

OPTICAL TRAPPING TECHNIQUES INVOLVING  
MICRORHEOLOGY AND TCELL TRIGGERING

By

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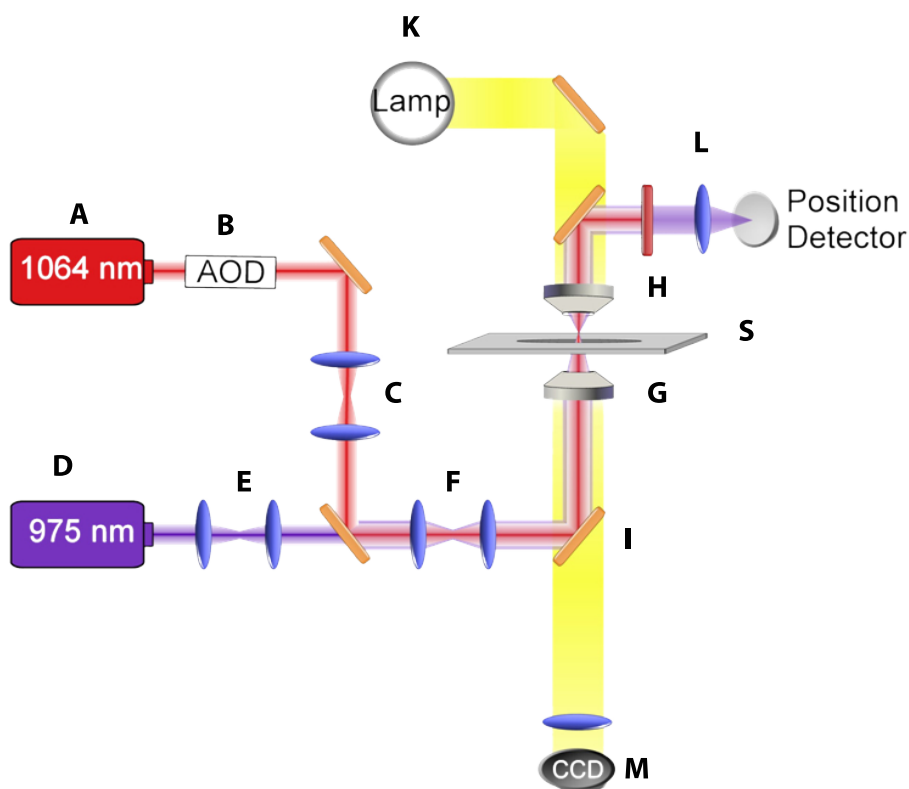
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## CHAPTER 1

### OPTICAL TRAPS

Optical traps, also known as optical tweezers, were first employed in the 1970s by Arthur Ashkin<sup>1-4</sup>. He demonstrated that a laser could displace and hold in place a micron-size dielectric particle in a solution. These devices are widely used in biological studies today due to their ability to apply piconewton forces while producing nanometer precision displacement<sup>5-25</sup>. Optical tweezers can trap a dielectric particle in place using a tightly focused laser beam, which generates a steep gradient of light intensity. Two forces are responsible for maintaining the bead's position. A scattering force pushes the particle towards the direction of the light propagation while a gradient force pulls the particle towards the center of the trap. These forces place the particle slightly beyond the focal point of the laser. A high numerical aperture objective is required to form a steep gradient powerful enough to overcome scattering forces. The force on the dielectric particle can be represented as a Hookean spring<sup>6-9</sup>.

The optical trap used for the experiments mentioned in later chapters is seen in Figure 1. It is composed of a 1064-nm laser that is focused with a high numerical aperture objective. The laser is steered by acousto-optic deflectors (AODs) and a telescope lens pair. The sample is controlled using a sub-nanometer precision piezostage. The position of the trap laser is monitored using a position sensitive device (PSD) or a quadrant photo diode (QPD) with high precision. Software coded by the Lang Lab is used to record the positions of the trap and the sample, as well as measure forces<sup>7</sup>.



**Figure 1.** A schematic of the optical trap used in the experiments. A) 1064 nm laser use to trap beads. B) Acoustic optic deflector used to guide the beam. C) Telescope lens pair use to align the trapping laser and increase the beam diameter. D) 975 nm position detection laser. E) Telescope Lens pair used to align the detection laser. F) Telescope lens pair used to align both lasers together onto the target. G) High numerical aperture objective. H) Condenser lens. I) Dichroic mirror used to integrate the trapping laser and detection laser into the transmission light path of the inverted microscope. J) Dichroic mirror used to direct the beams onto the position detector. K) Mercury arc lamp that serves as a bright field source. L) Position detection branch with a position detection sensor and filter that eliminates the 1064 nm laser beam. M) Camera used for imaging. S) Piezostage that holds the slide. A 532 nm laser absent from the diagram is also used for fluorescence. An extra PSD is used for active rheology experiments to monitor trap position. Figure modified from D. Appleyard and P. Tarsa.

The stiffness of the trap is determined by three methods, each of which will be discussed in detail. The first method measures the variance of a trapped particle that arises from Brownian motion. According to the equipartition theorem, the energy of a trapped particle due to Brownian

motion is given by  $\frac{1}{2}k_B T$  where  $k_B$  is Boltzmann constant and  $T$  is the temperature in Kelvin. The energy stored in the spring is equal to one half times the stiffness of the trap,  $k_T$ , times the variance,  $x^2$  where  $x$  is the distance from the center of the trap.

$$\frac{1}{2}k_B T = \frac{1}{2}k_T x^2 \quad (1.1)$$

This method is very useful because it requires no knowledge of the bead size or the viscosity of the solution<sup>6,7,9,10,11</sup>. The second method used to determine the stiffness of the trap is determined by drag force. By moving the stage at a given velocity,  $v$ , the stiffness is calculated by equation 1.2

$$k_T x = 6\pi r \eta v \quad (1.2)$$

where  $x$  is the displacement from the center of the trap,  $\eta$  is the viscosity of the solution, and  $r$  is the radius of the bead. If the bead is near the surface of the cover slip, it will experience boundary layer effects. This can be corrected by applying Faxen's Law seen in equation 1.3

$$\beta = \frac{6\pi r \eta}{1 - \frac{9}{16} \frac{r}{h} + \frac{1}{8} \left(\frac{r}{h}\right)^3 + \frac{45}{256} \left(\frac{r}{h}\right)^4 - \frac{1}{16} \left(\frac{r}{h}\right)^5} \quad (1.3)$$

The third method that is used to determine the stiffness of the trap is the power spectrum calibration method, which implies that the power spectrum of a trapped bead follows a Lorentzian profile. Therefore the stiffness of the trap is as follows:

$$k_T = f_0 2\pi\beta \quad (1.4)$$

where  $f_0$  is the roll off /corner frequency and  $\beta$  is the drag coefficient equal to  $6\pi r \eta$ .

## CHAPTER 2

### RHEOLOGY

Rheology is the science of deformation and flow of a fluid. It describes how a material reacts when a force is applied to it<sup>30-33</sup>. This is important for manufacturing companies who need their products to maintain a shape regardless of external conditions. It is also important for fluids, such as lubricants, that are made to handle constant stresses without losing their properties<sup>33</sup>. Fluids are generally placed in three main categories: shear thinning, shear thickening, and Newtonian<sup>30, 32, 33</sup>. Shear thinning fluids provide less resistance when more stress is applied; therefore the fluid has lower viscosity. Ketchup and whipped cream are two examples of shear thinning fluids. Shear thickening fluids have the opposite effect. That is, the fluid becomes more resistant and has a high viscosity as more force is applied. A mixture of cornstarch and water is an example of this phenomenon. Newtonian fluids are fluids that have a constant viscosity and provide the same resistance regardless of how much force is applied such as the case with water and ethanol.

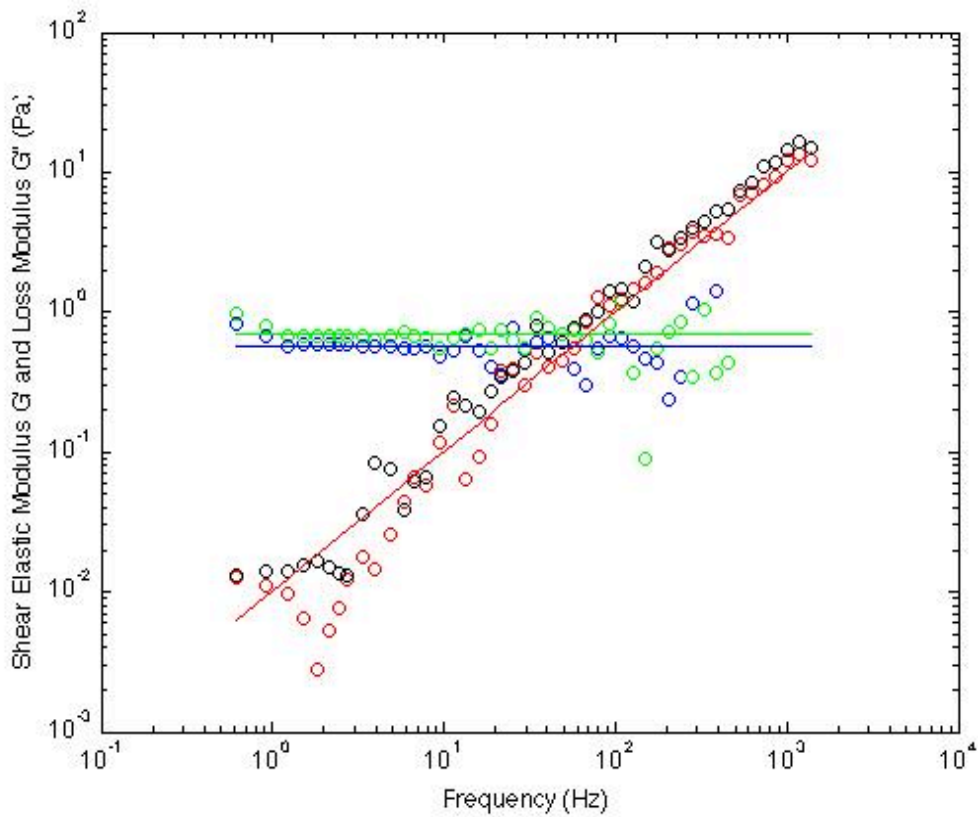
Fluids are generally described by their complex modulus<sup>11,30,33</sup>. The complex modulus includes a real part,  $G'$ , which represents the ability of a material to store energy while the imaginary part,  $G''$ , represents the ability of a material to dissipate energy. One method that people use to determine the viscoelastic properties of a material involves using a rheometer. Rheometers have 4 basic geometries. The cone and plate setup provides the best range for determining viscoelastic properties.



Optical tweezers were used to trap a bead in various concentrations of glycerol/water mixtures and xanthan gum/water mixtures to determine their rheological properties. For passive rheology, the thermal motion of the bead was recorded by the PSD and analyzed using the code found in the appendix. The storage modulus  $G'$  is determined by equation 2.1 and the loss modulus is determined by equation 2.2. As seen in Figure 2, these theoretical estimations are consistent with the experimental data.

$$G' = \frac{kT}{6\pi r} \quad (2.1)$$

$$G'' = 2\pi f \mu_{\text{water}} \quad (2.2)$$



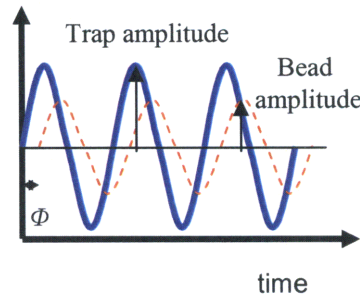
**Figure 2.** Passive rheology data for pure water. The red line is the predicted  $G''$  and the red and black circles are the experimental data points for  $G''$ . The green line is the predicted  $G'$  while the green circles are the experimental  $G'$ .

Passive rheology monitors only thermal fluctuations of the bead from the center of the trap; however, with active rheology the bead is displaced by the trap moving in an oscillatory motion according to equation 2.3, where  $x$  is the trap position,  $A_T$  is the amplitude of the trap displacement, and  $\omega$  is the input frequency<sup>11</sup>. Frequencies were tested from 0.1 Hz to 10 Hz.

$$x_{trap} = A_T \sin(\omega t) \quad (2.3)$$

The position of the bead in response to the trap is estimated by equation 2.4, where  $A_b$  is the amplitude of the bead's movement,  $\omega$  is the frequency and  $\theta$  is the phase delay.

$$x_{bead} = A_b \sin(\omega t - \theta) \quad (2.4)$$



**Figure 3.** A schematic of the trap moving in a sinusoidal motion along with the bead response. Courtesy of Ferrer's thesis.

The force on the bead is computed using Equation 2.5

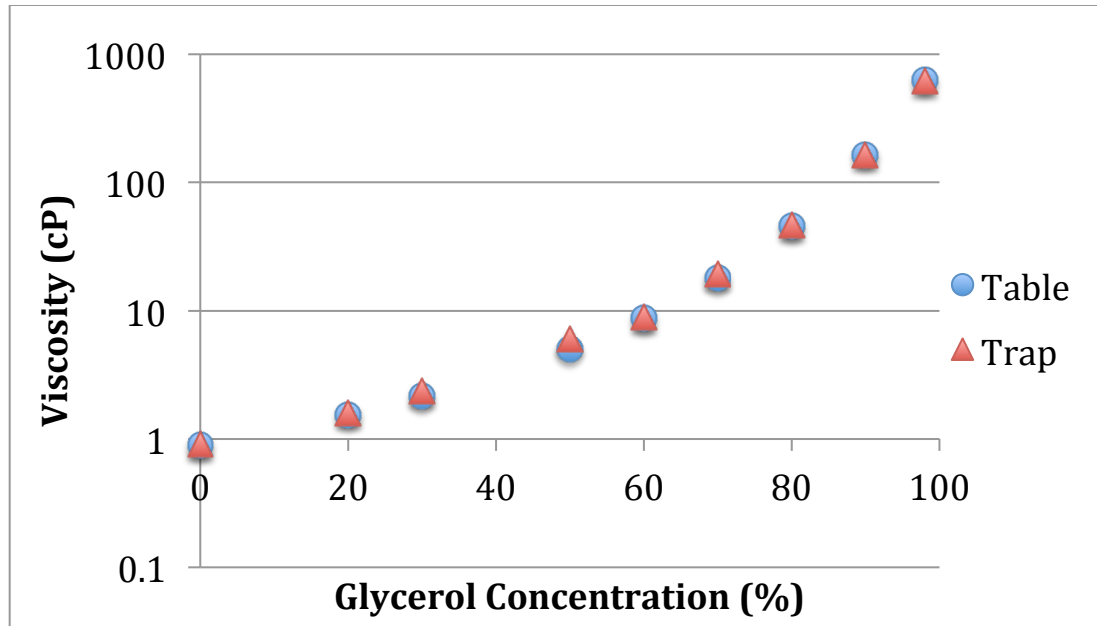
$$F = k_T [x_{trap} - x_{bead}] \quad (2.5)$$

The storage modulus and loss modulus are determined by equations 2.6 and 2.7 respectively, where  $\bar{F}$  is the magnitude of the forcing function,  $r$  is the bead radius,  $\bar{x}_{bead}$  is the amplitude of the bead's response, and  $\Delta\theta$  is the phase delay.

$$G' = \frac{\bar{F}}{6\pi r \bar{x}_{bead}} \cos(\Delta\theta) \quad (2.6)$$

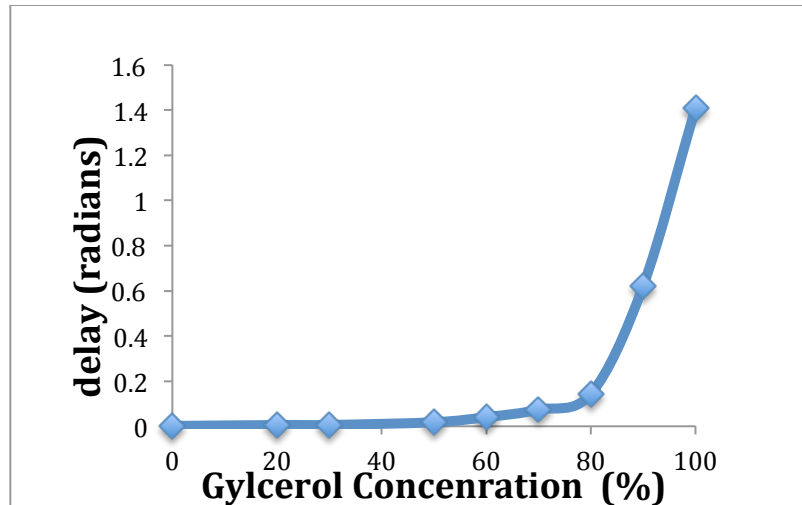
$$G'' = \frac{\bar{F}}{6\pi r \bar{x}_{bead}} \sin(\Delta\theta) \quad (2.7)$$

Glycerol is a colorless, odorless liquid that has many applications, which include usage as a sweetener, solvent, as well as a preservative<sup>11,34,35</sup>. Glycerol is also widely used as a humectant to help retain moisture and prevent drying. The viscosities of glycerol/water mixtures were tested at various concentrations ranging from 0% glycerol to 95% glycerol using optical tweezers. To conduct this experiment, a flow channel was created by placing two pieces of double-sided tape onto a glass slide with a narrow slit in between the two pieces of tape. A coverslip was next placed onto the tape and sealed. Beads were mixed at low concentrations (1ppm) with every glycerol/water mixture. The solutions containing the viscous mixture and the beads were flowed into the flow channel for active rheology. The experiments were conducted using the active rheology methods previously described with frequencies of 1, 5 and 10 Hz. Due to glycerol being a Newtonian fluid, the viscosity was constant at each frequency. The results from the optical trap were compared to the paper “*Glycerol Viscosity Tables*” published in Industrial and Engineering Chemistry as seen in Figure 4<sup>36</sup>.



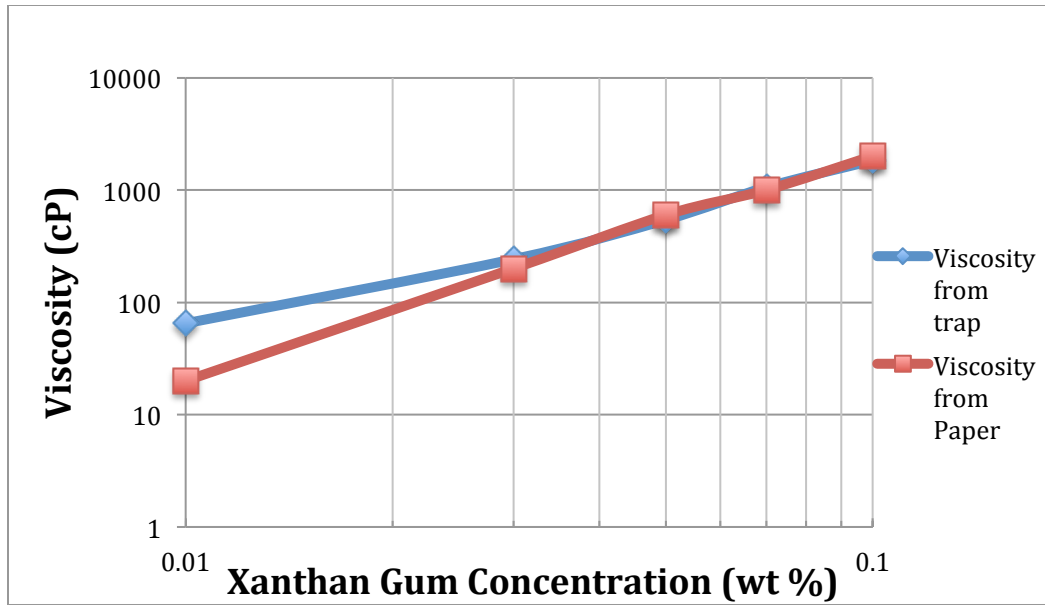
**Figure 4.** Comparison of the viscosity for glycerol concentrations from the optical trap and the paper “Glycerol Viscosity Table”. The error of the viscosity for each concentration is within the size of each data point.

As one might expect, the viscosity of the solution has a major impact on the phase delay. Pure glycerol is 1000 times more viscous than pure water<sup>36</sup>. Figure 5 provides a clear representation of the effect that viscosity has on the phase delay.



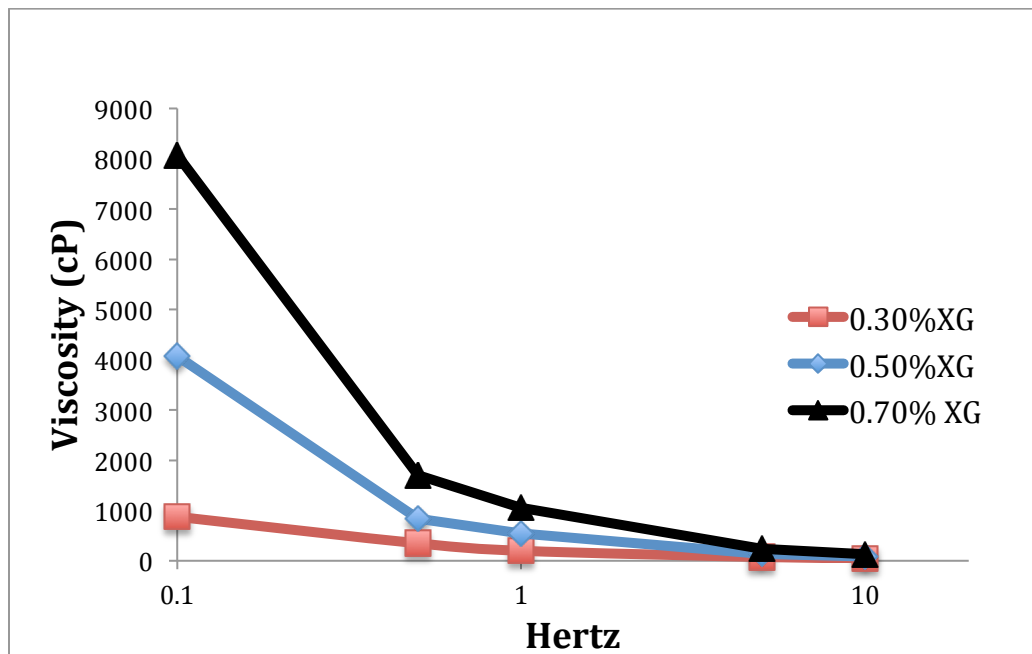
**Figure 5.** Phase delay vs concentration. The phase delay is calculated by subtracting the bead position from the trap position.

Xanthan Gum is a non-toxic food additive that was discovered by the USDA<sup>37,38</sup>. Adding just 1% of xanthan gum to water increases its viscosity by 100,000 times at low shear rates. For this reason, it is commonly used as a thickening agent in foods. Unlike glycerol, xanthan gum is non-Newtonian. While it is shear thinning, it is capable of recovering its natural viscosity instantaneously after a stress has been applied to the solution. Xanthan gum is used for temperature stability, pH stability, as well in the petroleum industry for oil recovery processes<sup>37,38</sup>. Xanthan gum powder, purchased from Sigma, was measured using a highly accurate lab scale to prepare solutions ranging from 0.01 wt% to 0.1 wt% in water. Once the correct amount was measured, the powder was placed in a beaker containing pure water. The solution was mixed overnight using a magnetic stir bar. Beads were added to the mixtures and flown into a flow channel for testing. The manufacture of the xanthan gum provided a paper that contains the rheological properties of xanthan gums at various concentrations. The experimental data taken at 1 Hz along with the data from the manufacture’s paper can be seen in Figure 6 for comparison.



**Figure 6.** Xanthan Gum viscosity at concentrations ranging from .01% to .1% measured by the optical trap along with the data provided by the manufacturing company Jungbunzlauer.

The xanthan gum solutions were tested at frequencies ranging from 0.1 to 10 Hz. As expected and seen, the viscosity decreases dramatically as the shear rate increases which can be seen Figure 7.



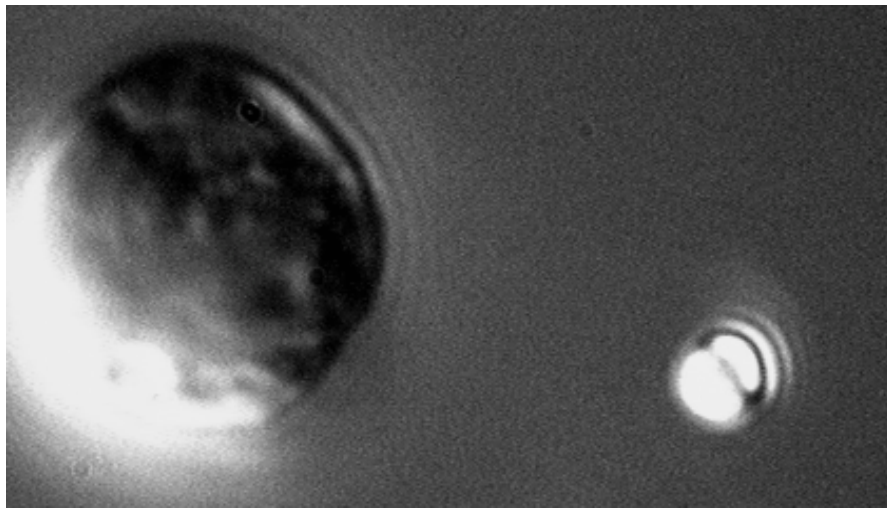
**Figure 7.** Viscosity of water with xanthan gum at 0.3wt%, 0.5wt%, and 0.7wt% in solution.

## CHAPTER 3

### T-CELL TRIGGERING

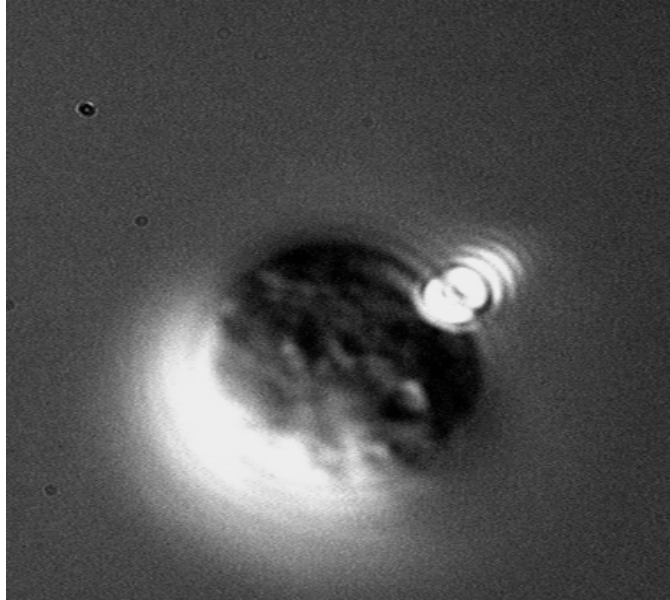
Humans, as well as other mammals, rely on thymus-derived lymphocytes to guard against viruses and other diseases<sup>39,40</sup>. These Tcells have receptors (TCR) that trigger intracellular signals when they contact specific peptide-major histocompatibility complex (pMHC) ligands. One example of intracellular signaling is initiating calcium fluxes. The TCR is a membrane bound protein that has a di-sulfide linked antigen binding domain<sup>39,40</sup>. To study the interaction of the TCR and pMHC in more detail, experiments were carried out using beads that were coated with pMHC and placed near a Tcell using an optical trap. The beads were coated with 2 different mAbs (2C11 and 17A2) created by Dr. Rienherz's lab at Harvard, which are discussed in greater detail in the paper "*The  $\alpha\beta$  T Cell Receptor Is an Anisotropic Mechanosensor*". Tcells from N15 transgenic mice were extracted for this experiment due to the fact they contain a TCR specific for vesicular stomatitis virus nuclear protein octapeptide bound to the H-2K molecule. The cells were centrifuged then loaded with calcium dye and media, as seen in the calcium dye protocol found in the appendix. The cells were next incubated for one hour then centrifuged once more to remove the excess dye from the solution. Next, the cells were then placed on slides that were etched and coated with poly-lysine to keep the cells attached to the cover slip. The slides were placed on the optical trap and casein was flowed in to block the beads from sticking to the surface as well as to remove any unattached cells. Beads that were coated with pMHC were flowed into the sample at low concentrations. A bead was then guided to an un-activated cell for experimentation after the trap was calibrated as seen in Figures 8 and 9. To determine if a cell was pre-activated, the 532-

nm beam was turned on and the fluorescence was monitored. Pre-activated and dead cells produced a high intensity, while non-activated cells produced little brightness. Once the bead was in contact with the cell, an image was taken every 30 seconds with the Andor camera. To avoid photo bleaching the cell, the 532-nm laser was blocked from entering the sample by keeping the shutter closed until one second before the Andor took the image. The shutter was briefly opened and then shutter was closed again.



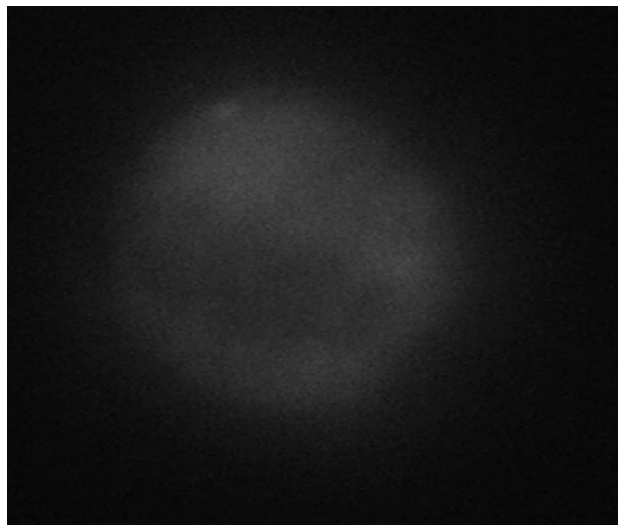
**Figure 8.** *Image from the bright field camera of a bead coated with 2C11 that is trapped in the vicinity of a Tcell to induce triggering.*



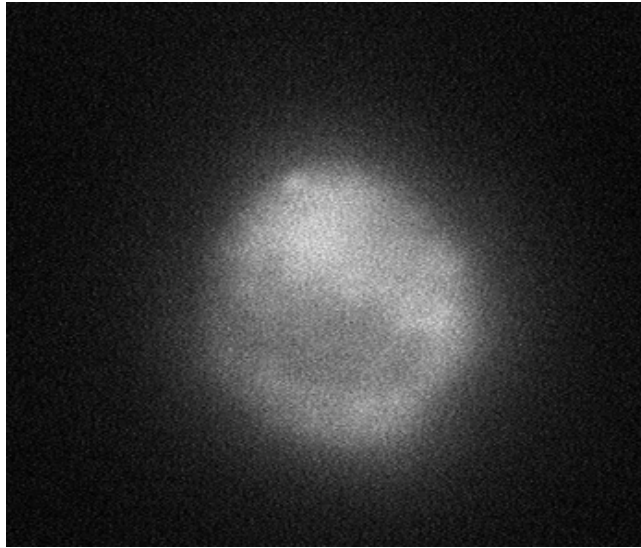


**Figure 9.** *Image from the bright field camera of bead coated with 2C11 brought in contact with Tcell.*

Beads that were coated with 2C11 activated the cell within minutes of contact. One can see by comparing Figures 10 and 11 the difference in the pre-activated cell and the activated cell. No force was required to activate the Tcell using 2C11.

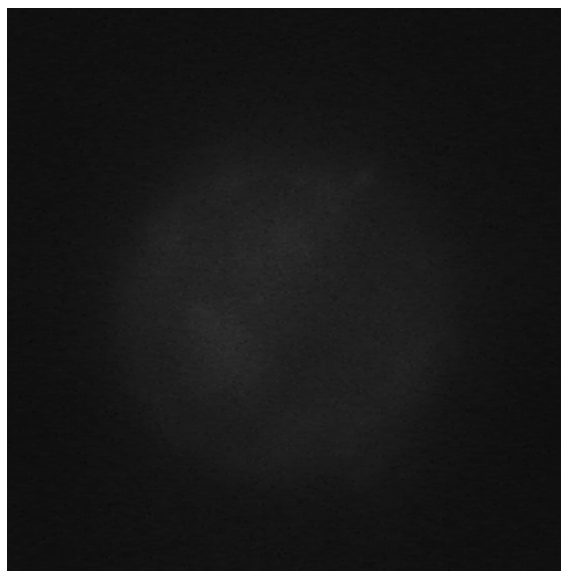


**Figure 10.** *Image from the Andor showing the fluorescence of a Tcell after the bead is brought in contact with the cell.*



**Figure 11.** *Image of the fluorescence of a triggered Tcell captured from the Andor. Note the increase in intensity as compared to Figure 10.*

Beads coated with 17A2, however, did not activate the cells immediately upon contact. 17A2 beads were oscillated tangentially to the cell to provide  $\sim 50$  pN of force upon the binding site. As seen in Figure 13, the cell was activated after  $\sim 5$  minutes of oscillation. The cells intensity increased very rapidly between four and five minutes. These results are in agreement with previous experiments performed by the Lang Lab at MIT<sup>40</sup>.



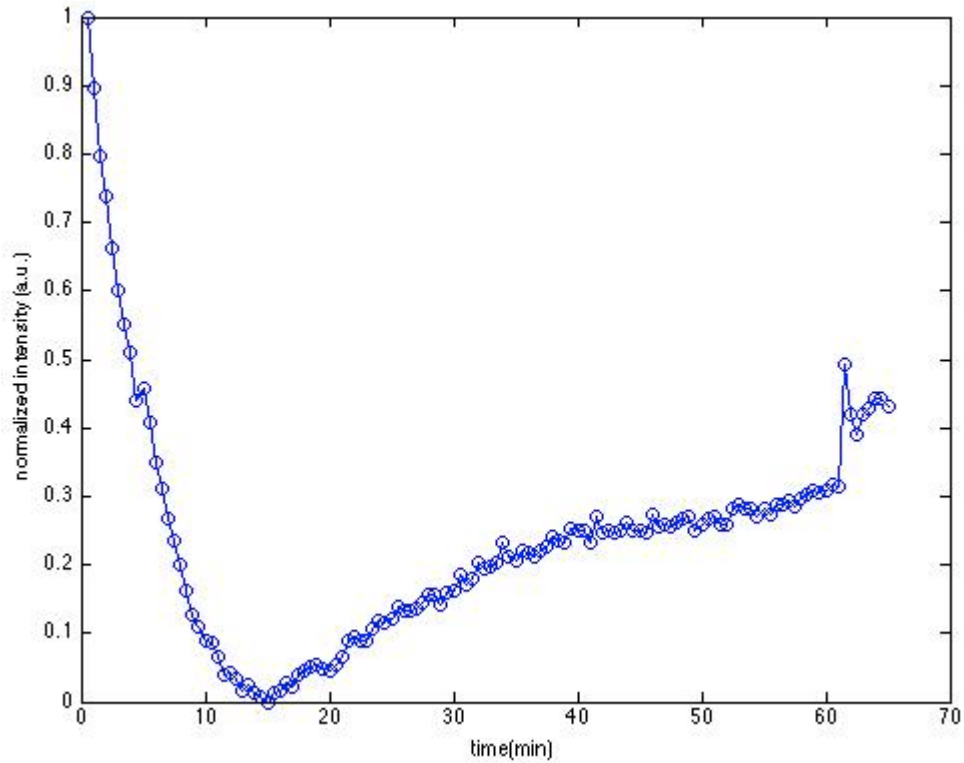
**Figure 12.** *Image of the fluorescence of the Tcell captured by the Andor before activation by 17A2.*



**Figure 13.** *Image of the fluorescence of the Tcell captured by the Andor after activation by 17A2 using oscillatory force.*

17A2 beads were flowed into the sample and brought into contact with the cell. The bead was then “stepped off” of the cell by moving the stage in 25nm increments while keeping the trap in place. This allowed the bead to apply a constant force to the cell as opposed to the oscillatory force

described previously. Although the cell was activated, its intensity increased very slowly unlike before. Figure 14 gives a representation of the intensity versus time for this experiment. The intensity was normalized using the initial brightness. One can see the intensity continuously drop until the cell activates, then the intensity slowly rises.



**Figure 14.** Plot of the intensity vs time of the fluorescence of a Tcell being triggered by 17A2 using the step and hold technique.

## CHAPTER 4

### CONCLUSIONS AND FUTURE WORK

Optical traps are an excellent tool for determining the properties of cells as they are capable of making very precise measurements. They have proven to be useful for protein measurements, DNA stretching, and for gaining knowledge and insights into how molecular motors work. In this thesis, rheological data was collected on a Newtonian fluid (glycerol/water mixture) and a shear thinning fluid (xanthan gum/water mixture) using optical tweezers. The data obtained highly accurately matched the data gathered using a rheometer. Optical traps, however, provide an advantage over rheometers with their small sample size requirement of 1 mL, as opposed to rheometers that could require up to 1 liter of material. They also have the advantage of being able to measure local viscosities for non-homogenous mixtures where rheometers can only give the overall or average viscosity of the mixture. T-cells were triggered using an optical trap by placing tension on the Tcell receptor using a bead coated with a known antigen. Using an oscillatory force of 50 pN, the Tcell was triggered within 5 minutes. Comparatively, the step and hold method took up to 30 minutes to trigger the Tcell. Additional experiments would need to be conducted on the antibody-antigen binding site using different forces to correctly identify the correlation between triggering time and force. These experiments would also have to be conducted on various antigen-antibody combinations as each pair would require different levels of force depending on their structure and binding sites. The last recommendation for future

experiments is to determine the chemical threshold for triggering T-cells by coating beads with various concentrations of the antigen.

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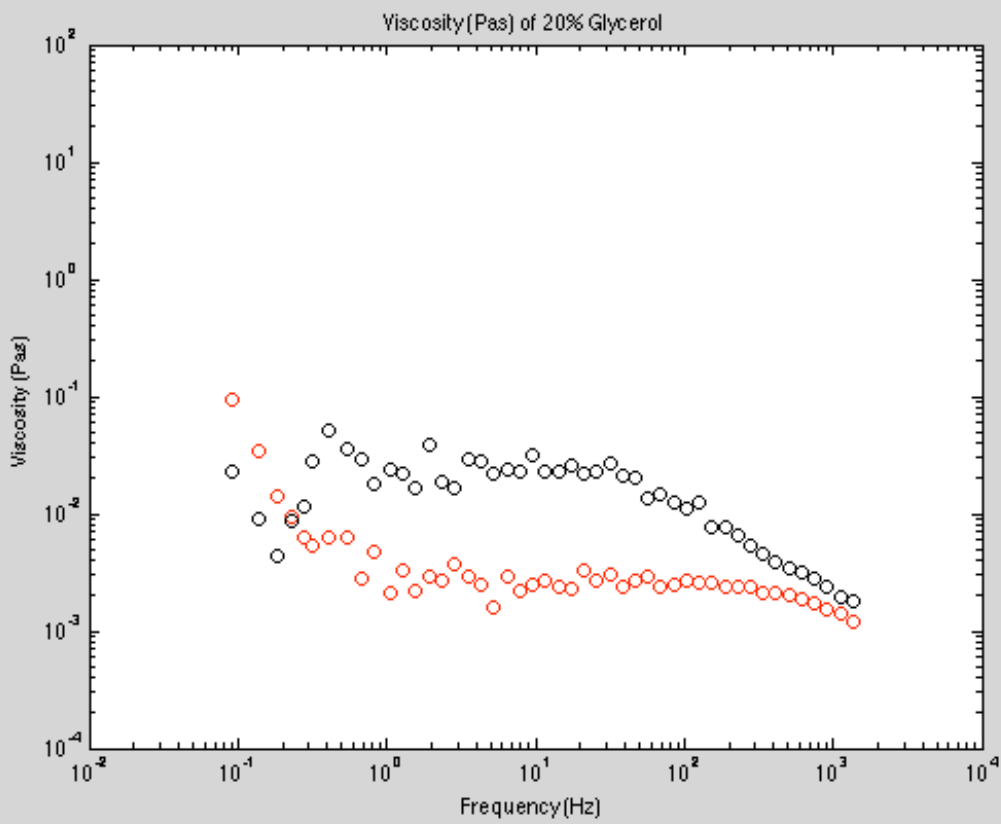
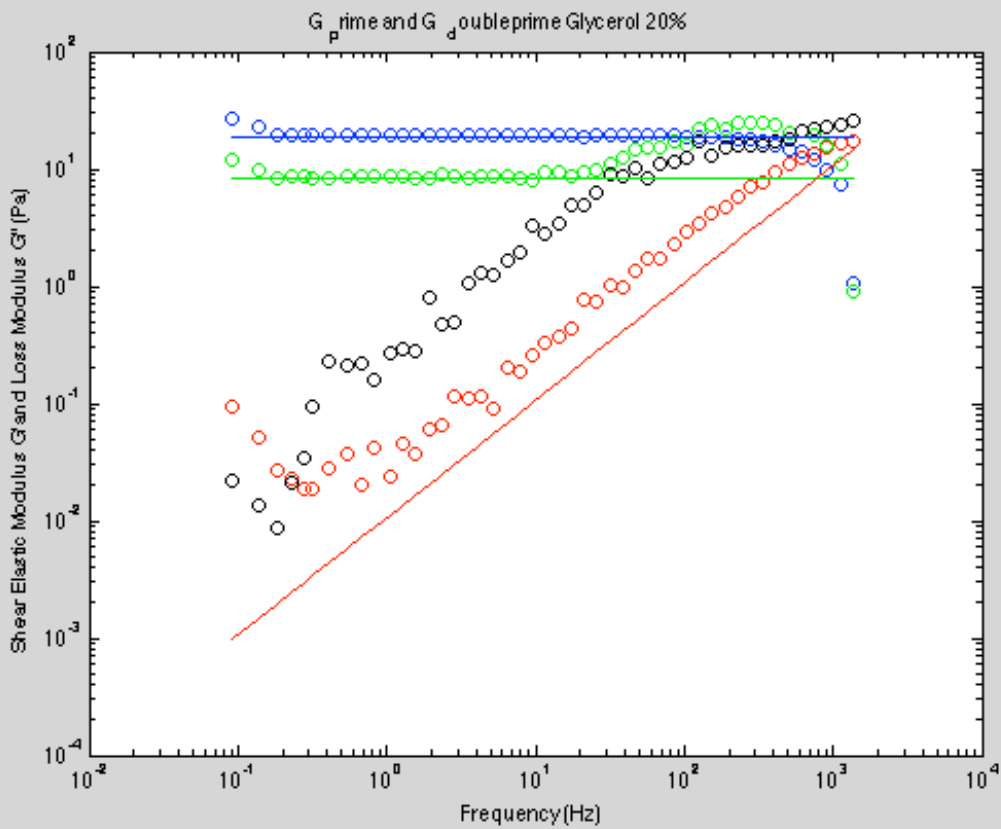
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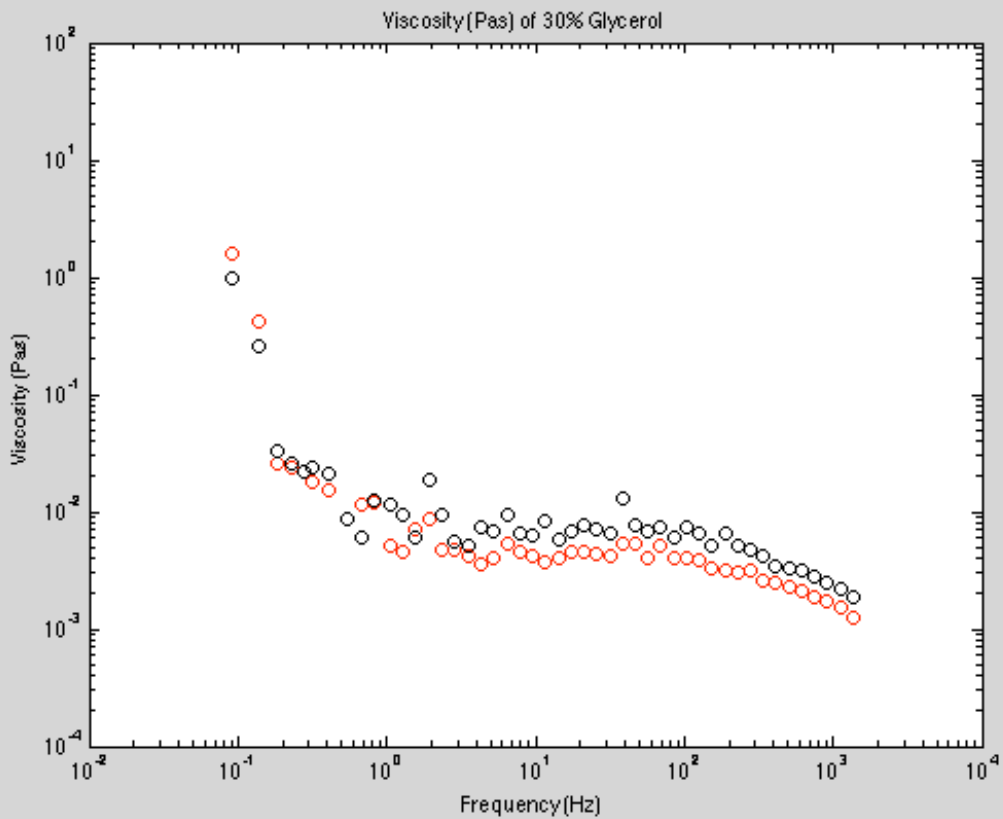
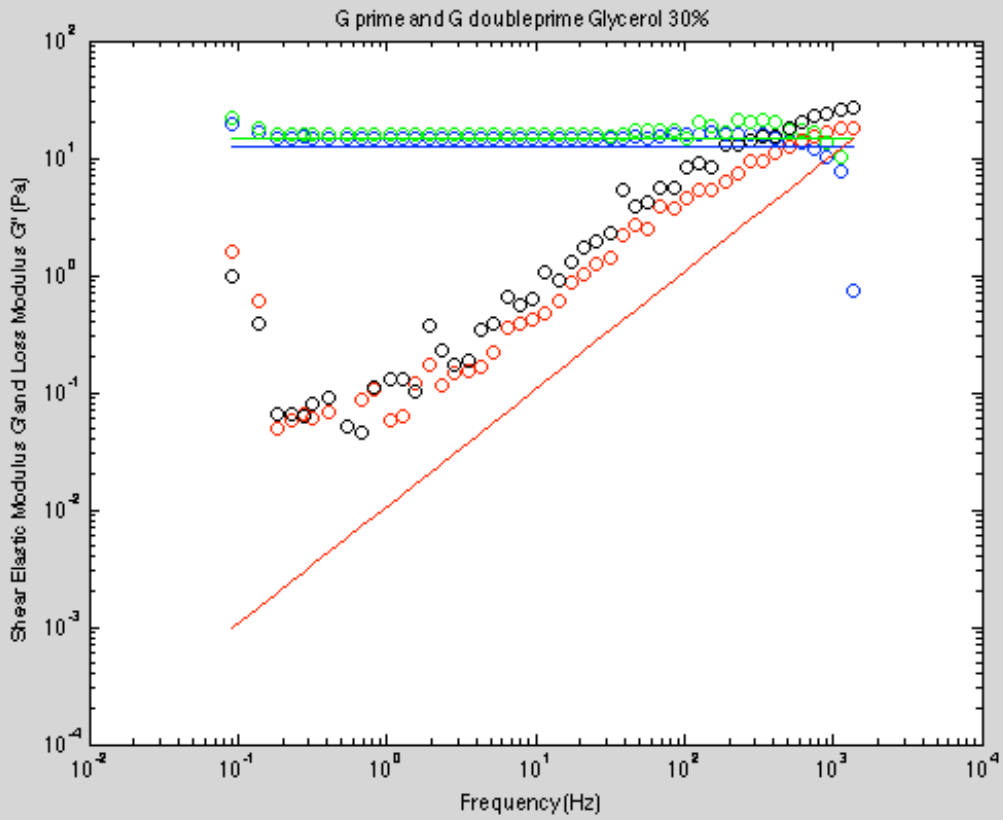
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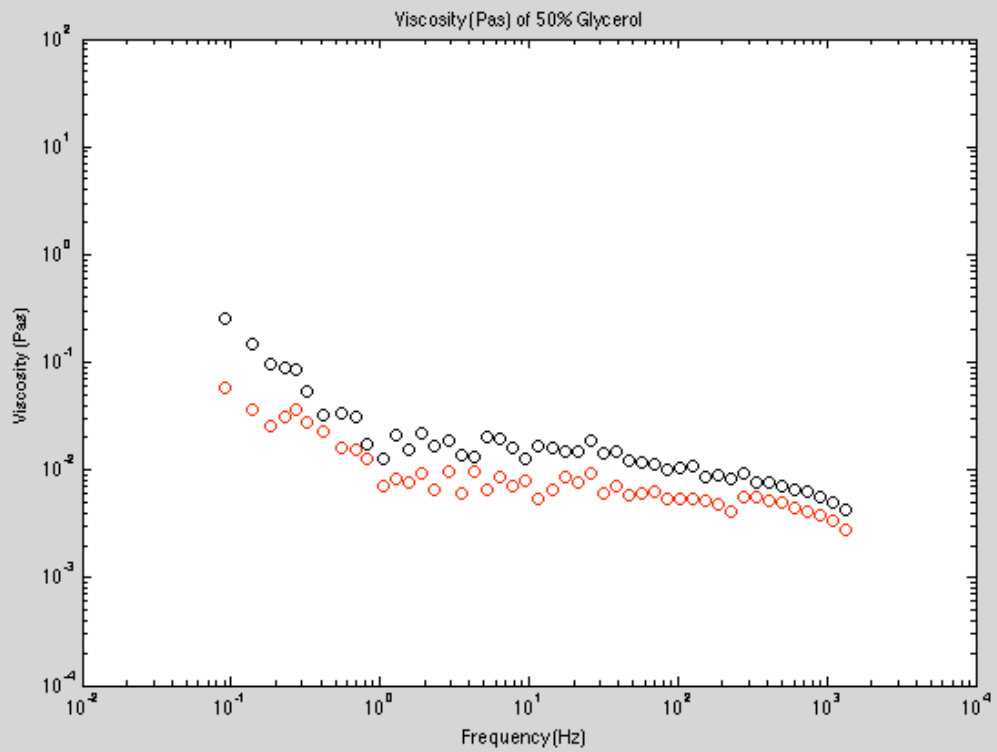
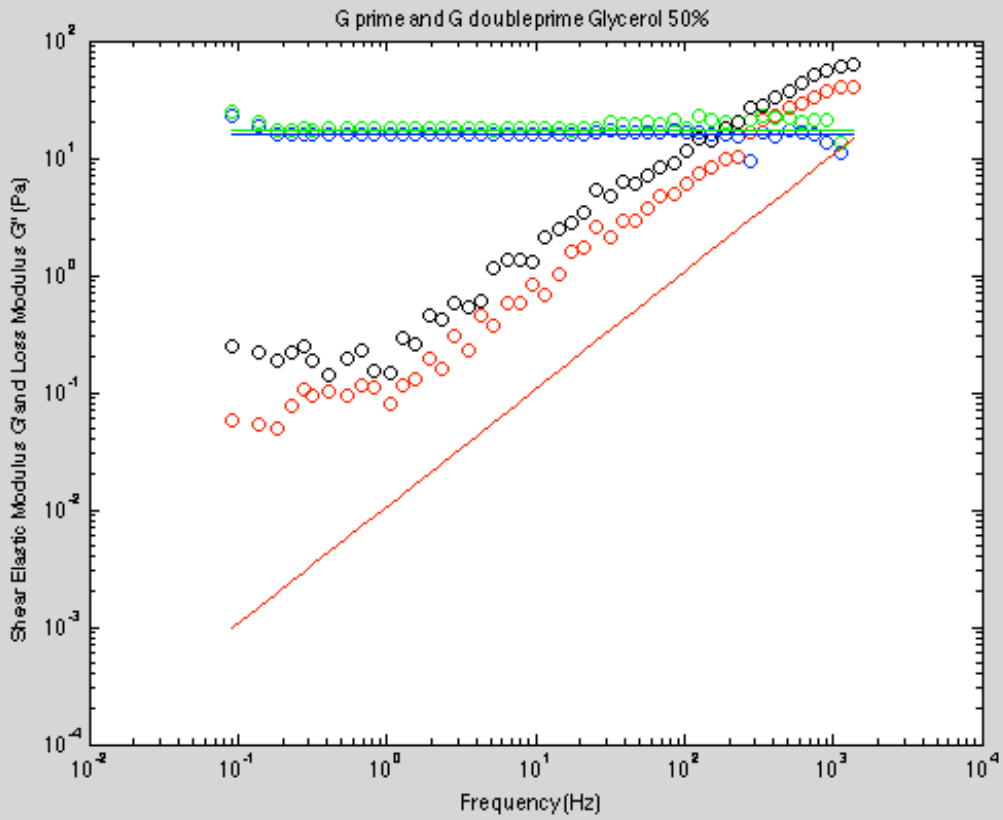


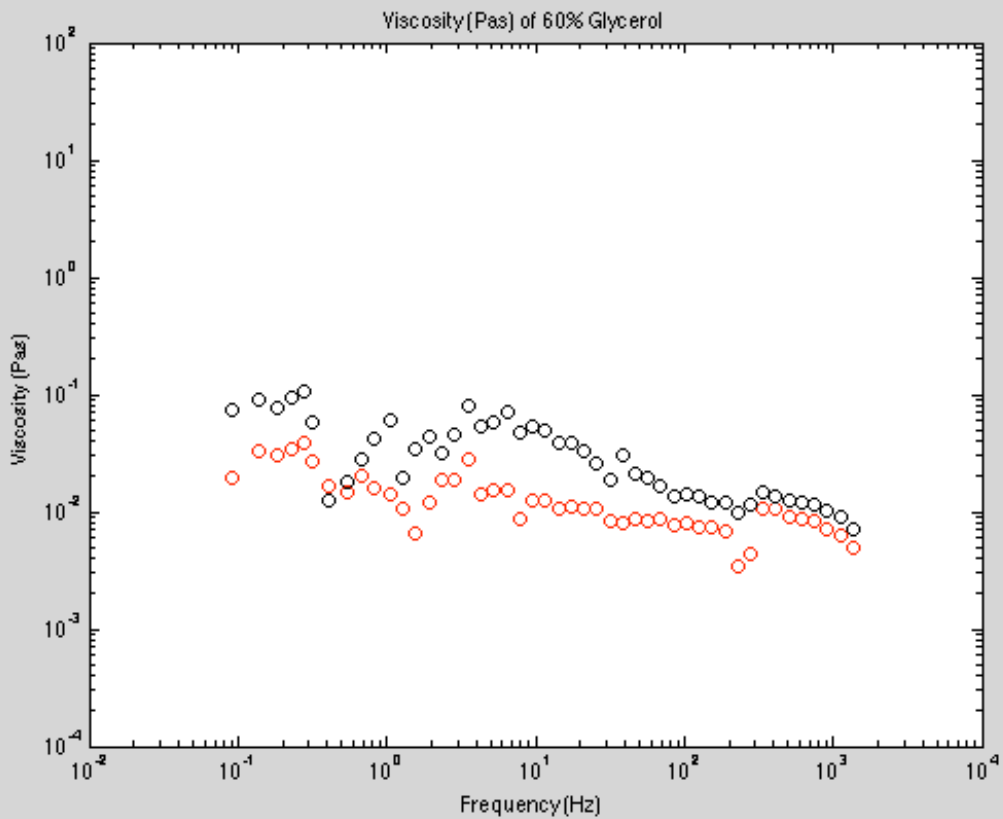
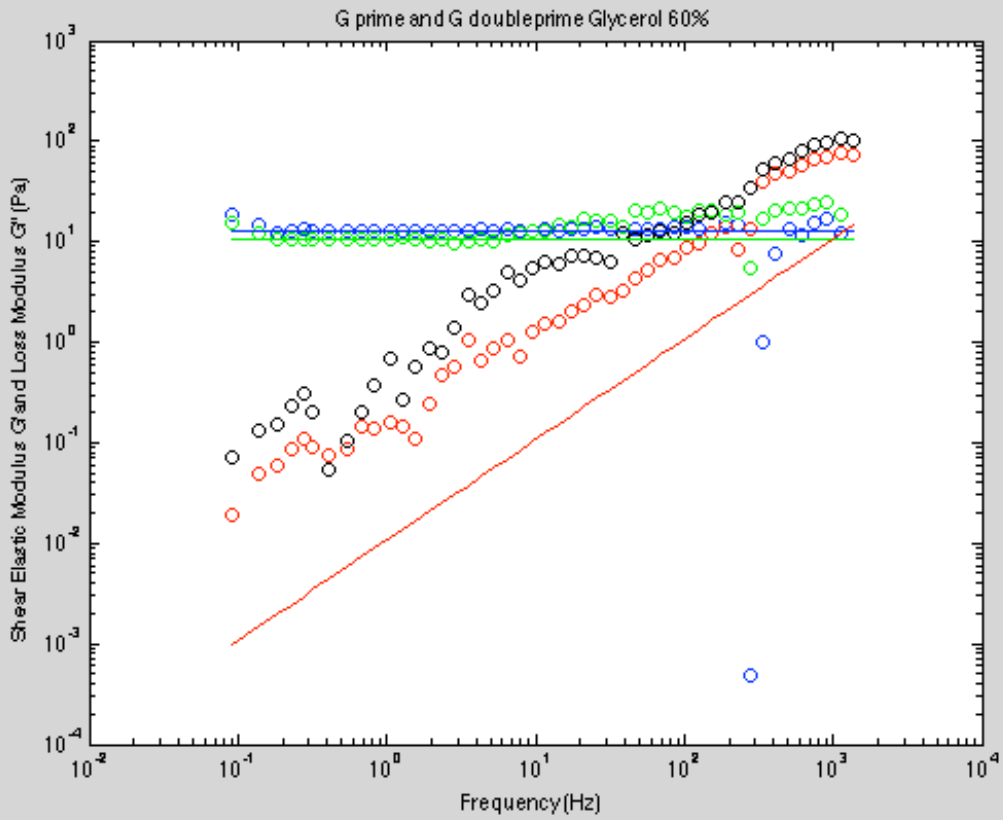
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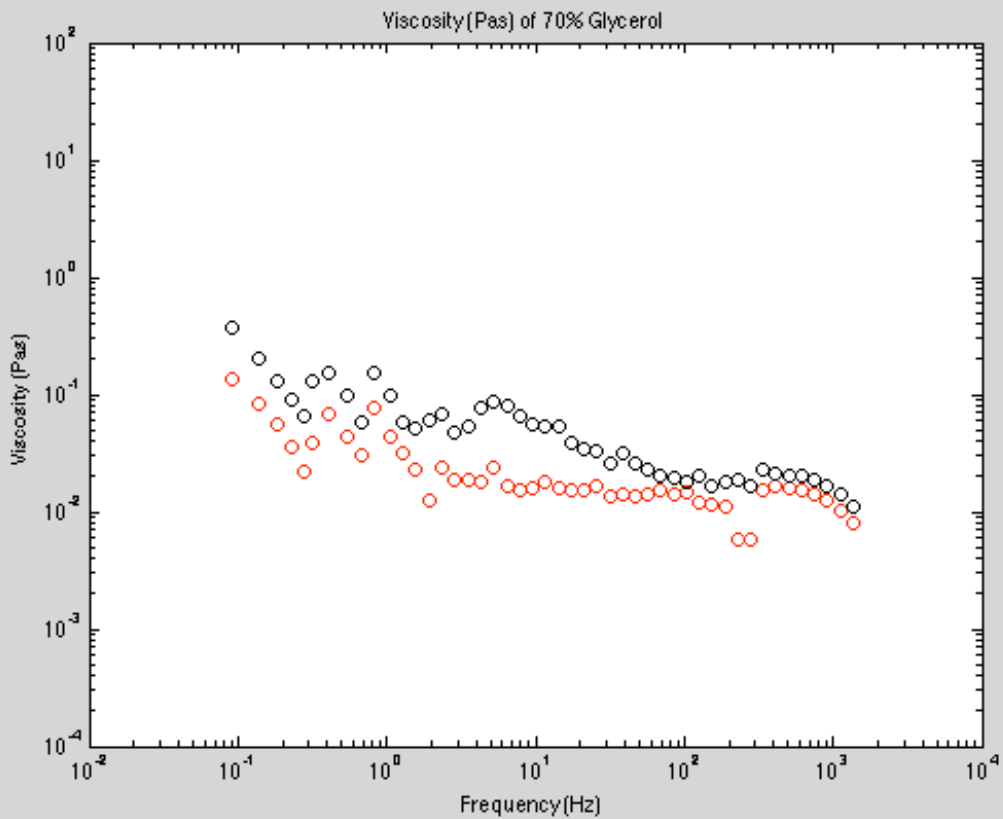
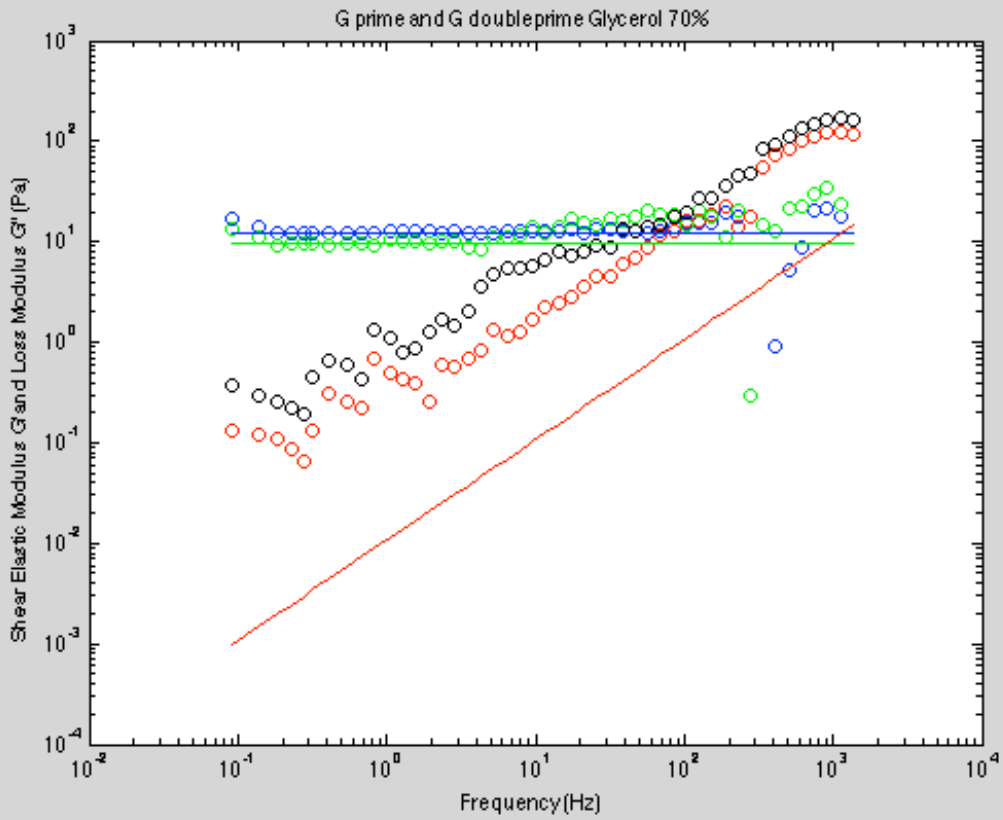
# **APPENDIX A: OPTICAL TRAP DATA**

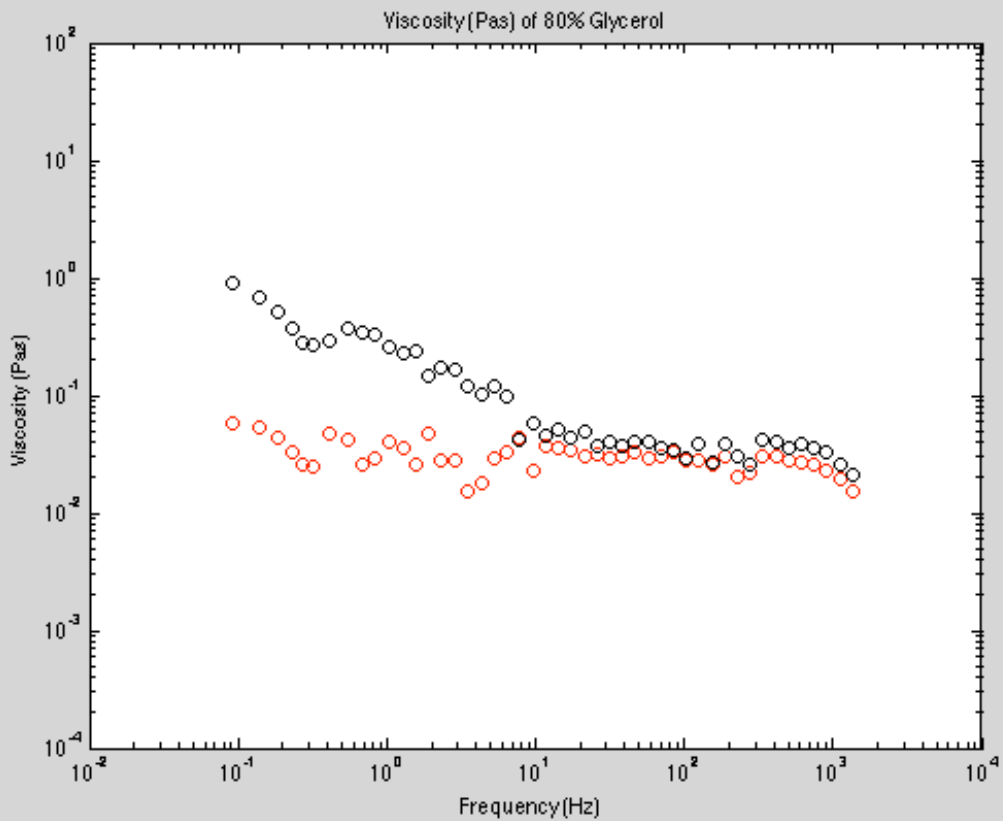
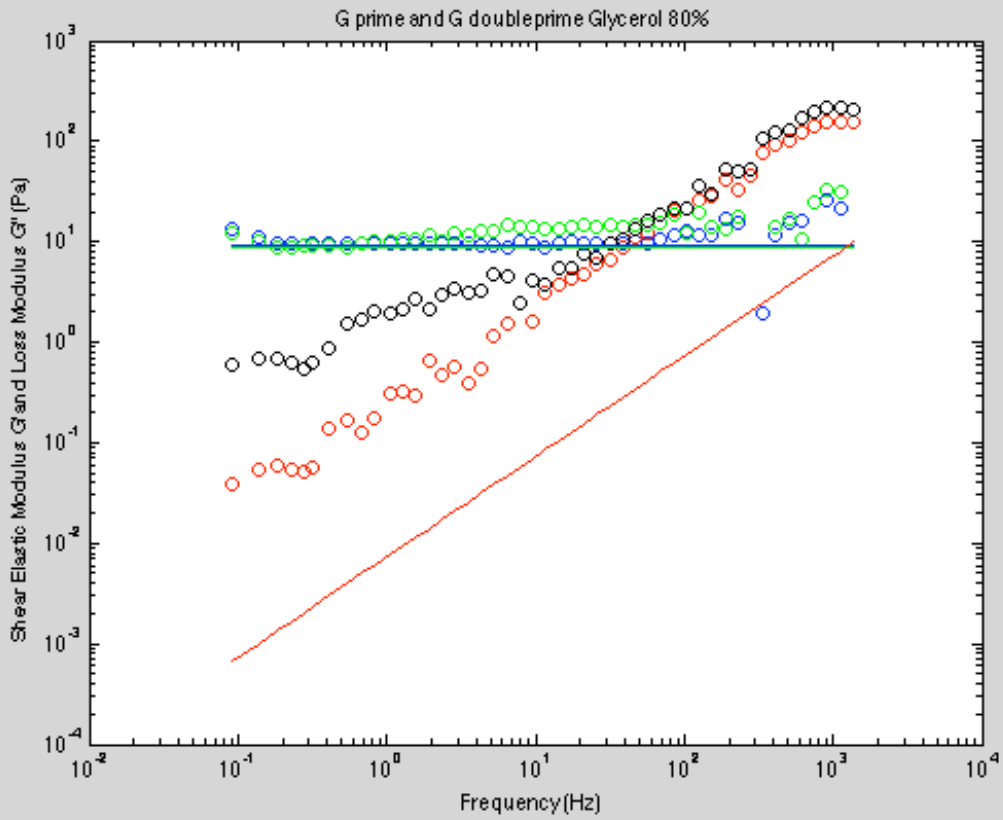




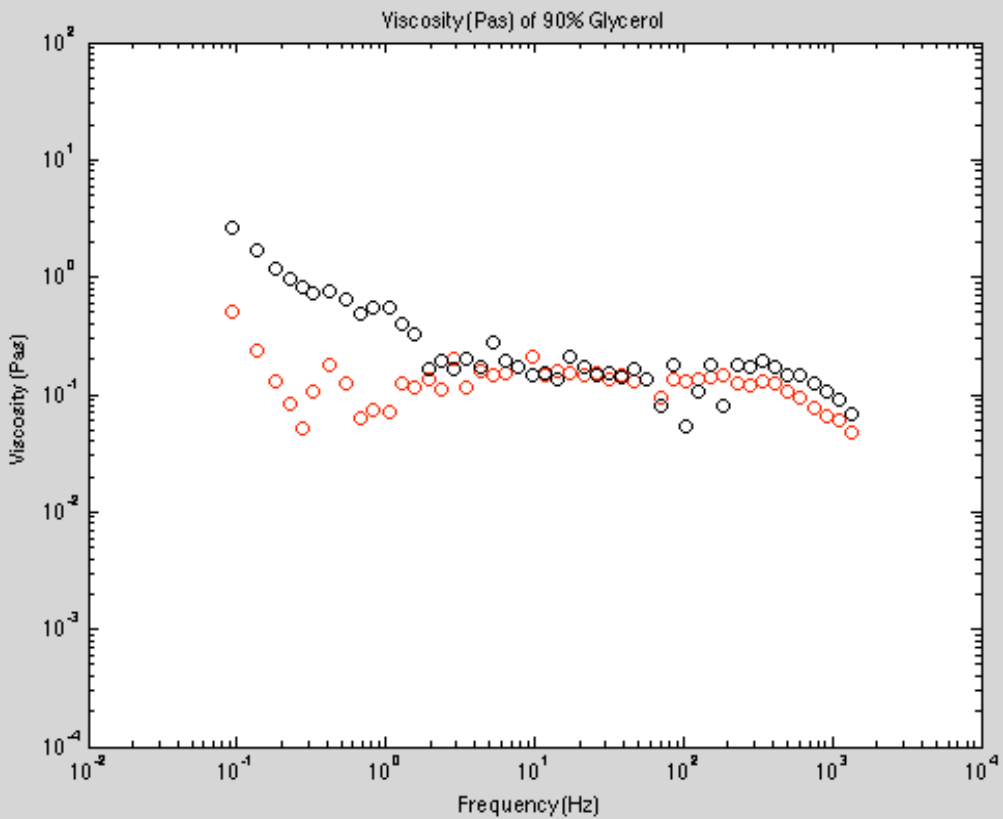
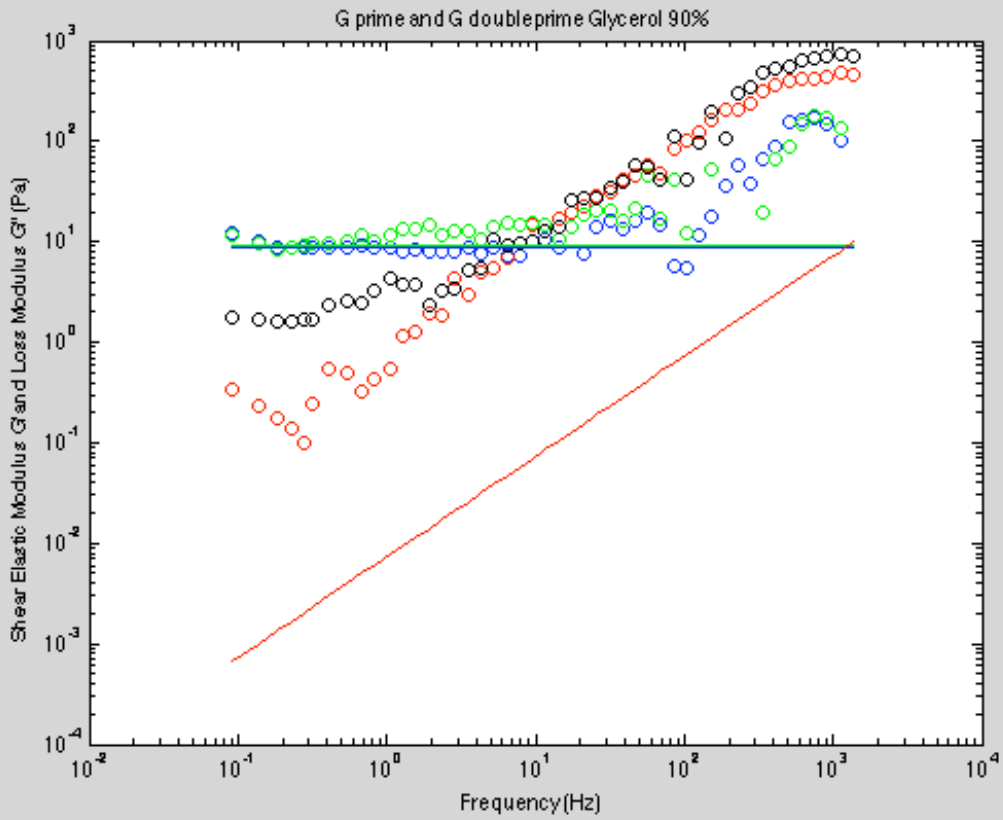


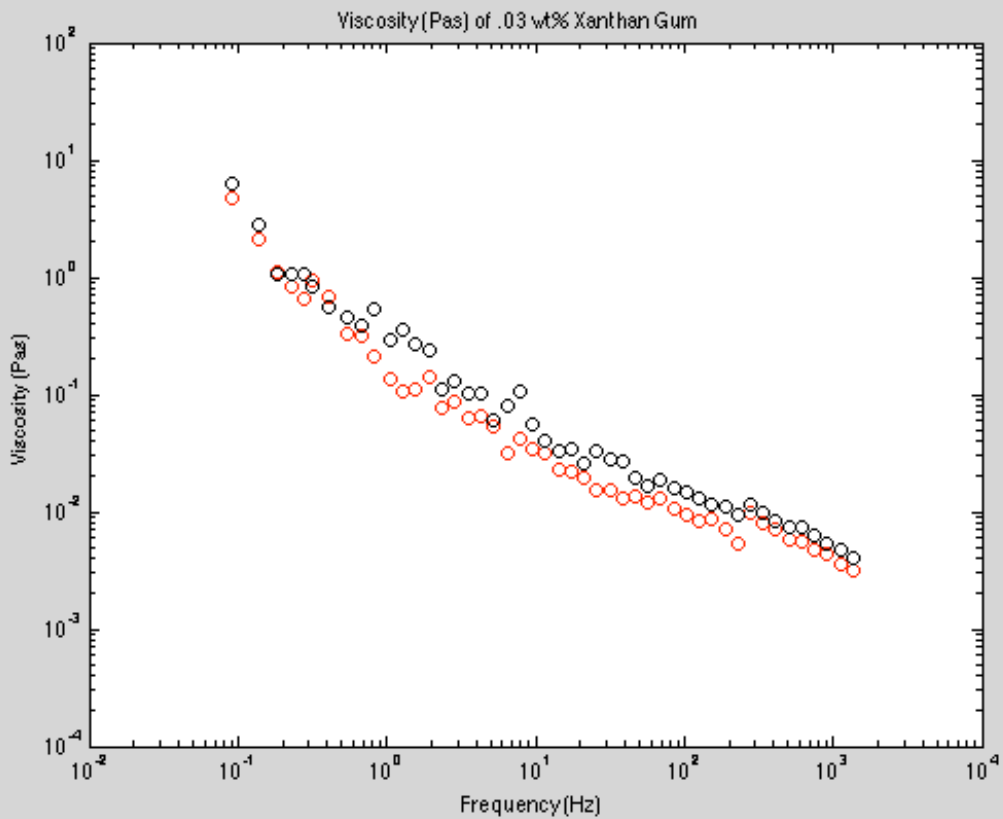
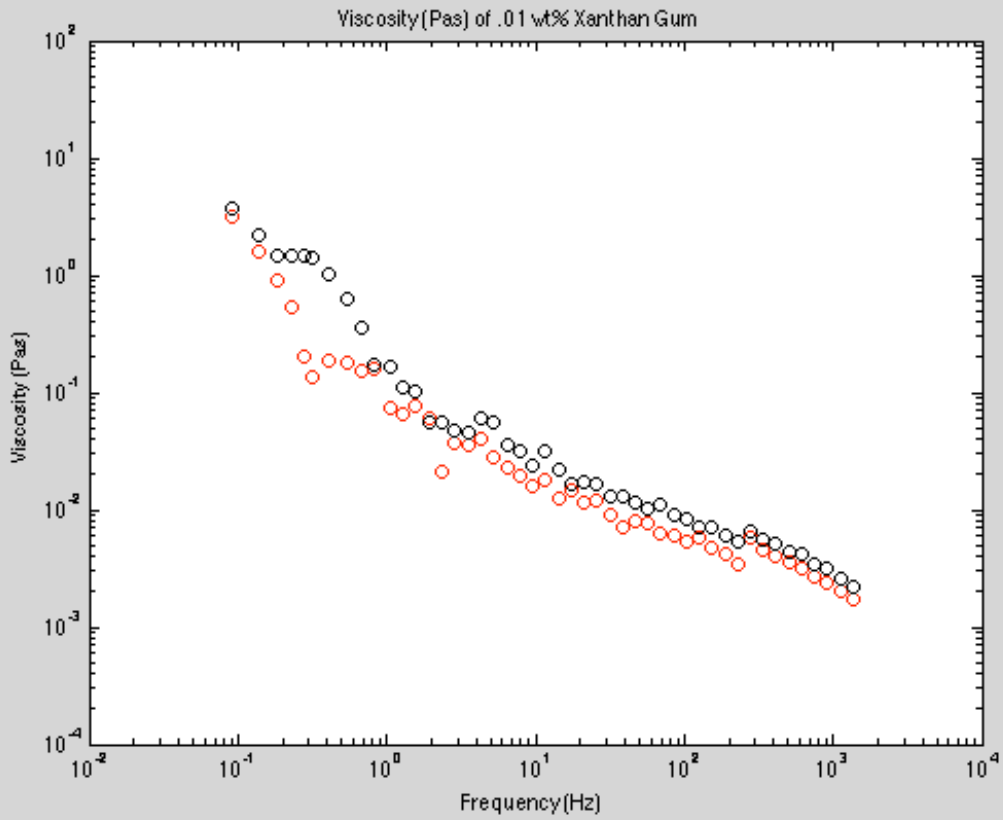


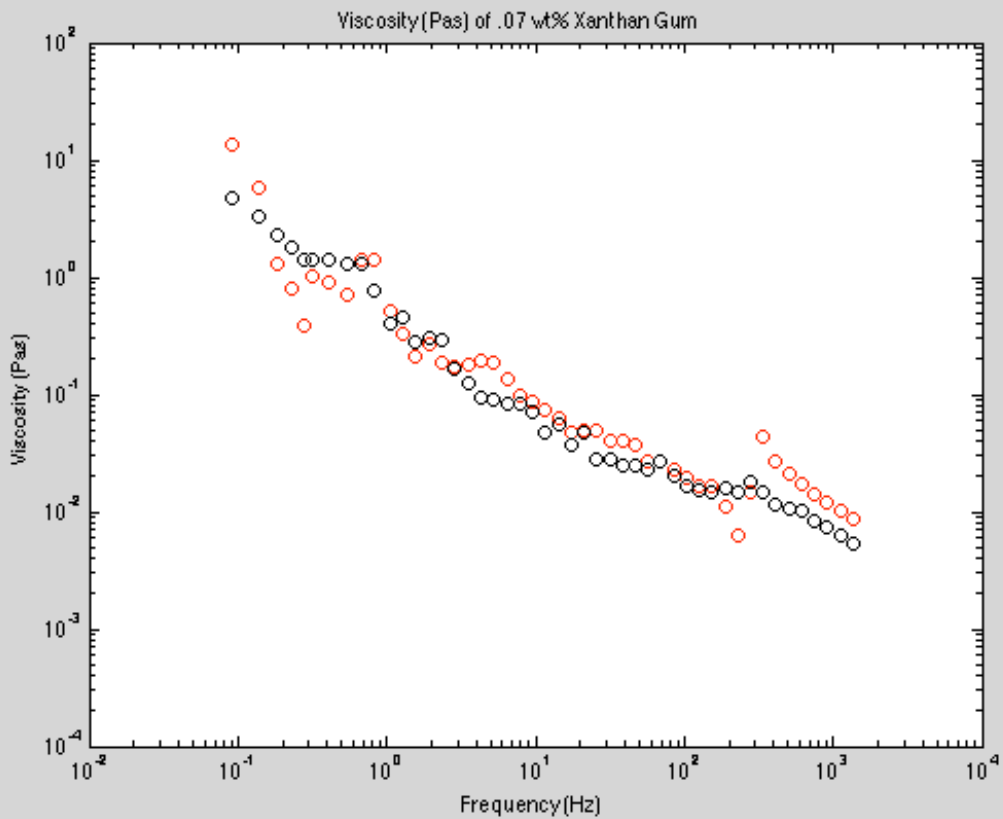
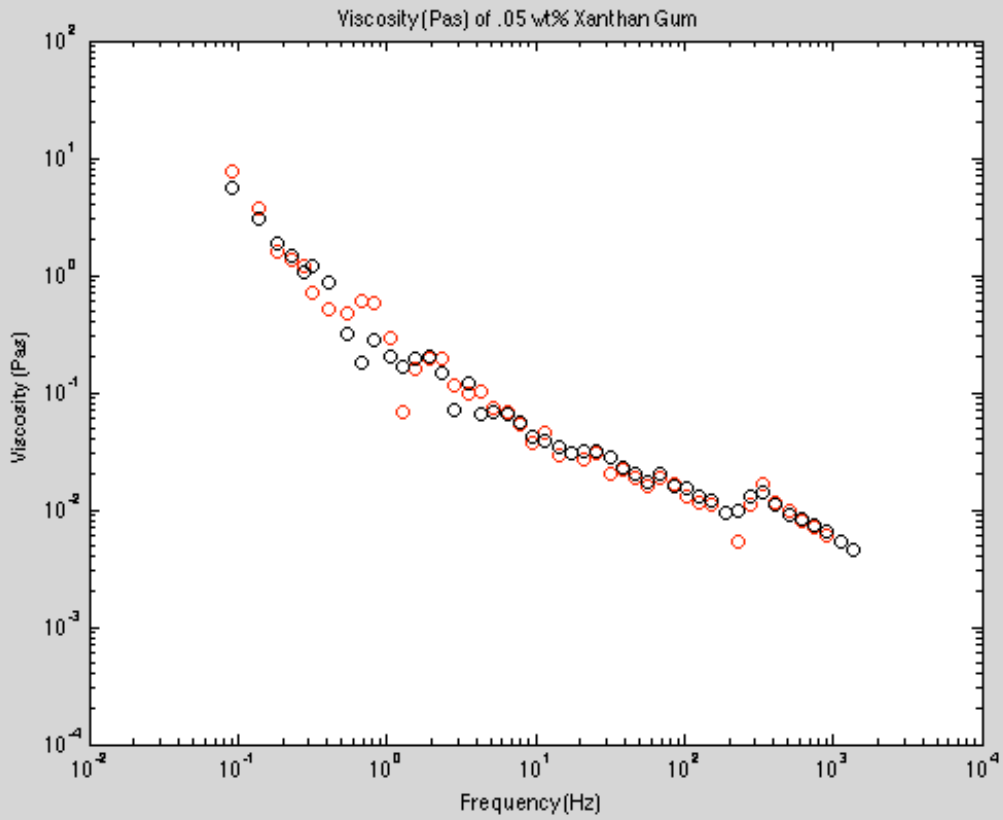


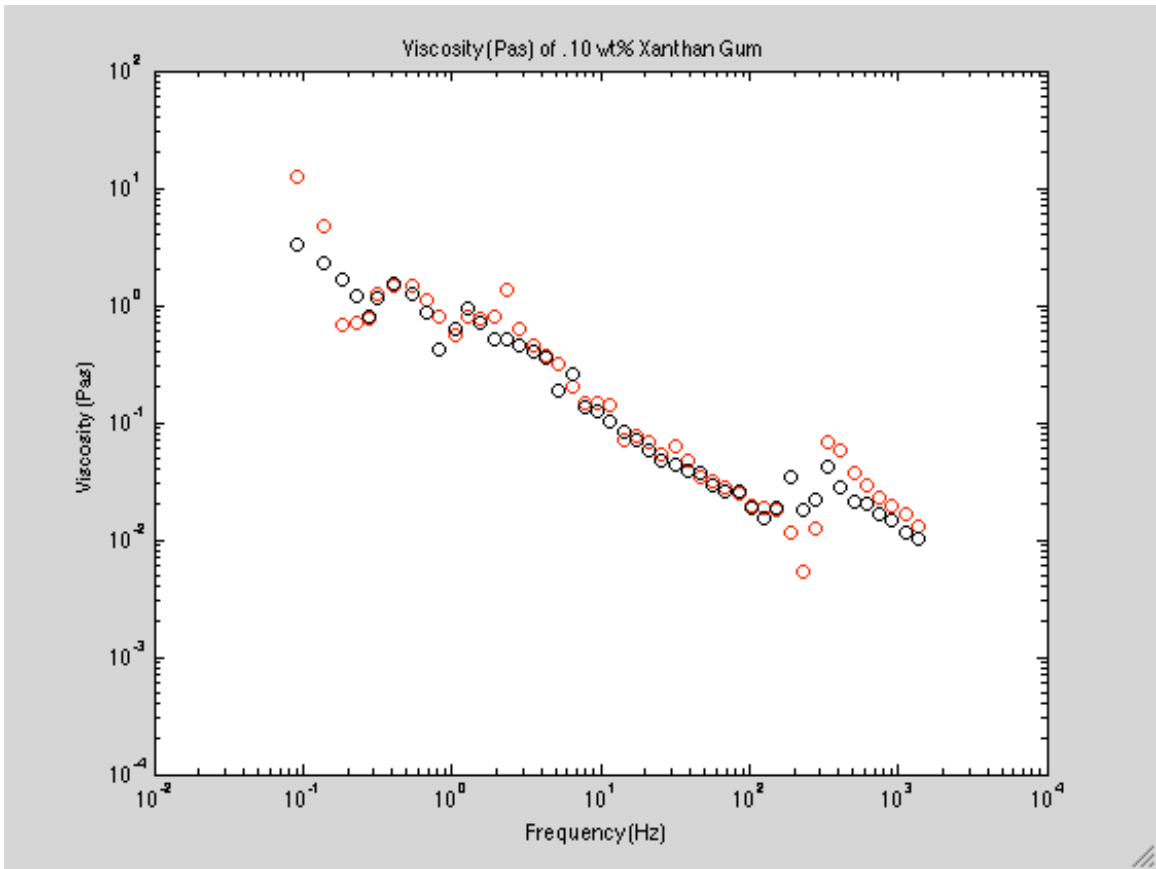












**APPENDIX B:  
RHEOLOGY CODE**

## Active Rheology Code

```
%Modified from Jorge Ferrer's code
% This program converts a stream of voltage data collected at a
specified
% sampling frequency (fs) to position using a 5th order
calibration file.
% The power spectral density of the position is obtained using
the command
% pwelch. Then, the PSD is fit to a Lorentzian to obtain the
corner
% frequency and determine the trap stiffness. The trap stiffness
is also
% obtained from the variance method. .From the PSD, it obtains
the complex
% modulus  $G = G' + iG''$  according to Addas et al (Phys Rev,
v70,2004).
% It also uses zero padding before the sine-cosine transforms to
obtain the
% compliance  $\alpha'$ . The data is averaged using geometric series
binning
% before obtaining the modulus G.
clear all
close all
clc
% ----- Parameters-----
----
fs=3000; %[Hz], sampling frequency, used in PSD (pwelch)
kb=1.3806503E-23; %[J/K], Boltzmann constant
T=300; %[K], temperature
a=500E-9; %[m], bead radius
mu=0.001; %[Pa*sec], viscosity of water
h = .5E-6; %[m], distance from surface to center of bead
denom = 1 - ((9/16)*(a/h)) + ((1/8)*((a/h)^3)) -
((45/256)*((a/h)^4)) -((1/16)*((a/h)^5));%correction factor
mu=mu/denom;%Height correction (Faxens Law)
beta = 6*pi*mu*a;%Drag coefficient corrected
% ----- Load file with voltage data-----
----
[calibration,pathname] = uigetfile('*.txt','Select the
CalibrationCoefficient file');
cal=load(calibration); %V to AOD Space Calibration Parameters
calx=cal(:,1);caly=cal(:,2);
[stiffness,pathname] = uigetfile('*.txt','Select the Stiffness
file');
stiff=load(stiffness); %V to AOD Space Calibration Parameters
xstif=stiff(1,1);
[voltdata,pathname] = uigetfile('*.txt','Select the data file');
```

```

trace=load(voltdata);
N=2^(floor(log2(length(trace))));%Determine the highest power of
2 corresponding to number of data points
Vx=trace(1:N,1);%Truncate number of data points to highest power
of 2 possible (N)
Vy=trace(1:N,2);
Vz=trace(1:N,3);
%%
clear trace cal pathname calibration
% ----- Convert voltage data to position-----
-----
% AOD 5th order
AODtonmx=1148.1*10^-9; %[m/MHz]
AODtonmy=1041.1*10^-9; %[m/MHz]
nmx=
AODtonmx*(calx(1)+calx(2)*Vx+calx(3)*Vy+calx(4)*Vx.^2+calx(5)*Vy.
^2+calx(6)*Vx.^3+calx(7)*Vy.^3+calx(8)*Vx.^4+calx(9)*Vy.^4+calx(1
0)*Vx.^5+calx(11)*Vy.^5+calx(12)*Vx.*Vy+calx(13)*Vx.^2.*Vy+calx(1
4)*Vx.*Vy.^2+calx(15)*Vx.^3.*Vy+calx(16)*Vx.^2.*Vy.^2+calx(17)*Vx
.*Vy.^3+calx(18)*Vx.^4.*Vy+calx(19)*Vx.^3.*Vy.^2
+calx(20)*Vx.^2.*Vy.^3+calx(21)*Vx.*Vy.^4);
nmx=nmx-mean(nmx); % [m]
nmy=
AODtonmy*(caly(1)+caly(2)*Vx+caly(3)*Vy+caly(4)*Vx.^2+caly(5)*Vy.
^2 +caly(6)*Vx.^3
+caly(7)*Vy.^3+caly(8)*Vx.^4+caly(9)*Vy.^4+caly(10)*Vx.^5+caly(11
)*Vy.^5
+caly(12)*Vx.*Vy+caly(13)*Vx.^2.*Vy+caly(14)*Vx.*Vy.^2+caly(15)*V
x.^3.*Vy+caly(16)*Vx.^2.*Vy.^2+caly(17)*Vx.*Vy.^3+caly(18)*Vx.^4.
*Vy+caly(19)*Vx.^3.*Vy.^2+caly(20)*Vx.^2.*Vy.^3+caly(21)*Vx.*Vy.^
4);
nmy=nmy-mean(nmy); %[m]
Nmz=nmz*10^9; %nm_bead

Nmz=110*(Vz-mean(Vz)); %nm_trap

%Sine Wave trap
mdl = @(a,x)(a(1)*sin(a(2)*x+a(3)));
%a = [1;3;2];
x=1:length(Nmx);
y = Nmz.';
a0 = [max(y);.002513;-1];
[ahat,r,J,cov,mse] = nlinfit(x,y,mdl,a0);
clear x y

%Sine wave response
x=1:length(Nmx);

```

```

y=Nmx.';
a0 = [max(y);.002513;-1];
[bhat,r,J,cov,mse] = nlinfit(x,y,mdl,a0);
clear x y

%Delay Measurement
delay=abs(bhat(3))-abs(ahat(3))

%G" = k/6pir*sin(delta_theta) in CentiPoise
Gdoubleprime=xstif/(6*pi*a)*sin(delay)*10^0
Coeff=xstif/(6*pi*a)*10^0;

%G' = k/6pir*cos(delta_theta) in CentiPoise
Gprime=xstif/(6*pi*a)*10^0*cos(delay)-xstif/(6*pi*a)*10^0

%mu=[(G'^2+G"'^2)^.5]/w
ViscosityScope=1/1*(Gdoubleprime^2+Gprime^2)^.5;
Viscosity=ViscosityScope*denom

```



## Passive Rheology Code

```
%Modified from Jorge Ferrer
%
% This program converts a stream of voltage data collected at a
specified
% sampling frequency (fs) to position using a 5th order
calibration file.
% The power spectral density of the position is obtained using
the command
% pwelch. Then, the PSD is fit to a Lorentzian to obtain the
corner
% frequency and determine the trap stiffness. The trap stiffness
is also
% obtained from the variance method. From the PSD, it obtains the
complex
% modulus  $G = G' + iG''$  according to Addas et al (Phys Rev,
v70,2004).
% It also uses zero padding before the sine-cosine transforms to
obtain the
% compliance alpha'. The data is averaged using geometric series
binning
% before obtaining the modulus G.
clear all
close all
clc
% ----- Parameters-----
----
fs=3000; %[Hz], sampling frequency, used in PSD (pwelch)
kb=1.3806503E-23; %[J/K], Boltzmann constant
T=300; %[K], temperature
a=500E-9; %[m], bead radius
mu=0.001; %[Pa*sec], viscosity of fluid
h = .45E-6; %[m], distance from surface to center of bead
denom = 1 - ((9/16)*(a/h)) + ((1/8)*((a/h)^3)) -
((45/256)*((a/h)^4)) -((1/16)*((a/h)^5));%correction factor
mu=mu/denom;%Height correction (Faxens Law)
beta = 6*pi*mu*a;%Drag coefficient corrected
% ----- Load file with voltage data-----
----
[calibration,pathname] = uigetfile('*.txt','Select the
CalibrationCoefficient file');
cal=load(calibration); %V to AOD Space Calibration Parameters
calx=cal(:,1);caly=cal(:,2);
[voltdata,pathname] = uigetfile('*.txt','Select the data file');
trace=load(voltdata);
N=2^(floor(log2(length(trace))));%Determine the highest power of
2 corresponding to number of data points
```

```

Vx=trace(1:N,1);%Truncate number of data points to highest power
of 2 possible (N)
Vy=trace(1:N,2);

%%
clear trace cal pathname calibration
% ----- Convert voltage data to position-----
-----
% AOD 5th order
AODtonmx=1148.1*10^-9; %[m/MHz]
AODtonmy=1041.1*10^-9; %[m/MHz]
nmx=
AODtonmx*(calx(1)+calx(2)*Vx+calx(3)*Vy+calx(4)*Vx.^2+calx(5)*Vy.
.^2+calx(6)*Vx.^3+calx(7)*Vy.^3+calx(8)*Vx.^4+calx(9)*Vy.^4+calx(1
0)*Vx.^5+calx(11)*Vy.^5+calx(12)*Vx.*Vy+calx(13)*Vx.^2.*Vy+calx(1
4)*Vx.*Vy.^2+calx(15)*Vx.^3.*Vy+calx(16)*Vx.^2.*Vy.^2+calx(17)*Vx
.*Vy.^3+calx(18)*Vx.^4.*Vy+calx(19)*Vx.^3.*Vy.^2
+calx(20)*Vx.^2.*Vy.^3+calx(21)*Vx.*Vy.^4);
nmx=nmx-mean(nmx); % [m]
nmy=
AODtonmy*(caly(1)+caly(2)*Vx+caly(3)*Vy+caly(4)*Vx.^2+caly(5)*Vy.
.^2 +caly(6)*Vx.^3
+caly(7)*Vy.^3+caly(8)*Vx.^4+caly(9)*Vy.^4+caly(10)*Vx.^5+caly(11
)*Vy.^5
+caly(12)*Vx.*Vy+caly(13)*Vx.^2.*Vy+caly(14)*Vx.*Vy.^2+caly(15)*V
x.^3.*Vy+caly(16)*Vx.^2.*Vy.^2+caly(17)*Vx.*Vy.^3+caly(18)*Vx.^4.
*Vy+caly(19)*Vx.^3.*Vy.^2+caly(20)*Vx.^2.*Vy.^3+caly(21)*Vx.*Vy.^
4);
nmy=nmy-mean(nmy); %[m]
Nmx=nmx*10^9;

% Rotate axis

% psdx=(nmx-nmy)*sin(pi/4); %[m]
% psdy=(nmx+nmy)*sin(pi/4); %[m]
% kxAOD=(kb*T/var(nmx));
% kyAOD=(kb*T/var(nmy));
clear AODtonmx AODtonmy
% ----- Trap stiffness from variance-----
-----
kxvar=(kb*T/var(nmx)); %[N/m]
kyvar=(kb*T/var(nmy)); % [N/m]
% ----- Power spectrum density of data-----
-----
n=log2(N)-1;%window data to 2^n points
[Pxx,freqx]=pwelch(nmx,2^n,[],[],fs);% Pxx [=] m^2/Hz; freqx [=]
Hz
[Pyy,freqy]=pwelch(nmy,2^n,[],[],fs);% Pyy [=] m^2/Hz; freqy [=]

```

```

Hz
%clear trace n fs h psdx psdy Vx Vy N nmx nmy
% ----- Cutoff frequencies-----
-----
flower=0.01; % [Hz]
fupper=1490; %[Hz]
% Find the index for the cutoff frequency
l=1;
while freqx(l) < fupper,
l=l+1;
end
l=l-1;
m=1;
while freqx(m) < flower,
m=m+1;
end
Pxx=Pxx(m:l); %[m^2/Hz]
freqx=freqx(m:l); %[Hz]
Pyy=Pyy(m:l); %[m^2/Hz]
freqy=freqy(m:l); %[Hz]
clear l m flower fupper N
% -----Fit the voltage data to Lorentzian-----
-----
% IG = [kb*T/(kxvar*pi^2) kxvar/(2*pi*beta)];%Initial guesses for
SO and corner frequency (f0)in PSD-x
% fitx = nlinfit(freqx,Pxx,@lorentzian_call,IG);
% fity = nlinfit(freqy,Pyy,@lorentzian_call,IG);
% SOxfit=fitx(1);%Initial value of PSD
% f0xfit=abs(fitx(2));%corner frequency in PSD-x
% SOyfit=fity(1);%Initial value of PSD
% f0yfit=abs(fity(2));%corner frequency in PSD-y
% clear fitx fity IG
% ----- Generate Lorentzian curve with fit parameters-----
-----
% Pxxfit=(SOxfit)*(f0xfit^2)./((f0xfit^2)+(freqx.^ 2 ));
% Pyyfit=(SOyfit)*(f0yfit^2)./((f0yfit ^ 2)+(freqy.^ 2 ));
% ----- Trap stiffness from corner frequency --
% kxfc=2*pi*beta*f0xfit; %[N/m]
% kyfc=2*pi*beta*f0yfit; %[N/m]
% ----- Plot the data PSD and the Lorentzian fit-----
-----
figure(1)
loglog(freqx,Pxx, 'b. ');
hold on;
loglog(freqy,Pyy, 'g. ');
xlabel('Frequency (Hz)')
ylabel('Power Spectral Density [m^2/Hz] ')
legend('Pxx', 'Pyy')

```

```

% ----- Get alpha' and alpha" according to Addas paper-----
-----
% alpha"
appx=(pi/(2*kb*T)).*freqx.*Pxx;%[m/N]
appy=(pi/(2*kb*T)).*freqy.*Pyy;%[m/N]
Nf=length(freqx);
% alpha' from the discrete cosine-sine transform with padding
pad=3; %Factor to multiply the number of points for padding
apx=sqrt(2)*dct(dst(appx,pad*Nf))/sqrt(pad*Nf);
apy=sqrt(2)*dct(dst(appy,pad*Nf))/sqrt(pad*Nf);
% Truncate apx to the same length of appx
k=length(apx);
apx=apx(1:k);
apy=apy(1:k);
clear k Nf pad
% ----- Complex compliance alpha-----
----
% alphax=apx+(j*appx);
% alphay=apy+(j*appy);
% ----- Complex modulus G-----
-----
% Glx=1./(6*pi*a*alphax);
% Gly=1./(6*pi*a*alphay);
% -----
-----
% Average PSD in bins of increasing width according to geometric
series
% Generate a vector of frequencies (of length Nf) from intial
freqx to last
% freqx in log space
Nf=50;%number of bins
fx=logspace(log10(freqx(1)),log10(freqx(length(freqx))),Nf);
% Determine bin with and parameters for geometric series
for q=1:length(fx)-1
bin(q)=fx(q+1)-fx(q);
end
clear q
a0=bin(1);% Initial bin width
ratio=bin(2)/bin(1);% ratio for geometric series
% Find the lower and upper indeces for each bin
sum(1)=0;
for k=1:length(bin)
sum(k+1)=a0*(ratio^(k-1)) + sum(k);
for m=1:length(freqx)
if freqx(m)<= freqx(1)+sum(k+1),
upper=m;
else
break

```

```

end
end
for q=1:length(freqx)
if freqx(q) > freqx(1) + sum(k)
break
else
lower=q;
end
end
flogx(k)=mean(freqx(lower:upper));%Average frequency (log space
bins)
Plogx(k)=mean(Pxx(lower:upper));
aplogx(k)=mean(apx(lower:upper));%Average alpha' (log space bins)
applogx(k)=mean(appx(lower:upper));%Average alpha" (log space
bins)
flogy(k)=mean(freqy(lower:upper));%Average frequency (log space
bins)
Plogy(k)=mean(Pyy(lower:upper));
aplogy(k)=mean(apy(lower:upper));%Average alpha' (log space bins)
applogy(k)=mean(appy(lower:upper));%Average alpha" (log space
bins)
end
clear bin k m q l upper lower fx Nf apx apy appx appy
figure(2)
loglog(flogx,Plogx, 'b. ');
hold on;
loglog(flogy,Plogy, 'r. ');
xlabel('Frequency (Hz)')
ylabel('Power Spectral Density [m^2/Hz] ')
legend('Plogx', 'Plogy')
% ----- Complex compliance (alpha) after average-----
-----
alphax=aplogx+applogx*j;
alphay=aplogy+applogy*j;
% ----- Complex modulus G after average-----
---
G2x=(6*pi*a*alphax).^-1;%[Pa]
G2y=(6*pi*a*alphay).^-1;%[Pa]
name=voltdata(1:length(voltdata)-3);
filename=['Results_',name, 'mat'];
save(filename, 'G2x', 'G2y', 'flogx', 'flogy', 'kxvar', 'kyvar', 'freqx'
, 'freqy', 'Pxx', 'Pyy', 'Plogx', 'Plogy')
clear filename Plogx Plogy alphax alphay f0xfit f0yfit Pxfit
Pyfit Pxx Pyy freqx freqy voltdata
% ----- Theoretical G' for trap + water only-----
-----
for s=1:length(flogx)
Gpthx(s)=kxvar/(6*pi*a);

```

```

Gpthy(s)=kyvar/(6*pi*a);
end
clear s
% ----- Theoretical G"-----
----
Gppth=2*pi*mu*flogx;
% ----- Theoretical alpha' for trap + water solution-----
----
% aptx=(kxvar/(12*(pi^2)*a*mu))*applogx.*(1./flogx);
% alpha2x=aptx+applogx*j;%[m/N]
% apty=(kyvar/(12*(pi^2)*a*mu))*applogy.*(1./flogy);
% alpha2y=apty+applogy*j;
%clear applogx applogy kxvar kyvar aptx apty
% ----- Plot G' and G" for all conditions (data and
theoretical)-----
figure(3)
loglog(flogx,real(G2x), 'bo',flogx,imag(G2x)*-1, 'ro')
hold on;
loglog(flogy,real(G2y), 'go',flogy,imag(G2y)*-1, 'ko')
loglog(flogx,Gpthx, 'b')
loglog(flogy,Gpthy, 'g')
loglog(flogx,Gppth, 'r')
xlabel('Frequency (Hz)')
ylabel('Shear Elastic Modulus G' and Loss Modulus G" (Pa)')
% legend('G' x', 'G" x', 'G' y', 'G" y','G"x theory','G"y
theory','G" theory')

for n=1:length(flogx)
    x(n)=flogx(n);
    y(n)=denom*real(G2x(n))/(2*pi*flogx(n));
    z(n)=denom*imag(G2x(n))*-1/(2*pi*flogx(n));
    a(n)=flogy(n);
    b(n)=denom*real(G2y(n))/(2*pi*flogy(n));
    c(n)=denom*imag(G2y(n))*-1/(2*pi*flogy(n));
end

figure(4)
loglog(x,y, 'bo',x,z, 'ro')
hold on;
loglog(a,b, 'go',a,c, 'ko')
loglog(flogx,Gpthx, 'b')
loglog(flogy,Gpthy, 'g')
loglog(flogx,Gppth, 'r')
xlabel('Frequency (Hz)')
ylabel('Shear Elastic Modulus G' and Loss Modulus G" (Pas)')

```

## **APPENDIX C: PROTOCOLS**

## **Lang Lab Protocol for Etching Glass Coverslips**

Adapted from Polly Fordyce Etching coverslips removes the wax layer on the glass, enhances adhesion, and reduces back-ground fluorescence.

- Materials

- a) 100 g KOH
- b) Ethanol
- c) MilliQ DI H<sub>2</sub>O
- d) Corning Coverglass (22 x 40 mm) No. 1.5

- Procedure

1. Dissolve 100g of KOH in 300 mL of Ethanol (takes about 30 minutes) in a 1 L beaker
2. Load teflon coverslip racks with coverglass
3. Fill 2 more 1 L beakers with 300 mL DI H<sub>2</sub>O and degas for 5 minutes
4. Fill one more 1 L beaker with 300 mL ethanol
  - (a) Degas this beaker and the ethanol/KOH beaker for 5 minutes
5. Submerge one coverslip rack in the KOH/ethanol solution and sonicate for 5 minutes
6. Wash coverslips by dipping the rack up and down and spinning it in the ethanol beaker
7. Wash coverslips by dipping the rack up and down and spinning it in the DI H<sub>2</sub>O beaker
8. Submerge coverslip rack in the second DI H<sub>2</sub>O beaker and sonicate for 5 minutes
9. Spritz coverslips with DI H<sub>2</sub>O bottle - use lots of H<sub>2</sub>O
10. Spritz coverslips with ethanol bottle - use lots of ethanol
11. Repeat steps 5-10 with each rack of coverslips
12. Dry all coverslips in the oven for at least 15 minutes
13. Store coverslips in racks inside sealed plastic nalgene containers



This protocol outlines the construction of the ubiquitous glass flow cell used for optical trap assays. A flow cell will have a volume between 10 and 15  $\mu\text{l}$  if constructed with a 4-5 mm gap.

- Materials

1. Etched coverslips A.2
2. Double sided tape (Cat # 909955, Office Depot)
3. Glass slides (Cat # 48312-068, VWR)

- Construction procedure

1. Place two pieces of tape along the short axis of the glass slides with approximately 5 mm separating them (the channel width). Remove all overhanging tape.
2. Place an etched coverslip on top of the two pieces of tape. Align the coverslip so that the long axis of the coverslip is perpendicular to the long axis of the glass slide.
3. Use a q-tip or eppendorf tube to gently press the slides together to seal the tape.
4. Initial loading of the flow cell will occur by capillary action

- Exchanging flow cell contents

#### A.4

1. Open the vacuum supply line
2. Place the liquid to load on one side of the coverslip in contact with the flow chamber
3. Place the vacuum line nozzle on the other side of the flow chamber and begin to suck the contents through
4. Change the location of the nozzle to control flow through speed

## Protocol for Triggering Cells

- 1) INITIALIZE the stage
- 2) Look for cell with mid to low fluorescence. Do this by locating a cell, turn up the gain, turn off the bright field, and turn on the fluorescence by flipping the switch on the control box
- 3) Trap a bead near a cell and run AOD line sweep
- 4) Run DVE program to center the bead at 26,26
- 5) Run the calibration
- 6) Open the Andor program and keep the shutter permanently closed
- 7) Open the joystick program and move the bead into contact with the cell
- 8) TURN OFF the bright field, move the mirror turn on the dark box.
- 9) Click on the wrench, and set the controls to Single and internal triggering with a .1 exposure time.
- 10) Click on camcorder icon and turn on fluorescence. Adjust the gain until the cell's fluorescence is clearly seen and distinguishable.
- 11) Turn off fluorescence, stop the Andor program and set the controls to Kinetic, External Triggering with 200 length.
- 12) Open Normalize Quad and run at 100-500 scans/sec
- 13) Use the relative move stage program and step the stage over 200nm
- 14) Click on the camcorder icon on the Andor and then run the shutter program at 30 sec intervals (30,000 msec)
- 15) Turn the detector on and off briefly to determine with time  $t=0$  is
- 16) Once a picture is clearly seen, click on the camera icon to start recording
- 17) Do whatever you please until the cell is triggered just periodically check the increase/decrease in fluorescence
- 18) Once triggering is achieved, stop the Andor program and click the save button. Confirm the file is saved properly by reopening it.

### Protocol for Loading Cells

- 1) Upon arrival of new cells, fill cooler with fresh ice and place the cells deep in the ice
- 2) Wearing gloves, remove one vial of cells and thoroughly resuspend the cells by hitting the sides of the vial with your hand (or by thumping continuously)
- 3) Place the cells in the hood and pipette up and down to resuspend the cells even more
- 4) Transfer the cells from the vial into an eppendorf tube and centrifuge at 4000 rpms for 5 mins
- 5) Remove the media from eppendorf tube with only the cells remaining attached to the surface
- 6) Determine a desired cell concentration and add  $\frac{1}{2}$  of the needed fresh colorless media to the cells and resuspend
- 7) Add the other half of colorless media mixed with 2.5 microMoles/Liter of probencid and 5 microMoles/Liter of calcium orange solution to the cells and resuspend thoroughly (see mixing protocol)
- 8) Place in incubator for 30 mins- 2 hours with the lid on the eppendorf tube opened
- 9) Centrifuge again at 4000 rpms for 5 mins and then remove the media from the tube
- 10) Add colorless media back to the cells and resuspend thoroughly
- 11) Place the tube back into the incubator and leave it there until needed for experiments

### Mixing Protocol

- 1) Take a vial (50 micrograms) of calcium orange out of the -20 and hold tightly in your hand to melt the solid into a liquid and keep it from being exposed to light. Once the calcium orange is a liquid mix it with 100 microliters of DMSO (concentration is now 1 milliMole/Liter or 200X ).
- 2) Take a vial (77mg) of probenic and mix it with 1080 microLiters of Cellgro. (concentration is now 250milliMole/Liter or 100X)
- 3) Determine a desire cell density and calculate the volume of solution needed (media + calcium dye+ probenicid)
- 4) Divide the calculated volume by 2. This is the amount of media you will add directly to the cells
- 5) In a separate eppendorf tube,  
Divide the calculated volume by 200 to determine the amount of calcium dye to add
- 6) Divide the calculated volume by 100 to determine the amount of probenicid to add
- 7) Subtract the volume of probenicid, calcium dye, and media added in the previous step from the calculated volume. This is the remaining amount of media to add.
- 8) Add the remaining media, calcium dye, and probenicid all in one eppendorf tube and mix thoroughly (by pipetting or vortex)
- 9) After the media solution is mixed, add the solution in with the media and cells solution from the previous step.

### Example 1

Shipment comes in with 2million cells/mL. After centrifuging there are 2 million cells attached to the surface. I chose I wanted 4 million cells/mL therefore I need .5 mL of media.

Step 1. Divide .5mL by 2 to get 250uL of colorless media and add it to the cells. Resuspend the cells thoroughly

Step2. Divide 500uL by 200 to determine calcium dye volume = 2.5uL of dye

Step 3. Divide 500uL by 100 to determine probenicid volume = 5uL of probenicid

Step 4. Determine the remaining amount of media to add.  
 $500\text{uL total} - 250\text{uL media} - 2.5\text{uL dye} - 5\text{uL of probenicid} = 242.5\text{uL of meida}$

Step5. Add the 242.5uL of media, 2.5uL of dye, and 5uL of probenicid in one tube and mix.

Step 6. Add the mixture in with the cells/media solution from step 1.

Step 7. Mix thoroughly by pipetting

### **Example 2**

Shipment comes in with 2 million cells/mL. After centrifuging there are 2 million cells attached to the surface. I chose I wanted 1 million cells/mL therefore I need 2 mL of media.

Step 1. Divide 2mL by 2 to get 1mL of colorless media and add it to the cells. Resuspend the cells thoroughly

Step2. Divide 2000uL by 200 to determine calcium dye volume = 10uL of dye

Step 3. Divide 2000uL by 100 to determine probenecid volume = 20uL of probenecid

Step 4. Determine the remaining amount of media to add.  
 $2000\text{uL total} - 1000\text{uL media} - 10\text{uL dye} - 20\text{uL of probenecid} = 1970\text{uL of media}$

Step5. Add the 1970uL of media, 10uL of dye, and 20uL of probenecid in one tube and mix.

Step 6. Add the mixture in with the cells/media solution from step 1.

Step 7. Mix thoroughly by pipetting