

Local viscosity probed by photonic force microscopy

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Abstract. The Brownian motion of 0.5 μm and 1 μm polystyrene spheres used as probes for a photonic force microscope is recorded at various distances from a surface with nanometer spatial and 25 μs temporal resolution. Analyzing time-windows of 0.4 s of the Brownian motion, the diffusion constant and local viscosity are determined. Using 0.5 μm spheres the viscosity can be measured in volumes as small as 1 μm^3 with $\pm 15\%$ precision within 0.4 s. The decrease of the diffusion coefficient due to the partial confinement near a surface is measured continuously over sphere–surface separations from 3 μm down to 200 nm. The increase in viscous drag is found to agree with Brenner’s analytical result [1].

The local viscosity of a fluid depends on parameters such as the chemical composition and temperature, but the probe of viscosity – the viscous drag on small particles – also changes, owing to spatial constraints. In the nano- to micrometer range, confined diffusion of small particles determines the function and properties of micro- and nanometer scale devices, owing to the increasing surface-to-volume ratio. Changes in the local diffusional behavior of small particles are especially important in molecular and cell biology; e.g., a vesicle close to a cell surface is partially confined and hence the particle’s diffusion coefficient is reduced. For these conditions Brenner derived an analytical formula [1]. Measurements of the local viscosity have so far had no three-dimensional control of the probe volume and were often temporally limited to video rate [2, 3]. In those experiments changes in the salt conditions were used to adjust the position of a particle close to a surface.

Here we report the continuous measurement of the diffusion coefficient of a colloidal particle near a surface with a temporal resolution better than 0.4 s using a photonic force microscope (PFM) [4]. A laser trap [5] keeps the Brownian particle in a confined volume, which is adjustable by the laser power. With the variation of the laser power the trapping potential is changed, and hence also the temporal resolution. The method is applied to measure the hydrodynamic corrections to Stoke’s law near a surface on a microscopic scale.

1 Brownian motion in a harmonic potential

A Brownian particle moving in a harmonic potential with spring constant κ through a viscous liquid with viscosity η is described by

$$\gamma \dot{r} + \kappa r = A(t). \quad (1)$$

In a locally purely viscous liquid the damping factor γ is frequency independent. For a sphere with radius ρ the Stoke’s friction reduces to $\gamma = 6\pi\eta\rho$. Inertial forces are negligible compared to the viscous drag for a system with low Reynolds number [1]. $A(t)$ represents the random force of the solvent acting on the particle. The resulting position ensemble can be described by the Boltzmann distribution [6]:

$$P(r) = c \exp \left[\frac{-\frac{1}{2}\kappa r^2}{k_B T} \right]. \quad (2)$$

Since κ depends on the laser power, the laser intensity determines the variances σ^2 of the position distribution along each dimension: $\sigma_i^2 = (2k_B T / \kappa_i)^2$. If the Brownian particle is a micrometer-sized polystyrene sphere inside the harmonic potential of a laser trap, the trap allows moving the center of the volume sampled by the particle to a desired place. The sample volume is given by the diameter of the bead plus its Brownian movement.

Assuming a trap stiffness of 10^{-5} N/m to 10^{-4} N/m the trapped particles move at room temperature with root mean square amplitudes of 20 nm to 200 nm respectively (see Fig. 1). One can use the measured position distribution to determine the trapping potential by plotting the distribution and fitting a Boltzmann distribution to determine the actual trap stiffness κ [7]. The damping factor γ can be computed from the autocorrelation function of the position fluctuations [8, 9]:

$$\langle r(0) r(t) \rangle = \bar{r}^2 \exp \left[-\frac{t}{\tau} \right] = \bar{r}^2 \exp \left[-\frac{\kappa}{\gamma} t \right]. \quad (3)$$

In bulk water, spheres with $r = 0.5 \mu\text{m}$ and $r = 1 \mu\text{m}$ trapped at $\kappa = 2.4 \times 10^{-5}$ N/m and $\kappa = 2.6 \times 10^{-5}$ N/m have autocorrelation times τ of 120 μs and 260 μs , respectively. Therefore, measuring the autocorrelation time and the trap stiffness

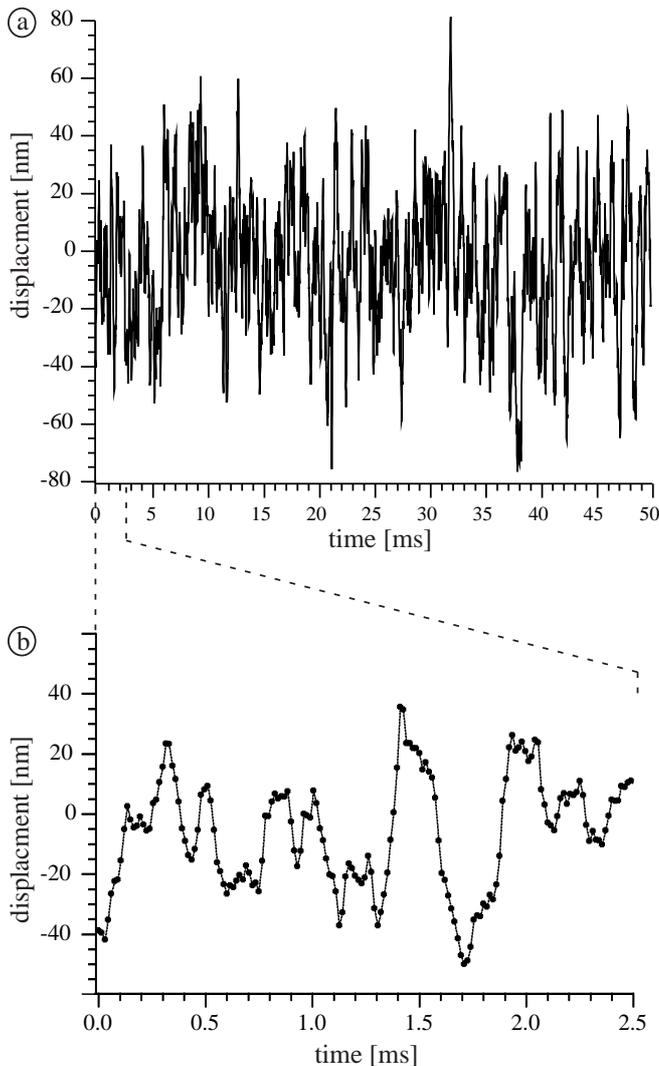


Fig. 1. **a** A $0.5\text{-}\mu\text{m}$ sphere held in the laser trap $6\text{-}\mu\text{m}$ above the surface shows position fluctuations up to 70 nm . **b** The first 2.5 ms of the trace proves that the sampling rate of 40 kHz is well above the frequency of movement randomness

allows us to determine the local viscosity η or diffusion coefficient $D = k_B T / \gamma$ of the sphere.

2 Experimental methods

A PFM [4] is a novel type of force microscope that uses nano- to micrometer sized particles in solution as a probe and measures the particle's position relative to the geometric focus of the laser trap with nanometer and microsecond resolution along all three dimensions. Using fluorescently labelled spheres and monitoring the fluorescence intensity excited by the trapping laser via a two-photon process provides an intrinsic, environmentally independent force sensor [10]. The PFM can be used to map mechanical and topological properties in mechanically sealed environments.

In our PFM a single-gradient laser trap [5] is part of an inverted microscope (Axiovert 35, Carl Zeiss, Oberkochen,

Germany). The laser is a Nd:YVO₄ laser ($\lambda = 1064\text{ nm}$) (T20-B10-106Q, Spectra Physics, Darmstadt, Germany) which operates in cw-mode and can be directed by two scanning mirrors (M2, General Scanning, Munich, Germany). The oil immersion microscope objective (Plan-Neofluor $100\times$, NA 1.3, Carl Zeiss) focuses the light into a sealed water-filled sample chamber. The laser focus is moved vertically by a piezo-driven objective lens (P721.00, Physik Instrumente, Waldbronn, Germany). The trap captures polystyrene beads in all three dimensions. The movement can be observed using the microscope equipped with differential interference contrast (DIC). During the experiments the temperature of the sample chamber can be held constant at $30\text{ }^\circ\text{C}$. The bead's lateral position with respect to the laser focus is monitored by detection of the forward scattered laser light using a four-segment diode (S5981, Hamamatsu). The signal is amplified and digitized with an AdWin-transputer board (AD-Win 5F, Jäger Electronics, Lorsch, Germany). The data analysis is done using IgorPro (WaveMetrics) [7].

Orange-fluorescent carboxylmodified polystyrene spheres (Molecular Probes) with radii of $215.5 \pm 6\text{ nm}$ and $515 \pm 14\text{ nm}$ suspended in distilled water and untreated prewashed glass coverslips (Fischerbrand, Fisher Scientific) were used. Different spheres were captured about $5\text{--}10\text{ }\mu\text{m}$ above the coverslip surface. The trap stiffness was adjusted to lateral root-mean square fluctuations of the particle of about 50 nm . The lateral position of the sphere relative to the laser focus was recorded every $25\text{ }\mu\text{s}$. The autocorrelation function was computed in time windows of $\Delta t = 0.4\text{ s}$ (Fig. 2), while continuously decreasing the sphere-surface distance. For each time-window the actual trap stiffness was computed by fitting a Boltzmann distribution to the position distribution [7]. To monitor the repulsive force between the sphere and the surface and to determine the bead-surface contact, the sphere's fluorescence intensity excited by the trapping laser via a two-photon process [10] was monitored.

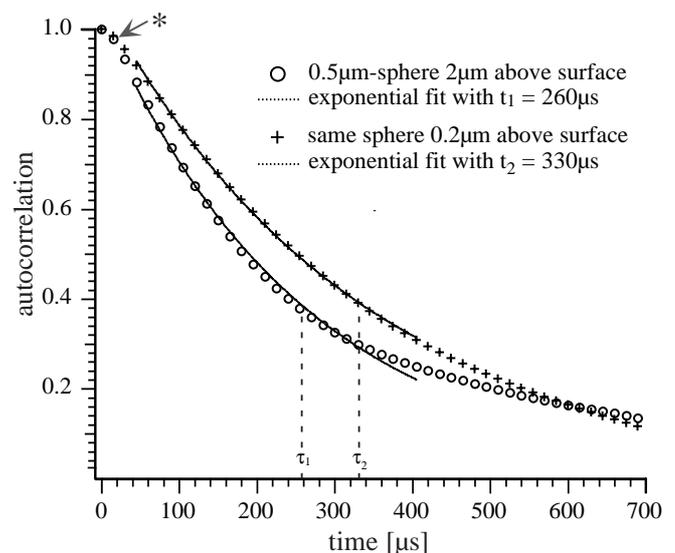


Fig. 2. Plots of the autocorrelation function of the same $0.5\text{-}\mu\text{m}$ sphere at two different surface distances, $2\text{ }\mu\text{m}$ and $0.2\text{ }\mu\text{m}$, show the increase in the autocorrelation time. The * in the upper left corner indicates an initial damping due to the limited bandwidth of the analog amplifier

3 Results and discussion

In Fig. 1a a time window of 50 ms of the position fluctuations of a 0.5- μm sphere in the laser trap held 6 μm above the surface is plotted. Trapped with a spring constant κ of 1.6×10^{-5} N/m, fluctuations up to 70 nm were detected and the autocorrelation time was $\tau = 180 \mu\text{s}$. Figure 1b displays the first 2.5 ms of the motion indicating that the data acquisition rate of 40 kHz is well above the frequency of randomness of the movement. Time intervals between the position measurements were about 1/10 of the correlation time and therefore the position measurements are not statistically independent. This confirms that the temporal resolution of the viscosity measurement is limited by the spheres motion and not by the bandwidth of the position detection. For reliable diffusion coefficient measurements, the observation interval Δt has to be several orders of magnitude greater than the correlation time τ . Using the estimated uncertainty of an exponentially decreasing autocorrelation given by Box and Jenkins [11] one can determine that the interval has to be at least $\Delta t > 1500\tau$ – i.e., 0.4 s – for a 10% precision in the correlation time. The temporal resolution of the diffusion coefficient measurement is given by the autocorrelation time which is about two orders of magnitude faster than what was available previously by video tracking, owing to the additional trapping potential. For time windows of the order of seconds, the relative variance of the measured bulk viscosity was less than $\pm 5\%$. For absolute measurements of viscosity the uncertainties in the sphere's radius (about $\pm 3\%$) and in the detector calibration (about $\pm 5\%$) have to be added.

The the autocorrelation time τ of a 0.5- μm sphere increases about 27% when it is moved from a sphere–surface distance of about four times its radius to roughly half of its radius. Figure 2 shows the corresponding normalized autocorrelation function. The measured positions are 15 μs apart, so the first two points show distortions because of the limited bandwidth (50 kHz) of the analog amplifier, but otherwise the function falls off smoothly.

In Fig. 3a the diffusion constant of a 1- μm sphere is shown as a function of sphere–surface distance. The diffusion coefficient normalized to its bulk value $D_0 = 6.9 \times 10^{-13} \text{ m}^2/\text{s}$ remains constant until the distance is in the order of the radius of the sphere. This remains true independent of the diameter of the sphere or the trapping potential, at least for diameters of 0.2–1 μm and spring constants of 10^{-5} – 10^{-4} N/m. The values are in good agreement with the analytical result of Brenner [1] for the lateral correction to the diffusion coefficient:

$$\frac{D(z)}{D_0(z = \infty)} = 1 - \frac{9}{16} \left(\frac{r}{r+z} \right) + \frac{1}{8} \left(\frac{r}{r+z} \right)^3 - \frac{45}{256} \left(\frac{r}{r+z} \right)^4 - \frac{1}{16} \left(\frac{r}{r+z} \right)^5, \quad (4)$$

where z is the distance between the surface of the sphere and the glass surface.

We have shown that the combined analysis of the thermal position noise by the Boltzmann distribution and the autocorrelation function allows the measurement of the local viscos-

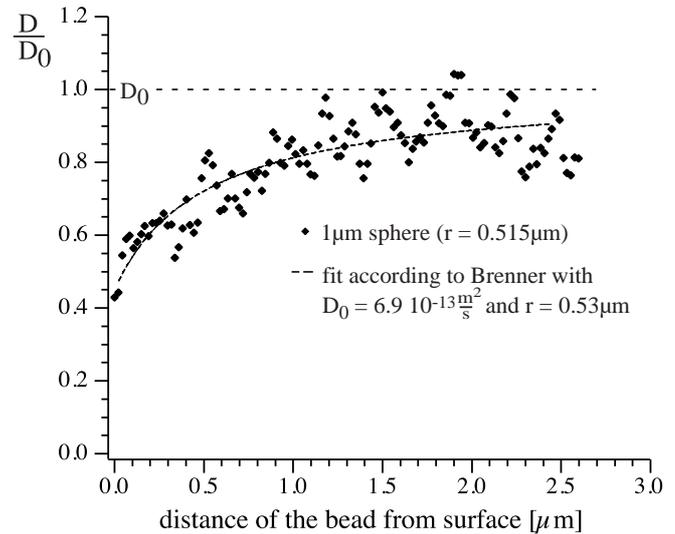


Fig. 3. The change in the diffusion coefficient of a 1- μm sphere for increasing sphere–surface separation

ity. The temporal resolution is given by the measurement of the autocorrelation time. The spatial resolution is given by the diameter of the probe used plus the thermal position noise. The spatial resolution is even increased when restricting the probe to two dimensions, e.g., by binding the particle to slow-moving cell membrane components. In this case the spatial resolution is given by the thermal position fluctuation and allows therefore a localized measurement in an area as small as 100 nm \times 100 nm.

We expect PFM viscosity measurements in the nano- to micrometer range to become a major tool especially for biological applications, measurement inside a cell are in principle possible, but also for the characterization of nano- to micrometer scale devices and materials.

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