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**In situ viscometry by optical trapping interferometry**

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We demonstrate quantitative in situ viscosity measurements by tracking the thermal fluctuations of an optically trapped microsphere subjected to a small oscillatory flow. The measured power spectral density of the sphere’s positions displays a characteristic peak at the driving frequency of the flow, which is simply proportional to the viscosity, when measured in units of the thermal power spectral density at the same frequency. Measurements are validated on different water-glycerol mixtures, as well as in a glycerol gradient, where no a priori knowledge of the solution is used to determine the glycerol concentration. © 2008 American Institute of Physics. [DOI: 10.1063/1.3020713]

The two main mechanical properties of a complex fluid are its elasticity and viscosity. Understanding and quantitative characterization of viscoelasticity in small volumes such as cells or microfluidic devices require the development of methods that can access these parameters in situ down to the nanoscale. Their measurement with optical tweezers was demonstrated qualitatively in a living yeast cell.¹ They have, in general, great potential for single-cell diagnosis, as for instance, viscoelastic cell deformability is sensitive enough to monitor changes occurring during cell alteration from a normal toward a cancerous state.² Changes in viscosity play a role in the regulation of diffusion-mediated processes, such as intercellular communication, signaling, and differentiation. Furthermore, it is a critical factor in the optimization of liquid photoresists used for stereolithography in microfabrication processes.³

To demonstrate quantitative in situ viscosity measurements, we here probe local viscosities with the thermal fluctuations of an optically trapped microsphere embedded in the fluid of interest. The sphere’s position is recorded with a quadrant photodiode by back-focal plane interferometry.⁴ Several similar so-called active micro rheological methods have been introduced before,⁵–¹¹ but they all rely on calibration against standards, for a preliminary determination of the detector’s sensitivity or of the local laser power at the sample, employing usually reference solutions with well-known properties. Therefore, they suffer limitations when truly quantitative in situ measurements are needed. A recently introduced detector calibration procedure¹²,¹³ is independent of such a priori assumptions on fluid properties and detector sensitivity. Instead, it measures them by considering the power spectrum of a trapped Brownian sphere exposed to a Stokes’ drag arising from a small oscillatory fluid flow. To validate the proposed method for in situ viscosity measurements, aqueous glycerol solutions at different concentrations are used as typical purely viscous model fluids of dynamic viscosity η and density ρ. A suspension of 10⁶ microspheres/ml fluid is loaded into a liquid chamber (size ≈20×5 mm² and thickness ≈100 μm) by capillarity. Subsequently, the liquid cell is sealed with vacuum grease. A piezoelectric stage moves the sample chamber with a sinusoidal oscillation x-drive(t)=A sin(2πf-drive t) at a given amplitude A and frequency f-drive, while the laser trap remains fixed. This creates a fluid flow around the trapped microsphere. Experiments are performed at room temperature (≈24 °C) with a polystyrene bead (radius a=0.5 μm) trapped with a Nd:YAG laser beam (λ=1064 mm, 500 mW). The Brownian sphere is typically positioned at a distance h=50 μm away from the glass surface to avoid any boundary effects.¹⁴,¹⁵ The equation of motion gives the positions of the bead in the lab-trap system as the sum of its hydrodynamically correct theory of Brownian motion,¹²,¹⁵ neglecting inertia would lead to an underestimation of more than 15%. We therefore use the a priori knowledge of the solution is used to determine the glycerol concentration. © 2008 American Institute of Physics. [DOI: 10.1063/1.3020713]
Due to the trapped particle, resulting in an oscillating displacement characteristic spike at around the trapping potential's minimum. The characteristic frequencies describing the “thermal background” arising from Brownian fluctuations. The characteristic frequencies \( f_m = \frac{3}{\pi} \eta a/m \), and \( f_v = k/(12\pi^2 \eta a) \), with \( m \) as the particle’s mass and \( k \) as the trap stiffness, are related to the particle, the fluid, and the trap, respectively. In practice, to avoid leakage, \( t_{\text{msr}} \) is chosen to be an integer multiple of \( f_{\text{drive}} \) so that the discrete experimental power spectrum \( P(f) \) features a Kronecker delta peak at \( f = f_{\text{drive}} \) (Ref. 12, Appendix C). The viscosity of the fluid follows then directly from Eqs. (1) and (2),

\[
\eta = \frac{k_B T f_v}{4\pi^3 a^2 |\triangle f_{\text{drive}}|^2 |P_{\text{therm}}(f_{\text{drive}})|^{1/2}}.
\]

FIG. 1. (Color online) (a) The superposition of Brownian motion and the applied oscillations become visible in the particle’s two-dimensional position histogram, when immersed in a solution of high viscosity such as 80% glycerol in H\(_2\)O (w/w). (b) If the viscosity is low, such as in pure H\(_2\)O, and the trap is strong enough, the bead’s fluctuations will remain centered around the trapping potential’s minimum.

The oscillating fluid imposes a viscous drag \( \gamma = 6\pi \eta a \) on the trapped particle, resulting in an oscillating displacement of its Brownian fluctuations about the trap’s center. Due to the laser counteracting on the particle’s oscillations, the amplitude \( A_{\text{resp}} \) of its response will be smaller than \( A \) and depends on \( \gamma \). To keep \( A_{\text{resp}} \) within the linear range of the detector, we set \( A = 200 \) nm, and \( f_{\text{drive}} = 10 \) Hz is chosen well below the resonance frequency of the piezostage. Furthermore, relatively strong trapping forces, i.e., highest possible \( f_c \) (typically between 1 and 1000 Hz), are adjusted by the incoming laser power to counterbalance the viscous drag on the bead. For a solution with high viscosity, for example, 80% (w/w) glycerol in water, the particle’s displacement with respect to the trapping potential minimum can be clearly detected in the two-dimensional histogram of the probability density of the particle’s position [Fig. 1(a)]. In a solution with relatively low viscosity, such as pure water, the sphere’s sinusoidal oscillations are masked by thermal fluctuations [Fig. 1(b)].

The measured \( P(f) \) is more sensitive to \( \gamma \) and features a characteristic spike at \( f = f_{\text{drive}} \) as can be seen in Fig. 2(a). At \( f \neq f_{\text{drive}} \), Eq. (2) fits the blocked values with an accuracy better than 6% between 100 Hz and 10 kHz, and even 2% between 10 and 100 kHz, yielding the corner frequency \( f_c \) [Fig. 2(b)]. \( P_{\text{therm}}(f_{\text{drive}}) \) is directly read off the fit in Fig. 2(b), and \( P(f) \) from the graph in Fig. 2(a), resulting in \( \eta \) by Eq. (3). The peak amplitude scales with the glycerol concentration and hence \( \eta \) (inset). As viscosity increases \( f_c \) drops below 10 Hz [lightest gray plot for pure glycerol in

\[
P_{\text{therm}}(f) = \frac{k_B T}{12\pi^3 \eta a} \left[ 1 + (\gamma f)^{1/2} \right] \left( f_c - \frac{f^2}{f_v} - \frac{f^2}{f_m} \right)^2 + \left( f + \frac{f^2}{f_v^2} \right)^2,
\]

FIG. 2. (Color online) (a) Power spectral density \( P(f) \) in different glycerol/water fractions (w/w) varying from 100% to 0% glycerol (from light gray to black plots) with \( A = 200 \) nm and \( f_{\text{drive}} = 10 \) Hz. Inset: zoom on the 10 Hz peaks to illustrate the differences in peak powers. For clarity, the peaks are shifted along both axes. Each curve is an average over ~10 consecutive measurements. (b) The raw \( P(f) \) is blocked in 50 bins/decade and fitted by Eq. (2), taking aliasing into account (red continuous line). The error bars indicate the standard error on the mean.

FIG. 3. Measured dynamic viscosity as a function of the glycerol concentration, fitted using Cheng’s model (Ref. 17) (continuous line) with a temperature of 35 °C. The error bars show the standard error on the mean of ~10 consecutive measurements, based on the standard deviation of the same 10 measurements.
induced heating in an optical trap in a medium of known viscosity, or more generally detect local temperature changes at the nanoscale.

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