers based on laser light in the regime of the infrared that is not absorbed by biological specimen. The straightforward implementation of optical tweezers in liquids is an advantage, an approach only recently developed by the AFM (Keller et al., 1997). Also, tweezers can invade e.g. a cell in a non-destructive manner, as the laser light is only focused inside the cell, and can be used for studies in a living cells. However, detailed investigations of

* Corresponding author.
† Permanent address: Department of Biology, University of North Carolina, Chapel Hill, NC, USA.

intracellular functions and especially molecular motors, have been performed by means of *in vitro* assays; one seminal example of this is the study of the molecular motor-molecule kinesin carried out in the group of Steven M. Block (see e.g. Svoboda and Block, 1993). Investigations of the elastical properties of DNA by stretching have been performed both by optical tweezers (Smith, 1996; Wang et al., 1997) and by a combined atomic force microscope and optical tweezers (Shivashankar and Libchaber, 1997). Manipulation of proteins in cell membranes which is the aim of our study has been carried out using AFM as well as optical tweezers. Due to the different force ranges of the two techniques, AFM has been used to study the vertical forces necessary to pull a protein out of a bacterial membrane (Müller, 1999), the adhesion of cells to a substrate (Sagvolden, 1999), and to explore the energy landscapes of receptor-ligand bonds which are found to be strongly dependent on the force load (Merkel et al., 1999), whereas optical tweezers have been used to study the motion of proteins laterally within the membrane (Edidin et al., 1991; Sako and Kusumi, 1995; Pralle et al., 2000).

EXPERIMENTAL SETUP

The optical trap is based on an inverted Leica microscope (Leica DM IRB HC) equipped with a motorized objective turret. The microscope is equipped with DIC optics and a 100 W halogen lamp. The setup is illustrated in Figure 1. The laser beam from a Nd:YVO_4 laser (10 W Spectra Physics Millennia, λ=1064 nm, TEM_{00}) is inserted in the optical part of the microscope through the epifluorescence port and deflected by a custom-made dichroic mirror placed in the epifluorescence prisms wheel. The laser output (diameter < 1 mm) is sent through a commercial beam expander (CASIX, China) and passed through a variable attenuator consisting of a rotatable halfwave plate followed by a beam-splitter polarizing cube. The beam is steered by two mirrors and a 1:1 telescope is used to move the trap in the specimen plane (Svoboda and Block, 1994). The telescope consists of 2 lenses with identical focal lenghts of 100 mm. The image of the laser on the first lens is imaged by the second on the back focal plane of the microscope objective. The first lens is mounted on a *xyz* translator. The manual translator along the beam axis (*z* direction) has a travel of 20 mm, while translation in *x* and *y* directions are motorized by the use of two orthogonally mounted Newport DC transducers (model M-MFN25CC) operated with a joystick control. The microscope objective (100x/1.4 NA oil plan apochromat infinity corrected, Leica) has its back aperture ($\approx$ 7 mm) overfilled with the laser beam, to ensure that the light converges to a tight, diffraction-limited spot (Svoboda and Block, 1994). The specimen is perfused in a chamber consisting of a coverglass with a thickness of 0.17 mm and a standard microscope slide separated by two layers of double-sticky tape. The Leica microscope stage has been substituted by a piezoelectric stage (PI 731.20, Physik Instrumente, Germany) with capacitative feedback control and nm position resolution mounted on a spring-loaded translation stage (750-MS Rolyn Optics, CA) for coarse movement of the sample. A CCD camera (Sony XC-75) is used for imaging the sample; its output is sent to a S-VHS video tape recorder or to a computer by a National Instrument frame-grabber (IMAQ PCI-1407). In order to detect the position of the trapped object with high temporal and spatial resolution we use a non-imaging technique, focusing the image of a trapped particle on a quadrant photodiode. We use the trapping laser as the source of illumination for the quadrant detector (Allersma et al, 1998), a configuration that ensures a high signal to noise ratio and keeps trapping and photodetection intrinsically aligned. After being focused by the microscope objective the laser beam is collected by a 1.4 NA oil condenser and projected by a dichoic mirror (Oriel) to a quadrant photodiode (S5981, Hamamatsu). The photodiode is positioned behind the focus point of the condenser which is aligned according to Köhler illumination. At the position of the photodiode the incident beam has a diameter of approximately 5 mm, half the size of the detector. The lateral displacements (*x* and *y* signals) of the trapped bead are obtained as the differences of the signal impinging on the two halves of the quadrant photodiode. As long as the

monitored motion is substantially smaller than the size of the bead, the signal from the photodiode is proportional to the displacement of the bead in the trap and the total intensity contains information about the axial displacement of the bead in the trap (Pralle et al., 1999). Signals from the photodiode enter custom-built electronics and are subsequently digitized by a 12-bit A/D board (PCI-MIO-16E-4, National Instruments). This multifunction board controls the piezoelectric stage as well. The analog bandwith of our system is higher than 100 kHz and is limited by noise in the electronics.

CHARACTERISATION OF THE OPTICAL TRAP

Lateral force calibration of the optical trap can be performed by the escape method (see e.g. Svoboda and Block, 1994). In this method the lateral force on an optically trapped spherical polystyrene bead is caused by a fluid drag which is gradually increased until the bead escapes the trap. Thus the measurement gives the maximum force which the trap is able to exert. The uncertainty of this calibration is around 20%. The fluid drag on the trapped bead is created by moving the liquid sample with respect to the trapped bead at a known velocity. The piezo stage used to generate the movement of the sample has a resonance frequency around 400 Hz and a travel of 100 μm. By applying computer generated triangular or sinusoidal waves with frequency up to 40 Hz and typical amplitudes between 1 and 10 μm, velocities of the order of a few hundreds μm/s are produced. For small velocities the drag force F on the bead is proportional to the velocity, v, $F = \gamma v$, where γ is given by Stokes Law:

$$\gamma = 6\pi\eta r, \tag{1}$$

where r is the radius of the sphere and η the viscosity of the suspending medium. Close to a surface Equation (1) must be corrected due to proximity effects by a factor k that can be approximated by Faxens law (Stout and Webb, 1998):

$$k = \left(1 - 9/16\left(\frac{r}{h}\right) + 1/8\left(\frac{r}{h}\right)^3 - 45/256\left(\frac{r}{h}\right)^4 - 1/16\left(\frac{r}{h}\right)^5\right)^{-1}, \tag{2}$$

where h is the distance from the center of the bead to the surface. Equation (2) is valid for $h - r > 0.02r$. The factor k takes values between 1 and 3. In our setup the distance from the coverglass is measured by the motorized turret of the Leica microscope which has a minimal step size of 0.05 μm.

For smaller forces a more precise calibration method uses the thermally excited position fluctuations of the bead in the trap, the uncertainty of this method is around 7% (Florin et al., 1998). In the plane perpendicular to the beam the intensity profile of the laser has a Gaussian shape, i.e., the dielectric bead will feel an approximately harmonic potential near the waist of the beam $U(x) = \frac{1}{2}\kappa_x x^2$, where x is the lateral position of the bead with respect to its equilibrium position and κ_x is the lateral spring constant of the trap. Analog arguments apply for the second lateral position coordinate y. The distribution of positions in the trap is described by Boltzmann statistics in a harmonic potential:

$$p(x) \propto \exp(-x^2/2\sigma_x^2). \tag{3}$$

Fitting this expression to a histogram of positions allows determination of σ_x. The equipartition theorem yields $\sigma_x^2 = \langle x^2 \rangle = k_B T/\kappa_x$, providing a determination of the trap stiffness κ_x. As the photodiode provides a voltage as measurement of the position of the bead in the trap, σ_x is not given in meters but rather in volts. The actual potential experienced by the bead can be measured directly by monitoring the movements of the bead, provided an independent calibration of the photodiode is available (Florin et al., 1998).

The motion of the bead in the trap is described by the Langevin equation

$$\mathcal{F}(t) - \kappa_x x - \gamma\dot{x} = 0, \tag{4}$$

where $\mathcal{F}$ represents the thermal forces and γ is the drag coefficient. As the Reynolds numbers of the

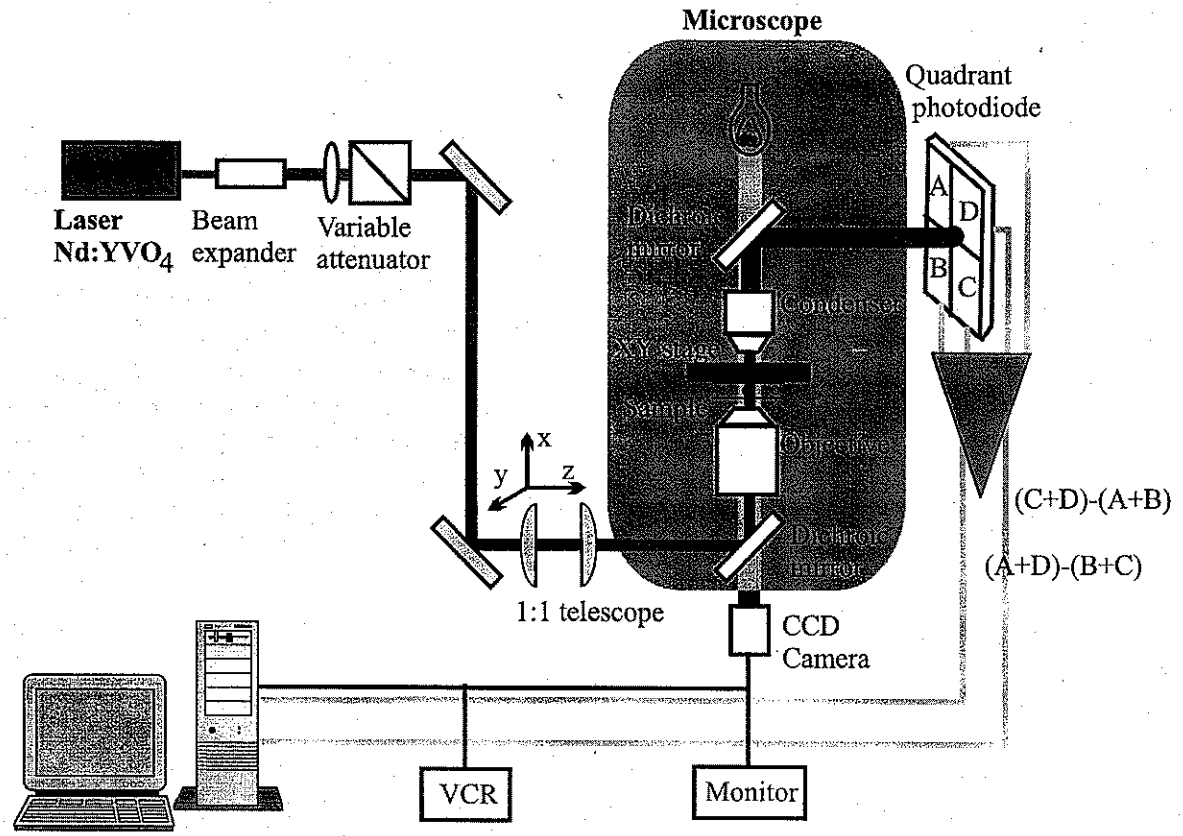

FIGURE 1 Schematic diagram of the optical tweezers setup. A Nd:YVO_4 laser is combined with a Leica microscope through the epifluorescence port. The trapped object is imaged on a quadrant photodiode for position detection with high temporal and spatial resolution

moving objects are very low inertial forces have been neglected in Equation (4). Taking the Fourier transform and the squared modulus of Equation (4) the following power spectrum is obtained:

$$S_x(f) = \frac{k_B T}{\pi^2 \gamma (f_c^2 + f^2)}, \qquad (5)$$

where the corner frequency $f_c = \kappa_x / 2\pi\gamma$ can be found by fitting Equation (5) to the power spectrum. The drag coefficient γ can be determined by Stokes's law Equation (1) and Faxen's law Equation (2) in which case Equation (5) provides a way of determining κ_x which is independent from the method suggested by using the Boltzmann distribution Equation (3). Comparing the two values of κ_x provides a method of calibrating the response of the photodiode (a voltage) to the position of the bead in the trap (a distance). Alternatively, κ_x found from Equation (3) can be used in the expression for f_c to find γ empirically if the photodiode is calibrated by other methods.

In Figure 2 a) a time-series for the position $x(t)$ is shown for a polystyrene bead with a diameter of 1.05 μm trapped approximately 5 μm above the coverslip. The sampling frequency is large enough to monitor the ballistic motion. Figure 2 b) shows the power spectrum obtained for the same data set as shown in Figure 2 a). The corner frequency is 420 Hz corresponding to a trap stiffness $\kappa_x = 2.6 \cdot 10^{-2}$ pN/nm. Comparing this value of κ_x to the one obtained from fitting Equation (3) to a histogram of the positions

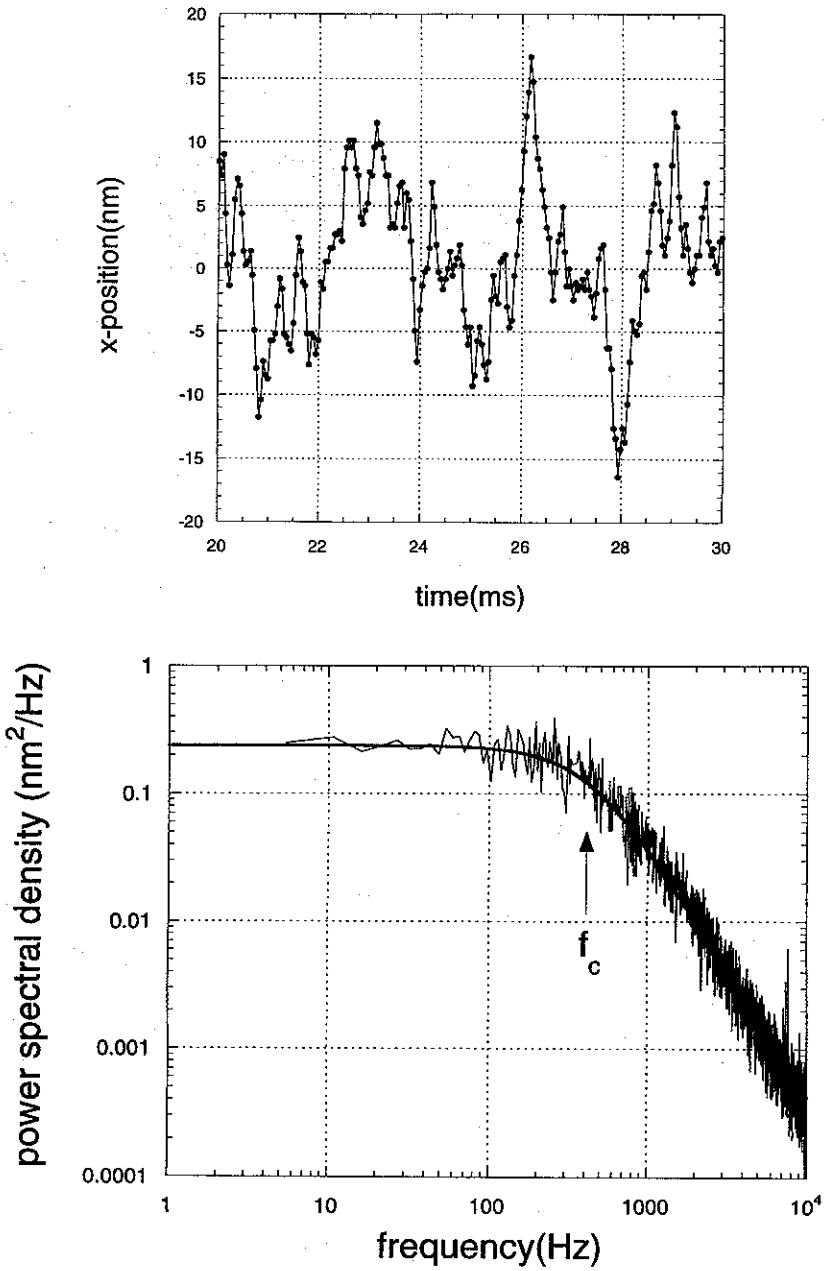

FIGURE 2 A polystyrene bead with a diameter of 1.05 μm is trapped 5.0 μm above a surface. The incident laser power in the trap is approximately 40 mW. **a)** A segment of the time-series for the lateral position of the bead. The sampling frequency is 22 kHz. **b)** Power spectrum of lateral position. The thick grey line is a Lorentzian fit to the data yielding a corner frequency $f_c = 420$ Hz. This corresponds to a trap stiffness of $2.6 \cdot 10^{-2}$ pN/nm

yields the calibration of the photodiode: 1.0 mV corresponds to 6.2 nm.

The properties of the trap in the z (axial) direction can be studied by measuring the total amount of for-

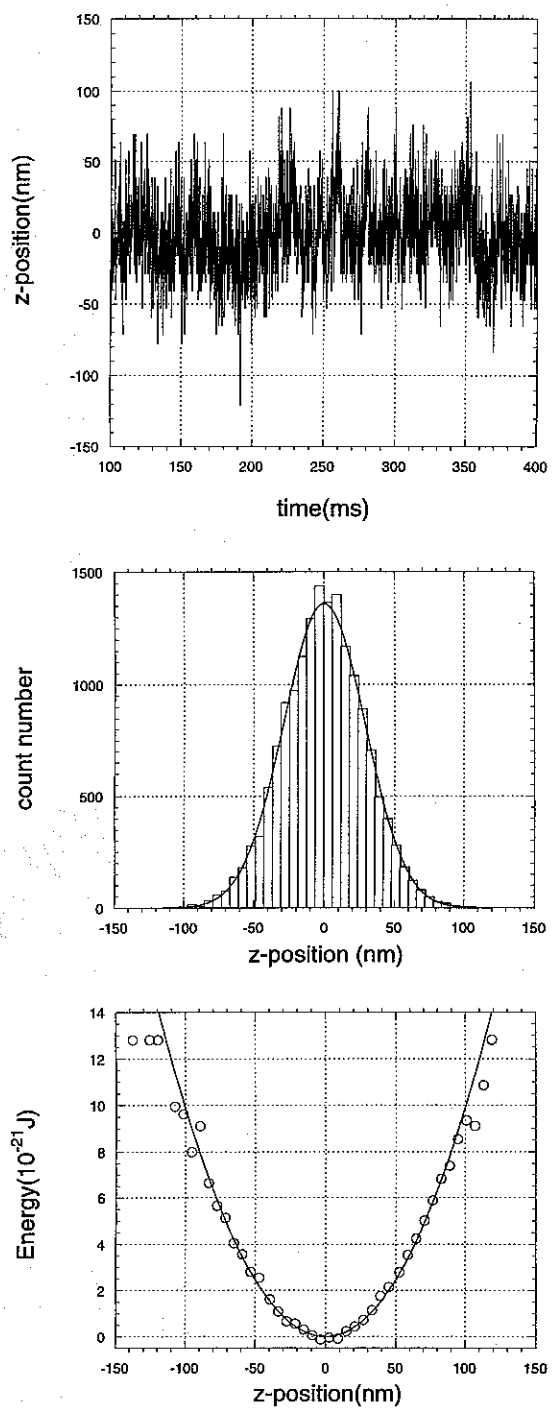

FIGURE 3 A polystyrene bead with a diameter of 1.05 μm is trapped 5.0 μm above a surface. The incident laser power in the trap is approximately 40 mW. The sampling frequency is 10 kHz. Total sampling time is 1.6 s. **a)** A segment of the time-series for the axial position of the bead. **b)** Histogram of the axial position fluctuations shown in **a)**. The full line is a Gaussian fit to the data. **c)** Axial potential experienced by bead. This is obtained by inversion of **b)** assuming Boltzmann statistics. The full line is a harmonic fit to the data

ward scattered light, as will now be explained. In (Pralle et al., 1999) the response of the photodiode current to the axial position of the bead in the trap was investigated. A fluorescent bead was glued to the coverslip and the focus of the laser trap was scanned through the bead by moving the objective of the microscope in the axial direction. The response of the photodiode to the total amount of forward scattered light was measured. Simultaneously, the laser-induced fluorescence of the bead was measured by a photomultiplier. It was found that the response of the photodiode is proportional to the axial displacement of the bead in the trap within a spatial range large enough to contain the thermal motion of a bead diffusing around the equilibrium (trapping) position.

Based on this result we now assume that the response of the photodiode to the total amount of forward scattered laser light is proportional to the axial position of the bead in the trap. We have recorded a time-series showing the z-position of a 1.05 μm bead in a trap. This is shown in Figure 3 a). Figure 3 b) shows a histogram of positions of the bead in the trap. The width of the bins does not indicate the resolution with which we monitor the spatial intensity profile of the beam, this resolution is of the order of the bead size.

Since the distribution $p(z)$ of axial positions in the trap can be described by Boltzmann statistics, we can find the axial potential felt by the bead by inversion of a relation similar to Equation (3):

$$U(z) = -k_B T \ln p(z) + C. \qquad (6)$$

This potential, obtained from the data set shown in Figure 3 b), is illustrated in Figure 3 c). The data for $z(t)$ are shown in Figure 3 a). We fit the potential with a second order polynomial to obtain a spring constant $\kappa_z = 2.0 \cdot 10^{-3}$ pN/nm (the conversion factor between voltage and position was obtained via a Lorentzian fit as described above for the x and y direction.)

As long as we deal with small displacements when using thermal motion for the calibration, the cross talk between the x, y, and z channels can be ignored (Pralle, 1999). The corner frequency for the trap in the z direction is typically 5–10 times lower than for the x and y directions, i.e., the trap is much weaker along the direction of the beam.

APPLICATIONS TO BIOLOGICAL SYSTEMS

We use our equipment to measure the lateral mobility of membrane proteins, both in eucaryotic and procaryotic systems. The mobility of a protein in the membrane can be used to determine the viscosity of the membrane. Currently, we are studying the mobility of the λ-receptor in the outer membrane of *E. coli* bacteria. In order to manipulate the λ-receptor using the optical tweezers, we attach a small polystyrene bead to the receptor protein. The λ-receptor is genetically modified such that it binds biotin to a particular extra-cellular site. The bead is coated with streptavidin and hence it binds specifically to the biotin on the λ-receptor. This binding is much stronger than the forces which are exerted by the optical tweezers. The efficiency of the biotinylation is very low thereby enhancing the probability that the avidin coated bead is attached to a single biotinylated λ-receptor. By studying the motion of a bead-protein complex, the motion of a single protein can be extracted.

The diffusion of proteins in membranes has been mainly studied by fluorescence and photobleaching techniques as described in a recent review (Saxton, 1999). In such experiments the average diffusional properties of the fastest molecules are monitored. With optical tweezers, single-molecule fluorescence microscopy (Schmidt et al., 1999), or single-particle tracking (SPT) (e.g. Saxton and Jacobson, 1997), this averaging can be avoided, and the motion of single molecules can be studied.

Assuming Brownian motion of the protein in the cell membrane, optical trapping techniques can be used to obtain an *in vivo* measurement of the local diffusion coefficient of the protein. The macromolecule of interest must be coupled to a bead to facilitate observation and manipulation. Two different approaches apply optical tweezers to measure lateral diffusion coefficients. In both approaches, the friction coefficient γ is measured, and the Einstein relation $D = k_B T/\gamma$ gives the diffusion coefficient D. In one

approach (Suzuki et al., 2000), the force necessary to drag the bead and the protein at constant velocity is determined, giving direct information on the friction coefficient γ felt by the combined bead-protein system. Since γ is averaged over a large surface area and is likely to be influenced by interactions with obstacles in the membrane the dragging procedure yields a non-local value of the friction coefficient. Often (e.g. Suzuki et al., 2000), the friction coefficient of the coupled bead-protein system is assumed to equal the friction coefficient of the protein in the membrane. However, a theoretical analysis of such a coupled system indicates that this is not always the case (Berg-Sørensen et al., work in progress). In another approach (Pralle et al., 2000), a more local measurement of the friction coefficient of the molecule is performed. Optical tweezers are used to spatially restrict the bead and thus the molecule in question. The friction coefficient is found by correlation function analysis of the stochastic motion of the bead-protein complex within the optical trapping potential. We use this second approach to find the local diffusion constant of the λ-receptor in the membrane of *E. Coli* bacteria.

CONCLUSIONS

A pair of optical tweezers is a powerful instrument for the study of biological systems at the macromolecular level. We have built a setup based on optical tweezers to manipulate dielectric beads coupled to macromolecules of biological interest. We resolve displacements at the nm scale and forces in the range of pN by the described methods.

We use our equipment to study the mobility of proteins in cell membranes in both procaryotic and eucaryotic cells. Currently, we study the λ-receptor situated in the outer membrane of *E. coli* bacteria and aim at determining the local diffusion coefficient of this receptor protein. To study the diffusional properties of the λ-receptor using optical tweezers, we have attached a dielectric bead specifically to the receptor.

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