Proof-of-principle demonstration of a Mueller matrix decomposition method for polarized light tissue characterization *in vivo*

Michael F. G. Wood*
Nirmalya Ghosh*
Eduardo H. Moriyama
Brian C. Wilson
Ontario Cancer Institute
Division of Biophysics and Bioimaging
University Health Network
610 University Avenue
Toronto, Ontario M5G 2M9 Canada
and
University of Toronto
Department of Medical Biophysics
610 University Avenue
Toronto, Ontario M5G 2M9 Canada

I. Alex Vitkin†
Ontario Cancer Institute
Division of Biophysics and Bioimaging
University Health Network
610 University Avenue
Toronto, Ontario M5G 2M9 Canada
and
University of Toronto
Department of Medical Biophysics and Radiation Oncology
610 University Avenue
Toronto, Ontario M5G 2M9 Canada

Abstract. We demonstrate the first *in vivo* use of a Mueller matrix decomposition method for polarization-based characterization of tissue. Collagenase is injected into a region of dermal tissue in a dorsal skin window chamber in a nude mouse to alter the structure of the extracellular matrix. Mueller matrices for polarized light transmitted through the window chamber in the collagenase-treated region, as well as a distal control region, are measured. From the measured matrices, the individual constituent polarization properties of the tissue are extracted through polar matrix decomposition. Large decreases in birefringence and depolarization are seen in the collagenase-treated region due to the destruction of collagen, showing the potential for this method to monitor the organization and structural anisotropy of tissue. This study represents the first *in vivo* demonstration of a Mueller matrix decomposition method for polarimetric tissue characterization.
© 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3065545]

Keywords: polarized light; collagen; Mueller matrix; tissue characterization; extracellular matrix; window chamber model.

Paper 08287LR received Aug. 15, 2008; revised manuscript received Nov. 19, 2008; accepted for publication Nov. 19, 2008; published online Feb. 4, 2009.

1 Introduction

Many tissue constituents, including collagen, muscle fibers, keratin, and glucose, interact with polarized light.[1] Changes in tissue structure, in particular collagen content and organization, are associated with a number of pathologies.[2,3] The ability to extract individual polarization properties from tissues has a number of biomedical applications, including measurements of tissue organization and anisotropy via birefringence, and the changes in these following various treatments, as well as determining concentrations of optical active molecules such as glucose via optical rotation. However, light scattering in the visible-near-infrared wavelength range complicates the extraction of important polarization information in tissue, due to the resulting depolarization and alteration of the incident polarized light. In addition, as several polarizing effects in tissue occur simultaneously, they must be individually decoupled and recovered from the overall measured polarization signals.

To perform quantitative polarimetry in tissue, a method of extracting individual polarization effects in the presence of scattering must be employed. We have previously reported a sensitive polarimetry platform[4] that measures low polarization signals from scattering media, and employs a polar Mueller matrix decomposition method to decouple the combined effects[5] from the experimentally obtained Mueller matrices. Presently, to demonstrate this approach *in vivo*, we report on intravital measurements in a dorsal skin window chamber mouse model, using collagenase to induce changes in tissue structure and anisotropy (birefringence). The skin window chamber model is suitable for birefringence modulation via collagen alterations, as collagens are the main skin component, accounting for ~60% of the dry weight.[6]

2 Materials and Methods

The polarization of light is well described through either Jones or Mueller matrix algebra.[7] However, the latter allows for depolarization interactions and so is preferred for tissue polarimetry. In this approach, a four-element Stokes vector $\mathbf{S}$ describes the intensity and polarization of the light beam. The transfer function of the Stokes vector for the light propagation through a medium that alters the polarization state is represented as a 4×4 Mueller matrix $\mathbf{M}$. The interaction with any medium is described by $\mathbf{S_o}=\mathbf{MS_i}$, where $\mathbf{S_i}$ and $\mathbf{S_o}$ are the input and output Stokes vectors, respectively.

To measure the polarization effects in optically turbid samples, including tissues, we have developed a sensitive Stokes polarimeter system.[5] Briefly, this employs polarization

*These authors contributed equally to this work.
†Address all correspondence to: I. Alex Vitkin, Ontario Cancer Institute, Division of Biophysics and Bioimaging, 610 University Avenue, Toronto, ON, Canada M5G 2M9. Tel: +1 416 946 2990; E-mail: vitkin@uhnres.utoronto.ca

1083-3668/2009/14(1)/014029/5/$25.00 © 2009 SPIE

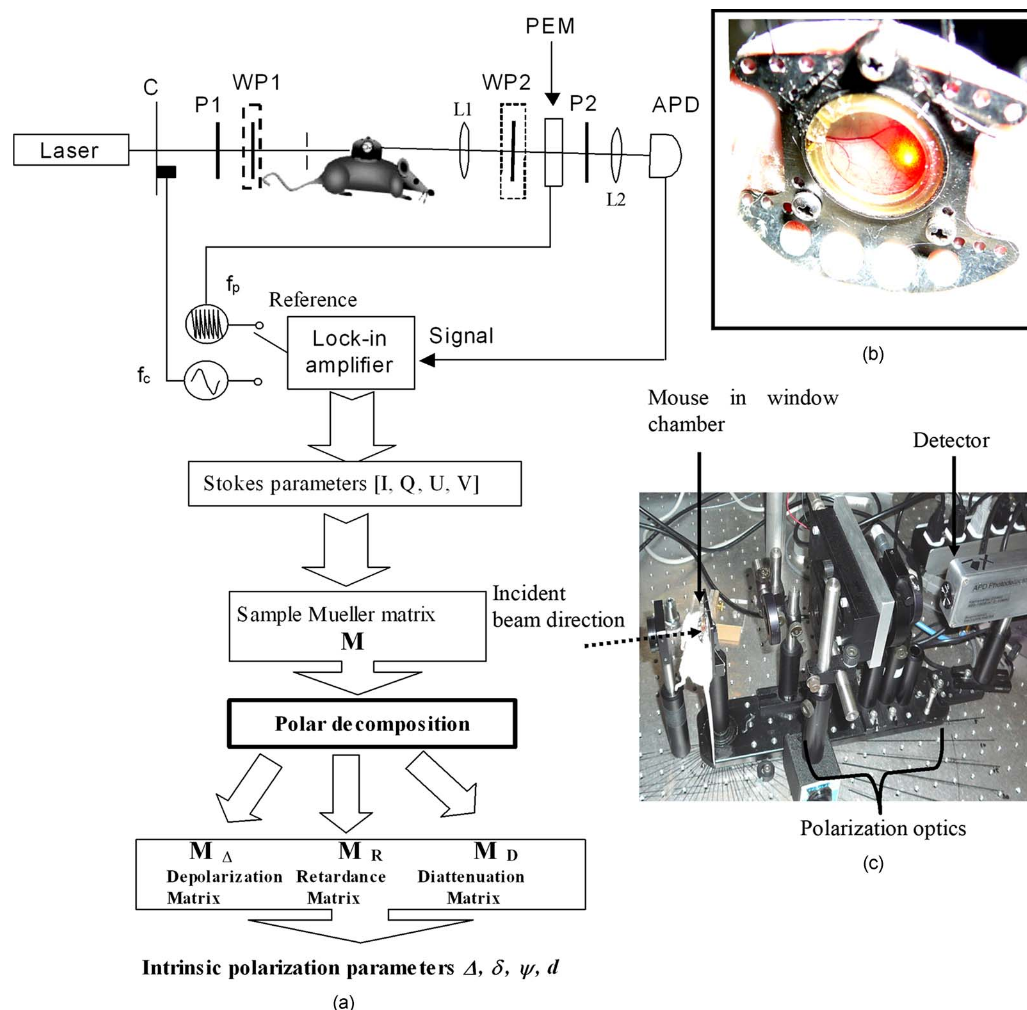

Fig. 1 (a) Schematic of the measurements system and the polar decomposition method: C, mechanical chopper; P1 and P2, linear polarizers; WP1 and WP2, quarter waveplates; PEM, photoelastic modulator; L1 and L2, lenses; APD, avalanche photodiode; f_c and f_p, modulation frequencies of mechanical chopper and PEM, respectively. (b) Photograph of the dorsal skin flap window chamber model in a mouse. Measurements were made in two regions (collagenase treated on the upper right, and control on the lower left) through the window chamber. In this photograph, the beam is incident in the treatment region showing the approximate region of measurement. Also note the increase in blood content in the treated region. (c) Photograph of the experimental system, showing the mouse with its implanted window chamber in the path of the interrogating beam.

modulation using a photoelastic modulator (IS-90, Hinds Instruments Hillsboro, Oregon) and synchronous lock-in detection (SR830, Stanford Research Systems, Sunnyvale, California), allowing sensitive low-noise measurements of the Stokes vector of the light interacting with a turbid sample. By cycling the polarization of the incident beam (HeNe laser, 15 mW, $\lambda = 632.8$ nm) using a linear polarizer and quarter-wave-plate combination, and measuring the output Stokes vectors, the Mueller matrix of the sample can be constructed.[5] A schematic of the experimental system is shown in Fig. 1.

However, in a complex medium such as biological tissues, several polarization-altering effects occur simultaneously, and the resultant measured Mueller matrix reflects these in a coupled complicated manner. Thus, a method to extract the individual effects is required to quantify contributions of individual polarization effects, such as birefringence, optical activity, and depolarization. To accomplish this, we have developed a polar decomposition method suitable for use in highly scattering samples,[5] where the measured matrix $\mathbf{M}$ is decomposed into "basis" matrices representing the individual effects. In this approach, a depolarization matrix $\mathbf{M}_\Delta$, a retardance matrix $\mathbf{M_R}$, and a diattenuation matrix $\mathbf{M_D}$ are employed, with $\mathbf{M} = \mathbf{M}_\Delta \mathbf{M_R} \mathbf{M_D}$.[8] From these decomposed matrices, useful polarization properties of interest can be decoupled and calculated. Specifically, the elements of $\mathbf{M}_\Delta$ can be used to calculate the net depolarization coefficient (Δ), the elements of $\mathbf{M_R}$ can be used to calculate the linear retardance (birefringence δ) and optical activity (ψ), and the elements of $\mathbf{M_D}$ can be used to calculate the diattenuation (d).[5,8] This decomposition process is summarized in Fig. 1.

We have previously validated this method theoretically with a polarization-sensitive Monte Carlo model and experimentally using tissue-simulating phantoms,[5] and performed measurements on *ex vivo* tissue samples.[9] To demonstrate its first use *in vivo*, we presently report measured changes in tissue structure using a dorsal skinfold window chamber mouse model.[10] In this model, the inner skin layer of an athy-

mic nude mouse (NCRNU-M, Taconic, Hudson, New York) was removed from a 10-mm-diam region on the dorsal surface and a titanium saddle was sutured in place to hold the skin flap vertically, as shown in Fig. 1. A protective glass coverslip (145 ± 15 μm thick) was placed over the exposed tissue plane. Thus, a thin layer of tissue ($\sim$500 μm) extends away from the animal, allowing for direct optical transmission measurements of polarized light through this layer, as shown in Fig. 1. This model enables accurate measurements in an *in vivo* setting, free of many of the challenges inherent in using fully 3-D tissue structures.

To induce changes in this skin preparation, 5 μl of collagenase (gelatinase) solution (Sigma-Aldrich, collagenase from *Clostridium histolyticum*) at a concentration of 1 mg ml^{-1} was microinjected into one region of the tissue. Collagenase cleaves collagen fibers by breaking the peptide bonds connecting the monomer peptide units.[11] Since collagen fibers are one of the primary sources of tissue birefringence and scattering, their destruction should change the polarization of the light propagating through this treated tissue region. Measurements of **M** were performed both in the region of collagenase injection and a distant control region, as shown in Fig. 1. Measurements were made with a 1-mm-diam laser beam before collagenase treatment and for 5 h postinjection at 30-min intervals, with an additional measurement at 24 h. An ink circle with a diameter of approximately 2 mm was marked around the control and treated regions so that the measurements could be repeated and correlated with subsequent histology. The treatment and measurements were carried out under general anesthesia induced by intraperitoneal injection of ketamine/xylazene (80- and 5-mg Kg^{-1}, respectively). Before each measurement, the animal was reanesthetized. Values for birefringence, depolarization, optical activity, and diattenuation were extracted from the experimentally derived Mueller matrices at each time point, through the prior polar decomposition approach. On completion of the measurements (24-h postcollagenase injection), the animal was sacrificed and the control and treated tissues were removed, sectioned laterally, and stained with hematoxylin and eosin (HE) for histological examination.

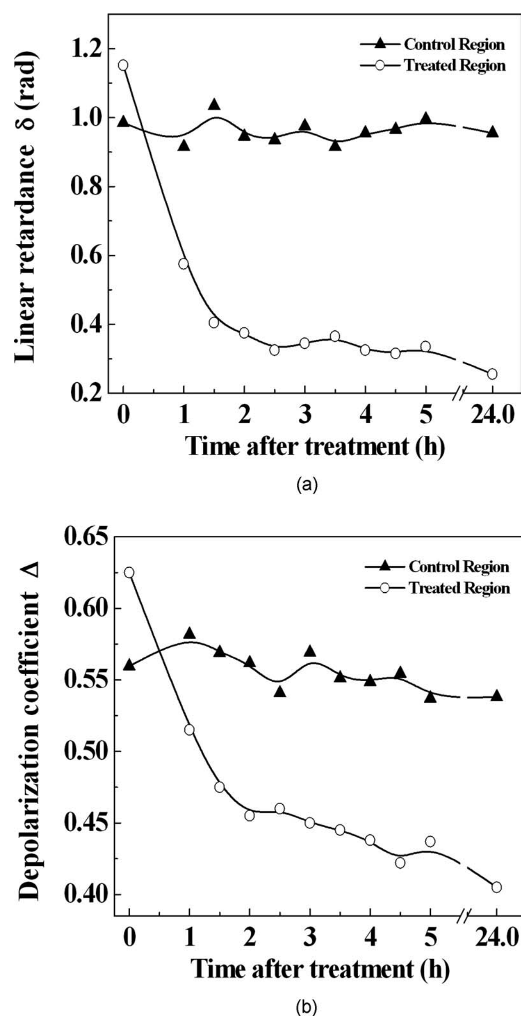

Fig. 2 (a) Birefringence and (b) depolarization before and as a function of time after collagenase injection in the treated and control regions. Symbols are experimentally derived values.

3 Results and Discussion

The derived birefringence and net depolarization values in both collagenase-treated and control regions are shown in Fig. 2. Values for optical activity and diattenuation were also extracted; however, these did not change appreciably with treatment and so the data are not shown. Histology (HE stain) of treated and control regions are shown in Fig. 3. As expected, injection of collagenase induced structural changes in the skin, seen as a loss of collagen fibers and the presence of inflammation and vasodilatation, which resulted in a more irregular dermis structure compared to the control tissue (Fig. 3).

In Fig. 2(a), the calculated values of the birefringence δ in treated and control regions are plotted as a function of time following collagenase injection, the former showing a range from $\delta\approx1.2$ rad to $\delta\approx0.3$ rad, in contrast to the control region where the values remain essentially constant at $\delta\approx1$ rad. (The small fluctuations in the control-tissue values give some indication of the reproducibility of the measurements over time.) The decrease in birefringence is likely due to denaturation of the collagen fibers, which reduces the structural anisotropy. This is confirmed by histology, where a reduction in collagen fibers is evident in the treatment region (Fig. 3).

Using the approximate light pathlength $l\approx500$ μm (estimated here as the thickness of the tissue), the intrinsic birefringence Δn (difference in directional refractive indices) can be estimated as $\Delta n=\delta\lambda/2\pi l$, where λ is the wavelength of the light (632.8 nm). Although the optical path length will be longer than the tissue thickness l due to scattering,[12] the polarization preserving light, which we are measuring, will have a shorter path length than the overall path length. Using this relationship, the birefringence values in the treatment region prior to treatment and in the control region were calculated as $\Delta n\approx2.4\times10^{-4}$ and $\Delta n\approx2\times10^{-4}$, respectively. After treatment, the value decreased to $\Delta n\approx0.6\times10^{-4}$. These birefringence levels compare reasonably well with those found in the literature for tissue birefringence; typical values are in the range of 1×10^{-3}.[13]

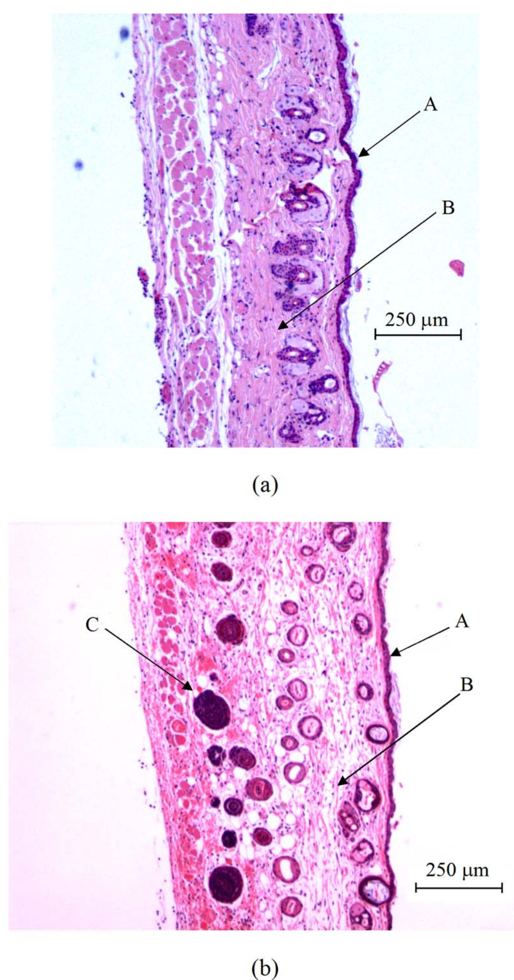

Fig. 3 Histology (hematoxylin and eosin stain) for (a) control and (b) treated tissue sections, showing skin surface A and collagen fibers in the dermis B. In the collagenase-treated region in (b), reduction in dermal collagen fibers is evident, as is the presence of blood vessels with increased diameter C as a result of inflammatory response. The sectioning was done laterally through the skin flap. The external surface of the skin is on the right and the internal surface in contact with the glass coverslip is on the left.

A decrease in the net depolarization coefficient Δ after treatment, from $\sim$0.63 to $\sim$0.45, was measured in the treated region and is plotted together with the values in the control region in Fig. 2(b), the latter again being essentially constant. This reduction in depolarization is also due to the destruction of the collagen fibers, since these represent one of the primary scattering structures in tissue. As with changes in birefringence, this is also confirmed by histology: see Fig. 3(b).

Using these changes in the depolarizing properties of the tissue, we were able to approximate the scattering coefficient of the tissue using our polarization-sensitive Monte Carlo simulation platform.[12] Simulations were run with a 500-μm-thick slab geometry, where the absorption coefficient (μ_a), scattering anisotropy (g), and birefringence were held constant at μ_a=2.2 cm^{-1}, g=0.9, and $\Delta n=4\times10^{-4}$ for control/pretreatment, while Δn was set to 1×10^{-4} for post-treatment (optical properties estimated for skin). The scattering coefficient was varied from μ_s=100 to 250 cm^{-1} in the simulations. Monte-Carlo-generated net depolarization values for those photons exiting the sample in the forward direction were calculated, from which the scattering coefficients corresponding to Δ=0.63 and Δ=0.45 were selected. Using this approach, μ_s was estimated as 182 cm^{-1} for the pretreatment tissue and as 134 cm^{-1} for the post-treatment tissue.

These results show the ability of the method to quantify changes in tissue structure using polarized light *in vivo*. The destruction of the collagen fibers in the extracellular matrix results in reduced tissue anisotropy and a reduction in scattering effects. These changes produce reduced tissue birefringence and depolarization, respectively, as measured via the experimental Mueller matrix, individually extracted through the polar decomposition method, and further quantified via polarization-sensitive Monte Carlo simulations. An important point to note is that, as seen in the upper-right photograph in Fig. 1, the treated region has markedly increased blood content compared to the control tissue, which would result in marked increase in the tissue absorption. Despite this, the Muller matrix decomposition technique is able to yield the underlying polarization parameters.

4 Conclusions

These results present the first *in vivo* demonstration of Mueller matrix decomposition-based polarimetric characterization of tissues. The interpretation of measured changes in values for birefringence and depolarization is consistent with histology results, and shows promise for the method's ability to accurately quantify biologically relevant tissue parameters such as scattering and birefringence. This initial proof-of-principle study is being followed up by more extensive investigations of the use of this method *in vivo*, both for noninvasive measurements of glucose and for monitoring the response of infarcted myocardial tissues to stem-cell therapies. These studies will investigate whether similar measurements can be made in reflection geometries on bulk tissues, considering the additional complicating factors present with such geometries (such as sampling volume calculations and the effect of additional tissue layers). Clearly, there are many other potential applications, both in tissue diagnostics and in treatment response monitoring.

References

1. L. V. Wang, G. L. Coté, and S. L. Jacques, "Special section on tissue polarimetry," *J. Biomed. Opt.* **7**, 278 (2002).
2. J. A. Pearce and S. Thomsen, "Thermal damage process in myocardium," *IEEE Eng. Med. Biol. Mag.* **20**, 256–258 (1998).
3. K. Schoenenberger, B. W. Colston, Jr., D. J. Maitland, L. B. Da Silva, and M. J. Everett, "Mapping of birefringence and thermal damage in tissue by use of polarization-sensitive optical coherence tomography," *Appl. Opt.* **37**, 6026–6036 (1998).
4. X. Guo, M. F. G. Wood, and I. A. Vitkin, "Angular measurement of light scattered by turbid chiral media using linear Stokes polarimetry," *J. Biomed. Opt.* **11**, 041105 (2006).
5. N. Ghosh, M. F. G. Wood, and I. A. Vitkin, "Simultaneous determination of linear retardance and optical rotation in birefringent, chiral, turbid medium using Mueller matrix polarimetry: combined Monte Carlo and experimental methodology," *J. Biomed. Opt.* **13**, 044036 (2008).
6. C. R. Lovell, K. A. Smolenski, V. C. Duance, N. D. Light, S. Young, and M. Dyson, "Type I and III collagen content and fibre distribution in normal human skin during ageing," *Br. J. Dermatol.* **117**, 419–428 (1987).

7. D. Goldstein, *Polarized Light*, Marcel Dekker, Inc., New York (2003).
8. S. Y. Lu and A. Chipman, “Interpretation of Mueller matrices based on polar decomposition,” *J. Opt. Soc. Am. A* **13**, 1106–1113 (1996).
9. N. Ghosh, M. F. G. Wood, S. H. Li, R. D. Weisel, B. C. Wilson, R. K. Li, and I. A. Vitkin, “Mueller matrix decomposition for polarized light assessment of complex media such as biological tissues,” *J. Biophotonics* (in press).
10. M. Khurana, E. H. Moriyama, A. Mariampillai, and B. C. Wilson, “Intravital high-resolution optical imaging of individual vessel response to photodynamic treatment,” *J. Biomed. Opt.* **13**(4), 040502 (2008).
11. J. L. Seltzer, A. Z. Eisen, E. A. Bauer, N. P. Morris, R. W. Glanville, and R. E. Burgeson, “Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin,” *J. Biol. Chem.* **264**, 3822–3826 (1989).
12. M. F. G. Wood, X. Guo, and I. A. Vitkin, “Polarized light propagation in multiply scattering media exhibiting both linear birefringence and optical activity: Monte Carlo model and experimental methodology,” *J. Biomed. Opt.* **12**, 014029 (2007).
13. W. Wang and L. V. Wang, “Propagation of polarized light in birefringent media: A Monte Carlo study,” *J. Biomed. Opt.* **7**, 350–358 (2002).