Polarized light imaging in biomedicine: emerging Mueller matrix methodologies for bulk tissue assessment

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\textbf{Abstract.} Polarized light point measurements and wide-field imaging have been studied for many years in an effort to develop accurate and information-rich tissue diagnostic methods. However, the extensive depolarization of polarized light in thick biological tissues has limited the success of these investigations. Recently, advances in technology and conceptual understanding have led to a significant resurgence of research activity in the promising field of bulk tissue polarimetry. In particular, with the advent of improved measurement, analysis, and interpretation methods, including Mueller matrix decomposition, new diagnostic avenues, such as quantification of microstructural anisotropy in bulk tissues, have been enabled. Further, novel technologies have improved the speed and the accuracy of polarimetric instruments for \textit{ex vivo} and \textit{in vivo} diagnostics. In this paper, we review some of the recent progress in tissue polarimetry, provide illustrative application examples, and offer an outlook to the future of polarized light imaging in bulk biological tissues. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE)

\textbf{Keywords:} polarized light imaging; Mueller matrix; bulk tissue microstructure.

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1 Polarized Light in Biomedicine

Optical imaging modalities in biomedicine offer insight into tissue structure (down to cellular or even subcellular level) and function (metabolic and compositional information, microvascular blood flow), often by providing bulk tissue properties, such as absorption or scattering, which can be used to detect and differentiate tissue pathology. To obtain this wealth of useful biophysical insights, an optical imaging modality measures a specific change in the amplitude, phase, wavelength, or polarization of light reflected, transmitted, absorbed, or remitted from tissue (often as a function of space and/or time). One of these optical characterization methods is polarimetry, which is the science of studying polarized light interaction with materials to infer information about their structure and composition. In addition to biological tissues, polarimetric methods have been extensively used in industry for characterizing thin films, semiconductor chips, chemical content of food/drugs, and for aerosol sensing applications.\textsuperscript{[12]}

One particularly important tissue property, well suited for polarimetric measurements, is its microstructural anisotropy (asymmetry). Many diseases are associated with microstructural alterations, such as changes in collagen content and organization, muscular hypertrophy/atrophy, or perhaps cellular orientations. These structural abnormalities can present changes in linear birefringence that can be detected by polarized light. Using polarized light to image collagen fibrils in thin tissue microscopy goes back to the 1960s.\textsuperscript{[13]} Today, many other uses of polarized light in biomedicine have emerged, most often as an add-on option (in combination with other optical imaging modalities), such as polarization-sensitive hyperspectral imaging, polarization-sensitive optical coherence tomography (PS-OCT), polarization-sensitive fluorescence microscopy, and polarization-sensitive multiphoton microscopy.\textsuperscript{[14]} For example, a well-established clinical application of polarized light is imaging the anisotropic structure of the retina with PS-OCT, whereby the damaged retinal nerve fiber layer (resulting from glaucoma, age-related macular degeneration, or diabetic retinopathy) can be quantified in terms of lateral and depth-dependent abnormal birefringence.\textsuperscript{[15]}

An emerging direction, in recent years, is the use of polarized light as a standalone modality for characterizing the structural properties of bulk biological tissues.\textsuperscript{[16]} Such bulk tissue polarimetry is challenging, since the heterogeneous and highly scattering nature of biological tissues causes extensive light depolarization and, thus, loss of polarization signal information. Short of getting rid of this problem altogether (as done in microscopy, through the use of thin slices) or partially negating it through depth gating (superficial layer imaging via OCT or confocal approaches), examining bulk tissues has to be done in the presence of severe light diffusion and, thus, polarization signal loss. Fortunately, sensitive measurement and data analysis methods have been developed recently to address this depolarization challenge. Most bulk tissue polarimetric methods can now yield well-resolved lateral images, but offer cumulative polarization properties through the interrogated depth direction. However, bulk tissue polarimetric methods have the advantage of being less complex, less expensive, sensitive to larger sampling depths, and easier and faster to implement.
for a large lateral field of view than the depth-resolved imaging modalities.

As explained in greater detail in the next section, many emerging approaches are based on measuring the bulk tissue’s complete polarimetric transfer function, known as its Mueller matrix. This is an information-rich tissue signature that contains the full polarization information reflecting its biophysical properties. However, its measurement, while important and often challenging, is only the first step, and given the abundance of information this matrix contains, further analysis must be used to isolate out the separate distinct biophysical polarization metrics. The Mueller matrix, thus, analyzed via various decomposition methods can then yield various optical effects, including the tissue’s linear retardance (proportional to linear birefringence/tissue anisotropy), circular retardance (proportional to circular birefringence/chiral molecule content), and depolarization. Depolarization abnormalities, arising from changes in tissue scattering and absorption properties (transport albedo), are related to alterations in the stroma (e.g., collagen remodeling) and cellular compartment disorders (e.g., nuclear enlargement). Given the fundamental and clinical importance of these and related changes, Mueller matrix polarimetry employing depolarization and retardance has been used in a variety of (mostly preclinical) studies. A partial list includes differentiating different tissue types, identifying skin cancer lesions, detecting oral precancerous tissue in animal models, visualizing cervical cancer margins in excised samples, locating myocardial infarctions and observing their regeneration following stem-cell therapy, finding the relation between the dispersion pressure and anisotropy in bladder wall, identifying bladder obstruction disorders, and imaging collagen structure in ex vivo tissue of animal models.

As evidenced by such variety, bulk tissue polarimetry holds much promise in biomedicine, but as with any new methodology, many significant challenges remain. In this paper, we summarize the status of the polarimetric field, outline possible emerging improvements, and speculate on its future deployment in biomedicine. We begin with an introduction to the Mueller matrix methodology, follow with a brief synopsis of recent developments in the instrumentation, and discuss the remaining challenges in the translation of polarimetry into a standalone diagnostic tool. Illustrative application examples and potential future directions of tissue polarimetry are provided throughout.

2 Mueller Matrix Polarimetry in Bulk Tissues: Methodologies and Illustrative Examples

The simplest polarimetric method to examine the properties of a turbid medium, such as tissue, is to illuminate it with a known polarization state (mathematically described by the four-elements incident Stokes vector $S_{in}$) and to detect the change in polarization of the light after the interaction (represented by the four-elements output Stokes vector $S_{out}$) [see Fig. 1(a)]. Stokes vectors can describe fully or partially polarized optical beams and, thus, are well suited for describing polarized light–tissue interactions. Comparing the polarization of the incident and the collected light after interaction with the sample can then yield some metrics (e.g., light depolarization) associated with various tissue properties. For instance, 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. Recently, Ghassemi et al. showed that different roughness in melanoma lesions leads to abnormal polarization changes and loss of the degree of light polarization. They used this criterion for in vivo differentiation of melanoma, benign nevi, and normal skin. Analogously, Kunnen et al. recently mapped the measured $S_{out}$ onto the surface of the Poincare sphere, which visualizes the Stokes vector in a three-dimensional (3-D) space with the axes being pure states corresponding to each of the three elements, to distinguish tumor from normal lung regions in ex vivo tissue samples [Fig. 1(b)]. These methods work well when comparing different tissue types or normal/abnormal tissues with the same thickness, and in the same detection geometry. In other words, the
experimental and sample parameters must be tightly controlled for meaningful results comparison and interpretation; this is because the readings are affected by both tissue parameters (quantities of interest) and experimental parameters (e.g., illumination polarization and geometry).

To characterize the polarimetric properties of bulk tissue without admixing uninteresting experimental parameters (e.g., illumination), and without any prior information (i.e., arbitrary and completely unknown turbid media, such as tissue), the full Mueller matrix $M$ should be measured. Mathematically, Mueller matrix $M$ is the medium’s polarization transfer function, a 4 by 4 element matrix related to the input and output light polarization by $S_{\text{out}} = M.S_{\text{in}}$. The Mueller matrices of optical elements, such as polarizers or retarders, can be theoretically calculated from the way they change the polarization of light. But the Mueller matrix of turbid inhomogeneous media, such as tissues, is unpredictable analytically because of the uncertainty in refractive index, size, and location (and nature!) of the scatterers that randomize the photons’ propagation paths and result in depolarization. In the special case of well-controlled scattering media (e.g., a turbid phantom composed of uniform-sized microspheres suspended in water), the elements of $M$ may be calculated or approximated, for instance, certain elements set to zero and others set equal (or equal in value and opposite in sign). However, simplifying assumptions are usually not justified in biological tissues, and their measured Mueller matrix elements are generally independent of each other and can have any numerical value from $-1$ to $1$ for a normalized matrix.

For all its advantages, the Mueller matrix is a lumped polarization transfer function of tissue and contains all the simultaneous optical effects, such as scattering, absorption, retardance, and optical activity occurring in the turbid media. The Mueller matrix elements on their own cannot provide detailed information about a sample of tissue with unknown properties. There are, however, several decomposition methods that help separate these convoluted intermixed effects from the Mueller matrix (more details below) and derive tissues’ structural and compositional properties. In recent years, a variety of Mueller matrix decomposition methods have been proposed. The very first approach that was successfully applied to bulk biological tissues was Lu-Chipman polar decomposition. Next, (2) the retardance matrix can be further decomposed to a linear retardance and a circular retardance matrices. The latter is related to the presence/concentration of biologically interesting chiral substances, such as glucose. From the linear retardance matrix, the tissue strength of the structural anisotropy and its orientation can be retrieved in terms of the optical phase retardance $\delta$ (proportional to birefringence) and the fast axis $\theta$. This is the aspect that is further pursued in this article in greater detail. Finally, (3) diattenuation is another polarization property of limited relevance/importance in biological tissues. Essentially then, Mueller matrix decomposition enables the separation of the depolarization effects resulting from multiple scattering and heterogeneities from other polarization properties, such as the retardance due to structural or compositional anisotropy.

Many groups have used Lu-Chipman polar decomposition of the Mueller matrix for developing diagnostic metrics based on the structural properties. Figure shows representative biomedical applications of Mueller matrix polar decomposition. As will be described in the next section, Mueller matrices were

![Flow chart showing the extended version of the Lu-Chipman polar decomposition and its outcome parameters. The tissue Mueller matrix $M$ is first decomposed to a product of a depolarization matrix $M_\Delta$, a retardance matrix $M_R$, and a diattenuation matrix $M_D$. From $M_\Delta$, three parameters, total depolarization $\Delta$, linear depolarization $\Delta_L$, and circular depolarization $\Delta_C$, can be obtained. The matrix $M_R$ can be further decomposed to a product of a linear retardance matrix $M_{RL}$ and circular retardance matrix $M_\psi$. From $M_{RL}$, the linear retardance $\delta$ and the dominant fast axis of anisotropy $\theta$ can be derived. From $M_\psi$, the concentration of the chiral molecules $\psi$ can be calculated. $M_D$ is the diattenuation matrix, which is of little relevance to the tissue.](https://www.spiedigitallibrary.org/journals/Downloaded From: https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 11/21/2018 Terms of Use: https://www.spiedigitallibrary.org/terms-of-use)
measured with different methods. Figure 3(a) shows an ex vivo distended rat bladder and the retardance images taken from the ventral side of the bladder marked with the black circle on the photo. As illustrated, the obstructed bladder shows high retardance compared with the normal bladder. This high retardance as suggested by the corresponding histology may be due to muscle hypertrophy or extracellular matrix reorganization/deposition. Locating these structural disorders is essential for optimal augmentation surgeries and monitoring the tissue functionality following with tissue engineering therapies. Polarized light imaging is the first method to localize these microstructural disorders induced by bladder outlet obstruction while the bladder is

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Fig. 3 Examples of biomedical studies showing the applications of Mueller matrix Lu-Chipman polar decomposition. (a) A picture of an ex vivo rat bladder; the black circle indicates the ventral region close to the urethra; retardance images (top row) from the ventral region on a normal and obstructed bladder; the arrows show the orientation of the fast axis; their corresponding histology (bottom row), stained with Movat stain. The Mueller matrix was imaged in reflection geometry, by mechanically moving polarizing components. (b) Retardance image of an ex vivo 1-mm-thick rat myocardium muscle and its respective histology slice. MT indicates malpighian tissue, C shows stroma, and CIN stands for cervical intraepithelial neoplasia. The pink and the blue color in the histology indicate the muscle and the collagen fibers, respectively. The Mueller matrix was imaged in transmission geometry, by mechanically moving polarizing components. (c) Photo, total depolarization, and retardance images from a patient ex vivo excised cervical tissue. The white lines show regions that were confirmed to be healthy and the red line shows the region that was reported cancerous by histology. The Mueller matrix was imaged in reflection geometry, by a liquid crystal (LC)-based system. (d) Retardance image of a 0.05-mm-thick fresh chicken tendon showing collagen fibers and their organization. The Mueller matrix was imaged in transmission mode by an LC-based system. (e) Retardance image of a fly larva (5x zoom). The Mueller matrix was measured in transmission geometry by a photoelastic modulator (PEM) based system, Exicor Microlmager (Hinds Instruments). Figures are produced with permission.
distended and intact; thus, it may have the potential of becoming an imaging modality in urology. Figure 3(b) depicts an \textit{ex vivo} demonstration of using Mueller matrix imaging and its polar decomposition, in detecting the myocardium muscle infarctions in a rat model. As shown, the loss of retardance (anisotropy) in the retardance image is well correlated with deposition of collagen, indicated by the blue color in histology, in place of muscle. This indicates the efficacy of polarized light as a research monitoring tool for evaluating the stem-cell therapy treatments for heart infarction, which is the number one cause of heart attacks. Figure 3(c) demonstrates a picture of an \textit{ex vivo} excised tissue of a patient with cervical cancer, and the retardance and the depolarization obtained from decomposing its Mueller matrix. The cancerous regions, indicated by the red lines as opposed to the healthy regions indicated by white, are associated with a loss of retardance and microstructural organization This suggests that polarized light imaging can be used as a rapid and accurate technique for detecting cancer margins during surgery, thus becoming a standard intraoperative imaging tool. Figures 3(d) and 3(e), respectively, show that the retardance images obtained from polar decomposition can be used for visualizing the well-organized collagen fibers (associated with high retardance) of 50-μm-thick chicken tendon and the different structure in a fly larva These two examples illustrate the capability of polarized light imaging/polar decomposition for wide-field microscopy and imaging detailed microstructures. From these examples, it is clear that polarized light imaging and polar decomposition of the Mueller matrix are powerful methods for characterizing various tissue microstructures, for various biomedical diagnostic/monitoring applications.

Bulk tissue polarimetry does not, in general, offer depth resolution/discrimination, so the two-dimensional depolarization and retardance images contain accumulated 3-D information over the interrogated depth. Similar to diffuse optical tomography, the optical voxel size of polarized light imaging is a function of light wavelength, measurement geometry, and tissue’s optical properties (scattering and absorption). Simulation results from polarization-sensitive Monte Carlo code (Pol-MC) suggest that average pathlength of the polarization preserving photons is ∼6 mm (in the visible wavelength range and for typical tissue properties). The interrogation depth then depends on the imaging geometry and the incident polarization state (circular, linear), and can be up to a few millimeters in transmission (∼4 mm) or in backscattering (∼2 mm). The lateral resolution (FWHM of point spread function of the polarization preserving photons in tissue for an incident pencil beam) is ∼200 μm. These numbers need further experimental validation with well-controlled phantom studies.

As mentioned before, there are a variety of methods to interpret a bulk tissue’s Mueller matrix. Polar decomposition is sequential, meaning that it models tissue as a sequence of a depolarization, retardance, and diattenuation. Perhaps somewhat surprisingly, this assumption works for tissues and model turbid media, as recently validated. However, polar decomposition fails to yield accurate results when the media is made of distinct layers of depolarizer, diattenuator, and retarder in a different sequential order than the polar decomposition sequence. For instance, Ortega-Quijano et al. showed that polar decomposition fails for a medium composed of a polarizer sandwiched between two layers of scattering media. Another approach, which has received a lot of attention recently, is the differential decomposition. Here, all the effects, such as depolarization and retardance, are modeled evenly throughout the entire sample and occur simultaneously (versus in sequence as per polar decomposition). In other words, all polarization effects occur simultaneously over infinitesimal propagation distance of light in the tissue. Some studies suggest that differential decomposition may be closer to tissue reality compared with polar decomposition, since it does not operate sequentially. To test this hypothesis, using Pol-MC code, we recently modeled many bilayered Mueller matrices of heterogeneous birefringent turbid media, with different orientation or value of anisotropy in the layers. The resultant Mueller matrices were then decomposed with both polar and differential decompositions. Our initial studies (manuscript in preparation) show that these two decompositions yield nearly identical results for a turbid medium that does not contain distinct layers of retarder/diattenuator (as is the case for biological tissues). Thus, it seems that differential decomposition does not offer a great advantage over polar decomposition for biomedical uses.

### 3 Polarimetric Instrumentation for \textit{Ex Vivo} and \textit{In Vivo} Tissue Diagnosis

Most current polarized light imaging systems that measure the full Mueller matrix of bulk tissues are at the research level. They have been used for \textit{ex vivo} characterization of tissues, intraoperative examination of excised tissues, and initial \textit{in vivo} examinations of retina and skin. Regardless of the application, a polarimetric measurement system contains a polarization state analyzer (PSA), which measures the Stokes vector of light after interaction with the sample (see Fig. 4). A complete polarimetric system for measuring the Mueller matrix should also have a polarization state generator (PSG) to control and modulate the polarization of the incident light, as shown in Fig. 4. Biological tissues are highly scattering and heterogeneous; thus, the collected light after interaction with tissue is significantly depolarized. To extract maximum polarimetric information in this challenging low signal-to-noise ratio (SNR) setting, the measurement system should be sensitive (able to sense small changes) and robust (measure the polarization changes reproducibly and accurately).

Several groups have shown that the polarization states generated and read by PSG/PSA can be optimized to yield more accurate and noise-robust recovery of the polarimetric signal. In addition to selecting the polarization states to optimize measurement accuracy, the measurement system should be carefully calibrated. The calibration procedures vary for different systems employing different polarization modulation components. However, in a rigorous study, Compain et al. suggested a general calibration procedure, applicable to any polarimetric system, regardless of the optics and optoelectronic modulation methods. With the novel advances in polarimetric systems, we believe that this procedure will be widely adopted in the next few years.

There are a variety of polarimetric measurement systems with different SNRs and sensitivities. Point measurement systems with low noise photodetectors and electronic lock-in detection are the most sensitive. For example, these point-sensing systems can be used for sensing small concentrations of glucose by measuring optical activity, or they can be used for detecting tissue microstructure with a point-by-point scanning approach with high sensitivity. However, imaging systems are more suitable for examining large areas (regions) of biological
tissues, which often show variations in the lateral direction. An ideal tissue polarimetry imaging system should be fast, robust, accurate, and provide high resolution over a large field of view. These characteristics depend on the modulating components in the PSG/PSA. Polarization can be modulated electronically by liquid crystals (LCs) or photoelastic modulators (PEMs), mechanically by rotating linear polarizers and quarter wave-plates, and by diffraction using polarization prisms, gratings, or graded index lenses. In recent years, the field has witnessed many advanced designs and approaches, and there have been several demonstrations of using these novel systems for imaging biological tissues (Fig. 3). Systems devoid of any mechanically moving parts are certainly preferred. LC- and PEM-based systems are both capable of imaging in millisecond time scales. LCs are easier to switch electronically and can be used for an arbitrary set of polarization states that result in improved measurement accuracy of the Mueller matrix. PEMs can only modulate the retardance in a sinusoidal fashion; however, they have larger clear aperture well suited for imaging over a large field of view. Snapshot systems, based on diffractive components, are the fastest, but they use spatial frequency filters, which reduce the amount of imaging information and may be suboptimal for high-resolution imaging over a large field of view.

One emerging direction in polarized light imaging is combining the measurement instruments with wide-field depth-resolved techniques. For example, a very plausible direction might be its combination with modulated imaging. Modulated imaging is a diffuse reflectance imaging modality to measure the optical properties of the tissue with limited depth-resolved capability in a relatively short time. Optical properties are beneficial for tissue differentiation and correction of fluorescence signals in image guided surgery. Since modulated imaging and Mueller matrix imaging both aim to characterize bulk tissues, one can combine them to potentially increase signal information content, specially for potential intraoperative biomedical diagnostics.

Finally, as mentioned, polarized light has been shown to effectively detect ex vivo tissue disorders of internal organs, such as the heart, cervix, and bladder. Reaching these organs deep within the body can enable in vivo deployment; therefore, polarimetric optical delivery/pick-up methods not based on free-space optics must be developed. These include various varieties of rigid or flexible waveguides, fibers, and catheters. This is challenging in that these optical delivery vehicles often distort the polarization states of the light they transmit; this will produce large signal artifacts that will likely overwhelm the weak tissue polarization signals these delivery vehicles are meant to relay. One promising solution to avoid this problem is the use of distal polarization components, which has been suggested by several researchers. Using distal polarizers facilitates accurate generation of polarized illumination and collection of the polarized light backscattered from the tissue. Using this concept, our group is in the process of fabricating a fiber optic probe with distal polarizing components for measuring the full tissue Mueller matrix (manuscript in preparation). This approach of using distal polarizing components has also been used in wide-field endoscopes; for instance, as shown in Figs. 3(a) and 3(b). Qi et al. incorporated moving polarizers at the proximal and distal ends of a commercially available laparoscope (1 cm outer diameter, rigid construction) to measure the linear part of the Mueller matrix. The first row and first column of the $4 \times 4$ Mueller matrix are, thus, not measured, often due to difficulties in generating and transmitting circularly polarized light. As illustrated in Figs. 3(c)–3(e), this modified laparoscope was used to measure the depolarization and the retardance of different organs in an open rat abdomen in vivo. As expected, the organs have different scattering, absorption, and morphological characteristics and, thus, can be differentiated with specific ranges of retardances and depolarizations. Another alternative is to use polarization maintaining fibers for delivery/collection of light and a PSA and a PSG at the proximal ends to modulate the polarization. For example, in a very recent report, Vizet et al. demonstrated full $4 \times 4$ Mueller matrix measurements through a single polarization maintaining fiber. To correct for random changes in the fiber optical axis, which changes the polarization at the sample compared with the proximal end, a switchable calibration mirror was used. This is similar to the calibration procedure applied in some PS-OCT systems that corrects the axis with the signal.
reflected back from the tissue surface. The details are not described in peer-reviewed publications yet, but are likely forthcoming. These developments will eventually enable in vivo polarimetry of deep-seated tissue pathologies in the near future, most likely in the cystoscopy/endoscopy/bronchoscopy settings.

4 Summary

United Nations has designated 2015 as the International Year of Light, and it is entirely fitting that a tissue assessment approach based on polarized light becomes an important player in diagnostic photomedicine in this time frame. After all, polarimetry has had a long and distinguished history in science, industry, and medicine, albeit primarily in transparent media and thin films; it is now poised to make its mark in examining thick biological tissues. The ability to retrieve structural properties of bulk tissues opens up many possibilities in biomedical applications, including fast intraoperative/laparoscopic diagnosis and simple low-cost cystoscopic/endoscopic screening. The most recent advances in the field are based on measuring (and decomposing) the tissue Mueller matrix, which contains all the relevant tissue biophysical properties accessible by polarimetry. The most promising directions in polarimetric imaging include applications, analyses, and instrumentation as outlined in this paper, demonstrating the resurgent interest and rapid progress in the field. Despite these recent advances, translating polarimetry in vivo and eventually into the clinic remains a challenge. Further advances that may facilitate this are development of more accurate Mueller matrix interpretation methods, improvements in measurement techniques and instrumentation (including optical delivery and pick-up probes that do not distort polarization upon transmission), combination with other wide-field imaging modalities, and applications to specific biomedical diagnostic problems.

References


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