

The first aim of this work is to create and characterize a colloid-polymer system that shows a tunable fluid-fluid phase transition. Prior to the awarding of this grant, my lab had worked with systems of various colloid-polymer systems that exhibited fluid-fluid phase separation. However, the goal of this grant is to have a *tunable* system that will undergo a phase transition in response to a change in temperature. Therefore, over the summer of 2017 I worked with an undergraduate student to synthesize temperature-sensitive microgel particles (poly(N-isopropylacrylamide), PNIPAM). We successfully made ~160-nm-diameter particles. Using differential dynamic microscopy—a digital Fourier microscopy technique that can be used to generate data similar to dynamic light scattering—we were able to determine the particle size as a function of temperature and found that at around ~32 °C, as expected, the particles shrink by about 25% of their radius. Using a mixture of such particles and xanthan we were able to create a system that displayed a temperature-sensitive fluid-fluid phase transition. This work led to the first student presentation resulting from work related to this grant at a regional conference: “Liquid-Gas Phase Separation in Colloid-Polymer Systems” presented by Andrew Boghossian at the University of California at San Diego’s Summer Research Conference in August 2017.

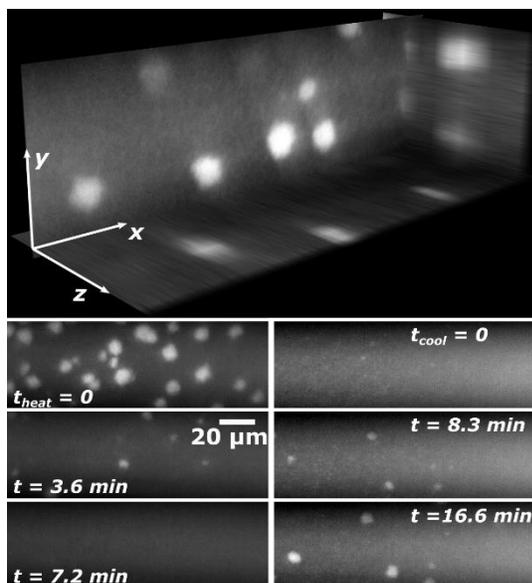


Fig. 1. (a) Using our light-sheet microscope we acquire images of our PNIPAM-xanthan sample over a volume of $145\mu\text{m} \times 48\mu\text{m}$ in x - y and $60\mu\text{m}$ in z (the optical axis). (b) As the sample is heated to 34°C , we observe colloid-rich droplets disappear (shown are z -projections over $60\mu\text{m}$). (c) Cooled back to room temperature, we observe droplet growth (same z -projections).

objective). In the summer of 2018, I used matching funds to purchase a new, faster and higher-resolution stage to position the sample within our microscope (Standa Ltd.). We are currently working to install that new stage. Lastly, over the summer of 2018 we upgraded the fluorescent filter set.

In addition to working to optimize the hardware of our light-sheet microscope, we have been developing computational methods to efficiently and precisely extract the dynamics of colloidal particles imaged with our system. While the grant proposal focused on the instrumentation needed to investigate fluid-fluid phase separation in a colloidal system, ways to quantitatively analyze our light-sheet-acquired images are also essential. During the summer of 2017, an undergraduate student and I developed methods to enhance differential dynamic microscopy. The methods allow us to capture faster dynamics and dynamics in all three-dimensions. We described our results in the first publication coming from grant-related work: Wulstein, D. M. & McGorty, R. “Point-spread function engineering

The temperature-sensitive PNIPAM particles were used during the 2017 fall semester in an advanced biophysics lab course I taught. Students studied fluid-fluid phase separation using a variety of microscopy techniques. An undergraduate student in that course, Caroline Riedstra, continued doing research in my lab over the following semester, this past summer and into this current semester. She described her work in the second student presentation resulting from this work at a campus-wide research symposium in April 2018: “Characterizing Liquid-Liquid Phase Separation.”

The first batches of PNIPAM synthesized in the summer of 2017 were not fluorescently labeled and therefore we were not able to observe them using our light-sheet microscope as planned. Therefore, during the summer of 2018 I worked with an undergraduate student to make fluorescent PNIPAM particles. We succeeded in making fluorescent particles that again exhibited the desired temperature-responsivity. We have been using these particles in our light-sheet microscope, following the plans described in the grant proposal, as well as in a laser-scanning confocal microscope. Data acquired with our light-sheet microscope is shown in Figure 1.

The second aim is to observe nucleating liquid droplets of the emerging colloidal fluid with optical microscopy. Specifically we are using light-sheet microscopy and the proposal detailed how light-sheet microscopy will allow for fast, 3D imaging and will be amenable to work with undergraduate students. Students and I have been working to optimize our light-sheet system. In the fall of 2017, I acquired a higher-quality imaging objective lens (an Olympus $20\times$ 1.0 NA water-dipping

enhances digital Fourier microscopy.” *Optics Letters* **42**, 4603–4606 (2017). The results were also described by an undergraduate, Devynn Wulstein, presenting at a regional conference, Frontiers in Soft Matter and Macromolecular Networks held at the University of San Diego in September 2017: “New microscopy techniques for probing soft materials.”

This grant spurred my lab’s work on optimizing a light-sheet microscope and accompanying image analysis software for the purpose of extracting dynamics. Such work and the results that followed led me and a colleague in my department to collaborate on a project to study the dynamics of DNA using my light-sheet microscope. We sought funding from NSF and the NIH for such work and were successful in securing an AREA grant from the NIH (R15 GM123420 “A novel in vitro microscopy suite to elucidate intracellular transport and conformational dynamics of nucleic acids”). Data acquired using my lab’s light-sheet microscope, supported by both this grant and the NIH grant, was used in two additional student presentations: a contributed talk by Devynn Wulstein, “Selective-plane illumination microscopy to characterize diffusion of DNA in cytoskeletal networks,” at the 2018 March Meeting of the American Physical Society and a poster presentation of the same title at a campus-wide research symposium in April 2018.

The third aim of this grant is to track growing nuclei and analyze the interfacial fluctuations to determine the surface tension. It is expected that this aim will be accomplished over the 2018-19 academic year and during the summer of 2019. Students have begun developing image analysis methods to extract the surface tension from droplet fluctuations. We have also been investigating other methods to extract the surface tension. A novel method we are currently developing and validating involves using differential dynamic microscopy to measure the dispersion relation of capillary waves in 2D (see Fig. 2). This involves using an inverted bright-field microscope and imaging the planar interface between the denser colloid-rich phase and the less dense colloid-poor phase.

In summary, this grant has led to **five student presentations** and **one peer-reviewed publication**. We have successfully synthesized temperature-sensitive microgel PNIPAM particles and optimized our light-sheet microscope. New methods for extracting dynamics have been developed and we have another novel method we hope to describe in an upcoming publication. We are excited to complete the final aim of this work in the second year of this grant and to publish the results. Furthermore, this grant has helped steer the research trajectory of my lab. It has generated data used in one **successful grant application to the NIH** and **two additional grant applications** which are currently pending (Cottrell Scholars Award and an NSF CAREER application).

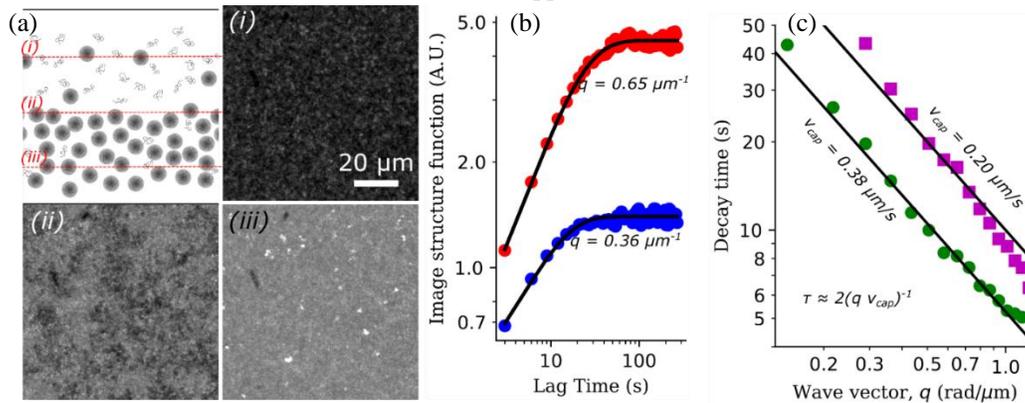


Fig 2. (a) Our PNIPAM-xanthan sample is sealed between a glass slide and coverslip. Hours after sealing such a sample, the denser colloidal-liquid phase settles to the bottom. The standard deviation (SD) of 200 frames are shown at three different planes of a colloid-polymer fluid-fluid demixed sample (same intensity scaling used for all three images). (i) The SD of images acquired in the gas phase is low and uniform as there are few particles. (ii) The SD of images at the interface show structure on the order of 10s of μm . (iii) The SD of images in the liquid phase is, like (i), homogenous though higher due to more particles present. (b) To analyze a time series of SDs of images we use differential dynamic microscopy to compute the image structure function $D(q, \Delta t)$, where q is the wavevector and Δt a lag time, and fit it to $D(q, \Delta t) = A(q)[1 - e^{-\Delta t/\tau(q)}] + B(q)$. Shown are $D(q, \Delta t)$ for two different wavevectors of the same sample and the fit. (c) For overdamped capillary waves in the regime where the product of the wavevector and capillary length is greater than one, we expect fluctuations of each mode with wavevector q to have a characteristic decay time $\tau_q = 2/(v_{cap}q)$ where v_{cap} is the ratio of surface tension to viscosity, γ/η . Our data follows this expectation and we show the determined capillary velocities for two different PNIPAM-xanthan samples. The capillary velocities found using this method agree with what we find from the analysis of droplets coalescing and are of the same order of magnitude as values reported in the literature of similar samples.