Optical Trap Based Microrheology Performed for Characterization of Microtubule Embeded Fluids

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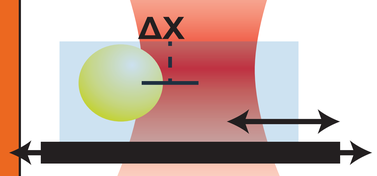
Abstract

Cellular substructures made of cytoskeletal elements organize into active materials through the consumption of ATP. Measurement and quantification of these materials can become quite difficult due to the intrinsic size of the system and inability to perform non invasive cellular measurements. During cell development and growth, microtubules polymerize and organize into different structures, and as such the cellular body changes its viscoelastic properties. To probe the materials present, we recreated these microtubule fluids outside of the cell. We then are able to trap inserted microspheres and drive in oscillatory motion created by an optical tweezer apparatus. Applying this methodology to water-glycerol solutions, we calibrate our system to find make measurements that result in the viscosity and complex modulus values of the fluid the bead is embedded in. Our results show that optical tweezer based microrheology has regions where materialistic properties are nonuniform based on the experimental apparatus. Further characterization of active microrheology and various limits at which valuable measurements can be determined would contribute to the implementation of this technology across systems and length scales. This technique has the ability to characterize materials in low volumes and size resolution, which would benefit materials where sample volume must be minimized.

Methods



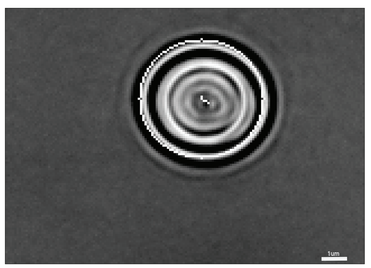
Active Microrheology:

- The optical tweezer laser beam (red) capture beads (yellow) in a material of interest (blue) on our stage (black).
- The trap provides a spring like force on the bead F=kΔx.
- We oscillate the stage to probe to extract viscoelastic properties of our system at $r_{stage} = A\sin(\omega t)$

Quantitative Image Analysis:

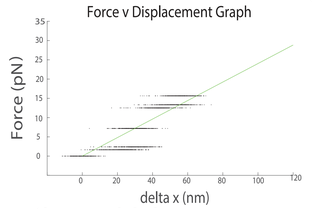
- Videos of the trapped bead as shown to the right are tracked using the Mosaic Suite plugin in the ImageJ software [(1)].
- This bead oscillates taking the form: $r_{bead} = r\sin(\omega t + \theta_1)$

- The displacement of the bead from the trap in reference fluid is then: $r_{s,b} = r_{bead} - r_{stage} = r_{max}\sin(\omega t + \theta)$



Calibration:

- We drive the bead at a constant velocity over many frequencies to solve for k
- K is the slope shown to the right. At 190mW this slope gives k = 193 pN/um.



- The k value changes with index of refraction, which we account for by scaling our k to match previously noted trends[(2)].

Theory

$$6\pi\mu R\frac{dr_{s,b}}{dt} + (k+\kappa)r_{s,b} = kA\sin(\omega t)^{(3)}$$

We start with Newton's Second Law for our system, with μ is the viscosity of our fluid, R is the radius of our bead, k is the spring constant of our trap, κ is the stiffness of our material, $r_{s,b}$ is the bead moving relative to the liquid, A is the amplitude of our stage, and ω is the driving frequency. We solve this for κ and μ to arrive at:

$$\mu(\omega) = \frac{Ak\sin(\theta)}{6\pi\omega R r_{max}} \qquad \kappa(\omega) = k\left(\frac{A}{r_{max}}\cos(\theta) - 1\right)$$

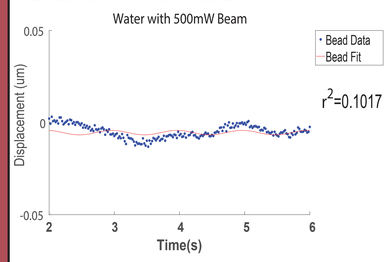
Which can be used to relate back to the complex modulus through the following equations:

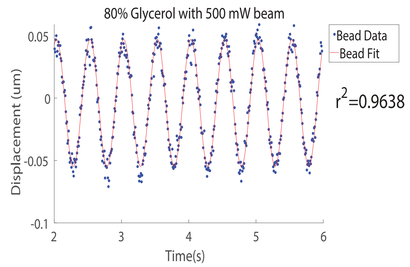
$$G'(\omega) = \frac{\kappa(\omega)}{6\pi R} \qquad G''(\omega) = \frac{Ak\sin(\theta)}{6\pi R r_{max}}$$

$$\tan(\delta) = G''(\omega)/G'(\omega)$$

Results

Optimal Conditions Deliver Measurable Data Trends





- Beam intensity, frequency, driving amplitude and viscosity impact measurement quality
- Oscillating less than .01um is indistinguishable from noise
- For fluids <0.001Pa•s, <200mW beam power should be used
- For fluids >0.001Pa•s, >200mW beam power should be used

Measured Viscosities of Water-Glycerol Samples

Glycerol-Water %	Viscosity $(\frac{Ns}{m})$	Accepted Values	κ$(\frac{N}{m})$	G′ $(\frac{N}{m^2})$	G″ $(\frac{N}{m^2})$	tan(delta)
60	0.0095± 0.007	0.0108	0.235 ± 0.001	0.00731± 0.00089	0.115± 0.088	36.7± 0.83
70	0.0147± 0.00115	0.0225	0.161± 0.00071	0.00507± 0.000632	0.199± 0.144	1372 ± 1.12
80	0.0272± 0.008	0.0601	0.206± 0.00071	0.00643± 0.00063	0.334± 0.099	242± 0.54

Relative Viscosities (Row/Column)

	60	70	80
60	1	0.646	0.349
70	1.547	1	0.540
80	2.863	1.850	1

- Viscosities of solutions made from 60%, 70%, and 80% glycerol to water by weight using 10 beads
- Relative viscosities show trend in increasing viscosity

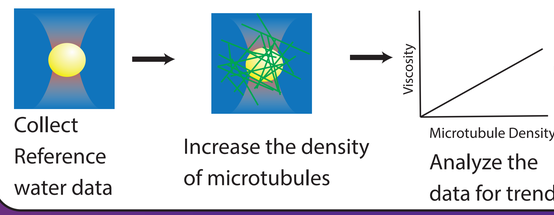
Discussion

We have found regimes where data acquisition is possible and have used this to make measurements of water-glycerol samples. Material property values from this do not align with accepted values of viscosity and carry a large amount of error as shown above.

Being misaligned by a frame when analyzing the data causes error shown to the right, which can be quite large. Such error can only be remedied by increasing the frame rate beyond standard cameras.

Predicted Viscosity	0.0239
1 Frame off ($\frac{1}{120}$ s)	0.0301
Error	25%

Our data follows the trend in reported viscosities. Characterization of microtubule networks can be found using the following plan:



Collect Reference water data → Increase the density of microtubules → Analyze the data for trends → Observe Microtubule Networks as More Viscous Fluids

References

1. I.F. Sbalzarini and P. Koumoutsakos *J. Struct. Biol.* (1995)
2. M.T. Valentine *et. al. J. Condens. Matter Phys.* (1996)
3. L.A. Hough and H.D. Ou-Yang *J. Nanoparticle Research* (1999)

Acknowledgments

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