

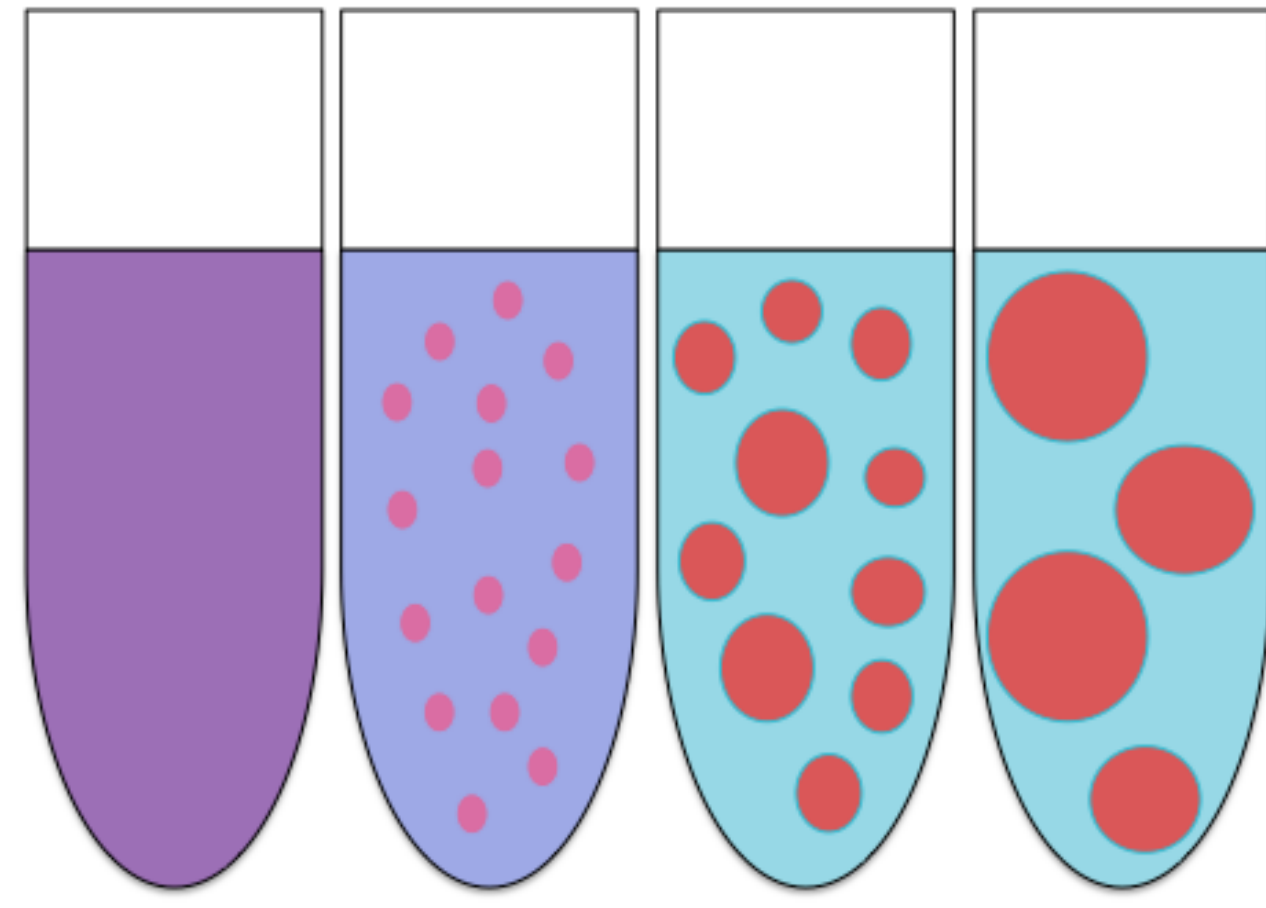
# Characterizing Liquid-Liquid Phase Separation

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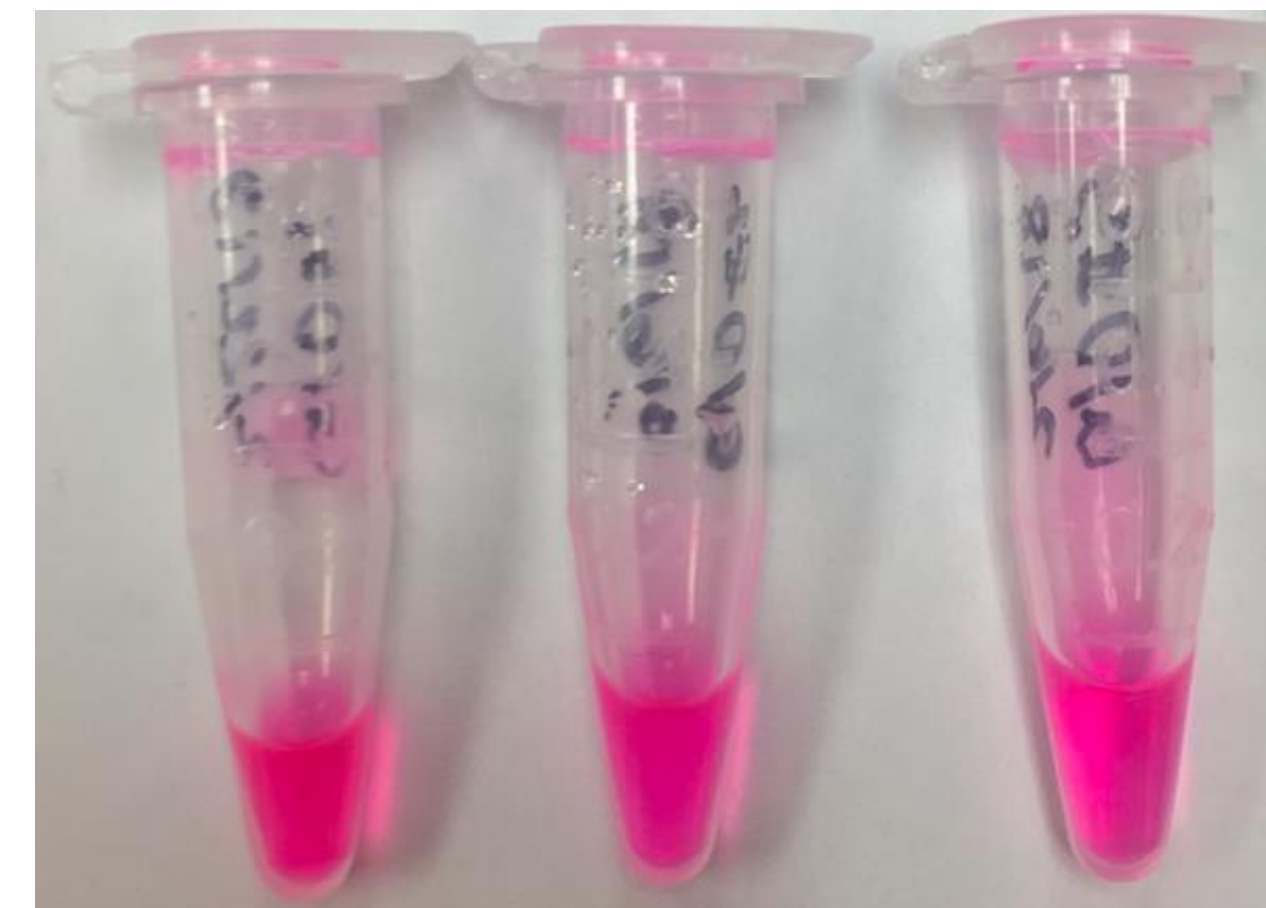
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**Abstract:** The process of liquid-liquid phase separation (LLPS) has been explored in polymer physics and colloidal science, and recently, researchers have studied this process to better understand intracellular organization. LLPS is the process by which a liquid mixture will separate into two fluid phases. Currently, many research labs are working to understand how and why certain cellular organelles are liquid bodies that are the result of phase separation. Investigations of how the cell uses phase separation to organize and compartmentalize has demonstrated a wealth of biological implications. We experimentally study the process of de-mixing in a model system in order to define better ways of analyzing and characterizing the physical properties of the two fluid phases. Our experiments utilize the following optical methods: phase contrast microscopy for video analysis of droplets merging to determine capillary velocity; fluorescence recovery after photo-bleaching (FRAP) to determine the diffusion coefficient; particle tracking to determine viscosity; and confocal z-scans to determine the density difference between two phases. By assessing methods to study the physical features of these droplets, like surface tension, viscosity and density, optimal methods can be applied to study membrane-less organelles assembled in cells via LLPS.

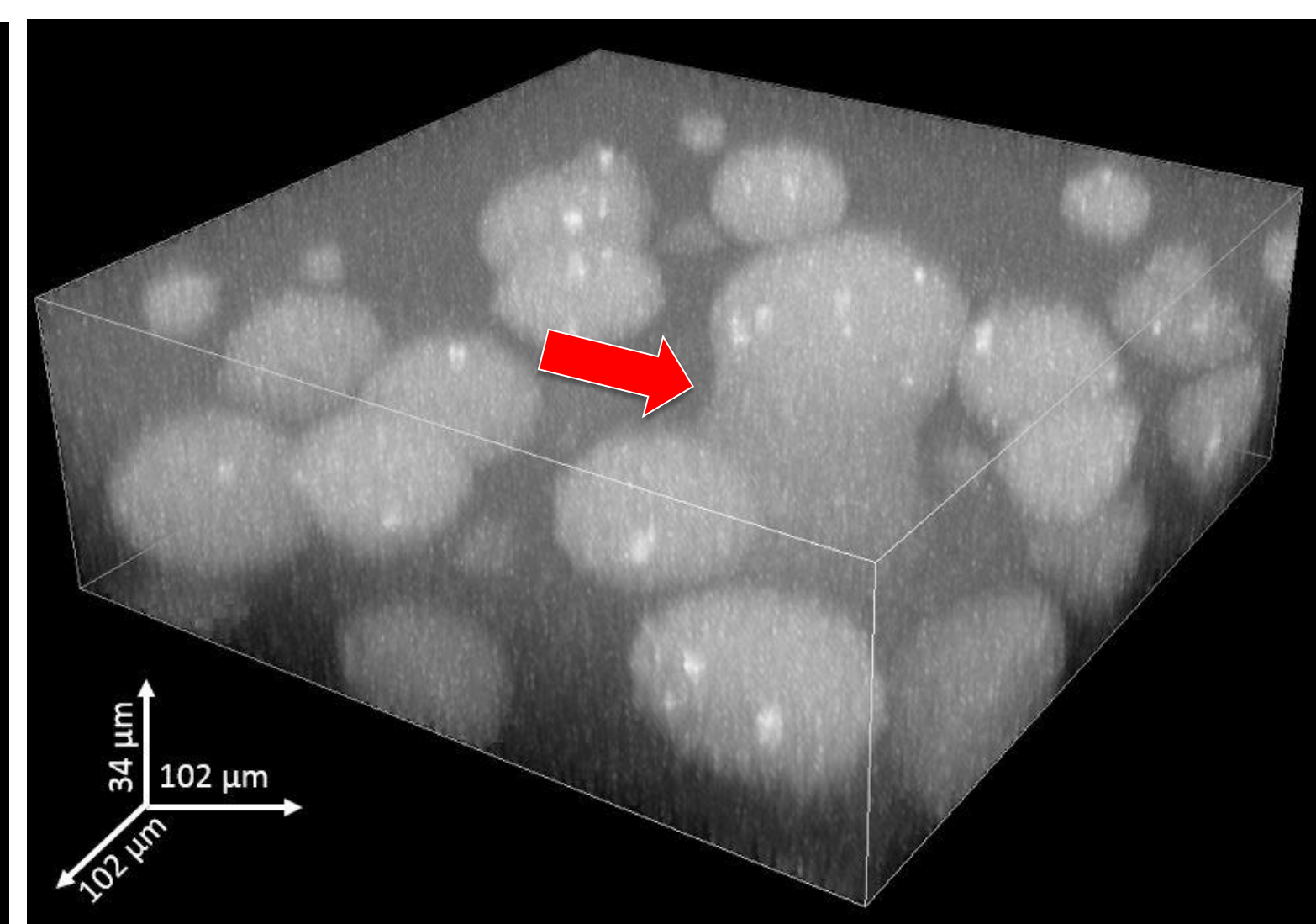
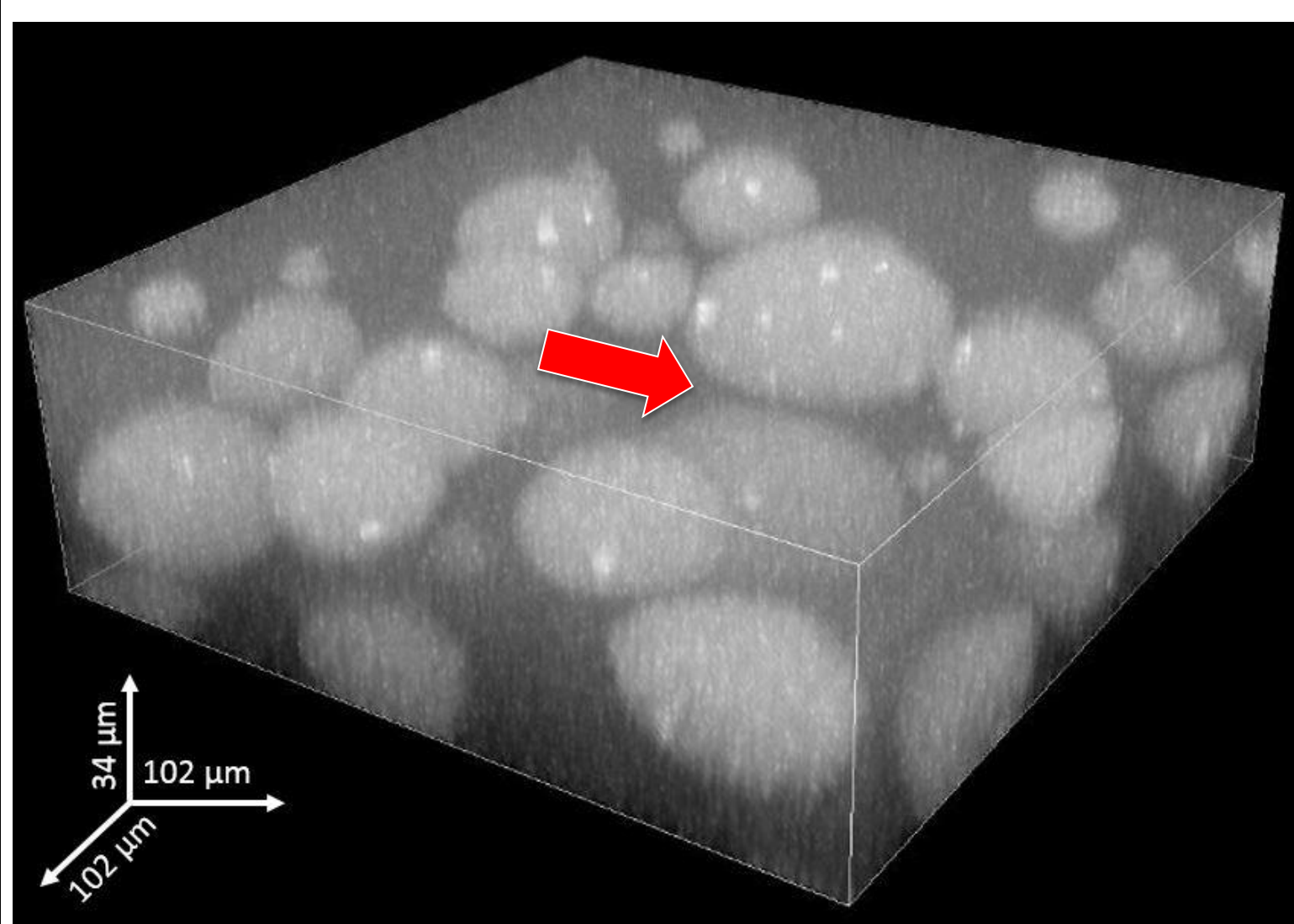
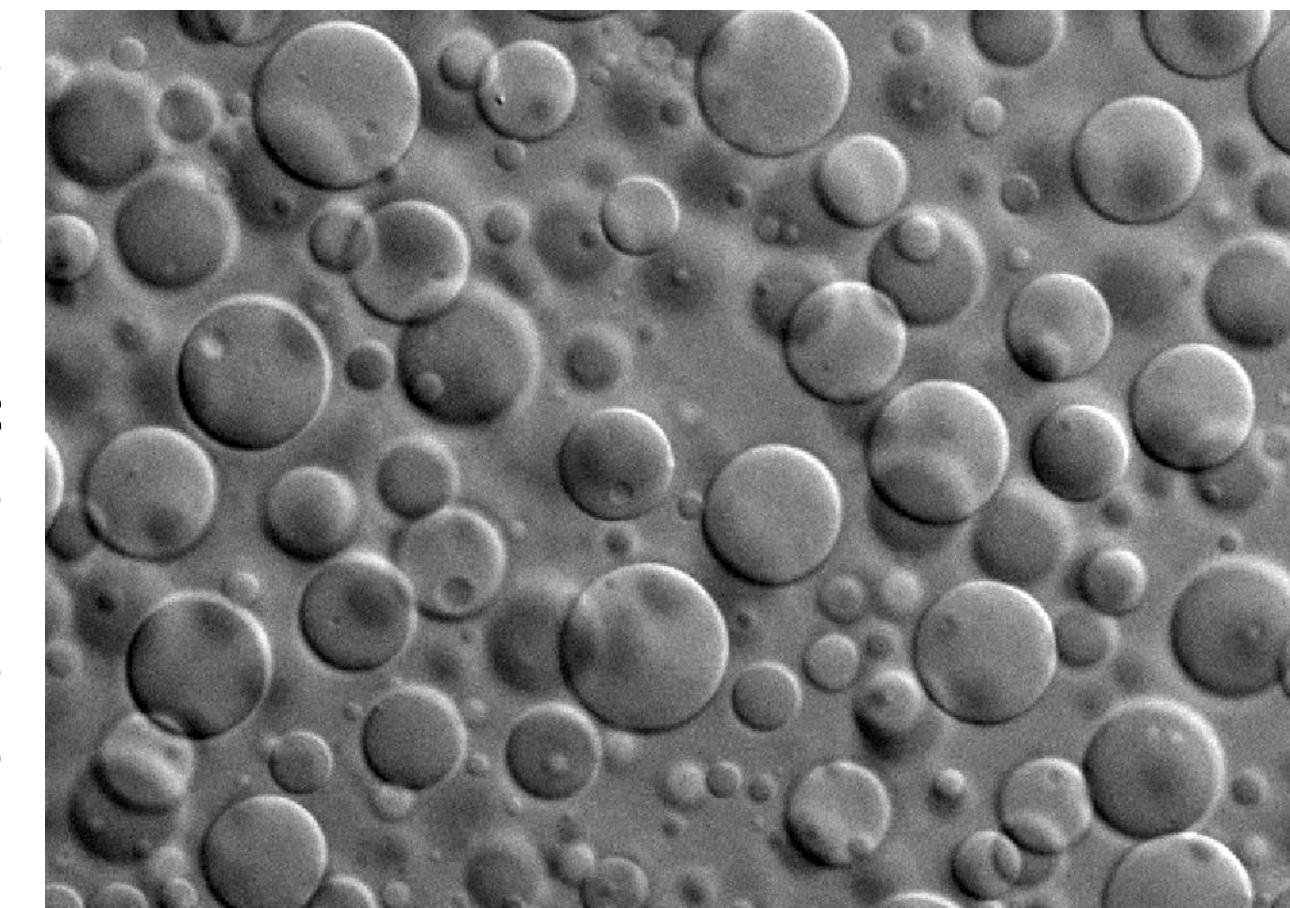
- LLPS is the process by which a liquid mixture separates into two phases based on concentration, density or various other physical properties.
- Recent biological studies have shown that phase-separated droplets pose a risk when they remain that way for a long period of time and can affect intracellular diffusive transport, thereby compromising the structure of the cell, possibly leading to pathological conditions.



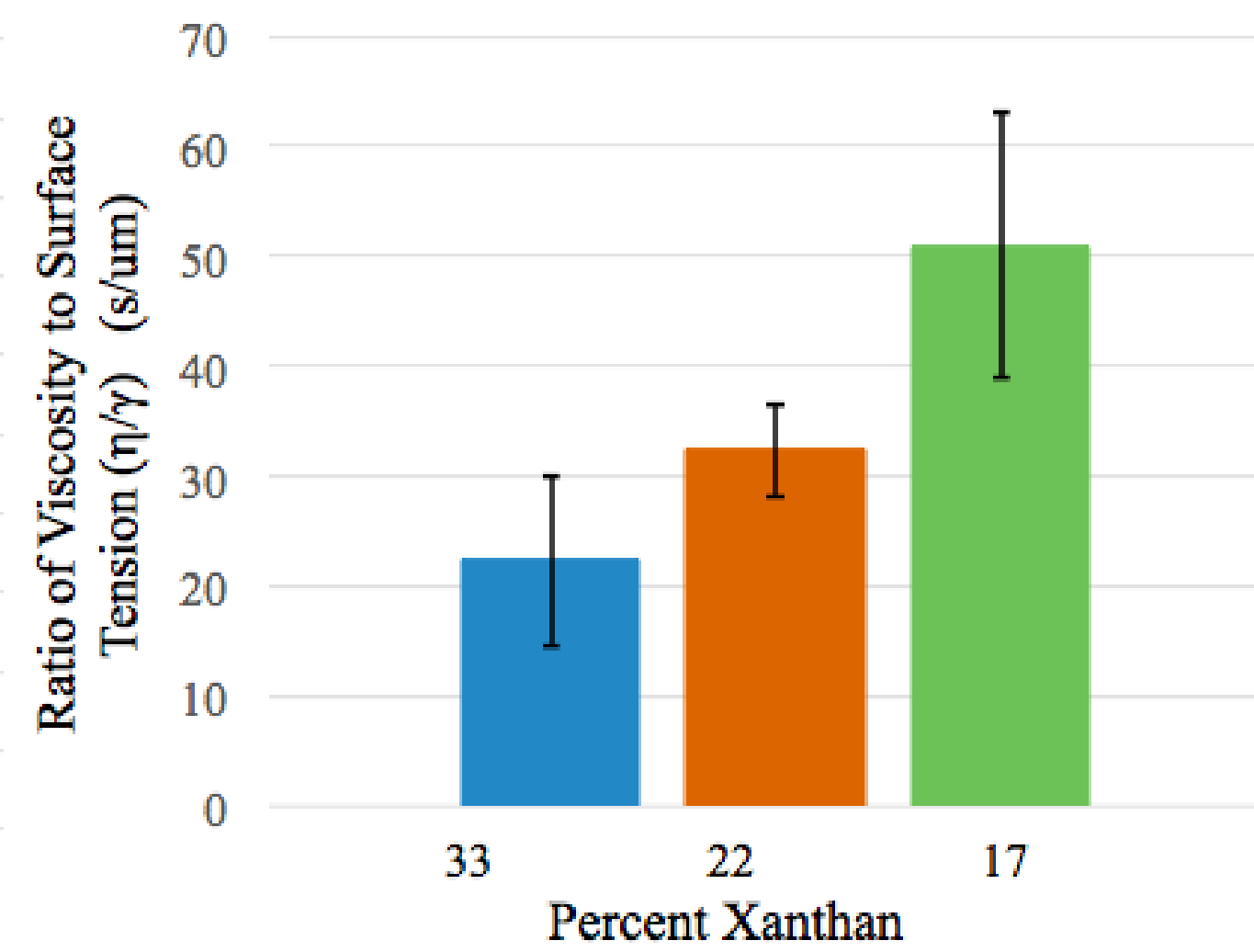
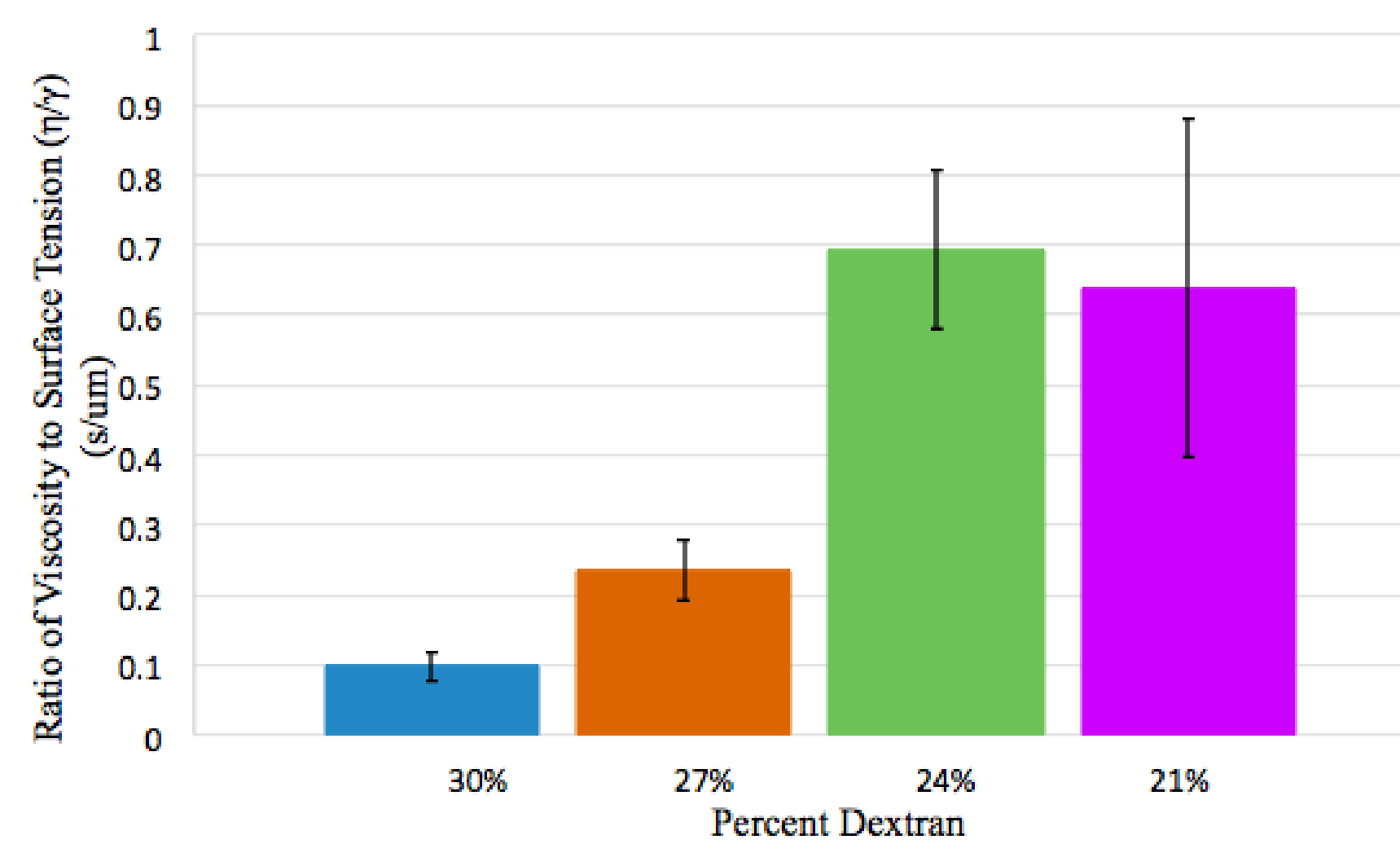
- We observe the process of de-mixing through models systems to demonstrate techniques that can be used in biological applications characterizing the physical properties of LLPS compartments.
- Even macroscopically phase separation is visible. From left to right we see the amount of dextran, a soluble biopolymer, decrease and the solution get progressively less clouded and more mixed.



- With DIC microscopy we see the already phase separated solution form droplets. After coalescence the regions maintain their spherical shape. We can use this characteristic to study the viscosity and surface tension of the solution.
- With temperature manipulation, we can watch the emergence of phase separated droplets.

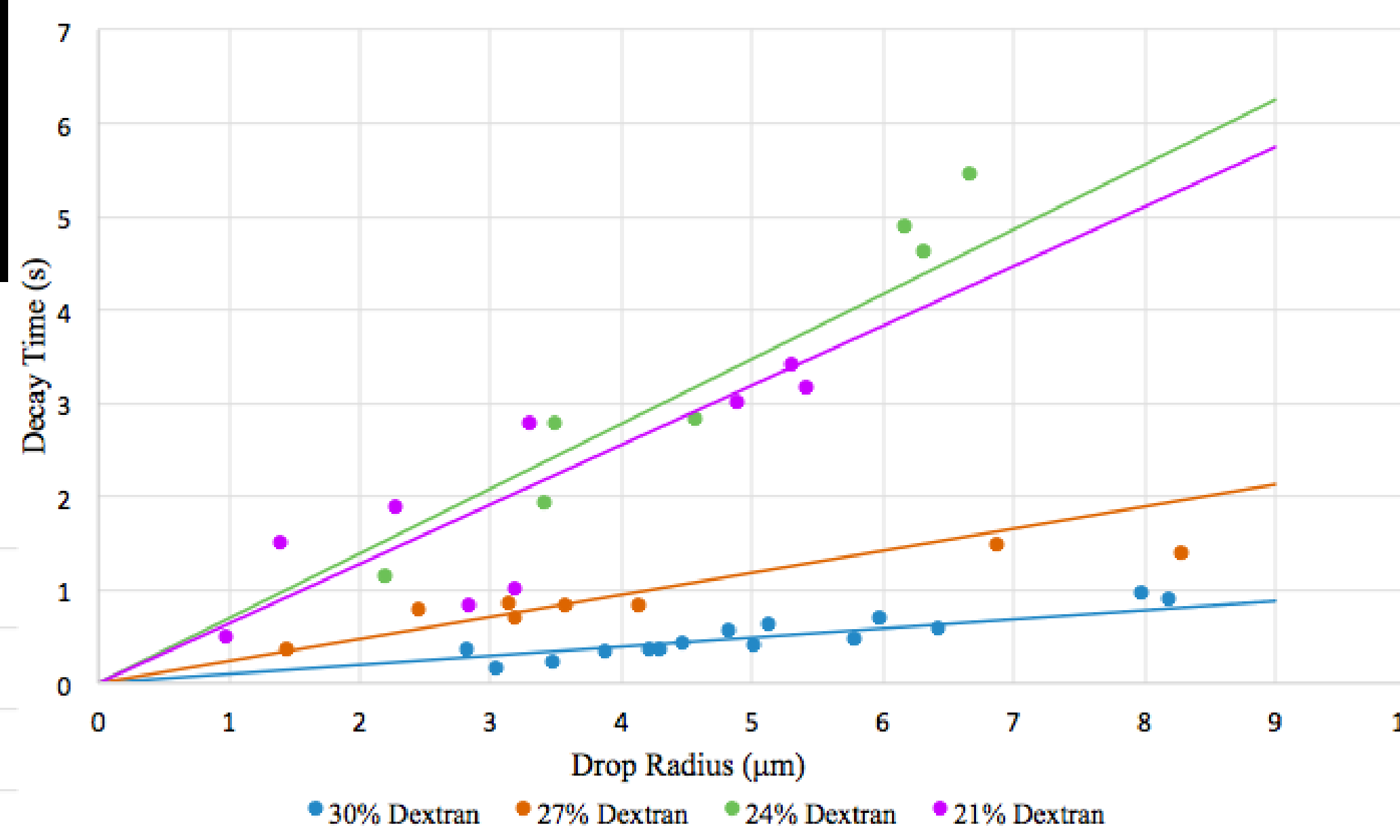


Confocal z-scans of our LLPS system. The gelatin-rich droplets contain fluorescent dye. In these 'before' and 'after' images we see two droplets of the same solution coalescing through the other liquid, along the z-axis.



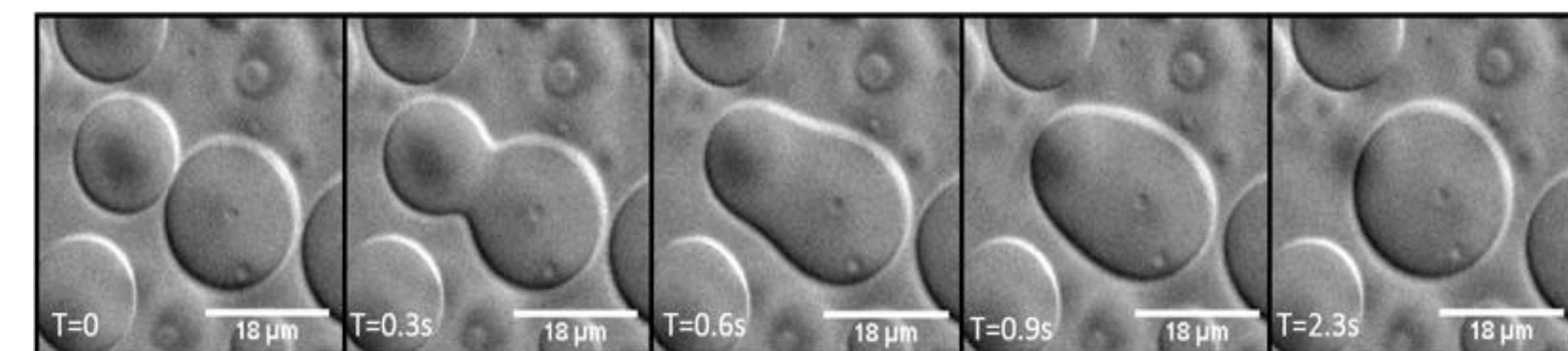
## Observations of coalescence reveal capillary velocity.

A plot of the decay time as a function of droplet radius across various dilutions demonstrates the relationship between the concentration of dextran and the decay time of droplet radii during coalescence. The speed of coalescence is given by the opposing forces capillary velocity driving fusion and the viscosity, which slows down the merging of two droplets.



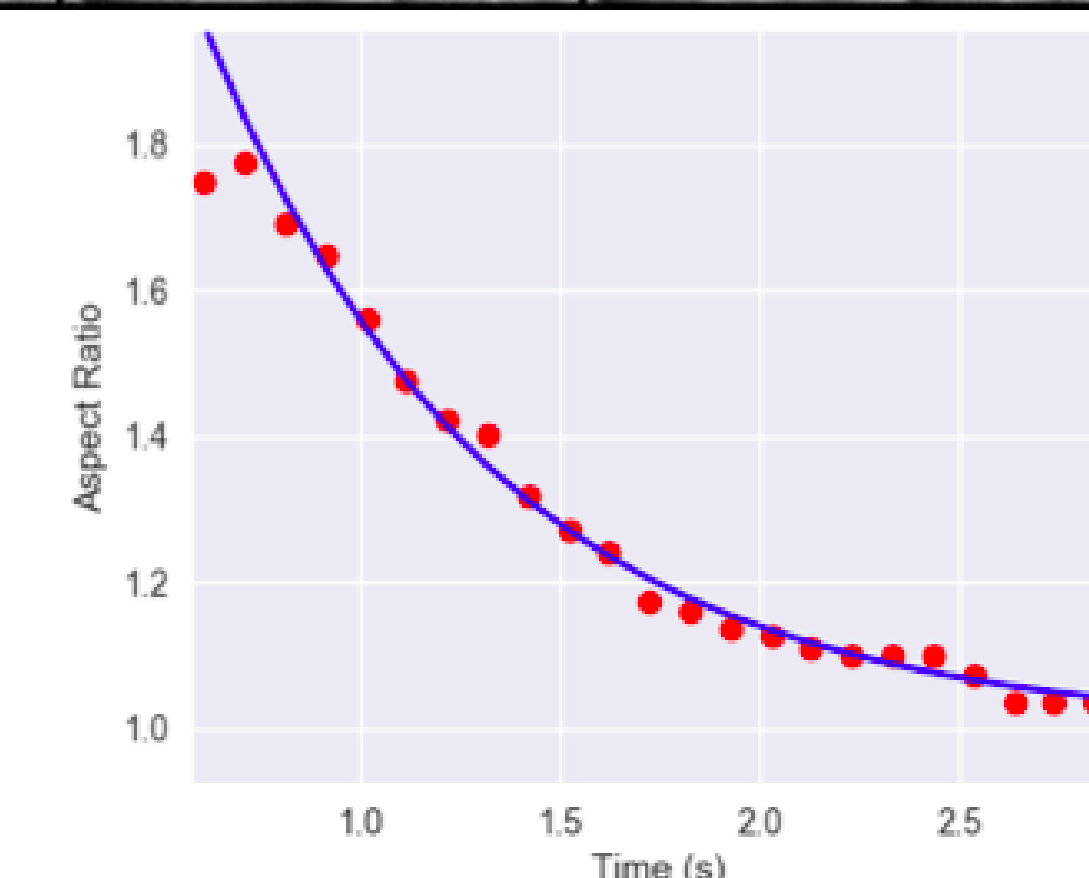
The bar graphs relate the inverse of the capillary velocity to the amount of dextran or xanthan present. As the weight per volume decreases, the ratio of viscosity to surface tension increases. The coalescence time scale ( $t$ ) is a function of the aspect ratio ( $R$ ), surface tension ( $\gamma$ ), and viscosity ( $\eta$ ).

$$t_v = R(\eta/\gamma)$$



With the DIC and phase contrast microscopy we measure the rate of coalescence – the merging of two droplets of like material, and can graph the relationship of aspect ratio to time during the coalescence of two droplets.

$$Aspect\ Ratio = 1 + Ae^{-(t-\Delta t)/\tau}$$



## Recovery of fluorescence leads to evidence of internal rearrangement and diffusivity.

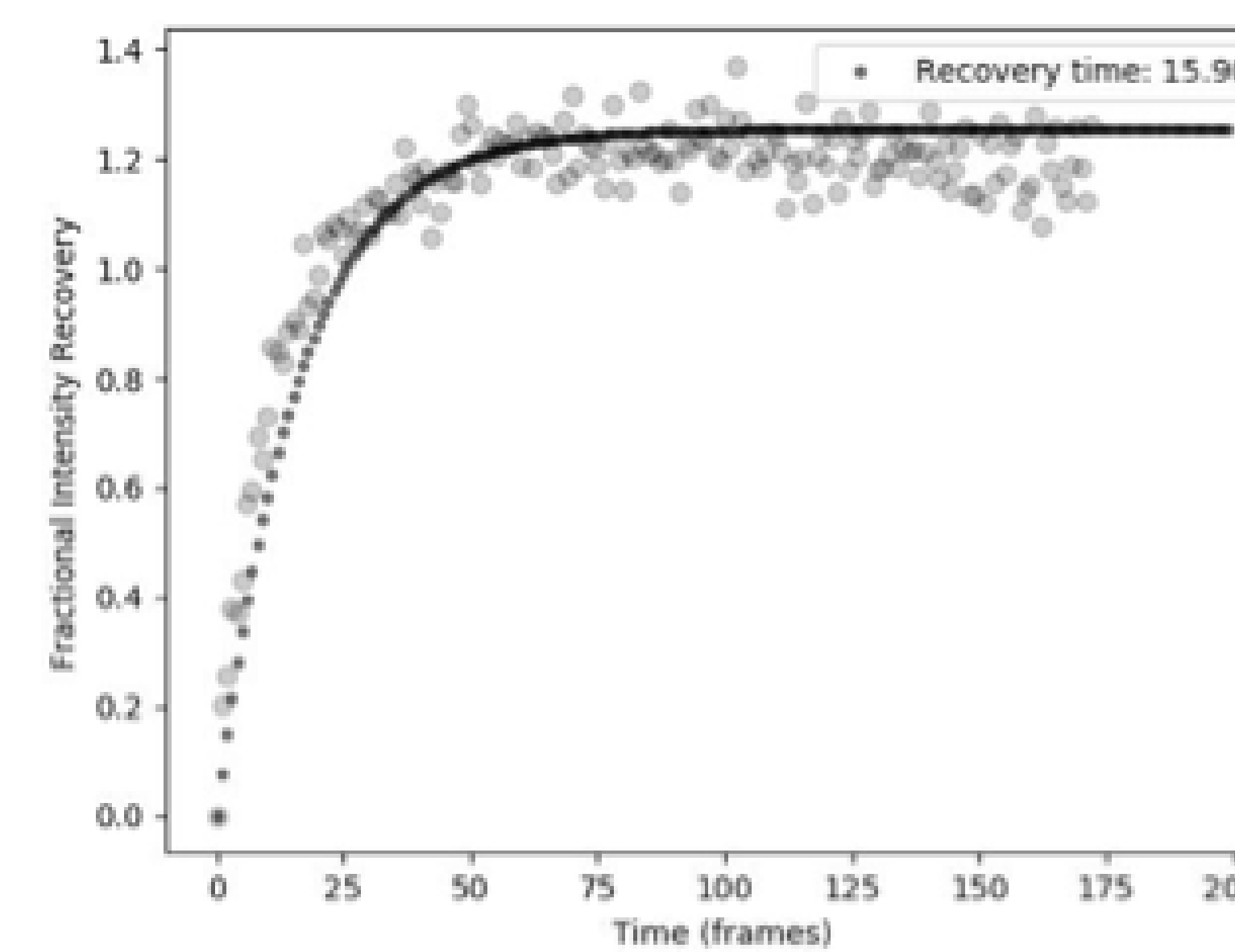
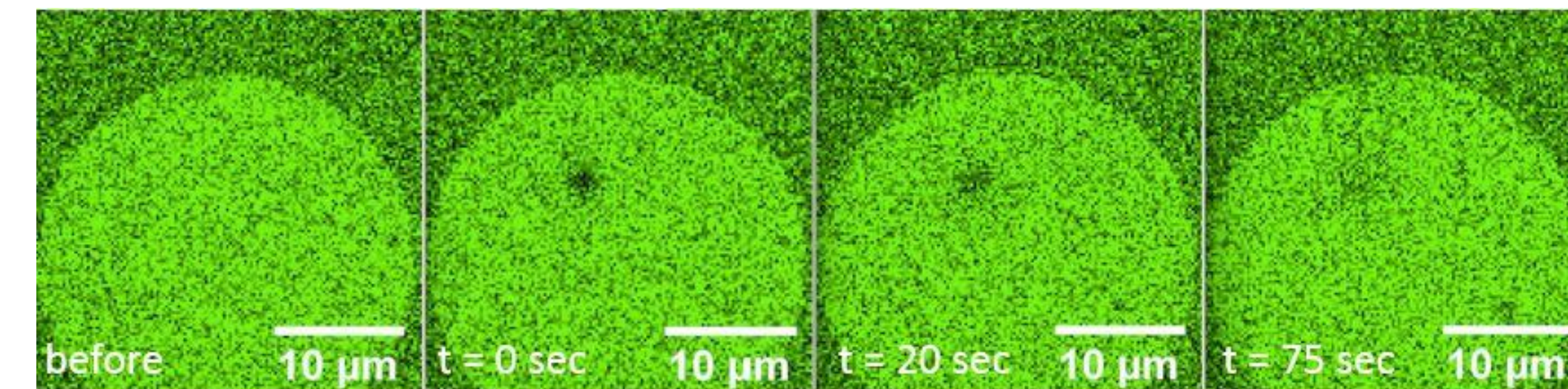
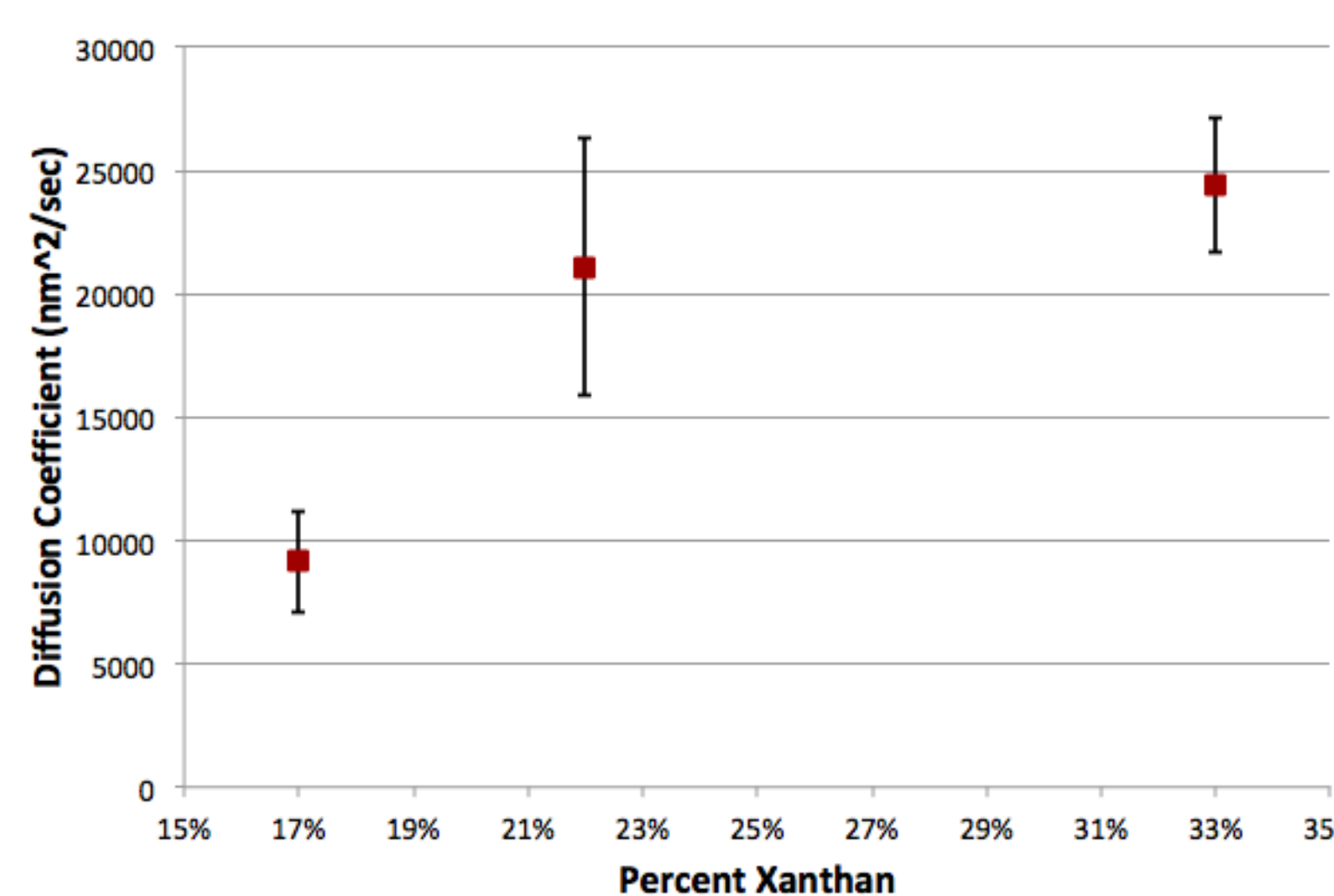


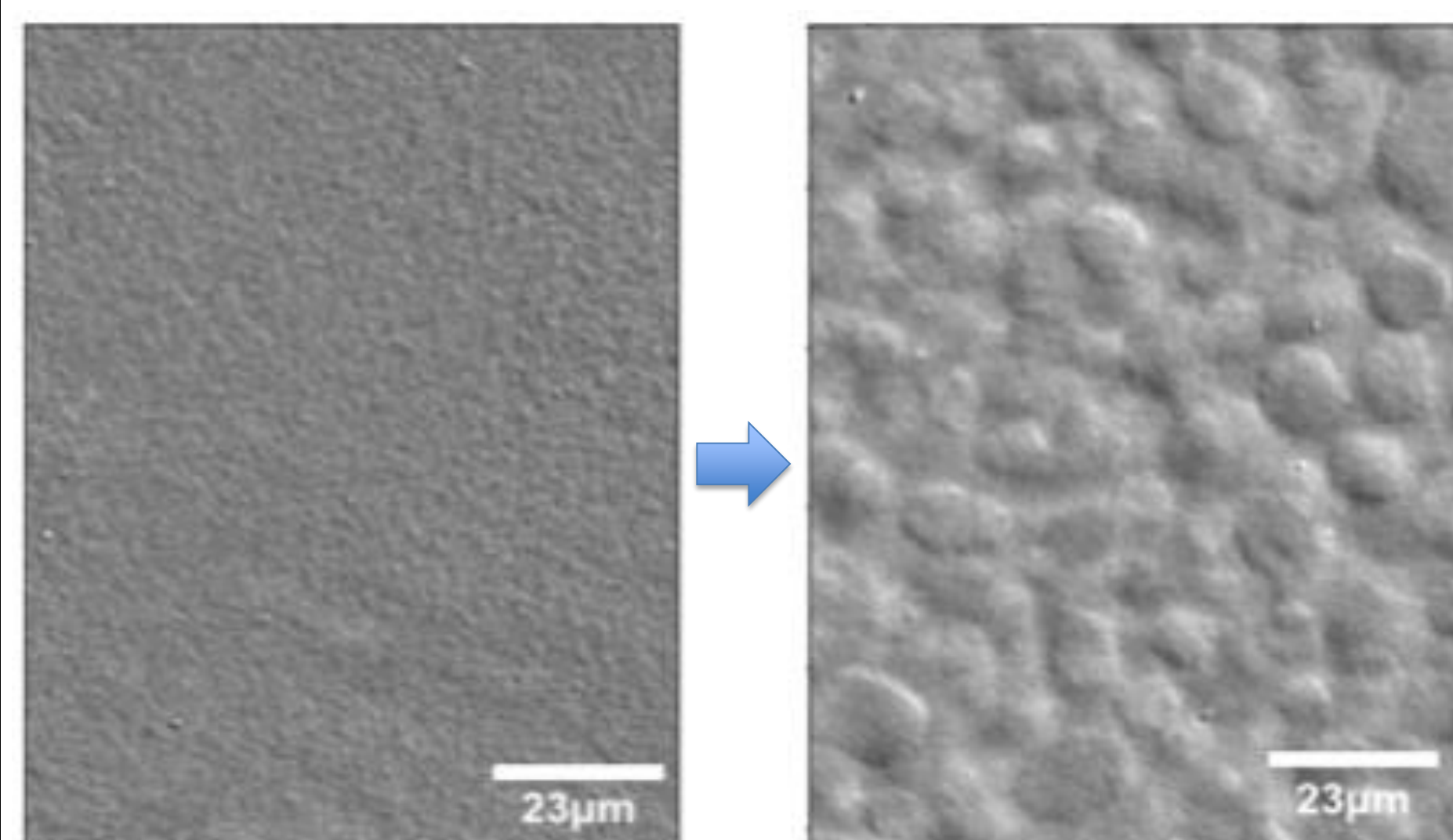
Photo bleaching followed by analysis of recovery was one method used to calculate the diffusion coefficient. By using fluorescently dyed molecules we can track the rate of internal rearrangement of the molecules within a droplet after a region has been bleached. This plot demonstrates the intensity of a region during the FRAP process.



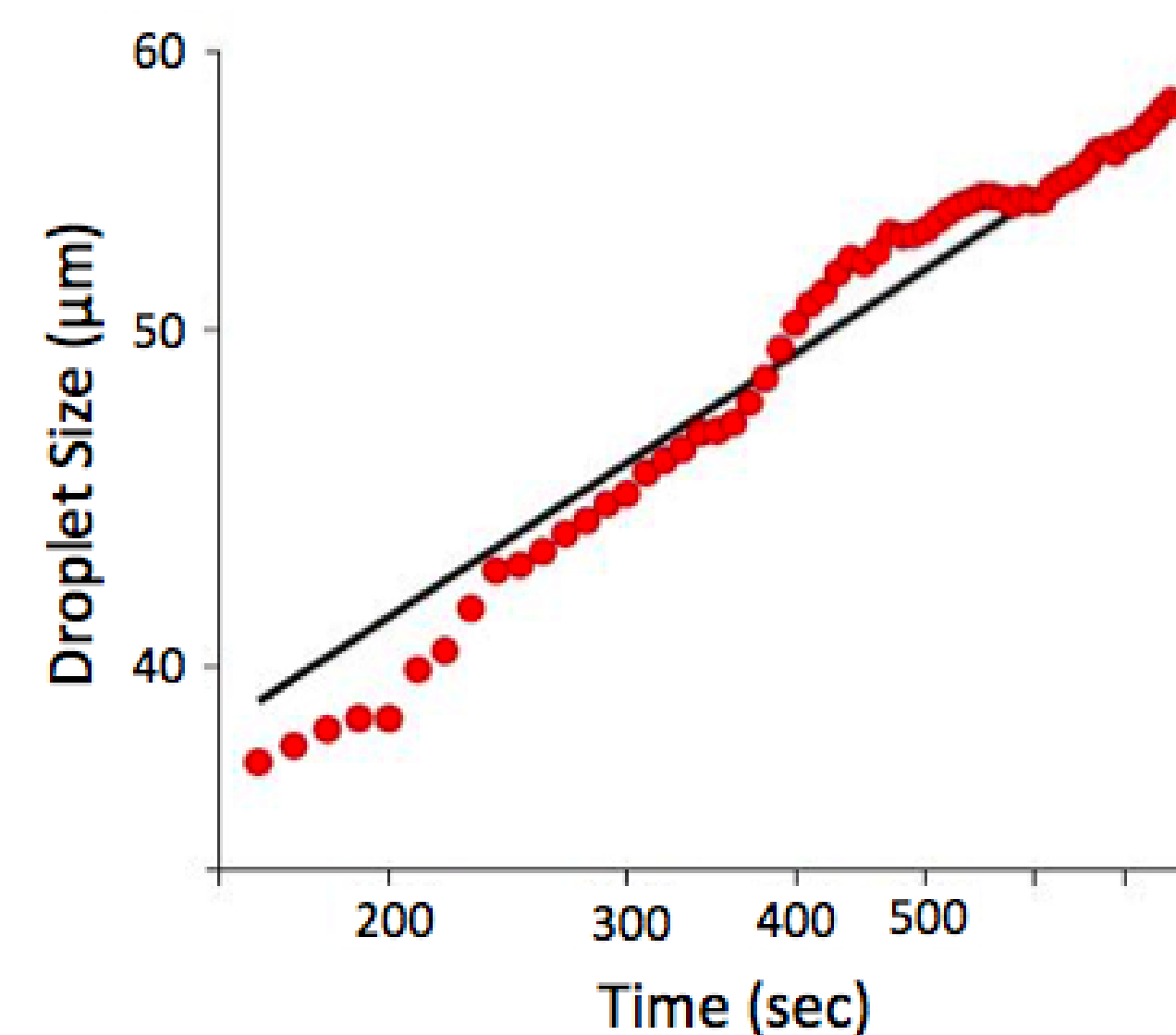
The mean diffusion coefficients obtained from the recovery data for the different dilutions in the colloid-polymer system. The diffusion coefficient is obtained with the following equation:

$$D = \frac{\lambda^2}{(2 \times N.A.)^2 \times Recovery\ Time}$$

## Temperature influences the size of the colloid and therefore the amount of free space available to the colloid to cause mixing.



The ~200 nm colloidal particles (PNIPAM) in the colloid/polymer mixture are temperature sensitive. By increasing the temperature of the system, the size of the PNIPAM beads decrease, causing the sample to mix. This mixture is seen on the left. As the system cools, the solution separates.



This log-log graph quantifies the power law dynamics of the growth of the characteristic size of the droplets. The characteristic size is determined by the Fourier transformation of images over time.

## References

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