Characterizing dual wavelength polarimetry through the eye for monitoring glucose

Bilal H. Malik* and Gerard L. Côté

Department of Biomedical Engineering, Texas A&M University, 337 Zachry Engineering Center, 3120 TAMU, College Station, TX 77843-3120

*malik@tamu.edu

Abstract: Diabetes is an insidious disease that afflicts millions of people worldwide and typically requires the person with the disease to monitor their blood sugar level via finger or forearm sticks multiple times daily. Therefore, the ability to noninvasively measure glucose would be a significant advancement for the diabetic community. The use of optically polarized light passed through the anterior chamber of the eye is one proposed noninvasive approach for glucose monitoring. However, the birefringence of the cornea and the difficulty in coupling the light across the eye have been major drawbacks toward realizing this approach. A dual wavelength optical polarimetric approach has been proposed as a means to potentially overcome the birefringence noise but has never been fully characterized. Therefore, in this paper an optical model has been developed along with experiments performed on New Zealand White rabbit eyes for characterizing the light path and corneal birefringence at two different wavelengths as they are passed through the anterior chamber of the eye. The results show that, without index matching, it is possible to couple the light in and out of the eye but only across a very limited range otherwise the light does not come back out of the eye. It was also shown that there is potential to use a dual wavelength approach to accommodate the birefringence noise of the cornea in the presence of eye motion. These results will be used to help guide the final design of the polarimetric system for use in noninvasive monitoring of glucose *in vivo*.

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1. Introduction

There are several researchers investigating various optical approaches towards completely noninvasive glucose sensing and, as a result, a number of different modalities have emerged [1–24]. Specifically, these completely noninvasive optical techniques include fluorescence spectroscopy [1,2], Raman spectroscopy [3,4], infrared (IR) spectroscopy [5–7], kromoscopy [8], optical coherence tomography (OCT) [9–11], optical polarimetry [12–21] and photo-acoustic spectroscopy [22–24]. While many of these devices probe the skin, interstitial fluid, or blood to ascertain the glucose concentration, because of the depolarization due to scattering and the presence of strong optically chiral molecules in skin and blood, optical polarimetry techniques have almost exclusively been used to probe the anterior chamber of the eye as a glucose sensing site. The eye provides a unique optical window, in which, absorption and scattering is minimal [25]. The major optical rotatory components in the aqueous humor are glucose, albumin, and ascorbic acid. The primary rotatory component is glucose which contributes to approximately 95% of the total observed rotation [26]. Moreover, it has been shown in humans that the glucose concentration of the aqueous humor is 70% of that found in blood [27] and a direct correlation exists between the glucose concentration in the eye and blood glucose concentration with an average time lag of less than 5 minutes [28].

Glucose sensing using optical polarimetry is based on the phenomenon of optical activity, which is the rotation of the orientation of plane polarized light passing through a solution of optically active molecules. In our case the solution is the aqueous humor of the eye, the optically active molecule is glucose, and the light is passed through the cornea and across the anterior chamber of the eye.

Birefringence is a property of materials in which atoms are arranged in a regular repetitive array. Such an arrangement can make the material optically anisotropic i.e. their optical properties, including the refractive index, are different depending on the direction of propagation of light, and the material is said to be birefringent [29]. The direction with the lower value of refractive index is the *fast axis*, and the difference between the principle refractive indices is a measure of the birefringence, and is usually called the *birefringence*. The origination of birefringence in cornea is attributed to the retarder like behavior of its constituent collagen lamellae which constitute more than 90% of corneal stroma [30,31]. The overall effect of these individual lamellae manifests as corneal birefringence, and is similar to the mechanism of form birefringence [32]. In polarimetric glucose sensing through the

anterior chamber of the eye, this corneal birefringence masks the optical rotation due to glucose in the aqueous humor, and therefore acts as a noise source. To date, this time varying corneal birefringence due to motion artifact and the index of refraction mismatch between the eye and air are the biggest issues limiting the development of polarimetric glucose sensing through the eye. Specifically, when the cornea moves due to eye motion, its birefringence changes the polarization of the light and this is a significant source of polarization noise. In addition, the index mismatch between the cornea and air causes the light beam to bend as it propagates and this complicates our ability to couple the light across the anterior chamber of the eye.

The effect of corneal birefringence has been studied since the early 1800s when Brewster reported on the depolarization of light passing through cornea [33] and, although some of the literature in this area includes characterization of birefringence in the peripheral regions of cornea [34–36], most of the work done towards detailing corneal birefringence has been focused on central region of the cornea [37–42]. Most of the commercially available scanning laser polarimeters, too, are limited to macular imaging and hence the corneal limbus region is usually not well characterized.

Recently, an eye model towards quantification of the effect of corneal birefringence on polarimetric glucose sensing was developed and published by our group in an effort to understand whether corneal birefringence is wavelength independent and, if so, where this might occur [43]. This is important to understand since the assumption is that by using multiple wavelengths this noise source may be able to be canceled out. Although through this eye model it was shown that eye coupling is optimal at the mid-point between the corneal apex and limbus, this model was limited since it was performed assuming a refractive index-matched environment. Such a limitation is adequate for preliminary rabbit experiments in which an eye coupling device is easily used [44], but for monitoring glucose through the anterior chamber in humans, a coupling device is not desired and thus the model is not adequate for this case. More specifically, although it might be possible to build a coupling device such as a scleral or contact lens for human use, a method that does not require index matching would be preferred in terms of patient acceptance since there would be no need to directly contact the eye. To this end, the eye model described in this paper shows both the propagation of light and the birefringence in an unmatched index of refraction case, which will aid in understanding the effects of corneal birefringence and ultimately enable the design and development of a polarization-based glucose monitoring system.

2. Materials and Methods

2.1 Eye Model

For the eye model, the corneal parameters and dimensions were taken from reference [45]. As illustrated in Fig. 1, the posterior and anterior corneal surfaces were modeled as spheres centered on the optic axis of the eye with radii of 7.7 mm and 6.8 mm, respectively. The central thickness was taken to be 0.5 mm which increased monotonically (as a conicoidal function) to 1.97 mm at 6 mm away from the center [46]. Outside the cornea, the index of refraction was assumed to be that of air ($n = 1.00$). The refractive indices of cornea and aqueous humor were taken to be 1.376 and 1.336, respectively. Unlike most tissues in the body, the cornea contains no blood vessels. Instead, it relies on tears and aqueous humor for nourishment [47]. The absorption and scattering in the cornea is minimal to maintain transparency for sight [25]. The transparency of cornea, as well as its shape and smoothness is pivotal to the proper functioning of the eye. The smoothness of cornea is defined by the liquid tear film formation over the cornea's posterior surface which makes the cornea behave similar to a perfectly polished optical lens [47].

Fig. 1. Representative dimensions and refractive indices of the eye model. The lower boundary of the anterior chamber indicates the position of pupil and lens.

As depicted in Fig. 2, a local Cartesian coordinate system is defined at an arbitrary point P on the posterior cornea such that the z-axis is normal to the spherical surface. With this reference, the x-y plane constitutes the tangential plane to that surface. If the fast axis is taken to be along the z-axis, the other principle axes lie in the x-y plane. A biaxial model of the cornea would dictate that the electric field vector of a light beam incident at point P would, in general, experience n_x , n_y and n_z , refractive indices along the principal coordinate axes. Van Blokland and Verhelst noted that $|n_z - n_y|$ is an order of magnitude larger than $|n_y - n_x|$ [39]. Hence, for light incidence at larger oblique angles, n_z becomes dominant and a uniaxial model will give a good approximation. Therefore, the cornea in our model was treated as a bent uniaxial slab in which, at each point on the corneal surface, the fast axis was coincident with the direction of the local normal at that point.

Fig. 2. Local Cartesian coordinate system at an arbitrary point P on the posterior corneal surface. The system is aligned such that the z-axis is coincident with the local normal, and the x-y plane represents the tangential plane at point P.

The most significant difference in coupling light through the anterior chamber of the eye compared to the index-matched environment is that the incident light must enter the eye at a relatively glancing angle with respect to the posterior corneal surface, as explained below. The difference in refractive index between the cornea and aqueous humor is on the order 10^{-2} and hence does not have a significant effect on the refraction at that interface. Without any index matching, there is a limit on the range of both beam position and angle for which the light beam of a given diameter and shape can be coupled in and out of the anterior chamber of the eye. To analyze and quantify these ranges, we assumed two coincident circular beams of

light at two wavelengths (532-nm and 633-nm) with diameter of 1 mm. The incident beam polarization was set at 45° with the horizontal axis in order to experience the worst case in our model, namely, the maximum change in the state of polarization, since it will experience both the fast and slow axes equally. Both the beam position and angle of incidence was varied to explore the behavior of corneal birefringence and to find a region of minimal change in its effect, if any. Although, use of dual wavelengths allows to accommodate for the effect of motion artifact, a beam position which minimizes the effect of corneal birefringence due to eye motion can reduce the overall noise due to the sample thereby increasing the signal to noise ratio.

All the optical simulations and calculations were performed in CODE V optical design software (Optical Research Associates, Pasadena, CA) and MATLAB (The MathWorks, Natick, MA). The CODE V software employs a polarization ray tracing method to solve for the optical path through the optical system, the details of which have been described elsewhere [48]. It has the ability to divide the optical surface into rectangular or circular grid like pattern, where the user has the ability to define the birefringence parameters i.e. the direction of fast axis and the birefringence ($n_e - n_o$, where n_e and n_o are the extraordinary and ordinary refractive indices, respectively) for each individual grid element [49].

2.2 Experimental Setup

The experimental measurements of the effect of birefringence were performed on three New Zealand White (NZW) rabbits' eyes. All experiments were performed on eyes < 4 hours postmortem, and the corneas were visibly transparent before, during, and after the polarimetric measurements. The plane of incidence was taken to be along the nasal meridian and the point of incidence was at the nasal side of the eye for all instances. As illustrated in Fig. 3, the optical sources were two lasers: a 633-nm He-Ne module (JDS Uniphase Corp., Milpitas, CA) and a 532-nm diode-pumped solid-state laser module emitting at 1 mW and 4 mW, respectively. Both beams were made coincident using mirrors on flip mounts (Thorlabs, Newton, NJ) and the output light was polarized (100,000:1) at 45° by employing a Glan-Thompson linear polarizer (Newport, Irvine, CA). The combined beam was then passed through the anterior chamber of the eye. In order to change the beam incidence position and angle, the eye stage was mounted on a combined translational (Thorlabs, Newton, NJ) and rotational mount (Newport, Irvine, CA). The optical train terminated in the input facet of a rotating waveplate-based polarimeter (Thorlabs, Newton, NJ) which was connected to a personal computer for real-time state-of-polarization measurements.

Fig. 3. Optical configuration for experimental measurement of corneal birefringence. Note that one of the mirrors was placed on a flip mount in order to couple either wavelength at a time.

3. Results and Discussion

3.1 Index-unmatched coupling of light

In index-unmatched coupling of light, there is a limit on ranges of both the available beam position (measured as the distance from corneal apex) and the angle of incidence, α , as illustrated in Fig. 4. For a given beam position, there is a limited range of incident angles for which the full width of the beam can be coupled back out of the anterior chamber. Similarly, regardless of the angle of incidence, there is range of beam position for which the whole beam is able to exit. For the physical dimensions and beam size used in our model, this range of input was calculated to be from 1.6 mm to 2.5 mm below the apex of the cornea. For any given set of incident beam position and angle, the output beam is divergent and the spot pattern looks similar to that of coma. The overall state of polarization of the output beam was represented as the mean of the states of constituent individual rays across the cross-section of the beam.

Fig. 4. Optical path through the anterior chamber of the eye for unmatched refractive indices. The angle of incidence, α , is measured from the horizontal. Note that light has to be incident at a relatively glancing angle with respect to the posterior corneal surface in order for the beam to exit the anterior chamber through the cornea. There is no visible difference (on the current scale) between the optical paths taken by the two beams at different wavelengths, and hence, a single beam path is shown.

3.2 Corneal birefringence model

Based on the above information a total of three different beam positions – 1.6 mm, 2.0 mm, and 2.5 mm below apex – were selected and the possible ranges of incident angles for which the full width of the beam can leave the eye were probed. The corresponding data values are plotted in Fig. 5. It is apparent that the largest and shortest range of incident angles is attributed to the beam positions of 2.5 mm and 1.6 mm below the apex, respectively.

Fig. 5. Angle of major axis of polarization ellipse as a function of angle of incidence for beam position at (a) 2.5 mm, (b) 2.0 mm, and (c) 1.6 mm below the corneal apex. . Note that the change in the angle of major axis of the output beam (i.e. the y-axis) represents the effect of corneal birefringence only. The angles are calculated assuming only aqueous humor without glucose or any other optical rotatory components present in the anterior chamber of eye.

It should be noted that, although the overall range of major axis angles associated with beam positions of 2.5 mm and 2.0 mm show larger deviations due to corneal birefringence, there is a region in which this deviation is minimized but only when larger angles of incidence are used. In comparison, the full available range of angles for a beam position of 1.6 mm below the apex shows minimal change in perceived major axis angle of the polarization vector for smaller incidence angles. It can be seen in Figs. 6b and 6c that for light coupled at

beam positions of 2.5 mm and 2.0 mm below apex, and at angles which show minimal change in the output beam's major axis, the beam tends to focus more towards the center of the anterior chamber and away from the opposite anterior corneal surface. Consequently, the beam at the exit is more divergent compared to that of incidence at 1.6 mm (Fig. 6a), in which the focal point is near to or in the cornea. Therefore, the beam incidence at 1.6 mm below the apex would allow for a smaller incident angle and thus relatively less complicated coupling when compared to incidence at 2.0 mm and 2.5 mm below the apex. Hence, the beam incidence at 1.6 mm below the apex appears to be the optimal position to minimize the effect of both corneal birefringence and the curvature of the eye despite a short range of available incident angles.

Fig. 6. Optical path through the anterior chamber of the eye for beam position and angle of incidence at (a) 1.6 mm and 16° , (b) 2.0 mm and 23° , and (c) 2.5 mm and 28° , respectively. The angle of incidence for each instance was chosen to be in the most stable region of change in major axis. Note that output beams in (b) and (c) are more divergent when compared to (a).

It should be noted that even in this most stable region of change of angle of major axis, a small change in incidence angle changes the output polarization vector by a magnitude much larger than that of due to change in optical path length (i.e. the distance traveled through the aqueous humor in the anterior chamber of the eye) and/or glucose concentration. For instance, as reported earlier [43], a maximum change in optical path length through the aqueous humor contributes to a net change of ~ 2.5 millidegrees in the angle of polarization vector. Similarly, a 10 mg/dL change in glucose concentration, which is approximately the current detection

limit in polarimetric glucose sensing, produces a net change of ~ 0.4 millidegrees for light at 633-nm wavelength. In comparison, if the incidence angle shifts from 15° to 16° due to motion artifact, the change in polarization vector due to corneal birefringence is ~ 195 millidegrees. This observation, shown later experimentally to be even more severe, clearly demonstrates that time varying corneal birefringence due to motion artifact is a significant source of noise that needs to be addressed to realize polarimetric quantification of glucose *in vivo*. That being said, our group has previously proposed a dual-wavelength polarimetric method to accommodate for the effect of corneal birefringence due to motion artifact for glucose sensing, which involves multiple linear regression (MLR) analysis [18]. In the case of a change in incidence angle due to motion artifact, such an analysis would benefit from a linear relationship between the change of angles of major axes for the two wavelengths as a function of incidence angle. MLR analysis of the respective data points in Fig. 5c demonstrates a highly linear relationship with coefficient of determination (R^2) value of 0.9999 across the full range of incidence angles. This value is higher than the calculated mean R^2 value of 0.996 in *in vitro* experiments reported by our group [18]. Hence, the change in major axis of the state of polarization due to a change in angle can potentially be accommodated using MLR analysis, and consequently this modeling indicates that a dual-wavelength optical polarimeter can potentially be employed to reduce the sample noise associated with motion artifact.

3.3 Corneal birefringence measurements

To establish the suitability of our eye model, experimental results were obtained. Birefringence measurements were performed on three NZW rabbits' eyes. The angle of incidence was set at 15 degrees with the beam position near the midpoint between the corneal apex and limbus, i.e. near or at 1.6 mm. As mentioned above, the output beam casts a coma-like pattern making it difficult to collect the full extent of the beam without using collection optics. To overcome this problem, the detector head of the polarimeter unit was placed within a few millimeters of the beam exiting the corneal surface. The large size of detector head (~ 9 mm) was also helpful in maximizing beam collection.

The measured mean angle of the major axes was observed to be within 7 degrees on average (-38.2 degrees) for the 633 nm wavelength and 9 degrees on average for the 532 wavelength (-41.8 degrees) when compared to the modeled data. Note that the alignment to overlap the beams was done manually and, as explained below, this small overlap mismatch may be responsible for generating these small deviations from the modeled values. As long as the difference within an eye is constant this should allow for compensation of corneal birefringence within that eye.

The variation in the effect of corneal birefringence as a function of angle of incidence within a single eye was measured. Measurements were taken at an angle of incidence of 15° , 16.5° and 18° , which covers most of the theoretically available range. As explained below, the optical alignment was done manually by visual inspection. This limited controlling the angle of incidence with precision, especially near the ends of the available range where a small degree of misalignment can limit the full extent of the beam from leaving the eye. Figure 7 shows the variation in the measured angle of the major axis as a function of incidence angle. It can be seen that there is a variation in the measured values when compared to the eye model. This can be attributed to the differences in the distribution of birefringence in the cornea of rabbits' and human eyes. Such a comparison has been previously reported by Wang and Bettelheim, who investigated the corneal birefringence in several species [50]. The maps of birefringence isochores in the human eye show highly centro-symmetrical behavior which is similar to that of our eye model, and gives us some confidence that the absolute numbers in the model are correct. In comparison, the birefringence isochore map of the rabbit cornea is relatively anisotropic. Therefore, even a small deviation from the nasal meridian can cause discrepancies between the modeled and experimental observations, and hence the absolute

numbers are slightly off between the human model and the rabbit eye data. It was noted, however, that the net difference between the measured values at respective wavelengths is relatively unchanged. MLR analysis of the respective data points in Fig. 7 shows strong statistical correlation between the variables with correlation coefficient of 0.9515. Thus, the above mentioned regression analysis can account for this variation by generating weighted coefficients, consequently, showing the potential of a multispectral polarimetric approach towards minimizing the effect of corneal birefringence and allowing quantification of glucose in the anterior chamber of the eye [18].

Fig. 7. Intra-eye variation of the measured angle of the major axis of polarization ellipse as a function of wavelength and angle of incidence. Note that the angle of the major axis changes significantly with change in angle of incidence, but the net change between the data points for respective wavelengths is relatively constant.

The dissimilarities between the measured values shown in Fig. 7 and values generated from the model can also be attributed to experimental limitations such as the light beam positioning, which was done manually by visual inspection. Hence, it is possible that the incident spot was not exactly coincident with the desired position of 1.6 mm below the apex and/or at the center with respect to the x-plane. This can have a significant effect on the effective corneal birefringence experienced by the light beam. For example, de-centering of 633-nm beam position by a millimeter in our model would change the output beam's angle of major axis by magnitude of ~16 degrees. Specifically, a + 1 mm horizontal change from the center placement changes the major axis angle to -51.4 degrees compared to the previous value of -35.2 degrees. This further highlights the significance of time varying corneal birefringence due to motion artifact as a source of noise, where such a small deviation from the theoretically modeled values can lead to a substantial change in experimental observations. Moreover, postmortem artifacts like stromal edema and corneal autolysis can potentially change the tissue ultrastructure thereby changing the effective corneal birefringence properties, which may further increase the disparity between the predicted and measured data. However, even though the absolute values are different for the model versus the experiment, the relative values for each wavelength and difference between wavelengths is relatively constant showing the potential for the dual wavelength approach to compensate for these changes even if they are time varying.

4. Conclusion

In an effort to fully understand and characterize the corneal birefringence properties in the eye under unmatched refractive index conditions, the change in the light propagation and state of polarization of transmitted light were both modeled and experimentally measured. The corneal birefringence was shown to vary significantly as a function of both position and angle of incidence, but regions of relatively minimal net change were also observed. It was demonstrated that change in the polarization vector due to corneal birefringence is at least an order of magnitude larger than that due to the change in optical path length and glucose concentration. Experimental observations ascertain the validity of our theoretical framework towards modeling of peripheral corneal birefringence and, although the absolute values did not exactly match the model, the relative values were consistent between the experiments and model. Both the modeling and experiments showed the potential of using a dual-wavelength polarimetric approach towards measuring the aqueous humor glucose concentration as means to minimize the corneal birefringence noise and thus quantify blood glucose concentration. Overall, the knowledge gained from these experiments and modeling is useful in understanding changes in polarized light as it traverses an index-unmatched eye and will provide a framework for building an index-unmatched coupling system toward the development of an optical polarimeter for noninvasive *in vivo* glucose sensing. For *in vivo* execution, the laser power will be reduced to be within laser safety standards (ANSI limits). This reduction of power does not pose a problem since we are looking at polarization changes and not intensity changes. Moreover, the proposed coupling scheme utilizes a lateral (or tangential) beam path through the anterior chamber which further reduces the light power at the retina.

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