Video Particle Tracking (Viscosity of 70% Glycerol Solution)

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1 Introduction

Video Particle Tracking is a passive micro rheological approach used to study the Brownian motion of probe particles added to a sample in order to analyze the rheological properties. In Passive microrheology, particles movement is due to thermal motion, which allows to measure various properties likes viscosity, elasticity, etc. Bead particles mixed in sample are labeled with fluorescent markers so that their random movement is recorded with video microscopy. Particles must refrain from interacting with each other or with the sample they're suspended in to ensure their motion remains unaffected by interactions[1]. To understand how this movement reflects the material's rheological characteristics, each particle's path is followed. Once the motion of these particles is captured, their positions in each video frame are determined by finding the center of their brightness, and then these positions are connected to trace their paths over time.

Mean Square Displacement(MSD) calculated as a function of lag time is a statistically relevant result that can be estimated from the motion of bead particles collected over numbers of frames in a VPT experiment. MSD measures how much a particle's position changes over time compared to its starting point[2]. It is commonly used to quantify the spatial extent of random mobility and a way to study the dynamical properties of the system.

We determine the viscosity of 70% glycerol solution by observing and tracking the motion of bead particles measuring 4.22 micrometers in diameter using video microscopy. The recorded movements of the bead particles were enhanced through optimization techniques such as tracking, filtering, and linking in ImageJ software, with specific parameters tailored to the analysis. We collected three different videos with different number (16, 19, & 23) of tracks for analysis. The trajectories of particles were graphically analyzed using python software tools. Plot of MSD as a function of time allows us to determine the diffusivity constant, from which the viscosity of 70% glycerol solution was estimated using Stoke Einstein's relation.

2 Theory

Video Particle tracking analysis to estimate the viscosity of glycerol solution is based on the diffusive motion of bead particles in the glycerol solution. Trajectories of bead particles are observed and analyzed based on various techniques; The movement of the center of mass of bead particles are determined by calculating the position vector R at each time interval based on the velocities of the individual particles[3].

$$R_{COM} = R_0 + \sum_{k=0}^{k} \bar{v_k} = R_0 + \sum_{k=0}^{k} (\frac{1}{N_k} \sum_{i=1}^{N} v_{i,k}) \Delta k \tag{1}$$

Where R_0 is the initial center of mass vector calculated by averaging the coordinates of all the beads in the first frame (k = 1),
$\bar{v_k}$ is the mean velocity of all the particles in frame k,
$v_{i,k}$ is the velocity of particle in frame k in units of μm/frame,
and N_k is the number of particles in frame k and Δ k is the frame difference.

Calculation of ensemble average and time average of the mean square displacement of beads as a function of lag time is based on equation 2[2, 3].

$$MSD(\Delta t) =< [x(t + \Delta t) - x(t)]^2 + [y(t + \Delta t) - y(t)]^2 > \tag{2}$$

where x(t) and y(t) are the coordinates of the bead at time t, and Δt is the time interval also called lag time. Further to extract the diffusion coefficient of the beads MSD plot is fitted using equation 3[4].

$$MSD(\tau) = 4D\tau^{\alpha} + N \tag{3}$$

where τ is the lag time, D is the diffusion coefficient and α known as diffusivity exponent gives the nature of diffusion, ($\alpha = 1$) represents normal diffusion, and N is the noise factor.
Using the value of diffusion coefficient estimated by fitting the MSD plot, viscosity η can be evaluated from Stokes Einstein relation given by equation 4[4, 5].

$$\eta = \frac{K_B T}{6\pi D R} \tag{4}$$

Where R is the particle radius, k_B is the Boltzmann coefficient, and T is the temperature in Kelvin.

3 Sample Preparation

4.5μml of 70% glycerol solution was mixed with 0.5 μml of 500X diluted bead solution. 70% glycerol solution was prepared by mixing 60 μml of water with 240 μml of glycerol solution. The solution was subjected to vortex mixer to mix the sample properly. Bead size taken was 4.22 μm in diameter. Before pipetting the sample to coated cover slip, three layer tape of 1-2 mm thickness was used as spacers on glass slide to set the height of the sample chamber in order to prevent wetting of sample on the glass surface.

4 Observation

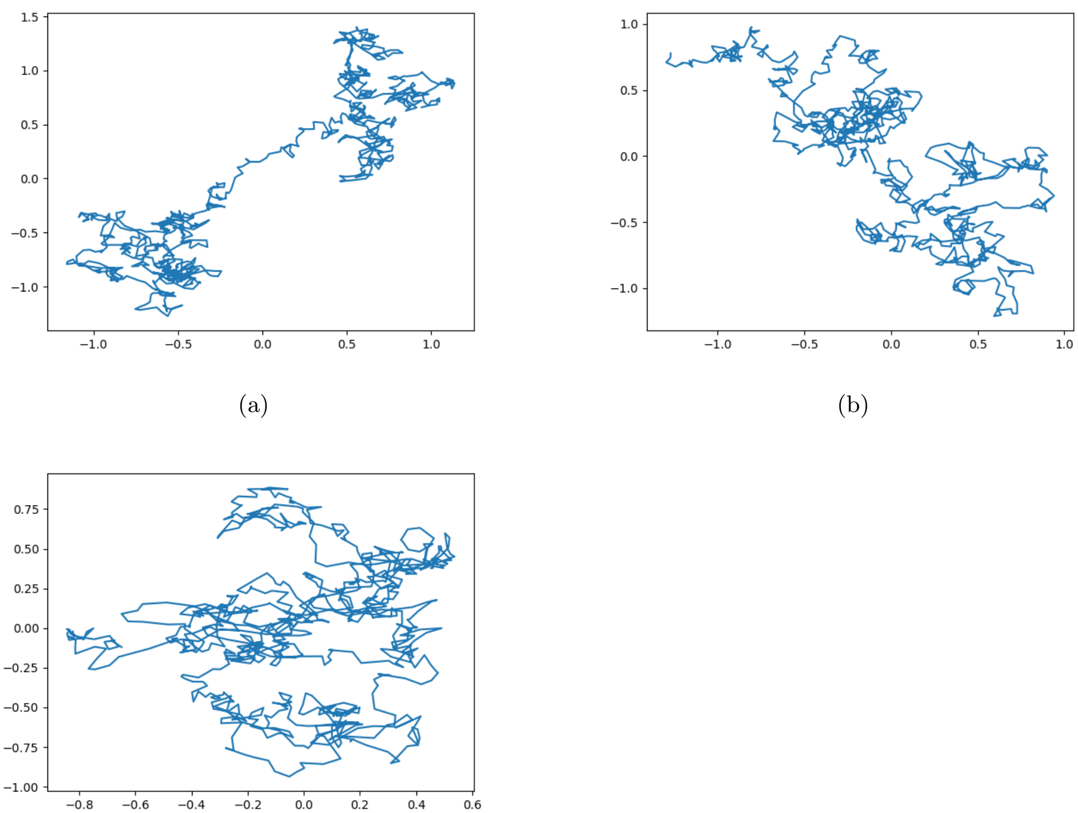
A 40X magnification objective lens was utilized to image the sample using fluorescence microscopy. Bead particles were followed using a suitable core and fine microscope adjustment, with an exposure length of 100 seconds for 1000 pixels recorded to follow the motion of the particles. With the aid of ImageJ tools, the motion of the tracked beads was further examined and evaluated.

5 Tracking and Filtering using ImageJ

The image scale is set to 0.172μm per pixel. A Difference of Gaussian (DoG) detector with a 6μ m object diameter and a quality threshold of 2 is applied to identify local maxima as detection spots. These spots are filtered based on quality and proximity, with a signal to noise ratio threshold. A median filter is used to mitigate extreme pixel intensities while retaining image details. Particle-linking consists of two stages; link particles to form track segments between frames, then closing gaps between segments[6]. Finally, the trajectory data is saved in an XML format for further analysis using Python tools.

6 Results and Discussion

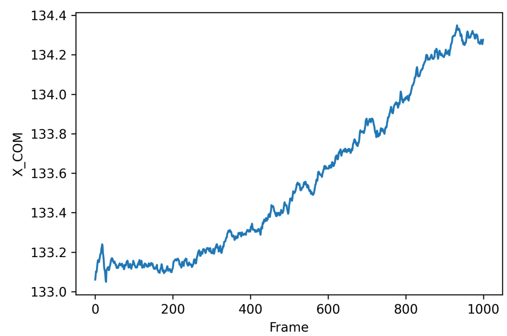
After tracking and filtering the trajectory data, Python tools were employed to analyze the diffusive motion of bead particles within a glycerol solution. Mean square displacement plots were generated and fitted to determine the diffusion constant. Utilizing this value, the viscosity of 70% glycerol solution was estimated based on three distinct trajectories, each comprising varying numbers of tracks.



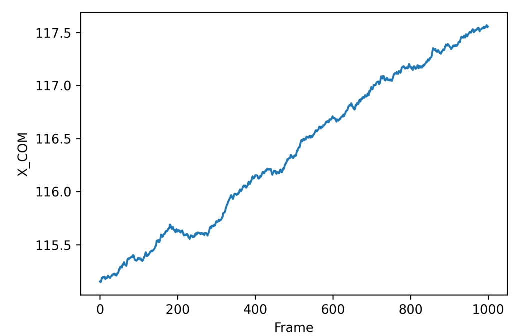
(a) (b)

(c)

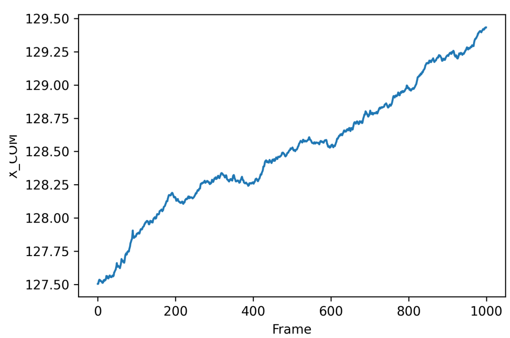
Figure 1: Figure (a) to (c) represents the trajectories of individual bead particles observed for different tracks taken. The bead particles in glycerol solution exhibit random motion as shown by the graphs.



(a)

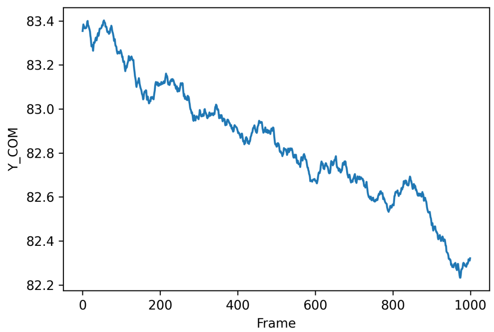


(b)

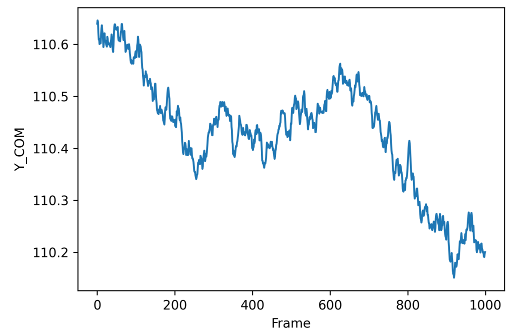


(c)

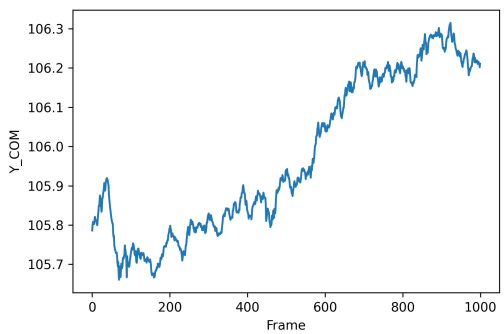
Figure 2: Figure 2(a) to (c) represents x_com of center of mass of beads at each time point computed for analyzing the diffusion of center of mass of bead particles. Equation to estimate the center of mass vector R at each time point is given by equation (1).



(a)

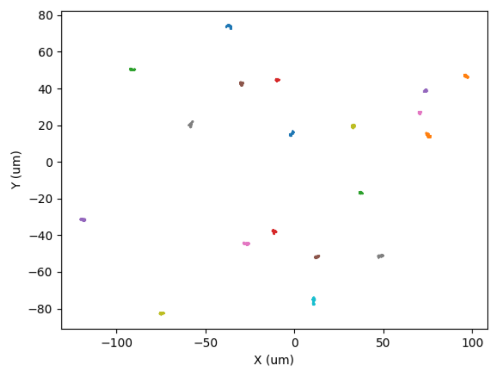


(b)

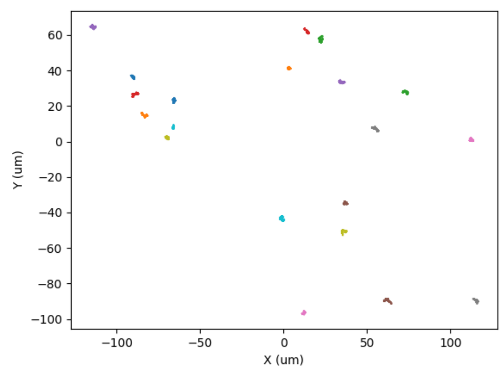


(c)

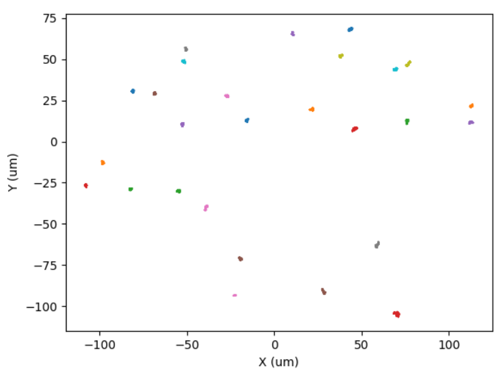
Figure 3: Figure 3(a) to (c) represents Y_com of center of mass of beads at each time point computed for analyzing the diffusion of center of mass of bead particles.



(a)

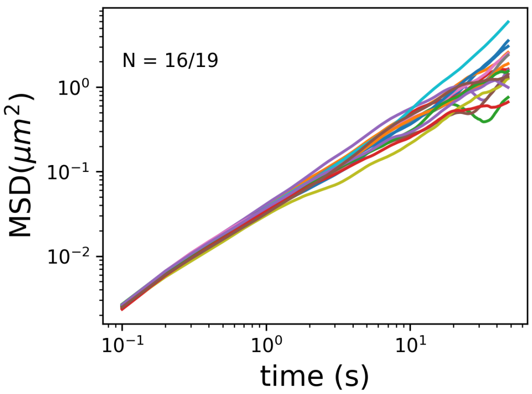


(b)

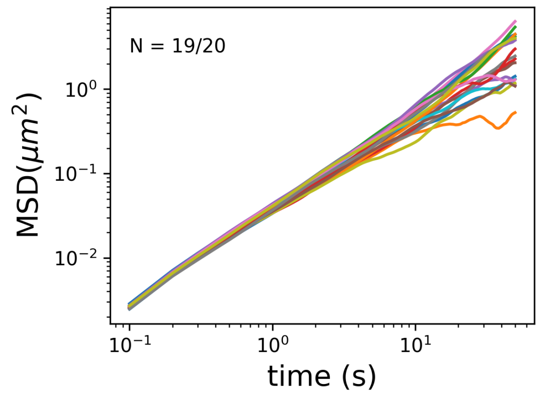


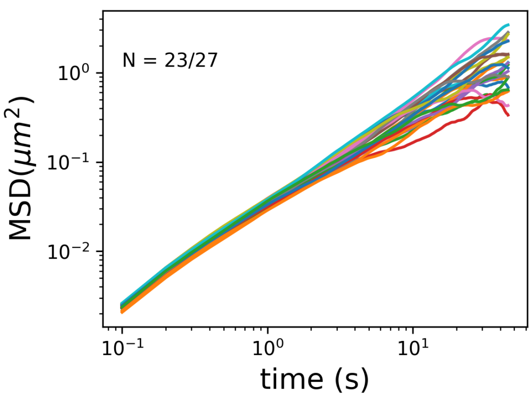
(c)

Figure 4: Figure 4(a) to (c) represents the trajectories of beads particle analyzed for different tracks with beads number 16, 19,and 23 respectively .



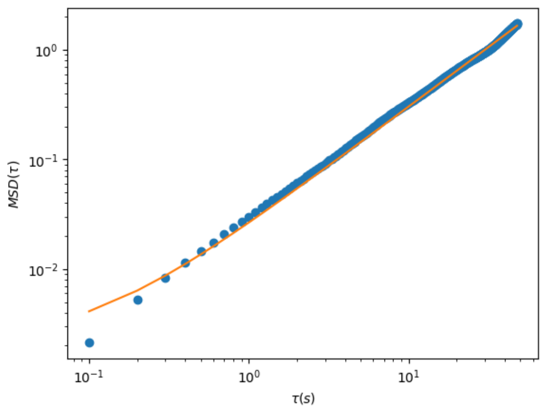
(a)

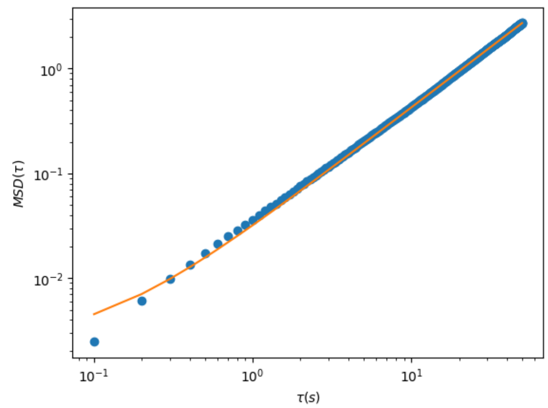




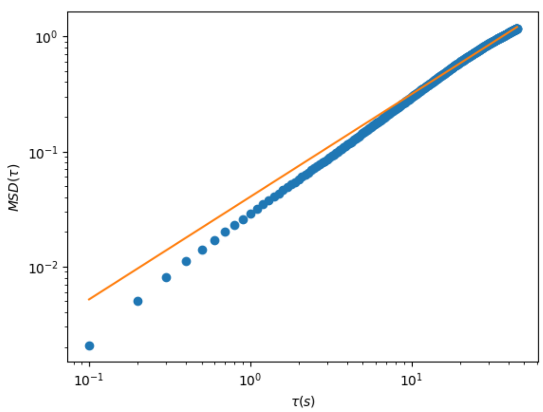
(c)

Figure 5: Figure 5(a) to (c) represents the ensemble and time averaged mean square displacement of bead particles as a function of lag time for different imaging series. Equation representing the mean square displacement as a function of lag time is given by equation (2). The plots show the linear relationship between the MSD and time t which allows for graphical methods to determine the diffusivity constant D.





(a)



(c)

Figure 6: Figure 6(a) to (c) shows the corresponding mean square displacement (MSD) as a function of time plotted for the trajectories in figure 1 (a) to (c). MSD of bead particles are then fitted by using equation (3) to estimate the diffusion coefficient values. Calculation of viscosity is carried out by Stokes Einstein's relation given by equation (4) at temperature 293K. Viscosity values obtained for three different plots are 0.01665 pa.s, 0.01378 pa.s,& 0.010085 pa.s respectively.

7 Conclusion

Study of viscosity of 70% glycerol solution is carried out with bead particles submerged in glycerol solution and is observed under florescence microscope with 40X objective lens. ImageJ software tools were utilized to track and filter a series of images to optimize the data for subsequent analysis of the random motion exhibited by bead particles. It is observed that the bead particles exhibit random diffusive motion from the trajectories of individual bead particles which is further corroborated by the plot of X_{com} and Y_{com} of center of mass of bead particles at each time point. Further the MSD plot with lag time shows linear trend which allows us to estimate the diffusion coefficient: the rate of material transport as a result of the random thermal movement of particles. Using the value of diffusion coefficient the average value of viscosity calculated form three different videos tracked is 0.0135 +- 0.0033 pa.s. This value is comparable with earlier estimated value (0.0225 pa. s) at temperature 273K[7]. The difference in viscosity value may be attributed to factors such as precise temperature control, accuracy in sample preparation and observation, etc.
The experiment conducted on video particle tracking to determine the viscosity of glycerol solutions offers insights into the random movement of particles immersed within the solution. This include a comprehensive process, starting from sample preparation through observation and analysis, culminating in the interpretation of tracked particle trajectories. These findings provide a foundational framework for delving deeper into the dynamic characteristics of condensates.

References

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