Polarized Light Imaging for Assessment of Anisotropy in Turbid Media: Instrumentation and Application in Urology

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Abstract

Assessment of anisotropy has many applications in tissue engineering and early detection of disease such as cancer or stroke. One of the emerging optical technologies for quantifying anisotropy in biological tissues is polarized light imaging. In this technique, the full Mueller matrix of the tissue is measured and then decomposed to yield optical retardance, which is proportional to the tissue anisotropy. The theory of polarized light imaging, its biomedical application and instrumentation are the subject of this thesis.

To retrieve more information from the Mueller matrix, a quantitative analysis of generic turbid media’s Mueller matrices beyond their decompositions was proposed. Two new metrics based on Mueller matrix were established in this thesis: 1) Tissue depolarization, calculated from the Mueller matrix, can be used to estimate its optical properties, and 2) Symmetry of the off-diagonal elements of a turbid medium’s Mueller matrix can be used to detect the axial heterogeneity of anisotropy.
Polarized light imaging, followed by Mueller matrix decomposition, was then successfully applied to locate the structural disorders induced by bladder outlet obstruction disease, in *ex vivo* functioning rat bladders. Motivated by the result of this study and to enable *in vivo* polarized light measurements of the bladder, a novel thin fiber based polarimetric probe which can measure the full Mueller matrix of the turbid media was suggested.

Finally, to enhance the speed of the *ex vivo* examinations of biological tissues and avoid artifacts, a new rapid benchtop polarized light imaging system based on photoelastic modulators (PEM) and a charge-coupled device (CCD) was proposed and implemented. The demonstrated scheme does not involve using mechanically moving components and thus reduces systematic errors. This imaging system proved to be the fastest high resolution polarimetric characterization tool for imaging turbid media to date.
To my parents,
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Structure of the thesis and list of resulting publications

The outcome of this project has been published in 6 journal articles, and the thesis structure is based on these peer-reviewed papers. The first chapter is an introduction to polarized light imaging for measuring anisotropy in turbid media. This introduction chapter also provides the motivation for the three main chapters in the thesis. Each main chapter includes 2 papers, with a preamble section (and a possible addendum), for each. Chapter 2 is focused on the theory and the quantitative analysis in polarized light imaging. Chapter 3 presents our studies on investigating the use of polarized light imaging as a diagnostic tool in urology. Chapter 4 introduces a new design and implementation of rapid polarized light imaging system. The final chapter summarizes the results and outlines the future work. One of the appendices explains a pre-established calculation method in polarimetry, and the other one describes our efforts to demonstrate a fiber-based polarimetric probe.

Publications appearing in this thesis:


Additional publication arising from this thesis:

1. Introduction

The goal of this thesis was to investigate the potential of polarimetry as a characterization technique in biomedical applications. In the first part of the introduction, I elaborate on the nature of the anisotropy in biological tissues and the importance of quantifying it. Then, the existing methods for quantifying anisotropy, among them polarimetry, are briefly reviewed. The final part of the introduction is dedicated to discussing the motivations of the thesis, which are: 1) To investigate polarimetric measurements from a theoretical point-of-view, to improve the interpretation and to derive biologically relevant parameters; 2) To develop an imaging modality for characterizing the structural disorders induced in the bladder outlet obstruction disease, and 3) To implement rapid polarimetric instruments.

1-1. Biological tissues with anisotropic structure

Anisotropy is a term used to describe structures with unequal physical properties along different directions. Qualitatively speaking, for the same thickness of tissue, a stronger anisotropy means stronger alignment of cells and fibers. There are two types of anisotropy in biology [1-3]: 1) Intrinsic anisotropy which arises from the asymmetric molecular structure. For instance, haemoglobin and collagen are anisotropic molecules; and 2) Form anisotropy that is due to the structural alignment of the cells and the extracellular fibers. For example, the radial arrangement of the nerve fibers on the retina causes a form anisotropy. In this thesis, we are characterizing the form anisotropy as a morphological property of the biological tissues. Particularly, we are using quantitative anisotropy imaging as a tool to identify morphological disorders in the bladder and introducing polarimetry as a potential imaging modality in urology.
Anisotropy is stronger in organs that perform a certain biomechanical function. For example, anisotropy plays an important role in the physiology of the bladder, heart, cartilage, tendon, skeletal muscle, retina, and skin [1-9]. Knowing the anisotropy values of these tissues during biomechanical cycles is valuable for tissue engineers who are interested in fabricating and designing scaffolds for artificial organs with optimum functionality. As such, a great amount of recent research has been focused on modeling and understanding the tissue anisotropy in heart, bladder and cartilage [10-17]. Tissue structural organization can also change in the early stages of many diseases such as cancer [18-20]. Thus studying and quantifying anisotropy may provide new tools and criteria for early diagnosis of the disease or better monitoring for drug development procedures.

To assess the micro-structure of the tissue, usually thin slices are obtained and visualized with microscopy after staining. Other types of microscopy such as scanning electron microscopy may also be used for similar evaluation [6-9,21-22]. Most of these microscopic techniques are limited to studying in vitro tissues. Therefore, they barely provide any insight into the three dimensional correlation of a tissue anisotropy and its function. Non-invasive in vivo assessment of anisotropy is usually carried out without direct visualization of tissues’ constituents and in terms of measuring their mechanical properties (elasticity, diffusion) [23-28], electrical properties (conductivity, permittivity) [29-30] or optical properties (nonlinearity, birefringence) [1-3,31-32].

One of the clinically available tools for studying the anisotropic behaviour of tissues is shear wave ultrasound which has been applied to muscle and breast [23-24]. This technology can measure the elasticity in different directions with high temporal and spatial resolution. Another clinical modality is diffusion MRI which measures the rate of water diffusion in tissues in three
dimensions. Diffusion MRI has been used to characterize anisotropic features such as the fiber orientations in tissues such as white matter, heart and rectum [25-27].

Recently, endoscopic and laparoscopic nonlinear microscopy have been developed and were used to examine the three dimensional structure of brain and oral cavity in in vivo rat models [31-33]. Multiphoton excited fluorescence (MPEF) can be used to visualize the structural components with fluorescence properties such as collagen and elastin. The higher harmonic (second and third) generation processes rely on the asymmetric properties of the tissue components such as collagen fibers in the extracellular matrix. Nonlinear microscopy techniques are depth resolved imaging methods, since the signal is generated with multiple photons impinging on a limited volume. These methods may be clinically adoptable when they are optimized in terms of imaging speed.

Another optical method is polarization sensitive optical coherence tomography which is a clinically established technology for evaluating depth resolved birefringence (proportional to anisotropy) and other morphological properties in the retina [34-35]. This technique has been used in many in vivo animal studies including early detection of bladder tumors [36]. However, optical coherence tomography is a relatively complex and expensive technology.

In this thesis, we focus on the potential of polarized light imaging as an independent tool to quantify the effective birefringence in tissues.

1-2. Polarized light and birefringence in non-turbid media

Polarization of light is important in all light/matter interactions and changes the outcome of phenomena such as reflection, refraction and scattering [37-38]. Polarization has been leveraged
for designing optical filters, implementing sensing tools, particle trapping, data coding in communication and many other applications [35,39-41]. Sir Isaac Newton coined the term polarization for light using particle theory, when trying to explain the double refraction from a calcite which was noticed earlier by Bartholinus [42]. The first complete explanation of polarization and light propagation as a dynamic electromagnetic wave was given by Maxwell in 1864 [43]. Quantum mechanics theory offered a different definition of the polarization of light based on the photons spins [44]. However, it has been shown that the algebraic tools defined in classic electromagnetic theory for polarized light can be applied successfully, and independently, in quantum theory as well [45]. In this thesis we work with the classical definition of polarized light.

Classically, light is an electromagnetic wave and its propagation governed by Maxwell’s equations. A simple monochromatic plane wave, propagating along the z axis of an isotropic nonscattering medium with refractive index n can be described as follow [46]:

\[
\vec{E} = \text{Re}\left( (E_x e^{-j\phi_x} + E_y e^{-j\phi_y}) e^{j\omega t - jkz} \right) \quad (1-1)
\]

where k is the propagation constant \(2\pi n/\lambda\), \(\lambda\) is the wavelength, \(\omega\) is the angular frequency, \(E_x\) and \(E_y\) are the amplitudes of the electric field and \(\phi_x\) and \(\phi_y\) are the phases of the electric field along the x and y axis in the Cartesian coordinates. Polarization of an electric field is the field’s evolution in time and space. The polarization of the plane wave, in Eq.1-1, can be described by Jones vector as [37]:

\[
J = \begin{bmatrix} E_x e^{j\phi_x} \\ E_y e^{j\phi_y} \end{bmatrix} \quad (1-2)
\]
As illustrated in Fig.1-1, as time passes by, a circularly polarized electric field sweeps a circular pattern in space and a linearly polarized electric field sweeps a linear pattern.

![Fig.1-1. Polarized light propagation in space. The spatial traces of the electric field for a linear polarized light (on the left), and a circular polarized light (on the right) are shown.](image)

The polarization of such a plane wave remains the same during propagation in an isotropic clear medium. An anisotropic medium is birefringent and exhibits different refractive indices along the orthogonal axes. When a plane wave propagates in a birefringent medium, the two orthogonal components of the electric field will travel with different speeds. This will result in an accumulative phase difference between them, known as optical retardance $\delta$. For a propagation distance of $d$, the optical retardance is equal to [37]:

$$\delta = \frac{2\pi}{\lambda} \Delta nd = \frac{2\pi}{\lambda} (n_x - n_y) d \quad \text{(1-3)}$$

$\Delta n$ is the birefringence, which is defined as the difference of refractive index along the two orthogonal directions in the plane perpendicular to the propagation direction ($n_x$ and $n_y$). The
optical phase retardance leads to a change in the Jones vector (Eq.1-2) or the polarization of light as it propagates.

The refractive index of a biaxial anisotropic medium varies in space according to the refractive index ellipsoid [47]:

\[
\frac{x^2}{n_x^2} + \frac{y^2}{n_y^2} + \frac{z^2}{n_z^2} = 1 \quad (1-4)
\]

where x, y, and z are the axes in the Cartesian coordinates, and \(n_x, n_y, \) and \(n_z\) are the refractive indices along those axes, respectively. A uniaxially birefringent medium have equal refractive indices of \(n_o\) along two axes (ordinary axes) and a different refractive index \(n_e\) along the other axis (extraordinary axis). Biological tissues are usually modelled as multiple layers of uniaxial material with the extraordinary axis being in the imaging plane. If \(\zeta\) is the angle between the propagation direction and the extraordinary axis (as shown in Fig.1-2), then one component of the electric field will experience refractive index of \(n_o\). The orthogonal component (along the projected direction of extraordinary axis on the perpendicular plane to the propagation direction which intersects the ellipsoid) will experience a refractive index of \(n(\zeta)\) calculated from [47]:

\[
\frac{1}{n^2(\zeta)} = \frac{\cos^2 \zeta}{n_o^2} + \frac{\sin^2 \zeta}{n_e^2} \quad (1-5)
\]
Fig.1-2. The refractive index ellipsoid. Here, the Z axis is the extraordinary axis (optical axis) with refractive index of $n_e$. X and Y are the ordinary axes with refractive index of $n_o$. The red solid arrow shows the propagation direction of an exemplary plane wave.

The Jones formalism relies on 2 parameters ($E_x/E_y, \phi_x-\phi_y$); therefore, assumes a 100% polarization when it is used to describe the polarization of a beam of light. In reality, a beam of light is composed of many plane waves which are not coherent [46]. Hence, a beam of light is not fully polarized and cannot be expressed by Jones formalism. To deal with such beams, it is common to use Stokes and Mueller formalism [37-38]. A Stokes vector can completely represent any polarized or unpolarized beam with 4 parameters as below:

$$
S = \begin{bmatrix} I \\ Q \\ U \\ V \end{bmatrix}
$$

(1-6)
where the components $I$, $Q$, $U$ and $V$ can be written in terms of the electric field in Eq.1-1 as:

$$
\begin{align*}
I &= E_x^2 + E_y^2 \\
Q &= E_x^2 - E_y^2 \\
U &= 2E_xE_y\cos(\varphi_x - \varphi_y) \\
V &= 2E_xE_y\sin(\varphi_x - \varphi_y)
\end{align*}
$$

(1-7)

To measure the Stokes vector experimentally, it is easier to rewrite (Eq.1-7) in terms of the intensities as:

$$
\begin{bmatrix}
I \\
Q \\
U \\
V
\end{bmatrix} = \begin{bmatrix}
I \\
I_0 - I_{90} \\
I_{45} - I_{-45} \\
I_R - I_L
\end{bmatrix}
$$

(1-8)

where $I$ is the intensity of the light, and $Q$ is the balance between the intensity of the horizontally polarized $I_0$ versus the vertically polarized light $I_{90}$, $U$ is the balance between the intensity of the linearly polarized light at $45^\circ$ orientation $I_{45}$ versus the linearly polarized light at $-45^\circ$ orientation $I_{-45}$ and $V$ is the balance between the intensity of the right circularly polarized $I_R$ versus the left circularly polarized light $I_L$. The degree of the polarization for a beam of light is defined by:

$$
dop = \sqrt{Q^2 + U^2 + V^2} / I
$$

(1-9)

Any phenomenon that may change the polarization of light from $S_{in}$ to $S_{out}$ can be described by a 4 by 4 element Mueller matrix according to $S_{out} = M \times S_{in}$ [46]. Mueller matrices of phenomena like single scattering from a spherical particle, refraction, reflection and transfer functions of optical components such as polarizers and waveplates can be theoretically calculated [46]. However, for a generic turbid medium, the 16 elements of the Mueller matrix are independent and analytically very difficult to calculate. Measuring the Mueller matrix is possible with variety of polarimetric methods that will be overviewed in section 1-5-3.
1-3. Simulating polarized light propagation in turbid media

Maxwell’s equations are ideal for studying the light propagation in clear media with well defined boundaries. However, biological tissues are heterogeneous and are composed of components (scatterers) with different sizes and different refractive index contrasts [48-49]. Exact analytical solution of light propagation in tissues with Maxwell’s equation is nearly impossible. The Maxwell solution of scattering by multiple particles can be approximated and extended numerically to obtain the light propagation behaviour in bulk tissue. However, this method is computationally expensive and time consuming [50-51].

An alternative analytical method is radiative transfer equation which is a function of the tissues’ bulk optical properties: the scattering and absorption coefficients ($\mu_s, \mu_a$) and the anisotropy factor $g$. $\mu_s$ and $\mu_a$ are, respectively, the probability of the photons being scattered and absorbed per infinitesimal pathlength and $g$ is the mean cosine of the scattering angle [49]. The radiative transfer equation formulates the balance of the energy radiance in tissues; with the energy radiance being “the energy carried by photons per unit time at a specific position and in a infinitesimal solid angle about a specific direction through an infinitesimal area” [49]. After propagation of few mean free pathlengths (multiple of $1/\mu_s$ cm), light experiences multiple scattering events. When photons are scattered enough to not have any preferred direction of propagation, the radiative transfer equation can be simplified into the diffusion equation which can then be solved analytically far away from the boundary and the sources [49,52]. In the diffusion regime, the propagation directions of the photons are random and thus light is unpolarized. Thus the diffusion approximation is not an appropriate tool to analyze polarized light propagation in scattering media. However, several numerical methods have been developed
for solving the radiative transfer equation which can be applied to polarized light. One of these techniques is the Monte Carlo approach [53-54].

Monte Carlo was first introduced by Metropolis and Ulam in 1947, as a statistical approach for solving differential equations [55]. The method is ideal for solving problems that include a large number of particles/users/trials [55]. In optics, photons are launched in random paths in the media; the individual behaviour of each photon is tracked and their average behaviour at the end is described as the bulk properties of light propagation though the media. Wilson and Adam were the first to implement a Monte Carlo code for simulating light propagation in tissue, in 1983 [53]. Later on, Wang et al developed a Multi-layered Monte Carlo (MLMC) which also models the light interaction at boundaries and interfaces [56]. Based on this model and by tracking the Stokes vector of each photon during propagation, several groups have implemented polarization sensitive Monte Carlo codes [54,57-59].

All the polarization sensitive codes have a similar algorithm as follow: Optical properties of the tissue are modelled by suspensions of scatterers of different sizes, refractive indices and concentrations. About 100 million to 1 billion photons are launched into the medium. Each photon goes through a scattering event after travelling a random distance. The random distance is selected from the probability distribution function \( \exp(-\mu d) \), where \( d \) is the distance and \( \mu \) is the extinction coefficient (the sum of the scattering and the absorption coefficient of the medium). Depending on the distance, the photon may hit the boundary and go through reflection or transmission according to a probability function dictated by the Fresnel coefficients. Upon each interaction, the corresponding Mueller matrix is applied to the photon and the photon polarization changes. After each scattering event, the direction of the propagation of the photon is determined by sampling the phase function of the single scattering event (known from Mie
The polarization sensitive properties of tissues such as their birefringence are modeled as a property of the background media. If the sample is birefringent, the Mueller matrix of a retarder is applied when the photons travel between any two interactions. When all the photons are absorbed, simulation ends and the photons at the surface of the sample or in the detector are counted to derive the output light polarization and intensity.

A polarization sensitive Monte Carlo (Pol-MC) has been developed, by Cote et al, in our lab [60-63]. The code can simulate the propagation of polarized light in turbid media with arbitrary defined linear birefringence and optical activity (circular birefringence) [60-63]. Optical activity is a property of a material with different refractive indices for the right and left circular polarizations. It arises from the presence of chiral molecules such as glucose [37]. The effect of the optical activity on linear polarized light is the rotation of the linear polarization around the propagation axis. Thus optical activity is modeled in terms of a rotation Mueller matrix with a rotation angle proportional to the volume of the chiral molecules in the sample [37]. The linear and circular birefringence are simultaneous optical effects, whereas matrix multiplication is not a commutative algebraic operation. Wood et al in our group overcame this problem by using the exponential sum of differential matrices called N matrices (based on Jones matrix formalism) and incorporated both effects in the Monte Carlo code [61]. The simulation results have been verified by extensive phantom studies [61-63]. Using this Monte Carlo code, we can investigate parameters such as the average photon pathlength, the degree of polarization of the light after interacting with the turbid media and the Mueller matrix of the sample in different geometries [61-63].
1-4. Polarized light imaging to characterize tissues’ anisotropy

Application of polarized light as a characterization tool in scattering biological tissues was first proposed by W. S. Bicker et al in 1976 [64]. Using a setup based on photoelastic modulators (PEM) and lock-in detection, they could show the sensitivity of the Mueller matrix elements to the size of different scatterers in cell lines [64]. The average anisotropy factor $g$ of tissues is about 0.9 and higher which implies a highly forward scattering regime [49]. Consequently, light that is scattered back from superficial layers will be more polarized than the light that has diffused further into the tissue and then backscattered. Based on this effect, later in 1997, Demos and Alfano suggested using co-polarized and cross-polarized intensity backscattered from the tissue, and introduced the ratio $(I_{\text{co}}(\lambda)-I_{\text{cross}}(\lambda))/(I_{\text{co}}(\lambda)+I_{\text{cross}}(\lambda))$ for a higher contrast backscattered image from superficial layers [65]. This definition of the degree of the polarization was later used by Jacques et al to find the margins of superficial skin cancer in vivo [66].

In another category of experiments, polarized light was used to infer some information about the refractive index of scatterers, the bulk scattering and absorption properties of different normal and malignant tissues [67-71]. For example, Backman et al developed a theoretical model of polarized light propagation in cells and experimentally showed that the spectrum of polarized light backscattered from the epithelial layers has a distinct fingerprint which depends on the size of the cells and nuclei, and their refractive indices [67]. Schmitt et al have studied the difference of depolarization of the light after interaction with tissue, when the illumination is linear or circular polarized light based on the refractive index of the non-homogeneities [69]. Vitkin and Studinski showed the dependence of the degree of polarization on absorption with in vivo experiment on skins with different pigmentations [70]. Sankaran et al showed that the degree of
the polarization of light is different in tissues with different scattering and absorption coefficients [70-72].

Despite the advances in measuring the depolarization, characterizing birefringence in bulk tissues was not fully feasible until 1995. The main challenge was separating the effect of polarization change due to multiple scattering from the polarization change due to birefringence. Few studies demonstrated measuring birefringence in optically thin media [1,3,73-76]. In optically thin samples, the effect of scattering is ignored which leads to two simplifications: 1) Optical pathlength is the same as the sample thickness, and 2) There is no depolarization and hence all the polarization change measured from the sample is due to the sample’s birefringence. Early studies showed that birefringence can be measured in the retina, because it was easy to maintain the polarization of light delivered to the retina through the non-scattering eye window [1,3]. In addition, in two separate studies Maitland et al and Snakaran et al reported a change of birefringence when the fibers in rat tail tendon were exposed to heat [73-74]. These assumptions are the working basis of polarized light microscopy [75-76]. Optically thin sample is also a valid approximation for polarization sensitive optical coherence tomography because in principle, only single scattered photons from each plane are detected [34-35,77-78].

A great step forward for accurate optical assessment of anisotropy in bulk biological tissues was polar decomposition of Mueller matrix suggested by Lu and Chipman in 1996 [79]. Previous methods were limited to interpretation of non-depolarizing Mueller matrices, whereas Lu-Chipman polar decomposition is unique for any depolarizing or non-depolarizing sample [79-80]. In Lu-Chipman polar decomposition, the Mueller matrix is decomposed into the product of three constituent ‘basis’ matrices: a diattenuator matrix $M_D$, a retarder matrix $M_R$, and a depolarizer matrix $M_A$. Mathematically, this can be written as [79]:
Although other multiplication orders are possible, the results for biological tissues are largely unaltered [81]. From $M_\Delta$, the depolarization of the sample can be obtained as:

$$\Delta = \frac{|M_\Delta(2,2)| + |M_\Delta(3,3)| + |M_\Delta(4,4)|}{3}$$  \hspace{1cm} (1-11)

and from $M_R$, one can obtain the sample’s optical retardance as [81]:

$$\delta = \cos^{-1} \left\{ \left[ (M_R(2,2) + M_R(3,3))^2 + (M_R(3,2) - M_R(2,3))^2 \right]^{1/2} - 1 \right\}$$  \hspace{1cm} (1-12)

where $M_s(i,j)$ are the elements of the matrix $M_s$. $\delta$ represents the cumulative relative phase shift incurred by the orthogonal light polarization components in traversing the sample.

Soon after polar decomposition was reported, Smith et al showed its application in an *in vivo* study to identify lupus (rapidly varying retardance) and skin cancer (less depolarization) [82]. Liu et al showed the efficacy of this method to provide diagnostic metrics in backscattering for strained tissue and melanoma [83]. Chung et al used polar decomposition to demonstrate the difference between the retardance and depolarization of oral cancer and precancerous tissues with high resolution [84]. Later on, Menhas et al extended Lu-Chipman polar decomposition a step further and decomposed the retardance matrix $M_R$ into the product of linear retardance matrix $M_{LR}$ and optical chirality matrix $M_{\phi}$ [85]. This allows separation of the effect of circular birefringence (optical activity) and linear birefringence [85]. The orientation of the anisotropy axis (fast axis), projected on the plane perpendicular to the incident beam, can be obtained from the $M_{LR}$ elements as [81]:

$$\theta = 0.5 \tan^{-1} \left( \frac{M_{LR}(4,2) - M_{LR}(2,4)}{M_{LR}(3,4) - M_{LR}(4,3)} \right)$$  \hspace{1cm} (1-13)
Our group was the first to adapt this type of polar decomposition to retrieve linear birefringence and optical activity from turbid media and verify it with Monte Carlo simulations and histology of thin samples [81,86-87]. In addition, a two angle measurement technique was developed to obtain the real 3D orientation of the extraordinary axis [88].

The feasibility of this technique for in vivo applications was first verified by monitoring the collagen change in the skin of a window chamber model of rat [87]. Li et al have also applied this decomposition to examine fiber orientations in stretched and relaxed samples of skeletal muscle [89]. One of the biomedical applications that attracted a lot of interest was the use of polarized light imaging followed by Mueller matrix decomposition to quantify optical retardance as a measure of the alignment strength in myocardium samples, identify the scar location and monitor its healing process with stem cell therapy [90]. Recently, Pierangelo et al also used the same imaging/decomposition method to investigate birefringence difference in ex vivo normal and cancerous cervical tissue samples [91].

Since applying polarized light imaging for detecting anisotropy in bulk biological tissues is relatively recent, extensive research project was needed to evaluate the full potential of this technique for biomedical applications. As such, this thesis contains three major chapters: 1) chapter 2: Quantitative analysis of Mueller matrix and its dependence on the various properties of turbid media; 2) chapter 3: Biomedical application of polarized light imaging in urology as a means to quantify and locate structural disorders in the bladder wall; and 3) chapter 4: Instrumentation and development of rapid benchtop polarized imaging systems for ex vivo tissue characterization. In the next pages of the introduction, I will discuss the motivation and the particular scientific aims in each chapter.
1-5. Thesis structure

1-5-1. Motivation for chapter 2: Developing new quantitative metrics from the Mueller matrix to characterize turbid media

The Mueller matrix is a transfer function of the turbid medium in a specific geometry (reflection or transmission). As discussed before, the Mueller matrix contains all the information related to a tissue’s non-homogeneities (size and refractive index of scatterers) and its structural organization (birefringence). Polarization sensitive Monte Carlo can be used to simulate the Mueller matrix of turbid medium with different properties in variety of geometries. However, the inverse problem of retrieving the properties of an unknown turbid medium from its Mueller matrix is very challenging. Lu-Chipman polar decomposition can provide valuable effective parameters such as the depolarization and retardance through the depth. This chapter is an attempt to derive more quantitative metrics from the Mueller matrix, beyond its Lu-Chipman decomposition.

1-5-1-1. Correlation between optical properties of tissues and the depolarization derived from their Mueller matrices

Unlike phantoms, biological tissues are not made of discrete, spherical, monodisperse particles. Hence, as mentioned before, biological tissues are usually described with their bulk scattering and absorption coefficients and anisotropy factors. There are various existing methods to experimentally measure these tissues’ properties; some examples are the integrating sphere, diffuse reflectance and cw radiance measurement [92-98]. The basics of all these methods are to have a theoretical model with some varying parameters, fit the experimentally measured reflectance or transmittance to the look up tables generated from the theory and derive the properties.
The optical properties dictate the fluence distribution of light in the media and hence the photon pathlengths and depolarization of light [98]. As a general rule of thumb, light is more depolarized when the detected photons travelled larger pathlengths. Several research studies have investigated the dependence of the degree of polarization of light after propagation in tissues and scattering media with different optical properties when the incidence is linear or circular polarized light [73-74,99]. However, this common approach depends on the polarization of the light impinging on the sample. Here, we examine the depolarization as measured and derived from the sample’s Mueller matrix. This depolarization $\Delta$ (Eq.1-11) does not depend on a specific illumination and only depends on the sample. Therefore, this Mueller matrix derived metric can be used to accurately compare the different tissues relative to each other, independent from the illumination.

Knowing the amount of the light depolarization is a substantial step of designing, calibrating and testing polarized light imaging systems. Thus our first aim in chapter 2 was to develop a quantitative correlation between the optical properties of tissue and the depolarization derived from its Mueller matrix in transmission and reflection geometry. We will also define and derive the linear and circular depolarization from the Mueller matrix to investigate the depolarization of linear and circular polarized light, respectively. To explain the observed trends, we refer to the Mie theory of scattering and confirm the results with previous studies.

1-5-1-2. Mueller matrix asymmetry and axial heterogeneity of anisotropy in turbid media

Biological tissues with special biomechanical function, particularly those which expand and contract periodically such as the heart and bladder are often composed of layers of cells or fibers with different alignments (different in both strength and direction) [16-17,100-101]. Optical
retardance as measured from Mueller matrix followed by decomposition, is in fact the effective retardance that light sees when propagates through all the layers in the tissue until it reaches the detector. Since orientation of the layers may be dramatically different (this is known for the myocardium muscle), the real birefringence of some of these layers may be higher or lower than the effective birefringence [100-101]. Measuring the birefringence of different layers is possible within a limited depth using depth resolved imaging methods such as polarization sensitive OCT or confocal measurements of the Mueller matrix [101-102].

In chapter 2, a new metric will be derived from the Mueller matrix itself to identify the axial changes of the birefringence in turbid media such as tissues. For a turbid medium, with no assumptions on the scatterers or other properties such as birefringence, the 16 elements of the Mueller matrix are completely independent. However, it has been shown by both simulations and phantom experiments that some of the elements are more sensitive to some properties. For example, the element in the fourth row and fourth column is the most sensitive element to size of the scatterers. Also, for a media with no optical activity or structural organization the absolute values of the diagonal elements are equal [103-104].

Images of the Mueller matrix elements carry a lot of information. Even if the sample is a suspension of particles, the Mueller matrix elements, even those of low value about the center of the illumination, vary in the image plane along the azimuthal and radial directions [105-107]. As such, examining the images of the Mueller matrix has been suggested for the overlay control process in the industry to insure correct alignment of the multi-layer thin films [105-110]. The variation in the Mueller matrix images usually appear as a butterfly pattern with several folds. Hielscher et al were the first time to study the dependency of these patterns (of cross polarized images), on the scattering coefficient, scatterers’ size and the anisotropy factor of the sample.
Later on, the symmetry between the patterns of the off-diagonal Mueller matrix elements of turbid media with no optical activity or birefringence was noted in several studies [109-111]. Despite all the extensive mentioned reports, there is no study on the properties of the Mueller matrix elements of anisotropic turbid media. Hence, our second aim in chapter 2 was to investigate the Mueller matrix images and their symmetry relations for homogenous and heterogeneous anisotropic turbid media. We will start from theory, then will develop a Monte Carlo code for multi-layered anisotropic media and will verify our findings with simulations to develop a metric for identifying axial heterogeneity of anisotropy in turbid media. Lastly, we will experimentally validate the application of the defined metric in multi-layered elastic and turbid phantoms.

1-5-2. Motivation for chapter 3: Bladder wall morphological disorders in bladder outlet obstruction disease

The urinary bladder is a hollow organ with a muscular membrane which is located in the pelvic cavity [112]. From an anatomical point of view, the bladder is connected to the kidneys with ureters and to the outside world with the urethra (Fig.1-3). For males the base is imbedded in the prostate. The top part of the bladder is called the dome and the bottom part is called the base, which includes the trigone (the region between the ureters and the outlet).
Fig.1-3. Anatomy of the human bladder. The ureters connect the kidneys to the bladder. The urine is emptied through the urethra.

The bladder wall structure is heterogeneous and is composed of several layers as illustrated in Fig.1-4. The inner layer, the Mucosa, has two distinct layers: the transitional epithelium (inner layer which is resistant to the urine toxicity) and the lamina-propria. The next layer is the submucosa followed by the detursor muscle (the thickest layer); the outer layer is called serosa [113]. The scaffold which provides structural support within and between these layers is the extracellular matrix (containing mostly collagen and elastin fibers). The other role of the extracellular matrix is to facilitate cell signalling and cytoskeleton tension [114].

Fig.1-4. The bladder wall heterogeneous structure is composed of several layers with different compositions and organizations.
The urological disease, investigated in this thesis, is bladder outlet obstruction (BOO). BOO is the partial or complete blockage of the outlet which stops or reduces urine outflow from the urethra [113]. BOO may develop in several conditions such as the following: bladder stones, neurogenic bladder (resulting from spinal cord injury or brain and nervous system disease), posterior urethral valves (anomaly in the urinary system of male new-borns), and most commonly, benign prostatic hyperplasia (BPH) (resulting from prostate overgrowth which affects 50% of men after the age of 60 years) [113-114]. The prolonged obstruction results in muscle hypertrophy or hyperplasia, fibrosis, elastin breakdown and decreased angiogenesis [114-116]. These morphological changes impair the bladder normal function which involves distension and contraction. The BOO induced bladder dysfunction, regardless of the etiology, triggers lower urinary tract symptoms (LUTS) including deterioration, acute urinary retention, urinary tract infection, and kidney failure which impair patient quality of life [116-117].

According to a recent study, the prevalence of BOO/LUTS will increase, reaching 1 billion patients, as the world population ages [117]. Currently, BOO/LUTS patients are treated with administration of anti-cholinergic drugs (to treat neurogenic bladder), intermittent catheterization (short term drainage with catheters) or surgical removal of the obstruction [114-116,118-119]. Unfortunately, none of these methods are able to fully reverse the bladder dysfunctions caused by prolonged BOO. Hence, in many cases, augmentation is the only viable method. Augmentation cystoplasty is the procedure of replacing the bladder wall, frequently the dome region, with parts of the autologous intestine/bowel wall or artificially engineered tissue [120-122].

Augmentation is traditionally performed on the dome to increase the bladder capacity. However, the artificial tissues are not fully functional and patients continue to suffer from LUTS in the
long term [10,123-124]. To better manage BOO with any treatment especially augmentation, it is essential to understand the BOO pathology including the location - which may be different than the dome - and the nature of the morphological changes in the bladder wall which lead to the bladder dysfunction.

As confirmed by several in vitro and ex vivo studies, the bladder wall morphological properties are not the same in different regions (dome, dorsal and ventral). When the bladder distends, different regions of the wall experience different mechanical strain and changes of structural properties [125-127]. Unfortunately, current in vivo imaging methods such as x-ray, ultrasound and MRI can only reveal the anatomical changes in the bladder [116,128]. Therefore, there is a need for a new in vivo imaging technique that can detect and quantify the regional structural characteristics of a bladder wall, while the bladder distends to different pressures. As such, in chapter 3 of this thesis, we propose using polarized light imaging followed by Mueller matrix decomposition, as a potential in vivo technique, to measure the bladder wall anisotropy as a morphological metric. Dr. Bagli’s urology lab at Hospital for Sick Children have developed rat models of the BOO disease and a procedure to harvest ex vivo bladders that can be distended to different physiological pressures [115]. Therefore, we suggested characterizing normal and obstructed bladders walls using polarized light imaging to first, quantify normal bladder microstructure upon distension, and second, to identify the morphological changes in distended obstructed bladders. A better understanding of the BOO structural disorders in distended bladders may help optimizing the augmentation surgery or developing guidelines to improve the functionality of the artificial tissue engineered bladders.
1-5-3. Motivation for chapter 4: Rapid polarized light imaging systems

The purpose of a polarized light imaging system is to measure the unknown polarization state of the light (Stokes vector) or the unknown polarization properties of a sample (Mueller matrix). As shown in Fig.1-5, a Stokes imaging system is made of a polarization state analyzer (PSA) which demodulates the different polarization states of light $S_{\text{out}}$ and projects them as intensity on the camera (at least 4 measurements, refer to Eq.1-8). As Fig.1-6 illustrates, a Mueller matrix imaging system has a second sub-system called polarization state generator (PSG) which modulates the polarization state of the incident light $S_{\text{in}}$ on the sample. The Mueller matrix can be calculated from knowing $S_{\text{out}}$ for at least 4 orthogonal polarized beams $S_{\text{in}}$ (at least minimum 16 intensity measurements). The number of the measurements to obtain a Mueller matrix and the selection of the polarized light illuminations $S_{\text{in}}$ have been the subject study of several articles [129-133]. The common theme among these papers is to minimize the noise sensitivity and the recovery error through minimizing the condition number of the system matrix - a matrix made of the different polarized light illuminations $S_{\text{in}}$ as its vectors.

![Fig.1-5. A Stokes imaging system, composed of a PSA and a CCD. The arrows show the propagation direction of light.](image)
Fig.1-6. A Mueller matrix imaging system, composed of a PSG (in the illumination) and a PSA in the detection. The arrows show the propagation direction of light. The system can be set up in transmission or backscattering geometry.

In addition to being accurate (having minimum recovery error), an ideal polarized light imaging system for characterizing biological tissues should have the following criteria: 1) Large field of view, since tissues are spatially heterogeneous 2) High resolution, since we are interested in the tissues’ micro-structure, 3) Rapid, to avoid movement artifacts, and 4) Applicable to turbid media, since tissues are scattering and often prior knowledge of their micro-structure is not available. These specifications vary among the different polarized light imaging systems. Based on the components that are used in their PSA and PSG, polarized light imaging systems are divided into four categories: 1) Imaging systems based on mechanical moving components [86,90,134]; 2) Snapshot imaging systems based on diffractive elements [135-136]; 3) Liquid crystal (LC) based imaging systems [137-138], and 4) PEM based imaging systems [139-142].

The first category of these systems is based on polarization filters and quarter waveplates that have to be mechanically rotated and translated in space to generate different linear and circular polarization states. Imaging with this type of systems is slow and may involve lots of systematic error such as mechanical drift of the rotational stages. However, they can have a large field of view and high resolution. They can also be applied for measuring any turbid media with no
simplifying assumptions and have been widely used for *ex vivo* biomedical applications [90, 134]. A schematic of these systems is shown in Fig.1-7.

![Fig.1-7. A polarized light imaging system based on mechanically rotational or removable polarizer and quarter waveplates (QWP). The arrows represent the beam.](image)

The snapshot imaging systems are based on diffracting the light beam into several beams with different polarizations in the PSG and combining them again in the PSA. At the end, to recover the different polarization measurements from the intensity registered at the camera, spatial frequency filters are used. The diffracting components can be polarization grating or Savarat prism as depicted in Fig.1-8. Since the polarization modulation and demodulation is based on diffraction and spatial filtering, these systems are the fastest and the imaging speed only depends on the camera. However, the spatial frequency filters in these systems, reduce the amount of the information in the image and degrade the resolution. Snapshot systems are appropriate for large field of view investigation and can be made compact, for industrial applications [135].
Fig.1-8. A snapshot polarized light imaging system based on diffracting elements (D1, D2, D3, D4), fixed polarizers and fixed quarter waveplates (QWP). The arrows represent the beam.

The third type of systems use LCs for modulating the polarization as illustrated in Fig.1-9. LCs can be easily controlled to change the retardance by varying their input voltages. LCs are fast and their switching time is about 30 milliseconds. However, LCs suffer from small acceptance angle and small clear aperture. Consequently, they are very sensitive to any misalignment which makes them difficult to use in an imaging system.

Fig.1-9. A polarized light imaging system based on LCs and fixed polarizers. The arrows represent the beam.

The fourth type of polarized light imaging systems, shown in Fig.1-10, is based on PEMs. PEMs are made of bulk crystals and have big clear aperture; thus are ideal for imaging with a large field
of view. PEMs modulate the retardance in a sinusoidal fashion in range of tens of kHz. The drawback is that these components oscillate in the resonance frequency of the crystal, therefore cannot be easily stabilized with respect to changes in temperature. The signal measurement and the Mueller matrix recovery, in the PEM based systems, are usually based on lock-in amplifier and photodetectors [141-142]. Therefore, to make an image, the beam or the sample has to be mechanically scanned which is time consuming [142]. Some groups have demonstrated imaging systems with PEMs and charge-coupled devices (CCD); however, these systems only measure two components of the Stokes vectors, and are only applicable for simple polarimetric measurements through non-scattering media [139-140].

![Diagram of a polarized light imaging system based on PEMs and fixed polarizers](image)

Fig.1-10. A polarized light imaging system based on PEMs and fixed polarizers. The arrows represent the beam.

Hence, our aim in chapter 4 was to develop a fast polarized light imaging algorithm based on PEMs and a CCD for characterizing turbid media. Such an imaging system is an ideal system for rapid polarimetric characterization of biological tissues and meets the criteria discussed in the beginning of this section.
2. Quantitative analysis of the Mueller matrix of generic turbid media beyond its polar decomposition

2-1. Quantitative correlation of depolarization and transport albedo

A discussed in chapter 1, depolarization of light after interacting with tissue can be used to approximate the size and refractive index of cells [68-72]. Depolarization has also been applied to visualize the subsurface layers and detect the margins of cancerous lesions [66,143]. Moreover, depolarization should be carefully measured when calibrating polarized light imaging systems; because, high value of depolarization will translate to polarimetric measurement with low signal to noise ratio. All previous studies measured the degree of the polarization, which is calculated from the Stokes vector of the scattered light from tissue, therefore, depends on the illumination [66-72]. In the following paper, “Quantitative correlation between light depolarization and transport albedo of various porcine tissues,” published in Journal of Biomedical Optics, a new approach of studying depolarization is presented. In this paper, we investigate the depolarization value only as a property of the sample and the imaging geometry, by calculating the depolarization from the Mueller matrix of the sample. This depolarization is thus independent from the illumination. Comparative experiments were run to explain the depolarization in a variety of biological tissues. To classify the tissue, we then used a parameter called transport albedo $\mu'_s/(\mu_s+\mu'_s)$, where $\mu'_s$ is the reduced scattering coefficient. Transport albedo has been used previously to explain the diffusion of light in turbid media but our work is the first to apply it for explaining the depolarization [49].
Swine models are used in most animal studies, because they share significant anatomical and physiological resemblance to humans [144]. Therefore, various \textit{ex vivo} porcine tissues are used for evaluating the performance of the imaging systems. In this chapter, we use various porcine tissues to study the quantitative relation between depolarization and the bulk optical properties of the tissues. A variety of highly anisotropic (heart, muscle, tendon) and nearly isotropic tissues (kidney, brain, liver) were chosen for this experiment. The bulk reduced scattering coefficients and absorption coefficients were measured with a diffuse reflectance spectrometer made by Kim et al in Dr. Wilson’s lab [95].

Samples of 1 cm and 2 mm thick of all the tissues were made, within 3 hours of obtaining the bulk tissues from the abattoir. The Mueller matrices of the 1 cm samples were imaged in backscattering geometry and the Mueller matrices of the 2 mm samples were imaged in both transmission and backscattering. The experiment was performed by me and Dr. Manzoor Ahamd. I analyzed the data and established the idea of correlating the depolarization to the transport albedo. Dr. Michael F. G. Wood a postdoctoral fellow in our group has built the imaging system, and provided guidance for accurate measurements and tissue preparation.
Quantitative correlation between light depolarization and transport albedo of various porcine tissues

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Abstract. We present a quantitative study of depolarization in biological tissues and correlate it with measured optical properties (reduced scattering and absorption coefficients). Polarized light imaging was used to examine optically thick samples of both isotropic (liver, kidney cortex, and brain) and anisotropic (cardiac muscle, loin muscle, and tendon) pig tissues in transmission and reflection geometries. Depolarization (total, linear, and circular), as derived from polar decomposition of the measured tissue Mueller matrix, was shown to be related to the measured optical properties. We observed that depolarization increases with the transport albedo for isotropic and anisotropic tissues, independent of measurement geometry. For anisotropic tissues, depolarization was higher compared to isotropic tissues of similar transport albedo, indicating birefringence-caused depolarization effects. For tissues with large transport albedos (greater than ~0.97), backscattering geometry was preferred over transmission due to its greater retention of light polarization; this was not the case for tissues with lower transport albedo. Preferential preservation of linearly polarized light over circularly polarized light was seen in all tissue types and all measurement geometries, implying the dominance of Rayleigh-like scattering. The tabulated polarization properties of different tissue types and their links to bulk optical properties should prove useful in future polarimetric tissue characterization and imaging studies. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.4.045004]

Keywords: optical properties; polarimetry; tissues.

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1 Introduction

In an effort to better visualize biological tissue structure, polarized light imaging has been used extensively to enhance spatial resolution and to provide contrast that cannot be achieved using ordinary light alone. In addition, polarized light-based techniques (polarimetry) have allowed tissue characterization via intrinsic properties such as birefringence (arising, for example, from fibrous connective tissue networks containing collagen and elastin), orientation (arising from structural organization), and optical activity (arising from the presence of chiral molecules such as glucose). Polarimetry-based metrics have demonstrated sensitivity to changes in tissue structure and composition and may be closely associated with underlying pathology. For instance, scar formation in the myocardium has been linked to a decrease in the birefringence signal. One of the limitations to polarized light-based techniques in optically thick turbid media (such as bulk tissue) is the effect of multiple scattering, which effectively randomizes the photon direction, phase coherence, and polarization. Consequently, only a fraction of the light emerging from a sample in particular detection geometry will remain polarized. Since polarimetry relies on measuring, quantifying, and interpreting the surviving polarized light fraction, examining the depolarization rates in different tissue types and relating these to tissue structure and pathology are of great importance.

Previously, several studies have concentrated on light depolarization in biological tissues. Demos et al. have suggested using the degree of polarization for discriminating between different types of tissues. Jacques et al. have used polarized light imaging to distinguish superficial backscattered light from the collagen fibers versus highly depolarized light from deep skin layers. The same group also has compared chicken liver and breast and porcine muscle and skin with a transmission mode polarimetry system, and it reported that linearly polarized light depolarizes faster in (anisotropic) tissues with birefringence, compared to isotropic tissues. Vitkin et al. have demonstrated in vivo that light that is diffusively scattered by human skin retains significant polarization in exact backscattering directions, and the degree of polarization increases with increased absorption. Sankaran et al. have investigated the degree of polarization in five porcine tissues (fat, tendon, blood, myocardium muscle, and artery) of different thicknesses (0.1 to 2 mm) in transmission geometry. They have shown that the Rayleigh-scattering regime is dominant for all the tissues except blood, with the degree of linear polarization exceeding the degree of circular polarization. More recently, Antonelli et al. compared the Mueller matrix in the backscattering direction from healthy and cancerous human colon biopsy samples, indicating that the cancerous samples were less depolarizing. A common theme in these studies is to quantify and understand the depolarization mechanisms in tissues and to use depolarization as a diagnostic tool. The current article thus describes our efforts in measuring, quantifying, and understanding
depolarization behavior in a variety of bulk porcine tissues in the context of their measured optical properties.

A commonly used approach to characterize tissues is by measuring their optical properties, specifically the reduced scattering coefficient \( \mu_s' \) and the absorption coefficient \( \mu_a \). The reduced scattering coefficient is the probability of the photons scattering in the forward direction per infinitesimal path-length. The absorption coefficient is the probability of the photons absorption per infinitesimal path-length. Several suitable methods have been developed, with their relative advantages, disadvantages, and ranges of validity closely examined.17 In the current study, we performed polarimetric and optical property measurements and analysis on optically thick samples from six different porcine tissue types. The isotropic (low birefringence) tissues examined were the liver, kidney cortex, and brain. The anisotropic (high birefringence) tissues were the myocardium muscle, loin muscle, and tendon. To quantify the dependence of the depolarization rates on source-sample-detector geometry, we performed polarimetric imaging in both transmission and backscattering modes. The paper is organized as follows: in “Materials and Methods” section, we outline sample preparation, the polarimetric imaging setup, and the methodology for measuring tissue optical properties; we also review Mueller matrix derivation, calculations, and decompositions. In “Results and Discussion,” we present the depolarization findings of the six different tissue types, correlate these with their measured optical properties, and discuss the meaning and implications of these correlative trends. Conclusions and future directions are presented in the final section.

2 Materials and Methods

We obtained fresh porcine tissues following sacrifice from a local abattoir. All the samples were imaged and characterized within three hours after sacrifice. Samples were moistened periodically with phosphate-buffered saline solution (0.02 M) to maintain freshness; this avoids potential complications associated with formalin fixation and other tissue handling or preparation methods.18 Three previously reported low-birefringence (~isotropic) tissues (liver, kidney cortex, and brain) and three highly birefringent (anisotropic) tissues (myocardium muscle, loin muscle, and tendon) were chosen. Liver was selected as an isotropic and highly absorbent tissue.17,20 In the case of the kidney, we examined the cortex, which is known to be optically isotropic.18,20 Brain was chosen as a highly scattering, low-absorbing medium considered to be non-birefringent.17,21 From the heart, we chose the myocardium, which is a highly birefringent tissue composed of oriented cardiac muscle cells.8,12 Loin muscle was another anisotropic tissue examined, where the myosin fibers are known to be loosely aligned.17,22 Tendon was chosen because it exhibits the same range of the optical properties as the brain but is anisotropic, stemming from parallel arrays of closely packed collagen.15

To measure tissue optical properties of the bulk tissues, a fiberoptic reflectance probe with multiple source-collector separations was used. The optical properties \( \mu_a \) and \( \mu_s' \) were extracted using the established technique of spatially resolved, steady-state diffuse reflectance (based on diffusion theory) as outlined by Farrell et al.23,24 Briefly, a multifiber probe with source-collector distances, \( r \), of 0.8, 1.5, 2.4, 4.0, and 5.3 mm was connected to an optical control system that consisted of a diode laser (Thorlabs, Newton, NJ) at 635 nm, an optical multiplexer (MPM-2000, Ocean Optics, Dunedin, FL), and a visible-light spectrometer (S2000, Ocean Optics).

The collector fiber was connected to the spectrometer. The multiplexer was used to index the laser beam from fiber-to-fiber so as to achieve the various source-collector distances required for the spatially resolved diffuse reflectance algorithm. The well-known diffuse reflectance equations were employed to model the reflectance at each \( r \).23,24 The probe was calibrated against a diluted Intralipid (Fresenius Kabi, Sweden) solution with known optical properties and validated against a series of Intralipid-dye phantoms with \( \mu_a = 0.5\text{−}5 \text{cm}^{-1} \) and \( \mu_s' = 3.8\text{−}11.5 \text{cm}^{-1} \). The two optical property coefficients, \( \mu_a \) and \( \mu_s' \), were computed from the five reflectance measurements by solving an inverse problem. It is worth noting that the algorithm did not utilize some of the reflectance measurements in the case of low \( \mu_s' \) because the diffusion theory reflectance equation breaks down at low \( \mu_s' \) for low \( r \), as outlined in Kim et al.25 Hence, the algorithm would omit the reflectance value at, say, \( r = 0.8 \) for \( \mu_s' < 5.75 \text{ cm}^{-1} \), and use only four reflectance measurements to solve in the inverse problem in this scattering range. With this probe, we measured the optical properties of the six different tissues, periodically examining a well-characterized scattering solution of Intralipid as a calibration/system validation control phantom.

For polarimetric measurements, two slabs of each type of tissue, one with 2 mm of thickness and one with 1 cm of thickness, were sectioned. No formalin fixation was used to avoid possible crosslinking-induced polarization property changes,18 so the results apply to freshly excised tissues. Cutting the fresh tissue with a blade to a uniform thickness is challenging; therefore, several sections were made and the three most uniform slabs with the desired thicknesses were chosen for the experiment. The resultant tissue slabs were placed between two glass slides. The 2-mm samples were imaged in both transmission and backscattering geometry, while the 1-cm samples were imaged only in backscattering mode (not surprisingly, complete polarization loss was observed in transmission for all 1-cm tissues).

The polarimetric imaging setup shown in Fig. 1 was used. A diode laser (Thorlabs) with a wavelength of 635 nm was used to illuminate the sample. The polarizer, P1, and the removable quarter waveplate, WP1, were used to generate different linear and circular input polarization states. The first lens, L1, was used to project a 1-cm-diameter spot size onto the sample. Light exiting the sample in a particular detection direction then was channeled through an output arm consisting of a polarizer, P2, a removable waveplate, WP2, and a collecting lens, L2, that focused the light onto a charge-coupled device (CCD)
camera (CoolSNAPK4, Photometrics, Tuscon, AZ). The output polarization was selected by P2 and WP2. Imaging was performed in two different geometries. In transmission mode, the output arm was placed collinear to the incident beam. In backscattering mode, light was collected by the output arm at an angle of 155 deg off-axis relative to the incident beam (25 deg off-axis from the exact backscattering direction). The camera field of view was set to a 1.5-cm-wide square, with the collected light spot in its center.

To characterize the interaction of polarized light with a sample of tissue for a specific measurement geometry, Mueller algebra was employed. In Mueller formalism, a polarized light beam is described by the Stokes vector \( S = [I \ Q \ U \ V]^T \), and its interactions are described by multiplication by a 16-element square \((4 \times 4)\) matrix. This matrix, called the Mueller matrix, represents a particular interacting entity (polarizer, wave plate, tissue sample, etc.). If the incident light beam is represented by the Stokes vector \( S_i \), and the output light beam vector is \( S_o \), then the Mueller matrix \( M \) acts as the polarization transfer function, such that \( S_o = M \cdot S_i \). To calculate the Mueller matrix for a particular tissue sample at particular detection geometry, we performed 24 sequential measurements. For each of four input polarizations \([H \ (\text{linear at } 0 \deg), \ V \ (\text{linear at } 90 \deg), \ P \ (\text{linear at } 45 \deg), \ \text{and } R \ (\text{right circular})]\), intensity profiles at six output polarizations \([H \ (\text{linear at } 0 \deg), \ V \ (\text{linear at } 90 \deg), \ P \ (\text{linear at } 45 \deg), \ B \ (\text{linear at } -45 \deg), \ R \ (\text{right circular}), \ \text{and } L \ (\text{left circular})]\) were measured. From these 24 measurements, Mueller matrices for each sample for that specific geometry (transmission/backscattering) were constructed, as described previously in several studies (e.g., Wallenburg et al.\(^{26}\).

A complicating issue in turbid polarimetry is the simultaneous occurrence of several polarized light-tissue interactions. This results in complicated cross-talk within and between the Mueller matrix elements and obscures the contribution of the individual sample polarization properties. Several mitigating strategies have been developed recently to deal with this problem.\(^{25,30}\) One approach is termed polar decomposition, originally proposed by Lu-Chipman and adapted by our group for tissue polarimetry.\(^{31-34}\) This decomposition method allows for extraction of individual properties of interest (birefringence, optical activity, depolarization, and diattenuation) from a single measured Mueller matrix.\(^{31-34}\) We have validated this approach using polarized-light Monte-Carlo simulations and phantoms with controlled polarization properties, as well as ex vivo and in vivo tissues.\(^{27,30,35}\) In this method, the tissue Mueller matrix \( M \) is decomposed into three constituent basis matrices—the depolarization matrix \( M_{\Delta} \), the retardance matrix \( M_{\Delta} \), and the diattenuation matrix \( M_{\Delta} \):\(^{27,30,35}\)

\[
M = M_{\Delta} M_{\Delta} M_{\Delta}.
\]  

This multiplication order (or its reverse) will lead to a physically realizable decomposition in which the basis matrices describe the polarization properties of the sample for the specific geometry.\(^{36}\) For instance, from the retardance matrix, one can calculate a retardance value (proportional to the linear birefringence) and the optical rotation (proportional to circular birefringence) that light experiences upon its interaction with tissue. Note that for different measurement geometries, the effective measurement sampling volumes (and hence the derived polarization characteristics) will be different.

The focus of this paper is on the depolarization metrics, which can be calculated from the depolarization matrix \( M_{\Delta} \).

Ignoring for the moment the other two derived basis matrices and the information that they contain, the depolarization matrix can be written in the following form:

\[
M_{\Delta} = \begin{bmatrix} 1 & \vec{0}_{\Delta} \cr \vec{P}_{\Delta} & m_{\Delta} \end{bmatrix},
\]  

where \( \vec{P}_{\Delta} \) is the polarization vector and \( m_{\Delta} \) is a symmetric sub-matrix. The diagonal elements of \( m_{\Delta} \) describe the preserved portion of each of the polarization states \([Q, U, V]\) in the Stokes vector of the light beam. The net depolarization factor, also called the depolarization power, can be defined as\(^{27,33}\)

\[
\Delta_{T} = \frac{1}{3} [m_{11} + m_{22} + m_{33}] - |m_{11}| + |m_{22}| + |m_{33}|.
\]  

where \( m_{11}, m_{22}, \) and \( m_{33} \) are the diagonal elements of \( m_{\Delta} \). Here termed the total depolarization, yields a quantitative average measure of how a material with this particular Mueller matrix depolarizes light. One can go further and define the linear depolarization, \( \Delta_{L} \), and circular depolarization, \( \Delta_{C} \), as follows:

\[
\Delta_{L} = \frac{1}{2} - |m_{11}| + |m_{22}|
\]  

\[
\Delta_{C} = 1 - |m_{33}|
\]  

Linear depolarization \( \Delta_{L} \) is a measure of the average depolarization of the \( Q \) and \( U \) components (linear polarization at 0 to 90 deg and 45 to 135 deg) of the Stokes vector interacting with the sample. Correspondingly, circular depolarization \( \Delta_{C} \) is a measure of the depolarization of the \( V \) component (circular polarization) of the Stokes vector. Using the resultant linear, circular, and total depolarizations allows quantitative comparison of the depolarizing behaviors of different biological tissues at a specific geometry, independent of the illumination polarization state (incident Stokes vector).

It is important to distinguish between depolarization metrics derived from the Mueller matrix of the sample and the degree of polarization calculated from the Stokes vector of the light. The former are intrinsic properties of the transfer function of the sample in a particular geometry (as embodied in the tissue Mueller matrix), whereas the latter depend on the sample and on the Stokes vector of the incident light. The derived Mueller matrices thus are more specific to the tissue being studied in that they factor out the confounding effects of the measurement system (incident light polarization). Further, the polar decomposition methods discussed previously permit one to isolate the depolarization effects from other polarizing interactions, something that is not possible with the Stokes descriptors alone. Mathematically, the total degree of polarization in the Stokes description is always higher than the linear and circular degrees of polarizations; an analogous relationship does not hold true for the Mueller matrix-derived depolarizations [Eq. (3) through Eq. (5)]. For the remainder of this paper, we discuss depolarization \( \Delta_{T}, \Delta_{L}, \Delta_{C} \) as derived from the decomposed Mueller matrices.

Following the decomposition procedure, we quantify the intrinsic depolarization parameters of the tissues in each geometry, as derived from Mueller algebra (independent of the incident light polarization state and other tissue polarizing interactions, as per the previous discussion). To gauge the uncertainty in the entire polarimetry methodology, a given Mueller matrix (each
with 24 measurements) was recorded three times for all samples in both geometries. Total, linear, and circular depolarization metrics reported in this paper are the average results of these measurements.

3 Results and Discussion

The measured optical properties ($\mu_s$, $\mu'_s$) of the swine tissues at 635 nm are shown in Table 1. The reduced scattering coefficient contributes to the extent of photon diffusion in tissue. It is related to the scattering coefficient $\mu_s$ and anisotropy factor $g$—the mean cosine of the scattering angle—through the relation $[\mu'_s = \mu_s(1- g)]$. The transport albedo $\alpha'$ is a dimensionless quantity defined as $[\mu'_s/(\mu'_s + \mu_a)]$. In addition to their role in interpreting tissue polarimetry results, these measured optical properties may contribute to recent tissue optics compilations. Polarized light imaging allows the construction of spatial maps in which the Mueller matrix derived parameters may be plotted. In Fig. 2, we illustrate the calculated total, linear, and circular depolarization of kidney cortex samples in the transmission and backscattering geometries. The data shown in each map is the average of three independent measurements. The average standard deviation over all the pixels in the image is 1.5%. This number is representative of the average uncertainty in the derived polarization metrics, characteristic of the system noise over the measurement period.

Several prominent features are visually evident from Fig. 2. First, linear depolarization is lower than circular depolarization for both transmission and backscattering geometries, as well as for both 2-mm and 1-cm kidney cortex samples. In other words, linear polarization is better preserved than circular polarization in this tissue type. Second, the total depolarization is observed to be lowest in transmission mode for 2-mm samples and greatest in the backscattering mode for the 1-cm sample. Such images have been obtained for all the examined tissues; to enable quantitative analysis and inter-tissue comparisons, we averaged the data along a 0.1-cm central strip in the $x$ direction, and plotted the total depolarization along the $x$ direction, as shown in Fig. 3.

Comparing panels (a) and (b) of Fig. 3, one can conclude that all the tissues except tendon and brain are less depolarizing in transmission geometry rather than in backscattering. For brain and tendon, the transmission mode is not as useful as depolarization becomes too severe (greater than 98.5% even for this relatively thin, 2-mm slab) and essentially all polarized light information is lost. Measurements with such large depolarization are not reliable and lie in the range of the polarimetry system’s noise level. The transmission geometry thus becomes untenable for tissue polarimetry as either physical thickness or optical properties increase sufficiently. Hence, the brain and tendon results are not included in Fig. 3(a). Such extensive depolarization is likely because brain and tendon both have transport albedos higher than 0.97 (Table 1). A large transport albedo signifies the predominance of multiple scattering, with its variety of long, zigzag paths that effectively depolarize the light. Furthermore, scattering in biological tissues is generally forward-peaked, with the anisotropy factor $g$ varying between 0.9 and 0.99. A large transport albedo and forward-peaked

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Reduced scattering coefficient (cm$^{-1}$)</th>
<th>Absorption coefficient (cm$^{-1}$)</th>
<th>Transport albedo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6.90 ± 0.10</td>
<td>4.10 ± 0.11</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>6.00 ± 0.10</td>
<td>1.20 ± 0.20</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>Myocardium muscle</td>
<td>8.22 ± 0.40</td>
<td>1.62 ± 0.35</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td>Loin muscle</td>
<td>4.00 ± 0.07</td>
<td>0.37 ± 0.04</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>Brain</td>
<td>11.27 ± 0.48</td>
<td>0.26 ± 0.10</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>Tendon</td>
<td>13.50 ± 0.54</td>
<td>0.35 ± 0.18</td>
<td>0.97 ± 0.06</td>
</tr>
</tbody>
</table>

Fig. 2 Depolarization maps of the kidney cortex derived from the polar decomposition of the measured Mueller matrices. The columns indicate the total, linear, and circular depolarizations, and the rows indicate the different detection geometries and sample thickness. The x and y axes indicate the length and width, respectively. The color bar indicates depolarization levels as specified on the percent scale, where dark blue signifies regions where light remains most polarized and deep red indicates regions where light has lost most of its polarization. The error bar size is equal to the line thickness.
scattering implies that photons detected in backscattering geometry have experienced fewer scattering events and on average have traveled shorter path-lengths compared to the photons detected in transmission geometry. In fact, most photons detected in the reflection mode in these types of scattering media have sampled mostly near-surface layers, as we have recently demonstrated by Monte Carlo modeling studies. As a result, less depolarization is observed for high transport albedo tissues in backscattering mode.

A general trend in the total depolarization values of the tissues emerges from a closer examination of Fig. 3. Liver has the minimum total depolarization in all measurement geometries, whereas tendon appears to have the highest total depolarization among all tissue types. The tissues arranged in the order of increasing depolarization are liver, kidney, loin muscle, brain, myocardium muscle, and tendon. Comparing Fig. 3 and Table 1, one notices a correlation between total depolarization rates and the transport albedos of the corresponding tissue type.

To explore this further, the minimums of total, linear, and circular depolarization (Fig. 3) are plotted against the tissues’ transport albedos (Table 1) in Fig. 4. As previously mentioned, brain and tendon data in transmission mode are not shown because they exhibit total depolarization greater than 98.5% in transmission mode, which is in the range of the system noise level. As a result, less depolarization is observed for high transport albedo tissues in backscattering mode.

A general trend in the total depolarization values of the tissues emerges from a closer examination of Fig. 3. Liver has the minimum total depolarization in all measurement geometries, whereas tendon appears to have the highest total depolarization among all tissue types. The tissues arranged in the order of increasing depolarization are liver, kidney, loin muscle, brain, myocardium muscle, and tendon. Comparing Fig. 3 and Table 1, one notices a correlation between total depolarization rates and the transport albedos of the corresponding tissue type.

To explore this further, the minimums of total, linear, and circular depolarization (Fig. 3) are plotted against the tissues’ transport albedos (Table 1) in Fig. 4. As previously mentioned, brain and tendon data in transmission mode are not shown because they exhibit depolarizations \( \sim 98.5\% \). Analysis of Fig. 4 reveals that tissues with higher transport albedos are generally more depolarizing. This trend appears to hold for all tissues, detection geometries, and sample thicknesses. The relations’ depolarization and transport albedo have been quantified with the correlation coefficient \( r \) between them, as shown in Fig. 4. These correlations include both anisotropic and isotropic tissues; as seen, anisotropic tissues show higher depolarization compared to the isotropic one with the same transport albedo. For example, myocardium muscle, whose \( \sigma' \) is not exceptionally high (close to the value for kidney cortex), exhibits very high total depolarization. In addition, when comparing tendon tissue depolarization to that of the brain, tendon exhibits a higher depolarization despite having a comparable \( \sigma' \). One possible reason for both of these observations could arise from the intrinsic anisotropy exhibited by the different tissue types. Myocardium muscle and tendon (and loin muscle, to a lesser extent) are known to be highly anisotropic relative to the other tissues. The underlying birefringence that causes this tissue anisotropy thus may contribute to depolarization over and above the transport albedo effect invoked previously. Specifically in tissues, birefringence magnitude and orientation may be spatially inhomogeneous, changing in different regions of tissues/microdomains; in these regions, polarized light undergoes additional randomization, and therefore the total depolarization increases. Note that we specifically concentrate on depolarization phenomena here and do not analyze the derived birefringence magnitude and orientation (derived from the retardance matrix \( M_3 \)), which are important in anisotropic tissues such as skeletal muscle; an ongoing study examining birefringence phenomena in greater detail (e.g., the effects of variable spatial domains of different magnitude/orientation of birefringence) will be reported elsewhere.

Another interesting trend evident from Fig. 4 is the preferential retention of linear polarization states compared to circular polarization states, in all tissues, for both geometries. Interestingly, the correlation coefficient values are also higher in
cases of linear depolarization. Better preservation of linear polarization is usually associated with Rayleigh-like scattering, whereas tissues are often considered Mie-like in their scattering behavior. Thus, identifying the appropriate dominant scattering regime in biological tissues is tricky. Conventional wisdom is that tissues are composed of large scatterers and thus exhibit Mie-like scattering behavior, consistent with the predominantly forward-peaked nature of the observed scattering phase function (high values of g, as discussed previously). While cells and subcellular organelles such as the nucleus and mitochondria are within the Mie scattering regime, extracellular matrix elements such as collagen fibrils fall in the Rayleigh scattering regime. In suspensions containing both small and large scatterers, Ghosh et al. have shown that scattering behavior is dominated by smaller particles. This was also observed in simulations whereby small organelles were shown to contribute significantly more to the total scattering than large ones. However, it is important to note that the exact mechanism remains unclear.

The other important determining factor is the relative refractive index. Based on another study by Ghosh et al., if a phantom has large scatterers and a high anisotropy factor but a small relative refractive index contrast, it does not fall in the Mie scattering regime (e.g., circular polarization is no longer preserved for high contrast tissue simulating phantoms). For the latter, they invoked the mechanisms of correlated (dependent) scattering engendered by dense packing to explain their findings. The preferential retention of linear polarization states observed in our study is also indicative of Rayleigh scattering, although the exact mechanism remains unclear. Given the complicated nature of tissue scattering, likely both small scatterers and large scatterers (with small relative refractive index and large anisotropy factor g), augmented by
compacting, are contributing. It is also worth mentioning that in all these studies, linear and circular degrees of polarization were based on the Stokes parameters of the light beam and thus depend somewhat on the input light parameters and are unable to account for other tissue polarizing effects; in contrast, our reported results are derived from the tissue Mueller matrix via polar decomposition and thus are free of these two confounding effects. Despite these differences, both approaches suggest the preferred preservation of linear polarization states over circular polarization states in biological tissues. We are currently conducting a controlled phantom study to explore further the linear and circular depolarization behavior as a method to ascertain the dominant scattering regime and the essential medium characteristics determining it.\(^{31}\)

4 Conclusions

In summary, we have measured depolarization and optical properties in a variety of biological tissues, and we have quantified the correlations between them. We used polarized light imaging and Mueller matrix polarization decomposition to extract linear, circular, and total depolarizations in several freshly excised bulk swine tissues (liver, tendon, kidney cortex, brain, myocardium muscle, and loin muscle). Both transmission and reflection geometries were used to examine 2-mm and 1-cm tissue slabs. Bulk optical properties of the different tissues were measured and related to the derived depolarization behavior. A linear correlation between transport albedo and depolarization was observed and quantified. Furthermore, in highly anisotropic tissues like cardiac muscle and tendon, birefringence increases depolarization over and above the multiple scattering effects, as determined by the high transport albedo (i.e., in spite of the relatively low \(a'\) of these birefringent tissues). Also observed was the preferential preservation of linear over circular polarized light in all tissue types and for all detection geometries. A tentative explanation invoking effective scatterer size and relative refractive index contrast was provided. Finally, for high-transport albedo tissues such as the brain and tendon, light experiences more depolarization through propagation in the forward direction (transmission mode) compared to the backscattering direction. In fact, transmission detection geometry becomes untenable for polarimetry applications for even 2-mm samples of brain and tendon because of extensive polarization loss. Detailed studies of depolarization behavior in various bulk animal tissues should help researchers understand the underlying biophysics of polarized light-tissue interactions, provide polarization properties of various tissue types that are generally unavailable in the literature, and assist in experimental design for polarized light imaging and for polarization-based tissue characterization studies.

Acknowledgments

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References

2-2. Mueller matrix asymmetry to identify axial heterogeneity of birefringence in turbid media

Mueller matrix decomposition provides effective values of different optical effects through the sampling depth; however, many anisotropic tissues are made of layers with different anisotropies. The following paper, “Detecting axial heterogeneity of birefringence in layered turbid media using polarized light imaging,” published in Biomedical Optics Express, is focused on the information that the Mueller matrix can provide on axial heterogeneity of anisotropy in tissues. In this paper, we first established definite symmetry relations between the off-diagonal elements of the Mueller matrix in turbid anisotropic media and then hypothesized that the axial heterogeneity decreases the symmetry. To confirm the derived theory with simulation, we used the Monte Carlo code. A polarization sensitive Monte Carlo (PolMC), in C++ platform, was developed by Dr. Daniel Cote in our group. Later, Dr. Michael F. G. Wood enhanced the code with incorporating the simultaneous occurrence of optical activity and birefringence using the N-matrix approach [61]. For the purpose of investigating heterogeneity of birefringence, I extended the PolMC code even more. The turbid samples in the new code can have two layers with different birefringence properties (values and extraordinary axis orientations). To my knowledge this is the first developed simulator that can model polarized light propagation in bi-layered turbid media. Using this code then, Mueller matrices of heterogeneous and homogenous anisotropic turbid media were simulated and investigated for symmetry properties. To validate the observed trends from simulation, I have used polyacrylamide phantoms. We have an optimized recipe of fabricating scattering polyacrylamide phantoms that can be stretched to produce a proportional birefringence along the stretching direction. They have been previously used by Dr. Michael F. G. Wood to calibrate polarimetric point measurement systems [61]. I
developed a new experimental setup for imaging these phantoms and further combined two layers to achieve bi-layered turbid phantoms with different anisotropic properties in each layer.
Detecting axial heterogeneity of birefringence in layered turbid media using polarized light imaging

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Abstract: The structural anisotropy of biological tissues can be quantified using polarized light imaging in terms of birefringence; however, birefringence varies axially in anisotropic layered tissues. This may present ambiguity in result interpretation for techniques whose birefringence results are averaged over the sampling volume. To explore this issue, we extended the polarization sensitive Monte Carlo code to model bi-layered turbid media with varying uniaxial birefringence in the two layers. Our findings demonstrate that the asymmetry degree (ASD) between the off-diagonal Mueller matrix elements of heterogeneously birefringent samples is higher than the homogenously birefringent (uniaxial) samples with the same effective retardance (magnitude and orientation). We experimentally verified the validity of ASD as a birefringence heterogeneity measure by performing polarized light measurements of bi-layered elastic and scattering polyacrylamide phantoms.

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OCIS codes: (170.3660) Light propagation in tissues; (110.0113) Imaging through turbid media; (260.1440) Birefringence; (260.5430) Polarization.

References and links


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1. Introduction

Diseases such as cancer, myocardium infarction and bladder obstruction involve microstructural alterations in the biological tissues [1–4]. One of these microstructural characteristics is anisotropy caused by fiber (and/or cellular) alignment which can be quantified as birefringence [5]. Birefringence is usually derived from the tissue’s Mueller
matrix and polarimetric measurements [3,4,6–10]. But tissue properties, including birefringence, may be spatially varying; in particular, layered tissue often exhibits depth-dependent properties in its different layers. Thus, the Mueller matrix measured via polarized light imaging contains contribution from the entire sampling volume, and the derived birefringence is the effective value. However, as revealed by microscopy studies, biological tissues are often composed of layers with different anisotropy [11,12]. The question then becomes whether the derived anisotropy metrics arise from a homogeneously anisotropic (uniaxial) material, or instead represent an effective average from a heterogeneously anisotropic one. One approach to resolve this ambiguity altogether is to perform depth-resolved acquisition of polarimetric data and from there derive the birefringence information of the different layers. Several groups have demonstrated Mueller matrix measurement of different tissues using polarization sensitive optical coherence tomography (PS-OCT) [13–15]. Although this technique has been used to map the birefringence values in the 3D structure of various tissues, it suffers from low penetration depth. A similar drawback is encountered in depth-resolved linear and non-linear microscopies with polarization-sensing capabilities [16].

While the depth resolve techniques can determine the birefringence magnitude and the orientations projected in the imaging plane within their axial resolution limit, the Mueller matrix itself obtained from the polarimetric depth-averaged measurements contains a lot of information, including potentially an answer to the above homogeneous versus heterogeneous question. The depth averaged Mueller matrix can be experimentally measured in biological tissues both in transmission and backscattering mode [17]. Depending on the tissue’s optical properties (absorption, scattering magnitude and anisotropy) and the detection angle, the Mueller matrix can be representative of few millimeters deep into the tissue [3,17,18]. Of great importance is the cumulative phase retardance \( \delta \) \((= 2\pi \cdot \Delta n \cdot d/\lambda)\) which is proportional to the birefringence \( \Delta n \) and the average pathlength of photons \( d \) at wavelength \( \lambda \). Retardance can be found from polar decomposition of Mueller matrix. In polar decomposition, Mueller matrix of the tissue is represented as a product of an equivalent retarder \( M_R \), diattenuator \( M_D \) and depolarizer \( M_\delta \) [19]:

\[
M = M_R M_D M_\delta
\]

From the retarder matrix \( M_R \), the retardance magnitude \( \delta \) can be derived as a measure of tissue anisotropy [1–4,19]:

\[
\delta = \cos^{-1}\left[\frac{\sqrt{2} \left( M_R(2,2) + M_R(3,3) \right)^2 + (M_R(3,2) - M_R(2,3))^2}{2 - 1}\right]
\]

As mentioned above, the underlying causes of the polar decomposition derived effective retardance are ambiguous, in that different combinations of \( \delta s \) from different depths are possible that yield similar results. The matrix \( M_R \) can be further decomposed to a linear part and a circular part [20,21]. From the linear part \( M_{LR} \), the retardance orientation (slow axis) can be obtained as \[2,3\]:

\[
\theta = 0.5 \tan^{-1}\left( \frac{M_{LR}(3,4) - M_{LR}(4,3)}{M_{LR}(4,2) - M_{LR}(2,4)} \right)
\]

\( \theta \) can be regarded as the anisotropy direction if the turbid media is homogenously birefringent (in this paper we assume positive birefringence and thus \( \theta \) is the extraordinary axis as well [20]). Another parameter derived from polar decomposition is the retardance ellipticity which may give an indication of birefringence (in)homogeneity [3,20,21]. However, without resorting to polar decomposition analysis and assumptions, we note that the Mueller matrix itself possesses interesting symmetry properties, depending on the sample’s composition and structure, and thus may shed light on the tissue birefringence (in)homogeneity problem. For example, several studies have shown that the images of off-diagonal elements of the non-
birefringent, homogenous scattering media. Mueller matrix are symmetric, in both transmission and backscattering [22–27]. These symmetric properties have been studied by polarization-sensitive Monte Carlo (MC) simulations and observed experimentally from Mueller matrices acquired from scattering liquid phantoms using polarized light imaging [11, 27]. Further, Mueller matrix of a non-scattering retarder is symmetric too, so it remains to be seen how these symmetries combine when one deals with a complex material such as tissue that exhibits both turbid and birefringent properties, and whether inhomogeneities in the latter can be ascertained.

In this paper, we derive the symmetry properties of the transmission Muller matrix of birefringent turbid media, and show that it does indeed exhibit symmetric properties. Next, we extend our polarization sensitive Monte Carlo (PolMC) code to model bi-layered anisotropic media with arbitrary anisotropy values and directions in the layers, to examine the heterogeneous birefringence case. To simplify the problem, in this paper we limit the numbers of the layers to two, and investigate the Mueller matrix properties of these bi-layered birefringent turbid media. From the simulation results, we demonstrate how heterogeneity affects the Mueller matrix elements and their symmetry patterns. Moreover, we define a symmetry metric to quantify the uniformity of birefringence in bi-layered anisotropic turbid media. Finally, we validate the simulation results with experimental images of Mueller matrices measured in transmission geometry from anisotropic (both homogenous and heterogeneous) bi-layered scattering polyacrylamide phantoms.

2. Mueller matrix of turbid homogenous (uniaxial) birefringent media from polarization sensitive Monte Carlo simulations

Recently, a polarization-sensitive Monte Carlo (PolMC) code has been developed by Wood et al. which can simulate photon propagation in birefringent and chiral turbid media [28, 29]. We use this simulation platform to examine the symmetries of the Mueller matrix from homogenous anisotropic turbid media, to be followed by extended PolMC simulations of heterogeneous bi-layered anisotropic media (different anisotropy orientations in the two layers). PolMC prediction will be validated experimentally with Mueller matrix measurements in scattering birefringent phantoms.

First, we analyze the symmetry properties of the Mueller matrix of a homogenously birefringent turbid media as is calculated from Monte Carlo. We model the uniaxial birefringent and turbid medium as a box with ordinary refractive index $n_o$, extraordinary refractive index $n_e = n_o + \Delta n$ (with $\Delta n$ being the birefringence magnitude) and extraordinary axis $\theta$ relative to the x axis as shown in Fig. 1a). Let us denote the forward transmission Mueller matrix at the detection facet by $M(x, y)$, as depicted in Fig. 1b).

To calculate $M$, many photons with different polarizations are propagated in the turbid media. Depending on the medium’s optical properties (scattering and absorption coefficients), photons are scattered and propagated along different trajectories as per standard MC modeling [28–30]. The scattering events are incoherent and not correlated, therefore, $M(x, y)$ is the sum of the Mueller matrices of all the trajectories:

$$M(x, y) = \sum_k M_k(x, y)$$  \hspace{1cm} (4)

where $k$ is the index of each individual trajectory. $M_k(x, y)$ will be proportional to the product of Mueller matrices $M_j$ of consecutive scattering events in each trajectory $k$:

$$M_k(x, y) \propto \left\{ \prod_{j=1}^{N_k} M_j(r_j, \xi_j, \phi_j) \right\}_{r, y}$$  \hspace{1cm} (5)

where $(r, \xi, \phi)$ denotes the polar coordinate system and $N_k$ is the number of scattering events in the trajectory $k$. For notational simplicity, we will write $M_j(r, \xi, \phi)$ as $M_j$. The symmetry
properties of $M_j$, the Mueller matrix between two scattering events, will influence the symmetry properties of $M_k$, the Mueller matrix of that particular path, and thus the final sample Mueller matrix $M(x,y)$. $M_j$ itself can be represented as a product of Mueller matrices which account for different optical effects acting on photons. Upon each scattering, the photon’s reference frame is changed to the scattering plane (rotation of $\phi_j$ degrees), and the single scattering Mueller matrix is applied [28–32]. To incorporate birefringence after each scattering event, the reference frame of the photons is rotated by $\beta_j$ degrees, for the parallel polarization of the photon to be parallel with the direction of maximum refractive index (which is the projection of orientation $\phi$ in the plane perpendicular to the propagation direction), as described in detail in Wood et al. [28]. Accounting for both effects, the total $M_j$ in the global coordinate (lab’s reference) frame can be written as

$$M_j = R(-\beta_j)M_{s}(g_j)R(\beta_j)R(-\phi_j)M_{s}(\psi_j)R(\psi_j)$$

(6)

where $M_{s}(g_j)$ is the retardance Mueller matrix and has the form [20]
\[ M_\delta(g_j) = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & \cos g_j & \sin g_j \\ 0 & 0 & -\sin g_j & \cos g_j \end{bmatrix} \]  

\[ g_j = \pi \Delta n(\phi) dz/\lambda \] is half the retardance over the short path length \( dz \) between the two scattering events and \( \Delta n(\phi) \) is the difference between the refractive indices seen by the photon calculated as [28]

\[ \Delta n(\phi) = n(\phi) - n_a = \frac{n_x n_y}{(n_x^2 \cos^2 \phi + n_y^2 \sin^2 \phi)^{1/2}} \]  

where \( \phi \) is the angle between the photon propagation direction after the scattering event \( j \) and the extraordinary axis. The total retardance \( \delta \) of the medium is the accumulation of the retardances \( 2g_j \) over the whole path length along the trajectory \( k \). \( R(\beta_i) \) and \( R(\zeta_j) \) are the rotation matrices which rotate the photon reference frames and can be written as (with \( a \) representing \( \beta_i \) or \( \zeta_j \)) [20]

\[ R(\alpha) = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos 2\alpha & \sin 2\alpha & 0 \\ 0 & -\sin 2\alpha & \cos 2\alpha & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \]

Scatterers in homogenous turbid media can often be identical spherical particles, hence, \( M_\delta(\psi) \) can be the single scattering Mueller matrix for a spherical scatterer, with \( \psi \) being the angle of the scattering. It can be written as

\[ M_s(\psi_j) = \begin{bmatrix} a(\psi_j) & b(\psi_j) & 0 & 0 \\ b(\psi_j) & a(\psi_j) & 0 & 0 \\ 0 & 0 & c(\psi_j) & d(\psi_j) \\ 0 & 0 & -d(\psi_j) & c(\psi_j) \end{bmatrix} \]

and its \( a, b, c, \) and \( d \) elements are calculated from Mie theory [23]. Thus knowing the form of the rotation, retardance and scattering matrices as per Eqs. (8)–(10), we can find the symmetric properties of the Mueller matrix \( M_j \). Following the approach presented by Rakovic et al. [24], we should find a projection matrix \( P \) which reveals the symmetry properties of the matrices in Eqs. (8)–(10). Let us define the diagonal matrix \( P \) as

\[ P = \text{diag}(1, 1, 1, -1) \]

and \( P^2 = I \) being the identity matrix. With direct calculation, it can be shown that

\[ [R(-\zeta_j)M_s(\psi_j)R(\zeta_j)]^T = P[R(-\zeta_j)M_s(\psi_j)R(\zeta_j)]P \]

\[ [R(-\beta_j)M_s(g_j)R(\beta_j)]^T = P[R(-\beta_j)M_s(g_j)R(\beta_j)]P \]

where \( M^T \) is the transpose of \( M \). From matrix product properties \( \text{transpose}(A \times B) = \text{transpose}(B) \times \text{transpose}(A) \), then plugging Eq. (12) into Eq. (6) results in

\[ M_j' = P[R(-\zeta_j)M_s(\psi_j)R(\zeta_j)][R(-\beta_j)M_s(g_j)R(\beta_j)]P \]

On the other hand, plugging Eqs. (8)–(10) into Eqs. (6) and (13), it can be shown that the order of the multiplication in Eq. (6) is not important; that is the order of applying scattering
or retardation over short pathlength \( dz \) will not change the total result on the photon. Therefore, Eq. (13) can be rewritten as

\[
M'_j = PM'_jP \tag{14}
\]

Using Eq. (14) in Eq. (5),

\[
M'_j = \prod_{j \in \mathcal{R}_j} M'_j = P(\prod_{j \in \mathcal{R}_j} M_j)P \tag{15}
\]

The reverse order of the multiplication in Eq. (15) is equal to a change of reference from \((x,y,z)\) to \((x,-y,-z)\), changing the extraordinary axis from \(\mathbf{\xi}\) in the old coordinate to \(\mathbf{\xi}\) in the new coordinate and switching the input/output plane as shown in Fig. 1.c relative to Fig. 1.a). Both these configurations result in the same forward Mueller matrix \(M(x,y)\). Hence, in a homogenously birefringent medium, the reverse order of the events in each trajectory is the same as the regular order and Eq. (15) can be rewritten as

\[
M'_j = P(\prod_{j \in \mathcal{R}_j} M_j)P = PM_kP \tag{16}
\]

Equation (16) reveals the symmetric properties of \(M_k\) along each trajectory \(k\) and dictates its general form. The general form of \(M_k\) that satisfies Eq. (16) is

\[
M_k = \begin{bmatrix}
M_{11} & M_{12} & M_{13} & M_{14} \\
M_{21} & M_{22} & M_{23} & M_{24} \\
M_{31} & M_{32} & M_{33} & M_{34} \\
M_{41} & M_{42} & M_{43} & M_{44}
\end{bmatrix}
\]

As can be seen, the off-diagonal elements in Eq. (17) exhibit certain symmetries (\(M_{12} = M_{21}; M_{13} = M_{31}; M_{23} = M_{32}; M_{642} = -M_{462}; M_{242} = -M_{424}; M_{142} = -M_{414}\)). The above form holds for the matrix \(M(x,y)\) as well, since it is the summation of all the \(M_k\) (all the trajectories). Therefore, the symmetry in Eq. (17) will be evident in terms of the symmetry in the images of the off-diagonal elements of \(M(x,y)\), denoted in the text by \(M_{ij(ej)}\). For example, images of elements \(M_{23}\) and \(M_{32}\) are positively correlated. Similarly, the spatial patterns in the elements \(M_{24}\) and \(M_{42}\) or \(M_{34}\) and \(M_{43}\) are negatively correlated. These symmetry properties thus describe the Mueller matrix of homogenously birefringent turbid media.

When we proceed to the more difficult case of heterogeneity in birefringence, the order of the multiplication will determine the final outcome, and hence the inhomogeneous matrix \(M_k\) will not follow the form of Eq. (17). As such, the symmetry properties of \(M(x,y)\) can be used as metrics for identifying axial birefringence heterogeneity in the material. Mueller matrix of a pure retarder has non-zero values for the 9 lower right corner elements [20]; as such, these elements are more correlated to birefringence even in a turbid medium. In fact, the asymmetry between the 6 off-diagonal elements in the lower right corner of the Mueller matrix can be a measure of birefringence heterogeneity in a turbid medium (with homogenous optical properties). Thus, we will use the asymmetry between the images of elements \((M_{24}, M_{42}), (M_{34}, M_{43})\) and \((M_{23}, M_{32})\) to detect the heterogeneity in birefringence, as described in the next section.

3. Mueller matrix of turbid heterogeneous (bi-layered) birefringent media from polarization sensitive Monte Carlo simulations

We now modify the above PolMC code [28] to enable simulation of bi-layered anisotropic turbid media. In the new code, each of the layers in the structure can have arbitrary uniaxial birefringence (magnitude and direction). For this study, we simulate bi-layered cubes of 2cm...
× 2cm × 2cm in dimensions; the two layers have the same thickness and optical properties (e.g., composed of homogenous uniform scattering microspheres, with typical tissue-like scattering properties); also no optical activity is assumed for simplicity [33–35]. For biomedical relevance, we mimic the kidney’s reduced scattering coefficient, reported in Alali et al. [17]. Optical thickness equal to 2 mm of kidney tissue was chosen for the simulations, by setting the phantoms total thickness to 2 cm and its scattering coefficient to 6 cm⁻¹ [17]. The absorption level was minimal, equal to water absorption at 635 nm [28]. The scatterers were modeled to be polystyrene beads with 1.2 µm diameter and refractive index of 1.59, to facilitate comparison with subsequent experiments.

The geometry of the bi-layered problem simulated with the extended version of PolMC is sketched in Fig. 1.d. As illustrated, the two layers can have different properties such as different anisotropy directions. A polarized pencil beam (~3 × 10⁸ photons) was shone on the center of the layer 1 and the polarization state of the photons at each pixel of the external facet (in transmission mode) of the layer 2 are tabulated. The sample was illuminated with 4 different incident polarizations (linear at 0°, linear at 90°, linear at 45° and right circular) and for each case, the number of the photons with 4 different polarizations (linear at 0°, linear at 90°, linear at 45° and right circular) were counted. From these 16 outcomes, Mueller matrix of the sample in transmission mode $M(x,y)$ was calculated, as described by several authors [21,29]. For these simulations, the magnitude of the birefringence in the two layers was kept same but the relative orientation was varied.

Prior to performing the Mueller matrix symmetry analysis suggested by Eq. (16), the obtained Mueller matrix was subjected to polar decomposition to determine the effective retardance $\delta_{\text{eff}}$ and orientation $\theta_{\text{eff}}$ [1–4,7–10,19]. To investigate the effect of heterogeneity, equivalent homogenous (EH) samples (same magnitude and orientation of birefringence in the two layers) resulting in the same value of $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$ were simulated in the new PolMC code. Each of the layers in the homogenous samples has the same anisotropy direction $\theta_{\text{eff}}$ and half of the effective retardance value $\delta_{\text{eff}}/2$. These simulations will thus highlight the differences in Mueller matrices of homogenous and heterogeneous samples as a function of birefringence orientation difference in the two layers. We are expecting the homogenous birefringent sample to show higher degree of symmetry between their off-diagonal Mueller matrix elements (as per Eq. (17)) compared to axially heterogeneous birefringent samples. To quantify the symmetries in equivalent homogenous (EH) and heterogeneous samples, we define the asymmetry degrees (ASD) based on Eq. (17), as the sum of normalized differences (or sums if there is a sign change) between the off-diagonal elements - excluding those of the first column and row because of their lower values as mentioned before—as follows:

$$\text{ASD} = \sum_{l} |\text{ASD}_l|, \quad l = 1, 2, 3$$

(18)

with

$$\text{ASD}_1 = \sum_{i,j} \frac{m_{ij}(x_i, y_j) - m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))} + \frac{m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))}, \quad i, j = 1, ..., N$$

$$\text{ASD}_2 = \sum_{i,j} \frac{m_{ij}(x_i, y_j) + m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))} + \frac{m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))}, \quad i, j = 1, ..., N$$

$$\text{ASD}_3 = \sum_{i,j} \frac{m_{ij}(x_i, y_j) + m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))} + \frac{m_{ji}(x_i, y_j)}{\max(m_{ij}(x_i, y_j))}, \quad i, j = 1, ..., N$$

(19)

where $m_{ij}$ is defined as the off-diagonal Mueller matrix element $M_{ij}$ normalized by $M_{11}$, $x_i$ and $y_j$ are the spatial position and $N$ is the number of the pixels in each dimension of the element’s
image. Normalization of $m_{ij}$ with respect to its maximum in the defining equations above will help quantify the difference in the spatial profile (patterns) in the image, rather than the differences in the magnitude of the off-diagonal elements. The ASD metric will give an indication of the sample’s axial heterogeneity in comparison to its EH counterpart.

In general, we expect ASD values to increase as we proceed from birefringently homogeneous to heterogeneous samples. However, it is important to note that ASD will vary even among different homogenous samples, for example depending on birefringence orientation or sample turbidity. In another words, two homogenous samples with different $\Delta \alpha_{\text{eff}}$ and $\theta_{\text{eff}}$ will have different ASD values. For example, if the values of the elements in the Mueller matrix of a pure retarder with retardance $\delta_{\text{eff}}$ and orientation $\theta_{\text{eff}}$ are large, then the symmetry in Eq. (16) will be more manifest, yielding lower ASD [21]. Otherwise, owing to relatively small magnitude of the off-diagonal elements of a pure scatterer, the spatial symmetry in the images will be less and the total ASDs will be higher. Thus, heterogeneity can be best gauged by comparing the unknown sample’s ASD to its equivalent homogenous ASD ($\text{ASD}_{\text{EH}}$). Moreover, depending on the values of $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$, different axial heterogeneities will yield different deviations from the corresponding $\text{ASD}_{\text{EH}}$.

4. Simulation results

4.1. Modeling heterogeneous anisotropic samples and their equivalent homogenous (EH) counterparts

Using Monte Carlo simulations, we investigate how varying birefringence direction with depth will change the ASD in a bi-layer turbid system compared to a uni-directional ASD $\text{EH}$. For this initial study, we chose to examine the effects of direction change only; the possible parameter space to explore is simply too large (orientation and magnitude values and their changes, varying layer thicknesses, varying optical properties in the layers, etc), so we start with simple, and biologically relevant, case of depth-dependent anisotropy axis change. The modeled samples I, II and III have the same constant birefringence magnitude and orientation in the first layer, but a different orientation relative to the first one (equal to $30^\circ$, $60^\circ$ and $90^\circ$) in the second layer. Table 1 shows the birefringence magnitude and orientation of the two layers in sample I, II and III. Also shown are the $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$ values derived from polar decomposition of the PolMC-generated Mueller matrix. $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$ listed in Table 1 are the mean value of the images ($\delta_{\text{eff}}(x,y)$ and $\theta_{\text{eff}}(x,y)$) obtained from polar decomposition.

Based on the derived $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$ from these birefringently heterogeneous samples, the next task was to generate Mueller matrices for EH samples (homogenously birefringent samples) which exhibit the same $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$. Obviously, the birefringence orientation in both layers of an EH sample should be equal to $\theta_{\text{eff}}$. But choosing the appropriate birefringence $\Delta n$ value is not trivial, since the samples are turbid and the overall pathlength is not known before simulations. So we ran several PolMC simulations, with birefringence orientation $\theta_{\text{eff}}$ and different birefringence magnitudes $\Delta n$. After some trial and error, the parameter values for the three EH samples (to match the heterogeneous samples I-III) were selected, as shown in Table 2 (compare its last two columns with those in Table 1). We now have the PolMC-generated Mueller matrices for the three heterogeneous samples and their EH counterparts, and can proceed with ASD analysis (Eqs. (15)–(17)).

Table 1. Birefringence magnitude and orientation in each layer of the heterogeneous samples and the bi-layered sample’s effective retardance magnitude and orientation calculated from polar decomposition

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta n_1$</th>
<th>$\Delta n_2$</th>
<th>$\theta_1(\circ)$</th>
<th>$\theta_2(\circ)$</th>
<th>$\delta_{\text{eff}}(\circ)$</th>
<th>$\theta_{\text{eff}}(\circ)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$1.15 \times 10^{-5}$</td>
<td>$1.15 \times 10^{-5}$</td>
<td>0</td>
<td>30</td>
<td>$108 \pm 3$</td>
<td>$8 \pm 1$</td>
</tr>
<tr>
<td>II</td>
<td>$1.15 \times 10^{-5}$</td>
<td>$1.15 \times 10^{-5}$</td>
<td>0</td>
<td>60</td>
<td>$59 \pm 4$</td>
<td>$32 \pm 3$</td>
</tr>
<tr>
<td>III</td>
<td>$1.15 \times 10^{-5}$</td>
<td>$1.15 \times 10^{-5}$</td>
<td>0</td>
<td>90</td>
<td>$23 \pm 4$</td>
<td>$90 \pm 5$</td>
</tr>
</tbody>
</table>
Table 2. Birefringence magnitude and orientation in each layer of the equivalent homogenous samples and the bi-layered sample’s effective retardance magnitude and orientation calculated from polar decomposition

<table>
<thead>
<tr>
<th>Samples</th>
<th>ΔQ</th>
<th>θ(°)</th>
<th>δ_{eff}(°)</th>
<th>θ_{eff}(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH I</td>
<td>9.4 × 10^{-6}</td>
<td>7</td>
<td>106 ± 3</td>
<td>7</td>
</tr>
<tr>
<td>EH II</td>
<td>5.53 × 10^{-6}</td>
<td>35</td>
<td>63 ± 4</td>
<td>35</td>
</tr>
<tr>
<td>EH III</td>
<td>2.7 × 10^{-6}</td>
<td>90</td>
<td>23 ± 4</td>
<td>90</td>
</tr>
</tbody>
</table>

4-2. Symmetry analysis of the Mueller matrix images

Figure 2 demonstrates the images of the off-diagonal Mueller matrix elements (normalized to M_{11}) of the sample I-III and their respective EHs respectively. As seen, the homogenous samples follow the symmetry patterns predicted in Eq. (17); however, as mentioned previously the symmetries are different among EHs. To quantify the symmetry of these Mueller matrix elements, we calculated ASD numbers from Eqs. (18)–(19) (Table 3).

Table 3. Asymmetry degrees of the axially heterogeneous samples and their EH counterparts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample’s ASD</th>
<th>EH’s ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.0084</td>
<td>0.0014</td>
</tr>
<tr>
<td>II</td>
<td>0.0429</td>
<td>0.0017</td>
</tr>
<tr>
<td>III</td>
<td>0.0902</td>
<td>0.0354</td>
</tr>
</tbody>
</table>

It can be concluded from Table 3 that the heterogeneous anisotropic samples show higher ASDs compare to their EHs. In other words, axial change in the extraordinary axis direction reduces the symmetry and increases the ASD, relative to a homogenous sample with the same effective retardance and orientation. Therefore, ASD of the Mueller matrix can be used as a metric to identify axial heterogeneity of anisotropy in turbid media. However, this measure is relative and should be used cautiously in light of the following considerations.

The spatial profile of effective retardance and orientation that are calculated from polar decomposition are not uniform over all the pixels, so their values are presented in terms of mean and standard deviation in Tables 1 and 2. As such, it is very difficult to simulate an EH sample which result in the exact desired image of effective retardance and orientation (equal to those of the heterogeneous sample). That’s why the mean values of the retardance and orientation of the samples and their EHs, listed in Tables 1 and 2, are close but slightly different. As mentioned before, homogenous variations in effective retardance and orientation leads to deviation in ASD numbers as well. For instance, uniform axial changes of ± 10% in δ_{eff} and θ_{eff} in the homogenous sample EH II causes a ΔASD_{EH} of ~15%. Given this uncertainty in ASD_{EH}, for a sample with effective values of δ_{eff} and θ_{eff} to be classified as heterogeneous, its ASD should obey the condition: (ASD_{sample} - ΔASD_{EH}) / ASDEH > 1. This condition will ensure that the ASD difference between the sample and its EH arises solely from heterogeneity. For example, the large difference between the ASDs in sample II and EH II, is due to heterogeneity and not from homogenous variations in δ_{eff} and θ_{eff}. This condition holds for samples I-III verifying their heterogeneity.

An additional important point to note is that the relative ratios of the ASD_{sample}/ASD_{EH} are different for each case and cannot be regarded as a measure of higher or lower heterogeneity. For example, ASD_{sample}/ASD_{EH} of sample II is about 8 times that of sample III, while the heterogeneity (in this case change of anisotropy axis) is larger in sample III.

Therefore, the heterogeneity strength cannot be inferred unless a look up table of heterogeneous samples ASD with the equal value of δ_{eff} and θ_{eff} are prepared. Generating such a table requires high computational power to run the PolMC code many times.

As will be discusses in the next section, ASD can be used in polarized light imaging to identify axial change of anisotropy. However, experimental availability of EH samples is challenging. In practice for biomedical diagnosis, one possibility would be to measure ASD of
Fig. 2. Simulation results of extended PolMC. Images of the Mueller matrices’ off-diagonal elements (normalized to M_{11}) of samples I, II, and III and their equivalent homogenous samples which result in the same value of effective retardance magnitude and orientation. The color bar shows the relative values of the element in x and y direction. The scale bar is 2 cm. Thickness of all the layers is 1 cm and the media’s scattering coefficient is 6 cm\(^{-1}\). Anisotropy properties of the samples can be found in Tables 1 and 2.

the normal and abnormal birefringent tissue, to detect axial change of fiber orientation in the latter. Yet to characterize the strength of orientation change, careful MC simulations (with the
tissue’s optical properties) should complement the experiment. Finally, ASD can be used to better interpret the results of depth resolved techniques which use Mueller matrix imaging. For example, the depth resolved birefringence map obtained by PS-OCT is limited to the OCT axial resolution and ASD can be used to identify sub-resolution heterogeneity/homogeneity of the detected birefringence at each depth in the tissue.

5. Experimental validation with turbid birefringent phantoms

To validate the simulation results experimentally, we used elastic polyacrylamide phantoms with controlled scattering properties (through addition of polystyrene beads) [28,35]. On average, these phantoms exhibit birefringence on the order of $1 \times 10^{-5}$ per 1 mm of stretch [28]. To make a bi-layered anisotropic media, two identical slabs ($6 \text{ cm} \times 4 \text{ cm} \times 1 \text{ cm}$) of polyacrylamide were fabricated, following the recipe explained in [35]. The phantoms’ scattering coefficient was set to 6 cm$^{-1}$, by adding polystyrene beads of 1.2 $\mu$m size and of refractive index of 1.59 using Mie theory [22,36].

Each slab was placed into a custom made puller (Fig. 3). The introduced strain induces a birefringence with the extraordinary axis along the stretching direction. To enable change of birefringence orientation, the pullers were made to rotate freely around their centers, as illustrated in Fig. 3. Each phantom was characterized using transmission mode polarized light imaging method described in [17]. From there the Mueller matrix elements and its effective retardance magnitude and orientation were calculated. The stretch and rotation angle of each phantom was modified to achieve the simulation parameters of the individual layers of sample II of Table 1. Experimental measurement values are listed in Table 4.

To make the heterogeneous bi-layered medium, the two phantoms in the pullers were placed against each other as depicted in Fig. 3(b). Following the same procedure, the sample EH II was made by changing the stretches in the two phantoms (till each yields half of the retardance $\delta_{\text{eff}}$) and rotating their orientation (till it is equal to $\theta_{\text{eff}}$). The properties of each slab and the effective retardance and orientation of the two layers together, found from polar decomposition, are listed in Table 4. As seen, the heterogeneous sample and its EH have a difference of about 20% in the experimental values of $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$. This is the closest we could achieve with this phantom system. Our simulation results indicate that 20% homogenous change in $\delta_{\text{eff}}$ and $\theta_{\text{eff}}$ will result in $\Delta\text{ASD}_{\text{EH}} < 25\%$. 

![Fig. 3. Experimental set up for heterogeneous anisotropy test.](image)
Table 4. Anisotropy properties of the layers in of the samples heterogeneous and its EH polyacrylamide phantoms, their effective values of retardance and slow axes and their ASD

<table>
<thead>
<tr>
<th>Phantoms</th>
<th>$\delta_1$ (°)</th>
<th>$\delta_2$ (°)</th>
<th>$\theta_1$ (°)</th>
<th>$\theta_2$ (°)</th>
<th>$\delta_{\text{eff}}$ (°)</th>
<th>$\theta_{\text{eff}}$ (°)</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterogeneous</td>
<td>60 ± 3</td>
<td>66 ± 2</td>
<td>0</td>
<td>60 ± 2</td>
<td>62 ± 4</td>
<td>35 ± 1</td>
<td>43.3870</td>
</tr>
<tr>
<td>EH</td>
<td>27 ± 2</td>
<td>33 ± 2</td>
<td>31 ± 1</td>
<td>31 ± 1</td>
<td>60 ± 5</td>
<td>31 ± 1</td>
<td>3.4808</td>
</tr>
</tbody>
</table>

The off-diagonal normalized elements of the Mueller matrix of the two bi-layered phantom samples are shown in Fig. 4. The relation between the images of the respective off-diagonal elements of the phantoms in Fig. 4 agrees well with those of the Monte Carlo simulations for sample II, shown in Fig. 2. However, the experimental results show higher asymmetry than the Monte Carlo simulations in both heterogeneous sample and its EH. This is most likely due to noise: experiments with layered scattering polyacrylamide phantoms of controlled and variable birefringence are challenging and prone to many errors. For example, these phantoms dehydrate slightly with time and as a result their birefringence increases over the course of the experiment. Therefore, there might be a slight change of retardance in the layers from the time that we characterize them individually to the time we measure them together.

The ASD numbers of the bi-layered phantoms, calculated from the images in Fig. 4 are listed in Table 4. As expected from the simulations, the experiments now confirm that the heterogeneous sample’s ASD is higher than its EH’s ASD. The measured ASD ratio is less than Monte Carlo predictions, largely due to experimental challenges with this phantom system. Nevertheless, the experimental ratio is large enough to satisfy our suggested condition: $(\text{ASD}_{\text{sample}} - \Delta \text{ASD}_{\text{EH}})/\text{ASD}_{\text{EH}}> 1$, and is consistent with MC. Hence, experiments and MC simulation agree in the trends and both give further credence to the proposed Mueller matrix asymmetry formalism for detecting and quantifying axial birefringence heterogeneity.

6. Conclusion
In this paper, we have developed a protocol to detect depth-dependent heterogeneity in birefringent turbid media using polarized light imaging. Employing polarization sensitive Monte Carlo simulation, we showed that forward Mueller matrix of a homogenous
birefringent turbid medium has symmetric properties about its diagonal. To further examine Mueller matrix symmetry, the polarization sensitive Monte Carlo code was extended to model bi-layered anisotropic media with different anisotropy orientation in each layer. Our simulations show that symmetry between off-diagonal elements of the Mueller matrix can be used as a metric to identify birefringence heterogeneity of a turbid bi-layered medium. To quantify these trends, a metric called asymmetry degree (ASD) was defined from the symmetry between the 6 lower right off-diagonal elements of the Mueller matrix. Based on our results, the procedure to detect birefringence heterogeneity is to first, calculate the effective retardance magnitude and orientation of the sample using polar decomposition; then, to simulate an equivalent homogenous (EH) sample which exhibit the same effective retardance and orientation (from polar decomposition); lastly, to compare the ASD of the unknown sample with its EH’s ASD. Our simulations show that ASD values are larger in heterogeneous samples compared to their EHs. We verified these ASD trends experimentally using polarized light imaging of bi-layered elastic polyacrylamide phantoms. The ASD metric developed in this paper and the procedure of using it for identifying axial heterogeneity of birefringence in turbid media may provide new criteria of characterizing biological tissues with different anisotropic layers.
Addendum

After publishing the paper, I realized that the visual changes in the Mueller matrix, due to the heterogeneity of anisotropy, are better observed if the elements are normalized to their maximum values in the field of view. The normalized images of Fig.2 of the paper are shown here in Fig.2-1. As can be seen, there is a good agreement between the experiment in Fig.4 of the paper (sample II of the paper) and Fig.2-1.

Fig.2-1. Simulation results of extended PolMC. Images of the Mueller matrices’ off-diagonal elements of samples I, II and III and their equivalent homogenous samples which result in the same values of effective retardance magnitude and orientation. The color bar shows the relative values of the elements in x and y direction. The scale bar is 2 cm. Thickness of all the layers is 1 cm and the medium’s scattering coefficient is 6 cm$^{-1}$. Anisotropy properties of the samples can be found in Tables 1 and 2 of the paper.
3. Polarized light imaging as a potential tool to quantify and locate structural disorders in the bladder wall

As discussed in chapter 1, BOO is a prevalent condition which leads to permanent morphological changes in the bladder. The motivation for this chapter is to introduce a potential imaging modality to identify these morphological disorders in terms of changes of anisotropy in distended \textit{ex vivo} bladders. Polarized light imaging which can quantify the anisotropy in terms of optical retardance seems to be an ideal candidate method. We collaborated with Dr. Darius J. Bagli’s lab at the Hospital for Sick Children, who developed a model of \textit{ex vivo} rat bladder (that can be distended) and a rat model of the BOO disease. We obtained \textit{ex vivo} rat bladders (both normal and obstructed) from Dr. Bagli’s lab, as fully described in the following two papers. The first experiment was to fully characterize the anisotropic properties of normal distended bladders.

3-1. Anisotropy assessment in \textit{ex vivo} distended rat bladders using polarized light imaging

A normal functioning \textit{in vivo} bladder goes through cycles of filling and voiding urine that are highly dependent on the neuromuscular interplay between the detrusor muscle in the wall and the sphincters on the outlet [112-113]. During filling, the detrusor muscle stretches and the sphincters contract; whereas in the voiding stage the detrusor contracts and the sphincters relax. The \textit{ex vivo} rat bladders behavior is solely passive (ATP and neural input independent) which allows the detrusor stretching (bladder distension) when there is a pressure difference inside and outside the bladder [112]. Of course, the active contraction of the muscle (ATP and neural input dependent) is not possible in the \textit{ex vivo} model [112].
There are few issues that have to be considered when characterizing the anisotropy of an *ex vivo* distended bladder. The first point is the different regional properties of a bladder. As has been shown by previous biomechanical studies, the passive response of the wall to distension is not the same in different regions of the bladder. For example, the dorsal region has a higher elasticity than the ventral region [16]. Hence, our experiment should be able to characterize the regional anisotropy of a bladder. The second issue is the interplay between the strain induced anisotropy and the ECM remodelling during distension. For instance as suggested by histological studies, the coil shaped collagen fibers become straight during the distension stage, allowing the detrusor muscle to stretch. This phenomenon will appear as a structural change when evaluated by polarized light and optical retardance measurement. At the same time, the stretch of the muscle layer (proportional to the wall thickness change) should induce a stronger alignment seen from the muscle cells and thus higher anisotropy when measured with polarized light. Therefore, the implication of a **regional anisotropy value** of a bladder distended at a specific pressure should be **structural alignment as a combined convoluted effect of the muscle stretch and the ECM remodeling**.

In the following paper, “Optical assessment of tissue anisotropy in *ex vivo* distended rat bladders,” published in the Journal of Bioomedical Optics, the imaging procedure I have developed for characterizing structural properties of *ex vivo* rat bladders during distension is described. Polarized light imaging, followed by Mueller matrix decomposition, was applied in the backscattering geometry to obtain regional optical retardance of the distending bladder walls. The observed quantitative microstructure trends of normal bladder walls were explained in collaboration with Dr. Bagli’s lab and are included in the paper.
Optical assessment of tissue anisotropy in \textit{ex vivo} distended rat bladders

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Abstract. Microstructural remodelling in epithelial layers of various hollow organs, including changes in tissue anisotropy, are known to occur under mechanical distension and during disease processes. In this paper, we analyze how bladder distension alters wall anisotropy using polarized light imaging (followed by Mueller matrix decomposition). Optical retardance values of different regions of normal rat bladders under different distension pressures are derived. Then optical coherence tomography is used to measure local bladder wall thicknesses, enabling the calculation of the tissue birefringence maps as a measure of the tissue anisotropy. Selected two-photon microscopy is also performed to better understand the compositional origins of the obtained anisotropy results. The dome region of the bladder shows maximum birefringence when the bladder is distended to high pressures, whereas the ventral remains roughly isotropic during distension. In addition, the average anisotropy direction is longitudinal, along the urethra to dome. The derived wall anisotropy trends are based on birefringence as an intrinsic property of the tissue organization independent of its thickness, to aid in understanding the structure-functions relation in healthy bladders. These new insights into the wall microstructure of \textit{ex vivo} distending bladders may help improve the functionality of the artificially engineered bladder tissues. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.8.086010]

Keywords: polarized light imaging; retardance; birefringence; anisotropy; bladder, urology; nonlinear microscopy; optical coherence tomography.

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1 Introduction

In several diseases such as bladder obstruction, spinal cord injuries and diabetes, normal bladder function changes as a result of bladder wall morphology alterations.\textsuperscript{1,4} Although these are known to be the main reason for bladder dysfunction, their exact relation to altered bladder functionality is still unclear.\textsuperscript{5} Bladder wall is composed of extracellular matrix (including collagen and elastin) and smooth muscle.\textsuperscript{7} During outlet obstructive diseases, the bladder wall experiences smooth muscle overgrowth (hypertrophy and hyperplasia) or changes in smooth muscle cell orientation, an increase in total wall thickness, and excessive extracellular matrix deposition and remodeling.\textsuperscript{1,5} In many cases, over the long-term these alterations become irreversible, decrease patient quality of life, and increase risk of kidney failure and urinary tract infections. One promising solution is replacing the bladder with engineered tissues.\textsuperscript{9,10} A major outstanding issue in this approach is ensuring comparable functionality through comparable engineered architecture, required for a healthy normal bladder. Thus, currently ambiguous relationships between distension-induced changes in the organization of smooth muscle and extracellular matrix tissues that comprise the bladder wall, and the resulting alterations in bladder function, warrant further investigation.

Several groups have taken a biomechanical characterization approach in quantifying the bladder wall displacement (strain) and correlating it to the liquid pressure inside the bladder. Korossis et al.\textsuperscript{10} mechanically stretched trabeculae from different regions of porcine bladder, and based on the resultant thickness changes in different directions, identified regions with distinct anisotropy and elasticity. To relate the anisotropy to the pressure inside the bladder, approximations such as a spherical bladder shape have been assumed, and Laplace’s law has also been invoked to describe pressure-shape relationships.\textsuperscript{10} In a recent study, displacement has been measured from the surface of the bladder wall while bladders were distended \textit{ex vivo} under controlled pressure and volume of liquid,\textsuperscript{11} from which regional anisotropy and strain have been calculated using Laplace’s law. Although such displacement measurements coupled with biomechanical models are useful to detect elasticity and anisotropy, they do not provide any information on the underlying tissue organization. Therefore, this missing organizational information about bladder wall morphology and microstructure is acquired from thin samples using optical microscopy techniques.\textsuperscript{10,12} Polarized light imaging in bulk intact tissues is beginning to be used for quantitative assessment of morphology in biological tissues.\textsuperscript{13,20} When the tissue polarimetric properties are properly decoupled with the help of polarization data analysis technique known as polar Mueller matrix decomposition,\textsuperscript{20,22} a particular parameter known as retardance emerges. Retardance is a...
measure of tissue anisotropy, which reports on its organized/disorganized nature. It is a product of tissue optical birefringence and sampled tissue thickness. Some tissues are known to be anisotropic (birefringent) in their normal functional state. For example, polarized light imaging has been utilized to determine the micro-structural organization in cardiac muscle, including its alterations due to infarct and post-infarct recovery induced by stem-cell therapies. Cardiac anisotropy was shown to decrease with infarct formation (disorganized collagen scar tissue), and recover towards near-normal anisotropic levels with various forms of regenerative stem-cell therapies.

Furthermore, birefringence can be induced by mechanical forces (strain) in most materials, including biological tissues. In fact, strain induces additional organization, which can be detected optically as birefringence. Birefringence in a distending bladder is a combination of both the birefringence due to intrinsic tissue organization and the strain induced organization.

In this study, we use polarized light imaging to obtain local maps of bladder birefringence (from polarimetry-derived retardance images, normalized by the bladder wall thickness measured with optical coherence tomography). This potential metric of bladder functional status varies spatially in normal bladders and in its regional response to bladder filling pressure, as characterized in this study. We also perform selected non-linear microscopic examinations of the bladder wall, to help understand the polarimetric findings of bladder anisotropy. We believe our results are the first systematic optical characterization studies of normal ex vivo bladder microstructural/organizational changes as related to distension pressure.

2 Materials and Methods

2.1 Ex vivo Bladder Harvesting and Distension

Female Sprague-Dawley rats were anaesthetised under isoflurane, the abdomen was opened and the bladders were exposed. A simple anatomical representation of the bladder and a photo of the ex vivo harvested bladder are shown in Fig. 1. The ureters were ligated with a 5-0 blue vicryl suture on the right side of the rat and with a black 8-0 silk suture on the left. This simple colour coding enabled proper orientation of the bladder and urethra were removed carefully from the animal, which was then sacrificed under anaesthesia according to an institutionally approved animal use protocol. The catheterized bladders were then placed in cell culture medium, phenol red-free Dulbecco’s Modified Eagle Medium (MEM), prior to distension.

To distend the bladders, we used varying pressure head, by varying the height of the liquid reservoir coupled to the bladder via the urethra [Fig. 2(a)]. To ensure that the polarimetric signal was coming from the illuminated spot on the bladder wall, we had to minimize the reflection and scattering from sidewalls and distal portions of the bladder wall; to do so, the liquid in the reservoir-bladder system was a mixture of crimson blue dye dissolved in ethanol (to prevent contributions from bladder regions other than the illuminated region) and oil (to prevent the dye from penetrating into the bladder wall and thus altering the wall tissue optics). This mixture has been optimized empirically, ensuring ∼1.5 h before the bladder wall begins to absorb the dye. For this reason and also to make sure that we are testing the bladders in their fresh condition, all imaging measurements were performed within 1 h. The density of the liquid mixture was measured to be 0.747 g/cm³. The pressure P at the top of the bladder (at the urethra) was then determined according to $P = \rho gh$, where $\rho$ is the oil-dye density, $h$ is the reservoir height and $g$ is the acceleration due to gravity. Three bladders (referred to as 1, 2, and 3 in the text) were distended at three different reservoir heights of 15, 30, and 45 cm, corresponding to pressures of 1.0, 2.2, and 3.3 kPa; this range of pressures in within the relevant physiologic range. With this method, we could image bladder 1 distended at 1.0 kPa, bladder 2 at 2.2 kPa and bladder 3 at 3.3 kPa. This was done instead of distending a single bladder to three different pressures, to avoid any possible hysteresis effects.

2.2 Polarized Light Imaging

To derive the retardance (and from that the intrinsic tissue birefringence, as a measure of its anisotropy) of the distended bladders, we used polarized light imaging in reflection mode [Fig. 2(b)]. A 635 nm diode laser (Thorlabs, Newton, New Jersey) was used as the light source. We generated four different incident polarization states (three linear and one right circular) using a...
waveplate and a polarizer for the sample illumination. The ex vivo bladders were placed on a rotational mount. The light spot on the bladder was ∼3 mm in diameter. Several spots in each anatomical bladder region (dorsal, ventral, and dome) were imaged by rotating the bladder. The imaging was not performed in the regions close to the sutures, since these likely exhibit strain anisotropy induced by the surgical preparation. The angle between the incident beam and collected backscattered light was 25 deg off the exact backscattering direction. Six different output polarization states (four linear and two circular) were collected using a polarizer and a waveplate. The polarized light images were detected by a CCD camera (CoolSNAPK4, Photometrics, Tuscon, Arizona).

From this 24-measurement methodology (four input and six output polarization states), Mueller matrices were calculated at different regions of the bladder at three different distension pressures. The measured Mueller matrix contains several sample polarization effects occurring simultaneously intermixed in a complex way in its 16 elements. One way to extract intrinsic constituent components of potential biological/biophysical significance is to use a technique known as polar decomposition. In particular, a Lu-Chipman decomposition approach has been used to decompose the Mueller matrix into its constituent basis matrices and extract individual polarization effects from these. We have adopted this method for biophotonic applications, validated it with phantoms and Monte-Carlo simulations, and successfully used it in biological tissues. The experimentally measured bladder Mueller matrix was therefore decomposed into the product of three constituent ‘basis’ matrices: a diattenuator matrix $M_D$, a retarder matrix $M_R$, and a depolarizer matrix $M_\Delta$. Mathematically, this can be written as:

$$M = M_\Delta M_R M_D.$$  \hspace{1cm} (1)

Although other multiplication orders are possible, the results for biological tissues are essentially unaltered. From $M_R$, one can obtain the sample linear retardance as:

$$\delta = \cos^{-1}\left\{ \left[ (M_R(2, 2) + M_R(3, 3))^2 \right. \\
+ (M_R(3, 2) - M_R(2, 3))^2 \right]^{1/2} - 1 \right\},$$  \hspace{1cm} (2)

where $\delta$ represents the cumulative relative phase shift incurred by the orthogonal light polarization components in traversing the sample. The retardance matrix $M_R$ can be further decomposed into the product of linear retardance $M_{LR}$ and the optical chirality $M_q$. $\theta$ the orientation of the anisotropy axis (fast axis direction), projected on the plane perpendicular to the incident beam, can be obtained from the $M_{LR}$ elements as:

$$\theta = 0.5 \tan^{-1} \left[ \frac{M_{LR}(4, 2) - M_{LR}(2, 4)}{M_{LR}(3, 4) - M_{LR}(4, 3)} \right].$$  \hspace{1cm} (3)
As mentioned, polar decomposition allows the extraction of the magnitude [Eq. (2)] and dominant orientation [Eq. (3)] of the linear retardance of the interrogated tissue. However, we are interested in bladder intrinsic birefringence (a measure of its anisotropy), and not just retardance, as the latter is influenced by thickness in addition to anisotropic (micro-organizational) changes. The governing equation relating retardance \( \delta \) to both sample intrinsic birefringence \( \Delta n \) and its thickness (in our case, the optical pathlength \( d \)) that the backscattered detected photons have traveled is:

\[
\delta = (2\pi/\lambda)\Delta nd, \quad (4)
\]

where \( \lambda \) is the light wavelength. Knowing the exact pathlength \( d \) in different measurement geometries is challenging, in part due to tissue multiple scattering effects (which preclude a unique value of \( d \), but rather yield a statistical distribution of photon paths, from which various averaged quantities of interest (e.g., average pathlength) can be derived). Hence, absolute birefringence results in backscattering, or most other geometries, are rarely reported. However, average pathlength of photons can be estimated using polarization sensitive Monte Carlo simulations knowing the tissue’s optical properties and the imaging geometry.\(^{32-34}\) We have measured the rat bladder’s optical properties using the spatially resolved steady state diffuse reflectance method described in Kim et al.\(^{35}\) The average value reduced scattering coefficient in normal rat bladders at 635 nm was \( \mu_s = 7 \pm 1 \text{ cm}^{-1} \), giving a transport mean free path of 0.14 cm.\(^{36}\) We have run polarization sensitive Monte Carlo simulations for slabs with the rat bladder optical properties and its anisotropy, and not just retardance, as the latter is influenced by thickness in addition to anisotropic (micro-organizational) changes. Thus the apparent birefringence \( \Delta n_{\text{app}} \) will be different at different detection angles \( \phi \).\(^{37}\) Here we keep the measurement angle \( \phi \) fixed at 25 deg reflection mode, and report differences in apparent tissue birefringence from different bladder regions and under different distension pressures. However, we have developed methods to derive the true \( \Delta n \) that require additional angular projections,\(^{37}\) and these will be applied to the bladder distension studies in future publications.

The linear retardance derived from Eq. (2) is the net retardance of the tissue. However, biological tissues are heterogeneous and the fiber alignments usually vary in spatial micro-domains, often as a function of depth (i.e., different anisotropy orientations in different bladder wall layers). Therefore, the detected net retardance might be smaller than the real retardance of each layer. Yet it is well known that a stack of retarders with different fast axis orientations results in a total nonzero circular retardance; giving an indication if a derived low net retardance value is due to true tissue isotropy or instead caused by this anisotropic heterogeneity effect. Retardance in general can be written as a combination of linear and circular contributions and can be described by the retardance vector as:\(^{22,38}\)

\[
\bar{R} = R(1, a_1, a_2, a_3)^T, \quad (5)
\]

where its magnitude and normalized fast axis can be calculated from \( M_R \) as:\(^{32,38}\)

\[
R = \cos^{-1}\left[tr(M_R) - 1 \over 2\right], \quad (6)
\]

\[
a_i = 1 \over 2 \sin R \sum_{j,k=1}^{3} \epsilon_{ijk} [m_R(j, k)], \quad (7)
\]

where \( \epsilon_{ijk} \) is the Levi-Civita symbol.\(^{22}\) Linear retardance is the sum of the retardances along \( a_1 \) and \( a_2 \); circular retardance (along \( a_3 \)) is not a real phase shift between polarization states and instead appears as a rotation (like an optical activity effect). This geometric phase, also known as Pancaratnam phase or Berry’s phase, occurs when the polarization states are transformed in different local coordinates.\(^{37}\) Knowing that the retardance vector itself can be regarded as a Stokes vector, we can define its ellipticity angle as:

\[
E = 1 \over 2 \tan^{-1}\left( a_3 \over \sqrt{a_1^2 + a_2^2} \right). \quad (8)
\]

The larger the magnitude of \( E \), the larger is the difference between the fast axis orientations of the layers in the heterogeneous tissue. Hence, we can use ellipticity angle to detect regions of the bladder wall with depth-dependent varying fiber alignments.

### 2.3 Optical Coherence Tomography

To measure the regional thickness of the bladder wall, we used our recently developed Fourier-domain OCT system.\(^{39,40}\)
The system is based on Mach-Zehnder interferometer; the source is a frequency domain mode locked fiber-ring laser with a center wavelength at 1310 nm and a sweep rate of 43 to 67 kHz. A polygon based filter is used to sweep a 112 nm wavelength range. The coherence length is 12 mm, the axial resolution is 9 μm in biological tissues, and the average power is 48 mW. We acquired the B-mode cross-sectional OCT images the bladder wall. The *ex vivo* distended bladders were placed under the OCT probe and several images from different regions were acquired by changing the bladder position. The catheter was inside the bladder in all the experiments and the bladder was filled with the oil-dye mixture to the predetermined distension level.

### 2.4 Multiphoton Microscopy

Multiphoton microscopy has been used in previous studies to investigate the morphology of the bladder wall.\textsuperscript{12,41,42} Similarly, we have used multiphoton microscopy to shed light on the underlying causes of the obtained polarimetric anisotropy signals and gain some information on interrogated tissue composition.\textsuperscript{43} A Zeiss LSM 510 microscope (META NLO, Oberkochen, Germany) with a 63x water immersion objective lens was used to focus a mode locked Ti:sapphire laser at 800 nm. Depth of field was approximately 2 μm and lateral field of view was 140 × 140 μm². The second harmonic generation (SHG) signal, purported to originate primarily from collagen, was collected at 400 nm, in a wavelength window of ±10 nm. The two photon excited fluorescence (TPEF) signal was collected from a spectral window of 430 to 570 nm, and likely represents both smooth muscle and elastin. To flatten the tissue for microscopic examinations, the distended bladder was cut along the apex to dome (see Fig. 1), and placed on a glass coverslip (no top coverslip to flatten the tissue was used, to avoid mechanical artefacts). In order to inhibit the nerve fiber response to slicing and consequently prevent smooth muscle contraction, Oxybutynin was added to the bladder prior to cutting.\textsuperscript{44} The images were taken in reflection mode, at a depth of about 50 μm below the outer surface of the bladder wall.

### 3 Results and Discussion

To quantify the distension pressure/wall thickness relationship, we performed OCT imaging of bladder 1 at 1 kPa, bladder 2 at 2.2 kPa and bladder 3 at 3.3 kPa. Figure 4 shows representative B-mode OCT images, from the dome region of the three bladders. The effect of decreasing wall thickness with increasing distension pressure is evident in these images.

The bladder wall thickness is not as uniform as may appear from the OCT images in Fig. 4. Moving several millimetres to a different spot but still remaining within the same anatomical bladder ‘zone’ (dorsal/ventral/dome) gave rise to ~20% wall thickness variation. The mean values of regional wall thicknesses averaged over ~3 × 3 mm² area of each bladder are shown in Fig. 5. Note the varying magnitude of this effect in different bladder regions; dome exhibit larger thickness decreases than the dorsal and the ventral zones.

As seen in Fig. 5, increasing distension pressure decreases dome thicknesses significantly, but has only minimal effect on the ventral and dorsal wall; specifically, the ventral wall becomes the thickest bladder wall structure at 3.3 kPa. We have examined additional distended bladders (results not shown); although actual numbers vary, the above trends are consistent. Now with this bladder thickness information in hand, we can look up the average pathlength that the photons travel in each bladder wall from Fig. 3 and use it to calculate birefringence from the measured retardance [Eq. (4)].

It is important to note the possible variations in choosing the location of the spots in each zone on different bladders and therefore the necessity for our future studies to evaluate the whole bladder wall anisotropy for larger number of samples to reach statistically significant and clinically relevant results. In this paper, we have chosen illustrative spots with the highest retardance from each region of the distended bladders. The measured retardances varied from 15 deg to 160 deg for the dome, from 10 deg to 78 deg for the ventral and from 8 deg to 114 deg for the dorsal regions. We did not observe phase-wrapping in any of the examined spots. The anisotropy was then calculated using Eq. (4) for each spot. Note that usually anisotropy is gauged from the thickness change or displacement with the approximate assumption of spherical or ellipsoid shape of the examined structure.\textsuperscript{10,11} In contrast, our approach enables the characterization of the bladder with respect to its optical anisotropy (birefringence), independent of its thickness and ‘regular geometry’ assumptions. Resulting images of regional anisotropy ∆n\textsubscript{app} are shown in Fig. 6. The images present 2 mm-diam circular fields of view, chosen from the highest signal to noise ratio (SNR) regions of each 3 mm-diam examined spot.
Maximal anisotropy appears in the dome region distended at 3.3 kPa. The dome is not the most anisotropic region at low and mid pressures (1.0 and 2.2 kPa), but becomes more anisotropic as the pressure increases. Conversely, the ventral wall is nearly isotropic at low pressures, and remains so as the distension is increased. On the other hand, the dorsal wall anisotropy is significant at mid and high pressures. It can thus be concluded that the pressure-anisotropy behaviour in dorsal and ventral regions differs from that in the dome region. Physiologically this seems reasonable, since the dome (the far region from the urethra) experiences high pressures when the bladder is nearly full and probably shows its maximum compliance at the final filling stages. Also, the dome is known to be the most physiologically vulnerable area of the bladder to acute pressure insults, and is most prone to rupture. Further, note that the regional anisotropy-pressure relationship does not follow the regional pressure-thickness correlation depicted in Fig. 4. The polarimetry data hence implies that mechanical strain which results in distension and thickness change is not the only factor leading to change in anisotropy. Most likely, other factors such as organizational changes that are occurring in the different layers and regions of the bladder wall are also responsible for the observed anisotropy (birefringence) changes.

The net birefringence orientation (represented by arrows in Fig. 6) also shows interesting trends. Dorsal and ventral regions both exhibit anisotropic longitudinal alignment along the dome to the urethra. The longitudinal alignment is known to be the dominant direction of the smooth muscle layer as shown previously. Therefore, we surmise that the dominant anisotropy in the bladder wall during distension is due to the stretching of the smooth muscle fibers. The orientation of the anisotropy in the dome follows the general orientation of the fibers in dorsal and ventral regions (note that the dome was examined in a horizontal position as shown in Fig. 2). However, at maximum pressure (∼pathological level) the anisotropy orientations in the dome deviate from the rest of the dominant orientations in bladder wall. In order to explore the reason we evaluated the retardance ellipticity at this spot; recall that this is a metric of depth-dependent birefringence heterogeneity. As shown in Fig. 7, the retardance ellipticity is high at those regions with abnormal orientations. This indicates existence of spatial micro-domain retarders with different fast axis orientations throughout the depth, resulting in small values of net retardance and random like orientations. The retardance ellipticity angle for all other spots in Fig. 6 is lower than ∼0.2 rad (about 20% of the ellipticity in Fig. 7), suggesting more uniform anisotropy orientation throughout those regions of the bladder wall. Another interesting point is that although the average thicknesses of dorsal and ventral regions are similar during different distension pressures (Fig. 5) and they both exhibit dominant longitudinal orientations, their anisotropy values are very different at mid and high pressure (Fig. 6). One possible reason might be the differences in the extracellular matrix organization in these two regions; because as previously has been reported, the extracellular matrix organization and orientation alter during the bladder filling. Thus, to elucidate the nature of the anisotropy–extracellular matrix micro-organization connection, we performed two-photon confocal microscopy at selected depths.
in the dorsal and ventral regions at 3.3 kPa (high pressure). We were not able to keep the curvature of the dome (when distended) with Oxybutynin after cutting the fresh bladder, so our microscopy results are limited to looking at the differences between dorsal and ventral regions. A normal rat bladder was distended up to 3.3 kPa and depth resolved two-photon microscopy images with 2 μm thick slices (depth of field) were acquired from representative spots of its dorsal and ventral regions. To get some idea of the depth dependence of the tissue structures, the Z-stacks of depth resolved slices were acquired around a central plane at 50 μm depth. 3-D rendering of these slices are shown in Fig. 8, where the green colour codes the SHG signal (likely emanating from the collagen fibers), while red shows the TPEF signal (likely from the elastin and smooth muscle compartments—see Materials and Methods for details). The collagen content is not very different in the two regions of the bladder wall (at least at the examined depths); however, as seen in the 2-D (maximum intensity projection) top views (1st column of Fig. 8), it appears more densely packed in the dorsal compared to the ventral regions. On the other hand, the elastin/smooth muscle network in the dorsal area is highly organized compared to the ventral zone (2nd column of Fig. 8). Comparison of Figs. 6 and 8 thus suggests that the organized and compact nature of extracellular matrix in the dorsal wall enhances its birefringence (anisotropy) compared to the ventral structures. Note that deriving a dominant orientation for the extracellular matrix (morphology) in its wall. This relation may prove to be useful in improving tissue engineered bladders function. For example, Helse et al., have shown that mechanical stimulation of the in vitro tissue engineered bladders triggers elastin production and improves bladder compliance. Knowing the regions of the bladder which possess high content of elastin (e.g., dorsal zone according to our results) and their organization, tissue engineers can apply the optimum mechanical stimulation. In addition, our results demonstrate that the different regions of ex vivo distended bladders exhibit very different (thickness independent) anisotropy. Achieving similar regional anisotropy/distension pressure trends in artificial bladders may serve to improve their functionality.

4 Conclusion

Using polarized light imaging for regional birefringence mapping and OCT for local thickness measurements, we examined organizational anisotropy in ex vivo distended rat bladders as a function of distension pressure. The dome region was seen to become most anisotropic with distension, whereas the ventral side remained roughly isotropic. The dorsal wall showed significant anisotropy at mid and high distension pressures. The dominant average direction of anisotropy in the bladders was longitudinal, along the dome-apex axis. As expected, depth resolved multiphoton microscopy of the ex vivo distended bladder revealed the difference in organization of the extracellular matrix in the dorsal and ventral regions of the bladder wall. Overall, the thickness—pressure relation, birefringence—pressure relation and microscopy data, all together suggest that anisotropy changes with micro-structural remodelling in the bladder wall as well as with mechanical strain (which is proportional to thickness change and distension). Since the bladder function (distension/contraction) is supported by the specific extracellular matrix organization of its wall, these initial results may prove useful in guiding the design and enhancing the functionality of tissue engineered bladders in order to assist bladder failure patients.
References

3-2. Identifying structural disorders induced by BOO in ex vivo distended rat bladders

As mentioned before, we use a BOO rat model that was developed in Dr. Bagli’s lab. The accuracy of the model was confirmed with histological examination of obstructed bladder and observing BOO induced structural changes in both the detrusor (e.g hypertrophy) and the ECM (e.g. elastin breakdown) [145].

In the following paper, “Assessment of local structural disorder of the bladder wall in partial bladder outlet obstruction using polarized light imaging,” published in Biomedical Optics Express, we characterized the structural anisotropy of obstructed bladders during distension and compared them to those of the normal bladders. The same procedure of polarized light imaging and Mueller matrix decomposition, explained in the previous paper, was used for characterization. To focus on the BOO structural disorders only, we chose to examine the regional bladder anisotropy patterns at one distension pressure. The goal of the paper is to prove the efficacy of the polarized light imaging for finding and quantifying the structural disorders induced in bladders by BOO in rat models. Again, the anisotropy difference of an obstructed bladder may be due to the intrinsic morphological changes caused by BOO or may be a secondary effect of those morphological changes, limiting the muscle stretching or the ECM remodelling during distension. Regardless of the origin of the observed abnormalities, having an imaging modality to accurately identify and quantify them is valuable for optimizing the treatment procedures for BOO.

I performed the experiment with the help of my colleague Adam Gribble. Dr. Karen J. Aitken and Dr. Annette Schröder, from Dr. Bagli’s lab, provided the clinical insight to explain the regional anisotropy abnormalities in the obstructed bladders.
Assessment of local structural disorders of the bladder wall in partial bladder outlet obstruction using polarized light imaging

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Abstract: Partial bladder outlet obstruction causes prominent morphological changes in the bladder wall, which leads to bladder dysfunction. In this paper, we demonstrate that polarized light imaging can be used to identify the location of obstruction induced structural changes that other imaging modalities fail to detect. We induced 2-week and 6-week partial outlet obstruction in rats, harvested obstructed bladders, then measured their retardances while distended to high pressures and compared them to controls. Our results show that the retardance of the central part of the ventral side (above the ureters) closer to the urethra can be used as a potential metric of the distending bladder obstruction.

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References and links

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1. Introduction
Partial bladder outlet obstruction (PBO) will affect about 1.1 billion people in 2018 [1]. PBO is the blockage of the outlet, which may arise from overgrowth of the prostate, posterior urethral valves or neurogenic bladder [2]. The outlet blockage will result in excessive urine accumulation in the bladder and high volume or high pressure that leads to the detrusor muscle hypertrophy and hyperplasia over time [2,3]. Other bladder wall properties affected by prolonged or chronic obstruction, indicated by microscopy studies, include decreased angiogenesis and changes in the extracellular matrix, including fibrosis (increase in elastin...
and collagen deposition) and elastin break-down into small fibers [3,4]. These compositional
and morphological changes in the bladder wall tissue impair the bladder’s capability to
contract and distend efficiently [2–5]. Due to this resultant bladder dysfunction, PBO patients
often suffer from urinary tract infection, incontinence and kidney failure [1–3,5]. In many
cases, the bladder wall damage is irreversible, necessitating augmentation surgery [6].
Augmentation cystoplasty is the procedure of replacing the bladder wall, frequently the dome
region, with parts of the autologous gastrointestinal/bowel wall or artificially engineered tissue
[6–10]. However, it is known that regional structural organization and biomechanical
properties of the bladder wall are region specific, and vary spatially depending on the
anatomical location of the tissue [11–13]. Hence, augmentation procedures should be
optimized to be specifically targeted to regions with the most morphological alterations.
Current in vivo imaging methods such as x-ray, ultrasound and MRI can only identify
anatomical changes in the bladder that are spatially nearly uniform throughout the bladder
wall [5,14]. Therefore, new in vivo imaging techniques are needed that can identify the
regions with the most pronounced morphological changes, to localize and optimize
augmentation procedures.

One of the morphological characteristics of tissue is its anisotropy (directionality), which
arises from the micro-structural organization (alignment) of muscle fibers and extracellular
matrix components. One appealing method to quantitatively assess anisotropy of biological
tissues is polarized light imaging [15–20]. Polarized light imaging can measure the media’s
anisotropy in terms of optical retardance, which is proportional to the tissue thickness
(sampling volume) and birefringence. However, extracting information from polarized light
after interaction with biological tissues is challenging, because tissues are turbid and light
depolarizes quickly after multiple scattering events during propagation [15–22]. However,
birefringence of optically thick tissues can be obtained by using Mueller matrix
decomposition, which separates different optical polarization effects [15–22]. For instance,
using polarized light imaging and Mueller matrix decomposition we previously demonstrated
regional anisotropy differences in normal ex vivo rat bladders under different distension
pressures [13]. Rats have been widely used as PBO models since they exhibit similar tissue
complications and urodynamics to humans [23–30]. In this study, we use obstructed rat
models and follow a similar polarized light imaging procedure we developed for normal
bladders [13]. We demonstrate that polarized light imaging can locate the obstruction-induced
morphological pathology changes in ex vivo distended rat bladders. As such, we believe
polarized light can potentially be used for in vivo regional evaluation of morphology in
bladder walls.

2. Materials and methods

2.1 Obstructed and control rat bladders

Female Sprague-Dawley rats with a mass of about 250 g were used in this work according to
animal use protocols at Sick Children’s Hospital (Toronto, Canada). The rats were divided
into two groups: one group for a 2-week obstruction study (6 rats: 3 obstructed and 3
controls) and one for a 6-week obstruction study (6 rats: 3 obstructed and 3 controls). Three
rats in each group underwent obstruction surgery as described previously [25]. Briefly, 1) the
abdomen was opened and the urethra was ligated with a 0.9 mm steel rod beside it to avoid
complete obstruction and attain consistency of the obstruction procedure, 2) the rod was
pulled out to enable partial obstruction, and the abdomen was closed. The other three rats in
the two cohorts served as controls. For these, the abdomen was opened and closed but no
obstruction procedure was performed. The rats’ bladders were harvested after 2 weeks and 6
weeks of partial or sham obstruction [13].

Despite the common surgical procedure, the resultant degree of obstruction differed
amongst harvested bladders in each group. This may have been due to the slightly different
sizes of individual bladders and their outlets, and slight differences in ligating the suture
during the surgery. To gauge the resultant obstruction in a rat model, the degree of
obstruction is usually quantified as the ratio of the obstructed bladder mass to the control bladder mass [29,30]. Here, we adopt the same definition to classify the harvested obstructed bladders. Since only the bladder mass itself is of interest and the ureters and urethra in each bladder may differ in mass and size, the ureters and urethra were removed prior to measuring bladder weight.

The bladders were distended using a liquid reservoir coupled to the urethra, as previously described [13]. The liquid is a mixture of Comas blue dye in ethanol and oil, so as to minimize light penetration to the opposite walls of the bladder. We examined all bladders at a 3.3 kPa pressure, which is close to the maximum physiological pressure experienced by a normal bladder (in both rats and humans). There were two reasons for choosing the highest distension pressure only: 1) the obstructed bladders were under high pressure due to obstruction inside the animal, and thus most would not distend under lower pressures, 2) we were looking for specific anisotropy differences due to obstruction, and performing varying pressure experiments would vary anisotropy due to strain and pressure induced micro-structural changes; as we were only interested in obstruction-induced morphological (anisotropy) changes, one maximum pressure would suffice.

2.2 Measuring anisotropy of bladder wall using polarized light imaging

The distended bladders were imaged in a backscattering geometry (25 degree off axis) using polarized light, as depicted in Fig. 1. As shown, each bladder was suspended such that the imaging plane was parallel to the plane passing through the ureters. The bladders were frequently sprayed with saline to prevent dehydration. The normal bladder volume is small (about 170 µL) and thus the pressure induced by the weight of the filling liquid was negligible compared to the kPa pressure head. Four anatomical regions of each bladder were examined (Fig. 1): dorsal urethral, dorsal dome, ventral urethral and ventral dome.

The imaging apparatus consisted of a light source (laser diode at 635 nm), a rotating polarizer and removable quarter waveplate in both the illumination and collection arms, and a charge coupled device (CCD). Since the collection arm is 25 degree off the excitation axis, no specular reflection reached the CCD. As previously described in detail, the Mueller matrix $M$ of each examined region was obtained from 24 polarimetric measurements (4 different polarization illuminations, with the backscattered light of each illumination analyzed under 6 different polarization collections) [13]. The resultant Mueller matrix, which represents all the polarimetric effects of the tissue, was then decomposed using Lu-Chipman decomposition into three sub-matrices, including the retardance matrix $M_{\delta}$ [16]. As explained in our previous papers, the retardance $\delta$ is calculated from the $M_{\delta}$ as:

$$\delta = \cos^{-1}\left\{\left[(M_{\delta}(2,2) + M_{\delta}(3,3))^2 + (M_{\delta}(3,2) - M_{\delta}(2,3))^2\right]^{1/2} - 1\right\},$$

(1)

where $M_{\delta}(i,j)$ is the element of $M_{\delta}$ in the $i^{th}$ row and $j^{th}$ column. To obtain the retardance orientation (optical axis) $\theta$ the matrix $M_{\delta}$ is further decomposed to the linear retardance matrix $M_{LR}$ and the optical activity matrix $M_{\omega}$. The direction of the optical axis projection on the imaging plane can be calculated as:

$$\theta = 0.5 \tan^{-1}\left\{\frac{M_{LR}(2,3) - M_{LR}(3,2)}{M_{LR}(3,1) - M_{LR}(1,3)}\right\},$$

(2)

retardance was then used as a measure of anisotropy in different regions of the bladder wall. However, it should be noted that $\delta$ represents the total effect of all anisotropy structures sampled throughout the imaged depth of the wall. Hence, by comparing the regional retardances of obstructed and normal bladders, we are comparing the total morphological changes through the depth; while the morphological changes in some layers might be higher or lower than the rest.
Fig. 1. a) Polarized light imaging set up for examining distended ex vivo rat bladders. The ureters and urethra are ligated and the bladder is suspended by the urethra. The CCD and detection arm are along the z-axis, and the illumination arm is $\theta = 25^\circ$ off angle with respect to the detection arm (it is $25^\circ$ away from the z-axis). $P_1$ and $P_2$ are polarizers, $QW_1$ and $QW_2$ are quarter waveplates and $L_1$, $L_2$ and $L_3$ are lenses. The image is not to scale. b) Schematic showing the anatomy of the dorsal region of the bladder. Two areas of the dorsal region were imaged: the dorsal urethral (DU) and the dorsal dome (DD). c) Schematic showing the anatomy of the ventral region of the bladder. Two areas of the ventral region were imaged: the ventral urethral (VU) and the ventral dome (VD).

Retardance $\delta = 2\pi \Delta n d / \lambda$ is proportional to birefringence $\Delta n$ and the sampling depth $d$ (related to tissue thickness, its optical properties, and experimental measurement geometry) and inversely proportional to the laser wavelength $\lambda$. In previous work, we calculated the bladder wall regional birefringence (by normalizing with optical coherence tomography-measured thickness and Monte Carlo simulated sampling depth) and correlated it with the distension pressure [13]. Here, however, we work with retardance because it is advantageous for potential in vivo studies where the thickness of interrogated tissue may be unknown.

3. Results and discussion

The mean masses of obstructed and control bladders in each group are reported in Fig. 2. The bladders in each obstructed group increased more than 150% in mass compared to the controls, which means our surgery resulted in a moderate degree of obstruction compared to other studies [30]. For example, Burmeister et al reported more than 200% increase in mass for the 2-week obstructed bladders relative to the controls, without further increase for the 6-week obstruction [30]. Similar trends from 2 to 6 weeks can be noticed in our results as well: as shown in Fig. 2, the mean mass of the 6-week obstructed bladders was more than the mean mass of the 2-week obstructed ones by only ~10%. This minimal increase in mass from 2 weeks to 6 weeks has been previously reported by other groups [29,30]. Another noteworthy finding in Fig. 2 is that the mean mass of the 6-week control bladder was higher than of the 2-week control group, probably due to aging of rats and consequent bladder growth.
Next, we investigated the measured regional anisotropy of the bladders from different groups. We emphasize here that the anisotropy detected by polarized light imaging has a distinct and different meaning from the anisotropy measured by biomechanical uniaxial / biaxial stretching tests [11,31]. The biomechanical anisotropy is a measure of how expandable the tissue is in different direction in response to applied forces. Optical anisotropy is an intrinsic asymmetry property of the examined tissue, regardless of whether the tissue is relaxed or stretched. In the case of a distended bladder, it is the total effect of the strain-induced directionality and the intrinsic structure's directionality. A more optically anisotropic bladder wall, at specific distension, may mean fibrosis (more collagen and elastin), more organized extracellular matrix, or muscle hypertrophy (thicker muscle layer) in the wall.

Representative retardance (anisotropy) maps obtained from polarized light imaging and Mueller matrix decomposition from one control and one obstructed *ex vivo* distended bladder in each of the 2-week and 6-week study groups are shown in Fig. 3. The retardance orientation is generally the same across each region, which shows that all bladders are exhibiting some anisotropy (a characteristic of distended bladder at high pressure as previously demonstrated [13]). However, the directionality (magnitude of the anisotropy) in the 6 weeks study seems to be stronger which indicates more organized micro-structure.
Fig. 3. Representative regional retardances (anisotropy) obtained from polarized light imaging and Mueller matrix decomposition of a) a 2-week control rat bladder, b) a 2-week obstructed bladder, c) a 6-week control bladder, d) a 6-week obstructed bladder. DU = dorsal urethral, DD = dorsal dome, VU = ventral urethral and VD = ventral dome (anatomically depicted in Fig. 1). Color bar shows retardance values in degree and the arrows show the orientation of the retardance (optical axis).

The retardance values were different between individual bladders within the same group, but common trends were present:

1) The control bladders in the 6 weeks study are more anisotropic than controls in the 2 weeks study.
2) Dorsal regions of the control bladder are more anisotropic than the ventral region.
3) Ventral regions are more anisotropic in the obstructed group than controls.
4) The ventral regions of the obstructed bladders are more anisotropic than dorsal regions. To observe the variation and consistency of these trends among all bladders, and to better identify the significant morphological changes, we quantified the maximum retardance (anisotropy) in all regions of each bladder. The maximum retardance for each region was calculated as follow: 1) each of the images were tiled into small regions of interest (ROI) of 0.2 mm by 0.2 mm; 2) the retardance in each ROI was averaged; 3) the maximum of these averages was used as the maximum retardance for the region. The mean of the regional maximum retardance for all bladders in each group (n = 3) was calculated and plotted, as shown in Fig. 4. The variation among bladders in each group was calculated from the standard deviation of the mean and is indicated by the error bars.

Fig. 4. Mean regional maximum retardance among different rat bladders in each group. The different regions are DU (dorsal urethral), DD (dorsal dome), VU (ventral urethral) and VD (ventral dome). The error bars are the standard deviation from the mean value.

The trend 1) implied from mass changes of Fig. 3 is clear in the anisotropy results of Fig. 4 as well. According to Siroky et al, aging bladders exhibit effects similar to those of obstructed bladders, such as detrusor thickening, fibrosis, and poor contractile response [32]. As discussed earlier, wall thickening and fibrosis can result in higher anisotropy values, which is in agreement with higher anisotropy detected by polarized light in the 6 weeks control bladders compared to the 2 weeks control bladders. This result suggests polarized light may have the potential to detect aging effects in bladders.

The results in Fig. 4 also reinforce the emerging trends 2) and 3). In addition, for both studies (and as expected) the mean value of regional retardance of obstructed bladder is higher than the regional retardance of the control bladders. To evaluate how well the obstruction can be detected using polarized light imaging, we calculated the p-value (from a two-tailed paired t-test) associated with mass and regional retardance differences between normal and obstructed bladders (Table 1).
Table 1. P-values (calculated from paired t-test) for differentiating normal versus obstructed bladders characteristics; \( \delta_{\text{VU}} \), \( \delta_{\text{VD}} \), \( \delta_{\text{DU}} \), and \( \delta_{\text{DD}} \) are the retardance of the ventral urethral, ventral dome, dorsal urethral and dorsal dome regions, respectively.

<table>
<thead>
<tr>
<th>Obstruction study duration</th>
<th>Mass</th>
<th>( \delta_{\text{VU}} )</th>
<th>( \delta_{\text{VD}} )</th>
<th>( \delta_{\text{DU}} )</th>
<th>( \delta_{\text{DD}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>0.04</td>
<td>0.03</td>
<td>0.18</td>
<td>0.92</td>
<td>0.66</td>
</tr>
<tr>
<td>6 weeks</td>
<td>0.02</td>
<td>0.03</td>
<td>0.14</td>
<td>0.42</td>
<td>0.39</td>
</tr>
</tbody>
</table>

The following conclusions emerge from the statistical results in Table 1.

1) The retardance of the ventral urethral region can be used to diagnose obstruction (p-value < 0.05).

2) Retardance values in the rest of bladder wall regions are not significantly different between the obstructed and control bladders.

The significant change of retardance in the ventral urethral is reasonable, since the ventral region can anatomically expand more to accommodate a larger volume of urine upon obstruction. In fact, regional differences in response to obstruction have been proposed before by Capelo et al [33] and Schröder et al [34]. For example, Capelo et al showed that the ventral sides of the obstructed bladders become less sensitive to contractile agents (NoRadermaline) [33]. Schröder et al have shown that the serosal layer of the ventral side exhibits greater thickening than that of the dorsal side following obstruction [34]. In another study, Sugimoto et al reported increases of short-length, coiled-shape elastin networks in the bladder neck (the region closer to the urethra) that do not contribute to elasticity; these extracellular matrix changes may be responsible for the anisotropy increase near the urethra that we are observing here [35].

Hence, our results demonstrate relevant significant regional anisotropy changes in the obstructed bladder walls, quantified using polarized light imaging and Mueller matrix polar decomposition. Recent studies show that polarized light can be delivered through endoscopic devices for tissue characterization [36,37]. We are currently developing a polarized light probe that can be used through a cystoscope. Having endoscopic polarized light imaging tools, one can potentially identify the regions with maximum retardance changes and then use this information for targeting/ pinpointing / optimizing surgical (augmentation) procedures. As mentioned earlier, currently most augmentation procedures target the dome part of the bladder, whereas our results show that the middle region close to the urethra has the most impaired tissue.

4. Conclusion

Polarized light imaging in combination with Mueller matrix decomposition was used to characterize local structural abnormalities of bladder walls after partial bladder outlet obstruction. A rat model was used for partial bladder outlet obstruction. 2 weeks obstructed (+ control) and 6 weeks obstructed (+ control) ex vivo bladders were harvested and distended to the same high (physiologic-level) pressures. Regional maximum retardance values were measured for each bladder wall in the retro-reflection geometry. As suggested by previous studies, the ventral region of the obstructed bladder behaves differently than the control bladders. Our results demonstrate that the retardance (anisotropy) of the ventral urethral regions increases significantly due to obstruction, and the increase follows a similar trend to the bladder mass increase. The increased anisotropy of urethral compared to dome regions may have implications for bladder augmentation surgeries that currently target the dome zones. Interestingly, control bladders of the 6-week study were more anisotropic than control bladders of the 2-week study. These obstructed bladder results are consistent with the presence of fibrosis, muscle hypertrophy and hyperplasia observed previously by other groups. Overall, these findings provide a foundation for investigating in-vivo applications of polarimetric imaging for regional pathology detection in urology.
Addendum

The presented paper showed that polarized light imaging can detect the BOO induced structural disorders in *ex vivo* distended rat bladders. An errata is sent to the journal to correct minor typos:

“1-0 the orientation of anisotropy, shown in all the pictures, is the fast axis direction (not the optical axis). This is true for the entire text. In fact, $\theta$ is calculated from (and not from Eq.2):

$$\theta = 0.5 \tan^{-1} \left( \frac{M_{LR}(4,2) - M_{LR}(2,4)}{M_{LR}(3,4) - M_{LR}(4,3)} \right)$$

2-To show the significance of the trends observed in regional anisotropy between control and obstructed bladders, the unpaired t-test (and not the paried t-test) was used. Consequently, the p-values, stated in Table.1, are calculated from the unpaired t-test (and not from the paired t-test).”

The next step is to image *in vivo* normal and obstructed bladders using polarized light. A clinically available instrument to visual the surface of the bladder is a cystoscope (Fig.3-1).

Fig.3-1. Commercial cystoscope, a picture and a schematic of the frontal view of a fiber based Olympus cystoscope tip. The proposed polarimetric probe will be inserted through the insertion tube, thus will minimally change the current cystoscopy procedure [146].
Cystoscopy is not very helpful for examining BOO, since the morphological changes associated with BOO may occur in the subsurface of the bladder wall [114]. The commercial cystoscopes fall into the two categories of flexible fiber based or flexible videoscopes (based on CMOS technology) [146]. The conventional ones offered by Olympus have a total diameter of 5.5 mm and a hollow working channel of about 3 mm (Fig.3-1).

To measure the tissue anisotropy *in vivo*, PS-OCT can be used. A PS-OCT flexible fiber probe can be inserted through the working channel of a cystoscope. This is feasible as a preclinical research project; however, OCT will be an expensive add-on technology to the cystoscopes. Implementing fiber based polarization sensitive optical measurement are challenging since propagation through the fiber based generally changes the polarization of the light. Using polarization maintaining fibers requires accurate alignment of their orientation axis relative to the sample, hence, they are not useful for sensing backscattered polarization changes from the *in vivo* tissue [147]. However, PS-OCT with fiber based probe is feasible now thanks to the several recently proposed schemes [148-150]. But, overall PS-OCT remains a complicated system, and there is still ongoing research for correcting the artifacts from polarization mode dispersion [151-152].

As demonstrated in the paper, we showed that polarized light imaging alone - without OCT- is enough to identify the effective structural disorders in the bladder wall. About a decade ago, Myakov et al and Turzhitsky et al demonstrated simple polarimetric measurements, consisting of co-polarized and cross-polarized readings. As shown in Fig.3-2, the polarization is selected at the distal end of the probe with a thin polymer dichroic sheet and the intensity in each fiber is treated as a polarization state [143,154]. This type of multi fibers flexible endoscopes, have been used
successfully for \textit{in vivo} polarization sensitive spectroscopy studies to detect the cancerous abnormalities and the mucosal blood flow in the oral cavity [143,154].

![Image](image.png)

**Fig.3-2. Simple fiber based polarized light probe [143].** The probe is made of three fibers; two of them are covered with the polarizer sheet and the third one is covered with the cross polarizer. a) The gap between the two polarizing films and two of the fibers are shown. b) The final probe tube with the three fibers.

Using the basic idea of filtering the polarization at the distal end, we propose a new design of a fiber based probe to measure the full Mueller matrix in appendix B. The design uses minimum number of fibers and micrometer size polarizers to insure a diameter of 2 mm. Using optical switches, each point measurement can be theoretically made in microsecond time scale. A first prototype of this probe was built and calibrated. The details of the design, fabrication/assembly and calibration can be found in appendix B.
4. Instrumentation and development of rapid benchtop polarized imaging systems for \textit{ex vivo} tissue characterization

4-1. A new algorithm for rapid Mueller matrix imaging with 4 PEMs and a CCD

The previous chapter was an example of a pilot study showing the efficacy of polarized light imaging for characterizing anisotropy in \textit{ex vivo} distended rat bladders. The imaging system used in those experiments was based on mechanically rotating polarizers and removable waveplates. Mechanically moving parts may induce some systematic errors in the measurements. In addition, imaging each region of the bladder took about 3 minutes (approximately, about 15 minutes total measurement time). This long time may cause dehydration of the \textit{ex vivo} tissues and change of their optical properties. Hence, in this chapter, I propose and implement a new rapid scheme of polarized light imaging based on PEMs without any mechanically moving parts.

As discussed in chapter 1, PEMs are optoelectronic components which modulate the retardance with a fixed frequency depending on their crystal size. These elements have large clear apertures and large acceptance angles of 40 degrees, as oppose to LCs, which make them ideal for imaging applications [155]. Previous to this work, PEMs have only been used for polarimetric point measurements of turbid media, which rely on photodetectors and lock-in amplifiers. For example, for a Mueller matrix point measurement, each of the Mueller matrix elements can be obtained from the amplitude of one of the harmonics of the PEM oscillations. To extend this scheme to an imaging system, Thompson and Fry proposed using a 2D array of photodetector each having their own lock-in detection systems [142]. It is not surprising that this expensive and
complicated solution was never implemented. Instead, to build an image with PEM based systems, the sample had to be scanned pixel by pixel, in a time consuming process [141].

As mentioned before, at least two PEMs should be used in the PSA or PSG to generate or select the necessary orthogonal polarization states of the light. PEMs oscillate continuously and, unlike the LCs, they cannot be switched on and off. A PEM frequency drifts with changes in temperature, mainly because of the small changes in the crystal size induced by thermal expansion. This frequency drift is not the same for each PEM, thus the main challenge of a PEM imaging system without lock-in amplifiers, is synchronizing them with a 2D camera (CCD or CMOS). On the other hand, even if the synchronization challenge is solved, continuous reading of intensity in time and filtering the harmonics in frequency domain (similar to the lock-in detection) will be very slow and defeat the purpose of a rapid imaging system.

In the following paper, “Optimization of rapid Mueller matrix imaging of turbid media using four photoelastic modulators without mechanically moving parts,” published in Optical Engineering, we proposed a rapid polarized light imaging system based on PEMs/CCD, with a new algorithm for retrieving the Mueller matrix elements. The main idea of this new scheme is to construct the full Mueller matrix with only 16 images captured by the CCD instead of the continuous reading of the intensity in time. Choosing the times that the intensity has to be captured and the optimum combination of the PEMs (different frequencies and orientations) is fully described in the paper. I developed the idea and performed all the simulations and optimizations.
Optimization of rapid Mueller matrix imaging of turbid media using four photoelastic modulators without mechanically moving parts

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1 Introduction
Mueller matrix analysis using polarized light is a powerful characterization technique with applications in thin film ellipsometry, aerosol characterization, and biomedical diagnosis. Imaging the Mueller matrix, as opposed to point detection, is highly desirable in biomedical applications, since most biological tissues are spatially heterogeneous. The Mueller matrix should be measured in the shortest time to avoid in vivo motion artifacts. The fastest polarimetric imaging scheme is the so-called snapshot systems. These techniques diffract different polarizations of the light beam and extract the polarimetric information from filtering the frequency content of the final image, at the expense of some image information loss due to filtering. The second category is the imaging systems that use switchable liquid crystals (LCs) to measure the sample’s Mueller matrix in few seconds. These systems can be optimized to decrease error sensitivity and are used for high-resolution imaging. Finally, the photoelastic modulator (PEM)-based systems are known to be the most sensitive due to the high and fast modulation of the PEMs, which enables synchronized detection. The PEM aperture is large, which makes them ideal for imaging application. Moreover, the modulation efficiency of the PEMs is superior to polarization gratings and fast LCs. Recently, Arteaga et al. have implemented four PEM polarimetric point detection scheme (originally suggested by Thompson et al.), which can measure the full Mueller matrix of turbid media. A corresponding system is now offered as a commercial product from Hinds Instruments, Hillsboro, Oregon. Nevertheless, it is time consuming to construct images from a point detection system, since mechanical scanning of the sample or steering the beam followed by image stitching will be necessary.

Here, we suggest a new method of recovering the Mueller matrix images using four PEMs and field-programmable gate array (FPGA)-assisted sequential time gating approach. The work further extends our recent theoretical formulation and experimental demonstration of the two PEM-based Stokes imaging technique. Here, we demonstrate how our proposed method analytically recovers the Mueller matrix images in time domain, within millisecond time frame, without sacrificing the image quality. Also, this method does not set any assumption on the examined sample and is applicable to arbitrary turbid media and biological tissues.

2 Theory: the System’s Matrix and Time-Gated Imaging to Calculate the Sample’s Mueller Matrix
Let us denote the 16 elements of the turbid media’s Mueller matrix by \( m_{ij} \) \((i, j = 1, \ldots, 4)\); these vary spatially and are, thus, functions of (\(x, y\)) unless otherwise noted. For an arbitrary turbid media, all 16 elements can be nonzero and are generally independent of each other; therefore, at least 16 independent equations are needed for their determination. A general four PEM-based polarized light imaging system is illustrated in Fig. 1, where the polarization state generator (PSG) enables different polarizations to impinge on the sample, and the polarization state analyzer (PSA) detects different polarization states of the light after interaction with the sample. As shown
in Fig. 1, we use two PEMs and a linear polarizer in each of the PSG and the PSA.\cite{15,16,19}

The polarization state of the light incident on the charged couple device (CCD) camera is described by a Stokes vector $\bar{S}_{in}$, which is calculated from

$$\bar{S}_{out}(t) = \begin{pmatrix} i(t) \\ q(t) \\ u(t) \\ v(t) \end{pmatrix} = M_{PEM2}(t)M_{PEM1}(t)\begin{pmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix}\begin{pmatrix} m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix} \times M_{PEM2}(t)M_{PEM1}(t)\bar{S}_{in},$$

where $i$ is the intensity, $q$, $u$, and $v$ are the linear polarization (at 0 deg or 90 deg), linear polarization (at 45 deg or $-45$ deg), and the circular polarization, respectively. $M_{PEM1}$ and $M_{PI}$ are the Mueller matrices of the $i$'th PEM and polarizer, respectively, and $\bar{S}_{in}$ is the polarization state of the light issuing from the polarizer $P_I$ in the PSG. The overbars signify a 4-element vector, capital symbols represent 4 x 4 matrices, and the rest of the symbols represent numbers.

The two PEMs and the polarizer in the PSG generate a time-varying Stokes vector $\bar{S}_s(t) = [g_1(t) \ g_2(t) \ g_9(t) \ g_4(t)]^T = M_{PEM1}M_{PSM}\bar{S}_{in}$. Similarly, the PEMs and the polarizer in the PSA detect a portion of light with the polarization that can be represented by a Stokes vector $\bar{S}_{o}(t) = [a_1(t) \ a_2(t) \ a_3(t) \ a_4(t)]^T$, which is the transposition of the row vector of the product of $M_{PSM}$ and the PSD functions as below

$$i(t_k) = [a_1(t_k) \ a_2(t_k) \ a_3(t_k) \ a_4(t_k)] \times \begin{pmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix} \times \begin{pmatrix} m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix} \times \begin{pmatrix} m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix},$$

where $i(t_k)$ stands for the integrated intensity on the CCD camera starting at time $t_k$ (assuming a short-integration time as small as nanoseconds, which we will discuss later). As seen, $i(t_k)$ can be considered as a weighted sum of the sample’s Mueller matrix elements modulated by the PSG and PSA functions as below

$$i(t_k) = \sum_{j=1}^{4} \sum_{i=1}^{4} (a_i g_j) m_{ij},$$

or in terms of matrix algebra as

$$i(t_k) = Z(t_k)\vec{M},$$

with the 16 elements of the row vector $Z(t_k)$ being

$$Z(t_k) = [z_1(t_k) \ z_2(t_k) \ ... \ z_{16}(t_k)] = [a_1(t_k)g_1(t_k) a_2(t_k)g_2(t_k) a_3(t_k)g_3(t_k) a_4(t_k)g_4(t_k) \]$$

$$a_4(t_k)g_4(t_k)],$$

and column vector $\vec{M}$ containing the 16 sample’s Mueller matrix elements that we seek to determine

$$\vec{M}(t_k) = [m_{11} \ m_{12} \ m_{13} \ m_{14} \ m_{21} \ m_{22} \ m_{23} \ m_{24} \ m_{31} \ m_{32} \ m_{33} \ m_{34} \ m_{41} \ m_{42} \ m_{43} \ m_{44}].$$

As mentioned, to find the 16 elements of vector $\vec{M}$ without ambiguity, 16 independent equations are needed. One way to do this is to acquire the intensity $i$ at 16 time points $t_k$ as follows:
The time points than error; basically, the error of recovering norm. The condition number sets an upper limit on the recovery, 

\[ \kappa(Z) = \|Z\|\|Z^{-1}\| \] 

where \( Z \) is a 16 \times 16 element matrix composed of \( Z \) row vectors evaluated at 16 time points \( t_k \)

\[
Z = \begin{pmatrix}
Z(t_1) \\
Z(t_2) \\
\vdots \\
Z(t_{16})
\end{pmatrix}
\]

We call \( Z \) as the system matrix. If we choose \( t_1, \ldots, t_{16} \) to yield a nonsingular system matrix \( Z \), we can calculate the sought-after sample Mueller vector \( M \) from direct product of the inverse of matrix \( Z \) and the acquired intensities as

\[
\bar{M} = Z^{-1} \begin{pmatrix}
\bar{i}(t_1) \\
\bar{i}(t_2) \\
\vdots \\
\bar{i}(t_{16})
\end{pmatrix}
\]

To ensure a stable solution for \( \bar{M} \) from Eq. (9), the determinant of matrix \( Z \) (which is a 16 \times 16 square matrix) should be far from zero, to avoid singularity. For \( Z \) to be less sensitive to errors, usually, the condition number of \( Z \) should also be minimized. Condition number is defined as \( \kappa(Z) = \|Z\|\|Z^{-1}\| \) with \( \| \) being the second-degree norm. The condition number sets an upper limit on the recovery error; basically, the error of recovering \( \bar{M} \) will be less than \( \kappa(Z) \) times the error of measuring the intensity \( \bar{i}(t) \). The time points \( t_1, \ldots, t_{16} \) should be, then, chosen to yield a nonsingular system matrix \( Z \) with minimum condition number.

3 Evolutionary Algorithm for Optimizing the System’s Matrix of Any PEMs Configuration

System matrix \( Z \) solely depends on the PSG and the PSA; in other words, \( S_y(t) \) and \( S_z(t) \) should be chosen carefully to result in a nonsingular \( Z \) matrix with minimum condition number. Each PEM in the incident and the detection arms introduces a time-varying retardance \( \delta(t) \):

\[
\delta(t) = \delta_0 \sin(2\pi f_t t + \phi_i),
\]

where \( \delta_0 \) is the maximum modulation amplitude, \( f_t \) is the oscillation frequency, and \( \phi_i \) is a phase. Most commercial PEMs are resonant devices with fixed modulation frequencies in the range of 20 to 100 kHz. Further, the modulation axis of each PEM can be differently oriented by tilting the device in the \( x-y \) plane. Hence, many different configurations of the system setup, as depicted in Fig. 1, are possible by selecting different values of \( f_t \) and \( \theta \) (the maximum retardance of each PEM, \( \delta_0 \), was chosen to be the same for all four devices; this was partly for simplicity and partly because judicious selections of the four frequencies and orientation angles were sufficient to recover \( M \) as below). For each \( (f_t, \theta) \), a configuration, one should find the 16 time points that result in a well-posed matrix \( Z \) with minimum condition number.

This is a large-scale problem that has no analytical solution and cannot be optimized by blind search in a short time. Such large-scale optimization problems can be tackled by heuristic approaches including genetic and evolutionary algorithms (EAs). These terms stem from the similarity with biological concepts viz. parent and offspring iteration layers, gene on-off alterations in the binary representation of the parent/offspring mathematics, sexual-like (two-parents to yield an offspring, mitosis) and asexual-like (single-parent to yield an offspring, meiosis) paradigms, and so on. In recent years, these algorithms have been used to solve various problems in optics such as focusing light through turbid media, designing optical thin films, focusing fields with desired fluence profile, shaping femtosecond pulses, generating nondiffractive beams, and even recently minimizing the condition number of LC and PEM-based polarimetric systems. Here, we use the EA initially proposed by Massoumian et al. Figure 2 is a simple schematic of how EA is applied to select the 16 time points that give the minimum condition number of the system matrix \( Z \); also, minimum difference of \( t_i \) and \( t_{i+1} \) is \( \Delta t \) and maximum difference of \( t_{16} \) and \( t_1 \) is \( T \), which is the common period of the PEM oscillations. The algorithm starts with a randomly generated solution vector of \( t_1, \ldots, t_{16} \). The solution vector randomly evolves by iterating via combinations of the two operators implemented in the algorithm: asexual parent–offspring mutation and sexual parent–offspring recombination. The solution vector, which minimizes the condition number of \( Z \) in each iteration, survives and evolves during the next iterations. The iteration ends when the condition number of \( Z \) (the objective function) does not decrease after some reasonable computation time (~3 min in our study).

4 Results and Discussion

By applying EA to a variety of configurations in Fig. 1, we found that there are many possible configurations with four PEMs that yield nonsingular matrix \( Z \). To reduce the number of variables for the demonstration purposes, we chose the maximum retardance amplitude \( \delta_0 \) to be \( \pi \) for all the PEMs. The phases \( \phi_i \) were set to zero for these examples; although, more discussion about this is provided below. Also, we chose the frequencies \( f_t \) to be an integer, which are multiples of 10 kHz; this insures a periodic behavior for the imaging system, with a time period of 0.1 ms. Therefore, the input variable \( T \) was set to 0.1 ms; in other words, EA was set to look for the fittest \( t_1, \ldots, t_{16} \) within...
0.1 ms. For all the EA trials, the smallest time difference between the points \( t_i - t_{i-1} \) was set to \( \Delta t = 1 \mu s \). Tables 1 and 2 present several preselected “reasonable” configurations and their resulting time points found by EA, which minimize \( \kappa(Z) \).

As seen from Tables 1 and 2, several variations of the frequencies and optical axes orientations of the four PEMs are possible for successful recovery of the Mueller matrix images. The resultant \( t_1, \ldots, t_{16} \) found by EA are unique for each setup and result in an invertible system matrix \( Z \). However, the condition numbers are not close to 1, which means that the recovery procedure will be sensitive to noise. Finally, these results are specific to the selected horizontal orientations of the polarizers \( P_1 \) and \( P_2 \); if these change, new optimal \( t_1, \ldots, t_{16} \) will be generated (results not shown).

This procedure is rigorous when the phases \( \phi_i \) are exactly known; whereas, in real life, the frequencies of the PEMs slightly drift, and \( \phi_i \) [in Eq. (10)], thus, randomly change in time.\(^{15,18}\) One solution to this experimental problem is to find the times at which the PEMs are in certain phase differences relative to each other. We have recently demonstrated practical feasibility of this method for two PEMs-based Stokes imager.\(^{18}\) We call our synchronization technique sequential time gating; briefly, an FPGA is used to sample the PEMs reference frequencies, to detect the rising/falling edges, or to send a trigger to the CCD camera whenever the rising edges (falling edges) happens at the same time. By extending the same approach to four PEMs, the intensity of the modulated light can be acquired to resemble the periodic behavior of the PEMs, regardless of the frequency drifts. In other words, in sequential time gating, the PEMs’ modulation follow Eq. (10) with fixed known phase \( \phi_i \).

To get some understanding of the experimental procedure and calibration, let us consider configuration \( V \) from Table 1 \((f_1, \theta_1) = (30, 30); (40, 60); (50, 60); (20, 30)\) as an illustrative example. In real experimental settings, the PEMs’ phases \( \phi_1, \phi_2 \) randomly change. To acquire the 16 images at known fixed \( \phi_i \), FPGA-assisted sequential time gating should be used along with the calibration process.\(^{18}\) To find the exact values of the phases through calibration: (1) several samples with known Mueller matrices, such as polarizers, are imaged; (2) the intensity \( i(t) \) incident on the CCD camera, then, can also be simulated via Eq. (2) for each sample and for different ranges of \( \phi_1, \phi_2 \); and (3) Then, the real values of \( \phi_1, \phi_2 \) can be found when the highest correlation between the experimentally acquired and simulated intensities occur.\(^{18}\) Once the correct phases are found, the times in Table 2 have to be recalculated. Here, without the loss of generality, and for demonstration purposes, we simulated the intensity for configuration \( V \) when all the PEMs are in phase \( \phi_1 = 0 \), keeping in mind that different \( \phi_2 \) will result in different waveforms for intensity \( i(t) \) that can be easily simulated. The simulated incident intensity on the CCD camera with no sample (air) and when the sample is a linear polarizer at 0 deg are presented in Figs. 3(a) and 3(b), respectively. As shown, different samples result in different intensity variation forms, as described in Ref. 18. Moreover, the periodicity in intensity \( i(t) \) [values being the same for \( r \) and \( r + T \) in Figs. 3(a) and 3(b)] implies that experimental averaging is possible, which confers a significant SNR boost to the Mueller matrix imaging approach while still enabling rapid measurements. The total time in which a complete Mueller matrix can be extracted depends on the CCD frame rate. For example, a top-end CCD (e.g., PIMAX-3, Princeton Instruments, Trenton, New Jersey) can be set to acquire images with nanosecond gating time and has a data storage rate of about 50 frames per second. This means that the 16 intensity images can be captured and saved in milliseconds time. Since this time is relatively short, the 16 images at \( t_1, \ldots, t_{16} \) [Eq. (9)] can be captured and averaged several times to boost up the SNR (by minimizing the effects of the random noise).

Next, we investigate on how acquiring the intensity at the EA-optimized 16 time points enables the extraction of the sample’s Mueller matrix image. In Fig. 4, we have simulated the performance of configuration \( V \) and its Mueller matrix recovery capability using the 16 time points optimized through EA (Table 2). To test the full ability of the Mueller matrix imaging procedure, we chose a complicated Mueller matrix, as illustrated in Fig. 4(a), from a heterogeneous bilayered turbid medium modeled by PolMC code, as described fully in Ref. 29. The phantom

### Table 1

<table>
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<tr>
<th>Configuration</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
<th>( t_5 )</th>
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<th>( t_{13} )</th>
<th>( t_{14} )</th>
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Note: The values of \( f_1, f_2 \) are in kHz, and the values of \( \theta_1, \theta_2 \) are in deg.

### Table 2

<table>
<thead>
<tr>
<th>Configuration</th>
<th>( t_1, \ldots, t_{16} ) (( \mu s ))</th>
<th>( \kappa(Z) )</th>
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<tr>
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</tr>
<tr>
<td>V</td>
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<td>8.4</td>
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</table>
was a $5 \times 5 \times 2$ cm$^3$ box with a scattering coefficient of $6$ cm$^{-1}$ and minimal absorption (equal to water’s absorption at 635 nm). Birefringence magnitudes in layers 1 and 2 were equal to 0.000115, and the extraordinary axes orientation differed by 30 deg in the two layers. Figure 4(b) shows the PolMC simulations of the CCD signal $i(t_k)$ from Eq. (2) at times suggested by Table 2 and the recovered Mueller matrix using the inverse of $Z$ via Eq. (9). As seen, the images of the Mueller matrix elements were fully recovered with $\sim 0$ error (considering four precision digits), which implies that the analytical formulation and EA optimization are self-consistent and yield a correct solution in ideal conditions without noise.

Although we demonstrate $\sim 100\%$ recovery in ideal conditions, the rather high condition numbers in Table 2 imply high sensitivity to noise. To investigate this further, 5% random noise was added to the registered intensities $i(t_1), \ldots, i(t_{16})$ which resulted in the recovered Mueller images, as demonstrated in Fig. 5. The fractional error of recovering Mueller matrix elements [except the elements with values close to 0 (first row and column)] is illustrated in Fig. 5(b). As seen in the bar graph, the mean value of error is less than 5%, and the maximum standard deviation is 12%. One way to reduce this sensitivity is to acquire more images in each period (increase the number of the time points to more than 16) and make the $Z$ matrix an over-determinant matrix. The condition number of such a matrix can be optimized to be closer to 1 and will result in less sensitivity to noise. This procedure will be explored in a future experimental publication.

Finally, one should note that there are some $(f_i, \theta_j)_{i=1}^{4}$ configurations which will not work in our scheme, i.e., they cannot generate a nonsingular $Z$ matrix. In such cases, EA
fails to find independent vectors \( Z(t_1), \ldots, Z(t_{16}) \). A few examples of such configurations are listed in Table 3.

The first category (cases A–C) are those whose PSG and/or PSA do not generate enough different Stokes vectors, due to the particular optic axis orientation \( \theta_i \) of the PEMs with respect to each other and the polarizers \( P_1 \) and \( P_2 \). To demonstrate the inadequate performance of these failed configurations, we present the corresponding Poincare sphere representations of the poor \( \theta \) states (A–C) in Fig. 6. As seen in Figs. 6(a) and 6(b), the Stokes vectors \( S_g \) or \( S_a \) sample limited regions of the Poincare sphere, compared with a “good” arrangement (configuration I, Table 1) of Fig. 6(c), where both \( S_g \) and \( S_a \) cover nearly the whole sphere surface.

The second category (cases D–F) contains the PSG and the PSA configurations that are highly correlated in time through inappropriate selections of the four PEM modulation frequencies. In such cases, the polarization states of PSG and PSA change together due to their frequency combinations, which result in insufficient temporal separation of the 16

<table>
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<tr>
<th>Configuration</th>
<th>( f_1 )</th>
<th>( \theta_1 )</th>
<th>( f_2 )</th>
<th>( \theta_2 )</th>
<th>( f_3 )</th>
<th>( \theta_3 )</th>
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Note: The values of \( f \) are in kHz, and the values of \( \theta \) are in deg.

Fig. 5 (a) Recovered Mueller matrix images of Fig. 4(a) when 5% random noise was added to the measured intensities. (b) The bars show the mean value of the recovery error in each element over the entire image in (a), while the error bars indicate the standard deviation of the recovery error.

Fig. 6 Temporal polarization trajectories of polarization states for different experimental configurations generated by PSA (red) and PSG (blue) states represented on the Poincare sphere. Shown in black are the reference circles on the Poincare sphere (equator and two of the meridians for better three-dimensional illustration). Poor \( \theta \) arrangements (Table 3) are shown in (a) configuration A and (b) configuration C, which demonstrate inadequate polarization state coverage. (c) A useful arrangement from Table 1 (configuration I) is shown.
5 Conclusion

In conclusion, we proposed a camera-based Mueller matrix imaging technique using four PEMS applicable to arbitrary samples including complex heterogeneous turbid media such as biological tissues. Unlike prior point-measurement systems that use synchronous detection to lock in on the four frequencies and their harmonics, we meet the challenge of imaging-based high SNR detection via temporal gating algorithm implementable on a CCD-based Mueller matrix polarimeter. Specifically, a practical approach based on an EA was developed to select the 16 optimal time points at which the camera-detected intensities should be recorded, and then, analyzed with matrix algebra to yield the sample Mueller matrix. The challenges of overcoming PEMS frequency drift were also foreseen and handled using previous experimentally demonstrated method with FPGA. The overall methodology was demonstrated for Mueller matrix inversion recovery using simulated turbid medium imaging data in ideal conditions and in the presence of noise. Four different PEM configurations with varying modulation axis orientations and modulation frequencies were examined and interpreted, in terms of their suitability for this method. As no filtering was needed in this approach, the spatial resolution and the contrast of the recovered Mueller matrix images were not compromised. Overall, the ability to rapidly and robustly obtain Mueller matrix images with PEM-based polarization modulation approach should prove advantageous in the rapidly expanding field of turbid Mueller matrix imaging polarimetry.

References


Sanaz Alali has an MS degree in optics and microwaves from Sharif University of Technology, Tehran, Iran, in 2006. She is currently pursuing her PhD in biophotonics (medical biophysics) at the University of Toronto, Toronto, Canada. Her research area focuses on studying polarized light interaction with biological tissues, including Mueller matrix analysis and developing new methods of measuring Mueller matrix.

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4-2. Experimental demonstration of rapid Stokes imaging with 2 PEMs and a CCD

To show the proof of concept of the proposed imaging scheme, in the following paper, “Rapid time-gated polarimetric Stokes imaging using photoelastic modulators,” published in Optics Letters, we report on an implementation of a Stokes imaging system using two PEMs and a CCD.

To synchronize the PEMs with the CCD, we used a field programmable gate array (FPGA). The FPGA counts the rising edges of the PEMs’ reference signals and determines the time when the PEMs get back in phase to trigger the CCD. Essentially, this protocol is the same as lock-in-detection in which a phase locked loop circuit is used. An undergraduate student from the Electrical Engineering Department was hired to program the FPGA. I put the rest of the imaging system together, performed the experiment and ran the analysis to prove the accuracy of the proposed imaging scheme.

The CCD used in this experiment has an electronic shutter with nanosecond gating capability and thus was very useful in gating the microsecond variations of the intensity. The common approach to gate the intensity in microsecond time is to use stroboscopic methods, in which a pulsed laser is used to illuminate the sample in microsecond time [140]. Stroboscopic methods are not applicable when there is no control on the source, for example for astronomical investigation. Thus, our imaging system may find useful applications in astronomy as well.
Polarized light is frequently used in biomedical applications for assessing tissue morphology [1,2]. For examining tissues that are spatially heterogeneous, large fields of view and high resolution are desirable, necessitating an imaging approach. Moreover, image acquisition time should be minimized to avoid ex vivo tissue alterations and in vivo motion artifacts. Recently, snapshot polarimetric imaging systems have been reported using gratings and prisms [3,4]. Although fast, these methods apply spatial frequency filters, reducing the information content of the image. Other potential rapid polarimetric imaging systems, based on photoelastic modulators (PEMs) or liquid-crystal (LC) modulators, offer higher resolution with large fields of view, and are thus more suitable for biomedical applications, using two photoelastic modulators (PEMs). A charge-coupled device (CCD) with microsecond time-gating capability was used to acquire the images. To synchronize the CCD with the PEMs, thus gaining signal-to-noise ratio advantage, a field programmable gate array was employed. After calibration, an evolutionary algorithm was used to select four time points from which the full Stokes vector can be recovered. Using the images taken by the camera at these four times (in ~80 ms), the images of the full Stokes vectors of different incident polarization states were accurately derived. © 2013 Optical Society of America

We report a rapid time-gated full Stokes imaging approach without mechanically moving parts, which is well-suited for biomedical applications, using two photoelastic modulators (PEMs). A charge-coupled device (CCD) with microsecond time-gating capability was used to acquire the images. To synchronize the CCD with the PEMs, thus gaining signal-to-noise ratio advantage, a field programmable gate array was employed. After calibration, an evolutionary algorithm was used to select four time points from which the full Stokes vector can be recovered. Using the images taken by the camera at these four times (in ~80 ms), the images of the full Stokes vectors of different incident polarization states were accurately derived. © 2013 Optical Society of America

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cw diode laser (Coherent) at a wavelength of 641 nm and a maximum power of 100 mW, and a spot size of about 2 mm was used. The polarization state entering the PSA was determined by another linear polarizer, a removable quarter-wave plate (QWP), and the sample polarization properties (if any). The CCD camera (PI-max III Princeton Instruments) registered 152 × 152 pixel images with a resolution of 50 μm per pixel. Defining the Stokes vector of light after interaction with the sample upon entering the PSA as $S_{in}$, the time-dependent Stokes vectors at the camera, $S_{out}$, can be written as

$$S_{out}(t) = \begin{pmatrix} I(t) \\ Q(t) \\ U(t) \\ V(t) \end{pmatrix} = M_{p2}M_{PEM2}(t)M_{PEM1}(t)S_{in}. \quad (2)$$

where $I(t)$ is the intensity, and $Q(t)$, $U(t)$, and $V(t)$ are the linear polarization at $0^\circ$ or $90^\circ$, the linear polarization at $45^\circ$ or $-45^\circ$, and the circular polarizations, respectively. $M_{PEM}$ and $M_{p2}$ are the Mueller matrices of the $i$th PEM and the polarization $P_{2}$. The PEMS and the polarizer in the PSA enable the CCD to image light with the polarization with a Stokes vector $S_{in}(t) = \begin{bmatrix} a_{1}(t) & a_{2}(t) & a_{3}(t) & a_{4}(t) \end{bmatrix}^T$, which is the transpose of the first row of the $M_{p2}M_{PEM2}M_{PEM1}$ matrix. Hence, $I(t)$ at any time $t$ registered by the CCD can be rewritten as

$$I(t) = [a_{1}(t), a_{2}(t), a_{3}(t), a_{4}(t)]S_{in}. \quad (3)$$

At different time points $t_{1}$, $t_{2}$, $t_{3}$, and $t_{4}$, we can rewrite the four time-dependent CCD-detected signal intensities in matrix form as

$$\begin{pmatrix} I(t_{1}) \\ I(t_{2}) \\ I(t_{3}) \\ I(t_{4}) \end{pmatrix} = AS_{in} = \begin{bmatrix} a_{1}(t_{1}) & a_{2}(t_{1}) & a_{3}(t_{1}) & a_{4}(t_{1}) \\ a_{1}(t_{2}) & a_{2}(t_{2}) & a_{3}(t_{2}) & a_{4}(t_{2}) \\ a_{1}(t_{3}) & a_{2}(t_{3}) & a_{3}(t_{3}) & a_{4}(t_{3}) \\ a_{1}(t_{4}) & a_{2}(t_{4}) & a_{3}(t_{4}) & a_{4}(t_{4}) \end{bmatrix} S_{in}. \quad (4)$$

By choosing $t_{1}$, ..., $t_{4}$ to ensure a nonsingular system matrix A, the Stokes vector $S_{in}$ is retrieved from the direct product of the inverse of matrix A and the acquired intensities as

$$S_{in} \equiv A^{-1} \begin{pmatrix} I(t_{1}) \\ I(t_{2}) \\ I(t_{3}) \\ I(t_{4}) \end{pmatrix}. \quad (5)$$

For such a system, the lower the condition number of A, the more accurate the recovered solution [13–17]. For an IC-based polarimetric system, Ans et al. used a genetic algorithm to optimize the LC’s parameters [15]. We use a similar approach here to find the times $t_{1}$, ..., $t_{4}$ (0 < $t_{i}$ < a common period of oscillation = 500 μs) that yield a non-singular matrix A with minimum condition number $κ(A) = ||A|| \cdot ||(A)^{-1}||$, with $|||$ being the second degree norm [16]. We use an adapted version of the evolutionary algorithm (EA) proposed in [18]. This EA starts from a random (parent) solution vector $t_{1}$, ..., $t_{4}$ and generates a population of offsprings using cross-mutation and self-mutation techniques [18]. In each step, the condition number of the matrix A is calculated for all the vectors in the population. If any vector is fitter (its $κ(A)$ is smaller) than the parent vector, it survives and becomes the parent solution vector.

However, selecting the times $t_{1}$, ..., $t_{4}$ is challenging because of the frequency drift of the real-life PEMS [8,10]. Due to this drift, the phases $φ_{i}$’s in Eq. (1) randomly change with time and change the system matrix A through Eqs. (1)–(5); indeed, selecting $t_{1}$, ..., $t_{4}$ to minimize $κ(A)$ is meaningful only when the phases of the PEMS’ modulation relative to each other are known. We address this challenge by employing an FPGA in what we are calling a sequential time-gating approach. An Altera DE2 FPGA was used to generate a trigger pulse for the CCD when the two PEMS were in certain fixed relative phase $φ_{o}$. Each PEM controller generates a 5 Vpp square wave at its modulation frequency. The square wave output of the PEMS’ controllers and camera’s external trigger were connected to an FPGA as shown in Fig. 1. A 32-bit 50 MHz FPGA counter was used to track the time of the rising and falling edges of each square wave. As shown in Fig. 2(a), a trigger pulse was sent to the camera if the rising edge of one of the square waves and the falling edge of the other one took place within three FPGA clock cycles (time difference of $Δt < 60$ ns), which determines the value of $φ_{o}$ when the trigger is sent [Fig. 2(a)]. The CCD should then acquire an image when it receives the trigger; however, there is another experimental variable, a short unwanted electronic delay $t_{e}$ before it starts acquiring an image as depicted in Fig. 2(b).

![Fig. 2. (a) Trigger pulse is generated by FPGA when rising edge and falling edge of the PEMS are within 60 ns. At the rising edge of the trigger, the PEMS have a phase difference of $φ_{o}$. (b) CCD gates the incoming intensity for $τ = 0.5$ μs in each exposure $n$. Gating times for consequent images are programed to be delayed with multiples of $dt = 0.5$ μs with respect to the trigger. $t_{e}$ is a short unwanted electronic delay of the CCD with respect to the trigger. Acquiring each image takes 20 ms.](image-url)
To capture the intensity variation in microsecond time, the CCD was programmed to gate (−embedded electronic shutter) the intensity for a time $\tau = 0.5 \, \mu s$, after a user-defined delay of $(n - 1) \times dt = (n - 1) \times 0.5 \, \mu s$ from the trigger pulse, in each exposure $n$, as illustrated in Fig. 2(b). As seen, while the trigger is generated rapidly, the CCD acquires each image after receiving the trigger and only if it is finished registering the previous frame. As an example, registering each $152 \times 152$ pixel frame takes about 20 ms. With this acquisition scheme, the time-varying retardances of the PEMs are

$$
\delta_1(t) = 3.142 \sin(2\pi \times 20.07 \times 10^5(t + t_o) + \varphi_o), \\
\delta_2(t) = 3.142 \sin(2\pi \times 42.06 \times 10^5(t + t_o)).
$$

To exactly measure $t_o$ and $\varphi_o$ in Eq. (6), the intensities of four known incident Stokes vectors were measured: the linear polarizations 0°, 45°, and 90° and the right circular polarization, all generated by rotating the polarizer $P_1$ manually plus adding a QWP for the right circular polarization through air (i.e., Fig. 1 with the sample removed). A sequence of $n = 1000$ images $[t = 0–500 \, \mu s$ in Eq. (6)] was acquired by the CCD. The registered intensities for the incident linear 45° and right circular polarizations are shown in Figs. 3(a) (Media 1) and 3(b) (Media 2). The mean value of 10 central pixels of the image sequence was calculated for these two cases, as plotted in black over 250 $\mu s$ in Figs. 3(c) and 3(d). By scanning different values of $t_o$ and $\varphi_o$, the maximum cross-correlation coefficient between the theoretical [from Eqs. (2)–(6)] and the measured intensities was obtained when $t_o = 87 \, \mu s$ and $\varphi_o = -1.2915 \pi$ rad. The correlation coefficients between measured and simulated intensities were 0.99 for 0° and 90° polarizations, 0.98 for 45° polarization, and 0.93 for right circularly polarized light. Corresponding theoretical predictions for $I(t)$ are plotted in red in Figs. 3(c) and 3(d), showing excellent agreement between experiments and simulations. The experimental results shown in Fig. 3 are robust, exhibiting ~1% variation over repeated acquisitions during different days. This verifies that the values of $t_o$ and $\varphi_o$ are ~constant regardless of the PEMs’ frequency drifts, and suggests SNR boosts via repeated acquisitions and averaging.

Next, the exact retardance of the PEMs from Eq. (6) was fed to the EA to obtain the optimum $t_1, \ldots, t_4$, which gave the minimum condition number $\kappa(A)$. The resulting values of $t_1, \ldots, t_4$ and $\kappa(A)$ are shown in Table 1; $\kappa(A)$ magnitude is comparable to condition numbers reported in [12,14], suggesting a stable solution. The system matrix $A$ was then used via Eq. (5) to recover the incident Stokes vectors for different incidences, using the images acquired at $t_1, t_2, t_3, \text{ and } t_4$ of Table 1.

Figure 4 presents the correctly recovered Stokes images when the incident beam polarization varies. Mean value of degree of polarization, $\langle (Q^2+U^2+V^2)^{1/2}/I \rangle$ of these Stokes vectors is 97%. Note that whereas we used the entire period (1000 frames) for the calibration purposes, here we only used four images that took a mere $4 \times 20 \, ms = 80 \, ms$.

To test system performance in a biomedicall setting, we imaged 0.6 mm thick of frozen chicken breast that is birefringent and has a transport albedo (= reduced scattering coefficient/reduced scattering coefficient + absorption coefficient) of 0.9 [19]. The degree of polarization of the incident Stokes vector (0° polarization) and that after interaction with the chicken breast were calculated and shown in Figs. 5(b) and 5(c). As expected, there is a significant depolarization after interaction with the scattering sample, and its magnitude fits well into the depolarization/optical properties trend established and validated in our previous study [20].

In conclusion, we demonstrated a fast dual-PEM imaging scheme for full Stokes polarimetry of turbid media. To account for experimental complications of PEM frequency drifts, we used an FPGA to trigger the CCD when the two PEMs are in certain phase relative to each other. Then, applying the EA, we determined the four

![Figure 3](image3.png)

*Fig. 3.* Experimental image sequence over 500 $\mu s$ (one period) acquired by the CCD, using FPGA assisted sequential time gating, when the incident light is (a) linearly polarized at 45° (Media 1), (b) right circularly polarized (Media 2). (c) Mean value of 10 central pixels in (a) over 250 $\mu s$ shown in black, and the simulation from Eqs. (2)–(6) when $S_0$ is linearly polarized at 45° plotted in red. We also illustrate how the entire signal is sampled sequentially (only three consequent gating times shown, each 0.5 $\mu s$), as suggested by Fig. 2. (d) Analogous figure for right circularly polarized incidence from the 10 central pixels of the time-varying image of (b).

| Table 1. Optimum $t_1, \ldots, t_4$ in Microseconds Derived from EA, and the Corresponding Condition Number $\kappa(A)$ |
|---|---|---|---|---|
| $t_1$ | $t_2$ | $t_3$ | $t_4$ | $\kappa(A)$ |
| 58.5 | 92 | 118 | 291 | 1.78 |
time points at which the matrix that describes the system has a low condition number. The CCD registered microsecond resolved time-gated images at those four times in synchronization with the PEMs. By multiplying these four time-gated images by the inverse of the system’s matrix, we recovered the full Stokes vector images, verified the system performance and accuracy for known input states, and demonstrated its operation in a typical biomedical imaging scenario. Importantly, this scheme can be expanded to a four-PEM Mueller matrix imaging scheme that can recover the full polarimetric images of the 16 elements in a millisecond time frame. Theoretical details of this approach are presented in a separate article [21].

References

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5. Summary and future work

5-1. Summary of the thesis

This thesis covered three streams of research in the field of polarized light imaging for assessment of anisotropy in biological tissues. The three streams are: theory and simulation of polarized light propagation in tissues; application of polarized light imaging as a potential diagnostic tool and instrumentation of polarized light imaging systems.

The focus of chapter 2 was on the Mueller matrix as the polarimetric transfer function of the sample in a specific geometry. Conventionally, the Mueller matrix is decomposed with mathematical techniques to decouple the depolarization, caused by multiple scattering of light in turbid media. The decoupled part contains optical retardance (proportional to anisotropy) and optical activity. We ran two separate studies to understand the Mueller matrix beyond its polar decomposition. In the first study, we quantitatively correlated the depolarization of different tissues’ to their transport albedos. The reduced scattering coefficient and the absorption coefficient of various isotropic and anisotropic bulk tissues were measured and their transport albedos at 635 nm were reported. Using polarized light imaging, Mueller matrices of optically thin and thick samples of those tissues in transmission and backscattering geometry were measured. The Mueller matrices were then decomposed to obtain the depolarization matrices. From the sum of the depolarization matrices’ diagonal elements, the depolarization parameters were calculated. A strong correlation between the depolarization parameters and the tissues’ transport albedos was found for both thin and thick samples in different imaging geometries. The observed trends confirm that higher transport albedo means that the photons will experience longer pathlength and thus light will be more depolarized after propagation in the tissue. It was
also noted that the anisotropic tissues show higher depolarization compared to the tissues with the same transport albedo, possibly because of axial changes of the anisotropy. In the second study, we established new metrics from the Mueller matrix to distinguish the heterogeneity of anisotropy in turbid media. The PolMC code was extended to bi-layered turbid media with arbitrary birefringence or extraordinary axis in the two layers. Mueller matrices of such homogenous and heterogeneous media in transmission were simulated. The off-diagonal elements of the Mueller matrices showed certain symmetry relations in the homogenous samples. These symmetry relations did not hold for the heterogeneous samples. Therefore, the sum of the deviation from those symmetric patterns was introduced as the asymmetry degree (ASD) for differentiating between homogenous and heterogeneous samples with the same effective retardance and orientation.

In chapter 3, we demonstrated that polarized light imaging is a viable tool for imaging the bladder wall structural anisotropy. First, we developed a polarized imaging procedure to assess the regional anisotropy of ex vivo normal rat bladder during different distension pressures. In summary, anisotropy increases with higher distension pressure. The direction of the structural alignment was measured to be from the base to the dome of the bladder. However, the strength of the alignment (anisotropy value) is not the same in the dome, dorsal and ventral regions of the bladder. The dome showed the highest anisotropy at high pressure. The dorsal region shows significant anisotropy which increases with pressure, whereas the ventral region is mostly isotropic at all pressures. The wall cross section of all the bladders was examined with OCT. It was found that when the bladder distends, the wall thickness decreases uniformly in all regions. This means that the anisotropy (structural alignment) in not only due to the muscle stretch but also caused by the ECM organizational remodeling. This hypothesis was confirmed by nonlinear
microscopy and visualizing the collagen and elastin fibers in representative \textit{ex vivo} relaxed and distended bladders. The same polarized light imaging procedure was used, in the second part of chapter 3, to characterize \textit{ex vivo} obstructed bladders, during distension. To concentrate only on the structural changes induced by BOO, the regional anisotropy of normal and obstructed \textit{ex vivo} rat bladders was measured at one high physiological pressure (40 cm H$_2$O). Two groups of 2 week and 6 week partial BOO were compared against their controls. Our results were in agreement with the previous reports, showing a high increase in the bladder mass during the first 2 weeks of the obstruction, and not much increase from the 2 weeks to the 6 weeks. It was found that the anisotropy of the ventral region of the bladder, closer to the urethra, significantly increases with obstruction. This is an important finding, which if confirmed \textit{in vivo}, should be considered in optimizing the treatment procedures. For instance, augmentation surgeries are mostly performed on the dome region of the bladder; but that may have to be changed, knowing the location with maximum structural disorders which impair the bladder normal function.

Chapter 4 was devoted to report a novel scheme for rapid polarized light imaging based on PEMs. The previous schemes used lock-in amplifiers and recovered the Mueller matrix elements from the different harmonics of the oscillations of the PEMs. The novel scheme, we proposed, is specifically designed to be used with a camera, and avoid lock-in amplifiers or mechanically moving parts. We demonstrated that the full Muller matrix images can be obtained from capturing the intensity at 16 time points in millisecond time. For the algorithm to work, the PEMs’ frequencies and extraordinary axes orientation should result in a polarization modulation that almost sweeps 2/3 of the polarization states on the Poincare sphere. In the second part of chapter 4, the experimental implementation of a Stokes imaging system based on 2 PEMs and a CCD was reported. An FPGA was used to synchronize the two PEMs in the PSA with the CCD.
The CCD had a microsecond gating capability which allowed us to register the full variation of the intensity in one period of the system, and fully calibrate the system. The calibration mainly involves measuring the phase delay of each component. The 4 images needed for recovering a full Stokes vector were acquired and saved in 80 ms time. The system was used to image various known Stokes vectors and unknown Stokes vector of light transmitted through thin samples of chicken breast as a turbid medium.

5-2. Future work

5-2-1. Further improvements on the quantitative analysis of the Mueller matrix

In chapter 1, we interpreted the Mueller matrix driven depolarization parameters with respect to the optical properties of the tissue. The reported values of optical properties of the various porcine tissues are important on their own, since these values have not been previously reported for some of the tissues. However, there is a wide range of variability in the reported optical properties of the same tissue type (for example the fresh loin muscle) [97]. Grabtchak et al summarizes some of the determining factors in the reported values as [97]: 1) Variation in the measurement methods, the look up tables and the fitting methods, 2) The water content, which may change when tissue dehydrates and eventually changes the tissue content concentration and scattering, and 3) The concentrations of deoxymyoglobin, oxymyoglobin, and metmyoglobin, which depend on the tissue freshness and change the absorption. A future work can involve measuring optical properties of tissues with different freshness and correlating them to their depolarization. The result will be valuable for calibrating polarized light imaging systems for \textit{ex vivo} studies.
The other novel finding in this thesis was the correlation of the Mueller matrix images to the axial heterogeneity of the turbid medium. Further image processing work is needed to perform pattern recognition in the Mueller matrix elements and identify the birefringence changes. Comprehensive look up tables of turbid media with different optical properties and different multi-layered structured of varying birefringence can be created for accurate fitting.

To experimentally verify the result of this study, multilayered birefringent phantoms should be used. Although, the turbid polyacrylamide phantoms used in this thesis are accurate models, they dehydrate and undergo birefringence changes on the order of 10 minutes. Thus, there is a need for novel durable scattering birefringent phantoms. Amouzegar et al, from Duke University, fabricated a new class of durable polydimethylsiloxane (PDMS) phantoms with ellipsoid particles [156]. These phantoms may be a candidate for phantoms with intrinsic birefringence, but should be modeled with a Monte Carlo code that can simulate ellipsoid particles.

Lastly, Lu-Chipman polar decomposition is a serial decomposition in the sense that it models turbid media as a sequence of an attenuator, a retarder and a depolarizer. In an attempt to achieve better accuracy in modeling the simultaneous optical effects happening in the turbid media, other decomposition methods such as symmetric decomposition, differential decomposition and parallel decomposition were developed [157-161]. Among these novel methods, differential decomposition was applied to Mueller matrices of scattering media and tissue samples [161]. In differential decomposition, all the depolarizing and non-depolarizing simultaneous effects over infinitesimal length are extracted in a single matrix. The elements of this matrix are then used to calculate retardance and depolarization. The retardance calculated from this method, for uniform homogenous media, shows less than 0.05 % difference from the one calculated from Lu-Chipman decomposition [161]. To study the advantages and shortcomings of each method, more
comparative polarimetric characterizations of heterogeneous samples followed by various decompositions is needed.

5-2-2. *in vivo* polarization enhanced cystoscopy for evaluating BOO disorders

Perhaps, the most obvious future direction of the thesis emerges from chapter 3 and appendix B, which is to validate the *ex vivo* observed disorders induced by BOO in *in vivo* animal model.

As mentioned before, PS-OCT with flexible probe may be used to carry on depth resolved anisotropy assessment of the bladder wall in conjunction with a flexible cystoscope. The probe can be inserted through the working channel of a cystoscope to examine the internal bladder wall *in vivo*. Alternatively, to demonstrate a more cost-effective add-on technology to the available cystoscopes, the design of the flexible polarimetric, probe proposed in appendix B, shall be pursued. The proposed probe can measure the full Mueller matrix of the tissue and provide effective anisotropy through the depth. To allow testing the probe in conjunction with the commercial cystoscopes, a bigger animal model such as a pig model must be used. Animal models of BOO in pigs have been previously developed and studied [119].

Once the feasibility is shown, polarization enhanced cystoscopy may find other applications, such as assisting in image guided surgeries to detect cancerous lesions in bladder cancer.
5-2-3. Rapid Mueller matrix imaging with 4 PEMs – Opportunities and experimental challenges

Currently, most biology labs use polarized light microscopes which detect birefringence of collagen and other thin materials in terms of different colors in the visible spectrum [162]. The 4 PEM imaging system, proposed in chapter 4, is an ideal replacement for polarized light microscopes, because it provides an accurate quantitative measure of the birefringence and does not depend on the user. Such a device will render intra-operative imaging or cancer screening using polarized light imaging.

We are currently working on the 4 PEM polarized light imaging demonstration with the FPGA assisted gating. However, time gating with 4 PEMs is very challenging. The frequency drift for 4 PEMs is much higher than the 2 PEMs, thus the imaging will be much slower. Our initial studies show that the 4 PEMs get back to a certain phase differences in about 1 second. Hence, the imaging speed will depend on the PEMs rather than the CCD. Any improvement in stabilizing the PEM oscillation will be valuable, since it is going to be a step closer to the fully automatic, millisecond speed, high resolution and large field of view Mueller matrix imaging.

PEMs are difficult to manufacture in small sizes. If miniaturized, they will be ideal candidate for modulating the polarization in future generations of polarimetric probes. With the current dimensions, PEMs can only be used at the proximal end of the endoscopes; for instance, in the polarimetric rigid endoscope proposed by Qi et al [147].
5-3. Conclusions and accomplishments

Polarized light imaging has been widely used in biomedical research. The common approach in polarized light imaging is to measure the Mueller matrix of a turbid medium and then decompose it to retrieve optical effects which correlate to the sample's structural/compositional properties. In the first part of this thesis, we concluded that the Mueller matrix can be analyzed beyond its polar decomposition to obtain some information about the uniformity or optical properties of the sample. The second accomplishment of the thesis was showing the efficacy of polarized light imaging as an imaging modality in urology. Currently, there is no imaging modality for studying the structure of a functioning bladder wall in vivo. This is a crucial need for developing new treatments for conditions such as outlet obstruction, which is common among the aging population. However, in this thesis, we showed that polarized light imaging can quantify the microstructure of functioning ex vivo bladders, and locate the structural disorders due to BOO.

To confirm the observed trends in vivo and enable polarization enhanced cystoscopy, a thin fiber based probe to measure the full Mueller matrix was suggested in appendix B. A fully automatic version of this probe can be used to evaluate the BOO induced structural disorders in in vivo animal models. Our other result, in this thesis, was the implementation of a novel polarized light imaging systems based on PEMs for ex vivo diagnostic purposes. The prototype was used for polarimetric characterization of turbid media, with large field of view in only 80 milliseconds time. This is the fastest polarimetric imaging system that allows high resolution characterization of any turbid sample.
Appendix. A. Constructing the Mueller matrix

The minimum number of polarimetric measurements in order to determine all the Mueller matrix elements independently is 16; however, usually more measurements 24, 36 or 49 are performed to increase the accuracy. Here, we use the 24 sequential measurements where for each of four input polarizations [H(linear at 0°), V (linear at 90°), P (linear at 45°) and R (right circular)], intensity profiles at six output polarizations [H (linear at 0°), V(linear at 90°), P (linear at 45°), B (linear at -45°), R (right circular) and L (left circular)] are measured. From these 24 measurements, Mueller matrix is constructed as [163]:

$$M = \begin{bmatrix}
m_{11} & m_{12} & m_{13} & m_{14} \\
m_{21} & m_{22} & m_{23} & m_{24} \\
m_{31} & m_{32} & m_{33} & m_{34} \\
m_{41} & m_{42} & m_{43} & m_{44}
\end{bmatrix} = \frac{1}{4}
\begin{bmatrix}
HH + HV + VH + VV & HH + HV - VH - VV & 2(PH + PV) - 4m_{11} & 2(RH + RV) - 4m_{11} \\
HH - HV + VH - VV & HH - HV - VH + VV & 2(PH - PV) - 4m_{21} & 2(RH - RV) - 4m_{21} \\
HP - HB - VP + VB & HP + HB + VP + VB & 2(PP - PB) - 4m_{31} & 2(RP - RB) - 4m_{31} \\
HR - HL + VR - VL & HR - HL - VR + VL & 2(PR - PL) - 4m_{41} & 2(RR - RL) - 4m_{41}
\end{bmatrix}
$$

(A-1)

To construct M from 16 measurements using Eq.A-1, one should replace the measurements XL with −XR and XB with −XP, where X can be H, V, P and R.
Appendix. B. Toward polarization enhanced cystoscopy

The current gold standard for the clinical assessment of BOO are urodynamic tests such as uroflowmetry, cystometry and pressure-flow studies [116]. In these tests, the pressure-flow dynamics of urination, residual urine volume and other parameters are measured to evaluate the bladder wall activity, compliance and urethra resistance [113]. Although clinically helpful for grading the obstruction and managing the symptoms, these tests do not provide any insight to the structure of the bladder wall. But as discussed previously, structural disorders are the underlying cause for bladder dysfunction in obstruction disease. Thus monitoring the wall micro-structure of obstructed bladders during distension\contraction may open up new ways for drug development and optimizing\localizing augmentation surgeries. As the ex vivo studies showed (chapter 3), the polarimetric signature of the bladder wall (the Mueller matrix) can be used to quantify the structural alignment of the bladder which changes dramatically in rat models with BOO. This imaging technique may be of great value if it can be used in vivo to locate the pathological micro-structure in the wall of a functioning bladder.

In the first part of this appendix, I describe the details of an ideal design for a 2 mm diameter flexible polarimetric probe that can be used in future polarization enhanced cystoscopy procedures. The second part of the appendix involves the design, fabrication and a suggested calibration procedure of a simpler version of the probe, the \(a\) prototype. My colleague Mr. Andras Linednmaier assisted me in the fabrication and assembly. The \(a\) prototype is not ready to use in practice in conjunction with a cystoscope yet; however, the prototype was used to demonstrate the proof of concept and to develop the calibration protocol for Mueller matrix
measurement. We are currently working with the UHN Technology transfer office to protect this IP and build the β prototype.

**B-1. Ideal design of a polarimetric fiber based probe to measure the full Mueller matrix**

As discussed in previous chapters, to derive the 16 elements of a turbid mediums’ Mueller matrix without ambiguity, we need to at least perform 16 polarimetric measurements. We propose a probe design, shown in Fig.B-1, with a hexagonal packing scheme to minimize the probe size, as suggested by Utzinger et al [164]. In this scheme, each fiber is sequentially used for illuminating the tissue and collecting the light backscattered from the tissue. The 16 measurements can be acquired with 7 fibers (each with either a single polarizer or a combination of a polarizer and a waveplate) at the distal end. The fibers are high numerical aperture multimode fibers. The diameter of the probe is about three times the diameter of each fiber (about 400 μm), approximately 1.5 mm, and can be guided easily through the working channel of a flexible cystoscope.

![Fig.B-1. Fiber arrangement in the proposed polarimetric probe to complement a cystoscope. The letters H, V, P and R indicate the fibers for filtering the polarizations linear at 0°, 90°,45° and the right circular, respectively. The distance D between the center to center of adjacent fibers in the probe is about 500 μm.](image)

The measurements consist of illuminating the sample sequentially, with 4 different polarization states: 1. H (linear horizontal), 2. V (linear vertical), 3. P (linear at 45°) and 4. R (right circular); the different polarizations of the backscattered light are collected by the rest of the fibers and the illumination fiber itself. The illumination/collection light cones from the fibers on the perimeter of the probe overlap with the central light cone from the central fiber H as shown in Fig.B-2.

An example of the overlap of the illumination and collection light cone on the tissue is depicted in Fig.B-2. This overlap changes with changing the distance of the probe from the tissue. Consequently, the measurement resolution changes depending on the diameter of the illumination spot on the tissue. For instance, as illustrated in Fig.B-3, when the probe is held 1 mm away from the tissue, the spatial resolution of our measurement will be limited to 0.86 mm (2 × 0.43 = 2 × tan (sin¹ (NA)). To estimate the collection efficiency of the fibers from the bladder tissue, Monte Carlo code was used. According to the simulation, and assuming isotropic bladder wall (reduced scattering coefficient $\mu'_{s} = 6 \text{ cm}^{-1}$), each of the fibers adjacent to the illuminating fiber collects 5% of the incident light power (NA of 0.4, without considering polarization). With this collection ratio, for an incident laser irradiance of 5 W/mm² (which is below the safety limit), about 3 mW power will be in the collection cone of the fiber. Assuming a coupling efficiency of even 1% for the fiber, hundreds of micro-watt power will reach the detector [165]; therefore, high SNR measurements are possible.
Fig.B-2. The fiber probe resolution dependence on the fibers’ illumination and collection. a) The illumination and collection spot size varies depending on the probe distance from the tissue. d is the spot size (radius of the lateral circle made from intersection of the illumination/collection cones with the tissue surface). The distance of the distal end of the probe determines the point measurements resolution. b) The illumination (orange circle) and the collection (blue) spots on the tissue when the probe is 1 mm away from the tissue. c) The illumination (orange circle) and the collection (blue) spots on the tissue when the probe is 2 mm away from the tissue. The numerical aperture NA is assumed to be 0.39. D is the distance between the centers of the adjacent fibers in the probe and it is 500 μm.

The illumination/collection scheme is depicted in Fig.B-3. The fiber H is used for illumination in the HX readings and for collection of light in the XH readings, where X can be any other polarizations. The X readings and all other readings involve illumination/collection with two fibers with the same polarization. For example, to measure VP, the tissue is illuminated with two V fibers and the backscattered P polarization is read from the average of the two P polarized fibers. The total polarization state of the backscattered light can be calculated from the average...
of the two fibers with the same polarization state. For example, the reading HP will be calculated from the average of the power collected by the two fibers with the filter P when the illumination is the fiber with filter H. By repeating the procedure for each different incident polarization state, the 16 polarimetric measurements will be performed and the Mueller matrix will be constructed, as described in appendix A.

Fig.B-3. Data acquisition steps with the fiber probe. The orange fibers show the illumination/collection from the same polarization at each step and the blue fibers represent the collection fibers for other polarizations. The measurement will be sequentially performed to acquire 16 readings. H is the horizontal polarizer, V is the vertical polarizer, P is the linear polarizer at 45° and R is the right circular filter made from a polarizer and a waveplate. a) HP, HV, HH and HR measurements, b) VH,VV, VP, and VR measurements, c) PH, PV, PP and PR measurements and d) RH, RV, RP and RR measurements.
Excitation and collection of the fibers are controlled by a system illustrated in Fig.B-4. A monochromatic laser diode is used for illumination. An optical switch (1×4) is used to excite the desired fiber with specific polarization illumination. Fiber couplers are used for dividing/recombining the intensity from each two fibers R, P, and V. Circulators are used to direct the backscattered light toward the detectors.

Fig.B-4. The proximal system to implement polarized light measurements. The sequence of the illumination and collection is controlled electronically. The diode laser is fiber coupled and connected to an optical switch to deliver light to different fibers sequentially. The dash lines from the circulator show the light path for the backscattered collected light to the detectors. This design is more plausible in infrared regime, since fiber based circulators are rare in the visible wavelengths range.
As discussed, with distal filtering, polarization of light can be read in terms of intensity through each fiber. However, if the fiber probe bends, the light in the fibers will have different polarization and thus the intensity transmitted out from each of the fibers will be different. For instance, if the polarization of the light in the fibers becomes circular, the intensity of the light illuminating the sample from the H fiber will be less than the intensity illuminating the sample with the R fiber. To account for this intensity variations in different illuminations, we need to calibrate the probe each time before measuring an unknown Mueller matrix. Hence, a switchable miniaturize mirror shall be added to the probe distal end, after the polarizers. The probe will be calibrated to obtain the known Mueller matrix of the mirror as will be described in section B-2-3. The mirror will be then flipped away to allow Mueller matrix measurement of the sample.

**B-2. First prototype**

**B-2-1. Simplified design**

For the proof of concept, a simple version of the proposed fiber probe, the α prototype was built. This probe is the first fiber based probe with distal end filters to measure the Mueller matrix. Making the following simplifications to the design, we demonstrated the proof of concept with minimum cost and components:

i) Only linear polarizer filters were used (as opposed to linear polarizer and quarter waveplates). Thus the probe can only measure the linear part of the Mueller matrix (a 3 by 3 matrix).
To measure the linear part of the Mueller matrix \( M_L \), 9 measurements are needed. One combination can be obtained from: illuminations of H (linear at 0°), V (linear at 90°) and P (linear at 45°) and collection from H (linear at 0°), V (linear at 90°) and P (linear at 45°).

Having these measurements, the linear part of the Mueller matrix can be calculated as [147]:

\[
M_L = \begin{bmatrix}
2m_{11} & HH + HV - VH - VV & 2PH + 2PV - 2m_{11} \\
1/2 & HH - HV + VH - VV & HH - HV - VH + VV & 2PH - 2PV - (HH - HV + VH - VV) \\
2m_{11} - 2(HP + VP) & 2m_{11} - 2(HP - VP) & 2PH + 2PV + 2HP + 2VP - 4PP - 2m_{11}
\end{bmatrix}
\]

(B-1)

where \( m_{11} \) is (HH+HV+VH+VV) and XY is the light collected by fiber Y when the illuminating fiber is X. For high scattering samples such as tissues where the backscattered light is highly depolarized, the full Mueller matrix \( M \) should be measured. The linear part of the Mueller matrix \( M_L \) does not have the effect of circular depolarization, which may lead to an overestimation of the retardance value. For example, decomposing \( M_L \) for ex vivo rat bladders yield higher retardance numbers, by almost 15%.

ii) To avoid circulators and switches, the illumination and collection were controlled manually. This means that backscattered light is never collected with the same fiber that is used for illumination. For easier measurements and avoiding the challenges of free space light coupling, the SMA connectors were kept on the proximal end of the fibers. The light delivery from the laser to the fiber, and from the fiber to the detector were then carried out through compatible SMA/SMA connectors as shown in Fig.B-5.
iii) To minimize the number of fibers, the first prototype is made to develop the calibration protocol and measure the Mueller matrix of a mirror. With this restriction on the sample, we could make the probe with only 4 fibers: Two H fibers with 0° polarizers, one V fiber with 90° polarizer and a P fiber with 45° polarizer. The fibers’ configuration is depicted in Fig.B-6. The HH reading is measured with two fibers with filters oriented at linear 0°. HV and HP readings are measured with the laser connected to the fiber with the H polarizer and the detector connected to the fibers with V and P polarizers, respectively. Since the mirror does not have any asymmetry properties, the combinations PP and VV are the same as HH. By a simple change of coordinates, the readings HV/VH and HP/PV are also the same.

Fig.B-5. The benchtop prototype and the experimental setup for testing it. The SMA connectors at the proximal end of the probe are used to deliver the illumination light from a fiber coupled laser, and backscattered light to a photodetector with an SMA connector. The sample is a silver coated mirror, used for calibration at a distance of 0.5 cm away from the distal end of the probe.
Fig.B-6. Configuration of the fibers at the distal end of the prototype probe. For simplicity, the fibers in the prototype are assembled in a larger distance with respect to each other and the total diameter of the probe is about 4.5 mm.

**B-2-2. Fabrication process**

The previous fiber based polarimetric probes, reported in the literature, were made with polymer based dichroic polarizers (extinction coefficients 1000:1), cut in millimeter sizes and attached to a plastic tube which had the fibers in as shown in Fig.3-1[143,154]. With three fibers (two co-polarized and one cross-polarized) the total diameter of the probe is about few millimeters. However, the three-fiber probe cannot be used to measure the sample’s birefringence (anisotropy). To accurately retrieve the turbid media’s anisotropy, the sample’s Mueller matrix (at least the linear part of the Mueller matrix) should be measured.

To make the probe as thin as possible, we took on a new challenge of cutting polarizer as small as the fiber cores. To have high collection of backscattered light, we used large core high numerical aperture fibers (Thorlabs, diameter =400 μm, NA=0.39), which means that we needed to cut polarizers as small as 400 micrometer. The micro-polarizers were cut from high quality polarization filters, nanoparticle embedded polarizers (Thorlabs LPVIS, extinction coefficients 10,000:1).
To cut the high quality polarizers in micrometer sizes, we used an automatic water jet dicing machine (Disco DAD3220) in the Device Development Facility, in Sunnybrook Hospital. The polarizer (LPVIS) is about 2 mm thick (2.067 mm). The polarizer is first attached with a UV detachable tape to the substrate of the machine. The machine was programmed to dice the polarizer in square shape pieces, into a side size of 500 μm, to cover the whole fiber area. A 100 μm diamond blade was used for cutting through the polarizer. Fig.B-7 shows a group of small pieces of the polarizer and an individual cube of them.

Fig.B-7. Micrometer size polarizers, the top row shows the top view and the bottom row shows the side view. a) The polarizer disk from Thorlabs. b) The same plate cut with the dicing saw into small pieces. c) A picture of a group of small polarizers attached to the UV tape; a 3D schematic of one of the micro-polarizers; and a picture of one small polarizer when it is tilted on its long facet. The task of dicing the polarizer is challenging; because, the height of each small piece is larger than its lateral size, and it may not stick to the substrate tape.
The polarizers were then attached to individual fibers with UV curable glue, while being visualized under a stereomicroscope. As show in Fig.B-8, a clear glue (Norland Optical Adhesive 85, with refractive index of 1.46 in the visible) was used in between the fiber core (fused silica, refractive index of 1.457 at 635 nm) and the polarizer (sodium silicate 1.52 at 635 nm). This glue should ensure minimal reflection of light about 3% from the fiber surface, but it is not hard enough to hold the polarizer. To enhance the durability of each fiber, another UV curable glue (Dymax 203-CTH-F-T, with refractive index of 1.58 at visible) was used on the perimeter of the junction. Both glues were cured with Dymax UVB light source.

![Diagram](image)

**Fig.B-8.** The assembly steps of each fiber in the probe. a) A schematic illustrating the steps of attaching the small polarizer pieces to the fibers, b) The picture shows a linear polarizer with 90° orientation held against the fiber with the H polarizer (with 0° orientation), thus the square cross section of the polarizer appears dark. The clear thick layer of the hard glue can be seen before the polarizer.
The last challenging stage of the fabrication is to hold the fibers in specific orientation and in equal distances with respect to each other. To have better control, we fabricated a special rotational mount that holds the core of the fiber and rotates it. The alignment of each fiber was then tuned with respect to a polarizer sheet in front of it. For example, to set the H polarizing fibers, polarizing sheet was held at 90° orientation and the fibers were rotated up to a point where they appear as dark squares (Fig.B-8). The fibers are then passed through a frame made of a plastic sheet to hold the fibers in equal distances from each other. The fibers were glued to the frame with UV curable glue (Fig.B-5).

**B-2-3. Calibration procedure**

Calibration of the α prototype was performed with a fiber coupled laser source (Thorlabs, 635 nm), and an avalanche photodetector (Thorlabs, APD110, 400-1100 nm) connected to a Tektronix Oscilloscope (see Fig.B-5). The first step of the calibration is to estimate the collection efficiency of the individual fibers in the probe. Although the fibers were all prepared with the same fabrication process, they transmit and collect the intensity differently. One reason may be the small cracks at the edges of the micro-polarizer pieces. We have learned how to eliminate these variations now and the micro-polarizers will have much better quality for the second prototype. The second reason might be the Dymax glue which was added for durability and has higher refractive index of 1.58. Since the glue cures in different shapes around the fiber or may contain air bubbles, some of the light may be coupled into the glue. That may introduce different losses for each fiber. However, as long as the ratio of the collection and transmission of the intensity is known for each fiber, the probe should be practical for polarimetric measurements. We measure these effects in terms of one collection function in a setup shown in Fig.B-9.
Fig.B-9. The setup for measuring the collection function of the individual fibers. The circular polarizer (a combination of a linear polarizer at 45° orientation and a quarter waveplate) is used to couple equal portion of the intensity into all three types of the fibers in the probe. The second linear polarizer is rotated during the experiment to measure the maximum and the minimum transmission of each fiber. The lens is used for expanding the beam waist to cover the whole distal end of the probe with almost uniform illumination.

A homogenous circularly polarized illumination was created and impinged on the fiber probe distal end. To measure the maximum and the minimum intensity transmitted by each fiber, a rotating linear polarizer was used before the fibers. The laser power was constant during all the measurements. As the data in Table.B-1 show, the collection function of the fibers (with the attached polarizer) can be very different from each other.
Table B-1. Relative max and min collected power for the four different fibers in the probe (Fig. B-6), when the illumination changes from co-polarization (x=1) to cross-polarization (x=0). x is a real number between 0 and 1 indicating the co-polarization proportion of the light collected by each fiber.

<table>
<thead>
<tr>
<th>Fiber</th>
<th>min (x=0)</th>
<th>max (x=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁</td>
<td>2</td>
<td>192</td>
</tr>
<tr>
<td>H₂</td>
<td>4</td>
<td>800</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>420</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>304</td>
</tr>
</tbody>
</table>

where x is the portion of the light co-polarized with each fiber. x is a real numbers between 1 (parallel to fiber’s polarization filter) and 0 (orthogonal to the fiber’s polarization filter). After normalizing all powers to 800 mV (the maximum transmitted power among all the fibers in Table B-1), the collection function of each fiber F can be expressed as:

\[ F_{H₁}(x) = 0.002 + 0.23x \]
\[ F_{H₂}(x) = 0.007 + 0.99x \]
\[ F_{P}(x) = 0.005 + 0.52x \]
\[ F_{V}(x) = 0.01 + 0.37x \]  \hspace{1cm} (B-2)

Once the collection function is derived, there is no need to repeat this step of the calibration anymore. However, as explained before, to account for the difference in the illumination
intensities among fibers and enable accurate Mueller matrix measurements, regardless of any bending in the probe, a switchable miniaturized mirror should be added to the distal end. The mirror is used to for the second step of the calibration which should be repeated every time before measuring an unknown Mueller matrix. This step of the calibration basically involves estimating the backscattered coupling efficiency between any two fibers, when one is used for illumination and one is used for collection of light from a non-diffusive reflecting surface. Although we have tried to be accurate in the assembly, the fibers may be located at slightly different distances from each other. The backscattered coupling efficiency thus includes the effects of the different illuminations intensities and the different coupling into the collection fiber. Here, we demonstrate estimating the backscattered coupling efficiencies using a 1 cm diameter silver coated mirror. The mirror reflects H and V polarization to H and V respectively, but flips the P polarization to B (which is still filtered out by the same P fiber).

The fibers have NA of 0.39 and they are located 2 mm apart from each other. Hence, to insure an overlap between the illumination/collection cones, the mirror was placed 5 mm away from the distal end. The powers $P_{H1H2}$, $P_{H1P}$, $P_{H1V}$, $P_{PH1}$ and $P_{PV}$ were measured, as explained before; where $P_{ab}$ indicates the light collected by the fiber b when the fiber a is illuminating the sample. The optical power $P_{ab}$ then can be described as:

$$P_{ab} = P_o K_{ab} F_b(x) \quad (B-3)$$

where $P_o$ is the laser power, $K_{ab}$ is the backscattered coupling efficiency of the fiber b when the fiber a is illuminating a mirror. This coefficient also includes the intensity illumination difference among the fibers at each calibration time. $F_b(x)$ can be calculated from the first step of the calibration according to Eq.B-2. As an example, for calculating $K_{HP}$, one needs to calculate $F_p(x)$.
for \( x=0.5 \). Since the illumination is H polarized, the P polarized light is half of the polarization reflected back from the mirror, which means \( x \) is 0.5. Following the same procedure for all readings, \( K_{ab} \) can be calculated as follow:

Table B-2. A calibration example of measuring the backscattered coupling efficiencies. The measured light powers \( P_{ab} \) for a mirror, when \( a \) is the illumination fiber and \( b \) is the collection fiber, and the calculated backscattered coupling efficiencies \( K_{ab} \). The laser power is constant for all measurements.

<table>
<thead>
<tr>
<th>Measured power, ( P_{ab} = P_o K_{ab} F_b(x) )</th>
<th>Backscattered coupling efficiency, ( K_{ab} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{H1H2} = P_o K_{H1H2} F_{H2</td>
<td>x=1} = 120 \text{ mV} ), ( K_{H1H2} = 0.6 )</td>
</tr>
<tr>
<td>( P_{H1P} = P_o K_{H1P} F_{P</td>
<td>x=0.5} = 144 \text{ mV} ), ( K_{H1P} = 2.77 )</td>
</tr>
<tr>
<td>( P_{H1V} = P_o K_{H1V} F_{V</td>
<td>x=0} = 116 \text{ mV} ), ( K_{H1V} = 59 )</td>
</tr>
<tr>
<td>( P_{PV} = P_o K_{PV} F_{P</td>
<td>x=0.5} = 200 \text{ mV} ), ( K_{PV} = 5.12 )</td>
</tr>
<tr>
<td>( P_{PH1} = P_o K_{PH1} F_{H1</td>
<td>x=0} = 144 \text{ mV} ), ( K_{PH1} = 6.15 )</td>
</tr>
</tbody>
</table>

For instance, \( K_{H1H2} \) then have effect of the transmission coefficient (including the variations due to any bending in the fiber) of the fiber \( H_1 \) and the coupling into fiber \( H_2 \), when the sample is a mirror.

To construct a Mueller matrix from polarimetric measurements, Eq.B-1 and the real values of \( HH, HV, HP, PV \) and PH reflected back from the sample should be used. The values of \( HH, HV, HP, PV \) and PH can be obtained from the experimentally measured powers \( P_{H1H2}, P_{H1P}, P_{H1V}, P_{PH1} \) and \( P_{PV} \). By plugging in Eq. B-2 into Eq.B-3, and knowing that \( x \) is the real value that we are interested in, Eq.B-3 for each reading can be expressed as:
Replacing the values of the backscattered coupling efficiency from Table.B-2 into Eq.B-4, the values of HH, HP, HV, PH, PP, PV, VH, VP and VV can be obtained. From these readings and Eq.B-1 the linear part of the Mueller matrix of the mirror is calculated as:

\[
HH = \frac{P_{HH}}{K_{HH}} - 0.007 \div 0.99
\]

\[
HP = \frac{P_{HP}}{K_{HP}} - 0.005 \div 0.52
\]

\[
HV = \frac{P_{HV}}{K_{HV}} - 0.01 \div 0.37
\]

\[
PH = \frac{P_{PH}}{K_{PH}} - 0.002 \div 0.23
\]

\[
PV = \frac{P_{PV}}{K_{PV}} - 0.01 \div 0.37
\]  \hspace{1cm} (B-4)

As can be seen, the Mueller matrix in Eq.B-5 is in close agreement to an ideal mirror transfer function, a matrix of with only a non-zero diagonal of (1, 1, -1). This step of the calibration should be performed using a switchable mirror before measuring the unknown Mueller matrix of the tissue. In a similar way, from Eq.B-4 and Eq. B-1 and measuring the intensity of the light reflected back from tissue then the tissue’s Mueller matrix can be calculated.
References


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