

Imaging microscopic viscosity with confocal scanning optical tweezers

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Received August 7, 2001

The techniques of confocal microscopy and optical tweezers have shown themselves to be powerful tools in biological and medical research. We combine these methods to develop a minimally invasive instrument that is capable of making hydrodynamic measurements more rapidly than is possible with other devices. This result leads to the possibility of making scanning images of the viscosity distribution of materials around biopolymer-producing cells. 100×100 images can be taken with $0.5\text{-}\mu\text{m}$ spatial resolution in 3 min. An image of the viscosity distribution around a pullulan-producing cell of *Aureobasidium pullulans* is shown as an example. © 2002 Optical Society of America

OCIS codes: 180.5810, 187.1790, 170.3880, 140.7010.

Viscosity is a fundamental parameter in fluid dynamics. On the microscopic scale, viscosity plays a major role in the dynamics of physical, chemical, and biological systems. For example, the dynamics of a protein molecule and its interaction with other molecules is affected by the viscosity of the medium around it. Understanding the diffusion and transport processes of macromolecules in cell biology requires knowledge of the viscosity in and around the cell. Creating a tool to physically probe and image these local viscosity gradients at high resolution should therefore be quite useful for researchers in these fields.

We describe here a method of probing the microscopic environment in fluids at micrometer-scale resolution in a way that is simple, sensitive, complies with scanning requirements, and does not require fluorescent probes. What would normally be the scanning beam of an inverted confocal microscope is used as optical tweezers to trap a microsphere near the laser beam focus. Only light scattered from near the focal point of the tweezers is confocally detected. We force the trapped microsphere periodically by moving the laser tweezers back and forth. The resulting particle motion is periodic, with a frequency equal to the driving frequency and a phase lag due to the hydrodynamic drag. By measuring this phase lag, we can determine the characteristic time constant of the motion, which is proportional to the medium's viscosity. The probe remains aligned even as it is scanned over the full field of view of the microscope and allows data acquisition rapid enough for a $50\ \mu\text{m} \times 50\ \mu\text{m}$, 100×100 pixel image to be acquired in just 3 min. There are other methods of making microscopic measurements of viscosity,¹⁻³ but, as will be shown elsewhere,⁴ these methods are not as fast as the method presented here.

Figure 1 shows the experimental layout. Focussing light from a cw Ti:sapphire laser at 815 nm by use of a high-N.A. objective lens (oil immersion, 1.25 N.A., $100\times$) forms the optical trap in the sample. An acousto-optic deflector sets the beam in periodic motion, causing the trapped probe microsphere to oscillate in the transverse direction. Backscattered light from the trapped particle is detected confocally

by placement of a pinhole near an image plane in front of a photodetector.⁵ As the particle moves about in the trap, the confocal signal varies. For a TEM_{00} Gaussian beam the signal is maximum at the center and decreases as the particle moves away from the focal region. Because of the hydrodynamic friction, the particle lags behind the oscillating beam. As a result, the beam traverses the particle twice in each cycle and the confocal signal peaks twice. A lock-in amplifier measures the phase difference between the confocal signal and the second harmonic of the spatial oscillation frequency of the trap. The phase is then used to determine the local viscosity with the theoretical model described below. The scanning mirrors in Fig. 1 have three functions. They can be used to form a digital image by x - y raster scan in the

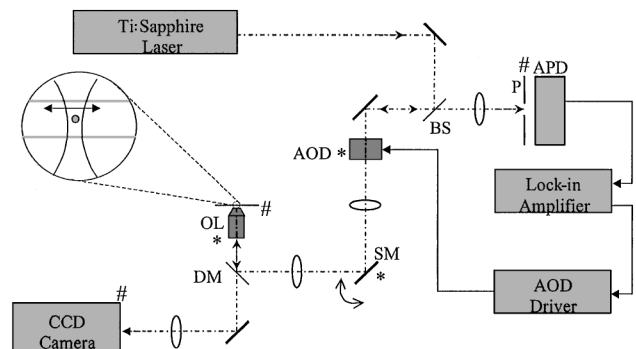


Fig. 1. Experimental layout. The pinhole (P) is placed near an image plane of the object, permitting confocal detection. This set of conjugate planes is indicated by #s. An acousto-optic deflector (AOD) is placed in an image plane of the back aperture of the objective lens (OL). This other set of conjugate planes is indicated by *s. Two orthogonal galvanometer scanning mirrors (SM) are placed in an intermediate image plane (*). A lock-in amplifier (SR850, Stanford Research Systems) drives the acousto-optic deflector with a sinusoidal waveform and measures the amplitude and phase of the avalanche photodiode (APD) signal at the second-harmonic frequency. The scene is viewed with a CCD camera in transmission through the dichroic mirror (DM), which has high reflectivity in the IR. BS, beam splitter.

conventional use of the scanning confocal microscope. They can be used to drag the probe particle in the sample and, with our measurement technique, obtain an image of the viscosity. We also use these mirrors to control and manipulate the laser trap's position by use of computer software that integrates the CCD camera, a frame grabber, and the galvanometer drivers to allow us to take measurements of the viscosity at particularly interesting spots in the field of view.

Below, we describe the main features of our physical model that relate the measured phase and the microscopic viscosity. A particle trapped by the light force finds itself in an approximately harmonic potential well. The one-dimensional equation of motion of a particle in a viscous Newtonian fluid undergoing Brownian motion in an oscillating harmonic potential is

$$\gamma \frac{dx}{dt} + \kappa[x - p(t)] = L(t), \quad (1)$$

where x is the particle's position, $p(t)$ is the time-dependent position of the trap, $p(t) = a \sin(\omega t)$, and γ is the hydrodynamic drag coefficient. For a sphere far from any surface, γ is given by the Stokes formula $6\pi\eta r$, where r is the radius of the sphere and η is the dynamic viscosity.⁶ $L(t)$ is the Langevin forcing function associated with Brownian motion, and κ is the tweezers spring constant. The inertial term (mass times acceleration) that would normally be included on the left-hand side of Eq. (1) can be neglected because in common fluids the motion of micrometer-sized particles takes place at a small Reynolds number and viscous drag dominates inertial forces.⁶ The assumption that the particle stays near the center of the trap also allows us to express the confocal signal as proportional to $1 - \{[x - p(t)]/\sigma\}^2$, where σ is a constant.

Ignoring Brownian motion for the moment, we solve Eq. (1) without the Langevin term and take the Brownian motion into account only later, in the discussion on the signal-to-noise ratio (SNR). By straightforward manipulation of the solution, we can show that the phase of the second-harmonic signal, φ_2 , from the lock-in detector is

$$\varphi_2 = 2 \cot^{-1} \omega \tau, \quad (2)$$

where $\tau = \gamma/\kappa$.

We used as a study case *Aureobasidium pullulans*, which is a yeastlike fungus whose cells secrete the high-molecular-weight polysaccharide pullulan.⁷ Figure 3 shows an image taken with our prototype scanning viscosity microscope. The viscosity gradient shown in the gray-scale map indicates the boundaries of the pullulan halo around a young blastospore. Note that, in comparison, the conventional bright-field microscopy image does not reveal any information on differences in the medium's properties in the vicinity of the cell.

To check the validity of our model, we measured the second-harmonic phase, φ_2 , as a function of the twee-

zers oscillation frequency, ω . Figure 2 shows the results of measurements on a 1.9- μm -diameter silica microsphere in an aqueous solution of glycerol of 24.1% by wt. ($\eta = 2\eta_{\text{water}}$). The laser power was kept constant (46 mW at the sample), whereas the oscillation frequency was varied from 100 to 600 Hz. Linear regression yields $\tau = 1.99$ ms (by use of the known viscosity, we get $\kappa = 1.66 \times 10^{-5}$ N/m). We also show the amplitude of the second-harmonic signal (A_2), which according to the model is proportional to $a^2/[1 + (\omega\tau)^{-2}]$.

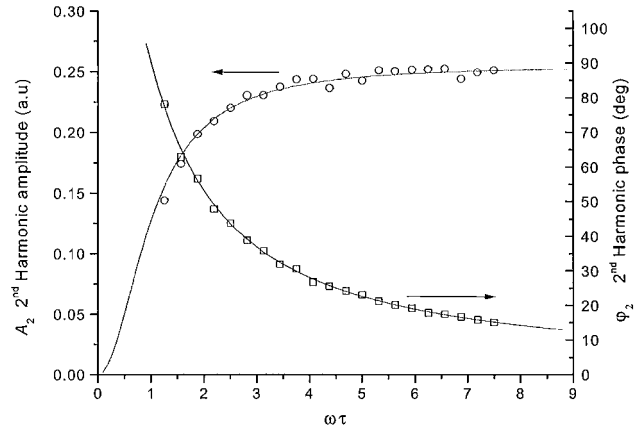


Fig. 2. Experimentally measured second-harmonic phase and amplitude for a 1.9- μm -diameter silica microsphere in an aqueous solution of glycerol of 24.1% wt. ($\eta = 2\eta_{\text{water}}$). The power of the laser was constant at 46.3 mW at 815-nm wavelength. From the linear regression to the phase data, we get $\tau = 1.990 \pm 0.006$ ms and a correlation coefficient of 0.9995. The solid curves were calculated from the theoretical functional dependence by use of the fitted τ ; for the phase, the fitting function is $2 \cot^{-1}(\omega\tau)$, and for the amplitude it is a constant multiplied by $[1 + (\omega\tau)^{-2}]^{-1}$.

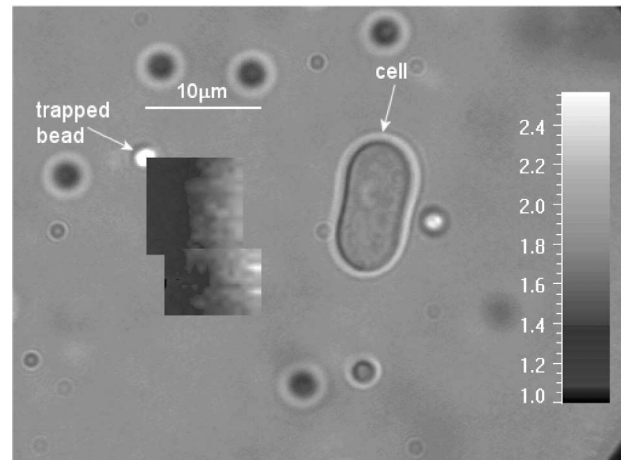


Fig. 3. Image of the viscosity gradient near a pullulan producing an *A. pullulans* blastospore (values are relative to the viscosity of water). The cell is shown in the middle of the image. The 1.9- μm -diameter silica bead that was used for the measurement can be seen in the upper left-hand corner of the map being held by the laser tweezers (there are a few other silica beads scattered around that were not part of the measurement). The bead was moved in steps of $0.53 \mu\text{m}$, and measurements were taken at each point to give a 16×16 image. The frequency of the trap's oscillation was 300 Hz, and the laser power was 19 mW at the sample.

There are important advantages to the method presented here, resulting from (a) the inclusion of confocal detection in scanning photonic-force microscopy^{8,9} and (b) lock-in detection, with its phase sensitivity and high SNR.

The SNR in the viscosity measurement can be determined by analysis of the effects of the Langevin forcing term, $L(t)$, whose autocorrelation function is given by $R_{LL}(t) = \overline{L(t)L(t')} = q\delta(t - t')$, where $q \equiv 2\gamma k_B T$, k_B is the Boltzmann constant, and T is absolute temperature. We find⁴

$$\text{SNR} = \frac{\gamma}{\Delta\gamma} = \frac{1}{\sqrt{8}} \frac{a}{\sqrt{k_B T/\kappa}} \frac{(\omega\tau)^2}{1 + (\omega\tau)^2} \frac{1}{\sqrt{\Delta\omega\tau}}, \quad (3)$$

where $\Delta\omega$ is the measurement bandwidth and a is the spatial oscillation amplitude. If we adjust the measurement bandwidth to be proportional to the tweezers' oscillation frequency, the SNR is maximized when $\omega\tau = \sqrt{3}$ or $\varphi_2 = 60^\circ$, and it slowly falls off at high frequencies as $1/\omega^{1/2}$. In the split photodiode method of Valentine *et al.*³ the SNR falls off more rapidly at high frequencies⁴ as $1/\omega^{3/2}$. The physical explanation for our method's high SNR at high frequencies is that at high frequencies the bead becomes almost stationary and there is a strong time-varying signal every time the trap crosses the particle.

Finally, we note that, while making the images of viscosity around the *A. pullulans* cells, we noticed that the bead became stuck in the entangled polymer matrix if it was brought very close to the cell, at which point our technique could not be used. Also, in such a medium our simple model, which assumes purely viscous Newtonian fluid, is not sufficient for quantitatively describing the dynamics. For such complex fluids, which exhibit viscoelastic properties, we need to modify the model to account for the medium elasticity.

We could do this by writing a generalized Langevin equation that incorporates a memory function for the time-dependent damping of the fluid, as was done by Mason and Weitz, and by calculating the autocorrelation function of the confocal signal from a stationary trap. Nevertheless, our technique is very useful in depicting the sharp gradients in viscosity surrounding the cells and their spatial structures.

The authors acknowledge helpful discussions with Amit Meller and Joe Noonan. M. Cronin-Golomb's e-mail address is mcroning@tufts.edu.

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