



Traction Rheoscopy

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October 5, 2022

Traction Rheoscopy

A DISSERTATION PRESENTED

BY

J. ZSOLT TERDIK

TO

THE DEPARTMENT OF SEAS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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IN THE SUBJECT OF

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Traction Rheoscopy

ABSTRACT

Micron sized colloidal particles, which interact with nearly hard-sphere potentials, can be used to form dense amorphous packings using a variety of well established techniques. Due to the large size and slow dynamics of colloidal particles, confocal microscopy can be used to directly measure the 3D structure and dynamics of these densely packed colloidal glasses. For example, previous results have directly visualized both the inhomogeneous particle level rearrangements that occur within a colloidal glass under applied shear strain, and the emergence of continuum strain fields surrounding these particle rearrangements as a function of applied shear strain. While microscopic visualization of colloidal glasses provides real-space information not readily obtainable in atomic systems, measuring the stress response of colloidal glasses, in addition to visualization, is a significant challenge. The large micron size of the colloidal particles and thermal interaction energies necessarily give rise to solids with exceptionally small elastic moduli on the order to 1 Pa . We introduce a new technique, traction rheoscopy, to directly measure the mechanical response of colloidal glasses while simultaneously visualizing microstructure. The method consists of a bilayer of colloidal glass sitting atop a well calibrated soft polymer gel of comparable shear modulus. The composite bilayer is sheared and the shear stresses are inferred from the displacement of embedded tracer particles in the calibrated polymer gel. Using these stress measurements, we show that under applied shear the colloidal glass

goes through as sequence microscopic rearrangements as the stress develops correlated spatial heterogeneities. The stress measurements with simultaneous real space visualization of the particle dynamics corroborate previously reported microscopic flow mechanisms and present new insight into how colloidal glass responds to applied shear. This thesis presents experimental techniques and protocols developed for traction rheoscopy, along with results on the mechanical response of colloidal glass under increasing applied shear strains ranging from $\sim 0.1\%$ to 10% .

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I DEDICATE THIS THESIS TO MY FATHER.
NAGYON SZERETLEK APUCI.

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0

Traction Rheoscopy

THIS CHAPTER OUTLINES the operating principle of traction rheoscopy and the primary experimental challenges that need to be overcome to realize traction rheoscopy measurements. It does not include protocols but those are included in the subsequent chapters.

0.1 WHAT IS TRACTION RHEOSCOPY?

Traction rheoscopy is a method to experimentally measure mPa-scale stress in colloidal systems undergoing mechanical deformation. The technique consists of placing a colloidal solid in mechanical contact with a soft elastic substrate with comparable modulus. The colloidal solid and soft elastic substrate form a bilayer, which is then sheared as shown in the x direction while imaged from below with a confocal microscope. A schematic cross sectional slice is shown in Figure 1. The modulus of the soft elastic substrate is measured on a traditional rheometer. Additionally, tracer particles are embedded in the soft elastic substrate. Using a confocal microscope, the displacement of tracer particles is measured as the bilayer is sheared. From these measurements and calibration of the soft elastic gel, the average stress across the bilayer is determined, and hence the average stress in the colloidal solid as shown in Figure 2. Mechanically, the bilayer is analogous to two springs in series under controlled strain deformation; in this analogy one of the moduli of the springs is known, while the other is the sample that is mechanically characterized. Confocal microscopy is also used to measure the position and displacement of particles within the colloidal solid which is used to determine the local strain within the colloidal solid. The combination of independent stress measurements along with single particle visualization of the internal structure and local deformation of the colloidal solid enable microscopic description of elastic and plastic deformation in colloidal solids. The technique is demonstrated using a strain ramp on colloidal glass.

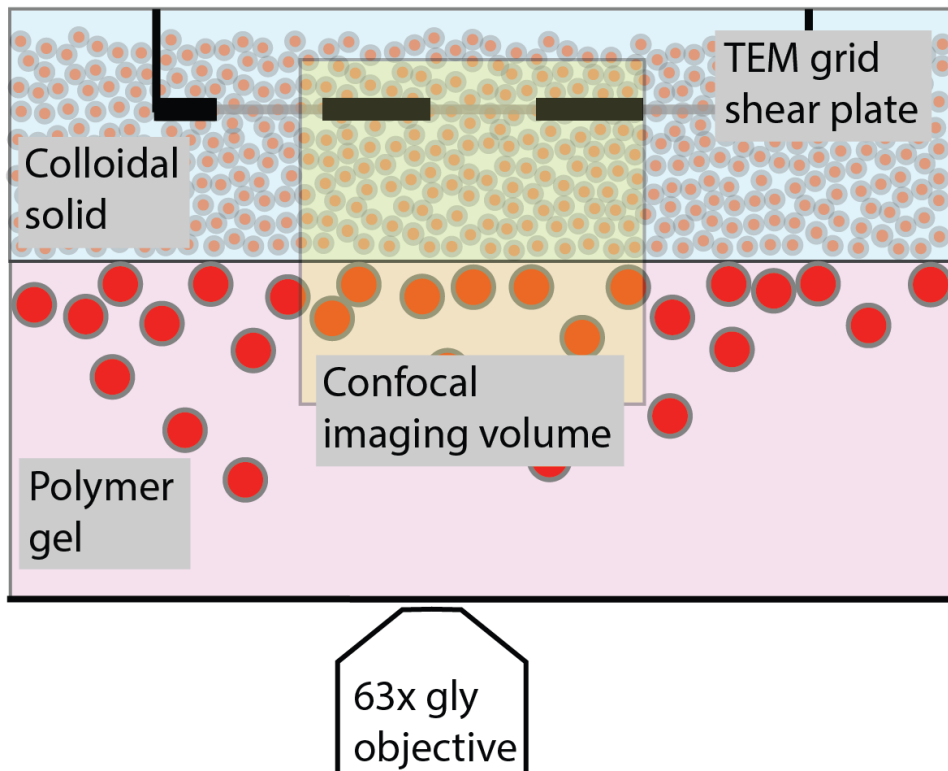


Figure 1: A schematic plot of the bilayer geometry used in traction rheoscopy. A soft polymer gel makes up the lower layer and is shown schematically in pink with red tracers. On top of the soft polymer gel, a colloidal solid is shown in light blue with smaller tracer particles. An externally imposed shear is applied from the top of the bilayer; a TEM grid is embedded in the solid and functions as a shear post. By measuring the deformation of the gel, we can determine the average stress in the bilayer. The geometry is conceptually similar to parallel plate rheometer, albeit the force transducer is polymer gel and the measurements of the deformation are made optically with a confocal microscope.

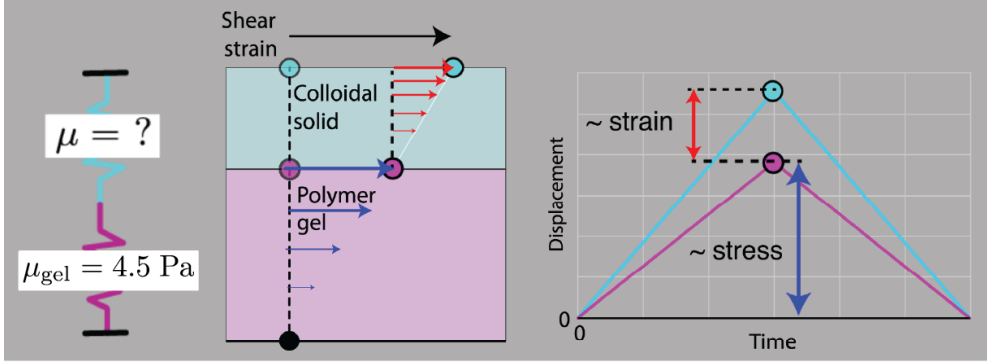


Figure 2: The operating principle in traction rheoscopy is presented in a simplified case of linear elastic solid with unknown modulus. The bilayer geometry used in traction rheoscopy is drawn. The deformation across the bilayer is directly measured using the displacement of particles in the gel and sediment layers. Using these displacement, the displacement of the top surface of the sample relative to the top surface of the gel is direct measure of the strain applied to the sample. The displacement of the top surface of the gel relative to the coverslip, which serves as no-slip boundary and is stationary, is a direct measure of the strain in the calibrated polymer gel, and hence the stress in the sample. This geometry and boundary conditions are analogous to displacement controlled extension if two springs in series, with a one spring having an unknown spring constant.

0.2 RESOLUTION

One central challenge in linking mechanical response to microscopic mechanisms is the stress scale typical for soft systems in which thermal energy is the relevant energy scale. Back of the envelope estimates of the required stress resolution in these systems can be made by computing the thermal energy over a typical volume²⁶. For colloidal materials, the typical volume Ω is taken to be the volume of single particle. Moreover, for colloidal systems where confocal imaging is crucial, a relatively large particle diameter is required to ensure a single particle locating resolution that is, ideally less than 1% of the particle diameter. In the work presented here I use $d = 2 \mu\text{m}$ diameter particles. At this size and energy scale typical stress are $kT/\Omega \approx 1 \text{ mPa}$, and the diffusive timescale $\tau = (D_0/d^2)^{-1}$ is 1 min, where D_0 is the diffusion coefficient in the dilute limit. .

Traction rheoscopy is developed with specific aim of measuring these minute stresses. Simple back of the envelope estimates are illustrative in estimating the sensitivity of the technique. For simple shear deformation (with the shear in the x direction), the stress is determined by displacement displacement the tracer particle divided the elastic gel thickness. The resolution in traction rheoscopy is, ultimately, limited by the smallest resolvable displacement obtainable from particle locating⁹.

$$\begin{aligned}\sigma_{xz} &= G \cdot \varepsilon = G \cdot \frac{\gamma_{xz}}{2} = \frac{G}{2} \cdot \frac{\delta x}{h} \\ &= \frac{5\text{Pa}}{2} \cdot \frac{20\text{ nm}}{150\text{ }\mu\text{m}} \\ &= 0.3\text{ mPa.}\end{aligned}\tag{1}$$

Many technical challenges have to be overcome to reach this resolution target including preserving the quality and signal to noise of the confocal images. The majority of this thesis is focused on the development of the novel materials, fabrication protocols, and technologies used in traction rheoscopy to achieve single mPa stress resolution along with single particle resolution of the colloidal glass. These technologies include the development of a novel pair of colloidal particle-solvent systems that can be dispersed in a pair of immiscible solvents with identical refractive indices; modular shear cell than can mounted on confocal microscopes; soft polymer gels with independently tunable refractive index and moduli near 1 Pa; fabrication protocols to form thin layers of near-critical polymer gels using sacrificial sugar glass capillaries; centrifugation protocols to control the phase behavior of the hard sphere colloidal particles by controlling the deposition flux of hard spheres; data

analysis pipelines to handle multi-Tb image data sets resulting from volumetric confocal stacks acquired during extended shear ramps; nonparametric estimates of noise distribution particularly for local strains where the fluctuation in strain are comparable to resolution limit of particle locating; and identification of dimers and other fluorescent defects that are not captured by particle locating routines.

In addition to these technological developments, I measure stress-strain curves for hard-sphere colloidal glass sample undergoing an sequence of increasing shear strains ranging from 0.1% to 10%. I identify local clusters of particles that undergo local strains significantly larger than the incrementally applied strain. Future work will be dedicated to relating the wealth of microscopic structural information in colloidal sediment with the mechanical properties measured with traction rheoscopy.

0.3 TRACTION RHEOSCOPY AND THE WIDER WORLD OF RHEOLOGY

The underlying principle of traction rheoscopy is identical to most other common mechanical testing: the material of unknown mechanical properties is placed into mechanical contact with a material with known mechanical properties, and then composite is deformed. By measuring the deformation of the calibrated body, you can infer the stress; by measuring the deformation of the unknown material you can determine the strain. The challenge for describing the mechanical response of soft materials is to image the internal structure of the material alongside stress measurement.

To address these challenges, several groups have combined mechanical testing in the form of bulk rheology with confocal microscopy by integrating a confocal microscope with a commercial

rheometer^{11,21} or using piezo strain gauge sensor attached to a custom shear cell²⁵. This work has typically focused on stiffer materials with smaller particle sizes²¹, attractive interactions, or fast shear in shear thickening regimes⁸. During the development of this work, a novel technique known as Boundary Stress Microscopy was developed by Dan Blair's group at Georgetown³, using essentially the same operating principle as traction rheoscopy: a polymer gel with embedded tracers was placed on a combined rheometer-confocal. By tracking the deformation of the gel, spatially resolved stress were measured in a variety of system including shear thickening suspensions^{28,29} and collagen networks³. Boundary Stress Microscopy is conceptually very similar to traction rheoscopy, albeit I have focused on using near-critical gels to achieve mPa stress resolution on considerably more compliant materials. Additionally traction rheoscopy does not involve a rheometer or any stress sensing apart from stresses inferred from the deformation of the gel.

1

Soft gels as force transducers

In traction force microscopy (TFM), the deformation of elastic substrate is used to measure the forces applied to the boundary of the substrate, while visualizing the sample above. Typically a transparent polymer gel with calibrated elastic properties is used as a substrate. Embedded tracer particles within the gel are located and tracked with a confocal microscope, that is also used to visualize the sample above the gel. Traction force microscopy and related techniques^{35,4}, have been used to

measure a wide range of biological and physical phenomena spanning orders of magnitude in stress including single-cell traction forces with magnitudes of 10 Pa and interfacial cracks of drying colloidal films with magnitudes of 100 kPa. This stress resolution, while spanning orders of magnitude, is nevertheless significantly larger than stresses encountered in rheology of soft solids. An order of magnitude estimate of modulus G of soft solids with length scale L can be made by assuming the interaction energies are thermal, $Gk_B T/L^3$. For a prototypical soft material such as a hard-sphere colloid dispersions of 1 μ m particles, this estimate gives the range of expected stress as 1 mPa. Extending TFM to this range of stresses requires compliant substrates and high resolution imaging to ensure measurably large displacements of the tracers from which to infer the deformation. In addition to limitation on the range of stresses that are measurable, existing elastic substrates are not typically index matched to the immersion oil of the objective. This refractive index mismatch, combined with the thickness of gel give rise to spherical aberrations that degrade the spatial resolution when imaging the sample through the substrate using confocal microscopy^{17,12}.

In this chapter, we formulate and characterize poly-dimethyl-diphenyl-siloxane (PDPS) gels with finely tunable elastic moduli approaching the gel point with independently tunable refractive index. The combination of mechanical and optical properties of these near-critical gels extends the range of stresses that can be measured with traction force microscopy to single mPa while minimizing optical aberrations encountered during confocal imaging through the substrate. Mechanically the gels exhibit true elastic solid response at low frequencies, with plateau storage moduli of single pascals and loss moduli that are approximately fifty times smaller. Conveniently, the near-critical gels are mixed from commercially available components and require no synthesis. Chemically, the gels differ from

poly-dimethyl-siloxane (PDMS) by substitution of dimethyl side groups for diphenyl groups along a siloxane backbone. Given the chemical similarity to commercial PDMS formulations used in TFM including Sylgaard-184 and CY52-276, both from Dow Corning, we propose them as substitutes in existing TFM protocols based on commercial PDMS formulations, especially in cases where more compliant substrate is desired or to reduce spherical aberrations that arise when imaging through the gel. The materials and methods outlined in this chapter are used to create soft elastic substrates used in traction rheoscopy measurements of colloidal solids.

1.1 FORMULATION PROTOCOL FOR NEAR CRITICAL PDPS GELS

PDMS elastomers at the gel points were first mechanically investigated by Henning Winter and Francois Chambon^{7,38}. In that work, a crucial experimental technique was developed to arrest gelation by poisoning the catalyst. This allowed for mechanical characterization of the gels arrested at the gel point. Subsequent work developed a second method of approaching the gel point by crosslinking the reactive components in the presence of non-reactive liquid filler³¹. We use this second approach with similar crosslinking chemistry consisting of platinum catalyzed vinyl-hydride addition^{24,23}.

The PDPS gels are formulated in three steps: formulation of stock solutions containing reactive components in nonreactive liquid filler, mixing of these stock solutions to prepare a pre-gel solution that contains all reactive components along with additional nonreactive liquid filler, and curing of the pre-gel solution by the addition of catalyst. Five stock solutions are required: (1) liquid filler

with the target refractive index, (2 and 3) base solutions of vinyl terminated PDPS polymers with refractive index lower, and higher, than the target refractive index, (4) hydride terminated crosslinker solution, and (5) platinum catalyst solution.

1.1.1 LIQUID FILLER

First liquid filler stock solution is prepared by mixing two nonreactive PDPS oils (PDM-1922 and PDM-0821, Gelest) that have larger and smaller refractive indices than the target refractive index. The variation in refractive index near the target refractive index for glycerol immersion objectives is plotted in Figure 1.1 as function of ratio of higher refractive index component. This liquid filler solution will serve as diluent for the remaining stock solutions, and will be added as component in the pre-gel solutions to tune the mass ratio of liquid filler. A stock solution of several hundred grams total is mixed at the target mass ratio; after mixing the refractive index is measured with Abbe refractometer and adjusted if necessary.

1.1.2 BASE SOLUTIONS OF VINYL TERMINATED REACTIVE POLYMERS

Second, a high molecular weight, vinyl terminated diphenyl-dimethyl siloxane with higher refractive index than the target (PDV-1641, Gelest) is mixed in the liquid filler stock solution. Third, a vinyl terminated base with lower refractive index (PDV-0541, Gelest) is mixed with liquid filler. The base solutions are slowly tumbled overnight to ensure adequate mixing of the viscous components. Dilution with liquid filler has the benefit of lowering the viscosity. The mass ratio of vinyl terminated polymers in these base solutions is set so that an even mixture of the two base solutions will yield a

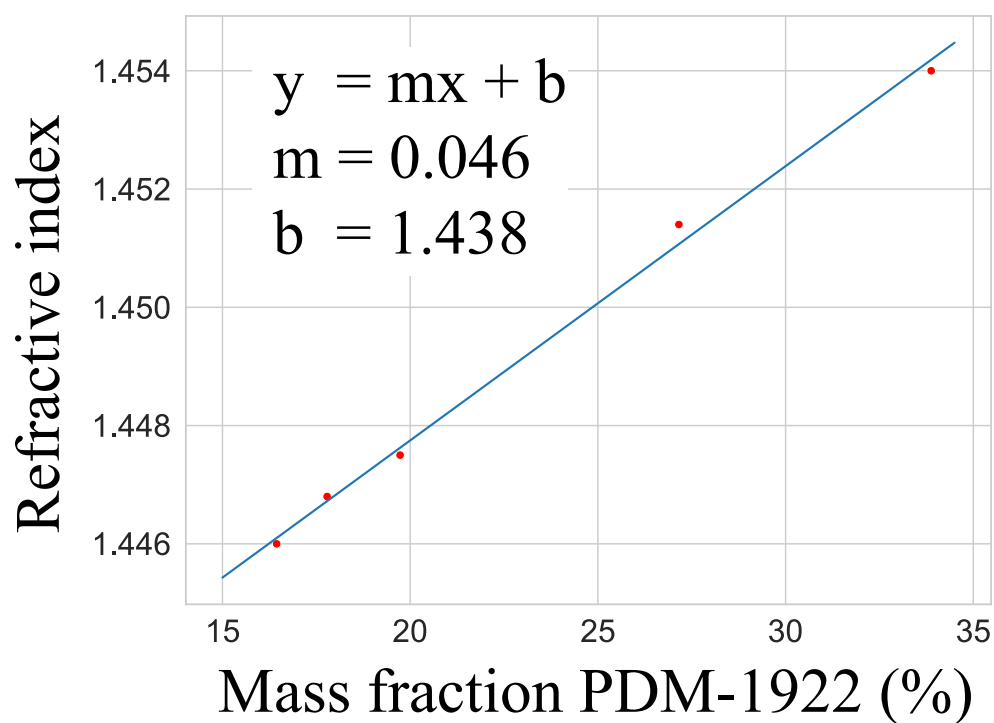


Figure 1.1: Refractive index variation of non-reactive diphenyl substituted silicone oils (PDM-1922 and PDM-0821). This calibration curve is used to determine precise mass fractions of silicone fluids required to achieve target refractive index of 1.45.

volume weighted average refractive index equal to the target refractive index. Hence, the mass ratio used to prepare the base solution will vary depending on the lot-specific refractive indices of the neat polymer. Note, that due to the high molecular weight of these reactive polymers, they are not miscible with each other, hence calibration measurements of refractive index, analogous to Figure², but with high MW reactive polymers in place of low MW silicone oils, are not possible. Nevertheless, the base polymers are soluble in the presence of liquid filler in the pre-gel solutions. Moreover they are expected to form random copolymers during gelation thereby homogenizing the refractive index. After gelation the pre-gel solutions form transparent networks indicating the absence of phase separation.

1.1.3 CROSSLINKER SOLUTION

Fourth, a polymeric, hydride terminated crosslinker (Gelest, HDP-111) is dispersed in liquid filler such that equal mass ratio of the two vinyl base solution and the crosslinker solution yields hydride to vinyl mol ratios of ~ 4 -5. Hydride to vinyl mol ratios larger than one, corresponding to an imbalanced stoichiometry of reactive components, is used to ensure that the vinyl terminated components are fully reacted and incorporated into the network. In principle, this ratio can be adjusted to maximize the ratio of G'/G'' in the cured gel⁷, however no significant variation was found for the gels used here; the measured ratio $G'/G'' > 50$ at low frequencies comparable to commercial PDMS formulations. The polymeric hydride terminated crosslinker is a minority component of the gel, making up less than 1% by mass. While at the lower concentration of the fully mixed pre-gel solutions, the crosslinker is soluble prior to gelation, the mass ratios used for the stock solution is beyond

the solubility limit as evidenced by cloudiness. A more dilute solution of crosslinker could be prepared and used by re-appropriating liquid filler from either the base stock solutions, thereby creating more viscous base solutions, or the final diluent step, at the expense of a larger minimum liquid filler mass fraction. Instead of these alternatives we opt to mix crosslinker solution, as needed, until a homogeneously cloudy solution is achieved.

1.1.4 CATALYST SOLUTION

Fifth, a room temperature catalyst (Gelest, SIP-6832.2LC) is dispersed 10% by weight in liquid filler. No inhibitors are used; addition of $\sim 10 \mu\text{L}$ of catalyst solution per gram of pre-gel solutions yields a work time of several minutes. Other platinum catalysts can be used for elevated temperature curing (Gelest, SIP 6833.2). Diethyl maleate can be added at ppm concentrations to extend the work time²³, however we find that near the gel point, addition of inhibitors increases the time for complete gelation, and gives with inconsistent mechanical properties. Decreasing the catalyst concentration is preferred method of increasing the work time; decreasing the catalyst concentration from $p=10 \mu\text{L/g}$ to $p=7 \mu\text{L/g}$ increases the work time by an order of magnitude. Representative curing time curves are shown in Figure 1.2

1.1.5 MIXING PRE-GEL SOLUTIONS

Using these stock solutions, a range of pre-gel solutions with varying liquid filler content and hydride to vinyl ratios can be easily prepared. We use convention of $b_1:b_2:c:\lambda$ to specify the mass ratios of the stock solutions. The first three components (b_1, b_2 , and c) are neat polymers of vinyl termi-

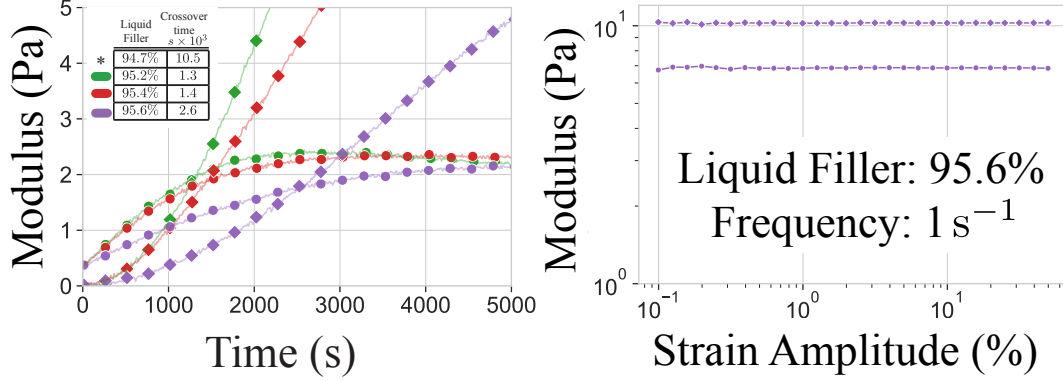


Figure 1.2: Time evolution of viscoelastic modulus during crosslinking of PDPS pre-gel solution. Diamonds correspond to storage moduli, while circles correspond to loss moduli.

nated base (b_1, b_2) and hydride terminated crosslinker (c) that have been diluted with liquid filler, while the fourth component λ is additional liquid filler used to achieve the desired mass ratio. Given the formulation of the stock solutions, for mass ratio of $b_1 = b_2$, the refractive index of the gel solution is independent of the amount of liquid filler added. Using this convention, we have the total liquid filler mass fraction ℓ as function of additional liquid filler λ :

$$\ell = \frac{(1 - 0.198) \cdot b_1 + (1 - 0.167) \cdot b_2 + (1 - 0.911) \cdot c + \lambda}{b_1 + b_2 + c + \lambda}, \quad (1.1)$$

where the numerical pre-factors correspond to the mass fraction liquid filler in the stock solutions. Finally, we compute the hydride to vinyl mol ratio r , using the hydride equivalent weights, $h_{eq} = 150$ for HDP-III and vinyl equivalents per kilogram $v_{1,eq} = 0.033$ and $v_{2,eq} = 0.027$ for

PDV-1641 and PDV-0541 respectively:

$$r = \frac{(0.011 \cdot c) \cdot h_{eq}}{(0.198 \cdot b_1 \cdot v_{1,eq} + 0.167 \cdot b_2 \cdot v_{2,eq})/1000} \approx 6.64 \cdot \left(\frac{c}{b}\right), \quad (1.2)$$

where the numerical pre-factors in the first equality correspond to the weight fractions of reactive polymer in the stock solutions. In the approximate and simplified equation, the ratio of base stock solutions is set such that $b_1 = b_2 = b$ in order to fix the refractive index. For the gels used here, $(b_1 : b_2 : c : \lambda) = (1 : 1 : 0.7 : \lambda)$ corresponding to $r = 4.6$ and $\ell = \frac{2.3}{2.7+\lambda}$

1.2 MECHANICAL CHARACTERIZATION

The gelation threshold is approximately 96% liquid filler by mass. Pregel solutions are prepared with target liquid filler fractions that are above and below this threshold. After tumbling overnight, 10 μ L of catalyst solution is added to 1 gram aliquots of the stock solution and tumbled by hand for 30 seconds. The gel solution is then loaded onto a on a strain controlled rheometer (Ares G2) with 50mm parallel plate. Typical gap heights are 250-350 μ m, and 25 μ m trim gap. Rheological calibration steps are (1) curing at 25deg C while monitoring the gelation with small strain oscillation (1%) for 24 hours, (2) frequency sweep from 10 rad/s to 10^{-3} rad/s, (3) additional curing step for 12 hours, (4) additional frequency sweep, and (5) strain sweep at frequency of 0.1 rad/s from 0.1% strain to 50%. As shown in Figure 1.2, the time of G'/G'' crossover occurs at approximately 1000 seconds, with a full cure after 48 hours. Longer cure times with typically crossover of 10,000 seconds are achievable with catalyst concentrations of $p=7\mu$ L/g.

Carrying out rheology tests on the two pre-gel solutions establishes the upper and lower boundaries of the shear modulus. Any target shear modulus between these boundaries can be reached by mixing these pre-gel solutions at variable mass ratios, measuring the corresponding shear modulus, and iterating the process with an adjusted the mass ratio. If the target shear modulus lies outside the boundaries, new upper and lower boundaries can be established using the existing pre-gel solutions, and treating pure liquid filler as lower boundary. This is a simple process of “leapfrogging” in which a new lower bound is established by diluting one of the existing pre-gel solutions, preferably the pre-gel solution with higher shear modulus, with liquid filler. Once a pre-gel solution of the target shear modulus is measured, the remaining pre-gel stock solutions are mixed to the corresponding target mass ratio. Rheological calibrations are repeated on an aliquot of this pre-gel solution with target mass ratio. If the modulus is unchanged, a third aliquot is prepared from which the TFM sample can be prepared and a final calibration. In my experience, if the modulus is not repeatable or the gelation threshold occurs at liquid filler mass fractions that are more than a few tenths of a percent different from previous formulations, the most likely culprit is insufficient mixing of the stock and pre-gel solutions; this is especially true of the base solutions. If the sample does not gel, the most likely culprit is degradation of the catalyst solution. Pre-gel solutions, without the addition of catalyst, can be stored for more than a month at room temperature without affecting the cured modulus.

The procedure outlined above was used to generate a series of gels with low frequency plateau modulus extending from 50 Pa to 5 Pa as shown in Figure 1.3. The frequency dependent loss modulus has an approximate $2/3$ power law dependence on the frequency f , consistent with relaxation

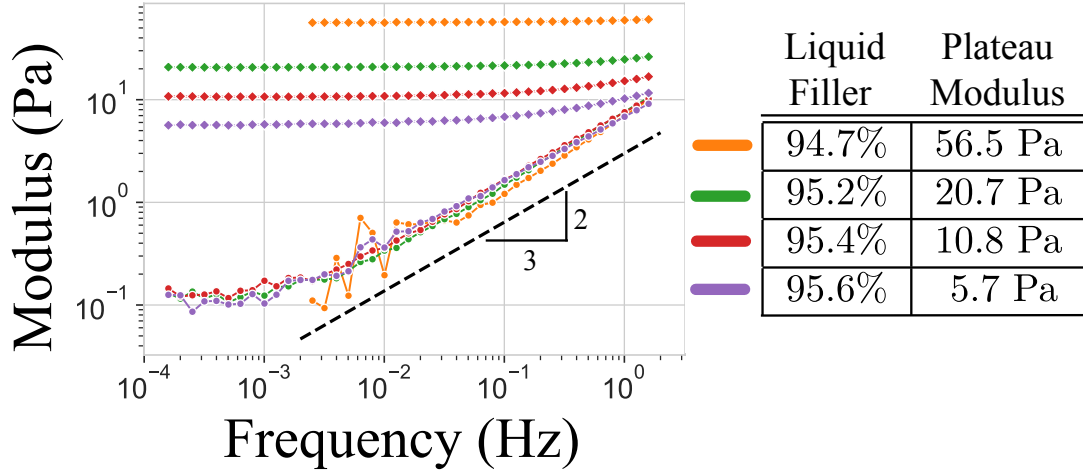


Figure 1.3: Frequency dependent viscoelastic moduli of near-critical PDPS gels. A function of mass percent liquid filler, the low frequency plateau modulus of the gels is tunable from 50 Pa to 5 Pa. Each gel exhibits true elastic solid behavior at low frequency with storage to loss modulus of >50 .

exponents in stoichiometrically imbalanced PDMS elastomers at the gel point^{38,31}. Importantly, the low frequency behavior of the PDPS gel presented here are comparable to commercial PDMS elastomers, and behave as true elastic solids at low frequency ($G'/G'' > 50, f < 10^{-3}$). The strain sweeps, conducted at frequency of $f = 1 \text{ s}^{-1}$ show no variation in modulus up to 50% strains as shown in Figure 1.2. Given these calibration results, a 5 Pa gel fabricated to 100 μm thickness can be used to measure stress over three decades in magnitude: 2.5 mPa to more than 2500 mPa by making use of the back of the envelope estimates of stress resolution with single particle locating.

1.3 EVALUATION OF OPTICS

Spherical aberration in confocal microscopy play a dominant role in image degradation for otherwise transparent samples, especially for deep confocal imaging^{17,12}. By index matching the gel to the

immersion oil of the objective, these spherical aberrations can be eliminated. To evaluate and quantify the improvement in optics obtained by using refractive index matched PDPS gels as substrates, we image a colloidal crystal sedimented onto index matched PDPS gel and commercial Sylgard formulation. The colloidal particles used in both samples are from identical batches and all imaging parameters, including laser intensity, exposure time, and confocal pinhole size, are held constant. We see a pronounced improvement in the signal intensity of the colloidal particles directly above the polymer gel substrates. Imaging through the PDMS gel, with mismatched refractive index, induces a three fold reduction in peak signal intensity as shown by comparing the pixel intensity through the center of the small crystalline patch directly above the elastomer substrate. Additionally, we estimate the signal to noise, by simulating an approximate gaussian blob with added uniform random noise. The distribution of the noise spectrum is adjusted until the simulated image matches raw experimental image. We see a 4-5 fold increase in the signal noise obtained by matching the refractive index. We note the uncertainty in particle locating is inversely proportional to the signal-to-noise ratio, and hence a 4-fold increase in the signal to noise is expected to reduce particle locating uncertainties by a factor of 4.

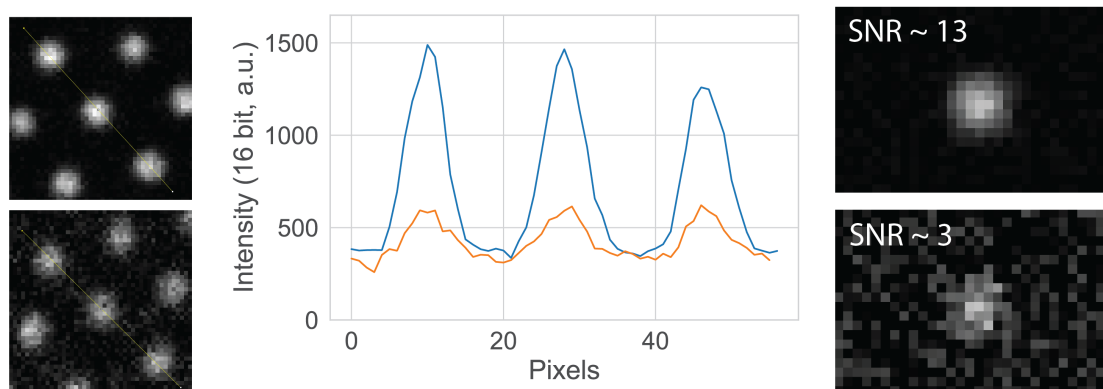


Figure 1.4: Comparison of optical aberration and signal to noise improvement obtained by matching the refractive index of elastic gel substrate to the immersion media of the microscope

2

Particle synthesis

2.1 INTRODUCTION

Traction rheoscopy consists of a compliant polymer gel phase, and a second phase consisting of the colloidal dispersion. Both phases contain colloidal particles that serve as tracers in the polymer gel, or make up the colloidal dispersion in the second phase. In addition to mechanical constraints to ensure that mPa scale stress induce measurable deformation on the polymer gel, there are chemical

and optical constraints to ensure that the two phases are immiscible, and that the refractive index of the two phases is closely matched. The combination of optical and chemical constraints poses a challenge when developing a colloidal particle systems for traction rheoscopy. Namely, we seek *two* colloidal particle and solvent combinations such that

- Each colloid is index matched to the respective solvent, which together constitute one "system"
- The refractive index of each system is equal and matched to the immersion media of the objective
- Each colloid can be well dispersed in matching solvent without aggregation
- The solvents are immiscible to prevent any swelling of polymer gel or mixing of the two phases
- (Ideally) The colloidal particles used in the condensed phase could be used to probe a wide range of phases included monodisperse hard sphere dispersion, monodisperse colloidal glass, crystals, and gel

In this chapter we outline the two colloidal particle systems used to simultaneously meet these constraints. As in other chapters, the emphasis of the main text is placed on the motivation and principles involved in selecting, synthesizing, and calibrating the particle systems, while specific step-wise instructions are included in separate protocol section which is referenced throughout the text.

2.2 OVERVIEW

The first and most crucial step is deciding (or rather finding) a pair of solvents that will satisfy the chemical and optical constraints listed in the introduction. Once this decision is made, colloidal particles can then be synthesized to have matching refractive indices by using identical monomer

solutions in the synthesis step, and dispersed in each solvent by altering the chemistry of the brush. Formamide and co-polymer blends of poly-diphenyl-dimethyl siloxane satisfy these chemical and optical constraints. Formamide is polar protic solvent, while PDPS is nonpolar, which suggests immiscibility; this is explicitly checked by vigorously mixing formamide and PDPS oil and looking for complete and rapid phase separation. We note in passing that the argument of polar protic is immiscible with nonpolar is insufficient as similar tests with water (the prototypical polar protic solvent) and the same PDPS oil do exhibit some phase separation and partitioning of one oil phase in the water. However the addition of 4M urea solution prevents this phase separation, suggesting a possible role of hydrogen bonding interactions with diphenyl substituted functional groups. Aqueous 4M urea solutions are extensively used in the preparation of traction rheoscopy samples.

With the target solvents of PDPS oil and formamide, we set out to synthesize index matched particles that can be dispersed in each solvent. The target refractive index is determined by the formamide, and solution of PDPS oil is mixed with matching refractive index. Index matching particles can be synthesized using free-radical dispersion polymerization of methacrylate monomers, following the well established chemistry of poly-methyl-methacrylate catalyzed by AIBN. The co-polymer blend of methacrylate functionalized monomers following reference [1]. Using the same particle composition to ensure index matching we create difference stabilizing brushes for the two solvent systems. First, following the work of Kodger et al, using surface initiated ATRP we grow a poly-electrolyte stabilizing brush to disperse the particle in polar protic solvent formamide. Following ref XX, in which PDMS stabilized particles were synthesized using graft co-polymer of PDMS and methacrylate, we synthesize a (novel) low MW methacrylate-PDPS graft co-polymer that will

function as the stabilizing brush in the PDPS solution.

Using this basic outline, several variations on particles are possible. First, the particle size can be tuned by changing the solvent composition used during synthesis. Second, fluorescent core and non-fluorescent shell particles can be synthesized with an initial dispersion polymerization using reactive methacrylate-rhodamine dye, followed by a second *seeded* dispersion polymerization in which the fluorescent particles from the initial step serve as heterogeneous nucleation sites for a second round of dispersion polymerization which occurs in the absence of additional dye. Third, the chemistry of the polyelectrolyte brush can be modified to achieve cationic and anionic stabilizing brushes; when anionic stabilized particles are mixed with cationic stabilized particles, an attractive gel is formed.

2.3 GEL TRACERS PDPS: STABILIZED MMA/FEMA

The synthesis procedure is composed of two basic steps: synthesis of methacrylate functionalized PDPS stabilizer, and particle synthesis. The full synthesis procedure is adapted from Ref [1, 2] with modifications for different refractive index target, particle density, and particle size. For traction rheoscopy, the tracer particles must be dispersed in PDPS solvent with refractive index of 1.45. Further, it is beneficial that the particles are not density matched as during the preparation of the gels, the concentration of tracer particles near the surface of the gel can be increased by simply curing the gels “upside down” so that the particles sediment to the interface during curing. Finally, the particle size should be as large as possible, while low polydispersity is not a goal of the synthesis.

With these modifications in mind, the particle composition was chosen to yield particles with target refractive index of 1.45 by adjusting the ratio of MMA to FEMA in monomer solution. As in Ref. [3], the refractive index of copolymer blends of MMA/FEMA can be predicted by the linear combination of homopolymer refractive indices weighted by the volume ratio used in the monomer solution. Additionally, to optimize the dispersibility of the tracers particles in the traction rheoscopy gels, the stabilizing brush was synthesized with low molecular weight, phenyl substituted PDPS polymers that were mixed to match the phenyl content of the traction rheoscopy gels. Using a single higher MW PDPS polymer with phenyl content below the average phenyl content of traction rheoscopy gels, resulted in a slight cloudy dispersion. Moreover, the cloudiness was temperature dependent: heating the solution a few degrees during sonication resulted in noticeably clearer solutions, while refrigerating the dispersion to 4°C resulted in a cloudy dispersion. This phenomena was attributed to preferential solvation of the brush by one of the components in the traction rheoscopy gel as the particle refractive index and refractive index of the polymer solution (at ambient temperature) were matched. Decreasing the MW of the stabilizing brush, and using a blend of two PDPS polymers which when mixed gave the target phenyl substitution resulted in good dispersibility and clear solutions in traction rheoscopy gels at room temperature.

2.4 POLYELECTROLYTE STABILIZED CORE-SHELL PARTICLES

The synthesis of core-shell polyelectrolyte stabilized particles involves four steps: synthesis of methacrylate functionalized rhodamine-B dye, synthesis of fluorescent core particles, growth of non-fluorescent

shell, and growth of polyelectrolyte stabilizing brush. The synthesis follows Ref [3], with modifications for monomer composition. The monomer composition used for this work is chosen to maximize the density of the particles subject to refractive index constraint of $n=1.45$. The maximum density is achieved by monomer compositions combining methyl methacrylate and trifluoroethyl-methacrylate without using *tert* butyl methacrylate.

2.5 PROTOCOLS

REAGENT ABBREVIATIONS AND BRIEF DESCRIPTION

- FEMA: 2,2,2,-trifluoro-ethyl methacrylate. Reactive monomer
- MMA: methyl methacrylate. Reactive monomer.
- AIBN: 2,2', Azobis(2-methylpropionitrile). Radical initiator.
- PDPS: *poly*-diphenyl-dimethyl siloxane. Reactive monomer or non-reactive oil
- SPMA: 3-sulfopropyl methacrylate. Crosslink-able stabilizer
- PVP K₃₀: *poly*vinylpyrrolidone. Steric stabilizer.
- PEG_{ini}: polyethylene glycol sacrificial initiator for ATRP growth
- HMTETA: 1,1,4,7,10,10-hexamethyltriethylenetetramine. Amine ligand used in ATRP.
- BIEMA: 2-(2-bromoisobutyryloxy) ethyl methacrylate. Reactive methacrylate polymer that is incorporated into the particle during dispersion polymerization and serves as initiation site for subsequent ATRP based brush growth.
- EDTA solution. 100 mM in water. Metal ion chelator

2.5.1 POLYELECTROLYTE STABILIZED COLLOIDS

Reagent list and brief description

- (Reactive dye) Rhodamine-B. Dye that is derivatized with methacrylate groups to form rhodamine-B methacrylate
- (Reactive dye) Toluene. Solvent for reactive dye synthesis
- (Reactive dye) Glycidyl methacrylate. Monomer of dye synthesis
- (Reactive dye) N,N'-dimethyldodecylamine
- (Reactive dye) *tert*-butyl catechol
- (Core) Rhodamine-B methacrylate solution in methanol. Reactive Rhodamine-B dye. See synthesis protocol
- (Core, shell) Monomer solution. 1:1.62 ratio of MMA to FEMA by volume. Ratio chosen to result in polymerized particles with refractive of 1.45.
- (Core, shell, brush) Methanol. Solvent for dispersion polymerization
- (Core, shell) Water. Solvent for dispersion polymerization
- (Core, shell) AIBN. Radical initiator
- (Core) SMPA. Charged, crosslinkable stabilizer
- (Core) Ethylene glycol dimethacrylate. Difunctional, reactive crosslinker.
- (Shell) PVP K₃₀. Steric stabilizer
- (Shell) BIEMA. 2-(2-bromoisobutyryloxy) ethyl methacrylate. Reactive methacrylate polymer that is incorporated into the particle during dispersion polymerization and serves as initiation site for subsequent ATRP based brush growth.
- (Brush) PEG_{ini}. Sacrificial initiator used to control MW of the brush
- (Brush) Dimethylacrylamide. Uncharged monomer for brush growth

- (Brush) 2-acrylimido-2-methyl-1-propanesulfonic acid solution. Negatively charge monomer for brush growth
- (Brush) HMTETA. 1,1,4,7,10,10-hexamethyltriethylenetetramine. Amine ligands for ATRP
- (Brush) Cu(I) and Cu(II) chloride. Reagents for ATRP.
- (Brush) Formamide. Solvent for brush growth.
- (Brush) EDTA solution, used to chelate metal ions during final wash step

2.5.2 REACTIVE DYE SYNTHESIS

1. Combine 1.48g of rhodamine-B, 150mL of toluene, 0.462g of glycidyl methacrylate, 32 mg of N,N-dimethyldodecylamine, and 10mg of t-butyl catechol in a 250mL round bottom flask
2. Heat at 120C under reflux for 48 hours. Given that the temperature is above boiling point of water, it is recommended to use a glycerol heat bath.
3. Filter cooled solution using glass wool.
4. Rotovap to remove excess toluene
5. Redisperse solid in methanol at 2 wt%.
6. (Optional) Syringe filter solution to remove aggregates and redisperse to methanol.

2.5.3 DISPERSION POLYMERIZATION OF CORE PARTICLES

Images of the dispersion polymerization of core particles is shown in Figure 2.1

1. In a 500mL round bottom flask, combine 91 mL of methanol, 22mL of water, 5.3mL: of monomer solution, 125uL of EGDMA, 110mg of AIBN, and 65mg of SPMA.

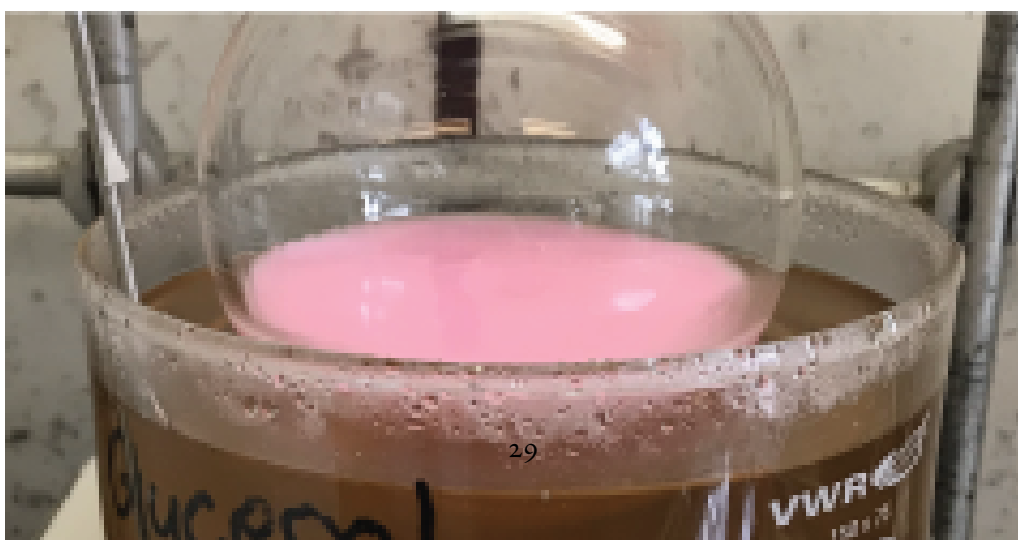
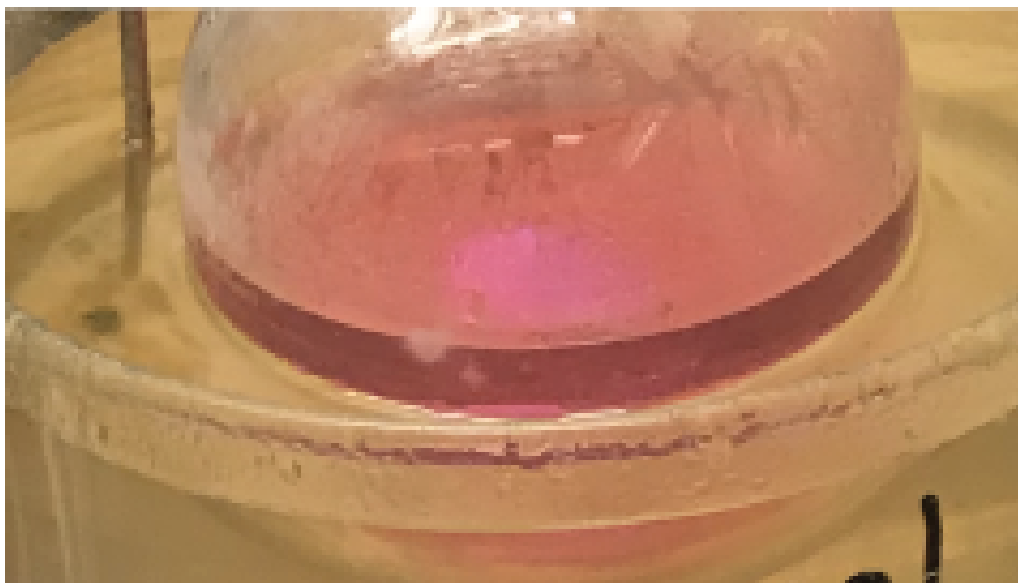


Figure 2.1: Experimental images during synthesis steps. Prior to polymerization, reaction solution is transparent pink solution (left). After polymerization of fluorescent cores and subsequent washing steps, solution is cloudy intense pink color indicating that the polymerization was successful and that the dye was incorporated into the particles (middle). After growth of nonfluorescent shell, solution off-white pink due to scattering through nonfluorescent shell (right).

2. Add 100 μ L of rhodamine-MMA dye solution (2 wt% rhodamine-MMA in methanol). Final solution should have a light cherry red color when viewed in transmission and a slight purple reflection from the top.
3. Position the flask in the heat bath so that the top surface of the solution in the flask is approximately 1 cm above the level of the heat bath as shown in top panel of Figure 2.1.
4. Heat flask to 80°C under reflux for 4 hours. Solution should become cloudy, as in middle panel of Figure 2.1, in less than 30 minutes.
5. After 4 hours at 80°C under reflux conditions, remove the flask from the heat bath, cool and filter solution with Whatman 4 filter paper.
6. Wash several times with water by repeated centrifugation of particle solution, each time discarded the supernatant and redispersing the particle solution in water. Centrifugation speeds should be 250g for 40 min. Washing should be repeated until supernatant is clear.
7. Concentrate particle stock into total volume of 65 mL, making sure to evenly distribute the particle solution if placed in multiple vials.
8. (Optional) Check the size of the particles on the confocal. To increase the size of the core particles, increase the amount of methanol relative to water used during the dispersion polymerization.

2.5.4 SEEDED DISPERSION POLYMERIZATION OF NONFLUORESCENT SHELL

1. In a three neck, 500 mL round bottom flask, combine 4.56 mL of water, 95 mL of methanol, 5.3 mL of monomer solution, 4.2g of PVP K30, 75 mg of AIBN, and 233 μ L of inimir BIEMA.
2. Add 10 mL of the core solution that has been consolidated to 65 mL total volume. This represents roughly 1/7 of the total yield of the core synthesis step. The solution should be cloudy, pastel pink color.
3. Attach a reflux condenser and rubber septum
4. Using a long non-coring needle inserted into the solution, bubble with Ar for 50 min.

5. Reduce the flow of Ar, and position the flask so that the surface of the solution is 1 cm above the surface of the heat bath.
6. Heat to 70°C under reflux conditions with additional flow of Ar for 20 hours. Given the long reaction time, it is important to minimize, but not eliminate the flow of Ar.
7. After 20 hours, the solution should have a dyed but off-white color indicating the growth of nonfluorescent shell as shown in bottom panel of Figure 2.1.
8. Cool the solution and filter through Whatman 4 filter.
9. Wash several (>3) times in 1:1 water:methanol solution to remove unreacted monomer and excess stabilizer. After several washes supernatant should be clear and sedimentation front should be sharp
10. Wash particles into 2:1 formamide:water at least 3 times.
11. As a final wash step, concentrate the particle density to 30 mL total volume. This should correspond to 10 mL sediment.
12. To adjust the size of the final particle, make small adjustments to the amount of core solution added. Fewer particles in the core solution will result in larger final particle size.

2.5.5 BRUSH GROWTH

1. In a 250 mL round bottom flask, mix particle solution (30 mL with 10 mL solid fraction), 600 µL of PEG₄₀₀, 4.36 mL of dimethylacrylamide, 16.26 mL of 2-acrylimido-2-methyl-1-propane sulfonic acid solution, 260 µL of HMTETA, and 57 mg of Cu (II) Chloride.
2. Bubble with Ar for 1 hour, regulating the flow of Ar so that foam does not form all the way to the top of the flask. If necessary reduce the flow of Ar at the end of an hour until any foam has subsided.
3. Under continuous flow of Ar, add 57 mg of Cu(II) Chloride. This step is technically challenging and there are likely dedicated glassware to accomplish this more easily. I accomplished by retracting (but not removing) the needle used for bubbling so that it is no longer

in contact with the solution but 1 in remains in the neck of the flask, remove the septum slightly using the retracted needle to keep the septum from falling off, funnel the Cu (II) chloride into the reaction flask using large weigh paper as make-shift funnel, and quickly replacing the septum. Any residual powder on the neck of the flask should be dissolved in the solution by shaking or swirling the sealed reaction flask so that the solution flows over the undissolved powder on the neck. Reducing the foam is also crucial to ensure that the reagent actually get into the flask.

4. Remove Ar needle, then remove pressure release needle, and finally turn off Ar gas.
5. Let reaction run at room temperature for at least 6 hours. The flask should be warm to hot to the touch, albeit the reaction volume is not very large and so this effect may be tricky to sense.
6. Wash the solution with 100 mM EDTA solution to remove Cu and reaction byproducts.
7. Repeat washing until supernatant is clear and undyed. Fast centrifugation speeds (2000g) are okay at this stage.

2.5.6 DISPERSING PROTOCOL

After particle synthesis, the particles are washed several times into deionized water. The total solid volume is measured by centrifuging into falcon tubes with graduated markings. Typical yield for the experimental protocols presented here are 2 mL of solid. The particle batch is then split to obtain two vials with roughly equal solid fractions, and these solid fractions are recorded. The particles are then washed into index matching formamide with 30mM KCL. After index matching to single component solvent of formamide, text can be read through 1 cm thickness of solid particles as shown in Figure 2.2 As will be discussed in the next chapter, an additional step necessary for form-

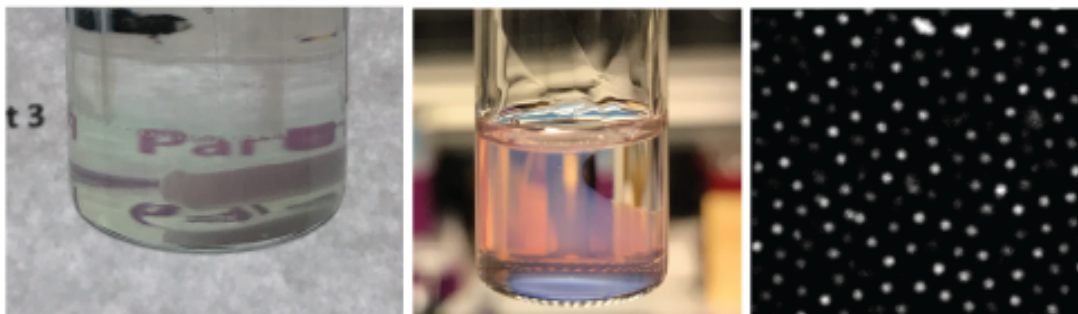


Figure 2.2: Experimental images indicating index matching of synthesized particles. The image on the left, text can be clearly read through a dense particle sediment. The particles in this image do not contain dye. In the middle image, core-shell particles are index matched and clearly transparent through dense sediment. In the rightmost image, a confocal slice of dense particle sediment is shown indicating that the cores are dyed with 800nm diameter, and an out diameter of $2\ \mu\text{m}$.

ing colloidal glass samples in traction rheoscopy, is to disperse the particle suspension 50% methanol-formamide solution.

2.6 POLY-DIPHENYL-DIMETHYL SILOXANE STABILIZED COLLOIDS

Reagent list:

- Monomer solution: 1:1.62 ratio by volume MMA:FEMA. This corresponds to a 32.4% MMA and 67.58% FEMA by mass.
- Reactive PDPS solution: solution of vinyl terminated poly-diphenyl-dimethyl siloxanes, 51.43% of PDV-0525 and 48.57% PDV-162 from Gelest.
- AIBN
- Hexane
- Pyrromethene 605 dye (Exciton)

2.6.1 STABILIZER GRAFT CO-POLYMER

1. 1.536 g of reactive PDPS solution is dissolved in 50g of hexane in three necked, 250mL round bottom flask affixed with reflux condenser, Ar inlet, and rubber septum for injection of reagents.
2. Reactive PDPS and hexane solution is boiled at 70C for 45 minutes and purged with Ar.
3. Under continuous flow of Ar, 0.307g of AIBN is added by removing the septum and funneling the dry reagent.
4. After boiling at 70C for an additional hour, 2.045g of PDPS solution is injected with a syringe.
5. The reaction is boiled for 12 hours
6. After 12 hours the reaction flask is allowed to cool
7. Solution is transferred to fluorinated centrifuge tubes and centrifuged at 10,000g for 15 min.
8. The supernatant is pipetted out and kept, while the sediment is discarded.

2.6.2 PARTICLE SYNTHESIS

1. The supernatant from stabilizer synthesis is transferred to 250 mL three necked round bottom flask. Additional hexane is added until the total mass is 60g.
2. This is heated under Ar at 70C for 30 min
3. In a beaker, dissolve 0.08g of AIBN, 6.87g of monomer solution, and trace dye. In this work, several mg of Pyrromethene 605 (Exciton) were added to achieve a deep red color.
4. Solution of monomer, dye, and initiator are added to the reaction flask with a needle
5. Heat at 70C for 12 hours. Solution should become cloudy in less than 30 min.
6. Cool, and filter through 25um Whatman 4 filter paper

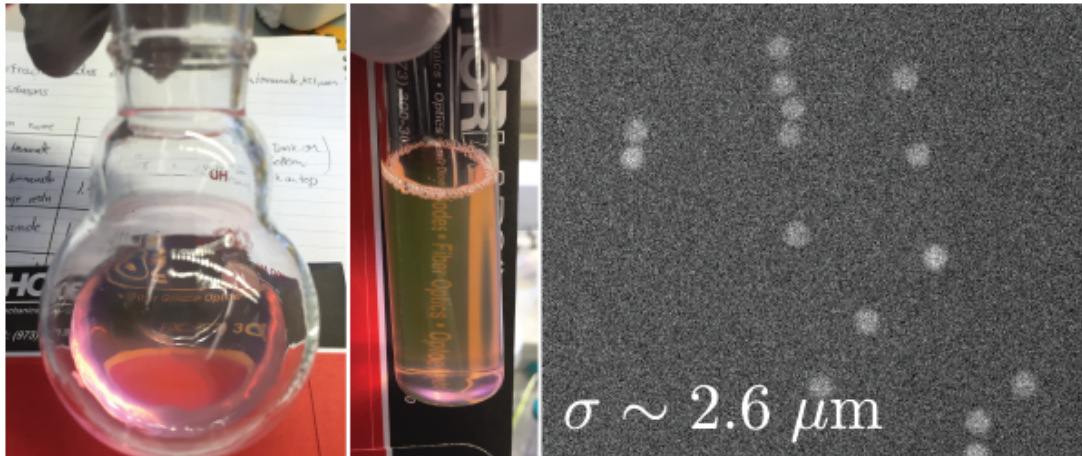


Figure 2.3: Experimental images indicating index matching of particles dispersed in PDPS oil; the approximate volume fraction of tracers in solution is 20%, and text can be clearly read through the dispersion. The confocal image shows PDPS stabilized tracers embedded in a soft polymer gel.

7. Wash several times in excess hexane to remove unreacted dye
8. Wash particle solution into index matching PDPS oil by centrifuging, removing hexane supernatant, and redispersing in PDPS oil
9. Remove residual hexane using rotary evaporation for 6 hours at 50°C.

After dispersing and removing residual hexane a transparent solution of PDPS stabilized particles is obtained as shown in Figure 2.3.

3

Shear cells, etc.

The chapter is a technical outline of the design and fabrication traction rheoscopy shear cells. The first section details the design of shear cells that can be mounted on confocal microscopes. The second section details the fabrication of thin polymer gel layers using sacrificial sugar glass capillary molds; these thin polymer gel layers are used to directly measure mPa stresses in traction rheoscopy experiments and are incorporated into the shear cells. Additional sections detail other parts of the

protocol including centrifugation with methanol, shear cell assembly and sealing, and functionalization of the surface of the soft gel with polyelectrolyte multilayers to create short range adhesive interactions between the sediment and the soft gel.

3.1 SHEAR CELLS

The shear cell used for traction rheoscopy is composed of several components each having distinct design goals and material choices. These components are modular in the sense that modifications to one component, or possibly a multi-component assembly, can be made without altering the design goals of the other components. The shear cell is shown in Figure 3.1, and SolidWorks design assemblies are available. First, the sample insert holder interfaces the piezo with sample cell holder with the microscope stage. Second, there is a three-point leveling apparatus use to align shear cell. It consists of several components: upper and lower plate, carbide pads that press fit and secured with thread-locker into the lower plate, three locking micrometer screws press fit and secured with thread-locker to the top plate, and several tension springs. Third, there is the shear post assembly consisting of the bracket, non-rotating micrometer head, and shear-post cap that can be slid onto the micrometer head. The shear post cap is designed to hold the TEM grid that functions as the shear post during the experiment. As such the piece has 3mm diameter rim, matching the diameter of standard TEM grid. This rim is raised 1mm above the sample to accommodate excess epoxy. The shear post cap and the micrometer head are in direct and continuous contact with the salt solution used in the traction rheoscopy experiment. The micrometer head is made of nickel plated steel. To

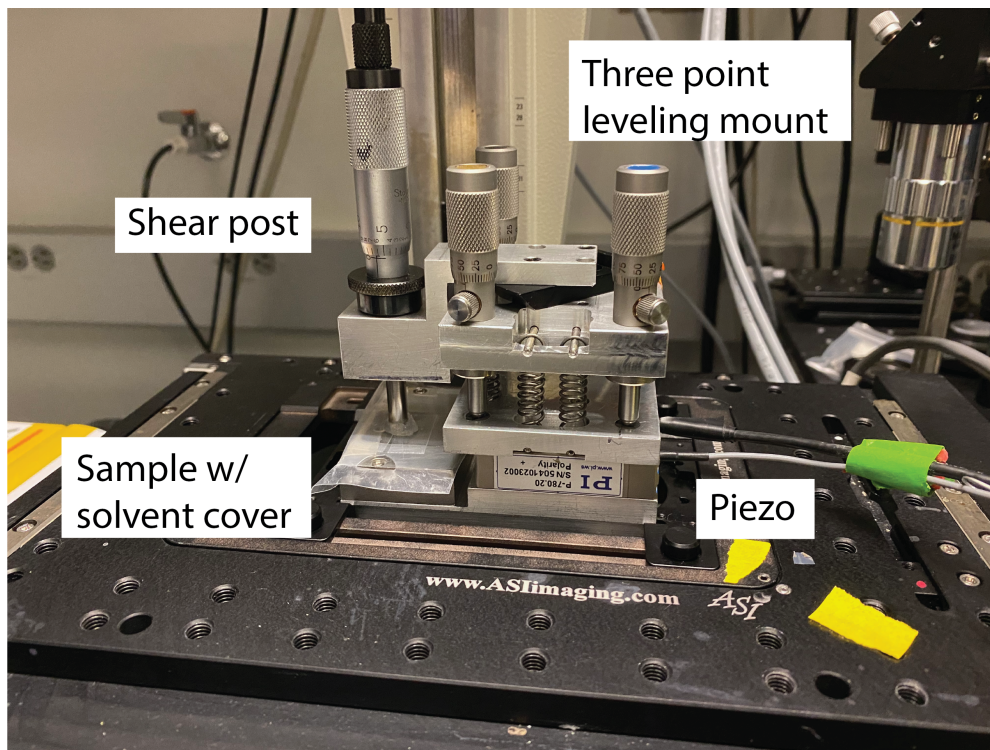


Figure 3.1: A image of the shear cell mounted on the microscope. The various components are labeled including the shear post consisting of the micrometer head and shear post cap which is embedded in the sample; sample cell with solvent cover; the piezo used to actuate the shear; and tension leveling apparatus with micrometer actuated three point adjustment. All components are aluminum expect the sample cell and shear post cap which is corrosion resistant 316 stainless as it is in continuous contact with salt solution during the experiment.

minimize galvanic corrosion between the shear post cap and micrometer head, the shear post cap is machined of 316 stainless steel that has similar galvanic potential as the nickel plating. Additionally, the junction between the shear post cap and micrometer head is sealed with PDMS or silicone-based adhesive gasket sealant. Finally, the sample cell insert (1) is used to fabricate the sugar glass molds, (2) hold the cover glass and spacer to create a partially sealed chamber for the colloid dispersion, and (3) contains reservoir volume of several mL, in addition to the primary volume used to hold the colloid dispersion, that is used carry out sugar dissolution and solvent exchange.

3.2 SUGAR GLASS CAPILLARY

A sacrificial sugar glass capillary is used to fabricate thin layers of PDPS gel without exposing the soft gel to a air-liquid interface, which would otherwise distort the gel. The sacrificial capillary is formed of filling one surface of the sample cell with a smooth sugar glass– thereby forming one surface of the capillary– attaching an adhesive spacer to the cell–thereby defining the gap– and using a coverslip attached to the adhesive spacer as the second surface of the capillary.

To form the sugar glass, a reusable reverse PDMS molds of the sample cell is created by sealing the bottom of the sample cell and pouring PDMS into the interior cavities of the cell as shown in Figure 3.2a-b. This mold cured, removed and trimmed removing a 1 cm thick disk of the mold that would touch the bottom of the sample cell as shown in Figure 3.2c-e. After trimming the mold is placed back into the sample cell and a sugar glass plug is formed by melting sugar in the small cavity with a blow torch. The shape of the sugar air interface and the amount of sugar deposited in the cavity can be controlled by heating the sugar past the glass transition with a blow torch and dipping a disposable wooden stir rod into the center of the sugar filled cavity. As the sugar cools it becomes viscous allowing for a slow drawing process that removes some sugar and forms a convex interface to the sugar as shown in Figure 3.4. After this done, a glass slide with a thin layer of PDMS is placed on the sample and serves a optically smooth mold for the sugar glass and as release substrate to prevent chipping of the sugar as shown in Figure ??c-d. The sample cell, PDMS molds, and PDMS glass slide are clamped in vise and placed in a vacuum oven above the glass transition temperature of the sugar for several hours. After heating for several hours, the heat is turned off while maintaining

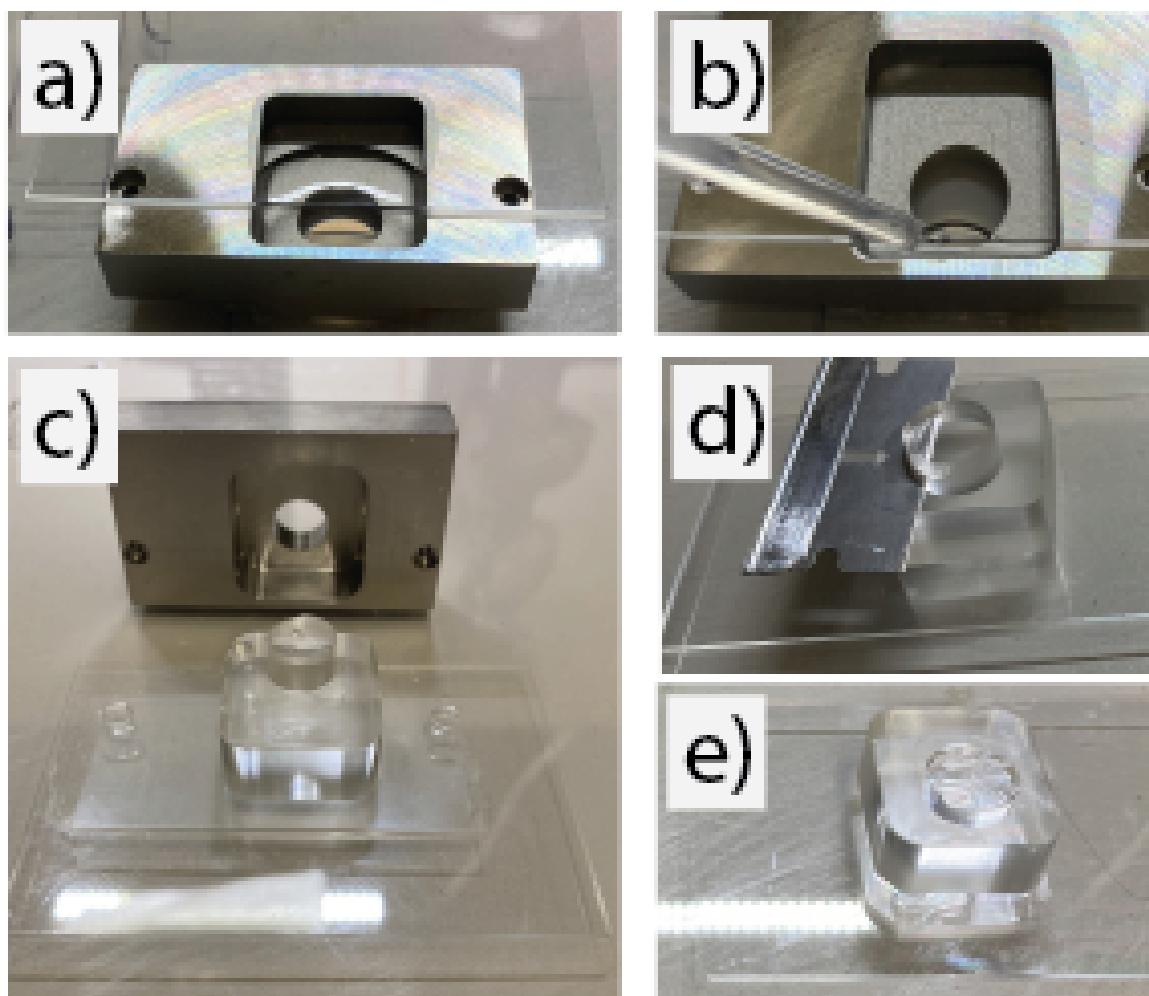


Figure 3.2: Fabrication of PDMS reverse molds for sugar glass formation.

the vacuum, and the oven is allowed to cool to room temperature. Once at room temperature the sample is removed, the PDMS glass slide is removed and the sugar glass is stored in a desiccator until the rest of the protocol is completed as shown in Figure 3.3e-f.

The shape of the sugar surface prior to placing the PDMS coated slide is crucial as the sugar needs to fill the cavity but not cause mechanical interference with PDMS slide. The sugar is hydrophilic, while the PDMS is strongly hydrophobic. Hence if too little sugar is placed in the cavity, an air bubble will form connecting the PDMS coated slide on the bottom and PDMS mold in the bulk of the sample, thereby minimizing the sugar-PDMS interface. If too much sugar is present, the cavity is overfilled and the sugar will leak out.

Finally, after the sugar glass is removed from the oven, and the PDMS-glass chip is removed, the surface should be optically smooth as evidenced by clear reflections off the sugar interface as shown in Figure 3.3f. If the surface is not smooth, it can be placed back in the oven without repeating the sugar glass formation steps. If the sugar leaks, the PDMS glass slide should be replaced and started anew. If insufficient sugar was deposited, additional sugar can be added and placed back in the oven.

3.3 ADHESIVE SPACER

Adhesive transfer tape, is die punched and cut to form as spacers in the sugar glass capillary. Forming the spacers is straightforward process of trimming and die punching. First, strips of adhesive transfer originally sold in rolls with release backing on one side, is used to create tape with double sided backing with a thin channel down the middle as shown in Figure 3.5a. This is done by plac-

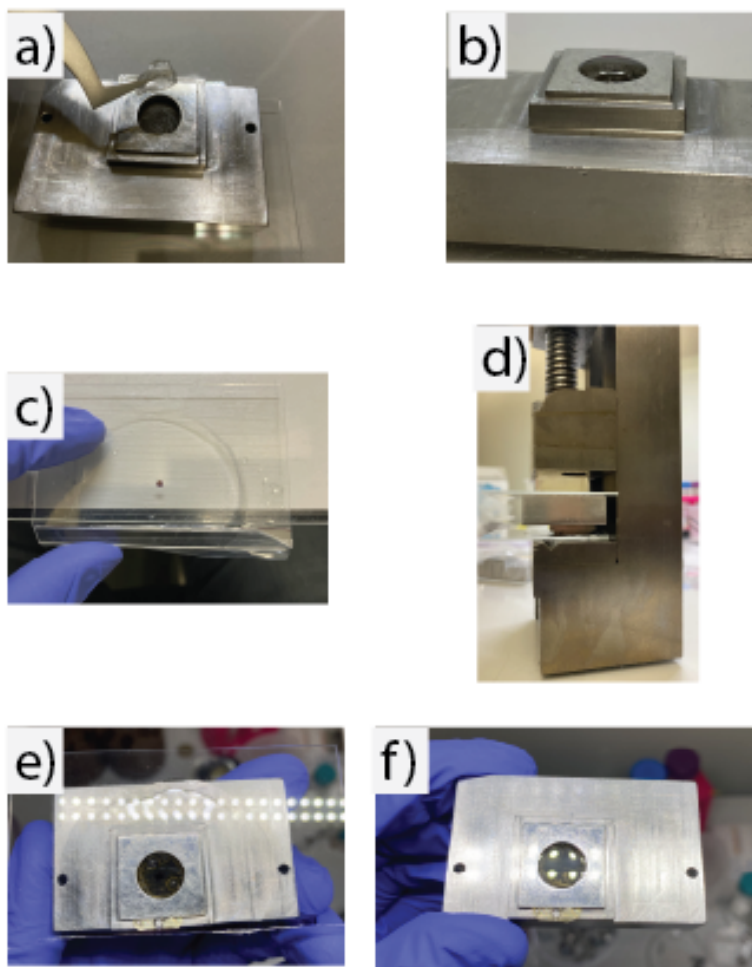


Figure 3.3: Fabrication of sacrificial sugar glass mold.

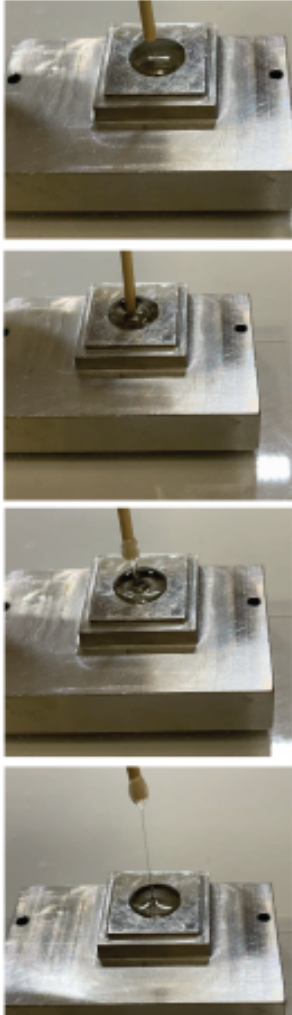


Figure 3.4: Pulling of liquid sugar glass to sure concave down interface.

ing the exposed sticky side of two thin strips separated by 3 mm onto a wide strip of release backing from which the adhesive tape was removed. The gap between the two strips defines the width of the capillary channel. Second, the exterior width of the strip is trimmed to be 1-2mm smaller than the width of the sample cell. Finally, the trimmed strip is placed on a die punch, and circular holes 1mm larger in diameter than the holes in the sample cell are punched at regular $3/4$ in intervals as shown in Figure 3.5b-c. When ready to use the strip is cut with scissors and each half of the adhesive tape is placed onto the sample cell.

3.4 LOADING THE GEL AND EPOXY SEAL

After the sugar glass capillary is formed, liquid pre-gel solution is loaded into the capillary. Typically, 1g of pre-gel is prepared by adding catalyst. Drops of this pre-gel solution are required for capillary loading, and the rest of the solution is used for rheological calibration. The capillary loading is accomplished by placing drops of the pre-gel solution on one of the capillary inlet and letting the solution wick into capillary. Care should be taken to prevent the pre-gel solution from wetting the exterior of the sample and potentially getting into the outlet. After the capillary is loaded, excess pre-gel solution is wiped off, and the gel is left to cure for 2 days during which the rheology calibration is carried out.

After curing, the gel remaining in the exterior perimeter of the sample cell, including between the coverslip and sample cell, is removed by repeatedly sliding a steel shim in the gap as shown in Figure 3.6a-b. This is necessary to remove the gel to ensure the epoxy forms a good seal with the

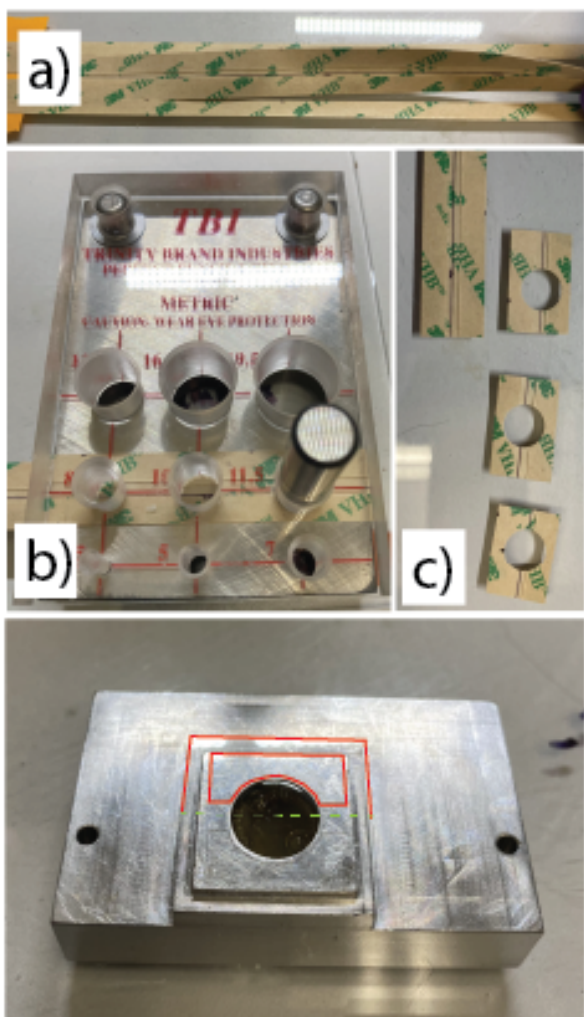


Figure 3.5: Fabrication of adhesive spacer used to form capillary between sacrificial sugar glass mold and coverslip. In figure a, two strips of adhesive transfer tape are placed, with a small separate distance of 1mm onto a release backing. The strips have uniform thickness of 0.002 in and are cut to 17mm width to be smaller than the 18mm side length of sample cell. Next, in step b, the strips of adhesive tape are placed onto a die punch with hole diameters of 11.5mm; this diameter is chosen to be larger than the central hole in the sample cell. In step c, several holes spaced approximately 1 in apart are die punched and cut into three small pieces, each containing a pair of spacers. Finally, the adhesive spacer are then placed onto the sample holder, trimmed, and coverslip is placed on top. The assembled capillary is shown in step d. The coverglass and adhesive spacer are transparent and difficult to visualize; to help with visualization, the outer perimeter of an adhesive spacer is outlined in red, along with the outer edge of the coverslip. No outline is shown in the bottom half of the image or the second half of the spacer. The adhesive spacer is only in contact with the metal sample holder and not with the sugar glass mold. Additionally, the outer perimeter of the adhesive spacer is trimmed to be fully within perimeter of the sample cell.

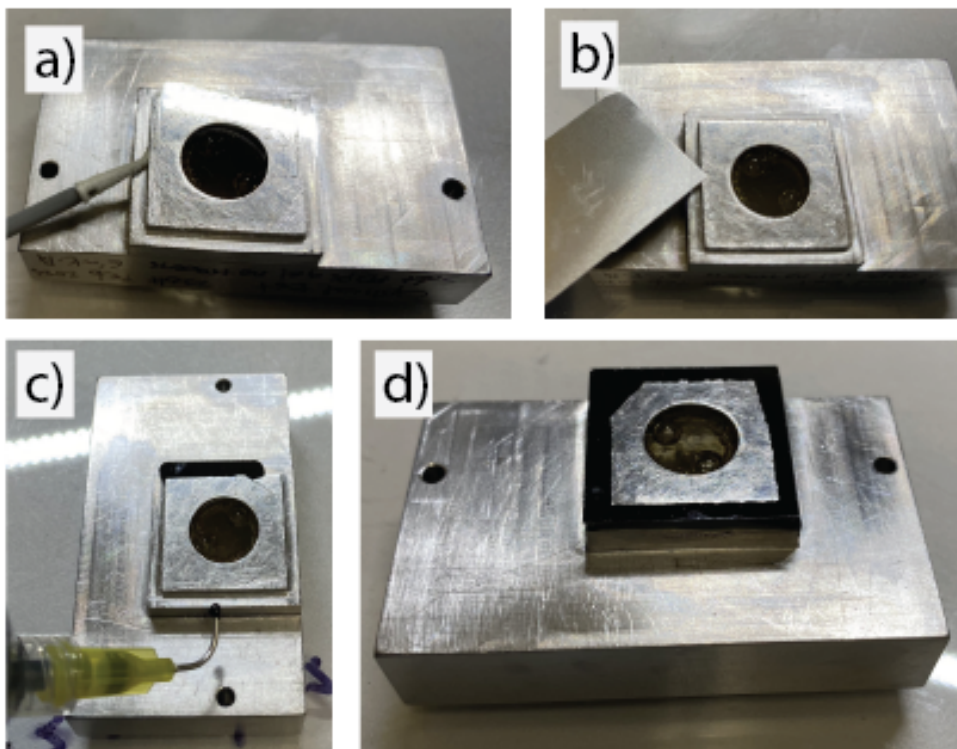


Figure 3.6: Cleaning and sealing of sample cell holder with low exotherm epoxy.

metal and glass. Next, low exotherm epoxy is entruded into the ledged gap between the sample cell and coverslip as shown in Figure 3.6c-d. Excess epoxy is wiped off and the sample is left to cure for 24 hours.

3.5 DISSOLUTION OF SUGAR GLASS AND POLY-ELECTROLYTE MULTILAYER

After the epoxy is cured, the sugar glass is dissolved by filling the central cavity of the sample cell with aqueous 4M urea solution. Urea is added to the solution because water causes some kind of phase separation in the gel leading to cloudiness, presumably by preferentially mixing with one of

the silicone oils that makes up the liquid filler. It was found that adding 4M urea prevented this effect. Formamide and methanol did not lead to phase separation of the gel, suggesting that hydrogen bonding between water the aromatic rings in the PDPS play a role; and hence urea solutions were tried. A simple and reliable test for solvent compatibility between the solvent and PDPS gel is to vigorously mix the solvent with liquid filler; if phase separation occurs and the resultant solutions are both clear, then the solvent is compatible and will not lead to swelling or demixing of the PDPS gel.

The urea solution is periodically gently pipetted and partially replaced with fresh, filtered urea solution, until all the sugar is removed. Crucially, the urea solution is never full pipetted out as the formation of three phase contact line would permanently distort the surface of the gel. Also crucially, and somewhat more obviously, do not stick the pipette into the gel. The progress is dissolving the sugar can be monitored by looking for refractive index variations when either mixing or pipetting in fresh solvent, or explicitly checking the refractive index of aliquot of the supernatant. Typically, I will use 50 mL or more of solvent in order to full dissolve the sugar.

After the sugar has been dissolved, a poly-electrolyte multi-layer (PEM) is deposited on the gel. The PEM layers serves to ensure that the colloidal solid and soft gel are in mechanical contact by providing an short ranged adhesive interface. This short ranged adhesive nature of the interface was confirmed by exposing the PEM to particles and attempting to pipette off the layer. Confocal microscopy showed that when the PEM layer was finished with opposite sign to the stabilizing brush on the particles, the particles were stuck to the surface and showed no Brownian motion, while residual particles a few diameters above the surface showed diffusive motion. If the PEM layer was the same sign as the particle, no particles were stuck to the surface. Additionally, without the PEM layer,

initial traction rheoscopy experiments showed clear interfacial depinning between colloidal crystal and the soft gel layer at applied stress of 10 mPa.

The anionic polyelectrolyte is polystyrene sulfonate, while cationic solution is poly(diallyldimethylammonium chloride) both dissolved in formamide. The deposition step is simply to soak the gel in the polyelectrolyte solutions for 10 minutes, followed by five washing steps with aqueous 4M urea solutions. The washing protocol is essentially identical to dissolving the sugar, albeit different solvents: keep the gel under liquid at all times and progressively dilute the solvent with repeated washing. It is crucial to use filtered polyelectrolyte solutions as this step is a primary source of non-fluorescent debris and contamination in the sample. Given the particles are stabilized with negative brushes, the last layer of polyelectrolyte should be positive. In the work presented here I used (-/+/-/+/-/+) sequence of layers.

3.6 METHANOL CENTRIFUGATION

In order to form a colloidal glass, the concentration of particles must be increased quickly enough to prevent the formation of a crystal. One simple way of doing this is to centrifuge a dilute suspension of the particles; at sufficiently high sedimentation flux, a colloidal glass is easily formed. Unfortunately, this is not straightforward with traction rheoscopy as the density of the solvent (formamide) is larger than the density of the silicone oils used in the gel. Naively centrifuging the sample will result Rayleigh Taylor instability between the formamide and silicone oil.

We solve this problem by centrifuging a dispersion of particles dispersed in methanol-formamide

solution that has a specific gravity less than the liquid filler. After centrifugation, the methanol is evaporated leaving the sole indexing matching solvent formamide as shown in Figure 3.7. Methanol is a near ideal solvent for this process as it has a low specific gravity and a much higher vapor pressure than formamide; it is a polar protic solvent in which the particles are easily dispersed and it does not swell the PDPS gel.

The centrifugation protocol is to replace the solvent in the sample with formamide-methanol solution with specific gravity less than the silicone oils, and seal the sample. Then, deposit a relatively concentrated dispersion of particles at concentration of ϕ 10% in formamide methanol into the central hole in the sample. Sufficient sample volume should be added so that the expected sediment thickness is 1 mm. Centrifuge buckets were modified, as shown in Figure 3.8, to hold the sample cell by the edges without placing force onto the coverglass or traction gel substrate during centrifugation. After centrifuging at 1000G for 15 minutes, the sample is removed and the solvent is very gently pipetted out without disturbing the interface and replaced with 30 mM KCl in formamide solution. Once this step is done, excess methanol is evaporated by leaving the sample unsealed for a few minutes. The refractive index should be restored leading to a transparent sediment.

After sedimentation restoration of the index match, the sample cell is sealed with weather sealing tape that is trimmed to match the dimensions of the sample cell and contains a central hole above the sample. This hole is sealed with additional piece of removable tape.



Figure 3.7: Evaporation of methanol from methanol-formamide solution to restore refractive index matching



Figure 3.8: Modified centrifuge buckets for holding sample cells during centrifugation.

3.7 SHEAR POST CAP AND ALIGNMENT

The TEM grid is glued onto the shear post cap the lining the outer perimeter of the cap with UV curable epoxy extruded with 27 gauge needle. After a thin layer is deposited onto the cap, the cap is flipped over and placed onto a glass slide. By removing the shear post cap from the glass slide, excess glue is removed in a process reminiscent of stamping. If the circular perimeter of the shear post cap is not fully intact, additionally glue is added and the stamping process is repeated. Next the TEM grid is gently placed onto the shear post cap, lining up the solid perimeter of the TEM grid with the ridge with thin epoxy film. The assembly is placed under UV lamp for at least 30 minutes. After curing, the integrity of the epoxy bond is checked by gently blowing compressed air over the top of the shear post cap.

The shear post cap is then placed onto the shear post and secured with a small dab of epoxy in place at the upper perimeter of the shear post cap. Additionally, the upper perimeter at the junction between shear post cap and the micrometer head is wrapped with tape with width of a few mm leaving a small empty cylindrical annulus with outer radii defined by shear post cap; inner radius defined by the micrometer post; and height defined by width of the tape wrapping the shear post cap. This gap is then filled in with PDMS extruded with a blunt tipped syringe. This final step is done to prevent any galvanic corrosion between the shear post cap and the shear post.

After attaching the shear post cap, the shear post is attached to the shear cell with an empty sample cell. Coaxial alignment between the shear post and hole in the sample cell is checked by lowering the shear-post through the central hole in the sample cell and making adjustments if necessary. Once

the axial alignment is set, the screws connecting the bracket to the leveling apparatus are tightened.

Next, the surface of TEM grid is aligned to be parallel to the optical axis by measuring a reflection signal off the TEM grid and adjusting the micrometer screws on the three point leveler. The flatness of the grid is not limited by the precision of the three point leveling apparatus but rather by distortions in the grid that presumably stem from the gluing process. Typically, the center of the grid is 10 μm below the outer perimeter. It is important at this stage to check that the holes of the grid are not occluded by epoxy. Once the grid is aligned, it is raised up and not removed from the shear cell. If the shear post is in the fully raised position, the sample cell can be slid onto the shear cell without removing the shear post.

3.8 SHEARING

After the sediment is formed, the final step is to lower the TEM grid and embed it into the sediment. This is done using two complementary techniques. First the sediment, as formed by centrifugation is substantially thicker $\sim 1000\mu\text{m}$ than the final shear gap $100\mu\text{m}$ in traction rheoscopy, and hence as the TEM grid is lowered it is lowered *into* an already formed sediment. Second, additional particles are co-sedimented onto the top of the grid. This is accomplished by loading a dense suspension of index matched particles into the hollow cavity within the shear post cap above the TEM grid. Prior to lowering the shear post, surface tension keeps the dense colloid fluid in place. As the grid is lowered through the solvent above the sediment, the dense suspension drips down through the holes in the TEM grid. Even immediately after lowering the grid, the sample is nearly fully dense and disordered.

After lowering the shear post, the interface between the trimmed weather sealing tape and the shear post are further sealed using additional pieces of tape placed at the perimeter but not in mechanical contact with post. Finally, silicone grease is applied to cover the few mm clearance between the post and weather sealing tape. The sample is left to consolidate on the microscope for 2 days prior to shear, with occasional imaging stacks taken every hour.

3.9 PIEZO CONTROL CODE AND DEFORMATION PROTOCOLS

The piezo used to actuate the strain is controlled with closed feedback control loop in which the piezo is told to move certain position at regular intervals of approximately 10Hz. For linear strain ramp, the step sizes are constant and adjusted to achieve the a target speed. The code employs multiple threads to ensure no systematic drift in the timing between subsequent steps, and allows for querying the position of the piezo during deformation. The position and requested position of the piezo are recorded at each time point. The code is set up so that new shear protocols, for example linear strain, hold for 10 min, then sin wave oscillation, can be sent to the piezo by simply programming a list of position that will be cycled through. Some simple functions including linear ramp up, linear ramp down, triangle wave, hold at given position for a given duration, and hold at a given position until interrupted are already written. The code is available along with the rest of the data and image analysis codes on github.

3.10 MISCELLANEOUS PROTOCOLS

3.10.1 CLEANING OF PARTS

The standard cleaning procedure for 316 stainless steel parts are scrub with non-marring metal bristles in a solution of Alconex soap, sonicate with Alconex soap, followed by sonication in 10 weight percent citric acid solution, and a final rinse in deionized water. Generally the pieces should be cleaned in a plastic tub, and not over the sink, to ensure that the small pieces do not fall down the drain. The UV cured epoxy is difficult to see and ensure you have removed. Hence the protocol relies on softening the epoxy with acetone and scraping with a dental probe.

SAMPLE CELL

1. Colloid dispersion is pipetted out and saved. Additional water is pipetted to redisperse remaining colloid solution. The process is repeated until the aliquots are clear indicating that a substantial fraction of the colloids have been recycled.
2. The sample cell is then rinsed with water.
3. The cover glass is removed by sliding a razor blade under epoxy sealing ring.
4. After removal of the coverglass, any excess epoxy or adhesive spacer is removed with a razor blade
5. An Alconex soap solution is prepared by mixing several tablespoons of Alconex soap powder 2-3 cups of water. The solution should be foamy upon agitation.
6. The sample is scrubbed with brass bristled brush in a solution of Alconex soap solution.
7. The sample is then sonicated for 30 min in Alconex soap solution and rinsed with water
8. The sample is then sonicated in 10 weight percent citric acid solution, and rinsed with water

9. Finally the sample is blow dried with compressed air and stored in a plastic bag.

SHEAR POST CAP

1. The TEM grid is removed with a pair of needle-nosed tweezers.
2. If present, residual epoxy film on the rim of the shear post cap is removed with tweezers. This can typically be done while preserving the epoxy film in a single piece; while not necessary, this is a good indication that all the epoxy has been removed.
3. Excess epoxy on the outer perimeter, if present, is removed by scraping with hard tipped dental probe.
4. Excess epoxy on the inner dimensions of the shear post cap are similarly scraped off with dental probe. This is done by inserting the probe into the hole previously covered by the TEM grid and scraping.
5. The shear post cap is sonicated in a sealed vial with acetone for 15 min.
6. After sonication, check for additional epoxy by scraping the dental probe over the inner and outer diameters of the shear post. The acetone swells the epoxy making the residual epoxy easier to identify and scrape off. Continue scraping until no material removed.
7. Acetone sonication and scraping are repeated until no material is removed.
8. Scrub with brash bristled brush in Alconex soap solution.
9. Sonicate in Alconex soap solution for 30 min and rinse with water.
10. Sonicate in citric acid solution for 30 min.
11. Rinse with water and blow dry.

3.10.2 REAGENT PREPARATION AND FILTRATION

Several sets of reagents are prepared including:

- stock solution for dispersing particles: 30 mM KCl in formamide and 30mM KCl formamide and pure methanol at 60-40 mass ratio
- Aqueous 4M urea solution used for sugar dissolution, washing, and solvent exchange.
- 20 mM polyelectrolyte solutions in 30mM KCL in formamide.

(1) used for dispersing particles, stock solutions of aqueous 4M urea for sugar dissolution, washing, and solvent exchange, and stock solution of polyelectrolytes in formamide. Generally all these solutions are prepared with volumetric flasks, vacuum filtered through Whatman 11 filter paper, and then dispensed through a 0.2 μ m syringe filter at time of use. Filtration of all reagents lowers the probability of debris occluding the field of view on the final sample; filtration of polyelectrolyte solution is especially important as trace cross-contamination will generally lead to aggregates in the solution.

3.10.3 SUGAR GLASS PREPARATION AND STORAGE

The sugar glass is prepared by mixing 4 parts of isomalt sugar with 1 part deionized water and heating until the temperature reaches 160C as measured with a candy thermometer. This takes over an hour. The viscous sugar solution is then poured on baking trays that have been that have been liberally coated with PDMS. After cooling, the sugar glass sheets are broken into small pieces using a ceremonial toy hammer, and stored in sealed plastic bags with dessicant.

3.10.4 PDMS FLAT MOLDS AND RELEASE COATING

Flat PDMS on glass slides are used to form smooth sugar glass surfaces. These are simply fabricated by depositing 200-500 uL drop of PDMS onto a large slide and letting the drop spread for at least 30 min followed by curing in a 60C oven. The central portion of the PDMS chip, that is several mm away from any edges, will be flat and of uniform thickness. After curing, the surface is covered with protective film for storage. Before use, the PDMS chip is trimmed so that it fits into the vise used to clamp the sugar glass molds when annealing in the oven. To trim the PDMS chip, mark the center of the chip using a marker and align the central hole in a sample cell above the marked spot. Trace the edge of the sample cell with a marker and score the glass along the traced line. Finally, break the glass using pliers along the scored edge, being sure to place the PDMS chip with the PDMS side down and the glass side up to ensure mode I fracture along the scored interface. This is shown in Figure 3.3.

4

Particle Locating: Sine qua non

4.1 INTRODUCTION

PARTICLE LOCATING is a collection of image restoration and analysis techniques to extract the information contained within real-space volumetric microscopy images taken during traction rheoscopy experiment. The microscope images contain all the information – principally the centers

of the colloidal particles and their subsequent displacement over time – that are the basis for *nearly all* of the measured quantities in a traction rheoscopy experiment. Example of measured quantities that depend exclusively on particle locations include:

- Average structural metrics such as pair correlation function of the colloidal sediment and interparticle separation distances
- Local average strain obtained by comparing the relative displacements of particles and neighboring shell over two time points
- Strain measurements in the polymer gel obtained from the position and displacement of embedded colloidal tracer particles over time, which are then used to infer the average *stress* across the bilayer

Particle locating algorithms were first developed by Crocker and Grier¹⁰. The algorithm was demonstrated bright-field images of dilute tracers imaged on charge-coupled-device (CCD) cameras. The algorithm has been applied with success to fluorescent tracer particles in 3D, even in densely packed environments. The original algorithm consists of several steps:

- Background subtraction using boxcar filters
- Noise reduction using low pass filter
- Selection of local maximum pixels
- Centroid refinement over predetermined size, including possibly merging candidate pixels.

Subsequent additions to the algorithm have included improved background subtraction²⁷, the addition of sub-pixel biasing correction¹⁶, nonlinear least squares fitting³⁶, and particle locating in iterative rounds¹⁸. The original algorithm has been implemented in various computer languages; in this work we use a python based implementation by TrackPy² along with open source deconvolution using DeconvolutionLab 2³⁰.

In this chapter, I present results on a particle locating and image segmentation pipeline that extends Crocker-Grier by correcting for correlated readout noise on modern sCMOS detectors, utilizes open source image deconvolution to replace band pass filtering steps resulting in improved signal to noise and z-resolution, a random forest pixel classifiers to detect fluorescent chunks including dimers, image segmentation between sediment and gel with different signal to noise ratios, and non-linear least squares fitting with per-particle uncertainty estimation. The framework is implemented in python using open source software packages trackPy², DeconvolutionLab2³⁰, and ilastik⁶. A key feature is the consistent use of embarrassingly parallel computations by splitting multi-Tb data sets into small volumetric chunks that are processed individually and in parallel. Each chunk requires with modest computational resources of 16Gb of RAM, and 4 processor cores. This allows the pipeline to be efficiently scaled to multi-Tb datasets on high performance computing clusters that typically have an excess of small partitions of unallocated resources. The efficient use of compute resources allows for image deconvolution and nonlinear least squares fitting—which are several orders of magnitude computationally more expensive than band pass filtering and centroid locating—to be applied multi-Tbs of image data and hundreds of millions of particle in 3D.

Given the central importance of particle locations in traction rheoscopy experiment, locating uncertainties and errors are likely *dominant* sources of uncertainties. Using this pipeline, for low signal to noise images, I demonstrate improvements over centroid based locating on important structural metrics such as pair correlation function. Additionally, I demonstrate the removal of fluorescent chunks that would otherwise lead to spurious conclusions. Finally, using the per-particle uncertainties that were measured using nonlinear least squares fitting I present a markov-chain monte-carlo

technique to re-sample particle configurations. Using these resampled configurations, I efficiently estimate the uncertainties in derived quantities, such as best-fit affine strain tensors, for which conventional uncertainty estimation is prohibitively difficult.

In addition to uncertainties in the particle locations, there are also particle locating errors in which, for example, a dimer is misidentified as a single particle or a particle is identified in a region in which there is just noise. These errors are typically the result of synthesis byproducts in the form dimers or small clusters of core particles that have aggregated prior to shell growth. While these byproducts can be reduced by lowering the dye concentration during synthesis, doing so comes at an obvious tradeoff in that the signal to noise of the particles is reduced.

Quantifying the uncertainties and identifying errors, especially rare errors that may, nevertheless, occur more frequently than rare events in the sample, are both complicated by the sheer size of the data sets. This large size precludes verifying locations by exhaustively visually comparing particle locations against the raw image data. I approach this problem in a hybrid fashion by first optimizing locating parameters on a subset of the data, and developing a systematic framework to check the locating results on a subset of the data as needed. To efficiently screen the located data for anomalies, the analysis pipeline retains information on the fluorescent images using a random forest pixel classifier which assigns a probability of a pixel belonging to several categorical bins such as colloid particle, nonfluorescent shell, fluorescent chunk, etc. When a particle is identified in the locating algorithm the pixel probabilities are integrated over the known size of the colloidal particle, and nonfluorescent shell. As the pixel probabilities across the entire image are an order of magnitude larger in file size than the raw data, only the value of these integrals are stored for later analysis. After data analysis on

the particle locations, if a cluster is suspicious – perhaps the pairwise distances are too small, or the strains too large – the integrated pixel probabilities are checked to see if the random forest classifier segmented the images as colloid or fluorescent chunk.

Additionally the key associated with the small chunk of raw data used to identify that particle is retained so that only that small chunk can be recomputed (without the use of a cluster due to modest compute requirements) and store *all* intermediate results for later analysis. These intermediate results for the small section in question can then be visualized and compared against the particle locations to check for errors. A simple code creates false color volumetric image of the located particles with an ellipsoidal 3D profile intensity profile at the located position. The color can be mapped to any selection or function of the particle locating data and then visually overlaid against the raw or deconvolved image data.

4.2 PIPELINE

The image analysis pipeline used in this work is outlined below along with the software implementation. All code is available on my GitHub: <https://github.com/tlozsolt>.

First the image undergo flat-fielding in which fixed pattern noise is removed and background is subtracted. Next the images are partitioned into small overlapping chunks of with dimensions $(t, z, y, x) = (1, 250, 450, 450)$ and assigned a unique hash value. After deconvolution, the images are thresholded to remove background and passed to neural network based pixel classifier. The results of the classifier are stored, but do not modify the underlying image. Finally, the image is passed

to trackpy to locate particles in several rounds of iterative locating. The results from centroid locating are used as initial guesses for least squares fitting of the particle spots. After locating, the particle positions are stitched back together, removing overlaps between particles.

1. Flat fielding. The fixed pattern noise in the image along with diffuse background is removed using measured pixel dependent gain and offset.
2. Spatial hash. After flat-fielding, the image, typical size is $(t, z, y, x) = (120, 850, 2048, 2048)$ is partitioned into small overlapping chunks with dimensions $(t, z, y, x) = (1, 250, 450, 450)$ and assigned a unique index. In addition the unique index (or hash values), the partitions are labeled as either sediment or gel. After flat-fielding, the sub-images are saved to separate files allowing parallel access.
3. Deconvolution. A single hash value is deconvolved using Deconvolution Lab 2, and theoretical point spread function generated by Huygens software.
4. Pixel Classifier. The deconvolved image is processed using a random forest pixel classifier to identify individual pixels as belong to one of several distinct channels (colloid particle, gel tracer, sediment background, etc). The image is unmodified in this step.
5. Thresholding. A hard threshold is computed and applied to the image to get a zero background.
6. Centroid locating. The particle locations are found using Crocker-Grier as implemented in TrackPy, with successive rounds of locating.
7. Least squares refinement. The centroid positions are used as initial guesses for least squares fitting of particle positions. The reported uncertainties correspond to the inverse curvature of the loss function used in fitting, and take into account the disk shaped fluorescence profile of particle cores that are somewhat larger than the diffraction limit.
8. Integration of pixel classifier. At each particle location, the each channel of the pixel probabilities are integrated to known fluorescent core and particle diameter.
9. Stitching. The particle positions are stitched together, and overlaps closer than 250nm are removed. Typical overlaps in particle positions on the edge of overlapping partitions are typically within the locating uncertainty of 20nm.

10. Tracking. The particle positions are tracked over time using Crocker-Grier tracking algorithm.

4.3 CHALLENGES

4.3.1 BIG DATA, AND THE NEED FOR SPATIAL HASHING

A crucial feature in traction rheoscopy experiments is the ability to acquire volumetric images of the colloidal solid and polymer gel during externally applied deformation over long time periods. These volumetric images can be obtained with a fluorescence confocal microscope because of careful index matching of the various components, and can be imaged over long times due to fluorescence stability of the dye and the use of low laser powers to minimize bleaching. The total size of the data sets obtained in traction rheoscopy are dictated, trivially, by the size of single stack measured in pixels $(z, y, x)_{px}$, and the number of stacks t in a time series. In this section I give a simple heuristic for estimating the size of data obtained in traction rheoscopy experiments under *optimal* imaging parameters of pixel size, magnification, and z-step size for a single stack, and reasonable parameters for number of required stacks to resolve deformations expected in colloidal solids. I found these simple heuristics useful in motivating the development of imaging pipelines that can process the resulting data sets.

Given the near optimal optical properties of the sample obtained by systemically matching all refractive indices of the various components, and the numerical aperture of the objective, we can place an upper bound on the size of volumetric stack by requiring Nyquist sampling in all dimen-

sion. On the one hand, without index matching and elimination of spherical aberrations, this upper bound would not be applicable as the point spread function would degrade with imaging depth. On the other hand, finer sampling than this upper bound is superfluous given the Nyquist sampling theorem. Given the imaging parameters of excitation wavelength $\lambda_{ex} = 561 \text{ nm}$, refractive index $n = 1.45$, and numerical aperture $\text{NA} = n \sin(\alpha) = 1.3$, Nyquist limited sampling gives:

$$\Delta_x = \Delta_y = \frac{\lambda_{ex}}{8n \sin(\alpha)} = 54 \text{ nm},$$

$$\Delta_z = \frac{\lambda_{ex}}{4n(1 - \cos(\alpha))} = 174 \text{ nm}.$$

The Nyquist sampling in x,y is quite small; undersampling, in principle, leads to loss of high frequency spatial components, albeit these components, are strongly attenuated by the presence of the pinhole. A typical rule of thumb for confocal microscopy is to under sample laterally by a factor of 2. For colloids experiments in particular, the loss of high frequency components can be expected to blur the edges of colloidal particles, which is problem for particle locating only if the fluorescent spots overlap as a result of the blurring. This problem is side-stepped through the use of core-shell particles.

We can approach Nyquist limited sampling using a z-spacing of 150nm, and, with no additional magnification in xy, a lateral spacing of 115 nm. Increasing the lateral spacing by binning the camera pixels affected image quality to an extent, but had a drastic effect on particle locating. With 800nm fluorescent cores, the number of pixels per fluorescent spot was only 3-4 after binning which is in-

sufficient sampling for centroid locating. Decreasing the lateral spacing (therby increasing the sampling rate closer to the nyquist sample) can in principle be achieved with the addition of magnification lens, however this introduced additional aberrations and was not used.

Using this sampling density over real space volume of (150, 235, 235) microns we obtain typically image stacks with pixel dimensions of $(z, y, x)_{px} = (1000, 2048, 2048)_{px}$ pixels. Somewhat larger volumetric stacks can be obtained by extending the z-range to the free working distance of the objective (220 μm); reducing the z-range would result in smaller volumetric stacks at the obvious cost of imaging fewer particles. Using 16-bit well depth, we end up with a typical stack size of:

$$1000 * 2048 * 2048 * (16\text{bit}) * (1\text{ byte}) / (8\text{ bit}) = 8.4\text{ Gb}$$

To systematically investigate the deformation over a range of applied strains, a volumetric stack should be acquired in strain increments sufficiently small to visualize individual rearrangement events. The target strain increment can be crudely estimated by assuming

1. the transformation strain for a single rearrangement in colloidal glass is $\sim 10\%$
2. all of the incrementally strain is carried by individual transformation
3. we want a target density of transformation of 1% in order to resolve individual transformations

Taken together, we end up with a target incremental strain of $\partial\varepsilon \sim 0.1\%$. This requirement has several benefits in mapping out the deformation in detail, and also ensures accurate tracking as the maximum displacement of any colloidal particle, which is assumed to be less than the displacement

of the shear post, is still much less a particle diameter. As a result, given a total deformation of $\sim 10\%$, 100 volumetric stacks are required on loading and unloading curves. The total data size, for a *single* shear deformation at 10% applied strain is then 1.6Tbs.

As argued above, visualizing the sample with high spatial resolution, fine strain increments, and over long times gives rise individual stack sizes of 10Gb and total time series of 1-10 Tbs. Thankfully, all of the image processing steps benefit from embarrassingly parallel computations in the sense that you can process chunks (of the data independently and then stitch the chunks back together. This process of splitting the data in overlapping partitions is known as spatial hashing. To determine partition size, image processing pipelines typically require memory requirements that are several times the size of the input image, where the size of the input image is determined by partition size used in the hashing step. To determine a reasonable partition size, we determine the step with most stringent memory requirements (which is the computational resource most in demand) and set the partition size to allow for computations of a single partition within a 16Gb allocations of memory.

For the pipeline described here, deconvolution step is empirically found to be the memory bottleneck and partition volumes of (250, 450, 450) give peak memory usage of 14-15Gb. Given this partition size, and requirement that the partitions share an overlap region twice the size of colloidal particle gives typically hash size (meaning total number of partitions) of (1, 7, 5, 5) or 175 partitions per time point. With the spatial hash established, each partition is associated with a hash value, and is processed along the entirety of the pipeline. If any single hash value fails only that hash value needs to be resubmitted or restarted. This allows for efficient use of the cheapest and most abundant com-

putational queue in which the jobs may be cancelled due to low priority, there is no guarantee or compute node speed, and there is no ability to perform distributed computing over multiple nodes. These restrictions make the queue abundant and cheap in high performance computing clusters as the heterogeneous and fragmented resources required for these low memory, image processing jobs cannot be allocated to most other computing needs. Even with near zero priority, I regularly run 500-1000 hash values in parallel, with a total queue size of 10k hash values.

4.3.2 LOW AND HIGH SIGNAL TO NOISE

Image processing in traction rheoscopy experiments requires separately processing colloidal sediment and polymer gel phases. In principle, distinct dyes can be used to identify the fluorescent tracers embedded in the gel and the colloidal particles that constitute the sediment phase, however in the work presented here only one color was used. Using a single channel for both phases has some minor advantages in that smaller total file sizes and simpler calibration procedures as the multiple cameras do not need to be aligned, nor calibrated. One serious drawback, however, is that out of focus fluorescence from the sediment phases lowers the signal to noise ratio in the gel phase. This is largely unavoidable as the sediment phase has dramatically more particles, and hence more diffuse fluorescence than the dilute tracers embedded in the gel.

A simple measure of the image characteristics in the raw, unprocessed images can be obtained by averaging the pixel intensities per slice and plotting as function of height in the sample. This is shown in Fig. 4.1. Several salient features are immediately apparent. First, the gel-sediment interface is clearly identified as a jump in the average fluorescence intensity. Second, the fluorescence intensity

in the gel phase increases up to the gel-sediment interface. Third, layering (without crystallization) of the colloidal particles in the sediment phases is apparent as oscillations in the average intensity. Fourth, the fluorescence intensity increases in the sediment phases before plateauing at a constant value. This increase is caused by out of focus fluorescent light from the sediment layers below the focus. Finally, and most trivially, there is a sharp decrease near the top of the sample as the shear post, which is non fluorescent, takes up a larger fraction of the area in each slice.

The slice-averaged characteristic of the image are complemented by looking at fluorescence profile of isolated gel tracers and colloidal particles as shown in Fig. ???. The signal to noise for gel tracers is lowest near, but below, the gel-sediment interface as the out of background fluorescence contributes to a larger spread in the noise as expected for Poisson noise. While this is not ideal for the traction rheoscopy experiments as the tracers near the top of the gel experience the largest displacements and hence, in principle, provide the most valuable information. However, the low signal to noise is compensated by deconvolution and large particle size.

4.3.3 FLAT FIELDING AND FIXED PATTERN NOISE

A source of correlated noise is the use of sCMOS cameras that have readout noise and gain that *varies* for each pixel as shown in Figure ???. As this source of noise is dependent on each pixel, it is *fixed* for each camera and hence can be removed. No comparable fixed pattern noise is present on point scanning confocals as the same photo-multiplier tube is used to generate intensity values for each pixel. We partially compensate for fixed pattern noise by implementing a flatfielding procedure making use of measured per pixel dark noise. The corrected image C is computed from the raw im-

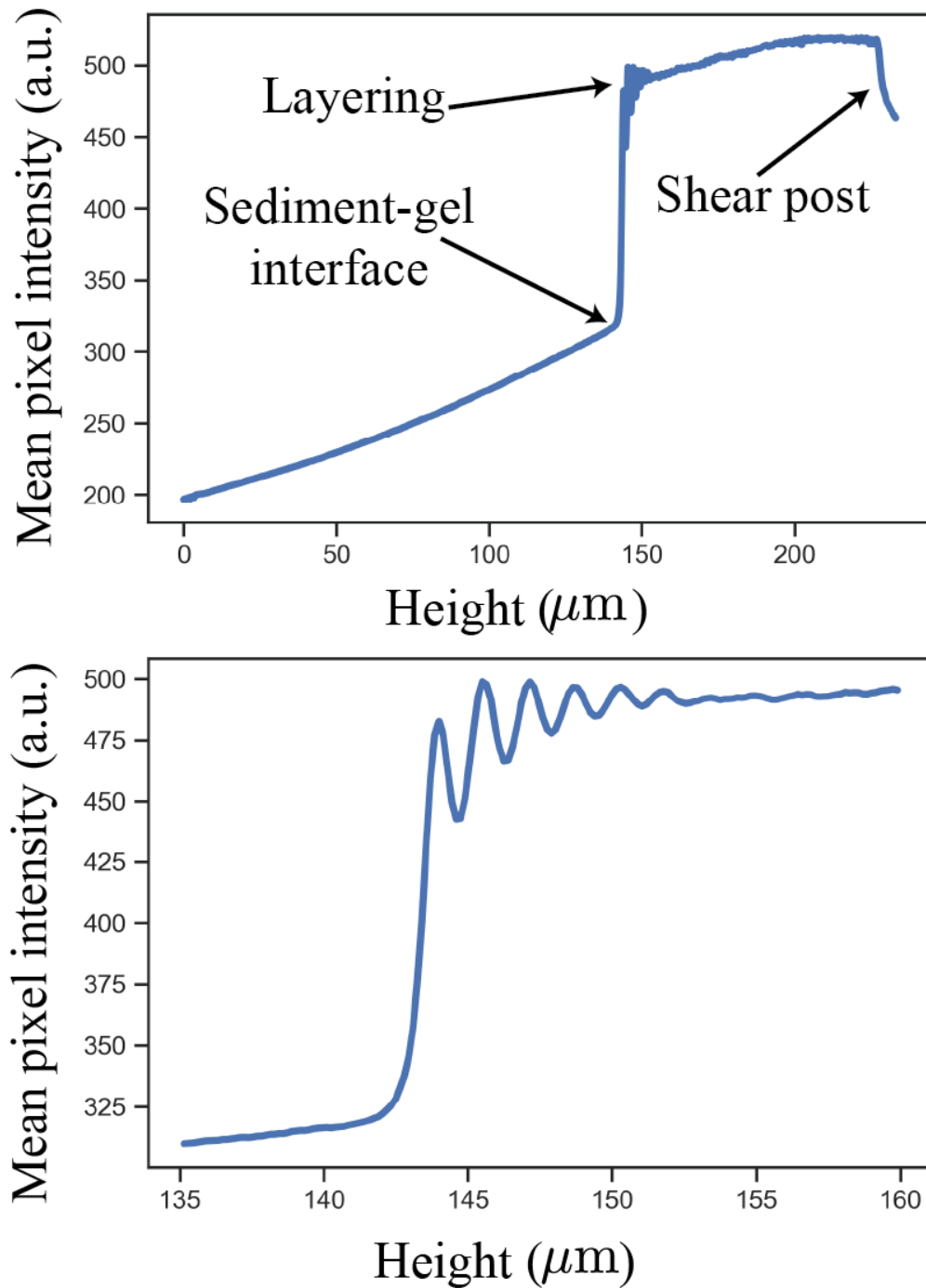


Figure 4.1: Pixel intensities of raw confocal gel sediment bilayer average over confocal slices. Several general features are apparent. First, the intensity of the image is much brighter in the sediment layer due to increased density of fluorescent particles; this fluorescent intensity is the dominant source of background in the gel, especially at upper layers and reduces the signal to noise in the sample. Second, there is a prominent layer of the sediment particles on top of the gel interface giving rise to oscillations in avg pixel intensities. These oscillations are caused by layering of the particle density near the flat interface, and made more prominent as only the core of the particles is dyed.

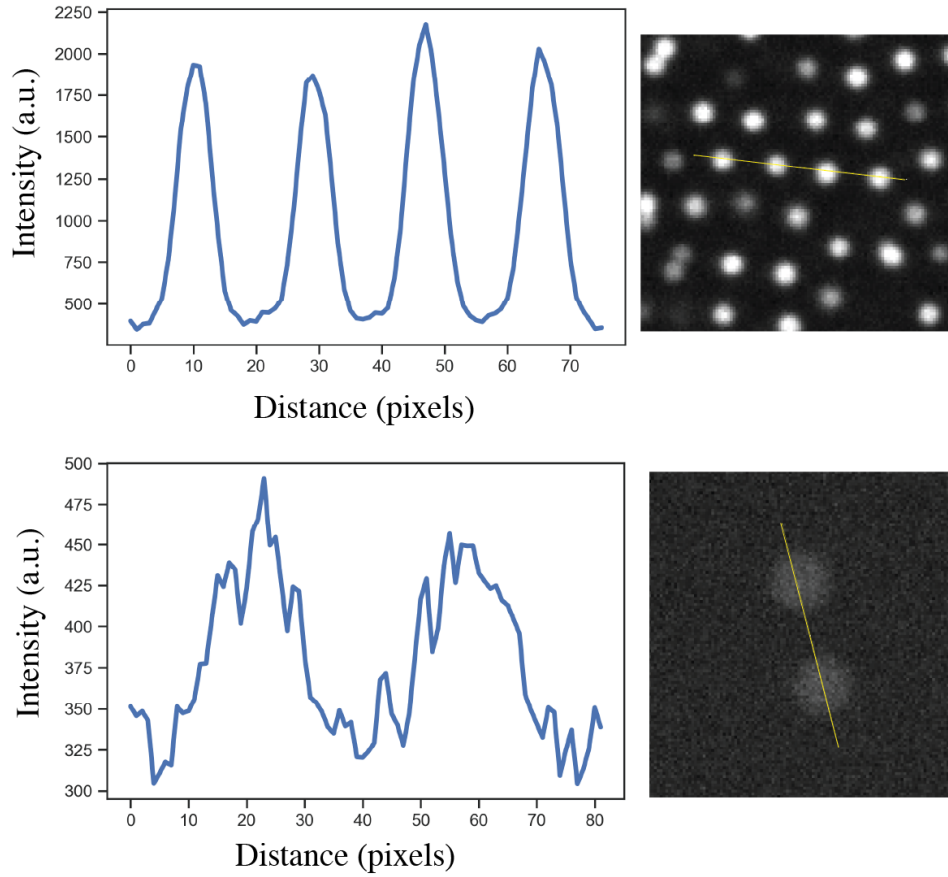


Figure 4.2: Pixel intensities of raw confocal images of gel tracers and sediment particles. The focus is set such that each particle center is in the focal plane. The raw pixel values across a line (depicted in yellow) is plotted. A measure of the signal to noise is given the peak height relative to the background fluorescence. The gel tracers have lower signal to noise ratio ($SNR \sim 1.5$) than the sediment particles $SNR \sim 4$.

age R , dark field image D , and flat field image F as:

$$C = \frac{(R - D) \times m}{(F - D)},$$

where m is the image averaged value of $(F - D)$, and all array operations are computed point-wise. The dark field image is obtained by a per-pixel average of time series of 15k images with the shutter closed. An approximate flat field image is obtained by low pass filter of the raw image. Future work is intended to estimate the per pixel gain ($m/(F - D)$) using a homogeneously dyed solution exposed at various laser intensities as part of improved denoising algorithm recently developed for sCMOS cameras?

4.4 DECONVOLUTION

After flat fielding, the images are saved with single tiff stack labeled by hash value. Next, each chunk is zero-padded and deconvolved using DeconvolutionLab2 using Richardson-Lucy with total variation regularization. This deconvolution algorithm was chosen after surveying the results of different algorithms applied to the data. In particular, total variation regularization preserves sharp edges in the image and tends to give regions of uniform intensity. Given the colloid synthesis, the image intensity should directly correspond to the distribution of dye in the colloid. Given the core-shell structure, we expect a near uniform distribution of dye within the core, followed by a sharp edge in the nonfluorescent shell that contains no dye. Hence, we expect Total Variation regularization to preserve these features, as opposed to introducing bias. Given the different signal to noise ratios in

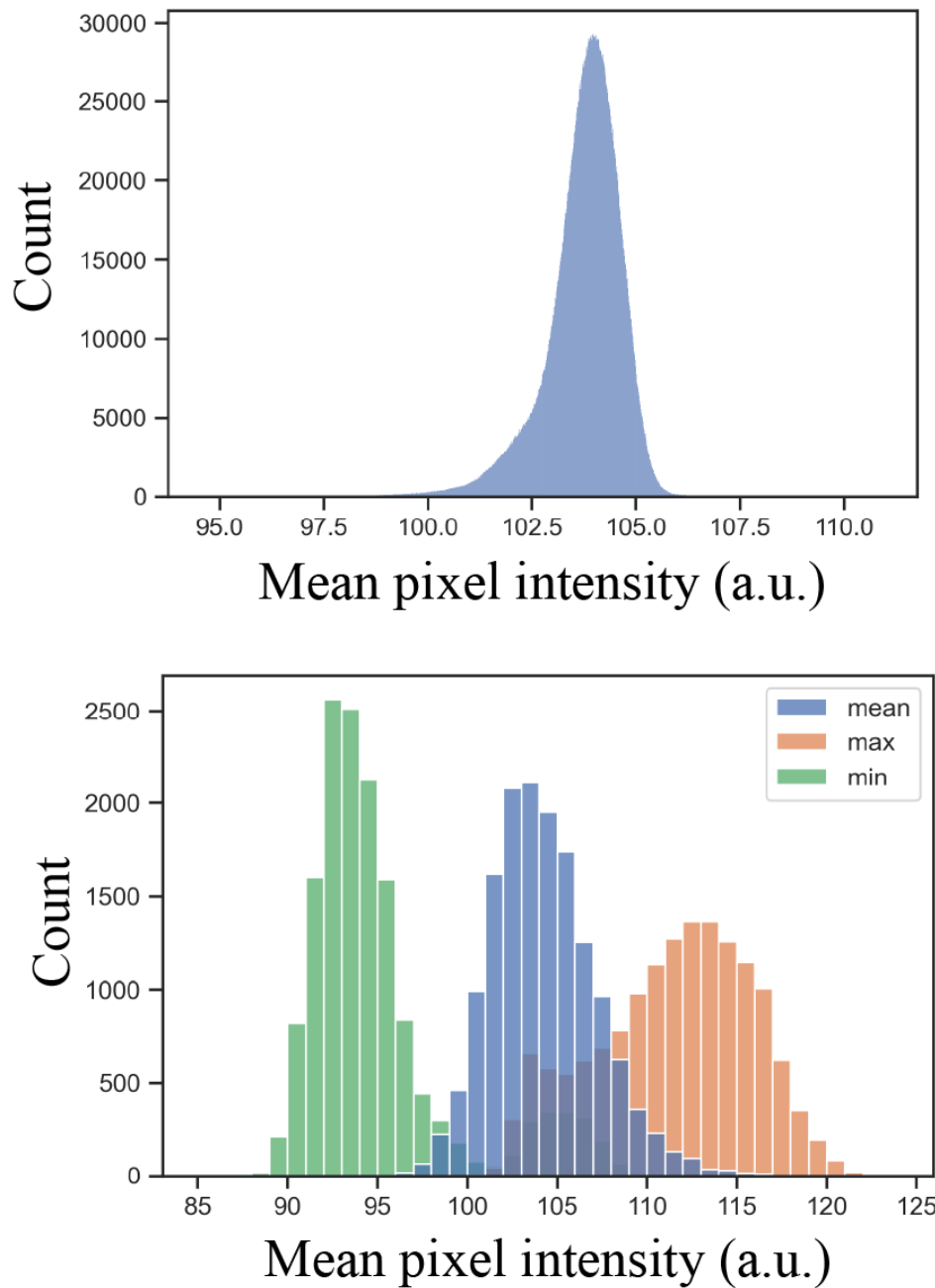


Figure 4.3: Distribution of pixel intensities on camera with no input light. A time series of 15,000 images is acquired in which the camera shutter is closed. The resulting pixel values represent temporally fluctuating and spatially correlated read out noise. The distribution of temporal averages, per pixel is plotted in top figure, indicating a typical read noise of 100 with 16 bit well depth. The distribution of temporal fluctuations for a single pixel whose average is minimum, mean, and maximum of all the pixels is shown in the bottom histogram

sediment and gel chunks, different regularization thresholds are picked.

Deconvolution using Deconvolution Lab 2 was found to require in memory computations of $\sim 10\times$ larger than the input file size; this was determined empirically by setting an input file size of $\sim 100 \times 100 \times 100$ pixels and determining the memory limit at which the program throws an out of memory error. The large memory overhead is likely caused by several factors including the need to pad the input image, increase the bit depth of the array, and store multiple arrays during the computation.

4.5 PIXEL CLASSIFIER

The output from deconvolution is used to train neural network based random forest pixel classifier. This technique requires sparse labeling of individual pixels into discrete channels. For the traction rheoscopy data sets, the channels and corresponding discrete colors were:

1. Colloid particle in sediment (Red)
2. Background of sediment (Aqua)
3. Tracer particle in gel (Blue)
4. Background of gel phase (Yellow)
5. Fluorescent chunks including dimers, fused cores, etc (Green)
6. nonFluorescent chunk including nonfluorescent debris, TEM grid (Pink)

Sparse labeling involved successive rounds of identifying individual pixels belonging to each of the categories, looking at the prediction maps, and adding more training data. The training data sets were small partitions of the data in both gel and sediment phase, and at different spatial locations

and time points. In total, sparse labeling is tedious and takes several hours– but not more than – to carry out and train.

After training, the network outputs pixel probabilities, that is, each pixel is associated with a vector of probabilities $\vec{p}$ such that the i -th component of the vector p_i corresponds to the probability that the associated pixel belong to the i -th channel. The vector is probability in the sense that $\sum p_i = 1$. Assigning a distinct color to each of the channels as described above allows for visualizing the results of the segmentation on typical slices from distinct hashvalues as shown in Figure 4.4.

The pixel probabilities are used to accomplish several distinct tasks in the image analysis pipeline: image segmentation of sediment and gel phases for hash values that overlap with sediment gel interface prior to particle locating as shown in Figure 4.5 and two distinct used post-locating: removal of false positives in gel tracer and identification and removal of fluorescent chunks in the sediment phase. The pixel probabilities are not used to modify the images prior to particle locating, and have no effect on the locating algorithm apart from initial segmentation of sediment and gel phase. The pixel output are used only *after* particle locating to filter the resulting locations.

First, the sediment and gel phases are segmented by requiring, for example, in the gel phase the a sum of pixel probabilities in gel tracers and gel background channels exceeds 50%. Analogously, the sediment phase is segmented, keeping the fluorescent chunks and and non-Fluorescent chunks in that category as well. At the partitions that contain sediment-gel interface, this segmentation is used to construct a mask to remove gel phase particles from the locating rounds on sediment and vice versa as shown in Figure 4.5. Importantly, this obviates the need to parameterize the interface which can drift. Note however, that finding and tracking the interface *after* particle locating is simple and

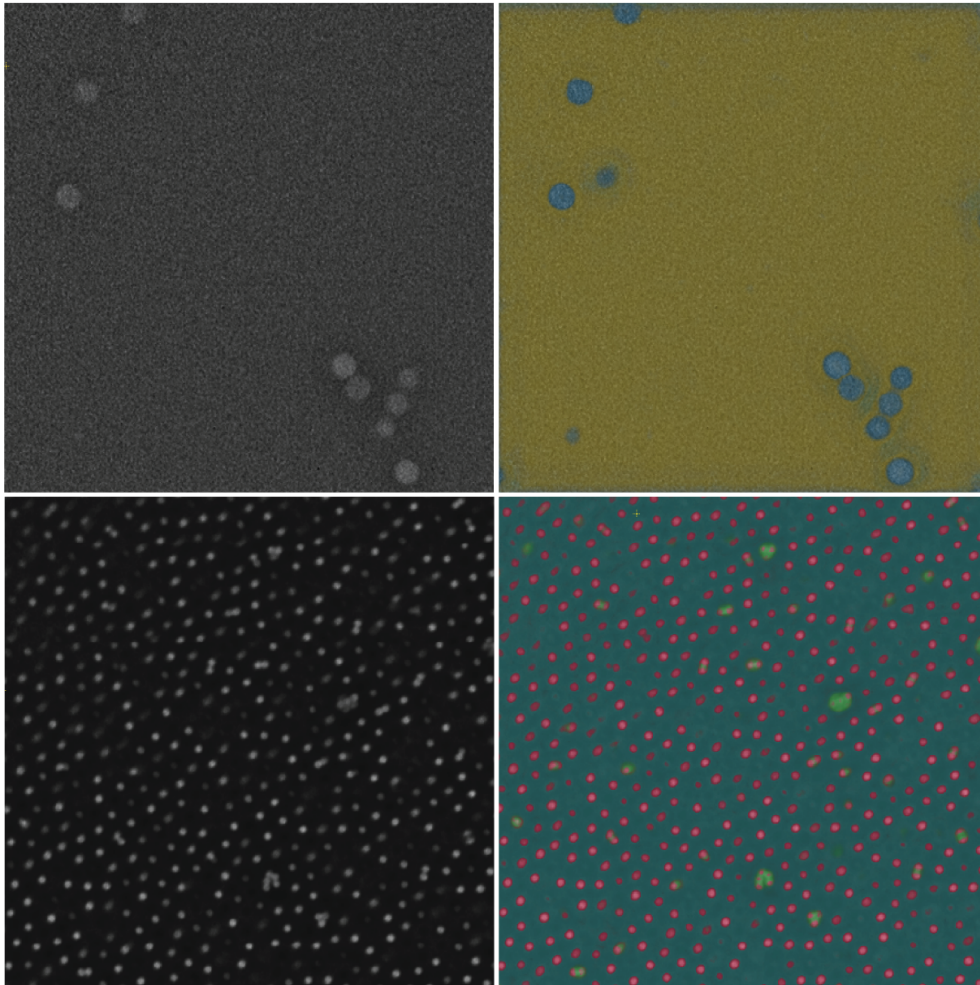


Figure 4.4: Segmentation of gel(top row) and colloidal sediment (bottom row) into particle and background channels using neural network based pixel classifier. A comparison is drawn between the deconvolved images used to train the neural network on the left columns and the output classification for sediment and gel phases separately. For the sediment phase, identification of dimers is shown by the labeling of regions between two fluorescent cores as fluorescent chunk channel. This process allows for the identification (and removal) of dimers when processing the particle locations.

does not require the pixel classifier; we simply identify the colloidal particles at the bottom of the sediment phase, and use the time evolution of the location of the particles to track the interface.

Given the low signal to noise ratio in the gel phase, it proved difficult to identify robust mass cut off parameters to exclude false positives in gel locating data. Setting the minimum mass too high, resulted in missed particles, while minimum mass parameters that were too low typically resulted in many false positives. This problem is very generic in particle locating, however in the gel phase it was exacerbated by low signal noise of the gel particles particularly in partitions that had few tracers. Without tracers in the subfields of the image, the hard intensity thresholding after deconvolution set the threshold too low thereby retaining noisy regions that had comparable mass intensity as the regions with tracers, albeit in those region the background was thresholded to zero. The pixel classifier had no such problems in identifying false positives and allowed the algorithm to safely set a low minimum mass threshold and require that the pixel probability integrated over the region of the gel tracer has larger probabilities of being a gel tracer than gel background.

4.6 PARTICLE LOCATING

The next step in the pipeline is particle locating using Crocker-Grier carried in several iterative rounds. Each round of locating in the sediment makes use of a mask size that larger the particle diameter as the mask can extend into the nonfluorescent shell of the neighbor. Between rounds, the location of particles found on previous rounds are masked out (ie set to zero intensity) and locating is then carried out on the modified images¹⁸. This process lowers the rate of missed particles at the

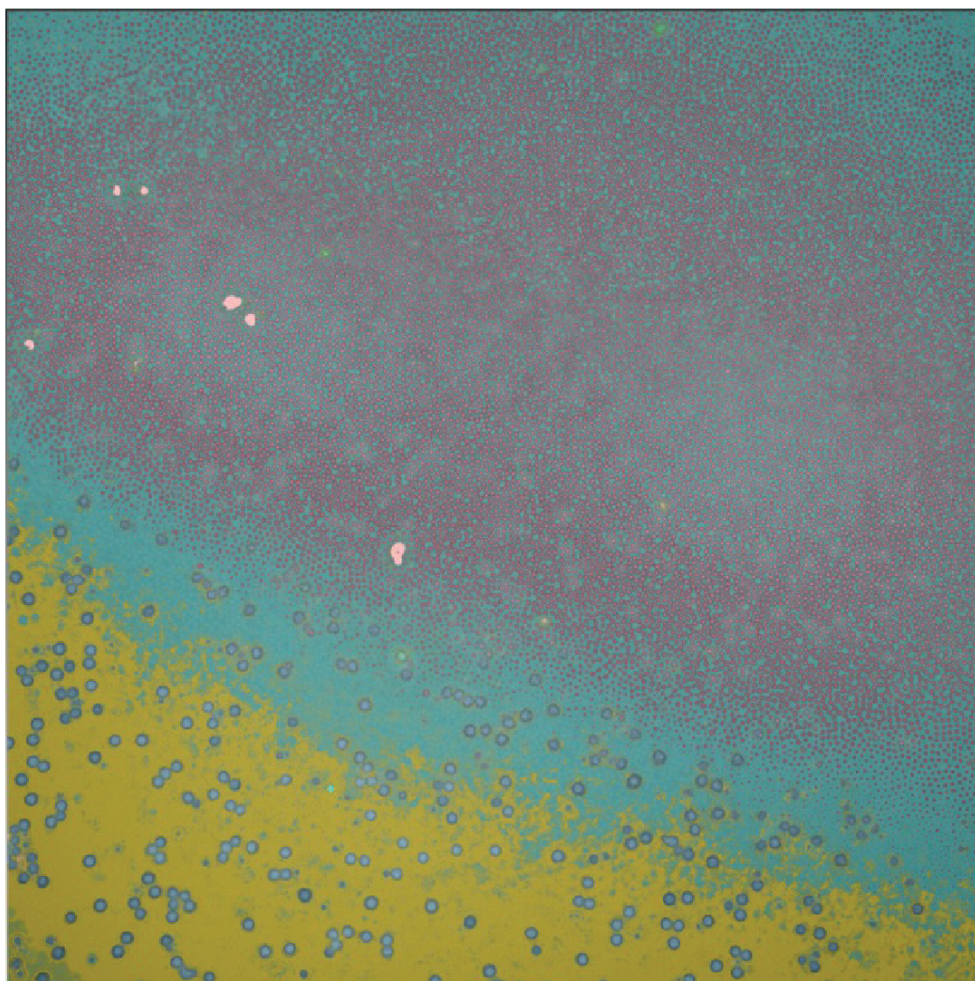


Figure 4.5: Segmentation of polymer gel and sediment interface using a neural network random forest pixel classifier. Separate channels were created sediment and gel phases, along with two channels for fluorescent and non-fluorescent contaminants in the sample. The sediment and gel channels were further split into colloidal particles and background yielding six total channels. The channels are labeled by color: gel background (yellow), gel tracer (blue), sediment colloidal particle (muave), sediment background (aqua), fluorescent chunk (green), nonfluorescent chunk (pink). The slice shown is a fixed distance above the coverslip, and the interface is tilted.

expense of introducing possible spurious particles that are located near fluorescent chunks that are not fully masked out between locating rounds.

In Figure 4.6 I show the integrated mass distribution of two times of the same separated by 6 hours or continuous imaging. The mass distribution clearly shifts to the left due to bleaching even after normalizing the image intensities. Further more, a diffuse peak at large integrated pixel intensities is found to correlate with ilastik pixel probabilities of fluorescent chunk, however fluorescent chunk channel includes lower mass 'particles' that lie within the tail of the mass distribution. The two peaks in fluorescence intensity, I believe, correspond to recycling particles that were imaged and partially bleached in previous experiments.

From the particle locations, the pair correlation function is computed and compared to Finney random ball model¹⁵. After normalizing for the diameter, the pair correlation functions show close agreement as shown in Figure 4.7. Further refinement of the pair correlation are made by using the results of the elastic pixel classifier to remove dimers. The discrepancy in the location and relative heights of the split second peak are tentatively attributed to polydispersity in the as smaller diameter particle that were additionally synthesized with less dye showed height variations in split second peak consistent with Finney's data. The polydispersity in these samples is estimated to be less than 4% however, as the suspension crystallize if sedimented slowly, and the first peak is sharp. Future work can determine the polydispersity for this specific batch of particles using SEM.

After successive rounds of centroid locating, the output positions are used as initial guesses for nonlinear least squares fitting of the fluorescenet spot^{1,37}. The shift positions due to least squares refinement show shifts that are systematic in each coordinate, with magnitudes that are nevertheless

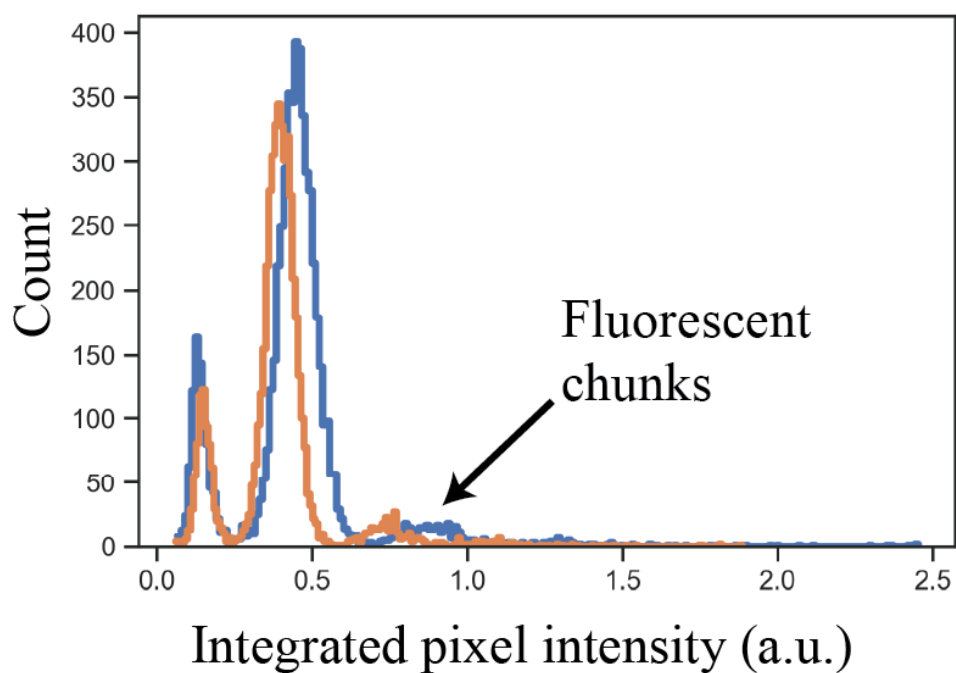


Figure 4.6: Distribution of integrated particle intensities for particles found over two time frames separated by 120 frames. The distributions show multiple modes: the highest integrated pixel intensities correspond to fluorescent aggregates in which multiple fluorescent are present in the fixed integration volume; the second and third peaks correspond to a (surprisingly) well separated group particles with different pixel intensities. The two populations of particles are obtained as a result of combining particle batches, some of which had been previously used in imaging experiments. The shift in intensities is caused by bleaching of the fluorescence, however the magnitude of bleaching is underestimated as the image intensities are normalized during the locating routine. The well separated mass intensities can be used to identify tracking errors by requiring that the mass of the tracked particle is is roughly constant over the experimental tracks.

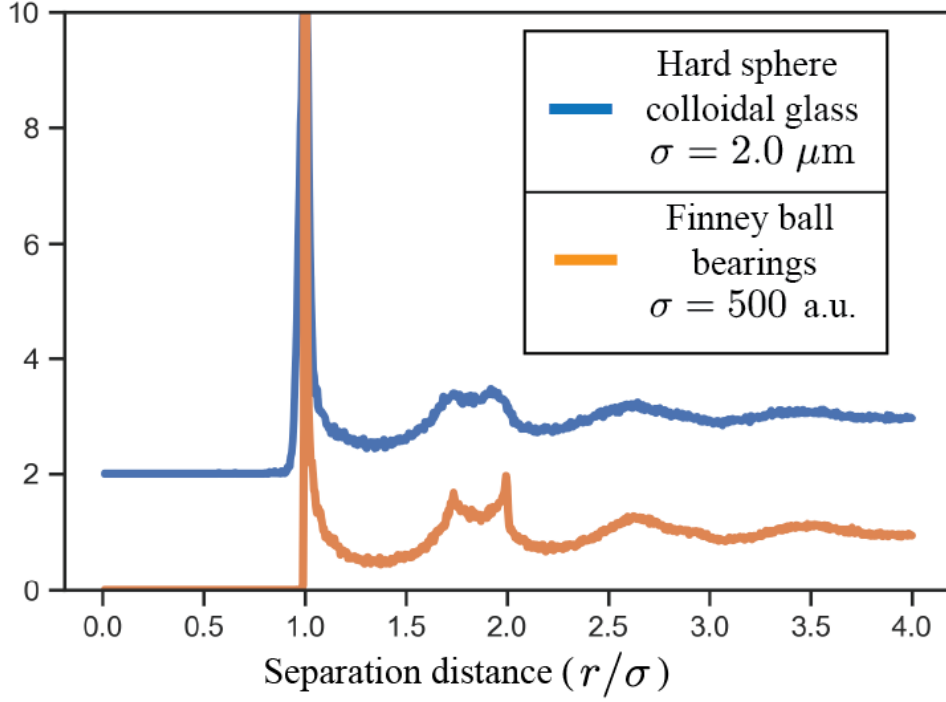


Figure 4.7: Pair correlation function of hard sphere colloidal glass used in this work, compared to Finney ball bearing bead pack. The location of the first peak was used to rescale the data and compare the results. The colloid data is offset vertically by two. The largest sources of discrepancy between the curves occurs at height of the first peak ($g(\sigma_{\text{Finney}})/g(\sigma_{\text{Colloid}}) \sim 30/9$), and a small shift in the locations and relative height of the split second peak; the colloid data is shifted to slightly smaller separation distance on the second split peak and shows approximately equal heights for the split second peak. Both differences are likely attributed to polydispersity in the colloidal sample.

comparable in the uncertainty as shown in Figure 4.9. For other batches of particles with less dye and smaller diameters, the shifts were larger (50nm) and systematic in z; this is presumably due to lower signal to noise in the particle.

In addition to the shifted positions that are corrected by least squares fitting, the *uncertainty* is also estimated using the inverse curvature of the loss function used in least squares fitting. These uncertainties represent 1 standard deviation uncertainties in coordinate position. The loss function includes subtle information on the spot size and shape including disk like structure due to 800nm cores being larger than diffraction limit. As expected, the distribution of uncertainties in x and y are nearly identical and centered at 8nm, while uncertainties in z are larger with a center at 12 nm. This difference is largely attributed to different pixel size as the uncertainties are nearly equal when expressed in pixels. Note that in addition to a larger typical uncertainty in z, the *distribution* of uncertainties is also broader in z. The long tail in locating uncertainties in all components is associated with locating fluorescent chunks as corroborated by ilastik pixel probabilities.

Finally, given the plethora of additional outputs in this locating pipeline we measure per particle correlations in the locating output to answer questions as shown in Figure 4.10. The matrix corroborates several conclusions obtained by moving down the columns of the matrix and examining correlation off the diagonal:

1. Location uncertainties are correlated with each other. If you locate well in x, you also locate well in z, for example
2. Location uncertainties are inversely correlated with integrated pixel intensity. Bright particles have higher SNR and hence lower locating uncertainty.
3. The iteration number is uncorrelated with any parameter. It does not matter in what round

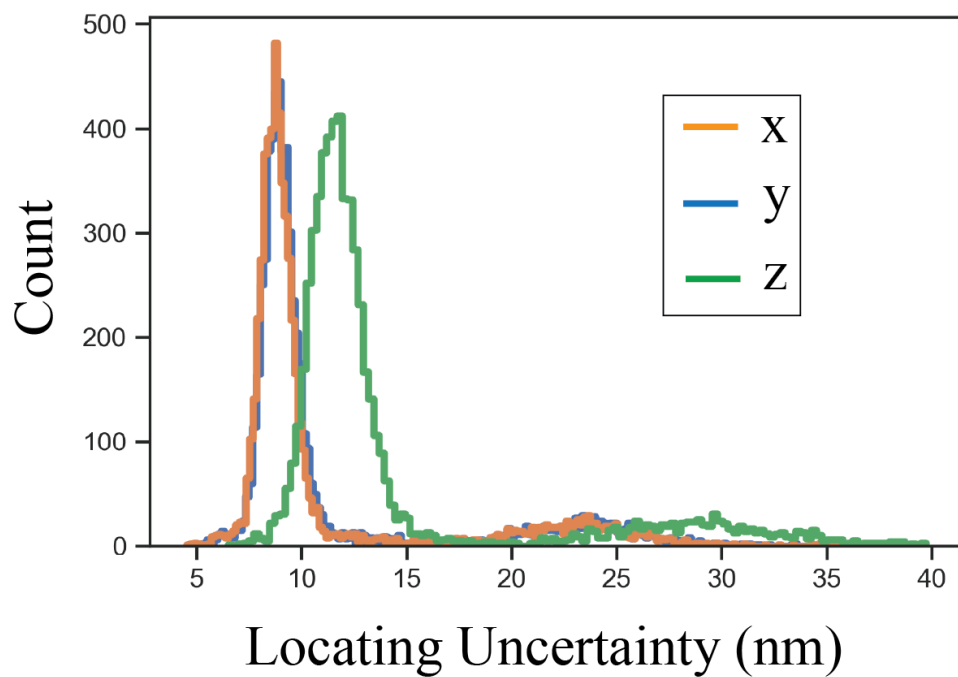


Figure 4.8: The distribution of uncertainties for each particle in a subset of the data. Typical uncertainties in z are larger than in xy as expected for confocal microscopy. Typical uncertainties are 10-20nm, although, there is a tail at larger uncertainties of 40nm. Larger uncertainty is correlated with lower integrated pixel intensity for the particle. Very large apparent uncertainties associated locating dimers or fluorescent chunks have been truncated in this plot.

Location refinement least squares fitting

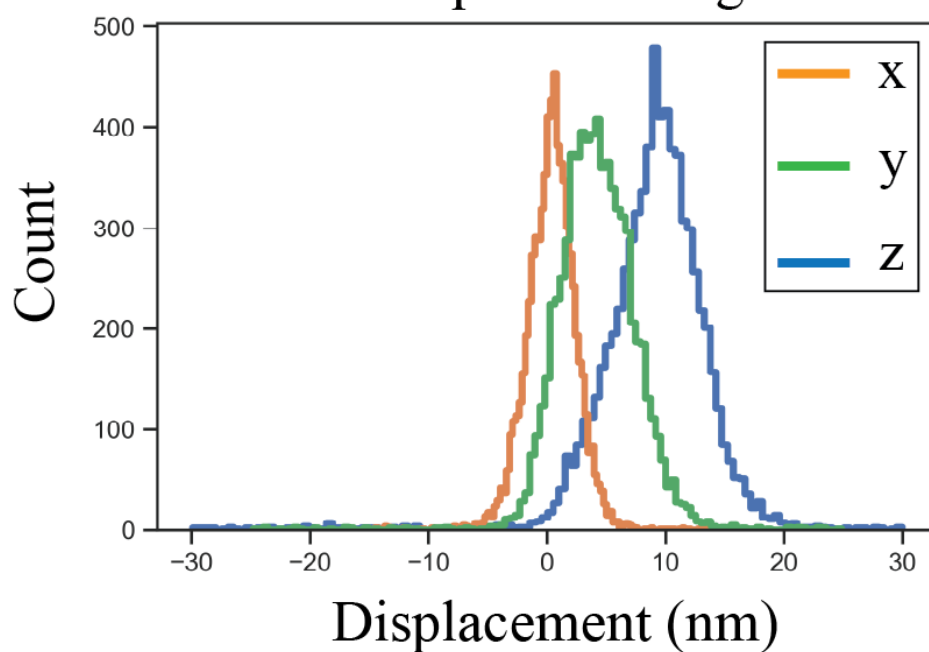


Figure 4.9: Shift in particle locations in using least squares fitting from initial centroid locations. The magnitude of the shifts are within the estimated uncertainty of individual locations. Nevertheless systemic shifts are detected in z, which are hypothesized to be attributed to asymmetry in the point spread function, and in y (but not x) which is hypothesized to be related to uneven magnification in the image. These hypothesis can be tested by further calibrations of the microscope, however the deviations are within the uncertainties of individual particle positions.

of iterative locating you found the particle.

4. Colloidal tracer channels are negatively correlated with background channels indicative of good segmenting in the ilastik image.
5. Weak correlation between nonfluorescent chunk channel and locating uncertainty are present, however due to the relative infrequency, the correlation is not strong over the whole sample.

4.7 ERROR CHAIN MONTE CARLO

Using the per particle location uncertainties, we develop a non-parametric technique to estimate the noise distribution of any quantity computed from the particle locations. This technique is termed Error Chain Monte Carlo (ECMC).

The basic principle of ECMC is to generate particle configurations by sampling the per-particle uncertainties found from particle locating. Once particle configurations have been generated the uncertainties in any derived quantity can be estimated by recomputing the derived quantity over the resampled particle configurations. It is monte-carlo scheme in the sense that "new" particle configurations are generated from the measured particle configurations, and it is a markov-chain in the sense that random displacement added to each particle center only depend on the particle position and not previous history. For the work presented here, the distribution of random displacements applied to the particle are derived from locating uncertainty and hence cannot be used to estimate, for example, uncertainties caused by particle polydispersity.

We demonstrate the use of ECMC to determine the distribution of local particle strains under the null hypothesis that *nothing* changes in the sample and all the strains are limited by locating un-

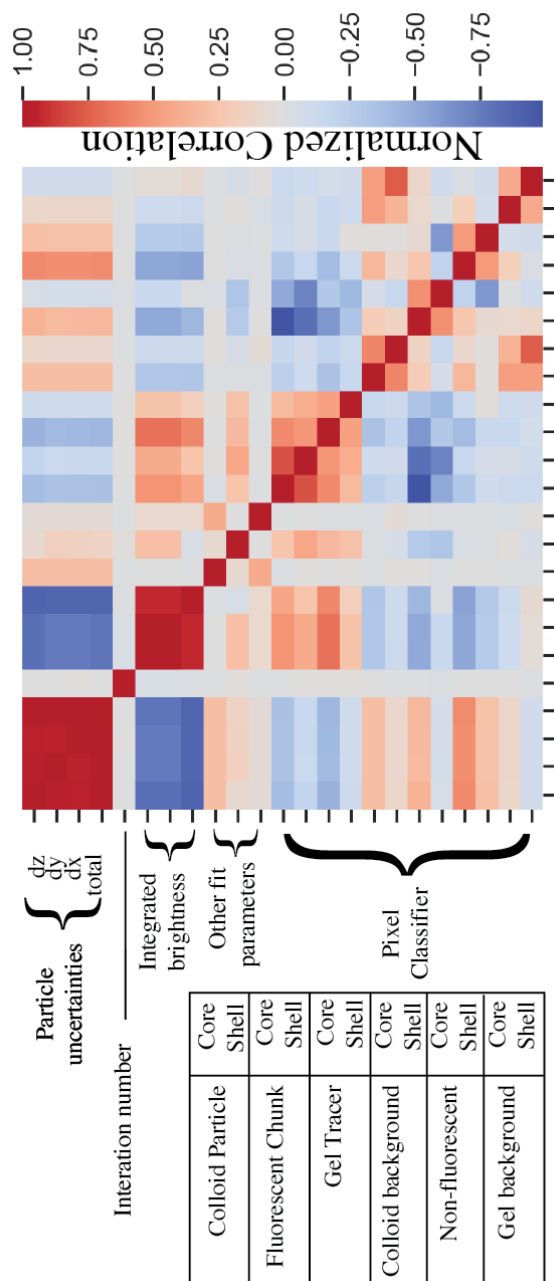


Figure 4.10: Correlation matrix of locating parameters including uncertainties from least squares fitting to particle position, and pixel probabilities obtained from neural network classification integrated over defined regions of particle core and shell.

certainty. As shown in Figure 4.11, typical strain components have standard deviations of 0.5%, with broader distributions in ε_{zz} and pressure terms. As we will be looking at rare large strain events in the next section, the tails of this distribution play a crucial role. The distributions are not altogether surprising given the locating uncertainty. A simple heuristic, supported by ECMC measurements is that locating strain uncertainty is equal to locating uncertainty δx divide by the typical separation distance in the cluster which can be taken to be 1 particle diameter. Using this heuristic would predict locating uncertainties of $20nm/2micron \sim 1\%$, which are order of magnitude larger than the applied incremental strains and comparable to the expected thermal fluctuations in the sample.

While heuristics are useful, the power of ECMC is the ability to estimate uncertainties in data analysis procedures where such heuristics will breakdown. As an illustrative example, important in the next section, What is the distribution in size of particles with high strain that have spatially clustered? If a locating uncertainty samples a rare 3σ displacement on a single particle, that displacement will affect the strain of nearest neighbor particles even if the displacement of the neighbors do not have large deviations. This potentially leads to cluster in local strain that can be difficult to confirm or reject using heuristics. However this poses no problem for ECMC: simply resample the particle configurations, compute whatever function on those resampled configurations and determine the distribution of the resulting quantities.

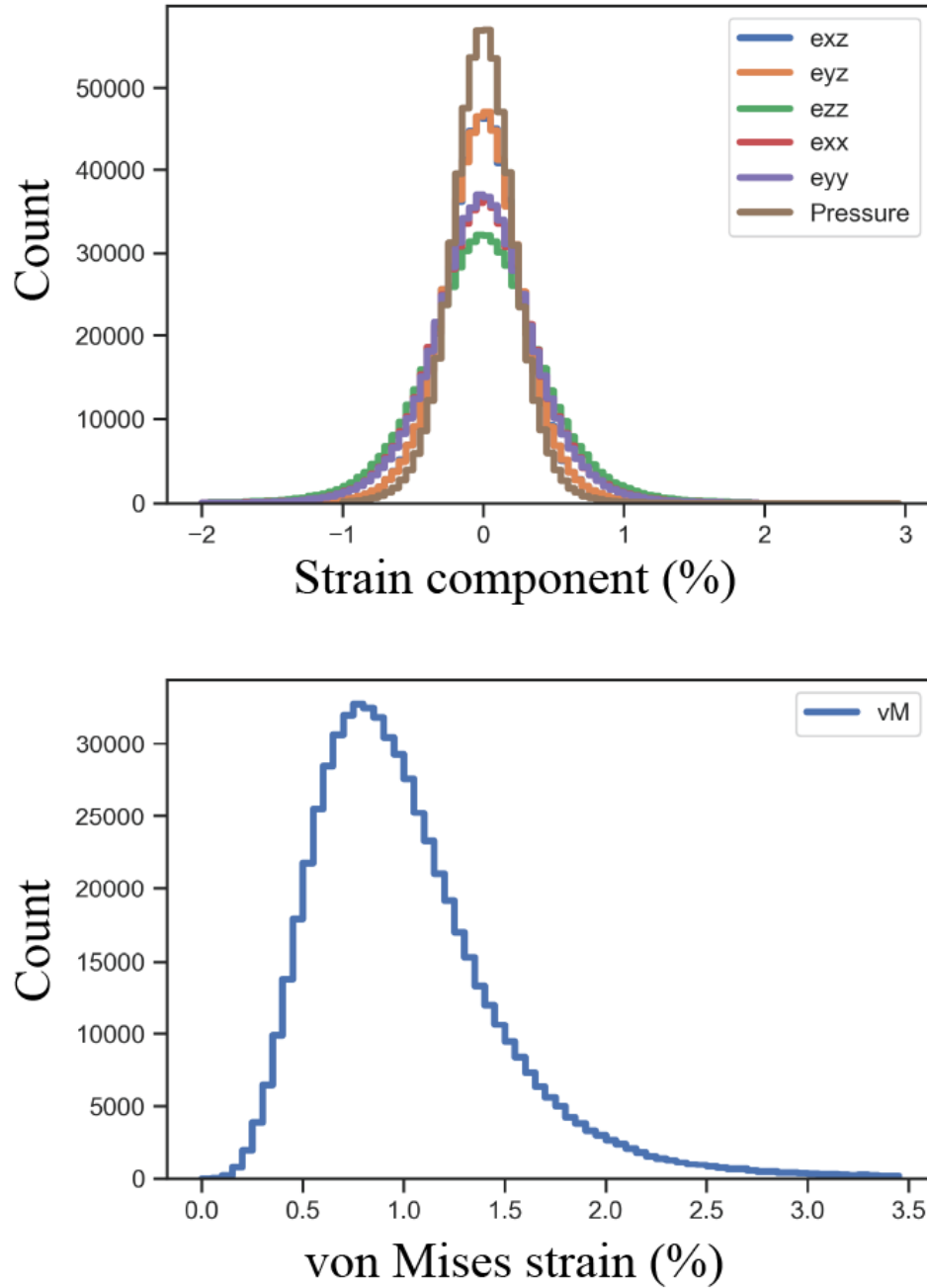


Figure 4.11: Distribution of local strain computed from per-particle locating uncertainties. This distribution represents an estimate of the measured strain distribution *in the absence* of any motion of the particles and hence represents a resolution limit in measuring the strain in the sample. Typical uncertainties in strain components are $\sim 0.5\%$. At this uncertainty level, and given the size of the data sets we should not expect to any strain fluctuations caused by locating uncertainty above 2%, but approximately 1 in every 20 particles will have a strain fluctuation caused by locating errors that is 1%.

5

Shear deformation of colloidal glass

In this chapter, I present experimental measurements on the mechanical response of hard sphere colloidal glass undergoing a sequence of simple shear deformations of increasing amplitude. The stress and strain are measured using traction rheoscopy. We observe an elastic shear modulus of 4 Pa, and an elastic to plastic transition at applied strains of $\gamma = 1.5\%$, and an nearly constant flow stress of 25 mPa at large cumulative applied strains. Additionally we observe a constant shear modulus

of 4 Pa on loading and unloading for each step in the strain deformation. During the deformation we identify clusters of high local strain as measured by von Mises strain invariant. These clusters constitute 1-2% of the volume of the sample and occur homogeneously throughout the bulk of the sample. We observe clusters even at the smallest applied strains, i.e. when the sample is deforming in the elastic regime, and between configurations in which the incrementally applied strain is less than 0.1%. The average shear strain within the clusters is typically at least 10x larger than the incrementally applied strain. At larger cumulative strain amplitude, a region near the shear post undergoes substantially larger strain than the bulk of the sample below. This region grows in spatial extent at larger cumulative strain amplitudes, and hence the strain in the sample develops mesoscopic inhomogeneities in addition to localized inhomogeneities distributed throughout the bulk of the sample. By separating the deformation into spatially distinct regions that show homogeneous and inhomogeneous deformation, we show significant average dilatation alongside larger local strains. Given the stress in the sample is constant and directly measured with traction rheoscopy, we link this dilatation and larger local strains to an overall softening of the material. These data, taken as whole, demonstrate the utility of traction rheoscopy in measuring the mechanical response of model soft materials and establishing the microscopic mechanisms by which the applied strain is accommodated within the material during deformation. These data also provide mechanistic insight into the flow and deformation of amorphous materials and, in particular, allow for independent measurements of the geometry of local rearrangements alongside stress measurements. The combination of independent measurements provides a more exacting test of theories of plastic flow in amorphous materials^{33,5,19}.

5.1 DATA ANALYSIS: STRESS

The stress is measured by locating and tracking the displacement of tracer particles in the gel, and then fitting the displacement profile to a linear deformation. The slope is direct measure of the strain in the gel, and is used, along with the calibrated shear modulus, to calculate the stress in the gel. The low frequency shear modulus of the gel used for this work is 4.9Pa. In a typical strain ramp protocol, the *imaging stacks* acquired during a deformation step include the top 30 μ m of the gel and full the gap between the top surface of the gel and the shear post. Prior to each deformation step, a *full stack* is acquired which includes the bottom coverslip, full gel, and the full sample. In addition to the imaging stacks that are primary data for determining the evolution of the stress during a deformation step, three micro-stacks that include small 10 μ m region around the coverslip-gel, gel-sediment, and sediment-post interface are also acquired. Future data analysis pipelines can include these stacks as second, complementary measure of the stress-strain curves as they include, in principle, all the information necessary to determine the stress and the average applied strain the sample in addition to correcting for drift at the coverslip.

The z-position of the imaging and full stacks, which have different pixel dimension and location in the sample, are correlated by using the location of the sediment-gel interface as a fiducial marker. Concretely, the first layer of sediment particles, which map out the flat gel substrate, are fit to an affine plane in both the imaging and full stacks. Then the offset in the affine plane used in the full stack sets the absolute gel thickness (with a typically small correction for the location of the coverslip in the full stack), and the positions and identities of the tracers that are common in both the imag-

ing and full stacks is determined by matching their positions relative to the sediment-gel interface. This procedure of using the sediment-gel interface as a fiducial marker is also used to correct z-drift during a deformation; while the absolute z-position of the particle is affected by microscope drift, the relative position to the sediment-gel interface is not. The strain in the gel is then determined for each time point during a deformation step by fitting the displacement of the tracers in the shear direction as function of the *absolute* height of the tracer above the coverslip, where the offset between imaging stacks and full stacks is determined once and relative changes in height are tracked using only the imaging stacks.

Two fitting procedures are used to determine the strain in gel and yield similar results. These fitting procedures, unlike ordinary least squares regression, were chosen as they are robust to outliers. Outliers were present in earlier work and when these analysis procedures were written it was unclear to me *why* the outliers were present, only that they were present and clearly spurious. Since writing these data analysis procedures, I have identified the underlying cause of outliers as excessively large maximum displacement in the tracking parameter that was used to compensate for rigid body drift in the sample. After improving the experiment to remove rigid body drift and re-adjusting the tracking parameters to be more stringent, outliers are much reduced and in many cases eliminated all together. Nevertheless I have continued to use linear regression that is expected to be robust to outliers, if present. Future work will go back to re-analyze the improved particle tracks with ordinary least squares regression. I do not expect this re-analysis to change the results of this chapter.

First, the trajectories are binned in z, and the average displacement is computed in each z-bin. Outliers in the displacement are removed using a simple "Tukey fences" in which any displacement

that is larger than 1.5 times the interquartile range computed over the bin is excluded. These outliers are rare and stem from tracking errors, which in principle, can be removed by imposing a more stringent displacement cutoff during tracking. Given the average displacement per bin, ordinary least squares fitting is used to determine the slope; the y-intercept is left as open parameter and is verified to be near zero as expected for no-slip boundary condition near the coverslip. The height dependent displacement profile obtained with binning are shown in Figure 5.1

I note in passing that any lateral displacement in the position of the coverslip is also explicitly checked by looking the auxiliary coverslip-gel micro-stack. Future data analysis pipelines could incorporate these microstacks to further reduce (or eliminate) uncertainty in the lateral drift, which is likely a dominant uncertainty in the stress measurement.

Second, a Theil-Sen robust linear regression is carried out on the displacement versus z-height data. Theil-Sen regression reports the slope of the linear regression as the median of set of slopes of lines going through every pair of points. Like OLS, it returns an unbiased estimate. Unlike OLS, Theil-Sen linear regression is not sensitive to outliers thereby obviating the need to filter and bin the displacements. Initial results comparing ordinary least squares regression to Theil-Sen regression show consistent and method-independent results on the fitted slopes, and hence the inferred stresses. Future work will consistently apply OLS in favor of Theil-Sen as the fitting procedure provides estimates on fitting uncertainties for the extrapolated displacement at the coverslip; the values of the extrapolated displacements are, however, small.

5.1.1 IMPROVEMENTS

The dominant source of uncertainties and signal loss during this step is a deleterious combination of comparatively low signal to noise of the gel tracers relative to the sediment particles, as discussed in the locating chapter, combined with the reliance on *complete* particle trajectories to map out the deformation over several frames. These two effects combine as the low signal to noise of the dimmest particles yields a large rate of missed particles. As a result, while approximately 3000 particles are located at each time frame in the stack (and somewhat paradoxically, this number remains nearly constant for all times), only 1k brightest, highest signal to noise particles are located and tracked for each time step. The other 2k+ particles, while located most of the time, are not located *all* of the time. The net result is incomplete particle trajectories that clearly agree with the deformation of the gel as a whole but are 'blinking' off when the particle is missed during a locating step. There are at least two data analysis procedures, and one experimental improvement that could be used to remedy this in the future. First, the particle trajectories could be interpolated between short stints of blinking off. A second, and in my opinion preferred data analysis procedure, is to map the deformation of the gel at each time back to the absolute reference configuration thereby completely eliminating the requirement of long coherent trajectories of particle. Given a time point, this could be done by first estimating the cumulative deformation of the gel up to that time point using all the trajectories that are complete up to then, then applying this estimated inverse deformation gradient to all the measured the particle positions to obtain an estimated reference configuration, and finally "tracking" the displacement of the tracers between the measured reference configuration, and the

estimated reference configuration. For each time, the inferred inverse deformation gradient can be applied to all particles including those that may not have been located at some prior time and hence do not possess complete trajectory up to the given time.

As discussed in the locating chapter, missed particles arise from low signal to noise of the tracers particle in part due to limited dye added to the particles, and significant out of focus fluorescent background caused by the much brighter and more densely packed sediment particles. Both of these limitations can be solved by spectrally resolving the gel and sediment phases into distinct channels, and adding substantially more, perhaps as much as $10\times$ more, dye to the tracer particle during synthesis. Changing the fluorescence of the gel tracers, as opposed to the sediment particles, is preferred as the synthesis does not require a polymerizable dye as in the case of core-shell particles used in the sediment phase.

Carrying out these improvements will likely yield somewhat lower uncertainties in the stress measurement, however if lower stress uncertainties is the primary goal, a more compliant gel can be used. I suspect that the most significant improvements gained by improving the data analysis of the gel tracers is the possibility of tracking the deformation of the gel over *multiple* deformation steps in an extended strain ramp, and dramatically improving the spatial resolution of resolved stress. With only $1k$ particles distributed in a volume of $30\mu m \times 235\mu m \times 235\mu m$, the mean spacing between the particles is at least $10\mu m$, but in some sections as large $30\text{-}50\mu m$, which is likely too large to resolve localized stress that may have spatial extent of only a few microns. In any case, future work based on improved particle locations of the gel tracers, should aim at implementing data analysis procedures developed for traction force microscopy, including finite element modeling, to determine the

spatially resolved stress using 3D resolved deformation available with traction rheoscopy.

5.2 DATA ANALYSIS: STRAIN MEASURES

The strain in the sediment measures the change in shape, and local configuration of the particles making up the sediment. There are several data analysis methods to determine the distinct but related measures of the strain from the particle trajectories. First, the *bulk strain* can be determined from relative displacement of particles at the upper and lower boundaries of the sample, and the shear gap which is defined as the separation distance between the upper and lower boundaries. Second, a more local but still averaged measure of the strain can be determined by fitting the displacement of particles in z-bins that are larger than single particles but smaller than the shear gap. In this work, I define this strain as the *mesoscopic strain*. If the displacement profile is linear over the whole sample, then the mesoscopic strain, calculated between two adjacent bins will be equal and equal to the bulk strain. Third, a *local strain* can be defined by analyzing the change in configuration of each particle and its nearest neighbor shell¹⁴. Each of these strain measures are related as the local strain, when averaged over the whole sample should match the bulk strain, and when averaged over bins should recover the mesoscopic strain. The mesoscopic strain and bulk strain are obviously related as the mesoscopic strain is just the limit of bin sizes extending to the whole sample.

To compute these strain measures, the upper boundary consists of colloidal particles near but below the shear post, while the lower boundary is defined by the bottom most layer of colloids just above the gel-sediment interface. The bulk strain in the sample is the relative displacement between

the upper boundary and lower boundary divided by the shear gap. Second, the mesoscopic strain is defined by binning the z-position of the particles throughout the sample, and fitting the displacement profile across the bin. In addition to these sample averaged measurements of the strain, we define a local strain measure using a symmetric part of the best-fit affine deformation for each particle as defined by Falk-Langer¹⁴. A slight variation on this is implemented in Ovito³⁴ whereby the computed best fit affine deformation gradient is decomposed to using a polar decomposition to obtain strain and rotation tensors, as opposed to symmetric and skew-symmetric decomposition used by Schall³².

After compute the particle level strains, the strain can be averaged over any set of particles, including spatial and non-spatial sets. For example, the local strain can be averaged over particles in distinct z-bins that were used to defined mesoscopic strain. As a hypothetical non-spatial example, the local strain can be averaged over of particles whose local strains were larger than some cut-off, or particles whose local coordination or structure met some criterion. These sets may not have some obvious, a priori spatial definition, but may have some underlying spatial structure that emerges from the data analysis.

The Falk-Langer strains are defined using equations 2.11-2.14 in Reference¹⁴, reproduced here

$$X_{ij} = \sum_n [r_n^i(t) - r_0^i(t)] \times [r_n^j(t - \Delta t) - r_0^j(t - \Delta t)] \quad (5.1)$$

$$Y_{ij} = \sum_n [r_n^i(t - \Delta t) - r_0^i(t - \Delta t)] \times [r_n^j(t - \Delta t) - r_0^j(t - \Delta t)] \quad (5.2)$$

$$\varepsilon_{ij} = \sum_k X_{ik} Y_{jk} - \delta_{ij} \quad (5.3)$$

$$D^2(t, \Delta t) = \sum_n \sum_i \left(r_n^i(t) - r_0^i(t) - \sum_j (\delta_{ij} - \varepsilon_{ij}) \times [r_n^j(t - \Delta t) - r_0^j(t - \Delta t)] \right)^2 \quad (5.4)$$

where $r_0^i(t)$ is the i -th component of the position of the central (ie 0-th) particle at time t , while analogously $r_n^j(t + \Delta t)$ is the j -th component of the position of a neighboring particle, indexed by n , at some later time $t + \Delta t$. The vector pointing from a central particle to a neighbor particle is termed a branch vector.

The physical meaning of local strain is that pressure changes (terms like ε_{xx} in which the direction of displacement is parallel to branch vector connecting the particles) correspond to dilatation or contraction of the particle's nearest neighbor shell, while shear terms like ε_{xz} , in which the displacement is perpendicular to the branch vector correspond to change in the angle between particle and its nearest neighbor. The deformation of the local environment is determined using a best fit affine deformation. Geometrically, an affine deformation is most easily visualized as a displacement that maintains parallel lines, that is parallelograms for example, are deformed into other parallelograms with perhaps different volumes and internal angles but they do get deformed into trapezoids. The residual term $D_{\min}^2$ is a measure of the non-affinity of the local strain is obtained by summing the residual displacement of the observed position of n particles (that is, terms like $r_n^j(t + t\Delta t)$ relative to

the best fit affine deformation ε_{ij} applied to the reference configuration (that is terms like $(r_n^i(t))$).

An interesting note on the local strain measures is that as they take into account the displacement of the particle relative to the neighboring shell a local strain by definition, will introduce short-range spatial correlations in the strain even when given a spatially uncorrelated vector field of random displacements; if one particle takes an apparent large displacement resulting from sampling the tails of the distributions, that particle will have a large strain, but the strain of its neighbors will also be large. The extent of this spatial correlation depends on the range of particles used to calculate the strain. In any event, any spatial correlations for random displacements arising from locating uncertainty can be estimated using error chain monte carlo.

Experimentally, the non-affinity can be the result of particle locating uncertainty or complicated local rearrangements and shifts in the particle positions that are not captured by locally affine deformations. As the spatial extent over which the local strain is computed is extending beyond nearest neighbor shells, more particles are included in the computation leading to a natural increase in the non-affinity. Moreover, as the spatial extent includes more particles, the affine deformation assumption breaks down with a subsequent reduction in the value of the local strain components and, likely, a diverging fractional error. In short, there is an optimal spatial extent to compute the best fit local strain at or around the first nearest neighbor shell. Admittedly this argument is just a heuristic, however future work will compute the fractional error of local strain measures under the assumption of no-applied strain and just locating uncertainty.

5.3 ELASTIC BILAYER

Using the data analysis procedures described in the previous, we start by computing the binned displacements as function of distance from sediment-gel interface as shown in Figure 5.1. This analysis is conceptually similar to the schematic diagrams used to motivate traction rheoscopy and serve important test of no-slip boundary condition between the colloidal glass and gel. We use bins of fixed width of δ for particles in the colloidal glass and gel, with a final bin that has some small variation in width to accommodate misalignment or z-motion of the grid. To specifically test for no slip boundary condition an interfacial bin is added at position 0 consisting particles that are at the interface of the sediment and gel. As discussed in the fabrication chapter, this first layer of particles is adsorbed to the gel using polyelectrolyte multi-layer.

As shown in three snapshots, the displacement profile of the gel is linear, while the displacement profile of the sediment is initially linear, before developing larger displacements at the top of the sample. This is indicative of stain localization at the top of the sample. Additionally, we see that the slopes of the sediment and gel layers are close, with the sediment being slightly softer. Using these slopes, one could infer the ratio in moduli of the sample and calibrated gel. As will be shown quantitatively in the next sections, this is consistent with the colloidal glass having a shear modulus of 4 Pa, while the calibrated polymer gel has a shear modulus of 4.9 Pa. Combining the salient material and geometric parameters of the gel – a fixed height of $145\mu\text{m}$ and shear modulus of 4.9 Pa – a useful relation is that a $1\mu\text{m}$ displacement of the top of the gel corresponds to a 17 mPa of shear stress.

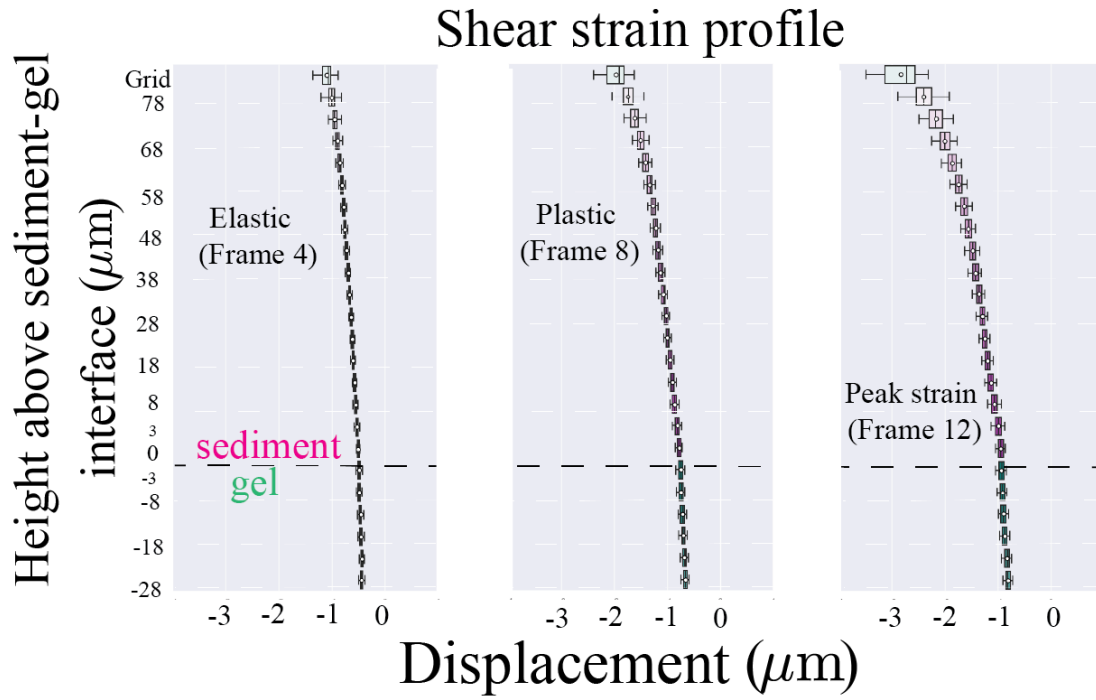


Figure 5.1: The displacement vs. height profile for three times in the elastic, plastic, and peak strain deformation of step d. The heights of the particles are computed relative to the gel sediment interface defined by fitting the first layer of particles to an affine plane. The gel tracers are computed as distance below the sediment-gel interface, and hence have negative values on this plots. The error bars represent the middle 90% of the distribution of displacements; the box represents the middle 50% of the distribution; the vertical line within each box is the median; the open white circle is the mean of the distribution.

5.4 STRESS AND STRAIN OVER TIME

5.4.1 STRESS

We next determine the evolution of the stress and strain over time a sequence of simple shear deformations in which the applied displacement is a triangle "bump": the post moves at constant velocity until a peak strain is achieved, and then it returns, at the same velocity, to the start position. The sequence of steps is deemed a "strain ramp" and each step is indexed by letters from a-f as the applied peak strain increases. The strain rate, strictly speaking, is not constant as it depends on the strain carried by the gel relative to the sample. Additionally, the initial steps, a-b have an order of magnitude lower applied strain rates to ensure at least 5 imaging steps on the ramp up to achieve the peak strain. Additional adjustments to the velocity of the shearpost are made so that the peak strain is reached at the end of an imaging stack.

As shown in Figure 5.8, for small applied strains in steps a-c, the stress increases linearly and then returns to zero. Additional imaging time of 10 frames is acquired at the end of strain deformation to measure any stress relaxations. At higher applied strains, steps d-f, the stress crosses zero prior to the shear post returning to the original position. This is indicative of plastic flow in the sample. Additionally, at even larger applied strain of e and f, there is an emergence of stress plateau indicating flow at constant stress. This is likely due to shear localization at the top of the sample.

The error bars in Figure 5.8 represent one standard deviation uncertainties in the fitted slopes of the gel. For step f, the magnitude of the uncertainties are plotted as function of time in Figure 5.3

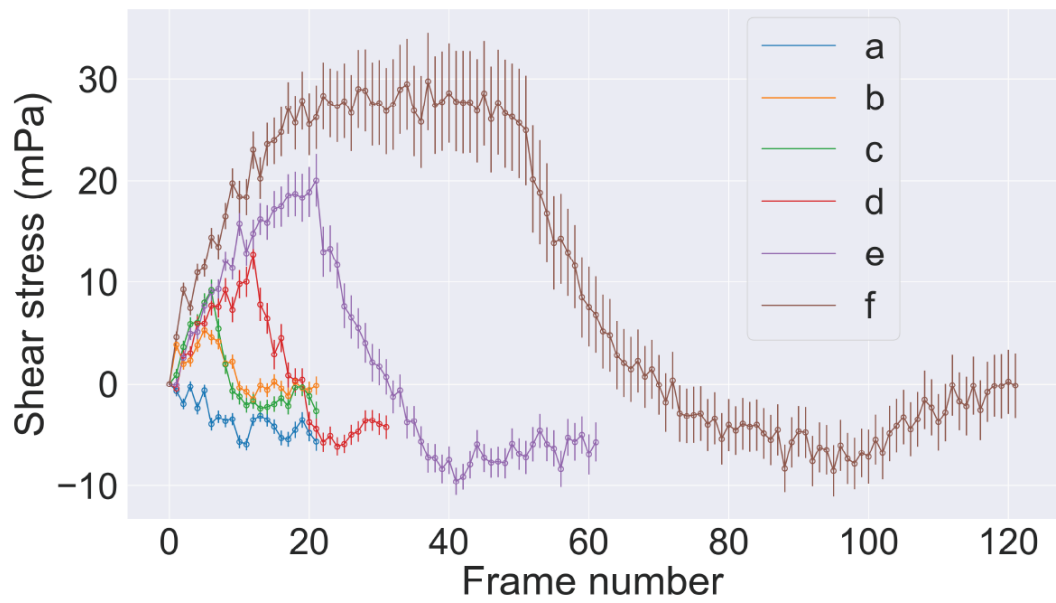


Figure 5.2: The time evolution of shear stress in the direction of the applied shear. The zero-stress configuration is treated as the initial frame at the beginning of each step (thereby ignoring any residual pre-stress from prior deformation). The error-bars represent one standard deviation error in the inferred slope from fitting the height dependent displacement of the tracers in the gel to a linear displacement profile.

Absolute uncertainty in stress measurement

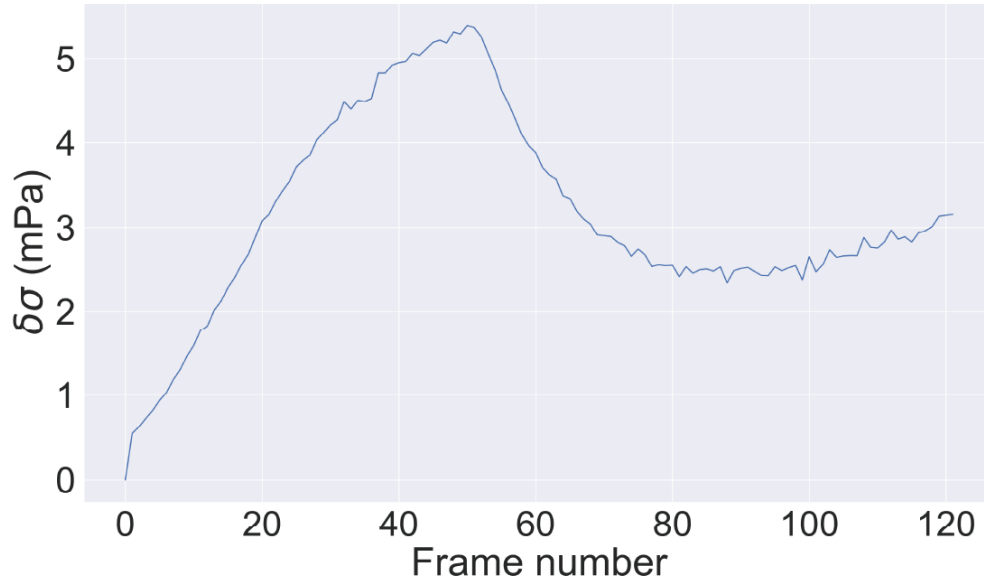


Figure 5.3: The calculated uncertainty in the stress measurement as a function of time for the final step in strain ramp deformation. The initial uncertainty is less than 1mPa, and at most 5mPa. Typical fractional uncertainties in the plastic flow regime are 20%.

The errors increase over time to roughly 10-20% fractional uncertainties in the plateau regime. Initial uncertainties, when the quality of fits is better due to higher signal to noise and more particles, are below 1 mPa.

Further improvements to the uncertainty can be attained by improving the signal to noise of the tracers in the gel phase, principally by separating the fluorescence of the gel and sediment phases into distinct channels, and increasing the amount of dye used during synthesis. Interestingly, the uncertainties go *down* when the shear is reversed. This is contrary several sources of uncertainty: loosing particle tracks and degradation of signal (either due to bleaching or index mismatch of the immersion oil due to evaporation) both *increase* with time independent of the applied strain and hence give

rise to progressively worse signal to noise. Moreover loss of signal would give rise to *additive* noise. One possible source of uncertainty that could explain the observed uncertainties in the strain is the presence of *fractional* uncertainty on the displacement of the particles. That is the positions of the particle are not corrupted by $x \pm \delta$, but rather corrupted by terms like $x \pm x \cdot \delta x$. The fractional uncertainties in displacement, could be caused by optical aberrations present in the sample. These optical aberrations come in the form of distortions of the pixel to micron conversion, along with possible shape distortion in the pixels, at the corners of the image. This distortion would give rise to spatially correlated *fractional* errors in the displacements of the tracers, which when propagated forward to the strain fits, could lead to fractional errors in the strain. Future work is examine the spatial dependence of the residual displacements of the tracers to test this possible source of uncertainty. If present, an important future improvement to traction rheoscopy is calibrate and remove this distortion. These further improvements (two channel fluorescence, improved SNR on the tracers, and microscope calibration) are likely necessary in order to resolve potential stress drops that may occur with magnitude approaching 1mPa. These improvements can be prioritized. Ignoring other sources of uncertainty and improvements due to averaging, current locating uncertainties of 150 nm in the gel give rise to uncertainties of ± 2.5 mPa in the measured stress. These uncertainties are in line with relative signal to noise of the gel tracers and sediment particles. If SNR of the tracer particles were improved to the level of sediment particles, I estimate that sub-mPa stress resolution, with additional spatial resolution of 5-10 can be achieved. Furthermore, improve the SNR of the gel tracers is the first step in calibrating any fractional errors due to optical aberrations.

5.4.2 STRAIN

We compute two independent measures of strain described in previous section as shown in Figure 5.4. We use $2\langle\varepsilon\rangle$ to denote volume average of the local strain compute with a fixed initial reference configuration, and γ to denote the strain computed from the relative displacement of the boundaries of the sample. The factor of 2 is a geometric factor relating the engineering strain γ to the strain ε . Consistent with linearly increasing and decreasing strain ramp, we see a relatively smooth increase in the strain as function of frame for steps a-d. The initial steps a and b show markedly different strain rates a direct result of different velocities of the shear post. As shown in Figure 5.5. At low strains we see close agreement of the boundary value strain γ_{xz} and the volume average strain ε_{xz} . However at steps e and f, deviations start to appear and rise to $\sim 2\%$, corresponding to the development of strain localization at the top of the sample.

In addition to the average xz strains, we plot the cumulative von Mises strain invariant as function of frame as shown in Figure 5.6. The von Mises strain is defined as the second invariant of the deviatoric strain and is a scalar measure of the total shear strain in the local volume over which it is calculated:

$$\varepsilon_{vM} := \sqrt{\frac{1}{2}[(\varepsilon_{xx} - \varepsilon_{yy})^2 + (\varepsilon_{yy} - \varepsilon_{zz})^2 + (\varepsilon_{zz} - \varepsilon_{xx})^2 + 3(\varepsilon_{xz}^2 + \varepsilon_{yz}^2 + \varepsilon_{zx}^2)]}, \quad (5.5)$$

where ε_{ij} is a component of the best fit affine strain tensor. The von Mises strain is tensor invariant and hence is independent of the basis in which it is computed. As a result, it is a useful measure find

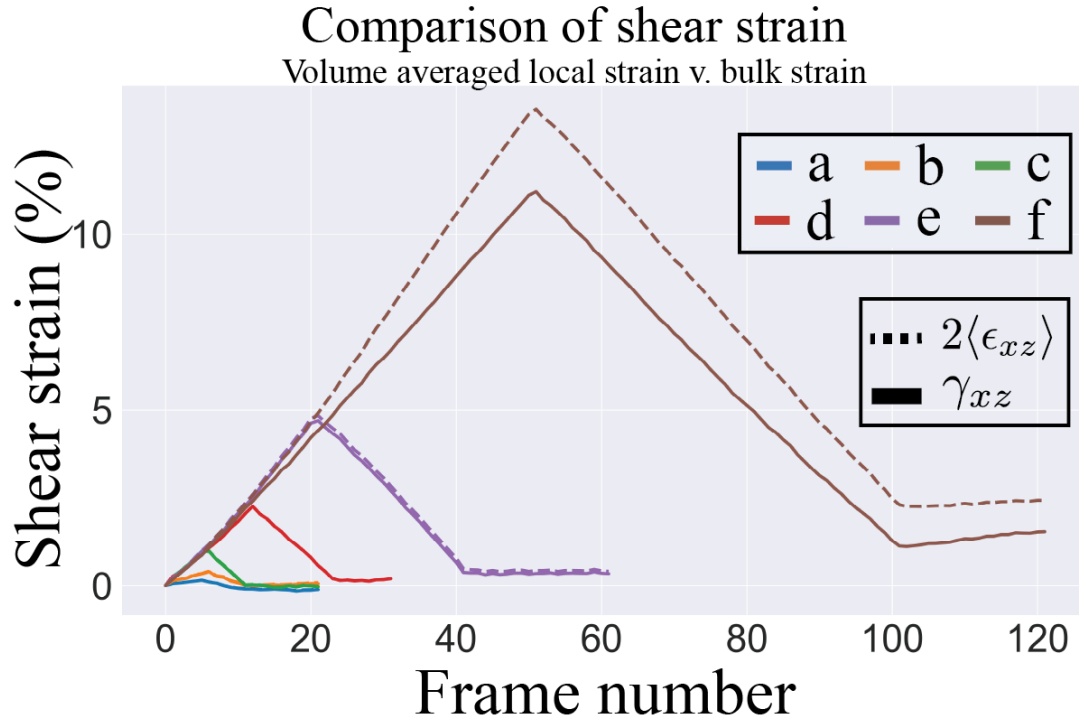


Figure 5.4: The time evolution of the shear strain component ϵ_{xz} for each step in a strain ramp experiment. The strain is computed in two distinct ways: First, a volume average of the local strain tensor $\langle\epsilon_{xz}\rangle$ where the average is computed over every particle in the sample. Second, the bulk applied strain γ_{xz} is determined by the relative displacement of the upper and lower boundaries of the colloidal glass. The strain measures agree at low strain, but, as the sample undergoes inhomogeneous plastic flow in steps e and f, the two strain measures show progressively larger deviations.

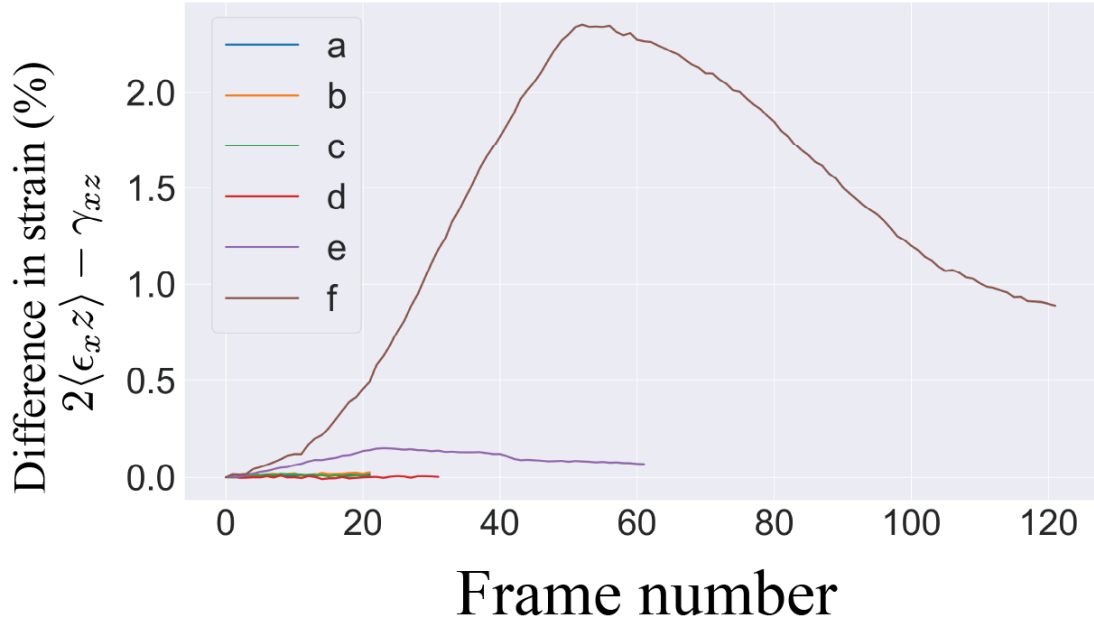


Figure 5.5: The time evolution of discrepancy between volume averaged local strain $2 \cdot \langle \epsilon_{xz} \rangle$ and boundary strain measures γ_{xz} . The differences accumulate at the onset of strain localization in steps e and f.

regions that undergo large shear strains where the direction of maximal shear, or equivalently the shear plane, does not line up with the globally defined shear direction. It is always positive and the expected noise distribution is roughly speaking a χ distribution (like the speeds in an ideal gas) with 6 degrees of freedom corresponding to the approximately normally distributed strain components, however these distributions are best described non-parametrically using error chain monte carlo.

Here, as with previous strain measures, the reference configuration is taken to be the first frame, and all subsequent strains are computed relative to this fixed reference. As a result, this a *cumulative* measure of the shear strain. When computed, averaged over the whole sample, plotted over the whole time series for each step, we observe several salient features. First, after the initial frame, for all samples, there is a roughly constant jump in value corresponding to locating uncertainty in

the sample. Second, there is a smooth increase in the von Mises strain with a linear trend 0.36% increase per frame. Third, and most interestingly, after the peak strain has been reached, the von Mises strain either increases more slowly (kink for step a-c), plateaus and then increases more slowly (d), or plateaus and then decreases to a minimum before increasing again. The location of the zero-stress crossover precedes the kink and precedes the location of the minimum in the von Mises strain. One hypothesis is to link these phenomena to an elastic to plastic transition. Under elastic loading, there exist some small population of clusters of large shear strain. When the applied strain is reversed a fraction of these cluster go back thereby *decreasing* the cumulative strain. Whether a the curve kinks or plateaus or decreases then depends relative contribution of two processes: cluster reversal decreases the cumulative von Mises strain while and accumulation of clusters of opposite sign, which nevertheless still have positive von Mises strain, lead to increases in the von Mises strain with a decrease in the shear strain components. As partially completed in the next section, these hypotheses can be directly tested by examining the local strain in the sample and isolating clusters of high von Mises strain. Finally, we also note that the von Mises strain increases even after the applied strain has been removed, and the sample is relaxing under the stress imposed by the elastic gel. Interestingly the rate of increase in the cumulative von Mises strain is roughly equal to the rate of increase under slow (and small in magnitude) applied shear strain in steps a and b suggestive of a creep phenomenon. As two potential future experiments motivated by these hypotheses, we suggest that it is possible to apply external deformation at rates comparable to creep rate in the sample thereby eliminating the kink independent of peak strain, as suggested by steps a and b and slow creep relaxation under 10 mPa of applied stress. Second, the presence of kink, plateau, or decrease depends on the

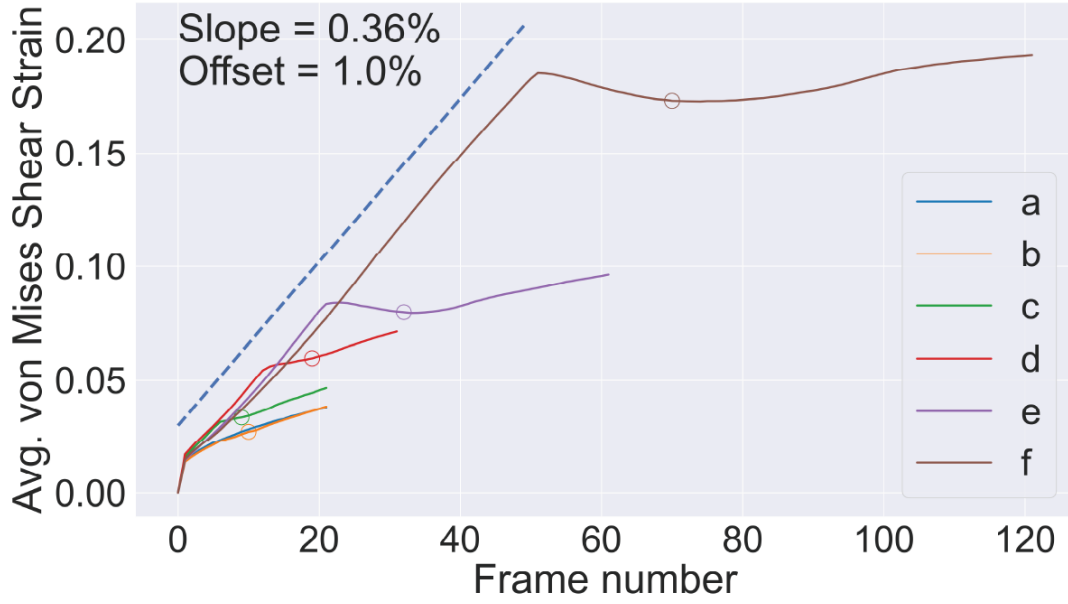


Figure 5.6: von Mises strain as a function of frame number. The von Mises strain at the closest zero-stress crossover on unloading curve is indicated by the open symbols for samples c-f, where the zero-stress crossover occurred at non-zero ε_{xz} strain due to plastic deformation.

rate of unloading: faster unloading turns kinks into plateaus and plateaus to decreases even at the same applied peak strain. These hypotheses can be microscopically confirmed or rejected by identifying and counting the number of high strain clusters as a function of applied deformation.

In addition to plotting the evolution of the average von Mises strain versus frame, we also show the time evolution of the distribution of von Mises strain for step e in Figure 5.7. With a fixed reference configuration, the von Mises strain increases cumulatively with the applied strain up to frame 19, near the peak applied strain for step e. Interestingly, the tail of the distribution at large von Mises strain values is essentially constant indicating that the strains, when exceed a threshold do not reverse. Decreases in the average strain are likely caused by a increasing population of particles that

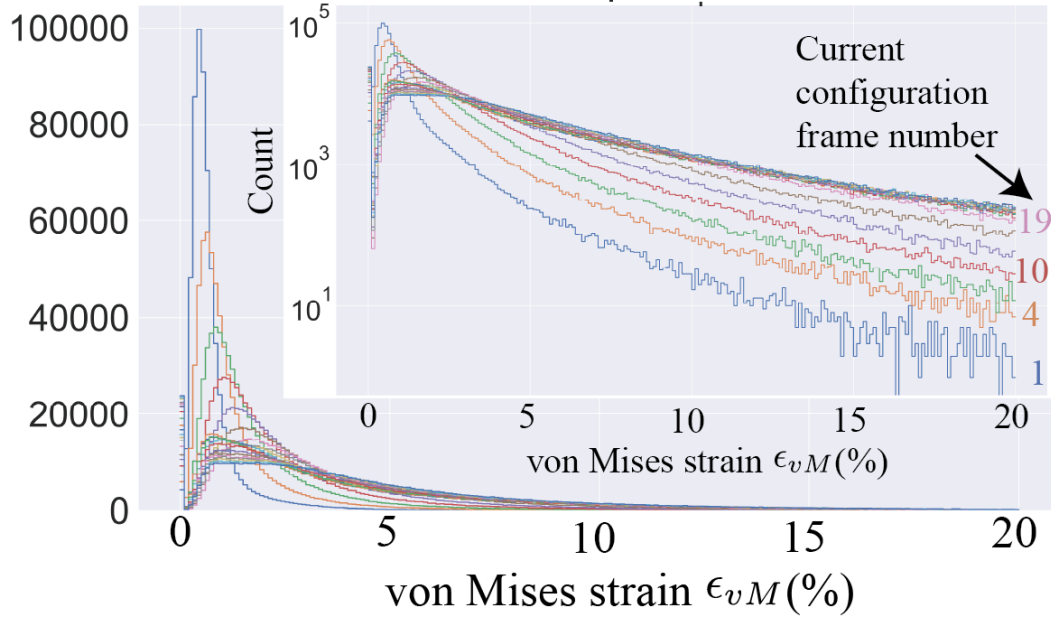


Figure 5.7: The distribution of von Mises strains computed using a fixed reference for step e. The von Mises strain is cumulative upto approximately frame 19, after which the strain distribution is stationary, indicative of plastic flow. Frame 19 is two frames prior to the peak applied strain for step e and roughly coincides with a flattening in the stress-strain curve.

exhibit near zero von Mises. Future work will examine the spatially distribution of these particle and test whether the decrease in von Mises strain is a spurious artifact (perhaps non-affinity is large indicating that strain is no longer a good measure) or if the strains have been reversed due to elastic relaxations.

Finally, we examine the time evolution of the distribution of von Mises strains calculated over adjacent frames, ie a moving reference frame of time gap $\delta t = 1$. This measure the change in the von Mises strain distribution, constantly updating the reference configuration. In contrast to cumulative strain measures, there is not obvious temporally ordering of the distributions, or the tails.

However, interestingly, tail of the distribution still varies by a factor of 5 across different snapshots suggesting varying amounts of plastic deformation. This variability is substantially *larger* than variability in the change in cumulative von Mises strain after 19 frames, suggesting once sufficient plastic flow is present, the fixed reference configuration is no longer meaningful measure for incremental changes to the strain; a simple additivity condition, $\varepsilon(t, t + \delta t) \approx \varepsilon(t, t + \delta t/2) + \varepsilon(t + \delta t/2, t + \delta t)$, of strain is not likely to hold for δt sufficiently large. Future work will examine this additivity condition in more detail by comparing the local strain over varying time windows, and over subsets of the particles. A natural refinement of this additivity condition is that the cumulative strain is additive provided that it remains below some threshold *regardless* of the frame interval. For the moment, these observations paired with deviations in the volume average of the local strain as compared with boundary strain at large peak strains in steps e and f, strongly suggest data analysis procedures that focus on shorter time intervals ($\delta t = 1$ or 2) over which local strain is reliably calculated and averaged. We also note in passing, that without an additivity condition, error chain monte carlo cannot be used to estimate uncertainties in cumulative strain as the method does not provide a proxy for the true reference configuration.

5.5 STRESS STRAIN CURVE

From the stress and strain time data, we create stress strain curves as shown in Fig. 5.9. The stress prior to shear at each step is assumed to be zero. From these curves we observe an initial start up shear in step a that is likely just noise. For steps b and c, we observe elastic behavior with modulus of

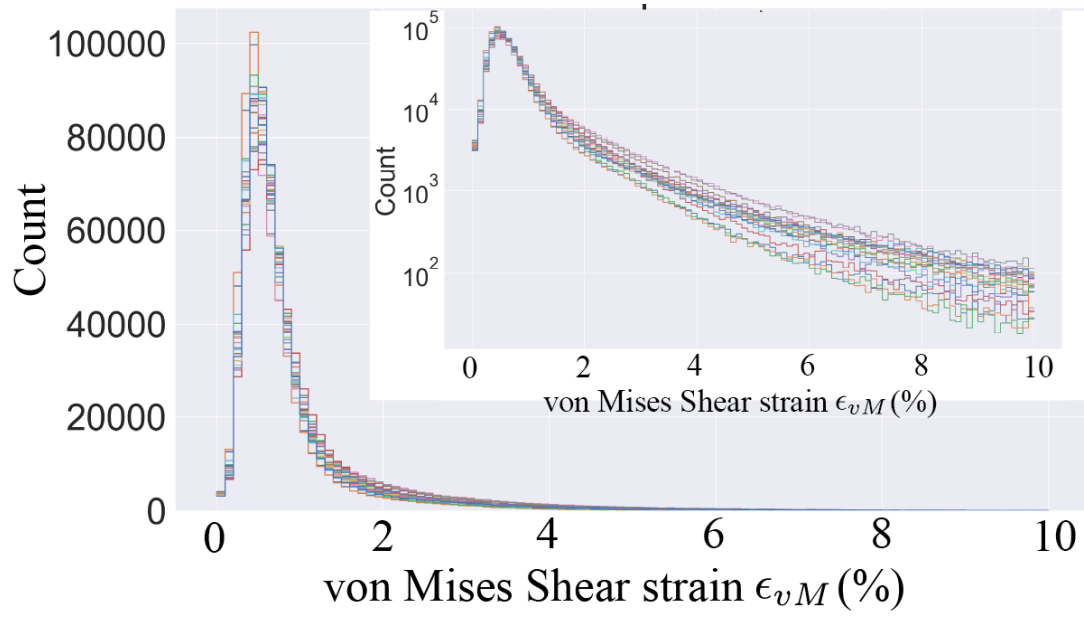


Figure 5.8: The distribution of von Mises strains computed using a rolling reference frame of $\delta t = 1$ for step e. The von Mises strain using a fixed reference is cumulative, the strain distribution computed using a rolling reference is, by contrast, fixed.

$\sim 4\text{Pa}$ and no hysteresis. Clear hysteresis is present on steps d with a zero stress cross-over at 0.5% shear strain. For steps e and f, there is the development of plastic flow with large strains at applied stresses of 20-30 mPa.

The initial frames on stress-strain curve provide an estimate of the shear modulus, but more precise measurements can be made on the elastic *unloading* modulus. Unlike the unloading curves, at the initial frames of each step, the shear only commences at the beginning of the start of the 2nd stack. As a result the stress are artificially lower than the strains. After the first frame however, sequential measurements of the stress and strain are separated by three minutes and hence equal amounts of displacement on the piezo. While simultaneous measurements of stress and strain would be ideal (and not attainable), the first frames are especially influenced by this mistiming. The unloading modulus however is not affected by mistiming.

We apply empirical shifts to the stress-strain curve to shift all the curves up to step f as shown in Fig. 5.10. All shear moduli are approximately 4Pa on loading and unloading. The unloading shear modulus on step e shows the largest difference, and 7 Pa when measured using the first two points on unloading. Unfortunately, the stress resolution is insufficient to determine whether there are stress drops during plastic flow regime.

The stress strain curves measured with traction rheoscopy are proof of concept that technique works and is sensitive to shear stress on the order of single mPa. These curves represent the first measurements of soft solids using this technique. The stress strain behavior is largely consistent with bulk rheology measurements on hard sphere colloidal glass, however direct comparisons are difficult due to uncertainty in the volume fraction and consistent use of polydispersity to suppress crystal-

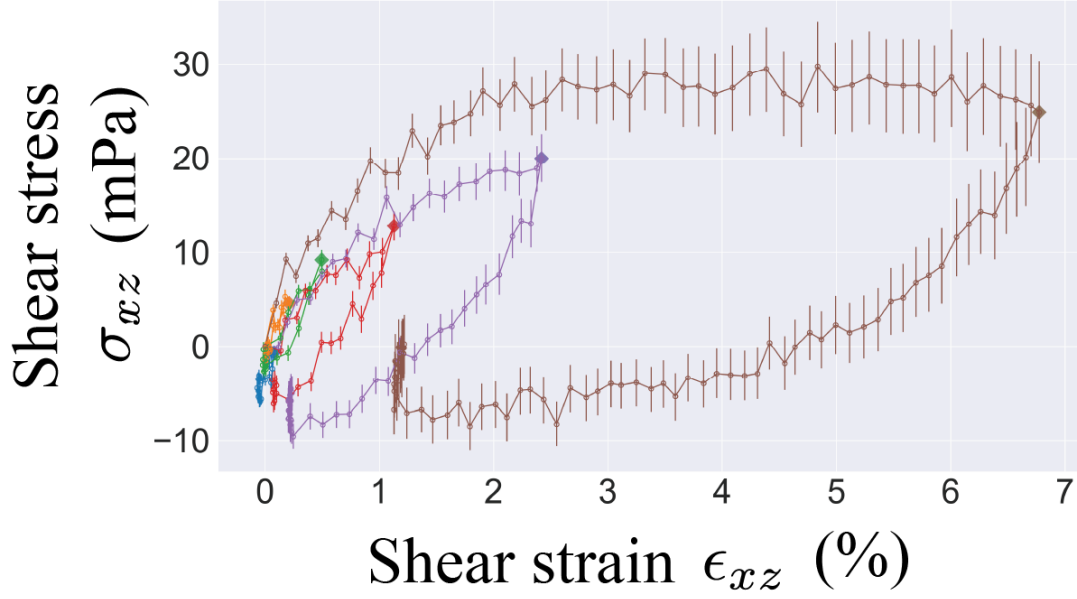


Figure 5.9: Stress strain curves measured using traction rheoscopy. The stress at the beginning of each step is implicitly zero as the initial confocal stack is treated as reference configuration. No shifts to the data have been applied.

lization. Additionally, unlike bulk rheology, the samples in traction rheoscopy do not have a solid plate covering the sample which likely contributes to fluidization and strain localization near the top. Given these caveats, to make direct comparisons with bulk rheology we compute dimensionless quantities often used in bulk rheology investigations for the data described here. The volume fraction in these samples is 62%, as measured by counting particles and using the diameter obtained from the location of the first peak in pair correlation function. The applied strain rates $\dot{\gamma}$, while not strictly speaking constant in traction rheoscopy experiments, are slower than conventional rheology $\dot{\gamma} \sim 0.1\%/(3min) \sim 10^{-6}s^{-1}$. The "bare" diffusion coefficient, that is the diffusion coefficient at

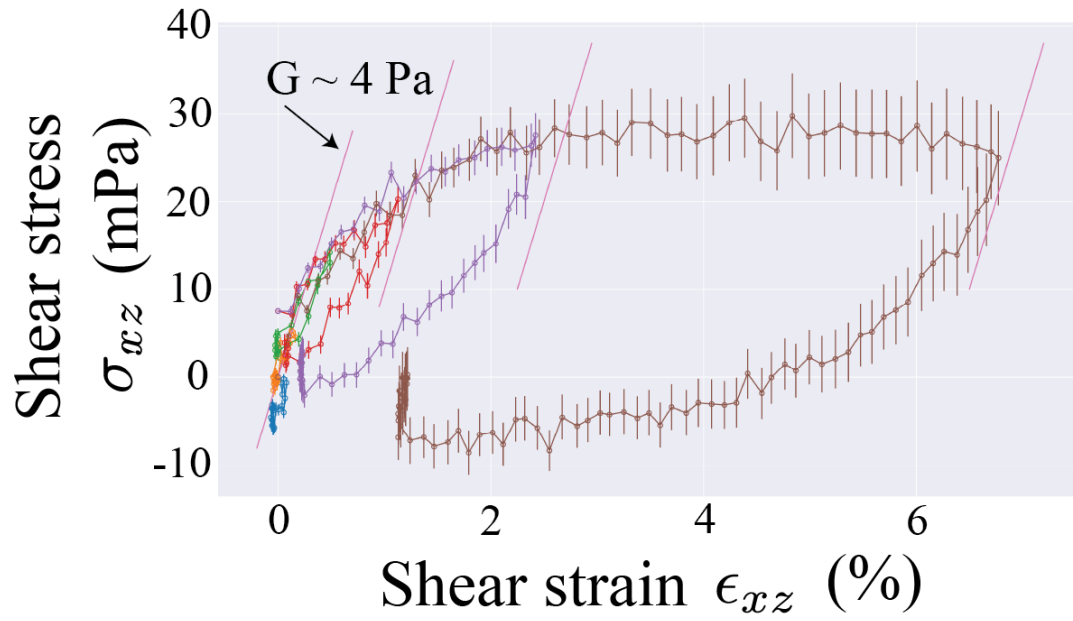


Figure 5.10: Stress strain curves for colloidal glass sample. The strain ramp was simple shear at progressively higher peak strains. The stress is computed using the deformation of the gel, while the reported strain is a volume average of the local strain component ϵ_{xz} . The curves have been shifted vertically to line up with the largest strain curve at step f with shift values listed in Table XX. The strain ramp shows the development of elastic to plastic deformation in the stress response.

	$r = 2d \text{ (}\mu\text{m)}$	cv (%)	ϕ^*	G'	kT/d^3	$G'/(kT/d^3)$
Le Grand <i>et al</i> ²²	0.183	12%	62 %	200 Pa	83.62 mPa	2400
This work	1.01	<4%	62%	4 Pa	3.98 mPa	1000

Table 5.1: Comparison of rheology measurements of dense hard sphere dispersions measured using traction rheoscopy and bulk rheology. The moduli, after rescaling by particle size, are comparable when measured with the two techniques, despite unscaled moduli differing by two orders of magnitude.

dilute conditions, is calculated using Stokes-Einstein equation

$$D_0 = \frac{kT}{6\pi\eta r} = 0.065 \text{ } \mu\text{m}^2/\text{s},$$

where the viscosity of formamide is $\eta = 3.3 \text{ } \text{cp}$ and the particles have a radius of $r = \sigma/2 = 1.01 \text{ } \mu\text{m}$. The diffusive timescale $\tau = (D_0/\sigma^2)^{-1} = 60 \text{ s}$. Using this, we obtain a dimensionless Peclet number $Pe_0 = \dot{\gamma}/(D_0/r^2) 10^{-5}$ which is an order of magnitude lower than bare Peclet numbers obtained in bulk rheology. These dimensionless quantities allow comparing the results of bulk rheology of different sized hard sphere particles.

Comparisons between traction rheoscopy experiments to bulk rheology experiments reported by Le Grand and Petekidis²² is shown in Table 5.1. By rescaling the measured modulus G' by thermal energy density for the particle suspension, the moduli measured with traction rheoscopy is a factor of two lower than the modulus measured with bulk rheology (and nearly equal if using the largest modulus measured during of step c). This comparison, however masks several differences between traction rheoscopy and bulk rheology. As mentioned earlier, the strain rates are typically at least an order of magnitude slower than the slowest reported bulk rheology experiments; even after taking into account slow diffusive dynamics due to comparably large particle size, the bare Peclet numbers

Pe_0 and Peclet numbers taking into approximate corrections $Pe(D(\phi))$ in traction rheoscopy are an order of magnitude lower than bulk rheology experiment. In bulk rheology, the reported yield strains are an order of magnitude larger than those found in traction rheoscopy, and the flow stress, even after normalizing by thermal energy density are also an order of magnitude smaller in traction rheoscopy. For the sample discussed in this work, the limited flow stress is a direct result of strain localization near the grid; while localization is a ubiquitous phenomena, the details of at what applied strain localization occurs will depend crucially on whether the applied pressure is large enough to suppress dilatation and fluidization. Additional steps, and perhaps a redesign of the sample loading mechanism to do away with TEM grids, may be taken in traction rheoscopy to suppress strain localization near the grid.

Taking these difference into account, traction rheoscopy probes a previously inaccessible deformation regime: slow shear, comparable to the relaxation rate of the system, alongside small applied strains and mPa stress. In this work the incrementally applied strains were as small as $< 0.1\%$ and there is no experimental limitation in going smaller. In traction rheoscopy, these rheological parameter are combined with direct visualization of local yielding phenomena at single particle level, which probes a dilute fraction of local relaxation events, that do not give rise to significant changes in pair correlation functions and hence may be difficult to detect with scattering experiments.

5.6 IDENTIFICATION OF HIGH STRAIN CLUSTERS

The central goal of traction rheoscopy is to relate the mechanical measurements obtained over the whole sample (average shear stress and total applied strain) to the local phenomena that can be visualized with confocal microscope at single particle resolution in the sediment. To this end, we introduce a novel algorithm based on single particle location data, to identify high strain clusters using von Mises strain invariant. We offer some suggestions and justifications for this algorithm over other alternatives. This algorithm makes use of the technological developments of error chain monte carlo to propagate locating uncertainties through the algorithm and ilastik integral pixel classifier to remove spurious clusters caused by locating *errors* as opposed to routine *uncertainties* in the location data. In many ways, early attempts at implementing this algorithm were the motivation for developing those technologies in the first place. ("Given the complexity of determining the local strain and clustering, how will I know if this algorithm fails? Am I just looking at noise?")

Additionally, an extension of the algorithm is given to segment sample into hierarchy of regions that undergo large plastic strain near the grid (termed inhomogeneous flow); homogeneous plastic flow consisting of localized highly strained clusters; and elastic matrix that undergoes affine deformation. In future work, this algorithm and segmentation procedure will be used to compute average quantities, such as strain components, over these subsets of the population of particles along with searching for possible local orientation of the highly sheared clusters. The end goal of this research is to relate the microscopic phenomena—such as locally affine deformations coupled with compact cluster of high strain – to bulk the mechanical response.

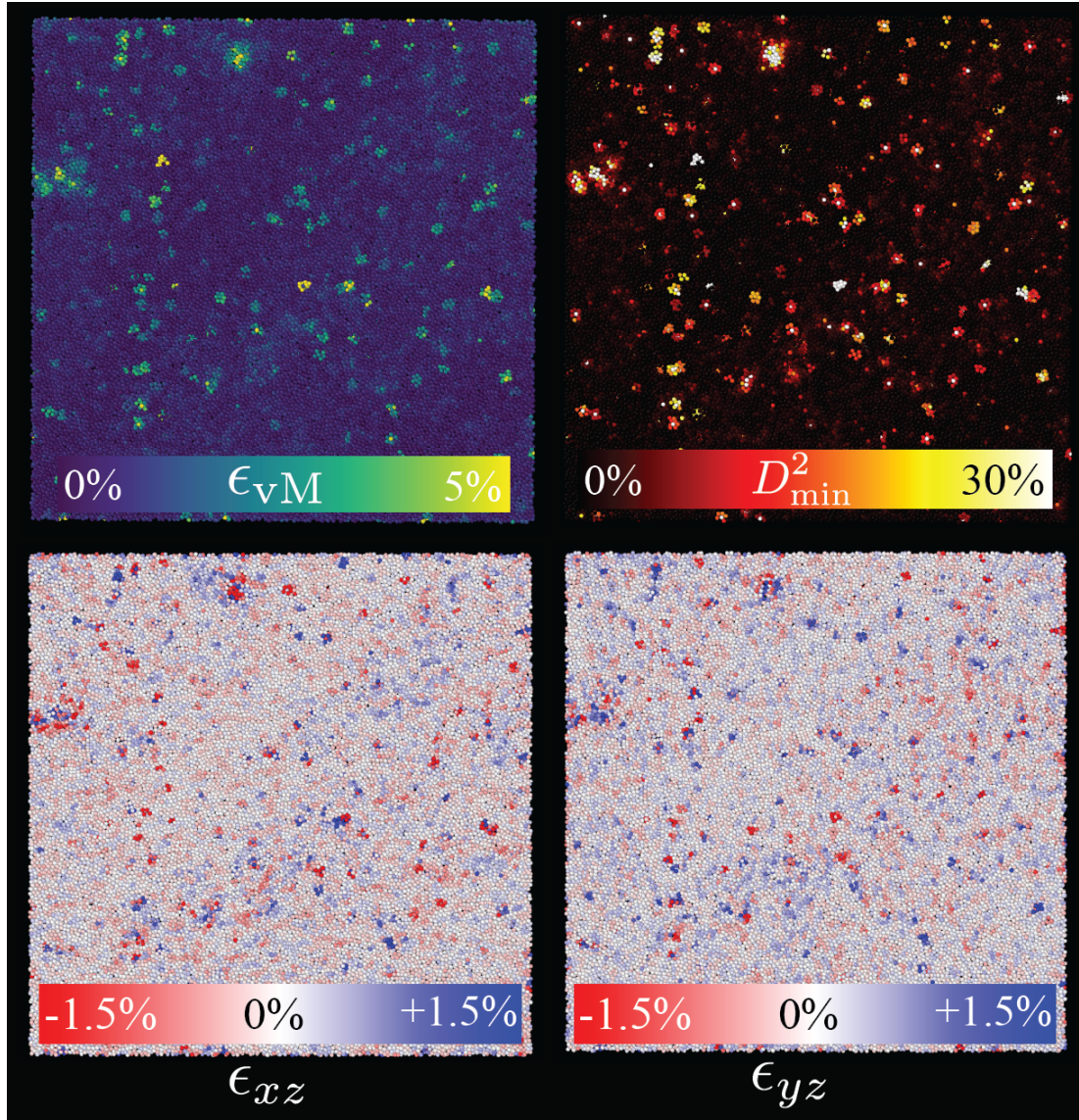


Figure 5.11: Analysis of spatial distribution of local strain measures and derived quantities in a sheared colloidal glass. Four local strain properties – von Mises strain invariant, local non-affine squared, and shear strains in the xz and yz directions – are computed and used to color the particles in a fixed xy slice. The von Mises local strain shows prominent clustering of high strain regions surrounded by a background that is close to the detection threshold. The local non-affine displacement shows clear spatial correlations with von Mises, indicating that local shear transformations captured by large values of von Mises strain are accompanied by non-affine displacements. Surprisingly, while the applied strain is simple shear in the x direction, regions of high von Mises strain do not exclusively consist of high ϵ_{xz} strain. Even within a single cluster, the sign of the shear strain can change, while most regions of high von Mises strain also have significant contributions of ϵ_{yz} . Nevertheless, a diffuse background of small negative shear strains in the ϵ_{xz} is consistent with the direction of the applied strain at the given time.

To motivate the algorithm we begin by visualizing the spatial distribution of shear strain components in a cross sectional slice of the data with the strain measured over consecutive frames. The applied strain is in the $-xz$ direction. As shown in figure 5.14, we see prominent cluster in von Mises strain invariant and non-affine squared displacement measures. Surprisingly, the strain components ε_{xz} and ε_{yz} *both* show spatial heterogeneity with measurably large positive and negative values. This is in contrast to the expectation given that the sample is not sheared in yz plane, and the $\langle \varepsilon_{xz} \rangle$ is negative. Furthermore, while the clustered regions in ε_{vM} correspond to large values of ε_{xz} and ε_{yz} , the values of the components can be either positive or negative, and often both in the same contiguous region defined clusters of large von Mises strain. These observations are at odds with the hypothesis that localized clusters of strain primarily, or exclusively, contribute to the microscopically applied strain, and hence we should see clusters with large shear strains exclusively in the xz components of the strain tensor.

Given these observations, one alternative hypothesis is that clusters of high strain are pure shear transformations with distribution of local orientations and shear-planes. Additionally, the signal to noise in the strain components is low as the locating uncertainty gives rise to noise distributions with standard deviations $\sigma \sim 0.5\%$. This is estimated with error chain monte carlo. With that noise distribution, a 3σ deviation, corresponding to $\pm 1.5\%$ strain measurement would saturate either end of the color bar in the figure. The high strain regions form correlated clusters with larger probability than expected for 3σ deviations, however the signal to noise in the background regions, outside the clusters, remains quite low. A back of envelope estimate suggests that averaging 100 particles, assumed to be homogenously deforming, would lower the uncertainty in the mean by a

factor of 10 thereby making the uncertainty in the average strain in magnitude to the incrementally applied strain. Carrying out averages like this would allow testing whether the strain the regions outside the inclusion cores are larger or smaller than the bulk applied strain.

We can identify the local clusters of high von Mises strain and segment into distinct regions using the following algorithm:

1. Threshold for high local von Mises strain with cutoff parameter $k = 2.5\%$
2. Carry out nearest neighbor clustering with a distance cutoff of 1.1σ where sigma is the particle diameter
3. Identify large clusters consisting of more than $N = 100$ particles. These are spatially located at the top of the sample, near the edges of the grid used to shear the sample.
4. Carry out a nearest neighbor closure, in which any particle with a neighbor that is highly strained is also considered part of the same cluster
5. Define *inhomogeneous* set $\mathcal{I}$ as the set of all particle that are within a cluster of size $N > 100$. This identification, when combined with nearest neighbor closure faithfully identifies highly strained region near the grid that give rise to strain localization.
6. Define *homogeneous* set $\mathcal{H}$ as everything else including particles within the bulk of the sample that have low von Mises shear strain.
7. Within the homogeneous set, define a cluster-core $\mathcal{C}$ as any particle with von Mises strain larger than $k = 2.5\%$ and define bulk set $\mathcal{B}$ as remaining particles that lie within $\mathcal{H}$ but outside cluster-core set $\mathcal{C}$
8. Note that the cluster-core set can be further indexed into spatially distinct clusters, and if desired a near-field elastic set $\mathcal{N}$ can be defined as nearest neighbors of high strain clusters.
9. In set notation, inhomogeneous and homogeneous sets are disjoint $\mathcal{I} \cap \mathcal{H} = \emptyset$. Bulk and cluster core set are disjoint subsets of homogeneous set: $\mathcal{B} \cap \mathcal{C} = \emptyset$ and $\mathcal{B} \subset \mathcal{H}$ and $\mathcal{C} \subset \mathcal{H}$

This algorithm is straightforward to implement with a sequence of set operations after the initial thresholding and clustering steps. The algorithm relies on a very limited number of physical

assumptions. These assumptions are that local von Mises strain measured over consecutive intervals is an accurate measurement of the local shear strain in that region, and that this quantity can be robustly thresholded that is to say, changing the threshold does not dramatically alter the results. Importantly, the algorithm makes no assumptions about the spatial distribution of elastic or plastically deformed regions, or the shape and spatial extent of strain localized regions. It does not assume that local clusters exist at all as the clustering output could produce a set of isolated single particle clusters consistent with noise distribution, or produce no clusters if the von Mises strains are below the threshold. Because it uses tensor invariant for the thresholding and clustering steps, it makes no assumption about the orientation of locally sheared regions or the strain components that contribute to that shear. It makes no assumption about the size or shape of the locally strained regions. As a result of the assumptions the algorithm *doesn't* make, it can be used to identify and track these quantities and examine the spatial evolution. With that said, the naming convention for the sets are merely suggestive as locally plastic or elastic deformations cannot be ascertained without testing for reversibility or providing additional assumptions in the form a local yield criterion.

Finally, a measure of the uncertainty and significance of algorithm output can be straightforwardly estimated with error chain monte carlo provided that the strains are computed over consecutive intervals. Future work will use error chain monte carlo to assess how robust the algorithm is to the choice of, for example, cutoff parameter in thresholding von Mises strain. An illustrative example is to assess the cluster size distribution assuming only particle locating errors using ECMC.

5.7 CLUSTER VISUALIZATION

Using this algorithm with $k = 2.5\%$ we segment the sample at a given time into disjoint sets as shown in Figure 5.12. First, the inhomogeneous set $\mathcal{I}$ forms a contiguous region outlining the grid used to deform the sample, consistent with the localization observed in the displacement vs. height plots in previous sections. Moreover, the highest strain regions occur near the internal edges of the grid, where diffusive motions of the particles are most prevalent in the absence of applied strain. The remaining particles constitute the homogeneous region and contain well separated compact clusters of high strain particles. The volume fraction cluster cores is 1.5-2% varying slightly with height in the sample. The nearest neighbor closure on high strain cores brings the volume fraction up to 7-8% also showing only small variations in height. Average clusters sizes are 10-15 particles in the region between the bottom surface and inhomogeneous set. At the bottom surface average cluster size is 30. Visualization homogeneous and inhomogeneous sets are shown in Figure 5.12 in a and c. Cluster-core set with nearest neighbor closure is shown in b and a cross sectional slice in c. In all cases the color scale saturates at $\varepsilon_{vM} = 5\%$ which is double the threshold used to identify clusters.

We next visualize a xz cross-sectional slice of the cluster-core particles and examine the distribution of other strain derived quantities on these clusters. We see strong correlations between von Mises strain used to threshold and identify the clusters and non-affine squared $D_{\min}^2$ displacement. Additionally, and surprisingly, the local shear strain show a range of positive and negative values in both the applied shear direction xz and the yz plane. As noted earlier, even within a single cluster individual shear strain components take on both positive and negative values. Given these results,

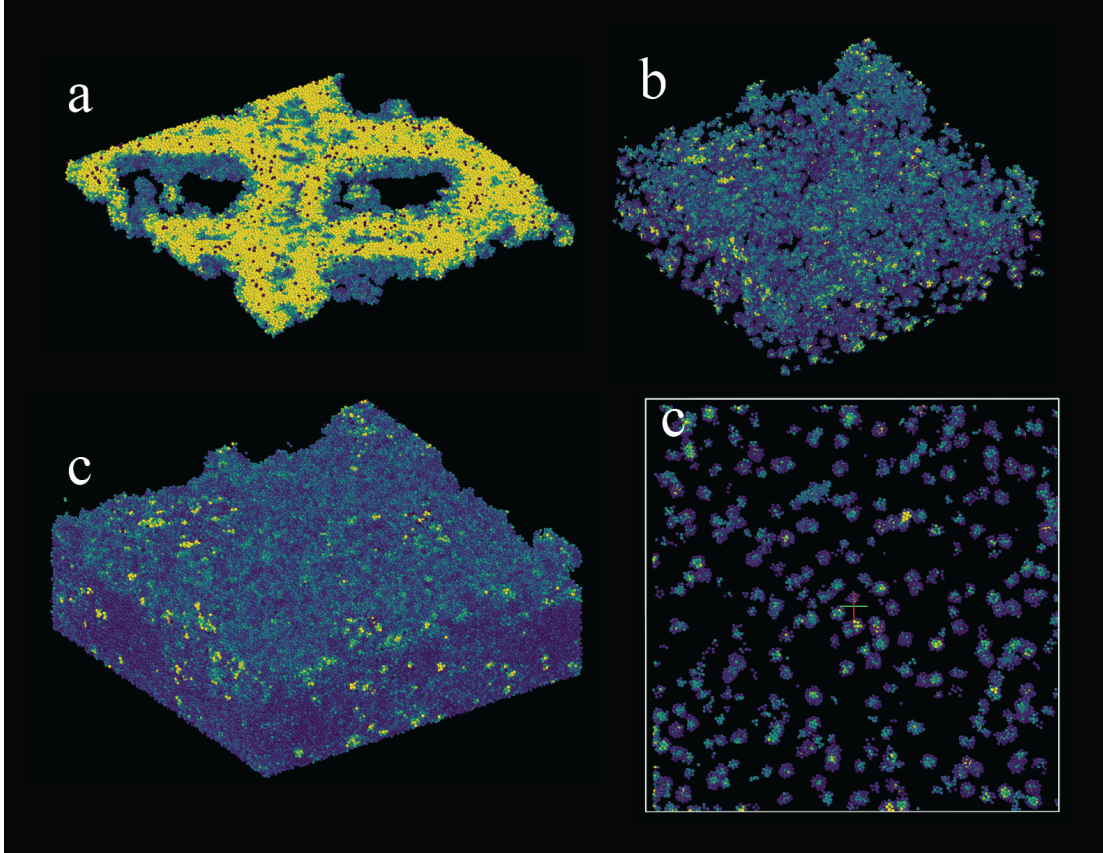


Figure 5.12: Segmentation of colloidal glass into inhomogeneous and homogeneous deformation regimes using clustering on von Mises strain. The local strain is computed for each particle over a frame interval of 1. When coloring by the von Mises strain invariant, two prominent features are evident. First, the top of the sample, near the shear post, undergoes large von Mises strain especially at the edges of the grid. Second, the strain in the remainder of the sample shows clusters of large von Mises that consists of several particles. An empirical threshold value of $\epsilon_{vM} > 2.5\%$ combined with a subsequent spatial clustering is used to identify each of these regions. The largest clusters, consisting of at least 100 particles, but typically 500 — 1000 occur near the shear post. The smaller clusters, with typical sizes < 20 , constitute the clusters within the bulk of the sample. This segmentation procedure is used to separate the data analysis, specifically sample averaged strains, as discussed in the text.

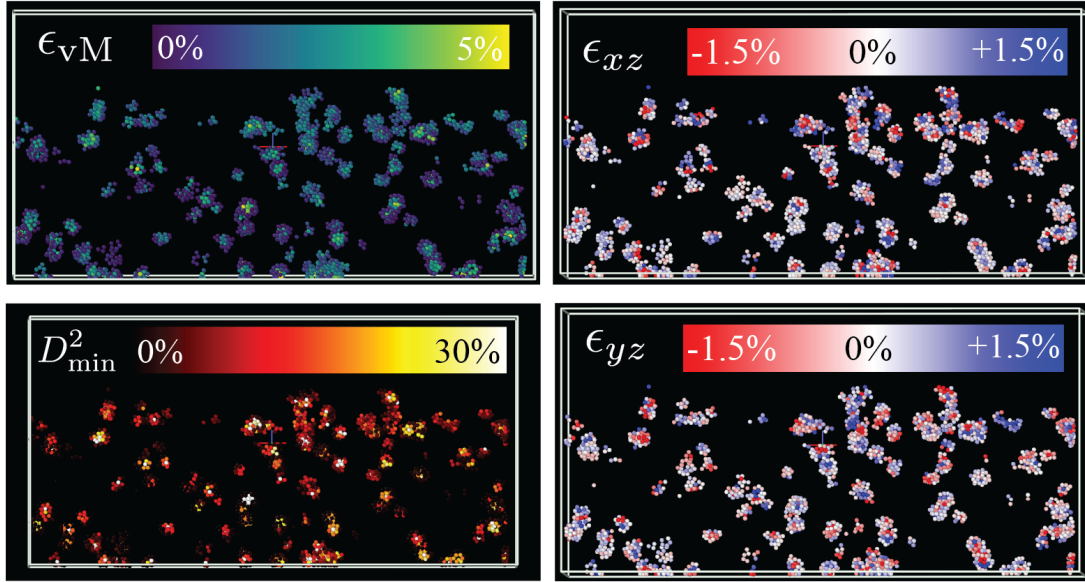


Figure 5.13: Visualizations of clusters of high local strain as measured by a threshold on the von Mises strain invariant. The particle clusters are shown in cross-sectional slice in the xz plane, and the shear applied in the $-x$ direction. Regions of high local von Mises strain clearly show clustering phenomena in which only a small fraction of the particles undergo large strains. Moreover, clusters of high local von Mises strain are correlated with high values of non-affine displacement. However the clusters themselves, while showing large shear stress components in ϵ_{xz} consistent with the applied shear direction, also so large shear values of opposite sign along with comparably large values in ϵ_{yz} . The data presented are representative of the data across the entire strain ramp and all steps. Specifically, the data presented here are computed for step f, with a current configuration set a t frame 12 and reference at frame 11; large clusters of deformation near that occur near the grid have been removed.

thresholding on individual strain components would yield somewhat similar clusters, or partial cores, some of the time.

5.8 ESHELBY INCLUSIONS

Using the clustering analysis, we observe small compact cores of high von Mises strain, but varying strain components within the core. One hypothesis is that the clusters of high strain constitute the cores of Eshelby inclusions¹³ that deform with constant shear strain^{32,19}, as the local strain corre-

lations in individual shear components show striking spatial auto-correlations consistent with the elastic fields of nearly spherical Eshelby inclusion. One of the central results in Eshelby's theory, is that within an ellipsoidal inclusion the strain inside the core is constant and produces anisotropic elastic fields outside the core. The working hypothesis given the clustering analysis is that the von Mises strains inside an individual core are approximately constant, but could vary from core to core. An additional hypothesis is that the unexpected variation in strain components within a core is primarily due to an *orientation* of an Eshelby inclusion relative to the globally defined axes used to compute the strain. Unlike von Mises strain invariant, the value of individual strain components depends on the choice of basis. If the strain components are calculated in a basis that does not line up principal axes of the inclusion, the values of individual components will be mixed and could take on both positive and negative values even within the core.

When computing the local strain a global basis lining up with the shear direction and imaging axis is used. This dependence on basis is a distinct *disadvantage* to use strain components when identifying individual inclusion. However, the primary advantage to using individual components is clear spatial signature in the anisotropic strain fields that are produced by the inclusion. To highlight this limitation, using Eshelby's theory we compute the expected strain field around a spherical inclusion that would extend to a particle and its first neighbor shell and a relaxed transformation strain of $\varepsilon_{vM} = 2.5\%$ obtained with simple shear in yz direction. This corresponds to an unrelaxed transformation strain of $\sim 6.5\%$. As shown in Figure 5.14, the von Mises strain fields exhibit only weak anisotropy, with a small four-fold pattern in yz and xy plane around the circumference of a cylindrically symmetric strain field. Additionally, given the small shear strain, the predicted strains at

distance extending to include the split second peak in pair correlation, approach the detection limit of the experiment. Using this analysis we draw some important conclusions:

1. The predicted shear strains in an Eshelby inclusion core of unrelaxed transformation strain of 6.5% are measurably large given the resolution limit.
2. The elastic fields surrounding the core are likely not measurably large as single measurements, however the strain field could be improved by averaging.
3. Within the spatial and strain resolution available, von Mises strain field is expected to produce only weak local anisotropy regardless of the orientation of the inclusion.

These conclusions, while qualitative are broadly in line with the empirical results of the clustering algorithm. We see little to no anisotropy in the fields surrounding the clusters, but clearly distinguishable compact cores.

In the near futures, we will implement an algorithm to assess potential alignment of inclusion cores. This will be done by identifying inclusion cores, removing any cores (likely small) that are predicted to be noise from ECMC, and diagonalizing the local strain tensor for particle within the core. We will check to see there are zero eigenvalue indicative of a pure shear transformation. Using the local eigenbasis for each core, we will align the eigenbasis and superimpose many inclusions to improve the strain resolution outside the core where elastic strain fields are predicts. This process will remove any dependence on local basis that would skew the averaging of individual strain components that would otherwise have mixed components. Finally, with each inclusion placed in a consistent local basis, we will average the strain fields and individual strain components surrounding the core. Further partitioning of the inclusion by size and average von Mises strain in the core will also be carried out to identify possible sub-populations of inclusion with different sizes and

transformation strains. The local orientation of the inclusion may be visualized with tensor field visualization²⁰.

5.9 DISTORTION STRAIN ENERGY

In this final section, we make some speculative arguments on the stress-strain curves and the observed inclusion clusters apparent at low strains. Using the stress-strain curves obtained with traction rheoscopy, we can estimate the concentration of defects by assuming an inclusion forms when distortion energy reaches a critical value. During the experiment, we observe localized defects at all times, even under small applied strains. This is likely due to the slow externally applied shear with $Pe \approx 0.01$. Using the first two points of the unloading curve for steps c-f, we measure the modulus μ_{xz} as the slope of the line connecting the points, and the yield stress σ_y as the drop in shear stress immediately after unloading. Using these two quantities we compute J_2 , an invariant of the stress tensor, and strain energy of distortion W_D :

$$J_2 = \frac{\sigma_y^2}{3}$$

$$W_D = \frac{J_2}{2\mu_{xz}} \quad (5.6)$$

The strain energy of distortion more properly a *density* of distortion strain energy. In this case it is the integral of the stress strain curve after unloading step. As we observe microscopic yielding, we treat this integral as strain energy of distortion and not merely a elastic energy density. We compute

Eshelby inclusion von Mises strain invariant

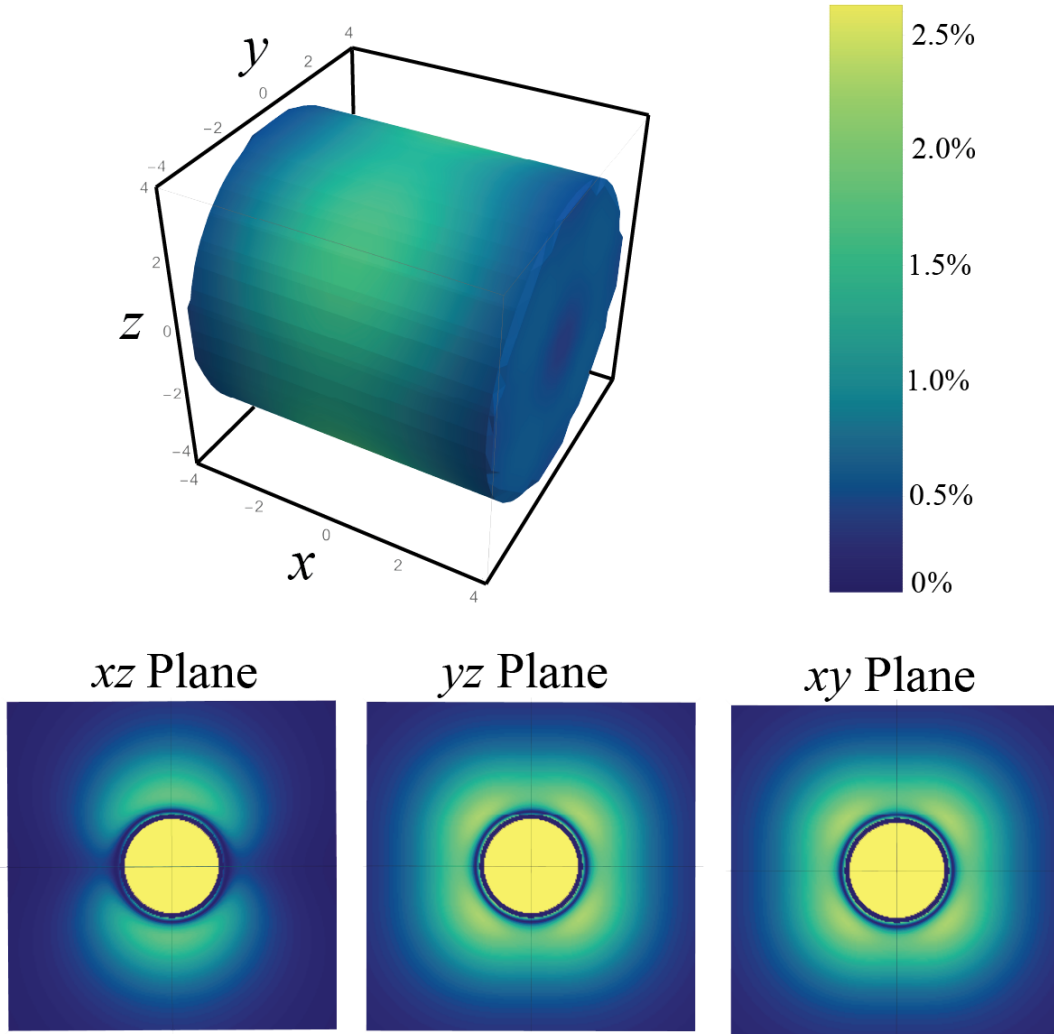


Figure 5.14: Strain fields for spherical esehbly inclusion with unconstrained transformation strain $\gamma_{yz} = 6.2\%$ and radius $a = 2$. These parameters were chosen to mimic the expected strain field for high strain clusters with von Mises strain of $\varepsilon_{vM} \sim 2.5\%$ in the core and inclusion sizes consisting of a central particle and its first neighbor shell. The von Mises strain is a tensor invariant and *constant* in the core of the inclusion, hence it is not dependent on the choice of basis or the transformation strain.

a typical volume over which we expect this distortion energy to be distributed by equating

$$W_D = \frac{kT}{N * \Omega},$$

and solving for N . The results are tabulated in Table ?? . Typical values are $N \approx 1000$. This argument does not give results on *how* the strain energy of distortion is distributed over N particles. A sample of particles could be homogeneously strained in which case the sample has not yielded, or only a small fraction could carry the majority of the distortion. If we further assume an inclusion core size that is 15 particles, thereby a particle and its nearest neighbor shell, we can estimate the concentration of defect cores of $\phi_{STZ} \sim 1.5\%$ in agreement, but smaller, than the measured volume fraction of inclusion cores of 1.5-2.5%.

This argument of equating the strain energy of distortion to thermal energy cannot be used to estimate the yield strain of an inclusion. Empirically, I find inclusions at concentration of 1.5-2% by thresholding the strain invariant $\epsilon_{vM} > 2.5\%$ and finding connected clusters using a nearest neighbor cutoff distance of $< 1.1\sigma$ where σ is a particle diameter. It is likely the measured frequencies, especially prevalence of cores consisting of a single particle, are partially corrupted by false positives. As shown in Fig 4.11, the von Mises strain has a small but non-zero tail above the threshold of 2.5% given the locating uncertainty in the experiment. Future work will use ECMC to estimate the false positive rate of inclusions with von Mises strain as a function of threshold value, along with false positive estimate of the cluster size distribution given the thresholding and clustering procedure used.

	c	d	e	f
Shear modulus μ_{xz} (Pa)	3.3	4.5	7.5	4.2
Yield Stress σ_y (mPa)	3.76	3.8	7.0	4.8
Strain energy of distortion W_d (mPa)	0.0007	0.001	0.001	0.001
J_2 (mPa) ²	4.7	8.1	16.5	7.6
$[W_d/(kT/\Omega)]^{-1}$ (#)	1361	1070	890	1063

Table 5.2: Strain energy of distortion is calculated for steps c-f at elastic unloading.

Analogous integrals of the stress strain curve, when applied to plastically flowing regime would produce much larger (100x) strain energies of distortion. However, in this regime, the deformation is heterogeneous with a large fraction of the strain carried by particles near the top of the grid. Future will carry out analysis similar to this at all time, while restricting the particles to the homogeneously deformation region.

6

Conclusion

I developed a experimental technique to carry out mechanical testing of soft solids, principally colloid dispersion, with combined in situ visualizations. This technique, termed traction rheoscopy, was demonstrated on strain ramp of colloidal glass sample. The experimental development of this technique required several technical innovations:

1. Design and fabrication of modular shear cell that can be mounted on confocal microscope
2. Two particle systems with a single refractive dispersed in immiscible solvents

3. Near critical gels with independently tunable elastic moduli and refractive index
4. Fabrication of thin gels suitable for optical microscopy, using sacrificial sugar-glass capillaries.

In addition to these technical innovations, I also developed and implemented image and data analysis program featuring:

1. Combined open source image deconvolution, single particle locating, and nonlinear least squares refinement of particle position
2. Random pixel classifier, with a novel integration scheme, to identify fluorescent chunks and colloidal dimers and other features in the image that give rise to spurious results in particle locating
3. Consistent use of distributed computing and embarrassingly parallel computations to process multi-Tb image data sets. Despite computationally expensive deconvolution and fitting procedures, multi-Tb data-sets can be processed in parallel with modest memory requirements of 16Gb.
4. Introduction of Error Chain Monte Carlo to carry out non-parametric estimates of noise distributions given the locating precision obtained using nonlinear least squares fitting
5. Software integration of the image analysis and traction rheoscopy specific data analysis code with existing open source data analysis tools including TrackPy, Ovito, and Freud.

The combination of these techniques enabled the experimental measurement of stress strain curves of a colloidal glass with $m \sim mPa$ stress resolution. The data show clustering of local strain regions as identified used local strain invariant, and strain localization paired with dilatation at larger applied strain in which the measured stress plateaus. Ongoing and future work will examine the relationship between the measured stress-strain curves and the microscopic information obtained through in-situ visualization. Several working hypotheses on this front were proposed.

Traction rheoscopy, as a technique, is capable of measuring the mechanical response and micro-structure of potentially a wide range of soft materials, each with distinct structure an mechanical

response. Some immediate extension to this work would investigate the shear deformation of colloidal polycrystals which are easily formed by ambient sedimentation (as opposed to centrifugation) of the same colloidal particles used to form a colloidal glass. Rich dislocation and grain boundary structures were seen during some initial investigations on this system, prior to the development of the centrifugation techniques used to form a monodisperse hard sphere glass. As another soft material, by adding polymer depletant and density matching the colloids, attractive gels can be formed. In both cases, the relevant microscopic deformation mechanisms (dislocation dynamics and strand bending and extension) are completely different than the colloidal glass. Finally, the operating principle of traction rheoscopy—namely measuring the deformation of the gel to infer mechanical forces in the sample above—can be used for skin friction measurements of flowing colloid suspensions which is also predicted to involve mPa scale stress fluctuations. These possibilities are only a small subset of the fundamental materials science, physics, and solid mechanics questions that be probed with traction rheoscopy.



Some extra stuff

All code and data analysis procedures are available on my GitHub: <https://github.com/tlozsolt>
under TractionRheoscopy repository.

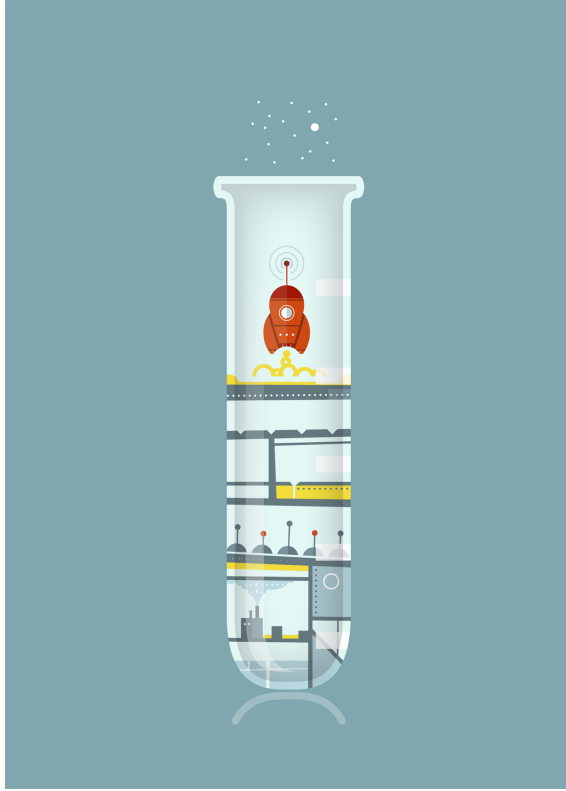
References

- [1] Allan, D., Caswell, T., Keim, N., van der Wel, C. M., & Verweij, R. (2021). soft-matter/trackpy: Trackpy vo. 5.0. *Genève: Zenodo*.
- [2] Allan, D., van der Wel, C., Keim, N., Caswell, T., Wicker, D., Verweij, R., Reid, C., Grueter, L., Ramos, K., & Perry, R. (2019). soft-matter/trackpy: Trackpy vo. 4.2. *Zenodo*, DOI, 10.
- [3] Arevalo, R. C., Kumar, P., Urbach, J. S., & Blair, D. L. (2015a). Stress heterogeneities in sheared type-i collagen networks revealed by boundary stress microscopy. *PLOS ONE*, 10(3), e0118021.
- [4] Arevalo, R. C., Kumar, P., Urbach, J. S., & Blair, D. L. (2015b). Stress heterogeneities in sheared type-i collagen networks revealed by boundary stress microscopy. *PLOS ONE*, 10(3), e0118021.
- [5] Argon, A. (1979). Plastic deformation in metallic glasses. *Acta metallurgica*, 27(1), 47–58.
- [6] Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., Schiegg, M., Ales, J., Beier, T., Rudy, M., Eren, K., Cervantes, J. I., Xu, B., Beuttenmueller, F., Wolny, A., Zhang, C., Koethe, U., Hamprecht, F. A., & Kreshuk, A. (2019). ilastik: interactive machine learning for (bio)image analysis. *Nature Methods*.
- [7] Chambon, F. H. (1986). *Linear viscoelasticity of crosslinking polymers at the gel point*. Thesis.
- [8] Cheng, X., McCoy, J. H., Israelachvili, J. N., & Cohen, I. (2011). Imaging the microscopic structure of shear thinning and thickening colloidal suspensions. *Science*, 333(6047), 1276–1279.
- [9] Crocker, J. C. & Grier, D. G. (1996a). Methods of digital video microscopy for colloidal studies. *Journal of Colloid and Interface Science*, 179(1), 298–310.
- [10] Crocker, J. C. & Grier, D. G. (1996b). Methods of digital video microscopy for colloidal studies. *Journal of Colloid and Interface Science*, 179(1), 298–310.

- [11] Dutta, S. K., Mbi, A., Arevalo, R. C., & Blair, D. L. (2013). Development of a confocal rheometer for soft and biological materials. *Review of Scientific Instruments*, 84(6), 063702.
- [12] Egner, A. & Hell, S. W. (2006). *Aberrations in Confocal and Multi-Photon Fluorescence Microscopy Induced by Refractive Index Mismatch*, (pp. 404–413). Springer US: Boston, MA.
- [13] Eshelby, J. D. (1957). The determination of the elastic field of an ellipsoidal inclusion, and related problems. *Proceedings of the royal society of London. Series A. Mathematical and physical sciences*, 241(1226), 376–396.
- [14] Falk, M. L. & Langer, J. S. (1998). Dynamics of viscoplastic deformation in amorphous solids. *Physical Review E*, 57(6), 7192.
- [15] Finney, J. L. (1970). Random packings and the structure of simple liquids. i. the geometry of random close packing. *Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences*, 319(1539), 479–493.
- [16] Gao, Y. & Kilfoil, M. L. (2009). Accurate detection and complete tracking of large populations of features in three dimensions. *Opt. Express*, 17(6), 4685–4704.
- [17] Hell, S., Reiner, G., Cremer, C., & Stelzer, E. H. (1993). Aberrations in confocal fluorescence microscopy induced by mismatches in refractive index. *Journal of microscopy*, 169(3), 391–405.
- [18] Jensen, K. E. & Nakamura, N. (2016). Note: An iterative algorithm to improve colloidal particle locating. *Review of Scientific Instruments*, 87(6), 066103.
- [19] Jensen, K. E., Weitz, D. A., & Spaepen, F. (2014). Local shear transformations in deformed and quiescent hard-sphere colloidal glasses. *Physical Review E*, 90(4), 042305.
- [20] Kindlmann, G. (2004). Superquadric Tensor Glyphs. In O. Deussen, C. Hansen, D. Keim, & D. Saupe (Eds.), *Eurographics / IEEE VGTC Symposium on Visualization: The Eurographics Association*.
- [21] Koumakis, N., Moghimi, E., Besseling, R., Poon, W. C., Brady, J. F., & Petekidis, G. (2015). Tuning colloidal gels by shear. *Soft Matter*, 11(23), 4640–4648.
- [22] Le Grand, A. & Petekidis, G. (2008). Effects of particle softness on the rheology and yielding of colloidal glasses. *Rheologica Acta*, 47(5), 579–590.

- [23] Lewis, L. N., Stein, J., Colborn, R. E., Gao, Y., & Dong, J. (1996). The chemistry of fumarate and maleate inhibitors with platinum hydrosilylation catalysts. *Journal of Organometallic Chemistry*, 521(1), 221–227.
- [24] Lewis, L. N., Stein, J., Gao, Y., Colborn, R. E., & Hutchins, G. (1997). Platinum catalysts used in the silicones industry. *Platinum Metals Review*, 41(2), 66–75.
- [25] Lin, N. Y. C., McCoy, J. H., Cheng, X., Leahy, B., Israelachvili, J. N., & Cohen, I. (2014). A multi-axis confocal rheoscope for studying shear flow of structured fluids. *Review of Scientific Instruments*, 85(3), 033905.
- [26] Lindsay, H. M. & Chaikin, P. M. (1982). Elastic properties of colloidal crystals and glasses. *The Journal of Chemical Physics*, 76(7), 3774–3781.
- [27] Lu, P. J., Shutman, M., Sloutskin, E., & Butenko, A. V. (2013). Locating particles accurately in microscope images requires image-processing kernels to be rotationally symmetric. *Optics express*, 21(25), 30755–30763.
- [28] Rathee, V., Blair, D. L., & Urbach, J. S. (2017). Localized stress fluctuations drive shear thickening in dense suspensions. *Proceedings of the National Academy of Sciences*, 114(33), 8740–8745.
- [29] Rathee, V., Blair, D. L., & Urbach, J. S. (2021). Dynamics and memory of boundary stresses in discontinuous shear thickening suspensions during oscillatory shear. *Soft Matter*, 17(5), 1337–1345.
- [30] Sage, D., Donati, L., Soulez, F., Fortun, D., Schmit, G., Seitz, A., Guet, R., Vonesch, C., & Unser, M. (2017). Deconvolutionlab2: An open-source software for deconvolution microscopy. *Methods*, 115, 28–41.
- [31] Scanlan, J. C. & Winter, H. H. (1991). Composition dependence of the viscoelasticity of end-linked poly(dimethylsiloxane) at the gel point. *Macromolecules*, 24(1), 47–54.
- [32] Schall, P., Weitz, D. A., & Spaepen, F. (2007). Structural rearrangements that govern flow in colloidal glasses. *Science*, 318(5858), 1895–1899.
- [33] Spaepen, F. (1977). A microscopic mechanism for steady state inhomogeneous flow in metallic glasses. *Acta metallurgica*, 25(4), 407–415.

- [34] Stukowski, A. (2009). Visualization and analysis of atomistic simulation data with ovito—the open visualization tool. *Modelling and simulation in materials science and engineering*, 18(1), 015012.
- [35] Style, R. W., Boltyanskiy, R., German, G. K., Hyland, C., MacMinn, C. W., Mertz, A. F., Wilen, L. A., Xu, Y., & Dufresne, E. R. (2014). Traction force microscopy in physics and biology. *Soft Matter*, 10(23), 4047–4055.
- [36] van der Wel, C. & Kraft, D. J. (2016a). Automated tracking of colloidal clusters with sub-pixel accuracy and precision. *Journal of Physics: Condensed Matter*, 29(4), 044001.
- [37] van der Wel, C. & Kraft, D. J. (2016b). Automated tracking of colloidal clusters with sub-pixel accuracy and precision. *Journal of Physics: Condensed Matter*, 29(4), 044001.
- [38] Winter, H. H. & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of rheology*, 30(2), 367–382.



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