In situ neutron scattering study of structural transitions in fibrin networks under shear deformation

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ABSTRACT

Fibrin is the major protein component of blood clots, forming a cross-linked network of fibers and is important in the blood coagulation process. The relationship of the structure of fibrin networks to their function is crucial to understanding the processes of haemostasis (the halting of bleeding) and fibrinolysis (the breakdown of clots once damage has been repaired). However, studying the structure of these materials has been difficult due to the wide range of sizes of such structures and the high turbidity of the materials. Small angle neutron scattering (SANS) will be used to examine the structure of such fibrin clots. We will examine the origin of the strain hardening in fibrin networks by directly measuring its structural response to simple shear deformation. A special Couette shear cell is used to simultaneously probe the structural (SANS) and rheological (Rheometer) properties of a fibrin clot under a range of steady shear stresses.

1. INTRODUCTION

1.1. Fibrin networks

Biopolymer networks have mechanical properties that are remarkably different from those of most synthetic materials[1]. These properties are directly related to the internal structure of the polymer fibers and of the network. Fibrin, the major protein component of blood clots, has drawn particular interest due to its important role in blood coagulation.[2] It has also been recognized that the relationship between the structure and function of fibrin networks is critical to our understanding of important biological processes including haemostasis and fibrinolysis. Despite its paramount importance, many structural parameters of fibrin have been difficult to measure in unperturbed samples. This is due to the high turbidity of the materials and the small size of these structural features.[5] Thus, previous research has been unable to probe the structure of these materials over a wide range of length scales and protein concentrations. In this experiment, small angle neutron scattering (SANS) along with rheological measurements will be used to characterize the structure of coarse fibrin clots formed in buffer solutions.

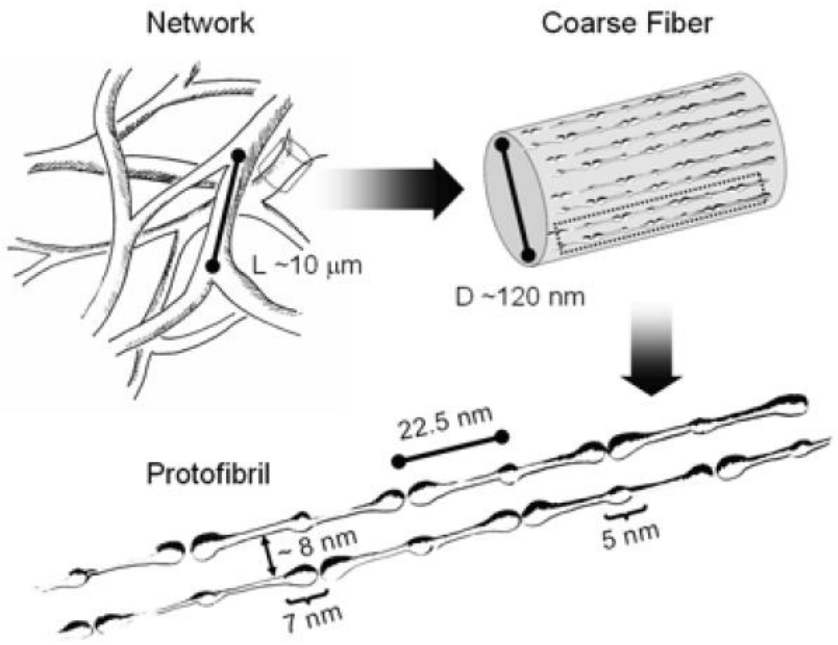

Figure 1: Schematic representation of the structural features found for coarse fibrin clots.

Figure 1 represents the three-dimensional structure of a coarse fibrin clot over length scales of 1 nm to 10μm. Fibrin clots are formed when the polymerization of fibrinogen is activated by the enzyme thrombin[2]. After activation, the proteins assemble into highly organized linear arrays called protofibrils. These protofibrils have a half-staggered linear structure with a repeat distance of half the length of the fully extended fibrinogen molecule (45 nm). Under certain solvent conditions, protofibrils can also aggregate laterally to form a larger coarse fiber.[2, 3, 4] It is also known that the inside of these coarse fibers is mostly composed of water that fills the space between the fibrinogen monomers.

"*Fibrin gels are highly elastic and exhibit strain dependent nonlinear mechanical properties that are unlike those of most synthetic polymer gels. Even at very small protein concentrations (<1 mg/mL), the fibrin network is very elastic and can effectively support haemostasis at the injury site. It is well accepted that the high elasticity and strain-hardening properties of fibrin are related to the structure of the individual proteins, the fibers, and the network. However, the origin of strain-hardening in fibrin and other biopolymers is still not fully resolved and experimental evidence directly linking structural transitions to mechanical properties could provide especially valuable information*"[6]

Furthermore, SANS coupled with rheology (Rheo-SANS) will allow us to study the unique mechanical properties of fibrin under shear deformation *in situ*.

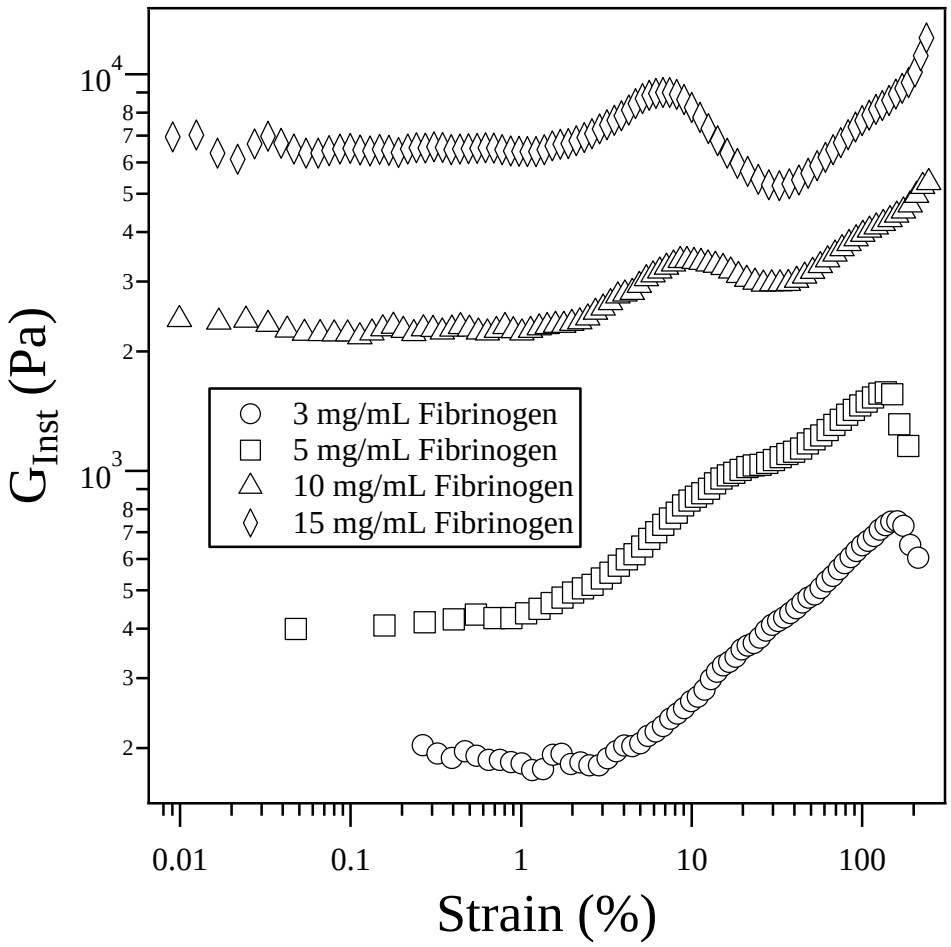

Figure 2: The instantaneous gel modulus (G_{inst}) as a function of strain for different Fibrinogen concentrations.

Figure 2 shows the strain hardening properties of different Fibrin gels. The instantaneous gel modulus is determined by taking the derivative of the stress as a function of the strain ($d\sigma/d\gamma$). In this case the stress and strain are determined from an applied shear stress ramp using a cone and plate geometry in a stress-controlled rheometer.

1.2. OBJECTIVES

Biopolymer networks represent an excellent case study in the emerging complement of techniques for SANS under shear (flow-SANS). In this study, we will attempt to measure the microstructure of a biopolymer network under strain as a function of the applied stress. Along the way, we will introduce the standard compliment of techniques available for flow-SANS at the NCNR, and use the results to demonstrate contemporary methods of data analysis for anisotropic scattering.

The model system for these measurements will be an aqueous solution of fibrinogen that gels with the addition of thrombin to form a fibrin network. The systems gels within minutes of

mixing requiring speedy and careful sample loading. While the system is well known to undergo strain hardening the microstructure under strain has until very recently been relatively unexplored.

These experiments will use Rheo-SANS measurements to investigate the microstructure of a strain hardening fibrinogen network as a function of the applied stress. In doing so, we hope to achieve a number of experimental objectives.

Determine the volume fraction of protein within the fibers. Making use of the fact that the absolute scattering cross section is obtained from the SANS experiment, the composition of the fibrin fibers will be determined given the known scattering length density of the fibrinogen and heavy water.

Determine, if possible, the fibrinogen radius and length at equilibrium. This will be accomplished by fitting an appropriate scattering model to the data at zero shear stress.

Determine whether the structure of fibrinogen is affected by an applied stress. This will be accomplished by analyzing the Q-dependent scattering using both circular and sector averaging.

Characterize the orientational distribution of fibrinogen fibrils in the scattering plane. This information can be extracted by calculating moments of the annular-averaged scattering profile.

Examine the effect of strain on the fibrinogen alignment. Parameterizing the alignment will allow for quick and efficient construction of a scattering "map" that shows how the microstructure evolves with the applied stress.

2. EXPERIMENTAL DESIGN

Successful neutron scattering experiments require a great deal of planning beyond simply how to analyze and interpret the data. Because of the complexity and time constraints involved with obtaining and using beam time at the NCNR, one must be sure to design experiments that will maximize the chances for success. This is especially true for flow-SANS measurements, which often require a significant portion of the beam time to set up before a sample is even measured.

2.1. Why SANS?

The geometries used in rheology (such as the Taylor Couette used in this experiment) require devices that can handle a wide range of torques and rotational speeds while in contact with fluids that have potentially very high viscosities, and in some cases abrasive characteristics. As such, the flow geometries are typically made from metallic materials of construction, such as aluminum and titanium (although in rare cases they can be made of quartz). This immediately prohibits the use of photon-based scattering method such as small angle light scattering and small angle x-ray scattering. Even in cases where special transparent flow geometries can be made, the types of fluids that exhibiting interesting microstructural transitions under shear are

often opaque or exhibit significant multiple scattering when using photon radiation sources. However, all of these materials are transparent to neutrons, such that existing flow devices can be easily adapted to be placed in a neutron beam, enabling SANS measurements under flow.

2.2. Scattering contrast

It is important to select the material and experimental conditions in order to make the most effective use of the beam time provided. In general, the time required for the experiments will be primarily determined by the amount of scattering from each sample, as a certain amount of scattering must be collected in order to ensure statistical accuracy of the data. As such, **it is desirable to maximize the rate at which a sample scatters neutrons** (provided, of course, that doing so does not compromise the material or the experimental objectives). This is especially important for flow-SANS measurements, as they typically require significant reduction in the size of the incident collimated neutron beam.

The **scattering intensity** is primarily influenced by two factors: the **neutron contrast** and the **concentration** (or density) of the sample. The neutron contrast, $\Delta\rho$, between a scattering object and the surrounding medium or solvent is given by

$$\Delta\rho = \rho_p - \rho_s$$

where ρ_p and ρ_s are the **scattering length density** (SLD) of the object and the solvent, respectively. The SLD for a component *i* is given by

$$\rho_i = \frac{\sum_{\{j\}} b_j}{V_i}$$

where V_i is the specific volume of component *i*, and the bound coherent scattering length for an atom or molecule *j*, b_j, is summed over all atomic or molecular components comprising component *i*.

The present experiments involve biopolymer networks of fibrinogen in aqueous solution. The SLD of the two phases (network and solvent) can be estimated assuming using the SLD calculator provided on the NCNR website. The SLD of Fibrinogen and water, as well as their deuterated counterparts, are listed in Table 1.

Table 1. Properties of the hydrogenated compounds and their deuterated counterparts

Compound	Mass density (g/cm^3)	SLD (Å^{-2} × 10^6)
Fibrinogen		3.17
H_2O	0.998	-0.56
D_2O	1.105	6.34

Table 1 suggests that the greatest neutron contrast is obtained from solutions containing hydrogenated protein and deuterated solvent, or vice versa. While deuterated protein could

probably be made D_2O is by far the less expensive of the two deuterated compounds, thus we choose to prepare our samples using hydrogenated protein in D_2O. The use of D_2O is also advantageous due to its decreased incoherent scattering compared to H_2O. However, it should be noted that the thermodynamic properties and phase behavior of many compounds (especially proteins) are sensitive to the presence of deuteration in the solvent. Fibrinogen is known to be less stable in D_2O therefore we must adjust our buffer conditions to account for this.

2.3. Sample selection and preparation

It is also important to select the samples for measurement that will best exemplify the behavior to be observed or the hypotheses to be tested. In this case, we would like to elucidate the microstructure of strain hardening Fibrinogen networks. Here, the sample we will choose to focus on primarily will be 10 mg/mL in D_2O, as this is the sample for which we can access appropriate strains (limited by rheometer motor torque) and still see the desired strain hardening behavior. We already know that this sample is strain hardening we also know from the literature that this sample will align under certain strain, which will be valuable in designing and analyzing the Rheo-SANS experiments.

The sample environments available for flow-SANS at the NCNR typically require anywhere between 3-20 mL of sample. Good preparation will involve determining the minimum sample volume required for the particular environment and flow geometry you will be using, and then bringing at least twice that amount in case a sample is compromised over the course of experiment either due to contamination, air bubbles, etc. In our case with a 10 mg/mL gel we would like to access the highest strain possible which is extremely limited by the couette geometry, for this reason we chose the smaller bob requiring only 3mL of sample, you may want this geometry especially when dealing with proteins as the tend to be expensive especially in the concentration range needed for SANS.

When to prepare the samples is also a key consideration while designing an experiment. Many samples which show interesting behavior in flow-SANS have rheology and microstructure that depend on the sample history, and so care must be taken to ensure that the sample is handled in a controlled, reproducible manner. If a sample is to be transported from another location, the effect of conditions during transport (such as temperature variations, bumps, and vibrations) must also be taken into consideration. For example, Fibrinogen must be loaded and the bob set in place within minutes before the gel forms. Therefore the sample must be mixed and immediately loaded at the beamline.

2.4. Selecting a shear environment

There are several sample environments designed for SANS measurements under shear at the NCNR. Each of these environments is primarily composed of a **Couette concentric cylinder flow cell**.

In Couette geometries (as in any rheometric flow), the flow field can be decomposed into three primary directions (Figure 3). The flow direction, also known as the 1-direction (θ-direction in cylindrical coordinates), is the direction in which the velocity or force is applied (in this case, the inner or outer wall). The gradient direction, also known as the 2-direction (r-direction in cylindrical coordinates), is the direction in which the velocity must vary in order to conserve momentum. The final direction is the vorticity direction, also known as the 3-direction (z direction in cylindrical coordinates), is the direction orthogonal to both the flow and gradient directions. For laminar flows, the velocity in the vorticity direction is zero (or nearly zero) everywhere within the flow field, and thus it is sometimes referred to as the "neutral" direction.

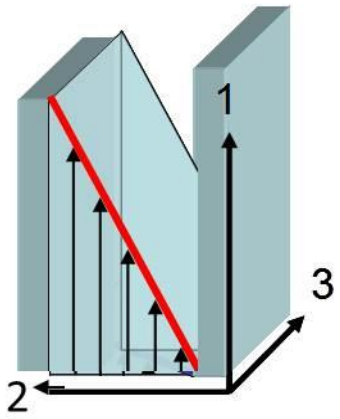

Figure 3. Illustration of the three primary directions of shear within Couette flow: (1) flow, (2) gradient, (3) vorticity. In the illustration, the left wall is moving, the right wall is stationary.

Due to the directionality of the flow field, the scattering plane probed in flow-SANS will depend on the direction of the incident beam relative to these primary directions (Figure 4). This is important because many fluids develop anisotropic microstructure under flow (i.e., structure that is not, on average, spherically symmetric), and thus will produce different two-dimensional scattering patterns depending on which configuration is chosen. For example, if the shear cell is configured such that the neutron beam is parallel to the flow direction (Figure 4a), the resulting scattering plane coincides with the gradient-vorticity (2-3) plane (this is often referred to as the "tangential" configuration). Therefore, the measured scattering will be a projection of the 3-dimensional fluid microstructure into the 2-3 plane. Similarly, if the neutron beam is parallel to the gradient direction (Figure 4b), the resulting scattering coincides with the flow-vorticity (1-3) plane (this is often referred to as the "radial" configuration). Finally, if the neutron beam is parallel to the vorticity direction (Figure 4c), the resulting scattering coincides with the flow-gradient (1-2) plane.

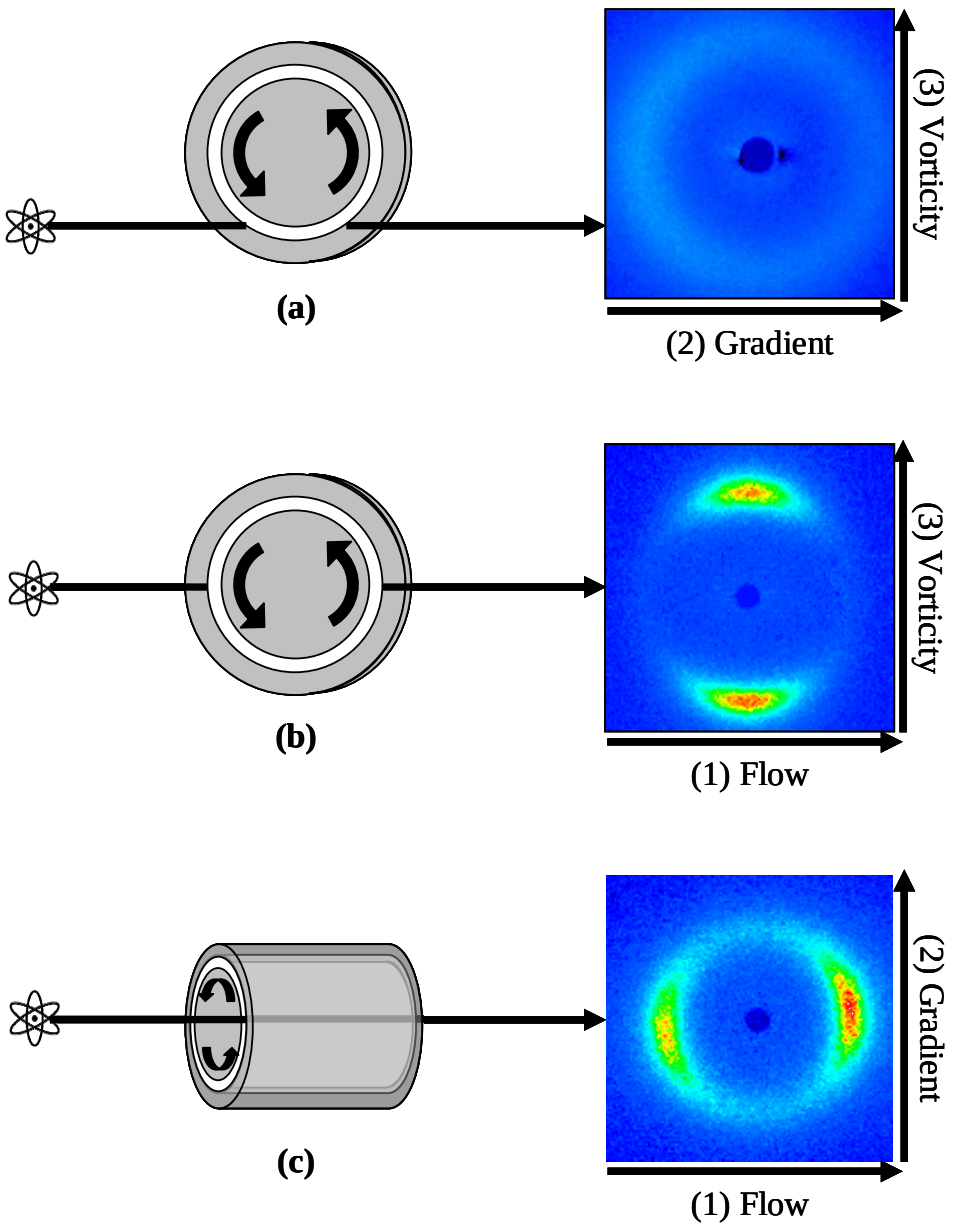

Figure 4. Beam configurations and resulting two-dimensional scattering patterns for flow-SANS measurements in the (a) gradient-vorticity (2-3) plane, (b)flow-vorticity (1-3) plane, and (c) flow-gradient (1-2) plane.

There are a few different shear environments that allow access to the different planes.

The **Rheometer** is a standard upright motor-transducer rheometer (Figure 5A) that allows for simultaneous rheological and SANS measurements (**rheo-SANS**). This is advantageous for applications where offline measurement of sample rheology is difficult (e.g. for gel or paste-like samples whose rheology is highly dependent on the sample loading). The Rheometer is equipped with a Couette cell that is specially designed for neutron scattering applications. There are both titanium and quartz bobs (inner cylinder) and cups (outer cylinder). As our gel is

extremely strong and difficult to remove we choose the much more rugged titanium cup and bob. Both the inner and outer cylinders come in various sizes to accommodate different sample volumes and maximum shear stresses/rates. These construction materials are transparent to neutrons and allow for the scattering and transmission of a sample to be measured using SANS while minimizing any signal from the sample environment. The temperature of the Couette cell can be controlled between -30 and 130 °C using the rheometer's native temperature control system. Because the Rheometer is… a rheometer, it is capable of performing a number of different shear protocols, including steady shear, oscillatory shear, and various shear transients, to name a few. The Rheometer can perform in both controlled shear rate mode up to 4800 s^{-1} and controlled shear stress mode up to 5579 Pa depending on the gap and size of the bob. In a typical configuration, the Rheometer is placed on the Huber table, allowing for horizontal translation between the radial (1-3) and tangential (2-3) configurations (see below).

The **Boulder Shear Cell (BSC)** is an upright, motor-controlled Couette cell consisting of a moving outer cylinder and a stationary inner cylinder, both constructed of quartz (Figure 3B). Both the inner and outer cylinders are available in different geometries, resulting in a number of possible gap sizes and curvatures depending on the desired sample volume and maximum shear rate. The temperature of the BSC is controlled by an external ethylene glycol bath. The strain (or strain rate) controlled motor can be operated in both steady and oscillatory shear modes. For the smaller gap configuration, shear rates of up to 3890 s^{-1} can be achieved. Although the BSC does not have the capability for online rheological measurement, it is sometimes preferable to the Rheometer, as the hardware is designed specifically to interface with the SANS data acquisition system. In a typical configuration, the BSC is placed on the Huber table, allowing for horizontal translation between the radial (1-3) and tangential (2-3) configurations (see below).

The **1-2 Shear Cell (1-2SC)** is a relatively new device and as its name implies is designed for flow-SANS measurements in the 1-2 plane of shear (see below). It consists of a horizontally-mounted, short-gap Couette cell driven by a strain-controlled stepper motor (Figure 3C). The motor drives an inner rotating cylinder constructed of aluminum, which is available in several radii for different gap sizes. The rotor is housed in an aluminum frame containing the outer cylinder as well as inset quartz windows through which the neutron beam is incident. The temperature of the cell is controlled by an external ethylene glycol bath. Although the motor is primarily intended for operation in steady shear mode (controlled shear rates up to 3500 s^{-1}), oscillatory shear capabilities are possible. In a typical configuration, the 1-2SC is placed in a mounted frame, which is then placed on top of the Huber table. The mounted frame contains a small translating stepper motor that translates a beam collimating Cadmium slit across the width of the Couette cell, allowing for spatially-resolved SANS measurements across the Couette gap (see below).

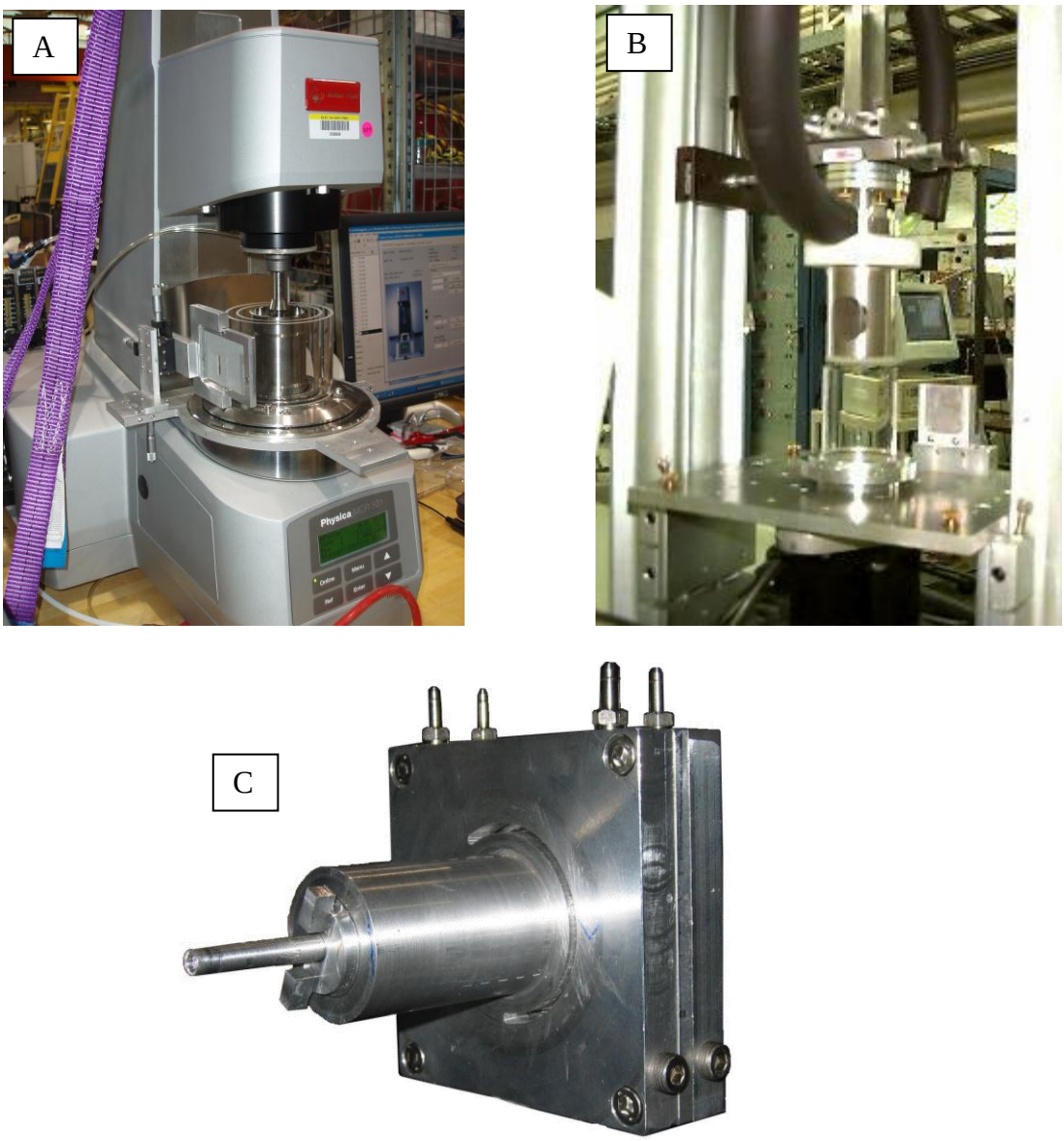

Figure 5. Photographs of the sample environments available for flow-SANS at the NCNR (A) Rheometer (B) Boulder Shear Cell (C) 1-2 Shear Cell.

The primary difference between the upright shear cells (Rheometer and BSC) and the 1-2SC is the scattering planes in which they permit flow-SANS measurements. It is clear from Figure 3 that the Rheometer and the BSC can be used for flow-SANS measurements in either the flow-vorticity or gradient-vorticity planes, whereas the 1-2SC can only be operated in the flow-gradient plane. Thus, the primary consideration in choosing between the two types of shear cells is the identification of the scattering plane that one wishes to measure the fluid microstructure in. In general, the most relevant scattering plane will depend on the particular material studied, and sometimes it is desirable to measure the scattering in all three planes of shear in order to fully characterize the anisotropic microstructure of the fluid.

In these experiments, we will use the Rheometer in the flow-vorticity plane configuration both for expedience and so that we can observe the sample rheology during measurement.

2.5. Selecting shear conditions

Once an appropriate shear cell environment is chosen, it is important to select the shear protocols and conditions that will be used for the flow-SANS measurement. These choices will affect not only the design of the measurement, but the analysis and interpretation of the data as well. A good experimental design for flow-SANS measurements will answer the following questions:

- Will the experiment be performed by controlling the applied shear stress or applied shear rate (or strain)?
- Will the experiment involve steady shear, oscillatory shear, or some other shear protocol?
- What particular shear rates or amplitudes will the SANS measurements be performed at? In what order will they be performed?

If the flow-SANS experiments are meant to elucidate the microstructural basis for an observed rheological behavior, the answers to these questions are typically dictated by previous experimental knowledge of that behavior.

In these experiments, we will perform flow-SANS measurements under steady stress. First, it is important to ensure that the sample has gelled. We will track the gelation by measuring the storage and loss moduli under very small strain 0.5%. After confirming gelation we will perform stress ramp measurements.

The experiments will be performed in order of increasing applied stress, beginning at 0.1 Pa until we reach the maximum stress limit of the rheometer (set by the 230 mNm torque limit of the motor)

3. DATA COLLECTION

3.1. Configuring the SANS instrument

A schematic of the 30-m SANS instrument is shown in Figure 6, and the instrument configuration parameters, and their allowed range, are listed in Table 2. In order to determine the instrument configurations we wish to use for the flow-SANS experiments, we will make use of the SASCALC tool available through the NCNR.

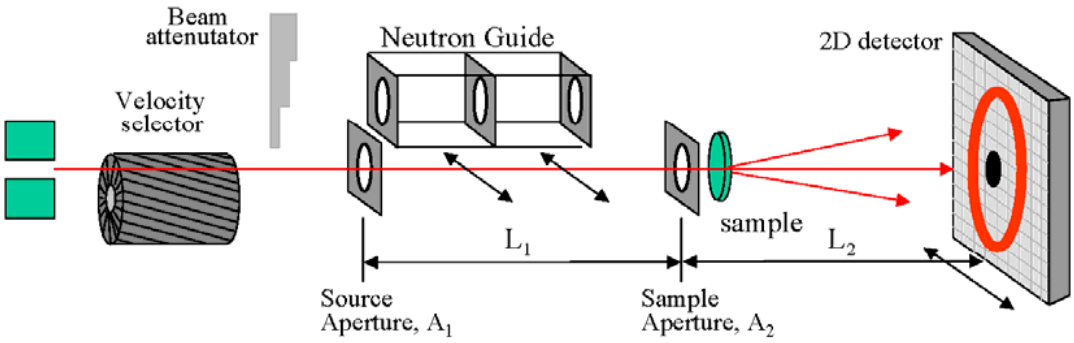

Figure 6. Schematic diagram of the components of the NCNR's 30-m SANS instruments.

Table 2. Instrument configuration parameters and their range of allowed values for the NG3 30-m SANS instrument.

Variable	**Allowed values**
Neutron wavelength	6-20 Å (determined by rotational speed of the velocity selector)
Wavelength spread (FWHM)	0.09, 0.11, or 0.22 (determined by inclination of the velocity selector)
Number of neutron guides	0-8 (determines the beam collimation by changing the distance of the source aperture from the sample)
Source aperture diameter	1.43, 2.20, or 3.81 cm for 0; 5.08 cm for 1-8 guides
Sample-to-detector distance	100-1530 cm
Detector offset	0-25 cm (detector translation perpendicular to beam to extend the Q-range covered at a given distance)
Sample aperture diameter	0-2.5cm
Beamstop diameter	2.54, 5.08, 7.62, or 10.16 cm
Beam attenuator	10 choices of beam attenuator thickness to reduce beam intensity for sample transmission measurements

There are several considerations that must be taken into account when determining instrument configurations for flow-SANS measurement. Since both the Rheometer and the 1-2SC will be placed on the Huber table, the actual sample-to-detector distance will be greater than that anticipated by the instrument configuration software. This additional distance is 55 cm for both the Rheometer and the 1-2SC, which is automatically added to the sample-to-detector distance input to SASCALC in order to obtain the true q-range.

For a given set of allowed parameters, SASCALC computes the corresponding q-range and the beam intensity (n/sec) on the sample. The q-range for a particular configuration is determined by the choice of wavelength, detector distance and detector offset. A wavelength of 6Å is customary for most SANS measurements, as it provides a large incident neutron flux, thus minimizing measurement time (longer wavelengths are typically used when very low q-values are desired). Similarly, a wavelength spread of $\Delta\lambda/\lambda$ of 0.12 provides an adequate balance of

flux and q-resolution. In general, we choose the largest number of neutron guides (allowed in the desired q-range) in order to maximize the beam intensity on the sample. The source aperture and beam stop diameters will depend on the detector distance chose.

All that remains is to select a combination of sample-to-detector distances and detector offsets that will yield the appropriate q-range for our measurements. To optimize intensity and q range, we will use SASCalc to achieved the correct q-range along with the highest count rate on the detector. The results from SASCalc are as follows:

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Source Aperture Diameter = 3.81 cm
Source to Sample = 1572 cm
Sample Aperture to Detector = 1460 cm
Beam diameter = 7.57 cm
Beamstop diameter = 3.00 inches
Minimum Q-value = 0.0027 1/Å /Q = 29.7 %)
Maximum Horizontal Q-value = 0.0197 1/Å
Maximum Vertical Q-value = 0.0197 1/Å
Maximum Q-value = 0.0279 1/Å (sigQ/Q = 5.5 %)
Beam Intensity = 281547 counts/s
Figure of Merit = 1.38e+07 Å^2/s
Attenuator transmission = 0.0232 = Atten # 4
****************** NG 7 ******************
Sample Aperture Diameter = 1.91 cm
Number of Guides = 0
Sample Chamber to Detector = 1400.0 cm
Sample Position is Huber
Detector Offset = 0.0 cm
Neutron Wavelength = 7.00 Å
Wavelength Spread, FWHM = 0.115
Sample Aperture to Sample Position = 5.00 cm
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Lenses are OUT

It is important to mention that the beam collimation for flow-SANS measurements is different than that for most other sample environments, as the beam must be confined to a small portion of the flow geometry. These additional beam collimations will affect the total count rate.

3.2. Configuring the shear environments

Both of the shear environments require additional configuration and considerations while setting up the experiment. These primarily include collimation of the beam to the appropriate size, and alignment of the beam relative to the flow field within the flow environment.

For the Rheometer, the beam is first collimated to an 18 mm × 30 mm area using a rectangular sample beam aperture on the snout prior to the Rheometer. For 1-3 plane measurements, the beam is further reduced using an additional 12 mm x 18 mm aperture or 6mm x 18 mm aperture

(depending on the size of cell used) affixed to the central diameter of the Couette cell. Alignment of the 1-3 plane is then accomplished by adjusting the tilt and translation of the rheometer alignment axis with respect to the sample aperture.

3.3. What measurements to make

In general, counts recorded by the detector with the sample in place can come from 3 sources: 1) neutrons scattered by the sample itself (the scattering we are interested in); 2) neutrons scattering from something other than the sample, but which pass through the sample; and 3) everything else, including neutrons that reach the detector without passing through the sample (stray neutrons or so-called room background) and electronic noise in the detector itself. To separate these three contributions, we need three measurements:

i) Scattering measured with the sample in place (which contains contribution from all 3 sources listed above), denoted $\boldsymbol{I_{sam}}$. This measurement will be made for each sample condition we are interested in (i.e. at a certain shear stress).
ii) Scattering measured with the empty Rheometer or BSC in place (which contains contributions from the 2nd and 3rd sources listed above), denoted $\boldsymbol{I_{emp}}$. This measurement will be made at only one shear rate (0 s^{-1}).
iii) Counts measured with a complete absorber ("blocked beam") at the sample position (which contains only the contribution from the 3rd source listed above), denoted $\boldsymbol{I_{bgd}}$. This measurement should be made only once for each flow environment with the flow cell in place (with or without sample).

In addition to these three 'scattering' measurements, the transmission (the fraction of the incident beam intensity that passes through the sample without being scattered or absorbed) of the sample and the sample cell must also be measured in order to correctly subtract the contributions to the background and to calibrate the scattering on an absolute cross section scale. The transmission is measured by inserting a calibrated attenuator in the incident beam (to reduce the direct beam intensity to an accurately measurable level) and measuring the direct beam intensity with and without the sample in the respective flow environments. The ratio of these two short measurements (typically 1-2 minutes each) is the sample (or sample cell) transmission. For Rheo-SANS measurements, it is important to measure the sample transmission at every applied shear stress that is measured. This information can be used in order to determine if the sample was compromised in any way during the course of measurement.

3.4. How long to count

A SANS experiment is an example of the type of counting experiment where the uncertainty, or more precisely the standard deviation, σ, in the number of counts recorded in time, *I(t)*, is $\sigma \sim \sqrt{I(t)}$. If the scattering is roughly evenly distributed over the SANS detector, then a good rule of thumb is that one should accumulate about 50,000 total detector counts per sample measurement. For example, if the accumulated counts are circularly averaged (see below), one obtains about 50 data points when plotting *I(q)* versus *q*. This amounts to about 1000 counts per

data point with a standard deviation of $\sqrt{1000} \sim 30$ or an uncertainty of about 3 %, which is good enough for most purposes.

A related question, is how long should the background and empty cell measurements be counted relative to the sample measurement. The same $\sigma \sim \sqrt{I(t)}$ relationship leads to the following approximate result for the optimal relative counting times

$$\frac{t_{background}}{t_{sample}} \sim \sqrt{\frac{Count\ Rate_{background}}{Count\ Rate_{sample}}}$$

Hence if the scattering from the sample is weak, the background should be counted for as long (but no longer!) as the sample scattering. However, if the sample scattering count rate is, say, 4 times greater than the background rate, the background should be counting only half as long as the sample scattering.

4. DATA REDUCTION

4.1. Data correction

Data reduction begins by correcting the measured scattering from the sample for the sources of background discussed previously, and multiplying the corrected counts by a scaling factor (to remove incidental differences between measurements such as the counting time and sample thickness) that puts the data on an absolute scale of scattering cross section per unit volume. The background-corrected neutron counts, $I_{cor}(q_x,q_y)$, recorded in a detector pixel in a time interval t are related to absolute cross section, $d\Sigma(q_x,q_y)/d\Omega$, through the expression

$$I_{cor}\left(q_x, q_y\right) = \phi\ A\ \Delta\Omega\ \varepsilon\ t\ d\ T\ \frac{d\Sigma\left(q_x, q_y\right)}{d\Omega}$$

where:

ϕ = the neutron flux (neutrons/cm2-sec) at the sample
A = the area of the beam incident on the sample
d = the sample thickness
T = the transmission of the sample (and its container, if there is one)
$\Delta\Omega$ = the solid angle subtended by one pixel of the detector
ε = the detector efficiency, and
t = the counting time.

The incidental instrumental factors can be lumped together into one constant, $K = \phi\ A\ \Delta\Omega\ \varepsilon\ t$, and the intrinsic quantity $d\Sigma(q_x,q_y)/d\Omega$, the differential scattering cross section per unit volume, is obtained by scaling the recorded counts

$$\frac{d\Sigma\left(q_x,q_y\right)}{d\Omega}=\frac{I_{cor}\left(q_x,q_y\right)}{KdT}$$

We now go over the specific steps involved in extracting $d\Sigma(q)/d\Omega$ from the raw data. The raw scattered intensity measured from the sample, I_{sam}, and the empty cell, I_{emp}, can be written as

$$I_{sam}\left(q_x,q_y\right)=KdT_{sample+cell}\left[\left(\frac{d\Sigma\left(q_x,q_y\right)}{d\Omega}\right)_{sam}+\left(\frac{d\Sigma\left(q_x,q_y\right)}{d\Omega}\right)_{emp}\right]+I_{bgd}\left(q_x,q_y\right)$$

$$I_{emp}\left(q_x,q_y\right)=KdT_{cell}\left(\frac{d\Sigma\left(q_x,q_y\right)}{d\Omega}\right)_{emp}+I_{bgd}\left(q_x,q_y\right)$$

where $T_{sample+cell}$ and T_{cell} are the measured transmission of the sample (in its respective flow environment) and the empty flow cell, respectively. From the above, the background corrected scattering, denoted I_{cor}, is given by

$$I_{cor}\left(q_x,q_y\right)=\left[I_{sam}\left(q_x,q_y\right)-I_{bgd}\left(q_x,q_y\right)\right]-\frac{T_{sample+cell}}{T_{cell}}\left[I_{emp}\left(q_x,q_y\right)-I_{bgd}\left(q_x,q_y\right)\right]$$

The corrected counts, I_{cor}, are proportional to the quantity of interest, namely the differential scattering cross section. From the above equations,

$$I_{cor}\left(q_x,q_y\right)=KdT_{sample+cell}\frac{d\Sigma\left(q_x,q_y\right)}{d\Omega}$$

The instrumental scale factor, K, will be determined from a measurement of the attenuated direct beam intensity,

$$I_{direct}=KT_{atten}$$

where T_{atten} is the transmission of a calibrated attenuator. The result of the data correction is a two-dimensional absolute, corrected scattering intensity $I_{cor}\left(q_x,q_y\right)$ as a function of the q-values (corresponding to detector pixels) q_x and q_y.

4.2. Data averaging

Once $I_{cor}(q_x,q_y)$, several types of data averaging can performed based on the desired application of the data. Visual examples of the various reduction protocols and their resulting SANS spectra for a sample two-dimensional scattering pattern are shown in Figure 7 for a sample under shear exhibiting anisotropic scattering. Circular averaging (Figure 7a) is used to compute the average, angle-independent scattered intensity, $I(q)$. This is done by averaging $I_{cor}(q_x,q_y)$ at a given radial q-value, q, over all azimuthal angles, and repeating this procedure for all values of q. In the

NCNR Data Reduction package for Igor, circular averaging is performed by selecting the "Circular" AVTYPE when building a reduction protocol. Note that this type of averaging eliminates the anisotropy from the data, which should be taken into account when analyzing the data.

Annular averaging (Figure 7b) is used to compute the angle-dependent scattering, $I(q^*,\phi)$, at a given radial q-value, q^*. This is done by constructing a centrosymmetric annulus centered at q^* with a width of Δ (in pixels). $I(q^*,\theta)$ is then computed by averaging $I_{cor}(q_x,q_y)$ on the interval $q=[q-\Delta/2,\ q+\Delta/2]$ for a particular value of θ, and then repeating the process for all values of θ. In the NCNR Data Reduction package for Igor, annular averaging is performed by selecting the "Annular" AVTYPE when building a reduction protocol, and defining the central q-value and pixel width of the annulus.

Sector averaging (Figure 7c) is used to compute the average, angle-independent scattered intensity, $I(q,\phi_{ref})$ over a given range of azimuthal angles $\Delta\theta$. This is done by averaging $I_{cor}(q_x,q_y)$ at a given radial q-value, q, over the set of azimuthal angles defined by $\Delta\theta$, and repeating this procedure for all values of q. In the NCNR Data Reduction package for Igor, circular averaging is performed by selecting the "Sector" AVTYPE when building a reduction protocol, and defining the azimuthal range $\Delta\theta$ and the central orientation angle, ϕ_{ref}. For Rheo-SANS data, is important to define ϕ_{ref} such that it corresponds to one of the principal directions of flow. For scattering in the 1-3 plane, $\phi_{ref} = 0^o$ corresponds to the flow direction, and $\phi_{ref} = 90^o$ corresponds to the vorticity direction. For scattering in the 2-3 plane, $\phi_{ref} = 0^o$ corresponds to the gradient direction, and $\phi_{ref} = 90^o$ corresponds to the vorticity direction. For scattering in the 1-2 plane, $\phi_{ref} = 0^o$ corresponds to the flow direction, and $\phi_{ref} = 90^o$ corresponds to the gradient direction.

5. DATA ANALYSIS

5.1. Characterizing the fibrin structure

The objective of this part is to characterize the fibrin structure "at rest". This will be accomplished by analyzing the circularly-averaged corrected intensity, $I(q)$.

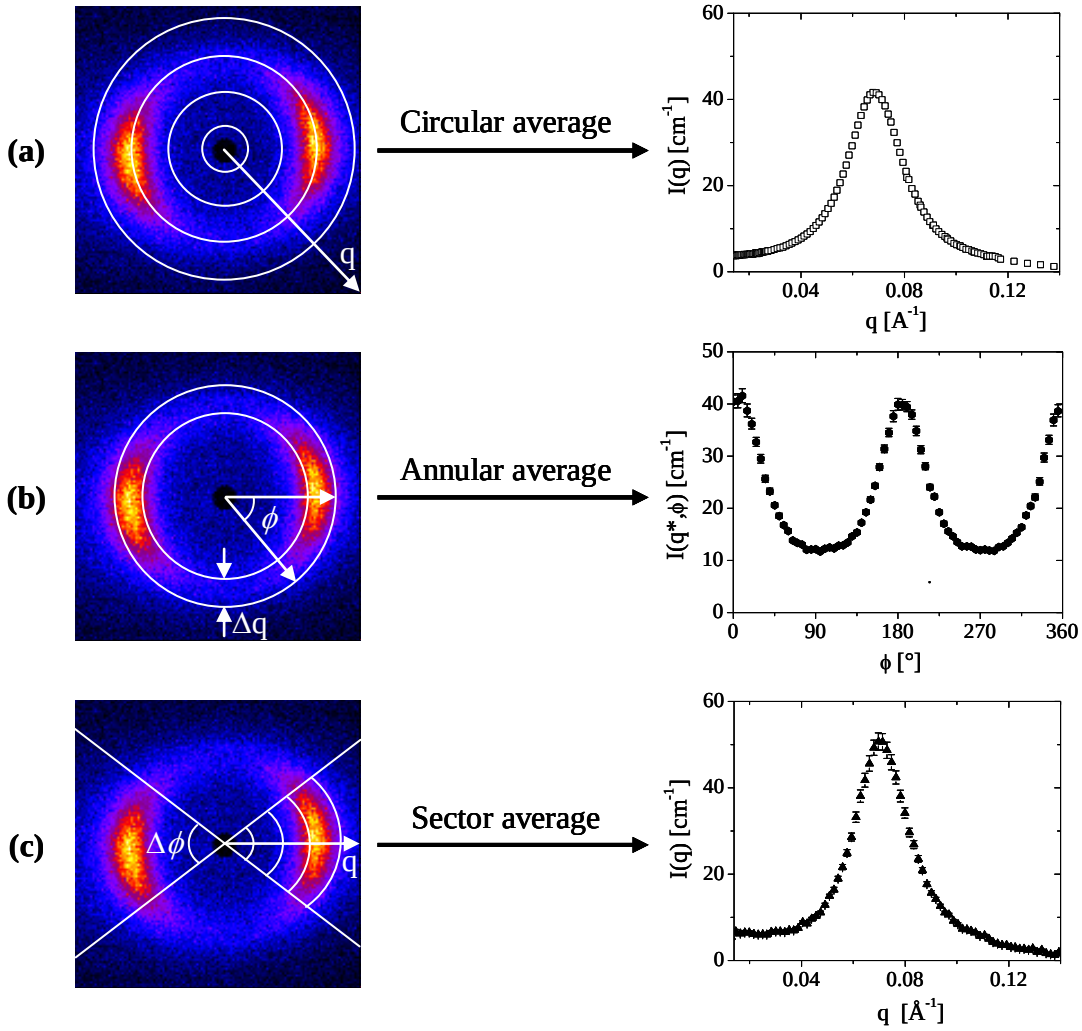

Figure 7. Schematic of SANS data reduction (left) and resulting absolute scattered intensity data (right) for (a) circular averaging, (b) annular averaging, and (c) sector averaging.

In the limit of $q \rightarrow 0$,

$$I(q) = I(0)\exp\left(\frac{q^2 R_g^{\ 2}}{3}\right) + I_{bgd}$$

where R_g is the average radius of gyration. This relationship (known as a "Guinier analysis") is typically valid for $qR_g < 1$.

Modified Guinier analysis can also be applied in the case of an elongated object such as a cylinder

$$I(q) = \frac{I_c(0)}{q} \exp\left(\frac{-q^2 R_c^{\ 2}}{2}\right) + I_{bgd}$$

where R_C is the average cross sectional radius.

- Can the radius of gyration of the fiber be resolved for these samples? The fiber length? The fiber radius?
- Can the interactions between fibers be neglected ($S(q)$=1), as in dilute systems?

5.2. Determining the effect of stress on Fibrin structure

The objective of this part will be to assess changes (if any) in the fibrin structure upon the application of stress. Scattering data will be analyzed to answer the following questions:

- Is the fibrin radius significantly changed by the application of stress?
- Which direction are the fibers primarily oriented in?
- Do the aligned fibers possess different structure compared to the isotropic fibers?

5.3. Characterizing the orientational distribution (2D fitting in SansView)[6]

In order to further quantify the alignment of the fibers it is necessary to define an orientation distribution function, F(q, ϕ). The azimuthally averaged scattering curves are best fit by a Legendre series expansion as in the paper by Burger et al. Due to the nature of even Legendre polynomials, it is necessary to apply a phase shift such that the maximum intensity of I(ϕ*) occurs at ϕ* = 0. (eqn (1))

$$F(q,\phi) = \sum_{n=0}^{\infty} a_n P_{2n}(\cos\phi^*) \tag{1}$$

where $\phi^* = \phi - (\phi_0 + \pi / 2)$, the values, a_n, are fitting coefficients and the functions, P_{2n}, are even Legendre polynomials. We use the first eight terms of the series to fit all of the angular distributions.

From F(q, ϕ) we determine the orientation parameter ($\overline{P}_2$) as a function of strain. $\overline{P}_2$ is used to quantify the degree of fiber alignment in the gel and is directly related to the a_1 coefficient from the Legendre expansion.

$$\overline{P}_2 = \frac{a_1}{5} \tag{2}$$

The value of this orientation parameter will vary between zero for random fiber distribution and one for perfectly aligned fibers. The orientation parameter is calculated for all measured strains. It is found to increase from zero when the gel is unstrained to a maximum value of ~0.35 at the largest strain (γ = 170%).

The diameter (D) of the fiber is also determined from the scattering data by fitting to a polydisperse cylinder model. Usually, for randomly oriented objects, the scattering intensity is radially averaged and fitted with a one-dimensional form factor, P(q). For aligned, or partially aligned objects, the scattering intensity, I(q), is anisotropic and is related not only the dimensions of the object, but also to the average particle orientation. In such a case, the traditional methods of analysis such as fitting to an analytical one-dimensional form factor or the use of a crosssectional. Guinier analysis for the determination of fiber radius are no longer valid. Therefore, a two-dimensional model is necessary to quantify the structure (orientation and dimension) of the fibers as a function of strain. This approach is challenging and computationally expensive but, as we will demonstrate, it can provide valuable structural information that is often inaccessible through other techniques. Here, the intensity I(q) at each detector pixel of the two-dimensional scattering profiles for each strain is fit with the 2D cylinder form factor, P(q) (eqn (3)) using SansView:

$$I(q) = \Phi \pi r^2 L(\Delta\rho)^2 P(q) + bkg \tag{3}$$

where q is the scattering vector, F is the volume fraction of fibers, r and L are the radius and length of the fibers respectively, $\Delta\rho$ is the scattering length density contrast term, and bkg is the incoherent background. P(q) accounts for the distribution of fiber orientations by averaging the cylinder form factor over the different angular distributions relative to the neutron beam.

6. REFERENCES

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