Calibration of trap stiffness and viscoelasticity in polymer solutions

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ABSTRACT

We present an experimental demonstration of a method using optical tweezers proposed by Fischer and Berg-Sørensen for measuring viscoelasticity using optical tweezers. It is based on a sinusoidal oscillation of the liquid in combination with force measurements using optical tweezers. We verify the method by applying it to measurements in water, glycerol and polyethylene oxide (PEO).

Keywords: viscoelasticity, optical tweezers, viscosity.

1. INTRODUCTION

Optical tweezers have been successfully used before to measure viscosity. Translational as well as rotational methods can be used to obtain the viscosity of liquids [1, 2]. Rotational measurements use birefringent particles that rotate in a circularly polarized optical trap and the exerted torque is used to obtain the viscosity [2]. When using a translational measurement technique, the trap is calibrated using the calibration method presented in reference 3. However, methods based on the observation of the thermal movement [3] of particles are only suitable for viscous liquids and not for viscoelastic liquids. Since most biofluids are viscoelastic, there is a need for the development of techniques that allow determination of the viscoelasticity. Here we describe such a method and verify its correctness by performing measurements n water and known concentrations of glycerol. We also use the method for measurements of PEO.

2. OPTICAL TWEEZER SET UP

The setup combines optical tweezers and back focal plane imaging. See figure 1 for a schematic picture of the setup, which comprises microscope designed by Dr. Gregor Knöner. Inverted microscopes provide stability and flexibility of multiple beam incorporation as well as easy access to the sample. An additional advantage in comparison to upright configuration is that the trapping force is stronger in the bottom of the slide, where we do most of the experiments.

The illumination system is based on the Köhler principle using a white light source (Olympus TGHM Spot macroilluminator). An Ytterbium doped fibre laser (1070 nm), with an output power up to 5W, serves as source of the trapping beam. Absorption by biological samples in this wavelength region is low and therefore heating of the sample is limited. The minimum stable operating power for this laser is 3W, so a polarizing beam splitter cube in combination with a half wave plate is used in order to reach a power in the order of tens to hundreds of miliwatts at the entrance of the objective. A small fraction of the laser power is reflected onto a power meter. The trapping beam is focused using an oil immersion 100x (Nikon) high numerical aperture objective (1.3 NA). A telescope is positioned to overfill the back aperture of the objective to achieve a tightest possible focus at the focal plane. Mirror M1 is imaged on the back of the objective to allow centering of the trapping beam with respect to the imaging beam in the sample plane. The sample is positioned on a 3D piezo-controlled micrometer stage with a precision accuracy of 5 nm.

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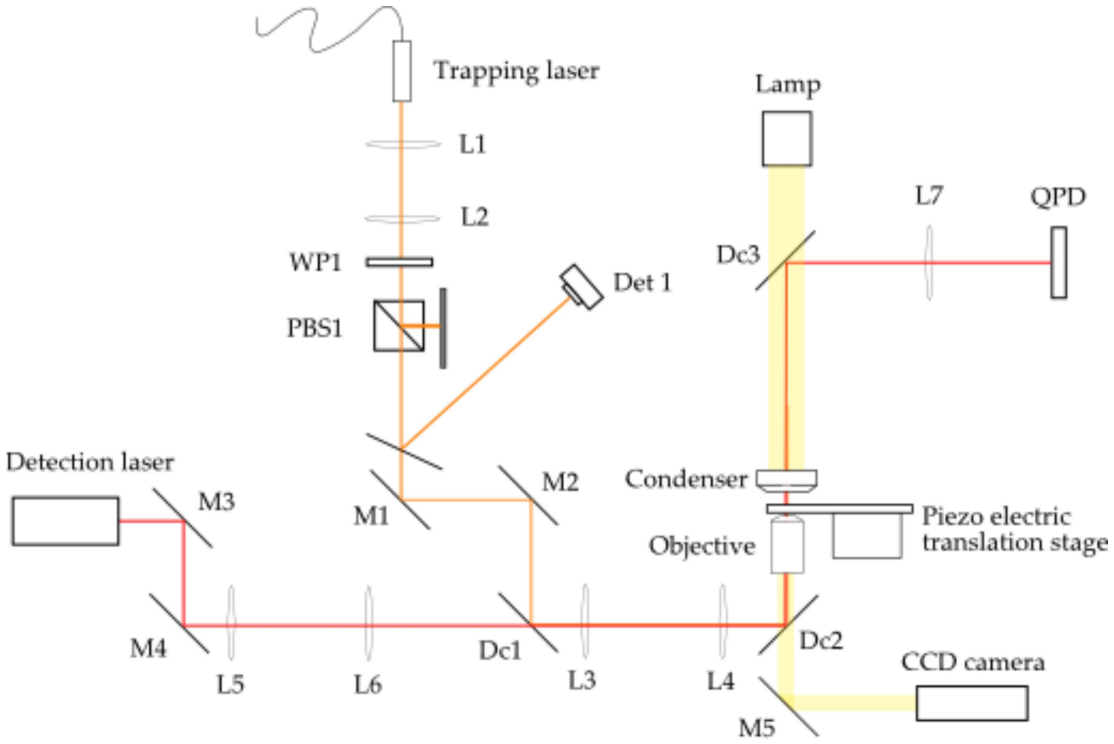

Figure 1 Setup for optical tweezers with position detection

A HeNe laser (Uniphase 1144P, 633nm) is coupled in through dichroic mirror M2 and serves as position detector beam. The transmitted light is collected by the condenser (Olympus 1.4 NA). The back focal plane of the condenser is imaged onto a quadrant detector (QPD). The four signals of the quadrant detector are acquired by a data acquisition system and further processed using Labview and Matlab.

3. THEORETICAL BACKGROUND

To obtain the complex viscosity of a liquid we generate a sinusoidal movement of the piezo stage where the sample is situated. Using this method we can determine the complex dynamic modulus (G), which is the strain response of a viscoelastic liquid to a sinusoidal stress deformation.

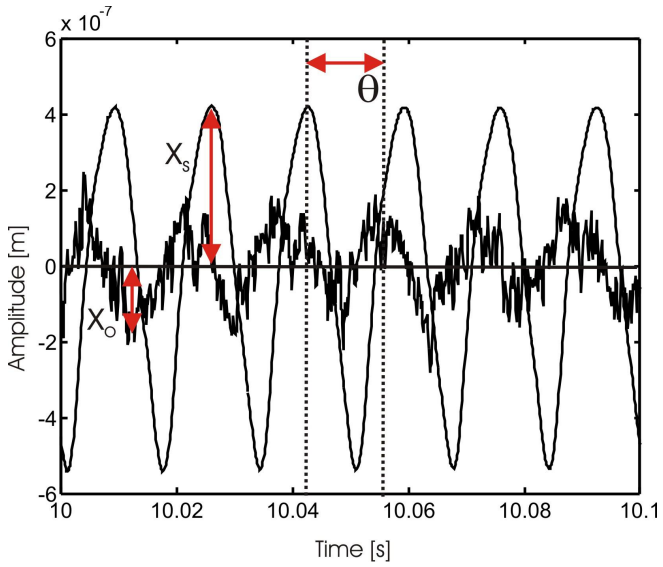

Figure 2 Sinusoidal input to the piezo stage and bead movement.

As an output we obtain the position of a trapped bead as a function of time. In figure 2 the input and output signals are plotted, where the detected position of the bead is a superposition of the thermal Brownian motion and the sinusoidal response to the stage oscillation of the amplitude $x_{s[}1]$. From these measurements we can obtain the phase lag (θ) between input and output and the amplitude of the bead in the liquid (x_0). These three parameters are used as an input to the model for calculating the viscoelastic properties of the liquid of interest [1], described by the complex modulus $G^*(\omega)$ of the medium. Below, we summarize the theory given in [1], and describe the procedure for determining the complex modulus $G^*(\omega)$.

The effect of the complex modulus on the motion of a particle (which will also be acted on by the optical trapping force, characterized by the optical spring constant *k*, thermal forces, and possibly an external driving force) is described by the *friction relaxation spectrum*,

$$\gamma(\omega) = \gamma'(\omega) + \mathrm{i}\,\gamma''(\omega) \quad . \tag{1}$$

Since here the real part $\gamma'(\omega)$ accounts for the effects of viscosity, which involves the loss of energy, and the imaginary part $\gamma''(\omega)$ accounts for elasticity, which involves the storage of energy, the relationship between the complex modulus and the frequency relaxation spectrum is

$$G^*(\omega) = \mathrm{i}\,\omega\gamma(\omega)\big/6\pi a \tag{2},$$

where *a* is the radius of the particle. Therefore, the spectral response, $\chi(\omega)$ of the particle to an external force *F*, defined by $x(\omega) = \chi(\omega)F(\omega)$, where *x* is the complex amplitude of the motion of the particle at frequency ω, is

$$\chi(\omega) = 1\Big/\left(k + \mathrm{i}\,\omega\gamma(\omega) - \omega^2 m\right) \tag{3},$$

where *k* is the spring constant and *m* is the mass of the particle.

A suitable way to drive the particle with an external force is to sinusoidally oscillate the stage. To a high level of accuracy, we can assume that the medium follows the motion of the stage exactly. In this case, the external force driving the particle is simply given by the friction relaxation spectrum, and the spectral response of the particle position to active driving by this external force will be

$$R(\omega) = \chi(\omega)\gamma(\omega) = \frac{x(\omega)}{\mathrm{i}\,\omega x_{\mathrm{S}}(\omega)}, \tag{4}$$

where $x_{\mathrm{S}}(\omega)$ is the complex amplitude of the motion of the stage. Since we are only interested in the relative phase between the motion of the trapped particle and the stage, we can introduce the phase lag θ between these two motions, and write the active response spectrum as

$$R(\omega) = \frac{x_0(\omega)\cos\theta}{\mathrm{i}\,\omega x_{\mathrm{S0}}(\omega)} - \frac{x_0(\omega)\sin\theta}{\omega x_{\mathrm{S0}}(\omega)} \tag{5}$$

where $x_0(\omega)$ and $x_{\mathrm{S0}}(\omega)$ are the magnitudes of the particle and stage motion respectively, with $x_0(\omega) = \left|x(\omega)\right|$ and $x_{\mathrm{S0}}(\omega) = \left|x_{\mathrm{S}}(\omega)\right|$. This conveniently separates the response into real and imaginary parts. The magnitude x_0 and phase lag θ at a particular frequency ω can be found from measurements such as those shown in figure 2.

At this point, we can use the definition of the response function $\chi(\omega)$, equation (3), to find an expression for $\chi(\omega)$, since we have $i\omega\gamma(\omega) = \left(1/\chi(\omega)\right) - k + \omega^2 m$ from the definition, and therefore

$$\chi(\omega) = \frac{1 - \mathrm{i}\,\omega R(\omega)}{k - \omega^2 m} \tag{6}$$

If $\chi(\omega)$ can be found, the friction relaxation spectrum can be found using equation (4), and hence the complex modulus $G^*(\omega)$.

However, it will first be necessary to determine the spring constant *k* of the optical trap, and the mass of the particle, if this is not already known or negligibly small (which will depend on how high the driving frequency is). For a particle of known size and composition, the mass will be known, and often is small enough to be ignored.

One of the most common methods of measuring the trap stiffness *k* is to find the power spectrum due to thermal motion, which is

$$P(\omega) = \left\langle \left|x(\omega)\right|^2 \right\rangle = \frac{2k_{\mathrm{B}}T\,\mathrm{Re}\left(\gamma(\omega)\right)}{\left|k + \mathrm{i}\,\omega\gamma(\omega) - \omega^2 m\right|^2}, \tag{7}$$

where k_{B} is Boltzmann's constant and *T* is the absolute temperature. However, we do not yet know the properties of the fluid, characterized here by $\gamma(\omega)$, and therefore cannot find the spring constant by the usual method of finding the corner frequency of the Lorentzian spectrum. However, $\gamma(\omega)$ here is the same as $\gamma(\omega)$ for the externally driven case, at least when the motion in both cases is in the same linear regime [1].

Noting that we can write the thermal motion power spectrum as

$$P(\omega) = 2k_{\mathrm{B}}T\,\mathrm{Re}\left(\gamma(\omega)\right)\chi(\omega)\chi^*(\omega) \tag{8},$$

and since

$$\mathrm{Re}\left(\gamma(\omega)\chi(\omega)\chi^*(\omega)\right) = \mathrm{Re}\left(R(\omega)\chi^*(\omega)\right) = \mathrm{Re}\left(\frac{R(\omega) + \mathrm{i}\,\omega\left|R(\omega)\right|^2}{k - \omega^2 m}\right) = \frac{\mathrm{Re}\left(R(\omega)\right)}{k - \omega^2 m} \tag{9},$$

we can eliminate the friction relation spectrum and find the optical trap spring constant *k* from the ratio of the real part of the active response spectrum $R(\omega)$ to the thermal motion power spectrum $P(\omega)$ using

$$k - \omega^2 m = 2k_B T \frac{\mathrm{Re}\left(R(\omega)\right)}{P(\omega)} \qquad (10).$$

Even if the mass is unknown, it should be noted that this will yield the entire unknown term $k - \omega^2 m$ in equation (6), allowing us to determine the viscoelastic properties of the fluid even if the spring constant, by itself, remains unknown.

4. MEASUREMENTS

4.1 Calibration

The Quadrant Photo Detector (QPD) is calibrated by monitoring the movement of a bead fixated at the bottom of a cover slip when the piezo stage is driven sinusoidal. The amplitude in nanometers is known and can be used to calibrate the QPD. For displacements that are small enough, the QPD behaves linearly.

4.2 Bead size

The measurements were performed on polystyrene beads with a diameter of 2±0.045 μm. We have calculated the effect of the size of the particle on the position in the trap as well as the trap stiffness using a full non paraxial vector wave treatment [4]. The result of the calculations is shown in figure 5a. These simulations were done for a NA of 1.3 and a trapping wavelength of 1064 nm. It appears that a small variation in size causes a large variation in z-position in the optical trap. This could be a problem because the position of the bead with respect to the imaging system changes, which means the calibration of the QPD changes and therefore the position we obtain in meters could be incorrect. In figure 5b the effect on the trap stiffness is shown. Again large variations can occur. To avoid problems with either of these factors we choose polystyrene beads of 2.077 μm with a standard deviation of 0.045 μm. For this size range the variation in z-position as well as trap stiffness appears to be minimal.

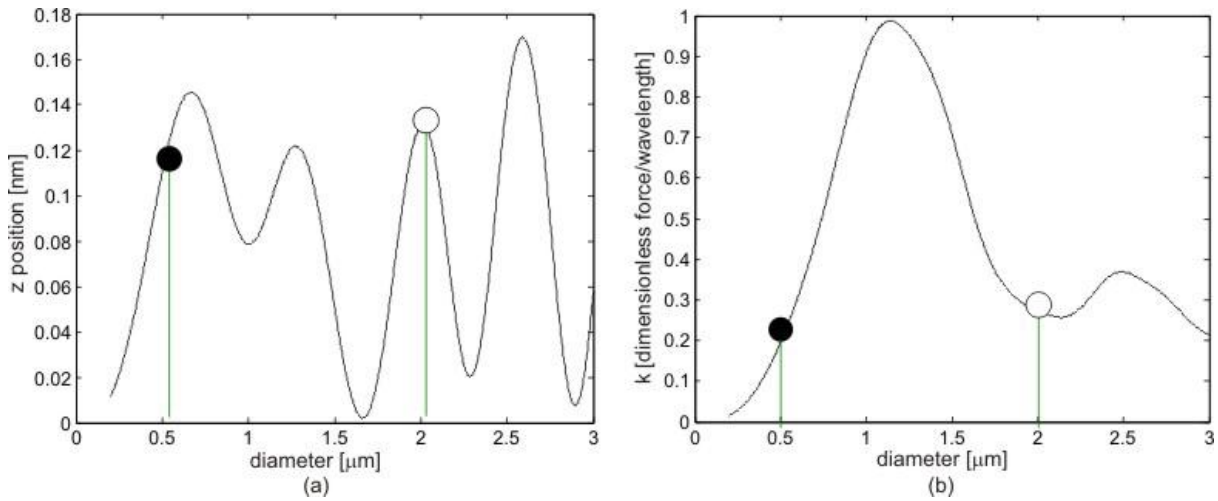

Figure 1 (a) variation in bead position in z-direction as a function of the bead diameter and (b) the effect of the diameter on the trap stiffness (dark circles represent a bead with diameter of 0.5 μm and clear circles a bead with diameter 2 μm).

5. MEASURING VISCOELASTICITY

The position of a bead in the optical trap as a function of time was obtained using a signal from the QPD. This was done first in passive mode, a situation where the stage was not driven, and after that several active measurements were performed when the stage was moving sinusoidally with an amplitude of 100 nm and a frequency of 60 Hz. From these input values the viscosity is obtained using equations 1 to 4. Figure 4.a gives an example of the raw data obtained from the quadrant detector during an active measurement. In the frequency domain this translates to figure 4.b, where the large peak is due to the driving frequency and the thermal background is visible from the Lorenz curve, where the corner frequency is related to the trap stiffness.

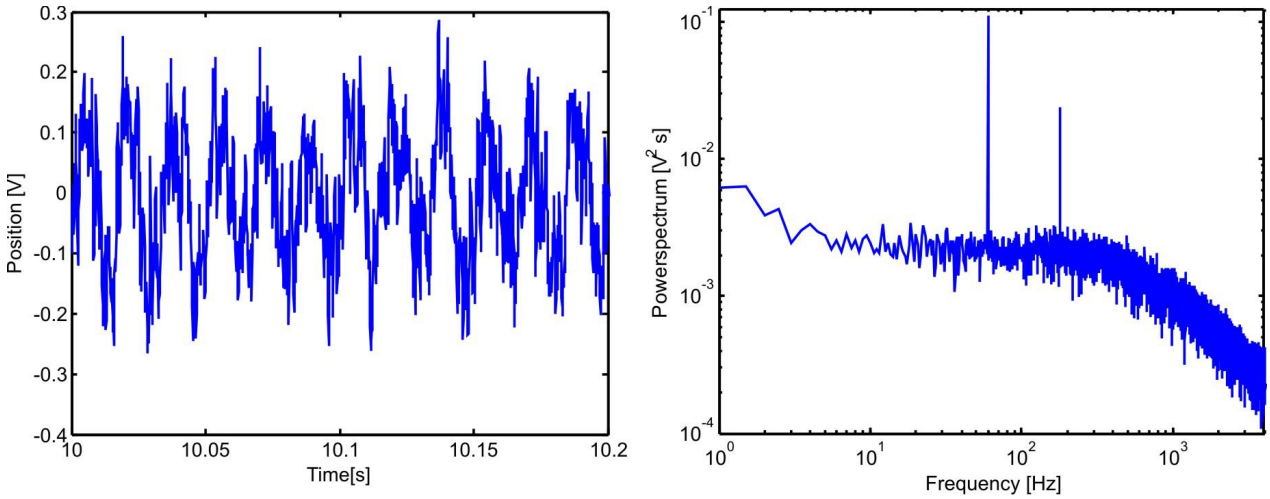

Figure 2 (a) Signal obtained from the QPD when the stage is driven sinusoidally. The obtained signal is an addition of Brownian motion and the sinusoidal stage input. (b) Power spectrum of the bead movement.

The method was tested in water and glycerol solutions. We found the viscosity in water to be 1.1 ± 0.1 Pa s with a variation of approximately 25%. Viscosity measurements on different concentrations of glycerol are shown in figure (5), which shows how viscosity of the solution increases with glycerol content, according to our expectations.

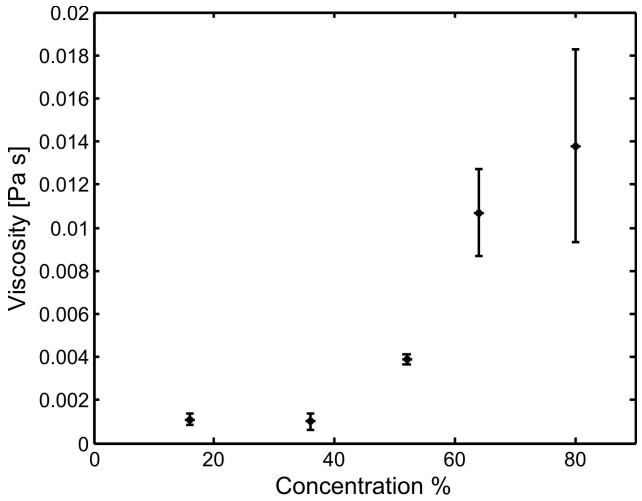

Figure 3 Measurements performed in glycerol solutions. The obtained viscosity is plotted as a function of the glycerol concentration.

6. CONCLUSION

In conclusion the method proposed by Fisher et al. [1] has been proven experimentally in water and glycerol. The size of the trapped particle affects considerably the trap stiffness and the particle's z-position in the optical trap. The obtained values of the viscosity of water are consistent as well the values found for the glycerol viscosity are proportional to its concentration. Future plans are to improve our calibration by using a liquid with a known viscosity, such as water, instead of a stuck bead to calibrate our QPD.

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