

The overarching goal of this work is to create a colloid-polymer system with temperature-responsive microgel particles (poly(N-isopropylacrylamide), PNIPAM) that exhibits a fluid-fluid phase transition tunable with temperature and to study that fluid-fluid transition using a custom-built light-sheet microscope. As described in the previous progress report, our lab has been successful in synthesizing PNIPAM particles with diameters between ~ 200 - 400 nm. As expected, the size of the particles is temperature-sensitive. Above ~ 32 °C, the particles' diameter shrink by about a factor of two. We add these particles to solutions of xanthan polymer. The polymer results in a depletion attraction between the particles. At high enough PNIPAM volume fraction and xanthan concentration, we observe a fluid-fluid phase transition: the sample becomes demixed with a colloid-rich (or liquid) phase and a colloid-poor (or gas) phase. We can control the state of the sample—either mixed or separated into liquid and gas phases—by changing the temperature. At or above 32 °C, the reduced PNIPAM particle size results in a weaker depletion interaction and a mixed phase. Below that temperature, we see a demixed sample.

We have also been successful in building and using a light-sheet microscope. As described previously, we have been able to clearly observe the different fluid phases using fluorescently-labeled PNIPAM particles and our fluorescence light-sheet microscope. Our light-sheet microscope has undergone a series of upgrades, supported by this grant and other sources. In the last year, we have incorporated a new motorized stage for positioning the sample within the microscope and added a new excitation laser. Additionally, upgrades to our microscope have allowed us to shear samples while being imaged.

As described in the research proposal, we seek to observe fluctuations of colloid-rich liquid droplets using the light-sheet microscope in order to quantify the liquid-gas surface tension. With our synthesized colloidal particles and working light-sheet microscope, we have been able to push forward on this aim. An undergraduate student, Caroline Riedstra, imaged colloid-rich liquid droplets and successfully ran edge-detection image analysis algorithms in order to quantify the fluctuations (Fig. 1). She was also able to do this for droplets that had just nucleated and were still growing by using temperature to bring the sample between mixed and demixed states. Riedstra presented this work at the American Physical Society's (APS) March Meeting in Boston in 2019. Her poster was titled "Quantifying the surface tension of non-equilibrium colloidal fluids" (Abstract L70.00179). Riedstra graduated in 2019 and is currently pursuing her master's degree at the University of San Francisco.

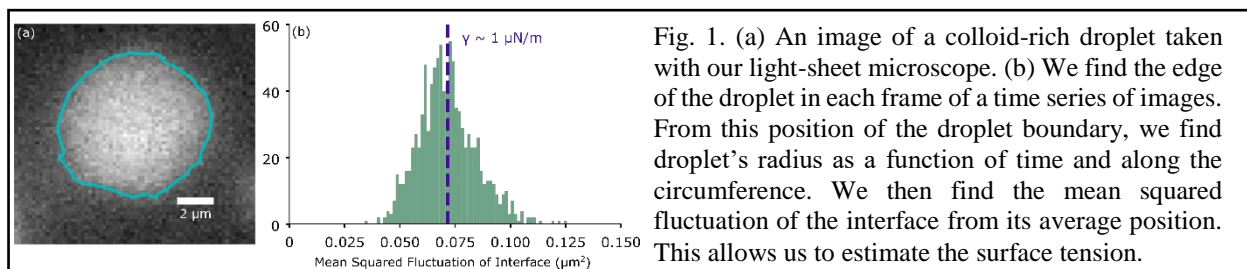


Fig. 1. (a) An image of a colloid-rich droplet taken with our light-sheet microscope. (b) We find the edge of the droplet in each frame of a time series of images. From this position of the droplet boundary, we find droplet's radius as a function of time and along the circumference. We then find the mean squared fluctuation of the interface from its average position. This allows us to estimate the surface tension.

Quantifying the interfacial fluctuations of liquid droplets has been challenging. Given that the surface tension between the colloid-rich and colloid-poor phases of our colloid-polymer mixtures are on the order of $1 \mu\text{N/m}$ we expect interfacial fluctuations to be ~ 0.1 - $1 \mu\text{m}$. As our work has shown, we are able to measure such fluctuations with our light-sheet microscope. However, observing interfacial fluctuations for newly formed droplets is challenging given that the optical contrast between the nascent droplet phase and the outer continuous phase is low. We have therefore been investigating alternative methods to study the interfacial fluctuations of our system as it transitions from mixed to phase separated. One promising method employs differential dynamic microscopy.

Differential dynamic microscopy (DDM) is a technique that extracts scattering-like data from a sequence of images that can be acquired using a number of standard microscopy modalities including bright-field and wide-field fluorescence. For the last couple of years, we have been trying to extend DDM to the measurement of interfacial fluctuations. This past year, an undergraduate student, Jing Wang, has led these efforts with great success (Fig. 2). At the APS March Meeting in 2019 she presented a contributed talk titled "Measuring the dispersion relation of capillary waves using differential dynamic microscopy" (Abstract R58.00012). Furthermore, we have recently published an article in *Soft Matter* with Wang as first author (Wang, J. and McGorty, R., "Measuring Capillary Wave Dynamics Using Differential Dynamic Microscopy," *Soft Matter* 2019 DOI: 10.1039/C9SM01508F).

Our published work on using DDM to study the interface between fluids in a demixed colloid-polymer sample has so far focused on equilibrium fluids. We have begun using this method to study the interface as we transition the sample from a demixed state to a mixed state. Some of these preliminary results were presented at the Frontiers in Soft Matter and Macromolecular Networks symposium held at the University of San Diego in September 2019 in a poster by Jing Wang titled “Probing the fluid-fluid interfacial dynamics of phase-separated colloid-polymer mixtures.”

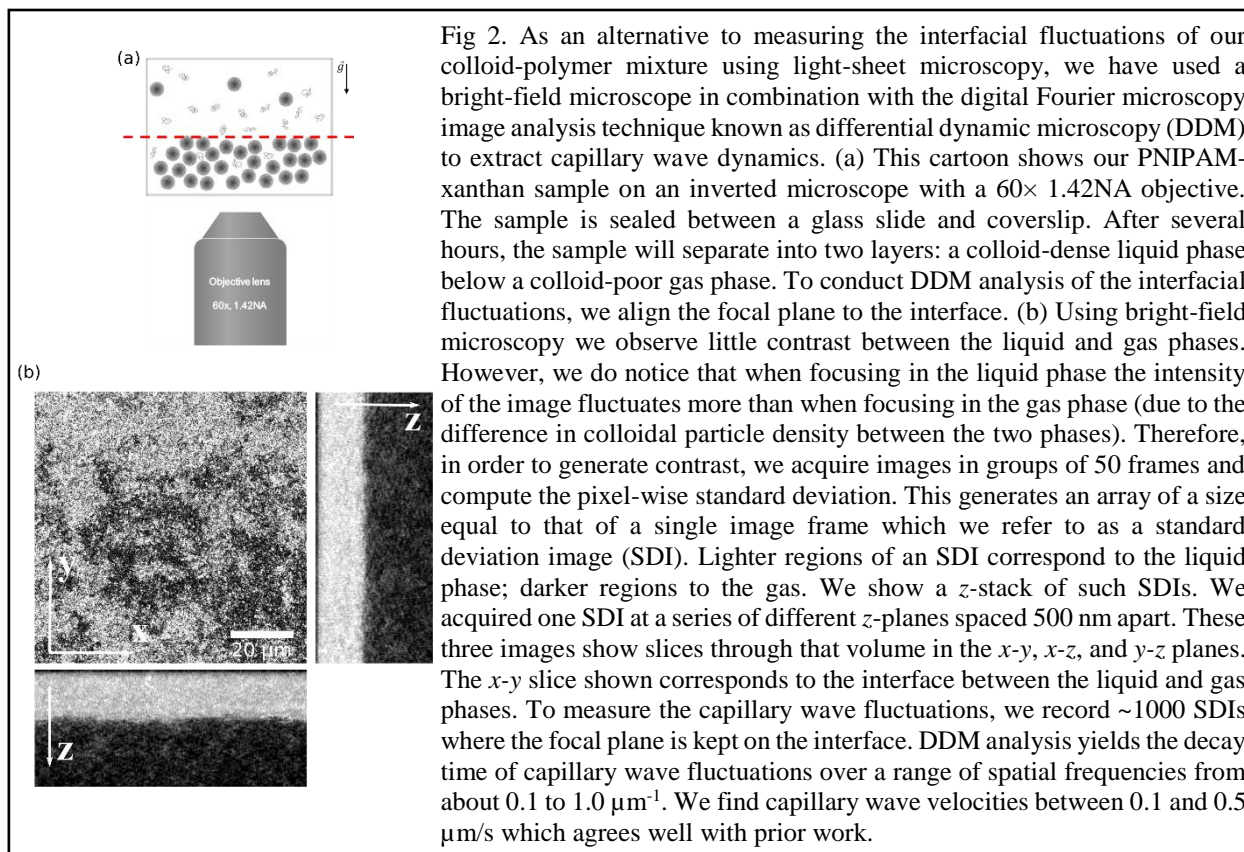


Fig 2. As an alternative to measuring the interfacial fluctuations of our colloid-polymer mixture using light-sheet microscopy, we have used a bright-field microscope in combination with the digital Fourier microscopy image analysis technique known as differential dynamic microscopy (DDM) to extract capillary wave dynamics. (a) This cartoon shows our PNIPAM-xanthan sample on an inverted microscope with a $60\times 1.42\text{NA}$ objective. The sample is sealed between a glass slide and coverslip. After several hours, the sample will separate into two layers: a colloid-dense liquid phase below a colloid-poor gas phase. To conduct DDM analysis of the interfacial fluctuations, we align the focal plane to the interface. (b) Using bright-field microscopy we observe little contrast between the liquid and gas phases. However, we do notice that when focusing in the liquid phase the intensity of the image fluctuates more than when focusing in the gas phase (due to the difference in colloidal particle density between the two phases). Therefore, in order to generate contrast, we acquire images in groups of 50 frames and compute the pixel-wise standard deviation. This generates an array of a size equal to that of a single image frame which we refer to as a standard deviation image (SDI). Lighter regions of an SDI correspond to the liquid phase; darker regions to the gas. We show a z -stack of such SDIs. We acquired one SDI at a series of different z -planes spaced 500 nm apart. These three images show slices through that volume in the x - y , x - z , and y - z planes. The x - y slice shown corresponds to the interface between the liquid and gas phases. To measure the capillary wave fluctuations, we record ~ 1000 SDIs where the focal plane is kept on the interface. DDM analysis yields the decay time of capillary wave fluctuations over a range of spatial frequencies from about 0.1 to $1.0 \mu\text{m}^{-1}$. We find capillary wave velocities between 0.1 and $0.5 \mu\text{m/s}$ which agrees well with prior work.

As mentioned in the last year’s report, an offshoot of the work supported by this ACS PRF grant involves using our light-sheet microscope to study the dynamics of macromolecules diffusing within crowded environments and is supported by an AREA grant from the NIH (R15 GM123420). We have been studying how ring and linear polymers as well as colloidal particles diffuse within different types of entangled or crosslinked networks made from actin and microtubule filaments. Details of this work can be found in an article we published in *Soft Matter* in January 2019 and two more articles currently in review. Multiple students have been involved in this work including two who left the University of San Diego in 2019: one to begin a PhD program at the University of Hawaii and the other a PhD program at Boston University.

In summary, this grant has supported this year **two student presentations at national conferences, one student presentation at a regional conference, and one peer-reviewed publication** (with one more in review). With partial support from this grant, an additional four student presentations have been given at national conferences and an additional one peer-reviewed article has been published (with two more in review). Furthermore, I have been invited to present the work described here at research colloquia at San Diego State University, California State University Fullerton and the University of California Merced. Support from this grant has continued to help steer the research trajectory of my lab. This past year I have received **two external grants**, both of which extend the research described here. One of those grants is a **2019 Cottrell Scholars Award** which will support using our light-sheet microscope to study our colloid-polymer sample when sheared. The other grant is an **NSF Major Research Instrumentation award** on which I am the lead PI and which will bring to campus a state-of-the-art rheometer for investigating rheological properties of our temperature-responsive colloid-polymer mixture (NSF Award 1919429). Additionally, this work this ACS PRF grant has supported is used in three grant proposals that are either pending or soon to be submitted.