EXPLORING MICROSTRUCTURAL HETEROGENEITIES IN CELLULOSE NANOCRYSTAL SUSPENSIONS IN THE REPULSIVE GLASS PHASE

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ABSTRACT

Cellulose nanocrystals are a plant derivative that has recently captured interest in the field of novel material development for their strong mechanical properties, sustainability, and abundance. Aqueous suspensions of cellulose nanocrystals (CNC’s) behave as liquid crystal systems and experience an isotropic-nematic transition and can form several elastic structures above this transition depending on the interparticle electrostatic interactions. I examined the behavior of aqueous suspensions of CNC’s in the repulsive glass phase which is described by a fully nematic system consisting of many locally aligned domains and strong electrostatic repulsion between individual particles. My research examined the effect of the aligned domains on the local mechanics of the system using wood-derivative CNC’s obtained from Forest Product Labs, a part of the USDA Forest Service. I first characterized the system through bulk rheology. The rheological signatures allowed for comparison to published results and identifying the phase diagram of the system. I next imaged the suspension at different concentrations using cross-polarization microscopy to characterize the isotropic-nematic transition as well as to investigate the characteristic length scales of locally aligned domains in the fully nematic state. I used a combination of a rheometer and a confocal microscope to image CNC suspensions under controlled shear. This experimental setup was used for two measurement techniques: boundary stress microscopy and using tracer particles to image flow heterogeneities. I used boundary stress microscopy to investigate the
local stresses on the stationary boundary of a sheared CNC suspension in the repulsive glass state. My results found that variations in the boundary stress resulting from the locally aligned domains are small enough to be below the noise threshold of the experimental setup. I imaged tracer particles suspended in CNC samples to examine the local flow behavior of CNC suspensions in the repulsive glass phase. This technique revealed significant non-affine motion within CNC suspensions for a limited range of applied shear rates. These results provide a novel window into the local behavior of repulsive glasses and have broad implications for the understanding and application of colloidal rigid rod systems.

**INDEX WORDS:** Cellulose, Nanocrystal, Nematic, Confocal Rheology, Cross Polarization Microscopy, Boundary Stress Microscopy, Repulsive Glass
Dedication

This thesis is dedicated to my grandfather, who taught me science even when I wasn’t listening.
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There are too many people who helped me get to this point to remember them all. In many stretches of this journey there were only one set of footprints while I got carried. For those of you who I forget to mention, you are forever appreciated, and know that I always see your name written next to mine on the title page.

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Chapter 1

Introduction

The search for environmentally responsible and economically feasible materials to replace or improve upon many of the currently used standards is motivating a refo-
cusing on plant-based materials development. The most common biopolymer on the planet, cellulose is the building block for the cell wall that gives plants the structure and flexibility to grow. Cellulose is biodegradable, renewable, abundant, and strong, making it a prime candidate for many research endeavors seeking new sustainable materials. One form of cellulose with potential for novel materials development is a nano-crystalline form that behaves as a rod-like particle. These cellulose nanocrystals (CNC’s) can be used in suspensions, gels, and thin films to create renewable materials with complex mechanical and optical properties. The thermodynamic diffusion and collective behavior of these rod-like nanocrystals when in suspension is similar to an ideal colloidal rod system as calculated by Lars Onsager in the mid-twentieth century, making CNC’s an interesting system both for practical materials development and for fundamental physics research. The entropically driven local ordering of the colloidal rod systems that emerges as a consequence of CNC diffusion is directly related to the interesting optical and mechanical properties of these systems. Understanding the dynamics of this local order is key to understanding the underlying mechanics of cellulose nanocrystal systems as a whole.

To investigate the dynamics of local order in cellulose nanocrystal suspensions, I examined these systems using several techniques, which are described in Chapter
3. A discussion of the current understanding of colloidal rod-like systems, CNC’s, and relevant literature is found in Chapter 2. I applied parallel plate rheology to determine the bulk material properties of CNC suspensions at a wide range of concentrations. Chapter 4 details the results of those experiments and the comparison to similar measurements of CNC suspensions reported in literature. The local order of CNC’s within a suspension was viewed through cross polarization microscopy, a method that allows for imaging a sample that affects the polarization of light, and the results are reported in Chapter 5. Through the bulk rheology and cross polarization microscopy, in comparison with other published investigations into the phase behavior of CNC suspensions, I determined that the focus of my research was the repulsive glass phase, a gel-like system at high CNC concentration with complex local ordering. This system occupies a point in the phase space of CNC suspensions that is not well understood and has the potential for interesting discoveries relevant not only to CNC material development but colloidal rod-like systems as a whole. I applied boundary stress microscopy to CNC suspensions in the repulsive glass phase to search for heterogeneities in the stresses at the boundary of a sheared sample, hoping to tie those back to the mechanics of locally ordered domains. The results of those experiments are reported in Chapter 6. CNC suspensions with suspended fluorescent beads were imaged with a confocal microscope while under shear to give a new lens into the local rearrangement within a sample under shear. Chapter 7 discusses the results of those experiments and the insights gained. These experiments revealed a fascinating behavior that should motivate future research into this and similar systems.
Chapter 2

Background

In order to better understand the context and results of the experiments described in this document, this chapter will aim to give an overview of the relevant physics and published literature for cellulose nanocrystals and colloidal rod-like systems in general. In the pursuit of developing cellulose-based materials, it is imperative to understand the underlying mechanisms that govern the macroscale properties [4][5][6][7]. A core concept of physics that is fundamental to the behavior of CNC suspensions is the occurrence of an isotropic-nematic phase transition, which is described in the next section. This behavior broadly describes liquid crystal systems and colloidal rod-like systems as a whole. This chapter will then discuss CNC’s: their origins, variations, and bulk material properties in published literature as measured through rheology. The observation of isotropic-nematic phase transitions in CNC suspensions is seen using a technique called cross polarization microscopy, and examples from literature are discussed. The complications arising from the electrostatic interactions of CNC’s and the consequences for their phase diagram are detailed at the end of this chapter, setting up the specific research reported in subsequent chapters.

The materials discussed in this chapter are described by some specific terms of which a reader without any background will need a basic understanding. Thermal fluctuations or diffusion in this chapter is the random movement of a small particle that is suspended in a fluid due to the temperature of the system. The higher the temperature, the more the particle will be bumped around by the fluid. Systems of
particles distributed in a fluid that don’t settle to the bottom or float to the top are referred to as a suspension. The particles in a suspension may be small enough to experience significant thermal fluctuations but that is not a requirement. A colloid is a system where one material is distributed within a second substance, with the distributed material being larger than a molecule but small enough to experience thermal fluctuations. And finally, the colloids of interest in this research are anisotropic, which means they are shaped such that one dimension is much larger than the others. For example, cellulose nanocrystals are much longer than they are wide or thick, making them resemble a rod. The relationship between length and width is called the aspect ratio, defined by $L/W$, which means that highly anisotropic particles have large aspect ratios.

2.1 Isotropic-Nematic Phase Transitions

![Figure 2.1: A cartoon of the Isotropic-Nematic transition in a suspension of colloidal rods.](image)

On the left, at concentrations below the transition, the average volume per rod is large enough for individual rods to entropically explore all translational and rotational degrees of freedom with minimal interaction with neighboring rods. On the right, at higher concentrations, the average volume per rod is reduced such that interactions between neighboring rods is greatly increased. The entropically favorable configuration of this system is for rods to align, which constrains their rotational freedom while still allowing for significant translational freedom.
In suspensions of highly anisotropic colloids that are rigid relative to thermal fluctuations (i.e. they don’t bend significantly while diffusing), neighboring particles can spontaneously align when the colloidal concentration is above a critical threshold. Below this threshold, the average inter-particle spacing is large enough that individual particles are free to entropically explore any orientation. At any given moment, the average orientation of the colloidal system is close to zero, and the system as a whole is described to be isotropic. Above this threshold, the average inter-particle spacing is too low to allow for the full rotational freedom of all particles to be explored individually. The entropically favorable state is for a local population of these rigid rod-like particles to align and decrease their average spacing, creating a region with higher particle density that allows the rest of the system to persist in the isotropic phase at a lower density. The aligned region is described to be in a nematic phase. At higher total particle concentrations, the percentage of the system that exists in the nematic phase grows until the entire system is locally aligned, though not necessarily globally aligned throughout the system. This behavior is called the Isotropic-Nematic phase transition.

The Isotropic-Nematic transition described above is concentration driven. It also assumes that electrostatic interactions between particles are not significant, since these interactions can drastically alter the behavior of a suspension [8]. There also exist systems that undergo Isotropic-Nematic phase transitions that are driven by temperature as opposed to concentration (Ref. [9] gives an example using altered fd virus particles). Those systems are not the subject of this thesis, and instead the focus will be solely on the lyotropic (concentration-driven) case. Among the systems that undergo a lyotropic Isotropic-Nematic phase transition, some are described as colloidal rigid-rod suspensions. In these systems, the suspended particles undergo Brownian motion, are larger than individual molecules, have high aspect ratios, and
are rigid compared to the thermally driven bending of the particle (also described as being shorter than the persistence length). In an ideal colloidal rigid-rod suspension, the critical concentration threshold for the Isotropic-Nematic transition is dependent only on the aspect ratio [10]. It is this category of systems that will be the focus of this thesis.

Not many physical systems behave as colloidal rigid-rod suspensions, therefore the number of directly comparable experimental systems is limited. The focus of this thesis is cellulose nanocrystals (CNC’s), which will be described in the next section. The nematic behavior of CNC’s has been observed through several methods including cross polarization microscopy and small-angle scattering [11]. The fd virus is another naturally occurring colloidal system that closely approximates an ideal rigid-rod suspension. The subject of current research, fd virus particles have sizes and aspect ratios comparable to CNC’s. In general, fd virus particles are longer than CNC’s by about a factor of 10 with similar diameters, and thus the higher aspect ratio induces nematic phase separation at volume fractions several times lower than in CNC suspensions. The rheology [12] and shear behavior [13] as seen through cross-polarization microscopy [14] yield similar behaviors to CNC’s and serve as a useful comparison for determining possible commonalities between rod-like systems. The tumbling and wagging behavior [15] predicted and observed for fd-virus suspensions [16, 17] may play a role in the flow behavior of CNC suspensions.

2.2 Cellulose Nanocrystal Origins

Cellulose is an abundant biopolymer that can be found in a variety of organisms, including plants, bacteria, and some marine animals. It is a building block of the cell wall in plants, helping to form the stiff but flexible structure that supports the
Figure 2.2: An image produced by Postek et. al. [1] that puts cellulose in the context of a tree. The top row progressively zooms in from a full tree to the cellular structure. The bottom row zooms further to examine the cell wall, which is made of a fibril-matrix structure, which in turn is made up of microfibrils. Cellulose nanocrystals are formed by separating the crystalline segments of the microfibrils from the amorphous sections. The final image on the bottom left is the chemical structure of cellulose. A cellulose nanocrystal is made up of bundles of cellulose polymer bonded to each other in a crystalline manner.

larger shape. Cellulose is made up of chains of repeating glucose segments that form fibrils through hydrogen and van der Waals bonds [18]. Fibrils collect to form larger fibers, which in turn form even larger fibers, creating a hierarchical assembly that ultimately packs to form structures like cell walls. The first step in this hierarchy, the fibrils, consists of ordered and disordered regions that create crystalline and amorphous segments of the fibril. The crystalline regions, when separated from the amorphous regions, become individual rod-like particles known as cellulose nanocrystals. The process for extracting the crystalline regions from bundles of cellulose involves
acid hydrolysis. While there are other methods for extracting larger sections of cellulose (e.g. cellulose nanofibrils), sulfuric acid hydrolysis is the prevalent method for obtaining cellulose nanocrystals. The sulfuric acid dissolves the amorphous regions faster than the rigid regions. There is therefore a duration of acid exposure after which the amorphous regions have dissolved away but the crystalline regions still exist. These now-disconnected crystalline regions are the desired cellulose nanocrystals. This process introduces sulfate esters to the surface of the CNC’s, thereby affecting the surface charge. A similar method of hydrolysis using hydrochloric acid can produce CNC’s of comparable size but without a significant surface charge [19]. Surface charge of CNC’s is discussed in more detail in section 2.7. CNC’s can form stable colloidal suspensions in water depending on factors such as the physical size of the crystals (which depends on the hydrolysis duration) and surface charge. Therefore, the sulfate esters contribute to colloidal stability of the CNC’s due to the increased electrostatic interactions between the rods. The CNC suspensions discussed here are suspended in aqueous solutions after extraction.

2.3 CELLULOSE NANOCRYSTAL VARIATIONS

Due to their relatively high aspect ratio and Brownian behavior, CNC suspensions can form nematic domains at concentrations above a threshold determined by the dimensions of the nanocrystals and the electrostatic interactions between individual rods. At certain concentrations these nematic domains become cholesteric and take on a helical structure. These cholesteric domains lead to optically active gels, glasses, and films[24]. These crystals are also relatively strong for their size with an axial modulus of about 110-220 GPa and a transverse modulus of approximately 10-50 GPa [18].
Table 2.1: A comparison of CNC samples and similar systems

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Mine</td>
<td>CNC</td>
<td>Wood Pulp</td>
<td>110+/-37</td>
<td>14</td>
<td>.42 to 1.69</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>[20]</td>
<td>CNC</td>
<td>Cotton</td>
<td>107+/-55</td>
<td>5.4</td>
<td>.16 to .91</td>
<td>.027</td>
<td>.11</td>
</tr>
<tr>
<td>[21]</td>
<td>CNC</td>
<td>Paper</td>
<td>115+/-10</td>
<td>16</td>
<td>.98 to 2.96</td>
<td>.05-.09</td>
<td>.07-.12</td>
</tr>
<tr>
<td>[22]</td>
<td>CNC</td>
<td>Wood Pulp</td>
<td>100+/-8</td>
<td>14</td>
<td>.14 to 2.14</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>[2]</td>
<td>CNC</td>
<td>Wood Pulp</td>
<td>214+/-10</td>
<td>14</td>
<td>.14 to 1.71</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>[23]</td>
<td>CNC</td>
<td>Wood Pulp</td>
<td>163+/-43</td>
<td>4.9</td>
<td>.15 to .45</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>[14]</td>
<td>fd</td>
<td>fd-virus</td>
<td>880</td>
<td>130</td>
<td>.22 to 1.94</td>
<td>0.0017</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

Each entry corresponds to a material used in a reference cited in this thesis. For each sample, the range of volume fractions used in the reference publication is listed as the volume fractions multiplied by the aspect ratio. The isotropic and nematic volume fractions listed refer to reported concentrations within the isotropic and nematic domains through the coexistence regime of the I-N transition.
Figure 2.3: A survey of the presence of ordered domains as seen through cross polarization as a function of CNC wt.\% by Lagerwall et. al. [25] The vertical axis describes the fraction of the sample observed to be in the liquid crystal phase through cross polarization microscopy. Several CNC samples from different origins are detailed. The black data points are obtained with CNCs from bacterial cellulose (BC), blue with filter paper-derived CNCs (FC) and red with fresh wood pulp-derived CNCs (PC). The suffix p indicates pure CNCs, without any additive. Representative cross polarization images are included on the left to demonstrate how the volume fraction of the liquid crystal phase is measured. The top graph is a zoomed-in region of the bottom graph, which seeks to show the vast range of unexplored concentrations. The sources for the data are as follows, in the order of the legend: [26] [27] [21] [28] [29].
Cellulose can be found in bacteria, plants, and even some animals (e.g. Tunicate). Cellulose nanocrystals vary in dimension depending on the source. CNC’s extracted through sulfuric acid hydrolysis can have lengths varying from less than 100 nanometers to larger than a micrometer, and widths from only a few nanometers to 50 nanometers [25]. Additionally, the duration of the sulfuric acid hydrolysis procedure affects both the sizes and the sulfur content of the resultant nanocrystals [22]. All these factors affect the effective aspect ratio that determines the concentrations at which isotropic-nematic phase changes occur. Table 2.1 shows a selection of CNC samples and their relevant properties as reported by publications referenced elsewhere in this thesis, as well as representative examples from comparable systems. Figure 2.3 shows the presence of nematic domains as a function of concentration, seen through cross polarization microscopy, for CNC’s originating from a variety of sources. As shown in the figure, CNC suspensions can reach a fully nematic state at concentrations spanning an order of magnitude depending on the source.

2.4 Cellulose Nanocrystal Rheology

The variation in CNC sizes and electrostatic interactions means that stresses measured by rheological tests depends on the specific sample[30]. Though the concentration at which certain behaviors occur is not uniform across suspensions of CNC’s from different sources, the general rheological signatures are relatively consistent. Figure 2.4 shows the typical dependence of linear oscillatory rheology on the concentration of CNC aqueous suspensions. At low concentrations, the loss modulus (G’”) dominates the storage modulus (G’), and the system behaves as a viscoelastic fluid. As the concentration of suspended CNC’s is increased, the storage modulus begins to dominate the loss modulus, and the suspension begins to behave as an elastic solid.
Figure 2.4: Oscillatory rheology of CNC suspensions by Urena-Benavides et. al. [20] The data shows the linear viscoelastic properties of suspensions at 17.3 vol% (red solid diamonds), 14.5 vol% (blue solid squares), 12.6 vol % (black stars), 12.1 vol % (orange solid triangles), 10.4 vol % (purple solid circles), 9.03 vol % (red open diamonds), 7.69 vol % (blue open squares), 6.30 vol % (orange open triangles), and 5.00 vol % (purple open circles). The two graphs on the left show the storage ($G'$) and loss ($G''$) moduli for each concentration as a function of frequency, and the graph on the right shows the relation between those two. A value of tan(δ) above 1 indicates a more viscous fluid, and a value below 1 indicates a more elastic system.

much like a gel. Figure 2.5a shows the typical steady shear behavior of CNC suspensions as a function of concentration. As expected from the oscillatory rheology, suspensions at low CNC concentration behave like Newtonian fluids, exhibiting a constant or near-constant viscosity over a range of applied shear rates. At high CNC
concentrations, the suspensions were continually shear thinning and acted like a yield stress material [31, 32, 33]. Figure 2.5b offers the complex viscosity as a function of frequency and concentration for comparison. While the behavior between the steady shear and complex viscosities are similar, lyotropic liquid crystals commonly do not obey the Cox-Merx rule, which is a principle that for many fluids the steady state shear viscosity is equal to the oscillatory dynamic viscosity at the frequency that corresponds to that shear rate [20]. Figure 2.6 shows a Cox-Merz rule comparison for 6.30 and 12.1 vol % from Figure 2.5. Lower concentration samples that contain more of the isotropic domain show a closer relationship between the steady shear and complex viscosities. Suspensions at higher concentrations, which predominantly consist of nematic domains, show significant deviation between the steady shear and complex viscosities.

The sample preparation procedures can significantly affect the rheology of CNC suspensions as shown by Shafiei-Sabet et. al. [22] in Figure 2.7. The effect of sulfur content on steady shear viscosity is compared between Figures 2.7 a and b. CNC-A was extracted in a higher concentration sulfuric acid solution (8.75 ml/g) than for CNC-B (6.00 ml/g), with all timings kept the same between both processes. The particles yielded through the process for CNC-A had an average length of 48.4 nm and a sulfur content of 0.85 wt%. CNC-B had particles with a similar average length of 46.0 nm but with a lower sulfur content at 0.69 wt%. According the analysis of Shafiei-Sabet et. al., the lower sulfur content caused a lower surface charge and electrostatic repulsion, which lead to more aggregation. Flow curves were measured for each sample before and after sonication at 1000 J/g. In both cases, the magnitude of the steady shear viscosities dropped, with a larger drop in the case of the sample with lower sulfur content. This is explained by the existence of more aggregates before
Figure 2.5: Steady shear (a) and complex viscosity (b) rheology of CNC suspensions by Urena-Benavides et. al. [20] The data shows the linear viscoelastic properties of suspensions at 17.3 vol % (red solid diamonds), 14.5 vol % (blue solid squares), 12.6 vol % (black stars), 12.1 vol % (orange solid triangles), 10.4 vol % (purple solid circles), 9.03 vol % (red open diamonds), 7.69 vol % (blue open squares), 6.30 vol % (orange open triangles), 5.00 vol % (purple open circles), and 3.07 vol % (black crosses).
Figure 2.6: A Cox-Merz rule comparison for CNC suspensions by Urena-Benavides et. al. [20] for concentrations of 6.30 (a) and 12.1 vol % (b). Steady shear values are represented by open symbols and complex viscosity values are represented by solid symbols.

sonication. This is a representative example of the range of possible rheological results for CNC suspensions depending on the preparatory procedures [34].
Figure 2.7: A comparison of steady shear viscosity curves for CNC suspensions with different preparations by Shafiei-Sabet et. al. [22] CNC-A was extracted in a higher concentration sulfuric acid solution (8.75 ml/g) than for CNC-B (6.00 ml/g). This yielded particles of roughly the same size (48.4/46.0 nm respectively) but with different sulfur contents (0.85/0.69 wt % respectively). Flow curves for each sample were measured before sonication (solid symbols) and after sonication (open symbols).
2.5 **Cellulose Nanocrystal Cross Polarization**

The domain structure of CNC suspensions as viewed through cross-polarization microscopy while increasing CNC concentration shows the transition from isotropic individual rods through a coexistence regime with isolated nematic regions to an eventual fully nematic system [3, 20, 35]. However, the fully nematic phase is heterogeneous, consisting of many domains with different nematic directors. This can be seen in 2.8, which shows the growth of nematic domains as CNC concentration increases as seen through cross polarizers.

![Cross polarization images by Haywood et. al. [23]](image)

**Figure 2.8: Cross polarization images by Haywood et. al. [23]** The optically active areas (any light, colored or not) are regions of nematic alignment of CNC’s. Color is dependant of domain orientation and the interaction of light with multiple stacked domains. The details of this sample are listed on Table 2.1 under citation [23].
Continuous shear of suspensions in different phases shows that CNC’s orient strongly in the direction of flow when below the fully nematic regime, then relax back towards an isotropic distribution of nematic domains [23][36]. Figure 2.9 demonstrates this behavior for CNC suspensions at concentrations below and above the gel-like transition. In this figure, the images labelled with capital letters are imaged through cross polarizers oriented at a 45 degree angle to the direction of shear flow, while the lower case letters indicate orientation in line with the direction of flow. Each letter (uppercase and lowercase) corresponds to a position on the curves graphing the optical contrast between the uppercase and lowercase images as a function of time. For concentrations fully within the nematic regime (H, I J), shear has a reduced effect on the alignment of the suspension, and these concentrations show only partial relaxation post shear. This behavior supports rheological measurements that show retention of stress after high continuous shear in suspensions within the gel-like regime, which will be discussed in the CNC Rheology chapter.

2.6 Cellulose Nanocrystal Surface Charges

Published measurements of the surface charge of CNC’s typically rely on one of two measurement techniques: elemental analysis or conductometric titration. Elemental analysis describes methods that quantitatively measure the mass of individual elements in a material. A common method of elemental analysis is optical atomic spectroscopy which can be very generally described as combusting the material and examining the emitted spectra to identify the contained elements and their mass fractions. In measurements of the surface charge of CNC’s produced through sulfuric acid hydrolysis, this method is used to identify the mass fraction of sulfur. The esterification of negatively charged sulfate groups onto the surface of CNC’s is assumed to be
Figure 2.9: Cross polarization images by Haywood et. al. [3] of CNC suspensions under 100 s$^{-1}$ constant shear. The optically active areas are regions of nematic alignment of CNC’s. Color is dependent of domain orientation. The images labelled with capital letters correspond to polarizers oriented with 45 degrees relative to flow, and the lowercase letters correspond to the polarizers oriented 90 degrees to the flow for the same frame. The graph shows the optical contrast between the two orientations for each of the three different concentrations as a function of time. The concentrations are: red=6.98% vol.(biphasic), blue=11.6%(liquid crystalline), and green=15.8% vol.(gel)

the dominant contributor to the surface charge of the individual nanocrystals, and it is assumed that all sulfur remaining in processed CNC samples is contained within these sulfate groups on the surface of the CNC’s. A further assumption in relating the sulfur mass fraction to the surface charge is that all sulfate groups are ionized.

Conductometric titration is a method of measuring the surface charge of suspended particles based on the change in the pH of the suspension as a known acid or base is added. This method is based upon many of the same assumptions as for elemental
analysis. As the charge carrier density within the suspension is altered, the interaction potential between the ionized sulfate groups and associated counterions (sodium in most cases) is altered. This can cause inflection points in the bulk pH of the suspension that relate to the total surface charge of CNC’s given a fixed concentration.

In my literature review, I have yet to find a published study that compares the results from both elemental analysis and conductometric titration on the same sample of cellulose nanocrystals extracted through sulfuric acid hydrolysis. The best estimate for the mass fraction of sulfur in the samples used in my experiments comes from the manufacturer. This mass fraction is 0.0095 of sulfur in CNC’s in the dried state. This value was confirmed by my collaborator Doug Fox at American University through his own measurements. In the sources described in Table 2.1, measurements of the mass fraction of sulfur were reported in references [22], and [2]. These measurements range from 0.0069 to 0.009 depending on the specific sample described. It may be useful to convert these parameters from mass fraction to the molar amount of sulfur compared to the mass of cellulose. Using this conversion, my samples contain 0.00029 moles per gram of cellulose. The same conversion for the range of mass fractions described in references [22] and [2] yields a range of 0.00022 to 0.00028 moles of sulfur per gram of cellulose, comparable values to my samples.

Publications including conductometric titration measurements on CNC systems report the results in a wider variety of methods. Reference [20] reports the molar amount of -SO3H per gram of cellulose to be $1.71 \times 10^{-4}$ mol/g. Reference [27], however, reports the molar mount of -OSO3 per gram of cellulose to be 15.3 mmol/kg or $1.53 \times 10^{-5}$ mol/g (it may be useful to note that the molar weight of -OSO3 is 1.16 times the molar weight of -SO3H). Other publications, such as reference [21], report a surface charge density (in this case 0.115 e/nm²) that has been calculated using the results of the conductometric titration and choosing an average CNC diameter
to divide over, assuming uniform charge distribution. For comparison, the fd-virus particles used in reference [14] were reported to have a linear surface charge density of 9 \( e/nm \). With a cross-sectional diameter of 6.8 \( nm \), this yields a surface charge density of 0.4 \( e/nm^2 \). Doing a similar calculation for my samples produces an average surface charge density of 0.5 \( e/nm^2 \), based on the average cross-sectional diameter of my CNC’s (7.8 nm, discussed in Section 3.1). The same calculation for the data reported by reference [20], previously described as an example for reporting molar amount of -SO3H, yields a value of 0.77 \( e/nm^2 \). Therefore we see that CNCs produced through sulfuric acid hydrolysis are measured to have Sulphur concentrations in the range of 0.00022 to 0.00029 moles of Sulphur per gram of cellulose, or surface charge densities in the range of 0.5-0.77 \( e/nm^2 \). This charge density is comparable to, although a bit larger than that found in fd-virus.

2.7 Cellulose Nanocrystal Phase Diagram

As discussed in Section 2.6, the behavior of CNC suspensions is dependent on the electrostatic interaction between rods [37, 38, 39, 40, 41]. The magnitude of the surface charge contributes to inter-particle repulsion and determines the critical concentration for nematic domain formation [22], while the screening of those charges suppresses that repulsion [42]. The effect is similar to a change in the effective aspect ratio of the rods [43]. The Van der Waals forces between rods causes an attractive interaction, but this attraction is only relevant once the repulsive forces have been sufficiently suppressed. When CNC’s extracted through sulfuric acid hydrolysis are suspended in an aqueous solution with no salts (beyond the sodium counterions that are a result of the production process) to screen the repulsive interactions, the repulsive forces dominate the attractive forces and the suspension is stable over long times (months).
As salts are added to the solution, the repulsive forces are suppressed and the rods begin to exhibit weak attractive interactions. As the repulsive forces are suppressed further the rods are able to come into contact and form attractive interactions with potential energies larger than $k_B T$. This can lead to aggregation or network formation. 

At certain concentrations, the presence of salts in the solution causes the suspension to form a gel-like substance. CNC suspensions can also enter gel-like phases at higher CNC concentration, though behavior still depends on electrostatic interaction. This was studied by Stokes and colleagues [2, 44] by varying the NaCl concentration in CNC suspensions and measuring the rheology of the results. They determined three general classifications of solid behavior: repulsive glass[45], attractive glass[46][47][48], and a gel phase (Figure 2.10). Similar states have been observed in colloidal Laponite particles [49].

![State diagram by Stokes and colleagues [2] for suspensions with varying CNC and NaCl concentrations.](image-url)
In the works cited above, the differentiation between each solid state was based on rheological measurements. The distinction between the gel and attractive glass states was based on creep compliance experiments, and was not determined to have a sharp transition from one state to the other. Similar methods have been used to identify gel and glass phases in other materials [50]. The repulsive and attractive glasses were distinguished by measuring the yield stress. A discontinuity in the yield stress as NaCl concentration was varied, as shown in Figure 2.11, was reported in reference [2]. This discontinuity was determined to be a transition from one glassy state to another. The onset of the transition shown in Figure 2.11 shows a sudden increase in the yield stress as NaCl concentration is increased. The authors suggest that this is due to the onset of attractive interactions between rods while they are still caged by excluded volume interactions due to repulsive electrostatic forces that have not been fully repressed.

Figure 2.11: Example of the discontinuity in yield stress observed by Stokes and colleagues [2] as NaCl concentration is increased for a 11.9% wt. CNC suspension. Yield stress was determined by the onset of a drop in viscosity as shear stress was increased.
The glass phase of rod-like colloids is seen in other systems besides CNC’s. A review by Solomon and Spicer [51] identified glass phases in rod colloidal suspensions consisting of hematite, gibbsite, ovalbumin, or β-lactoglobulin, among others. They recorded approximate boundaries for the transition into a glass phase for these systems when the product of the volume fraction and the aspect ratio ($\Phi*L/D$) was between 1 and 10. It may be important to note that glass phases are not universally observed in all rod-like colloidal systems [31]. Glass phases have been identified in fd virus systems as well[14]. Kang et. al. [52] observed fd virus suspensions using cross polarization microscopy and analyzed the image time-correlation functions as concentration was carried. The "texture" observed under cross polarization shifted over the course of 10’s of hours after loading onto the microscope for samples in the isotropic-nematic coexistence regime. There was a discontinuous change in this behavior well beyond the onset of a fully nematic system, in which the image time-correlation function became independent of time. This was observed as the cross polarization texture remaining unchanged over long time periods. For this system, with an aspect ration of 130, the sudden suppression of texture dynamics occurred at a concentration of approximately 11.7 mg/ml or volume fraction of 0.013. This can also be expressed as the product of the volume fraction by the aspect ratio which is $\Phi*L/D = 1.6$. For comparison, the onset of glassy behavior in my samples was observed around $\Phi*L/D = 1.2$.

These results further support the understanding that CNC suspensions above a critical concentration in the absence of salts form a solid phase that is dominated by repulsive forces [53, 54]. Stokes and colleagues [2] observe this behavior at a weight fraction of 9% (approximately 6% volume concentration), various studies by Virginia Davis and colleagues [3, 20, 23] find this critical concentration to range from volume fractions of 7% to 13%, and the CNC samples used in this dissertation exhibit a critical concentration around volume fractions of 8%. All of these values are far below
the critical concentration for hard spheres to form glassy states. While it is understood that high-aspect ratio colloids can form solids at low volume fractions, these aptly named “repulsive glasses” are forming structure in the effective absence of attractive interactions, and even exhibit elastic moduli of hundreds or thousands of pascals.
Before discussing the results of my research in subsequent chapters, it is important to understand the experimental methods and materials used in that research. This chapter gives an overview of the specific CNC suspensions tested and the techniques applied to measure the material properties. The measurement techniques fall into two categories: rheology, which measures bulk material properties, and microscopy, which can be applied in several ways to observe and measured the localized behavior of the material. In some cases these tools were combined to make microscopy observations while simultaneously performing rheological measurements.

3.1 Cellulose Nanocrystal Samples

Cellulose nanocrystal suspensions used in this dissertation were received from NIST after being produced by Forest Products Lab, which is a part of the US Department of Agriculture Forest Service. The CNC’s were extracted through sulfuric acid hydrolysis. The bulk samples were received at concentrations of 12% wt. Elemental analysis by collaborators at NIST determined that there is no appreciable amount of salt in the solution. Transmission electron microscopy (TEM) measurements made by NIST collaborators determined the size distribution of CNC’s from this source. The measured average diameter is $7.8 \text{ nm} \pm 1.3 \text{ nm}$ and the average length is $110.4 \text{ nm} \pm 36.7 \text{ nm}$. No salts were added to the samples (beyond the sodium counterions associated
with the CNC’s after processing), and therefore there is no significant screening of the charges on individual rods.

3.2 Parallel Plate Rheology

Rheology is a method of measurement that allows for analysis of how a bulk material responds to shear stresses or deformations. In the rheology discussed here, the sample under measurement is contained between upper and lower boundaries. One of those boundaries (typically the upper) is a sensitive rotating tool that can apply and detect torque. The tool can apply certain shear stresses or strains to the sample and detect the torques applied by the sample on the tool in turn. By applying torque in a variety of tests, the bulk properties of the sample can be ascertained.

Three types of rheology are discussed in this research: step strain, oscillatory, and continuous shear. Strain is a measure of the rotation of the tool relative to the thickness of the sample. When the tool has rotated such that the outside edge has traced a distance equal to the thickness of the gap between the tool and the bottom of the sample, the sample has been deformed by one unit of strain, also known as a strain of 100%. A step strain test rotates the tool to a fixed strain and holds there as the system relaxes from the built-up stress. In a step strain test, the typical variable measured is the total stress exerted on the tool by the sample. This is called the shear stress, calculated by the torque measured by the rheometer tool, and it represents the force per unit area that the sample and tool exert on each other.

Oscillatory rheology involves the measuring tool moving back and forth at a certain rate. The angle of rotation varies as a sine wave with a given frequency and amplitude. In other words, the tool begins at a point of zero strain, rotates in one direction to a certain maximum strain (amplitude), and then rotates the other direc-
tion through the point of zero strain until it reaches the same maximum strain. The tool repeats this process at a certain rate, completing a full cycle a certain number of times per second (frequency). An oscillatory sweep is a test that fixes one of those variables while varying the other. A frequency sweep varies the frequency with a fixed strain amplitude, and a strain amplitude sweep varies the strain amplitude at a fixed frequency. The bulk of strain amplitude sweeps described in this document are applied by varying the strain amplitude from 0.1% to 10% at a fixed frequency of 1 Hz. The bulk of the frequency sweeps are similar, varying the frequency from 0.1 Hz to 10 Hz at a fixed strain amplitude of 1%.

Continuous shear rheology is a continuous rotation of the measuring tool at a constant shear rate (amount of strain per second) or while maintaining a constant shear stress. When the shear rate/stress is varied, the resulting data is referred to as a flow curve. The bulk of the flow curves in this document were produced by controlling the shear rate and measuring the shear stress and viscosity.

Rheology is done on MCR301 and MCR702 Anton Paar rheometers. The bulk rheological measurements discussed here are done using either cone-plate or plate-plate geometry. Rheology in the absence of microscopy is performed on the MCR702. A 50 mm tool with either a 1 degree cone (CP50-1) or no inclination (PP50) is the upper tool while a slightly larger bottom tool (L-PP50/TD) allows for an oil trim that prevents evaporation. The oil trim for this setup, if used at all, is made out of mineral oil with 2% Abil polymer (a proprietary surfactant) to prevent the nanocrystals from collecting at the oil-sample interface. Additionally, the sample and tools are enclosed in a temperature control chamber to create a closed environment, though it was left at room temperature. The MCR301 is used in combination with a confocal microscope to image the local behavior of the sample while the rheometer applies controlled shear and measures the bulk response under parallel plate geometry. A 25 mm parallel plate
tool is used as the upper boundary and a glass cover slide as the lower boundary. A similar oil trim was used on this setup, but no temperature control chamber was used.

Before loading onto a rheometer, all CNC suspensions are sonicated for 35 minutes. If a test sample had been diluted to change CNC concentration, it is pipette mixed ten times before sonication. CNC suspensions are pipetted onto the rheometer in order to slightly overfill the volume between tools. The overflow is then trimmed by either a specialized plastic tool or kimwipes. The first rheological tests applied to all samples are oscillatory linear rheology profiles to determine initial bulk state. These are strain amplitude sweeps and frequency sweeps within the range of 0.1% to 10% strain and 0.1 Hz to 10 Hz. These ranges were determined to be within the linear regime through prior experimentation, and were used to test fidelity between samples.

3.3 Particle Image Velocimetry

Before discussing the microscopy methods used in this research, it is important to understand the primary image analysis tool that was applied to the collected data. Particle Image Velocimetry (PIV) is a method of particle tracking that does not focus on identifying individual particles but instead matches patterns between adjacent images in a sequence. The first step of PIV analysis is the division of the first image into a grid of subsections called "interrogation windows". Each interrogation window in the first image is then compared to a larger area in the second image known as the "search window." The search window is centered around the same coordinates in the second image as the interrogation window in the first image. The imaging information contained within the interrogation window is compared to every possible location within the search window through correlation functions. PIV then identifies the location within the search window with the maximum correlation function result.
The spatial vector between that location and the center of the interrogation window becomes the displacement of that region between the first and second image. The process is then repeated for each subsequent pair of images.

The resolution of the PIV analysis is set by the number of interrogation windows, so smaller windows increase resolution. However, the interrogation windows need to contain enough particles for the correlation function to be meaningful. An additional consideration in this research was that the interrogation windows needed to be significantly smaller than the local flow heterogeneities. Trial and error produced the parameters that were used in the results discussed in later chapters.

3.4 Confocal Microscopy with Rheology

A confocal microscope controls the light collected by the microscope such that the depth of field of collected light is significantly limited. When combined with a rheometer, this enables the imaging of individual layers of a sample while under shear. This setup, developed by Dutta et. al. [55], was built from a DMI4000b inverted microscope by Leica Microsystems and an MCR-301 stress-controlled rheometer by Anton Paar. A schematic of this setup is shown in Figure 3.1. The rheometer has a customized measuring cell which is recessed lower in the housing and requires an extended tool. The bottom of this measuring cell has a wide capsule shaped opening. This is positioned above the inverted microscope such that an installed microscope objective sits just below the opening. The rheometer can be translated in two dimensions to allow the objective to image different regions of the measuring cell. To conduct a rheological measurement, a glass slide is affixed to the bottom of the measuring cell by an O-ring clamp. The microscope was used for fluorescence imaging by emitting a laser and collecting light through the same objective. In the
experiments described in this dissertation, the glass slide is coated with a layer of PDMS gel with a modulus of 500 Pa. The components of this gel are mixed and then spin coated onto the class slide, then baked at 90° C for 2 hours. Fluorescent beads can then be attached to the surface of the PDMS through a combination of APTES and EDAC. Before spin coating a layer of fluorescent beads can be deposited on the surface of the glass slide as well. The PDMS is finally treated with a 5 mg/ml aqueous suspension of Poly-L Lysine (PLL) for 30 minutes. The PLL was selected after consulting with colleagues in our Chemistry Department about finding a way to facilitate interaction between CNC’s and the PDMS in order to reduce slip at that boundary.

3.4.1 PDMS Deposition

In most imaging experiments on the confocal rheometer setup, a PDMS layer is deposited on a glass slide to form a transparent bottom boundary. The use of ultrasoft PDMS gels is used in mechanobiology [56] and the same principles have been adapted for boundary stress experiments. The procedure for depositing the PDMS begins with mixing the PDMS in a small dish which is then vacuum desiccated for 30 minutes. This mixture is then spin coated onto a glass slide at 1000 rpm for 30 seconds and then at 1800 rpm for an additional 30 seconds. The glass slide is then carefully baked on a hot plate at 90 degrees C for two hours. This produces a PDMS gel with a typical thickness of 15 µm and a modulus of 500 Pa. These parameters are the maximum sensitivity achievable in my experience without losing surface uniformity on the gel. A softer gel would be able to detect smaller boundary stresses, but more surface roughness creates significant noise in the measurements.
3.4.2 Flow Field Imaging

Flow field imaging refers to the suspension and the imaging of fluorescent polystyrene spheres within a suspension of CNC’s. This technique uses these fluorescent spheres...
as tracer particles to track the motion of the surrounding material [57][58]. Spheres of 1 μm in diameter were suspended with a volume concentration of 0.2 vol% within a cellulose nanocrystal suspension. The spheres used in these experiments fluoresced with an absorption peak at 515 nm and an emission peak at 505 nm. The setup was prepared in the identical fashion as the boundary stress measurements except that the fluorescent spheres attached to the PDMS were optional. A 10x magnification objective was used to image an area of approximately 912 μm on a side with a depth of focus of approximately 10 μm. A sample image of the fluorescent spheres is shown in Figure 3.2. Similar to the boundary stress analysis, PIV was used to track the motion of groups of spheres to determine the local flow velocity of the sample.

Figure 3.2: A sample image of fluorescent polystyrene spheres suspended in a CNC sample. The spheres have a diameter of 1 μm and are suspended at a volume concentration of 0.2%. Brighter spots in the image correspond to aggregations of spheres.
3.4.3 Boundary Stress Microscopy

Boundary stress microscopy uses the combination of confocal microscopy and rheology instrumentation. The method focuses imaging on the glass slide and attached PDMS layer which forms the bottom boundary of the rheological cell. Prior to the measurement, a PDMS layer is cured on top of the slide. The top of this PDMS is then coated with fluorescent microspheres. A sample of CNC’s in an aqueous suspension is then deposited on top of the PDMS and then brought into contact with the rheometer measurement tool. The microspheres are imaged with the confocal microscope to track the deformation of the surface of the PDMS. This data is used to determine the stresses exerted on the PDMS by the suspension. The images are processed using a combination of Particle Image Velocimetry (PIV) and code written by Eric Dufresne [59] that converts the image displacements into stresses. The basics of the conversion into stresses involve taking the PIV output, which is an evenly spaced grid of average local displacements, and applying a Fourier transform to the data. This is done because a direct translation from local displacement to local stress in real space is difficult, owing to the coupling of neighboring regions that must be accounted for, but this translation becomes feasible in Fourier space. Therefore, after the Fourier transform, the data is converted into the Fourier transform of local stresses, which is then converted back into real space. For more information, see Reference [59].

From observations made using cross-polarization microscopy and other published observations [3, 20, 22], nematic domains in the soft solid CNC suspensions are on the order of 100 µm in length. Therefore a magnification of 40x was used in combination with a built-in 2x microscope magnification to capture a field of view of approximately 350 microns on a side. In order to produce detectable deformations, PDMS gel is made with a modulus of 500 Pa and cured to have a thickness of about 20 microns. This
modulus is the lower limit of feasibility for this process, and the thickness is chosen to balance the deformation of PDMS (and thus sensitivity) with the focal length of the objective.

3.5 CROSS POLARIZATION MICROSCOPY

Cross Polarization Microscopy is performed in connection with a rheometer in a similar manner to the boundary stress microscopy. Instead of a confocal microscope and glass lower boundary, the cross polarizing optical setup is used with an MCR702 system that has a transparent upper tool. The objective and camera are placed above the rheology cell and image vertically through the sample. A 20x objective is used to capture a wide field of view. Broadband light is projected through one polarizer and out through the objective into the sample. Then light reflected off of the bottom steel tool returns through the objective, through another polarizer that can be oriented up to 90 degrees relative to the first polarizer, then onto the CCD camera. A schematic of cross polarization microscopy in a translational configuration is shown in Figure 3.3.
Figure 3.3: A schematic of the cross polarization microscopy setup used in this research. The microscopy setup was combined with a rheometer to image sheared samples. The upper tool of the rheometer was made of a proprietary transparent material produced by Anton Paar. Broadband spectrum light passed through the polarizer and out the objective before passing through the sample. The light was then reflected off of the steel bottom boundary, which in this setup was another rheometer tool, and then passed back through the sample to be collected by the objective. The light path then split from the incoming light and passed through an analyzer before hitting the camera.
The first experiments performed on CNC samples received from NIST were rheological tests to characterize the material at different concentrations. Chapter 3 explains rheology and the experimental setup used in these experiments. The purpose of bulk rheology measurements was to identify the phase behavior of CNC suspensions at different concentrations in order to compare to the variety of CNC measurements in published literature. In the process of conducting these measurements a couple interesting behaviors were observed. A signature of significant slip at the boundary between the CNC sample and the bottom surface (PDMS or glass slide) was confirmed by microscopy observations. This slip was reduced by adjusting the chemical treatment of the boundary of the measurement cell. Additionally, significant residual stress after shearing was observed in CNC suspensions in the repulsive glass phase. A method for reducing the residual stress is described at the end of this chapter.

4.1 Oscillatory Rheology

Strain amplitude sweeps and frequency sweeps were taken at the beginning of, during, and after rheology experiments. The oscillatory rheology was consistent for a given sample after the sample had been sheared “hard”, typically above $100 \, s^{-1}$. Oscillatory tests before high shear consistently yielded moduli slightly higher than those after the high shear, though the overall behavior remained consistent. Oscillatory rheology
was consistent across samples from the same batch of CNC’s received from NIST and across batches as well.

![Graph showing frequency sweeps for CNC suspensions at three concentrations]

**Figure 4.1:** Frequency sweeps of CNC suspensions at three concentrations bridging the transition from liquid-crystalline fluid to repulsive glass states. The dominance of the storage modulus over the loss modulus for the 12% wt. sample demonstrates significant elastic behavior, indicating a gel-like material. The dominance of the loss modulus for the 6% and the relative similarity for the 8% indicate fluid and weakly viscoelastic behavior, respectively. Taken on an MCR702 rheometer in cone-plate geometry.

The frequency sweeps in Figure 4.1 demonstrate the transition from a liquid-crystalline fluid at intermediate concentration to a soft solid at higher concentrations. The highest concentration at 12% wt. (8% vol.) shows an order of magnitude dominance of the storage modulus over the loss modulus, indicating gel-like behavior. The two lower concentrations show the transition from more liquid behavior (loss modulus
dominating storage) through viscoelastic behavior (loss and storage modulus roughly equivalent) on the way towards the gel-like behavior.

Figure 4.2: Strain amplitude sweep of a 12% wt. CNC suspension. The consistent order of magnitude difference between the storage and loss moduli at lower strain amplitudes is the linear regime. This elasticity in the system persists until approximately 10% strain, at which point the system begins to deform irreversibly. The rise and subsequent fall of the loss modulus is repeatable across all samples and is consistent with expected behavior for colloidal suspensions. Taken on an MCR702 rheometer in cone-plate geometry.

The strain sweeps consistently demonstrate a linear regime out to just before 10% strain, followed by a drop in the storage modulus and a local maximum in the loss modulus as shown in Figure 4.2. The relative behavior of the two moduli is consistent with the weak strain overshoot behavior identified by Hyun et. al. [60] in their large amplitude oscillatory shear review. This is a generic signature of many soft glassy materials and is associated with weak or absent attraction between particles[61]. The
storage modulus in the linear regime is approximately 500 Pa for 12% wt. suspensions. This is consistent with my physical interactions with the material, in which it behaves as a soft gel that can flow with only minimal applied force. The end of the linear regime at approximately 10% strain indicates a yield stress of 50 Pa. Indeed, this yield stress is observed in flow curves of the higher concentration suspension.

4.2 Flow Curves

Flow curves are continuous shear tests at, in this case, many different shear rates. Each data point is a time average of the shear stress measured when the material is subjected to a fixed shear rate. The steady state value of the shear stress is measured by the rheometer’s internal algorithm. Looking at the measured shear stress as a function of shear rate as in Figure 4.3 shows the material response to continuous shear as the concentration of CNC’s is increased. The lower concentrations have an approximate slope of 1 which is a characteristic of Newtonian fluid behavior. This is consistent with the cross polarized microscopy imaging shown in the Background section that shows small, isolated nematic domains at low volume fraction. As the concentration of CNC’s is increased, the shear stress transitions to shallower slopes until 12% wt. where the slope has nearly disappeared. This higher concentration behaves as a yield stress material with a nearly constant stress required to shear the suspension as shear rate increases. The 12% wt. sample shows a yield stress of approximately 50 Pa, the same value determined by the end of the linear regime in Figure 4.2. The same data is plotted in Figure 4.4 in terms of viscosity to show the concentration-dependent behavior in a different manner. A Newtonian fluid should have constant viscosity regardless of shear rate, similar to the behavior of the lower
concentration CNC suspensions. For context, the viscosity of water is approximately 1 mPa·s.

Figure 4.3: Flow curves of CNC suspensions at concentrations from the Newtonian fluid state through the repulsive glass state. With the shear stress plotted, it is easy to notice the nearly horizontal stress for the 12% wt. that is characteristic of a yield stress material. Taken on an MCR702 rheometer in cone-plate geometry.

4.3 Comparison to Literature Values

The rheological measurements of suspensions of CNC’s depend not only on the mass fraction but also the specific length, width, and polydispersity of the nanocrystals. Therefore, in comparing the rheology in the previous section to other published results, it is important to note these factors.
Figure 4.4: Flow curves of CNC suspensions at concentrations from the Newtonian fluid state through the repulsive glass state. With the viscosity plotted, it is easy to notice the nearly constant stress for the lower concentrations that is characteristic of a Newtonian fluid. Taken on an MCR702 rheometer in cone-plate geometry.

To compare to a similar CNC system, the work of Stokes et. al. [2][44] uses CNC’s with approximately double the length of the ones described in the Methods and Materials chapter. However, both systems of CNC’s have an approximate average aspect ratio of 14 (see Table 2.1 for details). As shown by the frequency sweeps in Figure 4.5a, the Stokes et. al. 11.9% wt. CNC suspension exhibits storage and loss moduli similar to the 12% wt. CNC suspension in Figure 4.1. The lower concentrations 9% and 5% wt. in Figure 4.5a likewise have values comparable to the 8% and 6% wt.
concentrations shown in Figure 4.1. The 9% wt. concentration in Figure 4.5a shows a slight dominance of the storage modulus as opposed to the 8% wt. in Figure 4.1, which shows a slight dominance of the loss modulus. This is an expected difference since those concentrations are of the cusp of the transition from a viscoelastic suspension to a repulsive glass phase. Similarly, the flow curves from Stokes et. al. in Figure 4.6 show comparable shear stresses and viscosities to those in Figures 4.3 and 4.4.

For a comparison to a wider range of CNC suspensions, the work of Davis et. al. [3] involved CNC’s with a similar length to those described in the Methods and Materials chapter but with an approximate average aspect ratio of 7 instead of 14. The results shown in Figures 4.7 and 4.8 from Davis et. al. are differentiated by volume fraction instead of mass fraction, therefore for comparison note that the 12% wt. suspensions in Figures 4.1, 4.3, and 4.4 have a volume fraction of 7.7%. The data from Davis et. al. shows similar rheological behavior as described in the previous sections at approximately twice the volume fractions. A better value for comparison across CNC’s of different dimensions is the product of the volume fraction and the aspect ration ($\Phi \ast L/D$). For both my data and that of Stokes et. al., the onset of elastic behavior is seen at approximately $\Phi \ast L/D = 0.9$. For Davis et. al. the elastic behavior is seen at $\Phi \ast L/D = 0.84$. My samples at 12% wt. (7.7% vol.) and the Davis et. al. samples at 15.8% vol. have $\Phi \ast L/D$ of 1.08 and 1.11 respectively, with similar rheological behavior. This is consistent with the expectation that aspect ratio is the key variable in determining the behavior of rigid-rod suspensions.

4.4 **Repulsive Glass Phase**

The change in phase as a function of concentration in the flow curves can be plotted in a manner seen in the study by Urena-Benavides et. al. [20] (Figure 4.9) where the
Figure 4.5: Storage moduli ($G'$) and loss moduli ($G''$) for CNC suspensions ($L/D = 14$) by Stokes et. al. [2] over a range of CNC concentrations and added NaCl concentrations ("added" NaCl refers to the amount introduced to the suspension after the CNC production process, in excess of the counterions already in the system). Open symbols represent $G''$ and closed symbols represent $G'$. The CNC concentrations shown are 1 wt% (circles), 5 wt% (squares), 9 wt% (triangles), and 11.9 wt% (diamonds). The NaCl concentrations shown are (a) 0 mM, (b) 10 mM, and (c) 50 mM. The NaCl concentration modulates the attractive and repulsive electrostatic interaction between CNC particles. The rheology in (a) is analogous case to the data shown in Figure 4.1, which likewise has no added NaCl.
Figure 4.6: Flow curves by Stokes et. al. [44] over a range of CNC concentrations (L/D = 14) with no NaCl added beyond the bound counterions. The data shown in these graphs are comparable to Figures 4.3 and 4.4. Solid lines are power law fits to regions of the flow curves.
Figure 4.7: The storage modulus $G'$ (solid symbols), and loss modulus $G''$ (open symbols) of CNC dispersions ($L/D = 7$) at 11.6 vol % (squares) and 15.8 vol % (triangles)[3].

Figure 4.8: Steady shear viscosity of 3.27 vol% (filled diamond), 6.98 vol% (filled square), 11.6 vol% (filled triangle), and 15.8 vol% (filled circle) aqueous CNC dispersions ($L/D = 7$) as a function of shear rate. Error bars indicate the minimum and maximum measured values and are smaller than symbol size for most data. The inset highlights three region behavior of the 6.98 vol% dispersion[3].
Figure 4.9: The viscosity versus concentration plotted for steady shear rates of 0.1 rad/s (red diamonds), 1 rad/sec (blue squares), 0.1 s$^{-1}$ (orange triangles), and 1 s$^{-1}$ (purple circles) of CNC suspensions (L/D = 5) as measured by Urena-Benavides et. al. [20]. The solid lines indicate a phase transition to a fully nematic system as observed through cross polarization microscopy. The dashed line is an approximation of the gel transition.

viscosity at a few specific shear rates is plotted as a function of CNC concentration. Figure 4.10 shows the same data from Figure 4.4 plotted in this method. In both cases, the viscosities at these shear rates separate as the suspension passes through the liquid crystal phase into the soft solid phase. While Urena-Benavides et. al. names this soft solid a gel, the system in those experiments is a CNC suspension without significant screening of the surface charge, much like the system described in the Methods and Materials chapter. Therefore this soft solid state is more aptly described as a repulsive glass as described in the Backgrounds chapter.
Figure 4.10: The viscosity versus concentration plotted for steady shear rates of 0.1 \( s^{-1} \) (red), 1 \( s^{-1} \) (blue), and 10 \( s^{-1} \) (purple). The dashed vertical line indicates the approximate concentration when the storage modulus becomes larger than the loss modulus in the linear regime, and is my approximation of the gel transition shown in Fig. 4.9. The volume fraction at this point it approximately 5% vol. The CNC’s in my samples have an aspect ratio approximately 2.7 times that in Fig. 4.9, which matches the difference in the concentrations at which these transitions are observed (5 vs. nearly 14) The separation of the viscosities at higher concentrations matches the behavior observed in Figure 4.9.

4.5 Repeatability and Sample Lifetime

There was no significant difference between the results measured using cone-plate and plate-plate geometries. After loading and a high shear of around 100 \( s^{-1} \), rheological tests were consistent for a given sample, with a slight decrease over the span of hours due to evaporation. With the evaporation controls described in the Methods and Materials section, evaporation was not a significant factor for at least 5 hours.
Rheology results were consistent between samples with different loadings, with a shift in magnitude related to the degree with which the rheometer sample volume was over-filled or under-filled with the CNC suspension.

4.6 Dependency on Boundary Materials (Slip)

The flow profile within the sample is a significant factor in CNC suspension rheology. Using the confocal microscope-rheometer setup described in the Methods and Materials chapter, fluorescent beads distributed within sheared CNC suspensions were imaged in order to map the flow profile. In this setup, the top boundary is steel, as in the isolated rheology case, but the bottom boundary is a transparent glass slide instead of the steel base plate used for isolated rheology. Immediate differences were observed in the rheology, and measurements of the flow profile showed significant slip of the sample at the glass interface. Figure 4.11 shows the average bead velocity observed at different vertical positions in the sample. The velocity at the top of the sample (110 um) matched the velocity of the tool at that position. In the ideal case of no slip, the velocity of beads at the glass would be zero and would linearly increase vertically through the sample until matching the tool velocity at the top of the sample. However, bead velocities immediately above the bottom glass boundary were over half the velocity of the tool. This profile is indicative of significant slip at the glass boundary.[62][63]

To combat this slip, the glass slides were plasma cleaned for 5 minutes to determine if the hydrophobicity of the glass caused slip. However, observations of fluorescent beads suspended in 12% wt. CNC samples on hydrophilic glass (post-plasma) still revealed significant slip. This was supported by the bulk rheology. The flow curves for the CNC suspensions on both the plasma cleaned and default glass slides only
Figure 4.11: The velocities of suspended fluorescent beads in a 12% wt. CNC suspension as a function of vertical height through the sample. Note that the observed velocities just above the glass slide were more than half the velocity of the tool, indicating significant slip.

matched the data from samples between two steel tools at high shear rates, when the total applied stress has reached the yield stress of the suspension, and then quickly fell off towards a much lower curve. This is consistent with wall slip characteristics described by Cloitre and colleagues [62, 63]. In contrast, the 12% wt. suspensions of CNC’s sheared between two steel tools show only a gradual decrease in shear stress as the shear rate is decreased. The data in Figure 4.12 demonstrates these contrasting behaviors. In addition to the slip on the glass slide, a similar slip profile was observed at the PDMS surface used for boundary stress microscopy. After consultation with
colleagues in the Chemistry Department, specifically Dr. Steve Metallo, a solution was found by depositing a layer of poly-L-lysine (PLL) on the surface of the glass or PDMS to cause stronger adhesion between the surface and the sample. After application of PLL, both the glass and PDMS configurations demonstrated limited slip at the bottom interface and bulk rheology that more closely matched the results found in the steel-steel configuration (Figure 4.12). Further investigation revealed that 11% wt. suspensions yielded better flow profiles than the 12% wt. suspensions. While I was unable to determine a configuration that showed absolutely no slip at the bottom boundary, I was able to reduce the velocity of tracer particles at that boundary from 70% of the tool velocity down to around 10%. More discussion of flow profiles can be found in the Flow Profile chapter.

4.7 Residual Stress and Ring-Down Procedure

Upon loading a CN suspension onto a rheometer, a small residual stress can be measured by holding the tool steady. These measurements are made by applying a fixed shear rate of zero $s^{-1}$ and allowing the rheometer to measure the torque applied to the tool by the material. This stress is several orders of magnitude lower than the stresses measured upon shearing. In order to erase history in the sample between different rheology tests, the sample can be sheared hard at 100 $s^{-1}$ for one minute. As Figure 4.13 demonstrates, this leaves a residual stress (seen as the data labelled "100 $s^{-1}$ Shear 1 min") in the sample that is on the order of magnitude of the measured stresses (seen as the data labelled "3% Step Strain Over 10s") in rheological tests such as the step-strain tests. Therefore several methods of reducing the residual stress were explored. A successful method involved a strain amplitude sweep from high amplitude outside the linear regime to very low amplitude. This method is shown in Figure 4.13.
Figure 4.12: Flow curves comparing a range of 12% wt. CNC suspensions measured between two steel surfaces (filled circles) to an untreated glass slide and PDMS treated with PLL. The sharp drop-off in shear stress for the untreated glass surface is characteristic behavior for significant slip. The similarity between the treated PDMS and the steel base curves matches observations of suspended fluorescent beads in the sample, which show significantly reduced slip for the treated PDMS.

as well. The data labelled "Strain Amplitude Sweep 50%-0.01%" is the rheometer output for a strain amplitude sweep conducted at a frequency of 1 Hz with an initial strain amplitude of 50% and a final strain amplitude of 0.01%. For reference, the linear regime of the sample ends at 10% strain. The data output during this test is not straight forward, and doesn’t directly show the stress state of the sample, but the change in residual stress can be seen by comparing the data labelled "Wait 1
min" before and after the strain amplitude sweep. At these two points in the process, the rheometer tool is held fixed for one minute while it continues to read the stress exerted by the sample on the tool. As seen in the graph, this residual stress drops by an order of magnitude after the strain amplitude sweep is applied. This behavior is not seen if the sample is left stationary for the sample amount of elapsed time. This technique has been successful in reducing the stress to an order of magnitude below the desired measurement range.

Figure 4.13: 3% step strain test on 12% wt. CNC suspension. The preparatory procedure involves high shear for one minute to erase any previous history followed by a diminishing strain amplitude sweep starting well above the linear regime and ending well within it to reduce the residual stress. The two wait periods show the residual stress at each step of the procedure. Note the order of magnitude reduction in residual stress after the strain sweep. The jump in the data after the second "Wait 1 min" is the signal from the 3% step strain test applied over a 10 second interval. The tool is then held fixed for 3 minutes and the build up stress decays slowly. This stress did not decay to zero on any time scale observed.
Figure 5.1: A set of orthogonal indices of refraction, as can be found in a birefringent material. In this scenario, light is propagating into the page. Light polarized in any orientation other than the two axes defined by these indices of refraction will behave as a superposition of two orthogonal waves that are polarized along these axes. The difference in the indices of refraction, and therefore of the propagation speed of the two orthogonal waves, will be expressed as a relative phase shift once the light has passed through the birefringent material.

Cross polarization microscopy is based on the fact that birefringent materials have different indices of refraction depending on the polarization of incident light. Instead of a uniform index of refraction, such as in water, there is directionality to the indices of refraction within the sample, such as shown in Figure 5.1. In a case like that, where there are orthogonal indices of refraction, light propagating perpendicular to both indices will experience a phase shift and ultimately a rotation in polarization after passing through a birefringent material. In CNC suspensions, this is caused by the alignment of CNC particles which interact with light differently depending on the
relative orientation of the light polarization and the CNC particles collective orientation. In the cross-polarization setup shown in Figure 5.2, broad spectrum white light is passed through a polarizer, through the birefringent material, and then through a perpendicular polarizer that is referred to as the analyzer. The relative orientations of the polarizer, analyzer, and the birefringent indices of refraction are shown in Figure 5.3, with $\theta$ defining the relative orientation of the birefringent material to the polarizers.

![Figure 5.2: A schematic showing the setup of a cross polarization measurement.](image)

Incident broad spectrum light originates from the left side of the image. It passes through a polarizer and reaches the birefringent material with a uniform polarization set by the polarizer. After passing through the birefringent material, the polarization of the recombined light has been rotated, and the altered light then passes through an analyser oriented at a $90^\circ$ angle with respect to the polarizer. The component of the altered light that is able to pass through the analyzer is then collected by the camera.

5.1 Calculation of Maximum Signal for Given Polarizer/Analyzer Angle

Start with a broadband beam of light propagating in one direction, defined as the $\hat{z}$ direction for this calculation, where $\hat{x}$, $\hat{y}$, and $\hat{z}$ are perpendicular basis vectors. After passing through the polarizer, defined for this discussion to be oriented in the $\hat{x}$ direction, the light is uniformly polarized in the $\hat{x}$ direction. The wave equation for the light incident on the birefringent material is therefore
Figure 5.3: The relationship between the polarizer (P), analyzer (A), and the two indices of refraction within the birefringent material \((n_1 \text{ and } n_2)\) is shown here. In this image, the direction of propagating light is into the page. For cross polarization experiments, the polarizer and analyzer are always at a 90° angle with respect to one another. The angle between the orientation of the polarizer and the indices of refraction within the birefringent samples \((\theta)\) determines the intensity of rotated light that is passed through the analyzer. The peak intensity occurs when \(\theta = 45^\circ\).

\[
\vec{E}_{\text{init}}(z, t) = E_o \hat{x} e^{i\omega(t-(n/c)z)} \quad (5.1)
\]

The \(n\) in the wave equation is the index of refraction of the surrounding medium, in this case air. This can be expressed as a linear combination of two perpendicular wave vectors oriented along the directions of the indices of refraction of the birefringent material:

\[
\vec{E}_{\text{init}}(z, t) = E_o [(\cos(\theta)\hat{x} + \sin(\theta)\hat{y}) + (\cos(\theta)\hat{x} - \sin(\theta)\hat{y})] e^{i\omega(t-(n/c)z)} \quad (5.2)
\]

For the purposes of this description, \(\theta\) will be 45°, which is a typical orientation used in this research. This gives:
\[ \vec{E}_{\text{init}}(z, t) = E_o[((1/2)\hat{x} + (1/2)\hat{y}) + ((1/2)\hat{x} - (1/2)\hat{y})]e^{i\omega(t-(n/c)z)} \] (5.3)

Inside the birefringent material, the different indices of refraction affect the travel speed of light oriented in each respective direction, such that:

\[ \vec{E}_{\text{inside}}(z, t) = (E_o/2)[(\hat{x} + \hat{y})e^{i\omega(t-(n_1/c)z)} + (\hat{x} - \hat{y})e^{i\omega(t-(n_2/c)z)}] \] (5.4)

After passing through a birefringent material of thickness \( Z_o \), the net effect of the perpendicular indices of refraction is a phase difference between the two wave vectors equal to:

\[ \Delta \Phi = (\omega/c)Z_o(n_1 - n_2) \] (5.5)

After the birefringent material, the perpendicular wave vectors of the light have the form:

\[ \vec{E}_{\text{after}}(z, t) = (E_o/2)[(\hat{x} + \hat{y}) + (\hat{x} - \hat{y})e^{i\Delta \Phi}]e^{i\omega(t-(n/c)z)} \] (5.6)

When these wave vectors encounter the analyzer, only the \( \hat{y} \) components of the light are able to pass through. The result is that the final light captured by the camera has the form of:

\[ \vec{E}_{\text{final}}(z, t) = (E_o/2)[\hat{y} - \hat{y}e^{i\Delta \Phi}]e^{i\omega(t-(n/c)z)} \] (5.7)

If \( \Delta \Phi \) is zero, then no light is transmitted through the analyzer. This is the trivial case when \( n_1 \) and \( n_2 \) are equal, and thus the material is not birefringent. Given that \( \omega = (c/n)k \) and \( k = 2\pi/\lambda \), \( \Delta \Phi \) can be expressed as:

\[ \Delta \Phi = 2\pi Z_o(n_1 - n_2/(n\lambda)) \] (5.8)
There is an explicit relationship between the wavelength of the light passing through a birefringent material and the thickness of the material to the total phase shift. This means that in a material composed of many different birefringent domains, there will be a difference in the dominant transmitted color when viewed through cross-polarization microscopy.

5.2 Characteristic Domain Size

Cross-polarization microscopy was initially used to examine the characteristic domain size of CNC suspensions in the repulsive glass phase. Samples imaged ranged in thickness from 200 to 500 $\mu$m to capture the behavior of the suspension in the same conditions as the bulk rheology and flow profile measurements discussed in other chapters.

Figure 5.4: Cross polarization images for 12% wt. CNC suspensions at different sample thicknesses (left: 500 $\mu$m, right: 200 $\mu$m). The striations seen in the image are from the steel tool off of which the light is reflecting. The different colored regions represent different aligned domains; however, due to the nature of cross polarization microscopy, the colors seen are the result of the integration of all aligned domains through which the light has passed. Therefore, the color regions are not necessarily due to individual aligned domains, but rather give insight to the characteristic length scales seen in the system.
The signal in cross polarization images comes from the aggregate effect of birefringent material in the sample. It is non-trivial to extract depth-dependent structure from cross-polarization images, and it is not attempted in this research. However, cross-polarization images of CNC repulsive glass suspensions can be used to estimate the order of magnitude of the aligned domains. The color in cross-polarization images is the result of light passing through the birefringent domains. Regions of contiguous color in a cross-polarization image can be reasonably assumed to represent light passing through similar birefringent domains, if not the same domains. Figure 5.4 shows sample images of CNC suspensions in the repulsive glass phase after loading before high shear. The regions of contiguous color vary in size and shape depending on the sample, but they suggest that the cross-section of the aligned domains are on the order of 100 \(\mu m\).

5.3 Effect of Reverse Strain Amplitude Sweep Relaxation Procedure

The reverse strain amplitude sweep relaxation procedure described in the CNC Rheology chapter and shown in Figure 4.13 reliably reduced the residual stress in CNC suspensions in the repulsive glass phase. Cross polarization imaging was used to examine the effect on the structure of aligned domains in the manner discussed in the previous section. Figure 5.5 shows images taken at three points during the relaxation procedure: Figure 5.5a shows the domain structure before the procedure, Figure 5.5b was taken after the 100 \(s^{-1}\) shear, and Figure 5.5c was taken after the reverse strain sweep. Due to the shear, the sample has translated through the imaged region, and therefore these images are representative and do not show the change in a constrained volume of the sample. The regions of contiguous color in Figure 5.5b are larger than
**Figure 5.5:** Cross-polarization images showing the effect of the relaxation procedure. In these images, the angle between the polarizer/analyzer and the flow direction is $\theta = 45^\circ$. The image in (a) is taken before the procedure is initiated, after loading of the sample. The sample after the high shear step (b) shows larger and more uniform features due to the shear-induced rearrangements. The intensity is likewise higher due to a stronger signal, indicating that more of the CNC’s are aligned in the flow direction. After the reverse strain amplitude sweep, the intensity drops and there is an introduction in some more short-scale features, indicating that the aligned domains have lost some of the homogeneity in orientation along the flow direction. The intensity is also lower in Figure 5.5a and without the sharp edges, suggesting that the underlying aligned domains have been merged and that there is less distinction in the director of alignment between domains. The increase in granularity (i.e., the introduction of small scale texture as opposed to image-spanning regions of the same color) in Figure 5.5c suggests that the reverse strain sweep reduced homogeneity of the aligned domain structure introduced by the high shear.

Figure 5.6 compares the effect of the relaxation procedure with the cross-polarization setup at a 45 degree angle relative to the flow versus parallel and perpendicular to the flow. Figure 5.6 a and c are taken after the high shear in the relaxation procedure, while b and d are taken after the reverse strain sweep. The low signal in Figure 5.6c is due to the fact that the domains are predominantly aligned in the flow direction, minimizing the rotation of the polarization of the incident light.
Figure 5.6: A comparison of the effects of the relaxation procedure from cross polarization measurements made when the angle between the polarizer and the flow direction ($\theta$) is $45^\circ$ (a,b) and $90^\circ$ (c,d). The images in (a) and (c) are taken after the high shear step in the procedure. Images (b) and (d) show the sample after the reverse strain amplitude sweep is completed.

Figure 5.6d shows a minor increase in intensity, suggesting that while the reverse strain sweep may reduce the homogeneity in the aligned domain structure, it does not significantly affect the significant average alignment in the flow direction.

5.4 Discussion of Boundary Material Effect

The transparent tool used in the cross-polarization experiments was made of a proprietary glass-like material. The rheology chapter discusses the significant slip observed at the interface of glass boundaries, and slip was observed with this tool as well.
Therefore, efforts were made to replicate the boundary materials used in the boundary stress and flow profile experiments. A glass slide with PDMS was prepared in the same manner as in the boundary stress experiments and attached to the transparent tool using vacuum grease. This was only partially successful, as the slide would detach after significant shear, and the slide may have rotated relative to the transparent tool during a test. Therefore, it is difficult to directly connect the aligned domain size estimations seen through cross-polarization to the microstructure during boundary stress and bulk rheology experiments.
Boundary stress microscopy utilizes the confocal rheometer setup described in Chapter 3 to image fluorescent beads attached to the surface of a PDMS gel that forms the bottom boundary in a shear cell. CNC suspensions in the repulsive glass phase are placed in the measuring cell and sheared, deforming the PDMS gel. The relative displacement of the attached fluorescent beads can be measured to determine the local deformation of the PDMS which allows for the calculation of locally applied stresses. This method was used in combination with multiple rheological tests to examine whether there were heterogeneities in the measured boundary stress that could be connected to internal mechanics of the CNC repulsive glass phase.

6.1 Step-Strain Results

Initial boundary stress microscopy tests explored the buildup and relaxation of stress in CNC suspensions within the repulsive glass phase during a step-strain experiment. In these experiments, the sample is brought to a “zero stress” state using the reverse strain sweep procedure as discussed in Section 4.7 and shown in Figure 4.13, which minimizes the residual stress measured in the sample when not under strain, then the strain is increased at a fixed rate (typically 0.003 s\(^{-1}\)) to a point within the linear regime (<10% strain). The tool is then fixed at that strain while the relaxation of the built up stress can be observed. As discussed in Chapter 4, CNC suspensions in
the repulsive glass phase can retain stress for significant periods of time after high shear. These residual stresses could be as high as 10 Pascal. Step-strain tests typically yielded stresses on the order of several Pascal, which therefore required that the sample undergo a relaxation protocol to bring the residual stress down to fractions of a Pascal in order to get a reasonable signal in the boundary stress. This “zero stress” state was achieved through the reverse strain amplitude sweep relaxation procedure described in the CNC Rheology chapter and shown in Figure 4.13. The shear stress as a function of time for a typical step-strain test after the reverse strain amplitude sweep relaxation procedure is shown in Figure 6.1. The relaxation times of built-up stress observed in the bulk rheology can give information about the processes in which energy is stored and released within the sample. The rheological measurements for step strains were typically reproducible for repeated tests on the same sample and across different samples. Step strain tests have shown a phenomenological stress relaxation that is best fit by the sum of two exponentials as shown in Figure 6.1. The time constants associated with the fitted exponentials are approximately 20 seconds and 200 seconds. It remains an active area of research to connect these relaxation times with physical processes within the suspension. A possible source of these relaxation times is the rearrangement between nematic domains and rearrangement within nematic domains after shear. Interactions between neighboring CNC’s and between neighboring domains could both play a role in the buildup of stress in a sheared nematic glass.

The goal of these step-strain tests is to identify any heterogeneity in the elastic stress distribution through the sample to the underlying PDMS. By shearing to a maximum strain that is within the linear regime at a slow rate, step-strain tests isolate and examine the elastic properties of the system. Rearrangements of individual CNC’s

\[1\] Data currently inaccessible due to campus closure in response to the Covid-19 pandemic.
Figure 6.1: Shown on the left is the stress response from a typical step strain test. The strain is increased over a 10 second interval to a maximum within the linear regime (typically 6%). The tool is then fixed in place and measures the stress relaxation over a period of 3 minutes. The graph on the right is a zoomed-in look at the stress relaxation curve measured after the peak deformation has been reached. A sample fit has been overlaid in red. The fit takes the form of a double exponential \( \sigma/\sigma_{\text{max}} = Ae^{-t/T_1} + Be^{-t/T_2} \). For this specific sample and test, the constants are: \( A=0.047, T_1=28.6, B=0.948, T_2=250 \).

or aligned domains occur on time scales associated with the stress relaxation, which is much longer than the rate of the applied step. Observed heterogeneities in the stress applied to the surface of the PDMS can provide information on the characteristic size of microstructures in the suspension that conduct stress through the sample.

The boundary stress measurements from a sample step-strain test are shown in Figure 6.2. The image on the left (Figure 6.2a) shows the shear stress measured by the rheometer during the step-strain test (black) overlaid with the average boundary stress measured at each point in time (red). The shear stress measured by the rheometer has been scaled to the approximate radius at which the boundary stress measurements were made to account for the gradient in applied strain along the radius of a parallel plate tool. This is necessary because when a parallel plate tool
Figure 6.2: The boundary stress measurements observed at the peak of a step-strain experiment. The graph on the left shows the shear stress as a function of time for a 6% step strain applied at a rate of $0.003 \, s^{-1}$. The rheometer measurements (black) have been scaled to the radius at which the boundary stress was measured. The average boundary stress per frame (red) shows relatively good agreement with the rheometer data, taking into consideration the variation in PDMS modulus due to an unconstrained surface [64]. The colormap of local stresses on the right shows the spatially resolved boundary stress for the frame with the highest average stress. A contour plot has been overlaid onto the colormap for ease of reading.

applies a shear, the gap between the tool and the bottom boundary is the same at all radii, but the linear deformation is not. Therefore, the total strain applied varies as a function of radius, from 0 at the center to a maximum at the outer edge. The rheometer, however, reports the value of stress at the outer edge when using a parallel plate tool, and so the bulk data from the rheometer must be scaled to be directly compared to the local boundary stress data at a non-maximum radius. The time values are measured from the start of the test, including the preparatory procedure which lasts approximately 5 minutes. The image on the right (Figure 6.2b) is a heat map of the boundary stress in a 456x456 $\mu$m region of the sample at the peak stress of the step-strain. A contour plot of areas of constant stress is displayed on top of the heat map. The results of the step-strain experiments consistently showed that the
boundary stress was uniform to within approximately 20% of the average stress, and
the local boundary stresses decayed uniformly with the average stress.

6.2 Constant Shear Results

Additional boundary stress microscopy tests explored the effect of constant shear
on CNC suspensions within the repulsive glass phase. In a constant shear test, the
sample was sheared at a constant rate for a total applied strain well beyond the linear
regime of the sample. The applied shear rates were typically between 0.1 and 10 s⁻¹.
Similar to the step-strain experiments discussed previously in this chapter, it was
important to reach a state of “zero-stress” in the sample before beginning a boundary
stress measurement. Therefore, the same reverse strain amplitude sweep relaxation
procedure described in Chapter 4 and shown in Figure 4.13 was used in the constant
shear tests to prepare the sample before each test.

Figure 6.3: Colormaps of boundary stress measurements for constant shear
tests at 10 s⁻¹. The colormap on the left shows the total measured stresses, and
the colormap on the right shows the same stresses with the mean stress subtracted.
Contour lines are superimposed on the right plot for ease of reading.

Similar to the step-strain tests, the goal of constant shear tests was to identify any
heterogeneity in the stress distribution through the sample to the underlying PDMS,
but this time during continuous flow as opposed to an elastic deformation. Observed
heterogeneities in the stress measured on the surface of the PDMS could possibly provide information on the characteristic size of microstructures in the suspension that persist beyond the elastic behavior of the sample. An example of a microstructure that could exist during the continuous shear of a sample is an aggregate of clumped CNC’s. On the other hand, assuming a well-distributed sample without significant clumping of individual rods, these microstructures could be a result of interactions between aligned domains.

For a constant shear test, the average stresses are significantly higher than the heterogeneities. Figure 6.3a shows the stress map for a constant stress test at 10 s$^{-1}$, which typically has an average stress around 50 Pa. This average stress was relatively constant throughout the duration of the applied shear and repeatable across samples\footnote{Data currently inaccessible due to campus closure in response to the Covid-19 pandemic.}. Figure 6.3b shows the same stress map with the average stress subtracted. The heterogeneities in Figure 6.3b are on the order of 10% of the magnitude of the stresses in 6.3a. This is a consistent characteristic of the constant shear tests across applied shear rates.

6.3 Persistent Patterns in Stress Maps

Boundary stress measurements made for repeated tests within the same experiment sometimes showed persistent patterns in the structure of the stress heterogeneities. Figure 6.4 shows representative images from a series of six constant shear tests, one taken after another, on the same sample for three different shear rates. The stress heterogeneities appear to share structural similarities between subsequent tests even though the total applied strain in each test is on the order of 100 strain units (i.e. the sample has been sheared at the top boundary for a total deformation that is on the order of 100 times the thickness of the sample). There is a general gradient in the
stress from left to right that persists across all six tests as shown in the representative images. Additionally, the stress maps at 0.5 $s^{-1}$ and at 1 $s^{-1}$ share remarkably similar spatial features. The full stress relaxation procedure described in the CNC Rheology chapter and shown in Figure 4.13, including the 100 $s^{-1}$ shear for 1 minute, was completed before each test. Therefore, the heterogeneities seen in the stress maps do not appear to result from local stress propagation through the sample. Instead they are more likely to originate from local variations in the PDMS-CNC interface, as discussed in the next section.

Figure 6.4: Colormaps of representative images from boundary stress measurements for a series of six individual constant shear tests, taken in sequence. The tests were taken in pairs, starting with the 0.5 $s^{-1}$ tests and concluding with the 5 $s^{-1}$ tests. Each colormap has had the mean stress subtracted, and is overlaid with a contour map for the ease of reading. The similarity of patterns seen in the stress maps even after significant total elapsed strain suggests that the patterns originate from either the PDMS surface or structures attached to the PDMS surface. The gradient in stress from left to right is a persistent feature even after careful levelling of the plane of the PDMS surface to the imaging plane of the microscope.
6.4 Discussion of Boundary Stress Microscopy Results

The prevalence of persistent patterns within boundary stress experiments suggests that the signal was dominated by heterogeneities at the PDMS-CNC interface instead of within the sample itself. Interfacial heterogeneities could come from a number of factors; PDMS surface roughness, local PDMS modulus heterogeneities, and uneven PLL deposition are all possible candidates. Investigations into altering the PDMS substrate preparation procedure involved altering the PDMS baking time, PLL deposition time and concentration, and lack of PLL entirely. These experiments did not yield noticeable changes in the persistent pattern behavior seen previously.

To understand the role of systematic noise in the detection of boundary stress patterns, I performed boundary stress microscopy experiments on glycerol using the same experimental setup as the CNC trials. Glycerol was chosen due to its uniformity on all length scales probed and therefore uniform effect on the boundary stress measurements when the sample was under shear. Pure glycerol was used but the hydroscopic nature of the material meant that the sample always contained some fraction of moisture from the atmosphere. Rheological measurements compared to benchmark values for different glycerol mixtures confirmed that the samples measured were slightly below 100% pure glycerol. The viscosity of each test was not tightly controlled because the purpose was to determine the systematic noise of boundary stress microscopy measurements, not to investigate the rheological properties of glycerol mixtures. The glycerol samples were sheared at fixed shear stresses ranging from 10 to 100 pascals. The upper limit was determined by the practical limit before sample was ejected from the rheometer, and the lower limit was determined as an applied stress suitably below the shear stresses measured in CNC nematic glasses but still large enough to get a clean signal in the boundary stress microscopy measurements. For
comparison, CNC samples in the nematic glass phase exhibited a yield stress around 50 to 80 Pa, depending on the concentration, sample, and shear rate. As shown in Figure 6.5, glycerol sheared at the highest applied shear stress of 100 Pa was measured to have local variations in the boundary stress of approximately 1 Pa. This 1% variation in the boundary stress was characteristic across different tests of glycerol samples. Additionally, there were consistent patterns observed in the boundary stress measurements across repeated trials on the same glycerol sample, similar to the persistent patterns discussed for CNC samples.

Figure 6.5: The colormap of a representative boundary stress measurement for a constant 100 Pa stress applied to glycerol. The observed local heterogeneities in the boundary stress were about 1% of the applied shear stress across different glycerol samples and applied shear stresses. The patterns observed in the boundary stress remained consistent throughout a tests and showed strong similarities between successive tests.

The persistent patterns observed in the glycerol tests and CNC nematic glass tests suggest that the structure of the boundary stress measurements resulted from systematic errors in the the experimental setup. Microscopic surface roughness in
the PDMS substrates is a prime candidate for the source of these patterns due to the results of the glycerol experiments. Even though the boundary stress signal appears to be dominated by factors other than the presence of aligned domains within the CNC suspensions, these experiments provided an upper bound for the effect of the domain structure on boundary stresses. If any effect exists, it seems to be smaller than the stress heterogeneities measured in the boundary stress tests. In many cases, this means that any effect on the boundary stress from the aligned domain structure is less than 10% of the total applied stress. This is significantly larger than the 1% heterogeneities observed in the glycerol tests, suggesting that the heterogeneities observed in the CNC tests are not due to variations in the PDMS itself. It is therefore probable that the persistent patterns seen in the CNC tests are a result of the interaction between the CNC’s and the PLL layer coating the PDMS, in which variations in the strength of that interaction or eveness in the PLL coating lead to regions that couple more strongly to deformations applied to the CNC suspensions. I note, however, the existence of occasional boundary stress tests that yielded average stresses much higher than the bulk stress at the examined radius. These results were not repeatable and rare. Given the abundance of aligned domains in the repulsive glass phase of CNC suspensions, it seems likely that these outliers are anomalies due to systematic errors in the experimental setup, such as clumps within the sample or trapped air bubbles, but I cannot rule out that these outliers are due to significant structural rearrangement within the CNC nematic glasses. Given the magnitude of the measured boundary stresses, it is the conclusion of this thesis that if the aligned domain structure of a CNC repulsive glass suspension causes heterogeneities in the boundary stress of a strained sample, it is a small effect relative to the average stress on the sample.
Chapter 7

Flow Heterogeneities

To investigate the local velocity within CNC suspensions under shear, one micron
diameter fluorescent polystyrene beads were suspended and imaged using the confocal
microscope on a confocal-rheometer combined setup. The microscopy setup used in
the following experiments imaged a region of approximately one square millimeter.
The depth of that region in focus is approximately 10 micrometers. Beads were tracked
at a set height within the sample while under flow to determine the local flow velocity.
The set of average flow velocities at different heights throughout the sample is called
the flow profile.

7.1 Discussion of Flow Profiles

The significant slip discussed in Chapter 4 and shown in Figure 4.11 was reduced
through a combination of altering interfacial interactions and adjusting the total CNC
concentration of the suspension. PLL deposited on the surface of a PDMS gel reduced
the slip at the bottom boundary of the sample. Cutting the concentration from 12%
wt. to 11% wt. also reduced the plug-flow behavior that contributed to the slip.
Figure 7.1 shows the improved flow profile compared to the significant slip observed
in Figure 4.11. The 11% wt. sample shows significantly less slip at the bottom of the
sample with an average flow velocity around 10% of the tool velocity, compared to
70% for the 12% wt. sample without PLL applied to the bottom boundary. The 12%
wt. sample is also at a higher shear rate \((10 \text{ s}^{-1})\) compared to the 11% wt. sample \((1 \text{ s}^{-1})\). The higher shear rate typically reduced observed slip for the 12% wt. sample.

![Graph](image)

**Figure 7.1:** A comparison of the flow profiles for samples before and after slip at the bottom boundary was reduced. The 12% wt. CNC suspension (circles) showed significant slip at the bottom boundary and a minimal effective shear gradient throughout the sample, characteristic of plug flow. The 11% wt. sample with PLL applied to the bottom boundary (diamonds) showed a significant reduction in the slip at the bottom boundary and an approximately linear shear gradient throughout the sample.

The reduction in slip was seen throughout 11% wt. samples with PLL applied to the bottom boundary. Figure 7.2 shows the flow profiles for 3 separate samples under the same applied shear rates and boundary conditions. Each flow profile shows a significant reduction in slip at the bottom boundary compared to the 12% wt. samples. In some flow profiles, the general slope of increasing flow velocities as a function of increasing height is interrupted by a region of the same average flow velocity. The flow profile displayed in red diamonds shown in Figure 7.2 demonstrates such a region between 30% and 40% of the total height. The average flow velocities
recorded at both heights were nearly constant. This suggests non-affine behavior, which means that the local strain rate is not uniform throughout the sample.

Figure 7.2: Flow profiles for several CNC suspensions at 11% wt. with PLL-treated PDMS as the bottom boundary (same sample source, different loadings). Each sample is sheared continuously at $1 \, s^{-1}$ and the flow profile is measured at approximately two-thirds of the radius of the tool. Error bars are the standard deviation of average velocities per frame measured over a one minute interval.

The average velocities and accompanying standard deviations shown in Figure 7.2 are measured by applying particle image velocimetry to image sequences which recorded the position of fluorescent beads as they moved across the field of view. The PIV processing yielded the displacement between frames for regions $25 \, \mu m$ in length. For a given frame rate, this displacement is equivalent to the local velocity. For Figure 7.2, the local velocities were averaged over each frame and across all frames in the imaging measurement. However, when the average velocity in each frame is subtracted
Figure 7.3: A snapshot of fluorescent beads suspended in an 11% wt. CNC suspension at a depth of half the total gap during a shear of 1 $s^{-1}$. The arrows superimposed on the image are the result of PIV analysis comparing the displacement from the previous frame, with the average displacement subtracted. The collective regions of larger or smaller-than-average displacement, corresponding to faster or slower velocities, are visible by eye when viewed at 10x speed.
from each local measurement, interesting behaviors emerge. Figure 7.3 superimposes arrows representing the local velocity after the mean has been subtracted on top of the image of the suspended fluorescent beads. The average flow in this image is from right to left. The PIV shows regions of collective motion that are faster or slower than the average velocity, and in some cases with components in the vorticity direction. These regions are observable by eye when the image sequence is viewed as a movie. The magnitude of the difference in velocity between these regions and the average velocity in a given frame is approximately 10% of the average flow velocity. This is consistent at all heights in the sample. An important note is that this behavior is not perfectly repeatable. Some trials did not yield significant local deviations from the mean flow velocity for the same experimental conditions on different samples prepared through the same procedure. Additionally, this behavior was only observed within a restricted region of experimental parameters. Applied shear rates of 0.1 s$^{-1}$ and below as well as 5 s$^{-1}$ and above did not demonstrate local deviations from the mean flow velocity at the same scale as shear rates around 1 s$^{-1}$, even when scaled by total elapsed strain. Additionally, concentrations of 9% wt. and below did not show significant local deviations from the mean flow velocity at any applied shear rate.

Efforts were made to quantify and analyse these regions of collective motion. This work was greatly aided by David Gagnon and Miguel Ángel López-Castaño. Once local displacements had been determined through application of PIV, correlation functions were applied to examine the size and patterns of the regions of collective motion around the mean flow velocity. The PIV process outputs an average displacement for a small region of the image, calculated by comparing two successive images, the size of which is set by the user. The resulting data set can be thought of as an image sequence in which each frame represents the displacement between two frames of the raw data, and each "pixel" represents the local average displacement for a small region.
Figure 7.4: A sample of the results of a temporal correlation analysis applied to a set of videos of non-affine flow in an 11% wt. CNC sample. The applied shear rate is $1 \text{s}^{-1}$. Each curve represents a video taken at a fixed depth within the sample. The graph on the left is the result when the x-component of the PIV displacements is used, and the right graph shows the same analysis using the y-component.

of the field of view. Both temporal and spatial correlation functions were applied to these processed data sets. In a temporal correlation analysis, the displacement for a single sub-region is compared to every other displacement for that sub-region through time. In a spatial correlation analysis, the displacement for a single sub-region is compared to every other sub-region in the same moment in time. In practice, the x and y components of the displacement were usually analyzed separately. For spatial correlation analyses, the reference sub-region was only compared to other sub-regions along a single axis, either vertically or horizontally in the frame. Figure 7.4 shows the output from a temporal correlation analysis applied to a series of videos taken at different depths within a sheared CNC sample in the repulsive glass phase. The estimated length scales of the regions of non-affine flow are shown in Figure 7.5.
Figure 7.5: The results of the correlation functions in Fig. 7.4 were used to extract characteristic length scales of the non-affine regions of flow as a function of depth within the sample. The y axis lists the results in the units of pixels, which are 1.7 microns in length and width.

Several issues arose in this process. The first was the depth of field of the imaged area. The difference in mean flow velocity between the top and bottom of the imaged area was large enough to cause issues with the PIV, which does not account for a 3-dimensional system. Analysis of the correlation functions used within the PIV process did not result in the ability to extract depth-dependant information from the images\textsuperscript{1}. No further improvement was achieved in the compromise between a reduced depth of field and a large field of view of the microscopy setup in this research. A

\textsuperscript{1}Data currently inaccessible due to campus closure in response to the Covid-19 pandemic.
second issue was the systematic struggle with trapped air in the CNC suspensions in the repulsive glass phase. The preparatory procedure for suspending fluorescent microspheres within CNC samples introduced air into the sample, even with measures implemented to reduce this effect. Due to the high viscosity and stability of the CNC suspensions in the repulsive glass phase, trapped air bubbles did not escape the sample on time scales feasible for research applications. Methods to forcibly remove air, such as the application of a negative pressure, inadvertently caused the accretion of CNC films on the surface of suspensions in the repulsive glass phase, changing the concentration by an unknown amount. Therefore, data collection from these systems was complicated by the constant presence of disruptive air bubbles on the scale of the desired observable phenomena. Based on the current data available, we estimate the length scale of the regions of collective motion that deviated from the mean flow velocity were on the order of 100 micrometers\(^2\). This is a comparable length scale to the aligned domains observed through cross polarization microscopy and discussed in Chapter 5. Based on our observations we hypothesize that the local alignment of CNC particles in the repulsive glass phase are related to the local viscosity variations that are observed as local deviations from the mean flow velocity.

\(^2\)Data currently inaccessible due to campus closure in response to the Covid-19 pandemic.
Chapter 8
Conclusion

With so many fascinating aspects of colloidal rigid rod systems and cellulose nanocrystals in particular, my research aimed to shed light on a particular subset of that field that is as yet not fully understood. I examined CNC suspensions in a repulsive glass phase to better understand the high-concentration behavior of this system with the dual purpose of understanding the underlying physics as well as providing insights that will assist in the development of novel complex materials from CNC’s. In Chapter 2 I described the current state of understanding of the relevant physics as seen in published literature and well established theory. In the process of providing important background information to explain my subsequent experiments, I identified the lack of understanding of the microstructural behavior behind the repulsive glass behavior in CNC’s. Chapter 3 described the samples and systems that I then analyzed using four primary techniques, each of which was the focus of the remaining chapters.

In Chapter 4, I presented the finding of the bulk rheological tests for the purpose of comparing my samples to the well documented rheological measurements of CNC suspensions in the published literature. In Chapter 5, I applied cross-polarization microscopy to examine the microstructure of this system. The cross-polarization microscopy showed rich domain structure that was partially affected by shear. While the specific domain structure depended on the shear history of the sample, there were consistent patterns on the length scale of 100 micrometers, which informed my subsequent investigations.
Chapter 6 focused on boundary stress microscopy, which to my knowledge had not been applied to CNC systems previously. This did not yield boundary stresses that I could connect to the domain structure observed under cross-polarization microscopy. The primary issue was the occurrence of persistent patterns in the boundary stress even after significant shear had been applied to the sample. Using glycerol as a control sample, I believe that the patterns and dominant source of heterogeneities in the boundary stress are due to variations in the interaction between the PLL layer and the CNC’s. While there are possible confounding factors that contributed to the observed boundary stress patterns, the maximum boundary stress measurements were still significantly lower than expected, with a maximum of 10% of the average stress for the constant shear tests and 20% for the step strain tests. However, in Chapter 7, tracer particles revealed rich rearrangement behavior in sheared repulsive glass suspensions on approximate length scales of 100 micrometers. This suggests a connection to the domain structure observed under cross polarization microscopy, but more work is needed to analyze and understand the flow heterogeneities data.

These results open a new window into the local behavior of repulsive glasses, furthering the understanding of colloidal rigid rod systems in general and their practical applications in material development. With regards to cellulose nanocrystals specifically, my results contribute to expanding the capabilities of CNC based materials and supports the search for environmentally responsible and economically feasible materials on which we can rely on moving forward.
As part of our effort to investigate the potential utility of the morphological transitions for novel stimuli-responsive materials, we investigated the effect of temperature driven morphological changes on the rheology of dense suspensions of flagella. These experiments used the 1103 strain of Salmonella, which produce helical flagella in the “normal” conformation. These flagella were then suspended in a 62% Ethylene glycol solution with 35mM NaCl to induce a change into the “coiled” conformation, which has a larger radius and shorter pitch than the normal filaments. Added to this suspension was 400mM Tris to act as the driving agent for temperature dependent conformation change. At about 30 degrees Celsius, the flagella in this suspension are observed to undergo a transition to the “straight” conformation, appropriately named for its lack of an apparent helicity on relevant length scales.

Oscillatory rheology tests were performed on an Anton Paar MCR702 rheometer in TwinDrive mode using parallel plate geometry. The sample and tools were enclosed in a temperature control chamber. Oscillatory tests were conducted with constant strain of 1% and frequency of 1 Hz as determined by frequency and strain amplitude sweeps. We observed that the modulus changed dramatically and reproducibly when the temperature changed between 10 and 30 degrees Celsius for 10 to 20 minute periods in the temperature oscillation. The dominance of the storage modulus at low temperatures disappeared at high temperatures and both moduli dropped in magnitude, consistent with the expectation that an entangled network of coiled filaments was
Figure A.1: The storage ($G'$) and loss ($G''$) moduli of a dense suspension of flagella under oscillatory shear at 1% strain and 1 Hz while the temperature transitions between 10 and 30 degrees Celsius. Flagella are in a coiled configuration at low temperatures and straight at high temperatures. However, the gradual decrease of the moduli while the suspension was held at low temperatures and across cycles suggested that other phenomena might be involved. To test the internal temperature of the sample with respect to the reading given by the system’s thermometer, we performed the same tests over different time scales using pure glycerol as a control sample due to its known strongly temperature-dependent viscosity.
We found that the glycerol sample does not reach a constant viscosity until after at least 90 minutes, indicating that sample in the rheometer is very slow to reach the temperature setpoint. As a result of these findings, we are currently working to repeat the experiments from Fig. 1 with longer temperature cycles. We are also planning to investigate a second temperature-driven transition that changes normal filaments to straight filaments and back.

**Figure A.2:** The loss modulus of Glycerol under oscillatory shear at 1% strain and 1 Hz while the temperature setpoint is changed between 10 and 30 degrees Celsius. Duration between temperature changes is increased through 1, 1.5, and 2 hour intervals.

I began developing a largescale system for testing filament geometry effects on suspension rheology using 3D printing technology. The goal is to have a system that can independently confirm conclusions derived from flagella research, but where we have complete control over the parameters of the helical filaments. Through a collaboration with Prof. Ryan Sochol at the University of Maryland’s Department of Mechanical Engineering, we used a high performance 3D printer to experiment with various methods for printing helices at a small enough scale to allow for testing with our sensitive MCR rheometers. We designed a custom measuring cell that would allow
for a Couette geometry measuring tool with a much larger gap between the tool and the outer wall, allowing for much larger filaments. Our initial attempts at printing have found that while it is possible to print helices at scales small enough to fit into our custom measuring cell (Fig A.3a), the bonding between printed layers within a helix is not strong enough to withstand stresses generated from even minimal fluid flow (Fig. A.3b). We are currently working on alternative solutions to retain structural integrity for these printed helices.

Figure A.3: Images of 3D printed helices with a printed diameter of 300 microns. There is some loss of material through the removal process from the printed secondary support material. The left image shows different length segments that survive the removal process. The right image shows the separation of individual layers from the larger structure.
Appendix B

Internship at Scientific and Biomedical Microsystems

In 2017, I was on an apprenticeship at Scientific and Biomedical Microsystems (SBM) working as an engineering support contractor on a few different projects. The two primary projects were MAST, an in situ oil spectroscopy system using a high voltage spark, and Innovas, a smart-ball development project for health monitoring of industrial heat exchange systems. Both projects are named after the employer for the respective contract. On each project, I worked primarily as a research scientist with secondary exposure to program management, client communications, and product development. Here I describe briefly each project, the work I performed, and the lessons and skills learned.

B.1 MAST

The MAST project had the long term goal of making a small device, roughly the size of a deck of cards, which could take spectroscopic data from samples of oil from a running engine, ideally in a car. The phase of the project that I worked on had the goal to develop a functional tabletop system that fitted inside of a roughly 2 ft³ aluminum box that could be incorporated into a larger engine, such as that of a windmill or container ship.

The goal of this project was to detect wear elements associated with certain problems that can occur within an engine. We focused on detecting certain elements
selected for their relationship to these problems, as detailed in the table in Figure B.2.

The fundamental process for producing elemental spectra for data collection was Spark Induced Breakdown Spectroscopy (SIBS), which used a high voltage spark to oblate a small oil sample near an optical fiber which would collect the resultant emissions and send the information to a nearby spectrometer. This technique was chosen in a prior phase of the project when it was determined that oblation was required to get elemental spectral lines and the more precise laser technique (LIBS) was cost prohibitive for both the size and scope of the long term project goals. An overview of the process is detailed in the diagram in Figure B.3.
I focused especially on the flow cell, which was the component where the SIBS process occurred. This was a small 3D-printed chamber that housed the spark-producing electrodes, the optical fiber mounts, and the fluidic channel that would wet the electrodes with target oil samples. An initial design is shown in Figure B.4 in which the oil entered from the side and drained through the bottom. Optical fibers were mounted above, with the electrodes entering from the sides. The cathode was in the path of flow.
and theoretically would be wetted by the flowing oil. In my observations, I noticed an uneven flow over the electrode between iterations, resulting in a potentially inconsistent layer of oil on the cathode upon sparking. I attempted to remedy this with a new flow cell design that instead had a vertical cathode and a perpendicular anode coming in from the side of the cell. The oil entered the cell from four symmetric ports at the top of the cathode and ran down the cathode in an approximately even coat before draining. The cathode itself was coated in an insulating material except for a pinhole directly across from the anode, allowing for a more controlled spark location.

The data collection from the system was plagued by larger variance across successive sparks for the same sample and overlapping data between samples. I suspected
this was due to many factors, including the amount of oil on the electrodes, the plastic residue left after a spark, the location of a spark relative to the optical fibers’ field of view, and the wear on the electrodes. The design changes to the flow cell and various experiments with timing between oil flow and sparking attempted to reduce the oil amount variation. We instituted a “cleaning” cycle of rapid dry sparking between tests in an attempt to reduce residue on the electrodes. The insulating sheath on the cathode and flow cell geometry worked to reduce spark location variation. The electrode material was changed from graphite, which would deteriorate on the order of 10’s of sparks and absorbed oil, to tungsten, which deteriorated on the order of 100,000’s of sparks and did not absorb oil. However, tungsten’s spectrum is much noisier than graphite, which made data analysis more difficult. At the time of the
departure, though improvements had been made, variability was still a problem for the system. A sample of the results after a calibration trial using standard titrated samples and known “unknowns” is shown in Figure B.5. Rows in yellow represent elements that had unacceptable prediction errors.

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<th>Limit of Detection (3σ/SD(blank))</th>
<th>Calculated count for 300 ppm sample</th>
<th>% error</th>
<th>Calibration confidence interval</th>
<th>Calculated count for 700 ppm sample</th>
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<td>314</td>
<td>5%</td>
<td>12%</td>
<td>931</td>
<td>33%</td>
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</tr>
</tbody>
</table>

Figure B.5: A table displaying the results of the SIBS analysis of various elements infused in oils.

I learned several skills and lessons from this project. I learned about the fundamentals of spectroscopy, common spectral interpretation techniques, and can recognize at a glance elemental signatures for some of the elements of interest in this project. I learn in detail the data analysis technique of Principal Component Analysis (PCA), which is a tool for processing multi-dimensional data sets. I also learned how to use Soft Integrated Modelling of Class Analogies (SIMCA), which is a classification tool based off of PCA for multi-dimensional data sets. I refined my Matlab skills and learned the basics of LabVIEW. I learned what goes into the development of a product in design, such as part tolerances and stability testing criteria. Additionally, I learned
about how the success of a project is determined by the expectations, not necessarily about the results themselves. At the end of my time on this project, I believed that there was promising results for use as a monitoring system on large engines. But the results did not match the accuracy of laboratory spectroscopy equipment nor did the system move closer towards a car unit, and therefore the project was not necessarily seen as a success at this point. Additionally, I learned how difficult communication between a client, a contractor, and a sub-contractor can be, and the subtleties that arise as a result. And throughout it all, I was impressed with the importance of the phrase “perfect is the enemy of the good enough.

B.2 Innovas

I worked less on this project than on MAST. For this project, I helped with running data collection and interpreting results. Small “smartballs” of 15 cm in diameter with temperature and optical sensors were dropped into a model heat exchange system built as part of this project. Each ball recorded the temperature and light intensity as it travelling from the cold water reservoir, through the heat exchange system that ran the cold water pipes concentrically through pipes of hot water, and then back to the cold water reservoir. The data was then downloaded wirelessly once the balls had been removed from the system. Bright lights were placed on either end of the heat exchange portion of the system to give secondary confirmation of when the ball was passing through. Though the project was at a proof of concept phase, there were subtle expectations that a marketable product would be the result.

Unfortunately, several issues made data analysis difficult. The temperature sensor reaction time was on the order of the time that the balls spent in the heat exchange system, as we discovered with outside tests. Additionally, individual data points would
be lost in the wireless download. This made matching up the optical and temperature data, which were also collected at different rates, speculative at best. A graph of the data is shown in Figure B.6. The colored vertical lines are the light intensity readings, and the black curve is the temperature data.

Figure B.6: A sample graph of typical data produced from a smartball flow trial. The solid line is the temperature reading from the smartball as it moved through the heat exchanger setup. The colored lines are the optical readings from the smartball, used to detect two illuminated points in the system before and after the heat exchanger.

As the graph shows, the increase in the temperature from the time of releasing the balls into the water flow and removing them afterwards does not match up with the time the balls actually spent inside of the heat exchanger. This was thought to be a result of the temperature sensor response time. Again, the alignment of the thermal data with the optical data is speculative, due to unknown missing data points.

The primary lesson from this project is that program management is key to the success of the project. Much of the budget was spent on building the model heat
exchanger system and developing a unique electrical board for the smartballs. However, there were alternative experiments that were both cheaper and more controlled that could have yielded more conclusive results for the proof of concept phase of this project.
Bibliography


