Vaterite Twist
Microrheology with AOM Controlled Optical Tweezers

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ABSTRACT

Measurements of viscoelasticity in the microscopic regime are of interest in polymer solutions as well as in microscopic structures such as cells. Viscoelasticity can be studied using a localized microrheometer based on optical tweezers. We rotate a birefringent micron-sized calcium carbonate sphere crystallized in a vaterite structure. By applying a time-dependent torque or using the time-dependent thermal torque, viscoelasticity can be measured. The torque can be measured purely optically, by measuring the polarization state of the trapping beam after passing through the particle. We control the torque by controlling the relative amplitudes of two orthogonally circularly polarized components of the trapping beam with two acousto-optic modulators. This allows a wide range of oscillation frequencies to be used. We demonstrate applications of the methods on several systems.

Keywords: microrheology, viscoelasticity, rotational Brownian motion, optical tweezers

1. INTRODUCTION

Microrheology is the study of fluids on a microscopic scale. An application is, for example, when only a very small amount of fluid is available for testing, so that macroscopic rheometers can’t be used. This often occurs when bio-fluids, e.g., cytoplasm or the eye fluid, are of interest. Our new method meets many of the requirements for these research fields: First, most bio-fluids are viscoelastic polymer solutions. With the application of an oscillating torque or Brownian motion our method yields a complex shear modulus and thus both viscous and elastic properties. Second, viscoelastic properties are crucial for cellular processes like cell shaping, intracellular transport (of vesicle and other cellular components), cell locomotion, exocytosis, and endocytosis.\textsuperscript{1} Optical tweezers as a non-invasive measurement can be used inside cells. As Parkin\textsuperscript{2} showed in a proof of principle experiment, the vaterite sphere can be brought into a cell by cutting a hole into the cell membrane with a femto-second laser and pushing the vaterite through the hole with an optical trap. The hole closes automatically behind the vaterite. Third, viscoelastic properties can vary drastically with position. Our method represents a very local measurement: the bead has μm size and the shear decays with the distance cubed.\textsuperscript{3} Moreover, optical tweezers allow us to micro-manipulate small objects such as cell organelles, in all degrees of freedom and allow us to study the strength of linkages between molecules or forces of bio-motors simultaneously with the measurement of the viscoelastic properties of the surrounding fluid. Additionally it allows us to arrange the objects (e.g., move them away from unwanted influence like membranes or drag them into the region of interest) before measuring viscoelasticity. Besides all this, the optical tweezer setup is naturally combined with a microscope, so fluorescence observations are also possible. Last, cytoskeletal properties depend on the substrate stiffness,\textsuperscript{4} but as long as the substrate is transparent our measurement inside the cell is not influenced.

Why rotation? There are four schemes enabling measurement of viscoelastic properties:\textsuperscript{4} creep experiments, where a step force is applied and the strain is measured over time; relaxation experiments, where a step strain
is applied and the force relaxation is measured; oscillatory experiments, where the magnitudes of force, strain, and the phase shift between them are measured; and Brownian motion experiments.

Other approaches mostly focus on translational movement, because spherical probe particles are commonly used and on a sphere rotational movements are hard to observe. Some kind of symmetry breaking must be applied, which in the case of non-spherical particles, e.g., microdisks, complicates the calculation of the drag torque in the liquid. We use spheres with a non-homogeneous optical structure, which does not influence the drag torque and also allows a completely optical torque measurement.

An advantage of using rotational movement instead of translational movement is that if the particle is not an ideal sphere, it has less influence on rotation than on translation: if the relation of the major and minor axis of an ellipsoid changes from 1 to 5, the particle velocity changes threefold for translational movement, whereas for rotational movement the rotation frequency varies less than 10%. Apart from this, measurements in the rotational mode are more local. The fluid velocity at a distance \( r \) from a bead with radius \( a \) decreases with \( a/r \) for translational movement and with \( (a/r)^3 \) for rotational movement. And finally, dragging a particle through the cytoplasm may be more disruptive to the cell structure than rotating it in a limited volume.

2. METHODS

2.1 Vaterite

Vaterite is the least stable form of calcium carbonate crystals that appear in nature. It can crystalize in spheres from about one to ten \( \mu \text{m} \) size, which are highly birefringent. Bulk vaterite has a birefringence of \( \Delta n = 0.1 \), while the vaterite spheres have a overall birefringence of 0.06, probably due to the non-homogeneous structure and non-homogeneous optical axis. The actual structure is currently under research. This makes them suitable for our purpose. The spherical form makes it easy to calculate the drag force in the liquid when the particle rotates, and the birefringence makes it possible to detect the angular position, because it acts like a wave plate to the detection beam and on transmission changes the beam’s polarization state. It is possible to spin it with a circularly polarized trapping beam. One has to be careful not to introduce linear polarization, because then the vaterite will align to the electric field with its main optical axis in order to minimize energy. For a circularly polarized trapping beam, the vaterite will align with its optical axis in the plane perpendicular to the beam axis. However, it is highly desirable to be able to control the spin angular momentum content of the trapping beam, and to be able to have a low angular momentum without weakening the trap. This cannot be done by using elliptically polarized light due to the alignment with the plane of polarization noted above. Therefore, we use two beams, not mutually coherent, with opposing spin angular momentum, and combine these in the trap to create a beam with independently controllable power and spin angular momentum.

2.2 Setup

The setup is illustrated in figure 1. It is described in more detail in the other paper.

We use an infrared (IR) laser for three-dimensional trapping, which is split by a quarter-wave plate and a polarizing beam splitter into two equally strong beams with orthogonal linear polarizations. They are scattered by acousto-optic modulators (AOMs) to the first and minus first order respectively to introduce a 54 MHz frequency shift in order to avoid interference. The power of the two beams is varied by the AOMs following \( \sin^2 \) and \( \cos^2 \) functions respectively. (This results in a sinusoidal torque function as explained below.) For the Brownian motion experiment, the powers are kept constant. Although the half-wave plates in the two arms are theoretically not necessary, because the polarization which is deflected at the first polarizing beam splitter, should also get reflected at the recombining beam splitter, the half-wave plates are useful in our setup to compensate for imperfections. Also they allow us to scale the powers of the two beams, when the AOMs are not aligned perfectly and have different efficiencies. The total power of the trapping beam(s) is monitored by a power meter. For this a small constant percentage of the intensity is reflected from a glass slide (which is tilted the same angle in both polarization directions) to the power detector. A quarter-wave plate transforms the two linearly polarized components of the beam into right and left circularly polarized light. The beam is expanded using two telescope lenses to fill the back focal plane of the objective, to take advantage of the high numerical aperture of the objective for strong trapping. For the oscillation experiment (where we drive the vaterite sinusoidally) we
want to measure the optical angular momentum after the beam has passed the vaterite. After separating the trapping beam from the detection beam by a dichroic mirror, the right and left circularly polarized components of the trapping beam are separated with a quarter-wave plate and a polarizing beam splitter and the powers are measured.

We use a HeNe laser at 632.8 nm for the detection of the vaterite’s angular position. Its power is so small that the torque it exerts on the vaterite is negligible compared to thermal forces. It is focused at the sample by varying the distance between the telescope lenses (see figure 1). To compensate for the distortion at the “wrong” (i.e., designed for IR light) quarter wave plate, which the red light has to pass, we can adjust the angle at the “right” quarter wave plate. After passing through the vaterite, the power of one linear polarization is measured.

A microscope with Köhler illumination is used, a camera is focused on the vaterite, and the objective is mounted on an xyz-translation stage to enable manipulation of a vaterite sphere in order to bring it into the trap.

2.3 Angular Position Measurement

In general the initially circular polarization of the HeNe detection beam is converted to an elliptically polarized beam by the vaterite. The ellipse (i.e. the polarization direction with the most intensity) rotates with the particle. A power detector records the intensity after a linear polarizer, which has a \( \sin^2(\Theta) \) dependence, where \( \Theta \) is the time-dependent orientation angle of the vaterite. A similar angular position detection system was used by Wood et al.\(^{10}\) to measure Brownian motion superposed on uniform rotation.

2.4 Torque Measurement

The powers of the right and left circular polarized components (\( RC \) and \( LC \), respectively) of the trapping beam before the vaterite are known from calibrating the trap and monitoring the total power of the beam. The vaterite in the trap on the microscope slide transfers a certain amount of either polarization to the other one, according to its birefringence. After the beam is altered by the vaterite, the powers of the two circular polarized components are measured. The torque is calculated from the change in polarization before and after the vaterite:\(^{11}\)

\[
\tau = \frac{(LC - RC)_{\text{before}} - (LC - RC)_{\text{after}}}{\omega_{\text{Laser}}} \tag{1}
\]

where \( \omega_{\text{Laser}} = 2\pi c/\lambda \), \( c \) is the speed of light, and \( \lambda \) is the wavelength of the laser (1064 nm here for infrared).
2.5 Extraction of the Data

We use LabView in combination with a NI card to supply the AOMs with the radio frequency signal, and at the same time we sample the signal (voltage) from the four power meters (the total power before the sample, two times for the torque, and one for the position measurement) also with a NI card and write these signals into a file. For the data analysis we use Matlab.

As the electric field turns with the vaterite and is projected to one polarization at the linear polarizer, the signal of the power detector varies with the $\sin^2(\Theta)$ of the angle $\Theta$ of the particle. So we first record the signal with the particle rotating continuously in one direction and determine the offset (because the beam is elliptically polarized) and the maximal amplitude of the $\sin^2(\Theta)$. Then, when the vaterite is rotating freely according to Brownian motion or when (in the other experiment) it is driven sinusoidally, the angle is determined by

$$\Theta = \arccos \left( \frac{\text{signal} - \text{offset}}{\text{amplitude}_{\text{max}}} \right).$$

We then take the discrete Fourier transform of the processed angle data. This is the power spectrum for the Brownian motion.

For the oscillating experiment we cut off the offset, drift, and noise at 50 Hz and estimate frequency as the intensity-weighted sum over all frequencies and the magnitude as the total absolute value of the amplitude. The phase is estimated as the angle of the complex magnitude at the estimated frequency.

Now we have the torque magnitude, the angle magnitude, and the phase shift between them for each of the driving frequencies. We only need to measure the radius of the sphere in an image from the microscope and we can calculate the viscoelasticity according to different models for the fluid.

3. THEORETICAL TREATMENT

3.1 Viscoelastic Models

We now need a model for the viscoelastic fluid. Simple models such as the Kelvin–Voigt model (cf. figure 2a) or the Maxwell model (cf. figure 2b) have some disadvantages: the Kelvin-Voigt can’t explain stress relaxation and the Maxwell model does not allow decreasing strain rates at constant stress, so these are not sufficient to explain the behavior of polymer solutions. Bausch et al. showed that a more complicated model is needed and commonly a standard linear solid model (SLS) is used to model the polymer network with an additional dashpot in series for the fluid part (cf. figure 2c).

![Viscoelastic mechanical models](image)

Figure 2. Viscoelastic mechanical models of a) Kelvin body, b) Maxwell body, and c) SLS body with an additional dashpot in series

Even such more elaborated models (like the SLS with the dashpot) consist of two components: A dashpot represents the viscous part and will give a drag torque of

$$\tau_{\text{viscous}} = -\kappa V \eta \dot{\Theta}$$

where $\kappa$ is a shape factor, $V$ is the volume, $\eta$ is the viscosity and $\omega = \dot{\Theta}$ is the rotation frequency or in general the time derivative of the angular position. With the shape factor for the sphere the equation becomes

$$\tau_{\text{viscous}} = 8\pi a^3 \eta \dot{\Theta} = 8\pi a^3 \eta i \omega$$
where $a$ is the radius of the sphere. A spring represents an elastic part and the drag torque for a sphere is given by

$$\tau_{\text{elastic}} = 8\pi a^3 \mu \Theta$$

(5)

with $\mu$ being the shear modulus and $\Theta$ the angle. If two components are combined in a parallel manner, the torques are added, and if they are combined in series, the shear rates ($\dot{\Theta}$) will add. This way we can define a complex torque in the frequency domain: for the Kelvin–Voigt model this is

$$\tau = 8\pi a^3 \Theta (\eta i\omega + \mu) ,$$

(6)

for the Maxwell Model it is

$$\tau = 8\pi a^3 \Theta \frac{i\omega \eta}{1 + \omega^2 \mu} ,$$

(7)

and for our SLS with dashpot model it looks like

$$\tau = \frac{8\pi a^3 \Theta i\omega \eta_2 (i\omega \eta_1 \mu_1 + \mu_1 \mu_2 + i\omega \eta_1 \mu_2)}{i\omega \eta_1 \mu_1 + \mu_1 \mu_2 + i\omega \eta_2 \mu_1 - \omega^2 \eta_1 \eta_2} .$$

(8)

All the equations are of the form

$$\tau = \gamma \Theta$$

(9)

where $\gamma$ is the viscoelastic response of the fluid to the torque.

In the oscillation method we know the applied torque from the optical measurement and we measure the amplitude of the torque (and the phase shift) for different frequencies. The amplitudes (torque and angle) and the phase shift between them are obtained by the Fourier transform of the data (cf. section 2.5). With this the components of the model ($\mu, \eta$) can be fitted.

### 3.2 Rotational Brownian Motion

In the driven oscillation experiment the viscous and elastic components from the model can be directly fitted to the measurement data. In the Brownian motion experiment we don’t know the explicit torque, nor anything about the phase difference.

We will start with the equation of rotational motion for the particle and its consequences. $\Theta$ is the angle of the optical axis. The thermal torque $F_T$ on the particle is countered by an inertial term ($I$: rotational inertia), some complex response to the medium $\gamma$ (see equation 9), and some restoring force from the trap $k$.

$$F_T = I\ddot{\Theta} + \gamma \Theta + k \Theta$$

(10)

The motion is dominated by elasticity and viscosity, so we can neglect the inertia and our trap doesn’t restrict the particle rotationally, so $k = 0$. We assume white noise Brownian torque for the mean squared torque (i.e., the same torque for every frequency). This is justified, because the thermal impacts which cause Brownian motion can be assumed as independent delta functions, which yield an equal distribution over all frequencies. It can be written as

$$< F_T^2 > = 2k_B T 8\pi a^3 \eta .$$

(11)

We then can plot the logarithmic mean squared rotational displacement $\ln < \Theta^2 (\omega) >$ with $\gamma$ for the different models and obtain the theoretical power spectrum, which we can compare to the measured power spectrum.

For a simple dashpot the thermal torque $F_T$ must be equal the viscous to the drag torque, so all the energy is dissipated.

$$F_T = 8\pi a^3 \eta i\omega \Theta$$

(12)

$$< \Delta \Theta^2 > = \frac{k_B T}{4\pi a^3 \eta i\omega^2}$$

(13)

In a double-logarithmic plot against the frequency (power spectrum) this gives a slope of $-2$. 

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A single spring gives the thermal torque:

\[ F_T = 8\pi a^3 \mu \Theta \]  \hspace{1cm} (14)

\[ < \Delta \Theta^2 > = \frac{k_BT\eta}{4\pi a^3\mu^2} \]  \hspace{1cm} (15)

This is independent of the frequency and gives a plateau in the corresponding plot.

For the Kelvin-Voigt model at high frequencies only the spring is visible, because it gives an instant angle displacement whereas the dashpot needs time to show an effect. For low frequencies only the dashpot is visible, because the spring already is stretched and the dashpot still yields an increasing angle (cf. figure 3a)).

\[ < \Delta \Theta^2 > = \frac{k_BT\eta}{4\pi a^3(\mu^2 - \eta^2\omega^2)} \]  \hspace{1cm} (16)

For the Maxwell model, for high frequencies, the limiting component is the dashpot, so we can see it as a slope in the plot and for low frequencies the spring is limiting the achievable angle, so we observe the plateau corresponding to the spring (cf. figure 3b)).

\[ < \Delta \Theta^2 > = \frac{k_BT}{4\pi a^3} \left( \frac{1}{\omega^2\eta} + \frac{\eta}{\mu^2} \right) \]  \hspace{1cm} (17)

If more components (dashpots and springs) are added to the model, more slopes of \(-2\) and plateaus will appear in regions according to the arrangement in the model and their values like steps. For example in figure 3c) you can see the power spectrum for an SLS model with the added dashpot.

With the above derived equations we can determine the values of the springs and dashpots in our model from the heights of the slopes and plateaus.

![Figure 3. a) Power spectrum for the Kelvin model with \( \eta = 10^{-8}\)Pa·s and \( \mu = 10^{-6}\)Pa, b) Power spectrum for the Maxwell model with \( \eta = 10^{-6}\)Pa·s and \( \mu = 2 \cdot 10^{-2}\)Pa, c) Power spectrum for the SLS with dashpot model with \( \eta_1 = 2 \cdot 10^{-8}\)Pa·s, \( \eta_2 = 2 \cdot 10^{-6}\)Pa·s, \( \mu_1 = 10^{-4}\)Pa, and \( \mu_2 = 10^{-5}\)Pa](http://proceedings.spiedigitallibrary.org/)

### 3.3 Mean Squared Displacement

Instead of plotting graphs in the frequency domain, it is also common to plot the mean squared displacement \(< \Delta \Theta^2 > \) vs. time. This directly relates to Einstein’s diffusion theory (and theories developed in reference 14).

In order to average over a greater number of samples, each sample can be broken up into sub-samples (because Brownian motion is uncorrelated in time, which can then be averaged over: 15

\[ < \Delta \Theta^2 > (n\Delta t) = \frac{1}{N-n} \sum_{j=1}^{N-n} [\Theta(j\Delta t + n\Delta t) - \Theta(j\Delta t)]^2 \]  \hspace{1cm} (18)
where \( N \) is the total number of sampled points, \( n = 1, 2, \ldots, N - 1, N \), \( \Delta t \) is the time interval between two sample points, and \( \Theta(\text{time}) \) is the angle at a certain time \( t \). Application of this equation, already for small data sets, quickly leads to high computational effort, so we could only use it only on small data sets. For large sets, we only calculated a stochastic sampling from it.

4. RESULTS

We used a Nd:YAG trapping laser of 1 W output power at 1064 nm wavelength and a detection HeNe Laser of < 1 mW output power at 632 nm wavelength. The numerical aperture of the objective is 1.3 (oil immersion) and 1.4 for the condenser.

![Figure 4. RMS displacement for different polyethylene oxide PEO concentrations](image_url)

Figure 4. RMS displacement for different polyethylene oxide PEO concentrations

Figure 5 shows some preliminary results of the frequency spectrum of rotational angular fluctuations observed in solutions of PEO. At low frequencies, a \( 1/\omega^2 \) drop is observed, but at higher frequencies, a plateau, which is well above the detector noise floor, is observed. This can be interpreted in terms of the Maxwell model shown in figure 3b, and further measurements are underway.

5. DISCUSSION

The new method we propose proved to be challenging and it might not yet be directly applicable for certain fluids.

First, the vaterite crystals dissolve in acidid environments, so for examining acidic fluids the vaterite spheres might need to be enhanced, e.g., by coating. In non-acidic solutions, vaterite is stable for months.

Second, some fluids develop a depletion layer (however, our method is suitable for measuring a depletion layer) or, in the case of certain polymers, adsorb to the vaterite spheres, what can lead to wrong results for the bulk concentration.1
In our calculations we so far assume the vaterite’s mass as negligible,\(^3\) which might be significant at shorter time ranges of \(10^{-6}–10^{-5}\) s.\(^1\)

Concerning fluids, the probe particle has to be larger than the fluid molecules, which is valid for, e.g., PEO solutions, but not for, e.g., DNA solutions.

And because of the small order of magnitude in our application, even simple experiments quickly become tedious because of the necessary calibrations.

Note that in our measurements in figure 5 for concentrations 0.029%wt and 2.5%wt a second bead got caught in our trap, lowering the slope of the plotted spectrum.

6. CONCLUSION

We have investigated a novel method for microrheology, which allows us to apply variable torques to rotate a probe particle and examine the surrounding fluid. Very small torques can be applied down to practically zero, which allows the study of rotational Brownian motion. Alternatively, these torques can be automatically controlled by a computer, allowing phase shifts to be measured for a wide range of frequencies. We have shown results for different PEO concentrations, which are promising, with more experiments in progress.

The method developed in this paper is likely to find a number of very interesting applications, specifically for studies of cellular processes.

REFERENCES


