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POLARIZED SPATIAL FREQUENCY DOMAIN IMAGING OF HEART VALVE FIBER STRUCTURE

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Abstract

Our group previously introduced Polarized Spatial Frequency Domain Imaging (PSFDI), a wide-field, reflectance imaging technique which we used to empirically map fiber direction in porcine pulmonary heart valve leaflets (PHVL) without optical clearing or physical sectioning of the sample. Presented is an extended analysis of our PSFDI results using an inverse Mueller matrix model of polarized light scattering that allows additional maps of fiber orientation distribution, along with instrumentation permitting increased imaging speed for dynamic PHVL fiber measurements.

We imaged electrospun fiber phantoms with PSFDI, and then compared these measurements to SEM data collected for the same phantoms. PHVL was then imaged and compared to results of the same leaflets optically cleared and imaged with small angle light scattering (SALS). The static PHVL images showed distinct regional variance of fiber orientation distribution, matching our SALS results. We used our improved imaging speed to observe bovine tendon subjected to dynamic loading using a biaxial stretching device. Our dynamic imaging experiment showed trackable changes in the fiber microstructure of biological tissue under loading. Our new PSFDI analysis model and instrumentation allows characterization of fiber structure within heart valve tissues (as validated with SALS measurements), along with imaging of dynamic fiber remodeling. The experimental data will be used as inputs to our constitutive models of PHVL tissue to fully characterize these tissues' elastic behavior, and has immediate application in determining the mechanisms of structural and functional failure in PHVLs used as bio-prosthetic implants.

Keywords

Bio-prosthetic heart valves; cardiac tissue structure; fiber orientation mapping; polarized light imaging; structured illumination

1.) INTRODUCTION

Aortic valve disease is the most common heart disease associated with valve function, effecting more than 12% of the elderly population in developed countries¹. Of the nearly 67,000 valve replacement surgeries performed each year in the US alone, over 80% utilize bioprosthetic valves (BHVs) constructed from animal cardiac tissues². However, the longevity of these replacement valves is limited to 10–15 years before functional failure, primarily due to the constant, cyclical stress conditions to which the microstructure of BHVs is ill-adapted³. Studies suggest that collagen fiber misalignment and degeneration are the primary mechanisms of both short- and long-term valve failure^{4, 5}.

Several efforts have been attempted to predict *in vivo* performance of heart valves. Mechanical fatigue testing has long been a primary method for valve assessment, but is a destructive method that can only be used as a manufacturing control and cannot be performed on tissue which will eventually be implanted. Several imaging methods have been introduced to analyze the microstructure of heart valves. Small angle light scattering (SALS) has been developed to investigate heart valve tissue microstructure by Sacks et al, and remains a standard of analysis for our lab⁶. However, this is a transmissive imaging method, which requires optical clearing of the sample, leading to structural and chemical changes which prohibit implantation of valves imaged by this system. Additionally, it is a transmissive method, which is not capable of distinguishing the stratified valve structure without physical sectioning, and requires lengthy acquisition times (>30 minutes) for full valve imaging. Second harmonic generation (SHG) and polarization sensitive optical coherence tomography (ps-OCT) have also been used to analyze tissue structure^{7–9}. Although they provide detailed structural information, and additionally allow optical tomography up to a few millimeters into the sample, they also require lengthy imaging times, even for small (<1mm²) areas of interest. This severely limits its use to only small portions of the heart valve, and entirely restricts any sort of meaningful macroscopic functional analysis of full heart valve tissue.

Our group previously introduced Polarized Spatial Frequency Domain Imaging to address the current limitations of BHV microstructure assessment tools¹⁰. Linearly polarized light incident onto cylindrical scatterers exhibits a periodic response as the angle between the axis of polarization and the axis of the cylindrical geometry is changed, allowing us to identify the primary axes of structural orientation within tissue. The structured illumination (SFDI) component of the system allows rejection of diffusely scattered photons scattered from deeper in the tissue, providing a signal response representative of less diffuse scattering events which maintains the scattering profile of cylinders. Additionally, structured illumination allows inverse modeling of tissue optical properties and an ability to selectively limit the imaging depth. In our previous studies, we developed a proof of concept system

which detected the orientation of collagen fiber distributions within bovine tendon and porcine heart valve leaflets ex vivo. This present work expands upon both the rapid imaging capability of the wide-field system, as well as introduces an additional metric related to the anisotropy of the collagen fiber distribution.

2.) METHODS & INSTRUMENTATION

2.1. Polarized light theoretical model

To describe the back-scattered polarized light from the sample, we use a Mueller matrix formalism first described by Chenault and Chipman¹¹. Their model describes a linear rotating polarizer to investigate the optical anisotropies (diattenuation and retardance) in a sample. In our samples, the optical anisotropy stems from the structural anisotropy of collagen fibers. We therefore attribute the optical anisotropy within the sample to the microstructure of the collagen within. This model tracks the Stokes vector of light polarization based on all of the polarizing components in our system, including the instrumentation and the sample, in the following form:

$$\vec{S}_{out} = M_{sys} \vec{S}_{in} \quad (1)$$

Where S_{in} is the input Stokes vector of the illumination source, M_{sys} systems Mueller matrix, and S_{out} is the detected light and its polarization components. S_{in} and S_{out} are 4×1 vectors which fully describe the polarization of light as follows:

$$\vec{S} = \begin{pmatrix} I \\ Q \\ U \\ V \end{pmatrix} \quad (2)$$

Where I is the total intensity of the electric field, Q is the horizontal component of the field, U is the vertical component, and V is the circular component of a rotating electric field. For our purposes, we are illuminating using non-polarized light, so $S_{in} = I_{in} = 1$. This will reduce the mathematical complexity of our model moving forward.

Our system is composed of a rotating linear polarizer placed before and after the sample, and the sample itself. In Mueller matrix form, our system (M_{sys}) is a sum of the following:

$$M_{sys} = M_{pol} R_2 M_s(\mathcal{D}, \delta) R_1 M_{pol} \quad (3)$$

M_{pol} is the Mueller matrix for the polarizer, R_1 and R_2 are rotational matrices for the polarizer, and M_s is the Mueller matrix for the sample, expanded here:

$$M_{pol} = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad (4)$$

$$R_1 = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(2\theta) & -\sin(2\theta) & 0 \\ 0 & \sin(2\theta) & \cos(2\theta) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \quad (5)$$

$$R_2 = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(2\theta) & \sin(2\theta) & 0 \\ 0 & -\sin(2\theta) & -\cos(2\theta) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \quad (6)$$

$$M_s = \begin{pmatrix} 1 & \mathcal{D} & 0 & 0 \\ \mathcal{D} & 1 & 0 & 0 \\ 0 & 0 & \sqrt{1-\mathcal{D}^2}\cos(\delta) & \sqrt{1-\mathcal{D}^2}\sin(\delta) \\ 0 & 0 & -\sqrt{1-\mathcal{D}^2}\sin(\delta) & \sqrt{1-\mathcal{D}^2}\cos(\delta) \end{pmatrix} \quad (7)$$

In these equations, $\mathcal{D}$ and δ are the diattenuation and retardance of the sample, and θ is the angle of the polarizer. The Mueller matrix of the polarizer is identical because we are illuminating and imaging through the same polarizer, and the rotational matrix is inverted to account for the reflection of coordinates at the sample. As our input Stokes vector is unpolarized light, and we are only detecting unpolarized light at our camera, we can combine all of these equations and solve for the output intensity (I_{out}) detected by our camera:

$$I_{out}(\theta, \mathcal{D}, \delta) = \tau_{sys} \left[1 + \frac{1}{2} \left(1 + \sqrt{1-\mathcal{D}^2} \cos(\delta) \right) + \mathcal{D} \cos(2\theta) + \frac{1}{2} \left(1 - \sqrt{1-\mathcal{D}^2} \cos(\delta) \right) \cos(4\theta) \right] \quad (8)$$

This model is simplified by using coefficients of a simple Fourier series with three fitting coefficients:

$$I_{out}(\theta, a_0, a_2, a_4) = \tau_{sys} [a_0 + a_2 \cos(2\theta) + a_4 \cos(4\theta)] \quad (9)$$

Where

$$\mathcal{D} = \frac{a_2}{a_0 + a_4} \quad (10)$$

$$\delta = \cos^{-1} \left\{ \frac{a_0 - 3a_4}{[(a_0 + a_4)^2 - a_2^2]^{\frac{1}{2}}} \right\} \quad (11)$$

One point to note is that in its present form, θ is the angle of the polarizer and assumes that the sample anisotropy is aligned along $\theta = 0^\circ$. Therefore, we can modify this for any orientation of sample anisotropy in our final polarization equation:

$$I(\theta, \mathcal{D}, \delta) = \tau_{sys} [a_0 + a_2 \cos(2(\theta_p \pm \theta_s)) + a_4 \cos(4(\theta_p \pm \theta_s))] \quad (12)$$

Where θ_p is the polarizer orientation and θ_s is the sample orientation. We use this model to fit to our imaging data. We can identify the primary axis of anisotropy by finding the peak of the curve at $\theta_p - \theta_s = 0$, thus identifying the primary orientation of the fibers within the sample area.

Our last step is to determine the amount of structural anisotropy detected by our signal, which we call our Degree of Anisotropy (DOA).

$$DOA = 1 - \frac{a_0}{a_0 + a_2 + a_4} \quad (13)$$

In addition to giving us information about the optical anisotropies, this metric is decoupled from the optical properties themselves by normalizing it to the total average reflectance of the sample. A manuscript regarding this metric and its derivation in detail is currently in preparation for peer review, but we adapt it here for our processing purposes.

2.2. Spatial frequency domain model

We use the standard demodulation technique for the spatial frequency domain images, originally developed by Cuccia et al¹². A spatial pattern with frequency f is projected onto the sample, and an image is collected. The pattern is imaged at three different phases of the spatial frequency: 0° , 120° , and 240° . These images are demodulated to their respective DC and AC components:

$$I_{DC} = \frac{I_{0^\circ} + I_{120^\circ} + I_{240^\circ}}{3} \quad (14)$$

$$I_{AC} = \frac{\sqrt{2}}{3} \sqrt{(I_{0^\circ} - I_{120^\circ})^2 + (I_{120^\circ} + I_{240^\circ})^2 + (I_{240^\circ} + I_{0^\circ})^2} \quad (15)$$

The DC component image captures all of the diffuse reflectance from the sample, including highly diffuse photons which have traveled deeper within the sample. The AC component image rejects the majority of the highly diffuse photons, and instead is composed of only those which have scattered relatively fewer times near the surface of the sample. This effort is twofold: we limit the influence of multiply scattered photons from deeper within the sample, allowing control of the imaging depth for samples which have layer-dependent structure, and we also limit our measurements to photons which have been scattered fewer times and therefore more closely reflect our polarization model. The polarization image processing described previously is applied to both the AC and DC component images after demodulation.

2.3. PSFDI instrumentation

A new PSFDI system has been constructed to allow higher speed acquisition than was previously accomplished. The illumination system consists of a 490nm LED and a condenser lens system. A spatial frequency projection mask is printed onto a 35mm film, which is placed directly behind the condenser lenses. The film is mounted onto a linear stage which can translate the film along the axis of its spatial pattern to shift the phase of the pattern. A projection lens projects the image of the film onto the sample through a linear polarizer with a 2 inch aperture. The polarizer is mounted on a Zaber high speed rotational stage, which has a maximum rotational speed of 300 RPM. A Basler acA1300-60gm camera images the sample with a f/1.4 imaging lens through the same rotational stage, offset from the optical axis of the projection system by approximately 10°. The camera has a resolution of 1.3MP, and a total field of view of 32mm X 38mm, and the exposure time is set to 10ms. The entire system is run controlled through a custom Labview VI. The camera captures a sequence of frames as the polarizer rotates continuously and the projection mask is static. A total of fifteen frames are captured over the entire 180° rotation of the stage, at which point the spatial frequency is shifted by 120° and a second set of fifteen images is captured (this is repeated again for the third spatial frequency phase). The entire set of 45 images is acquired in 12 seconds.

2.4. Electrospun fiber phantom validation

To validate the system, we fabricated structural phantoms made from electrospun polymer fibers. The process for achieving specific fiber morphology and alignment has been described in detail in many instances^{13–15}. A grounded aluminum mandrel with a 2inch diameter is rotated at high velocities. A syringe with polycaprolactone gel (PCL) is kept at a high voltage (7kV) while the plunger is depressed, ejecting high voltage polymer fiber which is then attracted by strong static force to the mandrel. The mandrel collects the PCL, and the speed of the mandrel rotation determines the strength of structural anisotropy in the fibers – low speeds result in randomly oriented fibers, and higher speeds result in highly aligned fibers. Samples were prepared with the mandrel at velocities ranging from 0 RPM

and 3000 RPM. The samples were also imaged using Scanning Electron Microscopy, and the distribution of fiber diameter and orientation was quantified using image processing¹⁶. The same samples were also imaged using our custom PSFDI system and processed in Matlab to measure the degree of anisotropy.

2.5. Porcine heart valve leaflet imaging with PSFDI and SALS

Porcine heart valve leaflet tissue was imaged with the PSFDI system, and then subsequently prepared for Small Angle Light Scattering (SALS) analysis, which has been previously described as a separate method for determining major fiber orientation and an orientation index related to the strength of structural anisotropy⁶. A 5mW He-Ne laser at 632.8nm is directed onto the target, and the beam transmitted through the sample is projected onto a screen. This screen is imaged by a camera, and the digitized beam profile is analyzed to describe the fiber orientation quantitatively. The samples were prepared in set of four solutions of glycerol in 1x phosphate buffered saline (PBS), with glycerol concentrations ranging from 50–100%. Because the samples are translucent, the results are an average measurement through the entire thickness of the sample at each point. The leaflets were then placed between two glass slides and scanned by the system. The beam width, which approximately represents the full final pixel size, was 250 μ m, and was raster scanned at 250 μ m steps. The entire SALS data collection took approximately 30 minutes to cover the entire sample.

2.6. Dynamic realignment imaging of bovine tendon

Several biomechanical studies of structural tissues have been performed by our lab associates^{17–21}. These can be used to measure the elastic moduli of the sample based on stress strain curves. The sample is attached by small metal hooks to two axes of a stretching device, and one or both of the axes is extended and retracted to induce axial strain. Load cells in the motor arms measure the subsequent stress, and from this functional relationship, mechanical elasticity can be computed. In our experiment, we used bovine tendon which was attached on four sides to the motor arms. Each arm was retracted until the suture wire was taught. The sample was oriented such that the hooks were pulling 45° from the axis of the collagen fibers. The sample was then subjected to strain in a single axis, with the other axis held constant. Steps of 50 μ m were taken, and in between each step, the PSFDI system took full measurements. This was repeated in identical intervals up to at least 200mm total. The stress/strain curves were not recorded for the purpose of this preliminary experiment.

3.) RESULTS

3.1. Electrospun fiber phantom validation

Our fabricated electrospun fiber phantoms exhibited obvious preferential alignment as the rotational speed of the mandrel was increased. The image analysis showed that the fibers had an average diameter of 0.6–1.0 μ m, which was within the range of our modeled fiber size. Figure 2 details our results. The SEM images had a qualitatively clear difference in fiber distribution as well. The quantitative distribution of fiber orientation quantified to show a major peak in anisotropy for the fiber phantom fabricated at high speed, with a random distribution apparent for the fiber phantom fabricated at lower speed. The PSFDI data

showed almost no anisotropy in the 0RPM sample, while the 2200RPM sample showed a degree of anisotropy metric as high as 0.3, indicating well aligned fibers. A study investigating intervals of mandrel speeds will be conducted to further establish the ability to fabricate phantoms with controlled orientation distributions and preferential alignment.

3.2. Porcine heart valve leaflets: PSFDI vs. SALS

The AC image of the porcine heart valve leaflet showed much stronger contrast as compared to the raw DC image, particularly in the superficial upper layers. In the AC image, the leaflet appears less transparent, likely due to the rejection of the highly diffusive photons which may travel through the entire thickness of the sample. In the microstructural analysis, the leaflet showed a regional variation in both the orientation and degree of anisotropy of its structure, to correlating to the directions and alignments of the collagen fibers within the tissue. The SALS data showed similar results, although at a lower resolution. The top of the leaflet wall showed the highest degrees of anisotropy in both imaging modalities, indicating a structural buffer where the leaflet is attached to the heart wall and experiences high degrees of flexural stress. Throughout the rest of the leaflet, the collagen fibers seemed to be oriented circumferentially to the shape of the deployed leaflet, which is due to the surface pressure applied to the closed valve. These results are consistent with previous analysis of heart valve tissues^{6, 10, 17, 22}.

3.3. Dynamic fiber realignment in bovine tendon

The PSFDI images of bovine tendon showed that the tendon initially had a moderately high degree of fiber orientation, all oriented in the same direction along the long axis of the fiber. As the fiber was stretched, the fibers reoriented in the direction of the motion. Initially, this somewhat weakened the fiber anisotropy, but as the stretch became pronounced, the anisotropy also increased. However, the results were somewhat obscured by a surface reflection on the sample, and a subsequent imaging study will need to be performed to see more detail.

4.) DISCUSSION

The electrospun fiber phantoms showed a relatively uniform degree of anisotropy across the entire sample, with some regions being obscured by folds in the phantom material. An increasing degree of anisotropy was apparent as the fiber phantom was prepared with a high rotational speed. This also agreed with the decrease in orientation standard deviation of the analyzed SEM images as a function of increasing mandrel rotational speed, both features indicating a more highly aligned fiber structure. Although the degree of anisotropy metric is useful in correlating absolute anisotropy, further work is needed to translate this metric into an orientation standard distribution to fully describe the fiber distribution.

In comparison to the SALS imaging method, we saw that PSFDI was able to produce qualitatively similar maps of both major fiber orientation. Additionally, the color maps for areas of high alignment (described as degree of anisotropy and orientation index for the PSFDI and SALS images, respectively) showed good correlation, although it should be noted that only the relative value, and not the scalar values, were comparable. Of important

note was the visible increase in resolution, which was obtained more than two orders of magnitude faster than the SALS data. The samples also did not require any special preparation, unlike the glycerol treatment required to optically clear the samples for SALS imaging. Overall, the PSFDI system showed complementary information at much higher speeds and resolutions with much lower influence on the native tissue structure.

The dynamic tissue imaging showed an ability to track changes in both the fiber orientation and degree of alignment as the tissue responded to induced strain. The fibers preferentially realigned with the axis of strain to counteract the force, and additionally the degree of anisotropy increased, both likely physiological attempts to provide stiffer resistance to the strain. Additionally, the degree of anisotropy metric remains relatively stable over short intervals. This means that it could be used as a local registration for pixel-resolution strain mapping in the tissue. However, the imaging system needs to still be improved to be able to detect viscoelastic behaviors, including those related to stress-relaxation and creep.

Compared to previous PSFDI instrumentation, our current iteration has the capacity for much higher rates of imaging. Most SFDI systems use a digital micromirror device (DMD) to project the spatial frequency pattern onto the sample. While this method allows high speed switching and easy electronic control of any spatial frequency desired, it has a major impact on integration time. Because each of the mirrors in the DMD only has a 1 (on) or 0 (off) state, to create a grayscale pattern, the mirrors flicker on and off at different frequencies, which over extended integration times result in a smooth pattern. However, high speed imaging requires reduced exposure times, at which point the frequency of the mirrors is too slow to produce a continuous, smooth grayscale. Alternate methods, including binary square wave projection and more complicated spatial patterns, have been presented as options for increasing acquisition speed, which we would also like to explore for use in the future^{23, 24}. However, our current method of using a projection film mask results in instantaneous pattern projection, and has the capacity to allow sub-second acquisition times once the system has been fully optimized by installing hardware triggers, rather than the current software triggers, which are slower. The spatial pattern projection does still represent the main imaging bottleneck in our system due to the time needed for shifting of the spatial frequency phase. However, this lateral shift is very small, and easily achievable by a variety of small-scale piezoelectric translational stages.

5.) CONCLUSIONS

PSFDI has the capability of detecting tissue microstructure on a macroscopic scale. We demonstrate its ability to determine fiber orientation and degree of anisotropy in acquisition times several orders of magnitude faster than comparable imaging modalities, and show promise for even further improvement. We validated our measurements using an electrospun fiber phantom with controlled orientation distributions. The results of PSFDI analysis showed that it was comparable to SALS measurements with much faster acquisition time, higher resolution, and similar contrast in both fiber principal orientation and anisotropy. Finally, we demonstrated dynamic imaging of the microstructure in bovine tendon undergoing mechanical stretching, showing reorientation and fiber recruitment to resist induced strain. These results provide a sound basis for which to build an even higher speed

imaging system on the order of single second acquisition times. Future studies will further optimize the hardware for sub-second image acquisition. The ultimate goal of this technology is for use in analyzing bioprosthetic heart valve tissue undergoing dynamic mechanical analysis and predict functional failure of the valve based on microstructural features, allowing longer lasting prosthetic heart valves.

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Figure 1.
PSFDI System Schematic.

Figure 2. Left column: SEM Images of electrospun fiber mats at 0 and 2200 RPM. Middle Column: Fiber distributions obtained through image processing of SEM data. Right Column: Degree of Anisotropy maps obtained through PSFDI processing