View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Queensland eSpace

Viscoelasticity Measurements inside Liposomes

Shu Zhang*, Lachlan Gibson, Daryl Preece, Timo A. Nieminen, Halina Rubinsztein-Dunlop
School of Mathematics and Physics, The University of Queensland, Brisbane, QLD 4067 Australia

ABSTRACT

Microrheology, the study of the behavior of fluids on the microscopic scale, has been and continues to be one of the most important subjects that can be applied to characterize the behavior of biological fluids. It is extremely difficult to make rapid measurement of the viscoelastic properties of the interior of living cells. Liposomes are widely used as model system for studying different aspects of cell biology. We propose to develop a microrheometer, based on real-time control of optical tweezers, in order to investigate the viscoelastic properties of the fluid inside liposomes. This will give greater understanding of the viscoelastic properties of the fluids inside cells.

In our experiment, the liposomes are prepared by different methods to find out both a better way to make GUVs and achieve efficient encapsulation of particle. By rotating the vaterite inside a liposome via spin angular momentum, the optical torque can be measured by measuring the change of polarization of the transmitted light, which allows the direct measurement of viscous drag torque since the optical torque is balanced by the viscous drag. We present an initial feasibility demonstration of trapping and manipulation of a microscopic vaterite inside the liposome. The applied method is simple and can be extended to sensing within the living cells

Keywords: Microrheology, Liposome, Optical tweezers

1. INTRODUCTION

Optical manipulation exploits optical methods to manipulate process and detect micro meter-sized objects with important potential application for biology, chemistry, material science and physics. Many of the most powerful optical manipulation techniques are derived from single-beam optical traps known as optical tweezers introduced first by Ashkin [1-2]. It is known that optical tweezers could be not only for simple manipulation but also used as a tool to probe microscopic properties of fluid [3], since light can carry angular momentum as well as linear momentum, the transfer of angular momentum can be used to produce optical torque [4]. This method is not influenced by the size or shape of the particle or the laser beam geometry. Apart from this, rotational mode is more local since no translational motion is needed. However, no matter by displacement of the trap centre or application of a constant torque to an intrinsically or shape birefringent particle with circularly polarized incident beam, it is difficult to rapidly resolve the local viscoelastic properties of a small volume of fluid across a wide frequency range [5]. In our experiment, a birefringent particle will be trapped by linearly polarized dual beam, which is less sensitive to boundary effects and more accuracy.

By now, optical manipulation has been widely applied in many fields of biology and physics. Both the experiment and theory are devoted to investigate and extend the technique for different kinds of micro particles. Such analytical optical tweezers have become an important tool to sort and organize cells, control bacteria motion, measure torsional forces and index of refraction of micro-particles, and has transformed our understanding of numerous facts of cell biology [6-8]. In order to assess the local viscoelasticity in cells, we first ought to figure out the microrheology inside liposome. Giant unilamellar vesicles (GUVs) have been widely used as simple models for studying different aspects of real cell as they vividly mimic the feature of cell membranes which are primarily composed of phospholipids.

Correspondence and requests for materials should be addressed to H.R. –D. (halina@physics.uq.edu.au)

Optical Trapping and Optical Micromanipulation XI, edited by Kishan Dholakia,
Gabriel C. Spalding, Proc. of SPIE Vol. 9164, 91642O · © 2014 SPIE
CCC code: 0277-786X/14/$18 · doi: 10.1117/12.2060938

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

2. MEASUREMENT OF VISCOELASTICITY

Here we describe three different methods to test viscoelasticity of the fluid in experiment. The set-up is illustrated in Figure 1.

2.1 Passive Brownian motion

The frequency-dependent behavior of viscoelasticity fluid is expressed in the form G*(ω) = $G'(\omega) + iG''(\omega)$, where $G'(\omega)$ is the frequency-dependent elastic modulus and $G''(\omega)$ is the frequency-dependent viscous modulus [9]. It is known that the viscosity of the medium around a trapped particle is the main factor affecting the statistical distribution of the particle's time-dependent position. We adopt a generalized Langevin equation Eqn. (1) to model the particle's position in three dimensions.

$$I\ddot{\phi} = \tau_{TH}(t) - \int_{-\infty}^{t} \xi(t - t')\dot{\phi}(t')dt' - \chi\phi \quad (1)$$

The angle ϕ is chosen to be the angle between the polarization of the trapping beam and the optic axis. The angular position evolves under the effects of the Hookean optical restoring torque with effective stiffness χ , the zero-mean thermal torque τ_{TH} and the viscoelastic memory function ζ .

By the analogy with Preece et al [10], we could get G*(ω) = $\left[I\omega^2 - \chi\omega\tilde{A}(\omega)/\left(i + \omega\tilde{A}(\omega)\right)\right]/8\pi a^3$ from Eqn. (1), where $\tilde{A}(\omega) = \int_0^\infty A(\tau)e^{-i\omega t}d\tau$ and $A(\tau) = \langle\phi(t)\phi(t+\tau)\rangle / \langle\phi(t)^2\rangle$ is the normalized angular position autocorrelation function (NAPAF). So with the measurement of $\phi(t)$ in our experiment, the viscoelasticity of the sample could be obtained, and for this experiment, only one AOM as shown in Figure 1 is needed to control the intensity of transmitted laser.

2.2 Sinusoidal Oscillation

In this experiment, the two circularly polarized beams that are mutually incoherent and of opposite handedness are incident on the particle in order to trap it, and then the particle will rotate at an angular velocity of $\omega = \theta/\Delta t$, where θ is the displacement in angular position of the particle within time Δt .

The mean squared angular displacement within time Δt is expressed as $\langle\theta^2\rangle = 2D_R\Delta t$, where D_R is the rotational diffusion coefficient, given by the Stokes-Einstein relation as $D_R = k_B T / C_R$, where k_B is Boltzmann constant, T is the absolute temperature, and is related to the rotational viscos coefficient. For a spherical particle, $C_R = 8\pi\eta a^3$ where η is the viscosity of the surrounding fluid and a is the radius of the sphere.

Therefore, the viscosity η can be measured via detecting the mean squared angular displacement of the rotation particle, $\eta = k_B T\Delta t / \left(4\pi a^3 \langle\theta^2\rangle\right)$ [11].

For this purpose, a 633nm probe laser with circular polarization is used to detect the angular displacement of particle Figure (1). When the circular polarization beam is incident on the particle, the polarization will be changed to be elliptical due to the birefringence of the rotation particle. The dependence of the polarization change on the angular displacement would be measured by the angular power detector placed after a linear polarizer, that the detected power P is determined by P~ $\cos^2\theta$.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

2.3 Wiggler-Waggler

In terms of this measurement, the particle is trapped by linearly polarized dual beams. Brownian motion will be tested in each trap to make sure consistency between two traps. Then the particle starts to rotate with a single-beam trap and switching to dual-beam trap controlled by two AOM [5]. So while the particle is rotating back to the original position, the flip is recorded. In a similar way, the complex shear modulus is defined by G*(ω) $=\left[I\omega^{3}-\chi\omega\tilde{A}(\omega)/\left(i+\omega\tilde{A}(\omega)\right)\right]/8\pi a^{3}$, where $A(\tau)=\left|\langle\phi(t)\rangle/(4/\pi)\right|$, since the trap polarization is rotated by $\pi/4$.

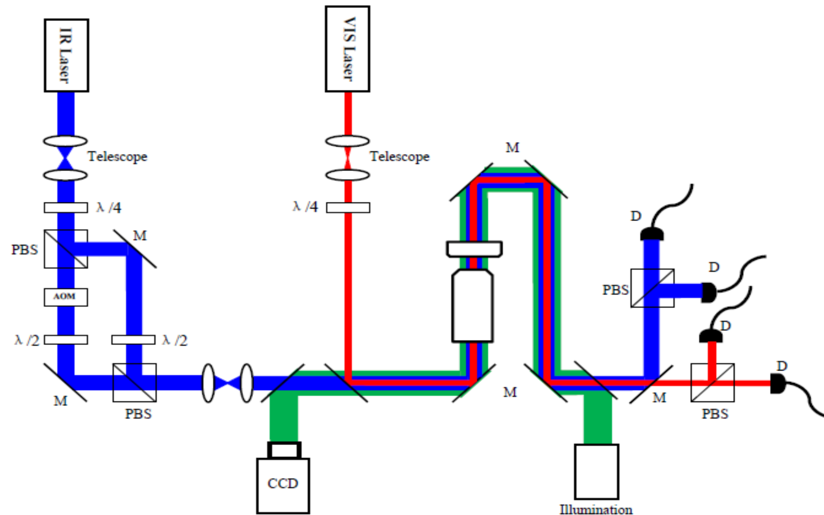

Figure 1. Experimental setup for the measurement of viscoelasticity.
AOM: Acoustic-optic-Modulator, PBS: polarizing beam splitter; M: reflection-mirror; D: photo detectors; CCD: camera

3. LIPOSOME PRODUCTION

In order to assess viscoelasticity of the micron volume fluid by trapping a particle inside the liposome, we need to put particles into the liposome as well as make liposomes. The particle could get into the liposome by both optical and chemical method. The particle can be brought into the cell by cutting a hole into the cell membrane with a femeto-second laser and trapping the particle through the hole with an optical trap [12, 13]. We test different methods of making liposome to find as discussed below Figure 2.

3.1 Dehydration-hydration method

A lipid film is prepared by evaporation of a lipid-containing organic solvent, and then the liposomes would be formed when the film is exposed to the buffer solution with vaterites immersing in it. The advantage of this method is high yield of making liposomes while the disadvantage of this method is less controllable such as the size and layers of the membrane.

3.2 Modified electroformation procedure

1nstead of originally preparing a dry lipid film deposited on an electrode, the hydrogel stamps are used to pattern lipid deposits on ITO slides [14]. During the procedure of lipid formation, we produce a large number of monodisperse giant

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

liposomes, the birefringent microspheres are added to buffer solution and finally are effectively encapsulated inside liposomes.

3.3 Water/oil emulsion

The inverted emulsion droplets with vaterites are transformed into giant unilamellar vesicles when they pass through the water/oil interface. The interface is prepared by using the microfluidic channels [15].

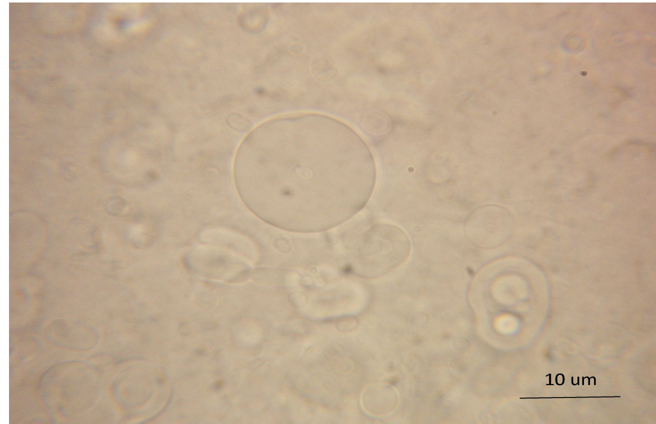

Figure 2. Liposomes

4. DISCUSSION

Boundary walls in microscopic scales sample have a non-ignorable influence on the flows of fluid and drag force on moving objects [16, 17], so it is necessary to consider the wall effects when calculate the viscoelasticity of the fluid in the liposome. We treat the liposome as a rigid sphere, which means velocity become zero at boundary. The flow between two uniformly rotating concentric spheres has been calculated:

$$\vec{\upsilon}(\vec{r})=\frac{1}{\frac{1}{a^3}-\frac{1}{b^3}}\left[\left(\frac{1}{r^3}-\frac{1}{b^3}\right)\vec{\omega}_1+\left(\frac{1}{a^3}-\frac{1}{r^3}\right)\vec{\omega}_2\right]\wedge\vec{r} \quad \text{(b>a)} \tag{2}$$

where $\vec{r}$ is the position vector, with its origin at the center of the bead.

Considering a particle rotates inside a liposome, $\vec{\omega}_1=\omega\vec{u}_z, \vec{\omega}_2=0$, then the hydrodynamic torque is equal to $\vec{T}=\vec{T}_0/\left(1/a^3-1/b^3\right)$ and $\vec{T}_0=8\pi\mu\omega a^3\vec{u}_Z$, So the viscosity

$$\mu^*=\frac{\mu}{\left(\frac{1}{a^3}-\frac{1}{b^3}\right)} \tag{3}$$

The drag on an isolated sphere in an infinite fluid is $\tau_0=8\pi\eta a^3\omega$. This drag will increase if there is a surrounding stationary surface. We can write the torque in terms of an increase relative to the isolated sphere torque: $\tau=A*\tau_0$. There are two simple cases. First, when the vaterite probe is at the center of the liposome, our increase in torque is given by [18]: $A_{concentric}=1/\left(1-\left(a_{vaterite}/a_{liposome}\right)^3\right)$.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

Second, if the vaterite is close to the liposome surface, and the liposome is much larger than the vaterite Figure 3, we can approximate this situation as a sphere rotating next to an infinite plane wall. While we do not have a simple formula for this case, it is straightforward to calculate the increase in drag numerically [19]. Both of these cases are shown in Figure 4. These cases provide upper and lower limits for the increase in drag for a vaterite eccentrically rotating within a liposome.

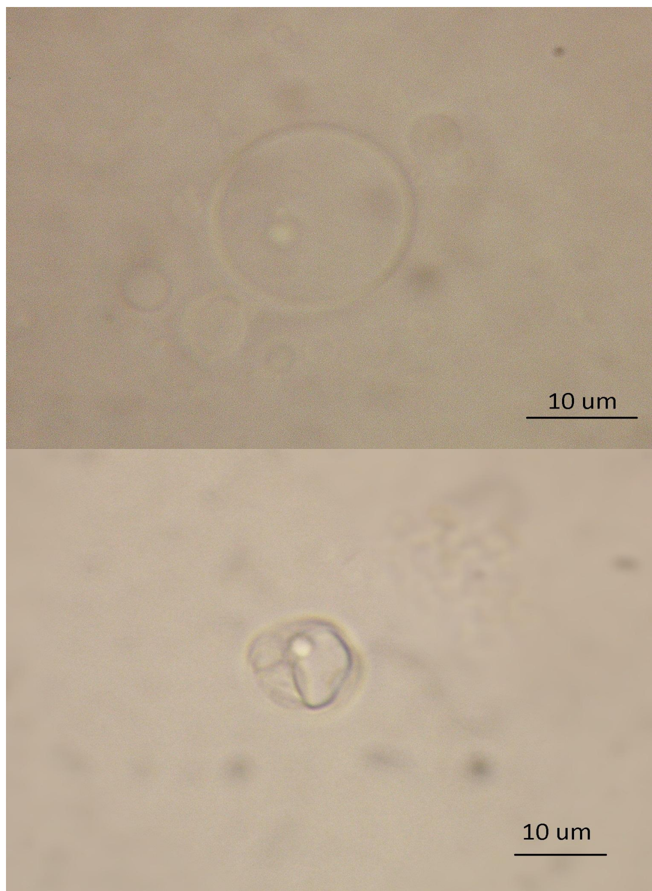

Figure 3. Liposome with a particle inside

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

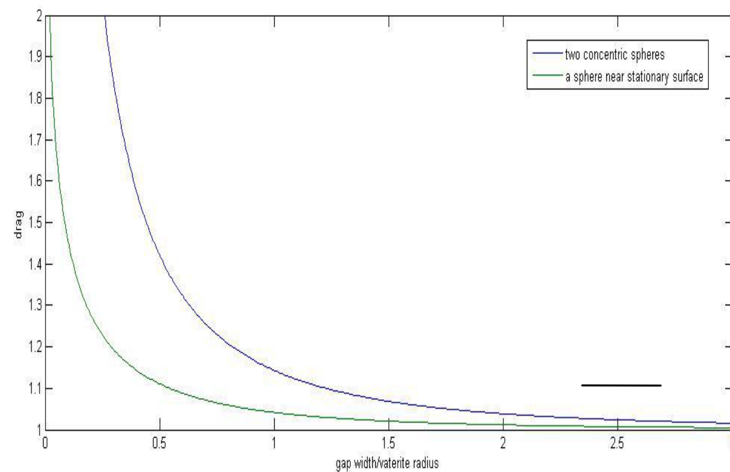

Figure 4. Viscous drag in two simple cases. The blue line is the case of two concentric spheres, and the green line is the case of a sphere surround stationary surface.

5. CONCLUSION AND FUTURE PLANS

We present an initial feasibility demonstration of trapping and manipulation of a microscopic vaterite inside a liposome. The applied method is simple and can be extended to sensing within living cells.

In the future, we intend to complete our measurements with different approaches in order to optimise optical tweezers as more ideal tool access to cells and gain a better understanding on Microrheology. Furthermore, the theoretical model will improved with the goal of more precisely calculating the properties of the medium inside cell.

REFERENCES

[1] Ashkin, A., “Acceleration and trapping of particles by radiation pressure”, Phys. Rev Lett. 24, 156-159 (1970).
[2] Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E. and Chu, S., “Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles”, Opt. Lett. 11, 288(1986).
[3] Bennett, J. S. et al. "Spatially-resolved rotational microrheology with an optically-trapped sphere”, Sci. Rep. 3, 1759; DOI: 10.1038/srep01759 (2013).
[4] Nieminen, T. A., Heckenberg, N. R. and Rubinsztein-Dunlop, H. “Optical Measurement of Microscopic torques”, Journal of Modern Optics, 48(3), 405-413 (2001).
[5] Bennett, J. S., Brousse, E., Baudisch, B., Preece, D., Nieminen, T. A., Heckenberg. N. R. and Rubinsztein-Dunlop, H., “‘Wiggler-Waggler’-Towards Optical Measurements of the Complex shear Modulus” Proc. SPIE 8097 (15), 1-10 (2013).
[6] Ashkin, A. and Dziedzic, J. M., “Optical Trapping and Manipulation of Viruses and Bacteria, Science 235, 1517 (1987).
[7] Knoner, G., Parkin, S., Nieminen, T. A., Heckenberg N.R. and H. Rubsztein-Dunlop, “Measurement of refractive index of single microparticles”, Physics Review Letter 97, 157402 (2006).
[8] Wu, T., Nieminen, T. A., Mohanty, S., Miotke, J., Meyer, R. L., Rubsztein-Dunlop, H. and Berns, M. W., “A Photon-driven micromotor can direct nerve fiber growth”, Nature Photonics, 6, 62-67 (2012).
[9] Ferry, J. D., [Vicoelastic properties of polymers], Wiley, New York (1980).
[10] Preece, D. et al., “Optical tweezers: wideband microrheology”, Journal of Optics 13, 044022 (2011).

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

[11] Funk, M., Parkin, S. J., Stilgoe, A. B., Nieminen, T. A., Heckenberg, N. R., and Rubinsztein-Dunlop, H., "Constant power optical tweezers with controllable torque", Opt. Lett. 34(2), 139-141 (2009).
[12] Parkin, S., "Angular Momentum in Optical tweezers", PhD thesis, The University of Queensland (2008).
[13] Muhammad Waleed, Sun-Uk Hwang, Jung-Dae Kim, Irfan Shabbir, Sang-Mo Shin, and Yong-Gu Lee., "Single-cell optoporation and transfection using femtosecond laser and optical tweezers", Biomedical Optics Express 4(9) 1533-1547.
[14] Kang, Y. J., Wostein, H. S., and Majd, S., "A Simple and Versatile Method for the Formation of Arrays of Giant Vesicles with controlled Size and Composition", Adv. Mater. 25, 6834-6838 (2013).
[15] Matosevic, S. and Paegel B. M., "Stepwise Synthesis of Giant Unilamellar Vesicles on a Microfluidic Assembly Line", J. Am. Chem. Soc. 2011, 133, 2798-2800 (2011).
[16] Leach, J., Mushifique, H., Keen, S., Di Leonardo, R., Ruocco, G., Cooper, M., and Padgett, M. J., "Comparison of faxen's correction for a microsphere translating or rotating near a surface" Physical Review E, 79, 026301, 1-4 (2009).
[17] Kim, S. and S.J Karrial., "Microhydrodynamics-Principles and Selected Applications", Butterworth-Heinemann, Stoneham, (1991).
[18] Landau, L. D., [Fluid Mechanics], Pergamon Press (1959).
[19] Chaoui, M. and Feuillebois, "Creeping flow around a sphere in a shear flow close to a wall", Quarterly Journal of Mechanics and Applied Mathematics 56(3), 381-410 (2003).

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 12/09/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx