Corneal birefringence measured by spectrally resolved Mueller matrix ellipsometry and implications for non-invasive glucose monitoring

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Abstract: A good understanding of the corneal birefringence properties is essential for polarimetric glucose monitoring in the aqueous humor of the eye. Therefore, we have measured complete 16-element Mueller matrices of single-pass transitions through nine porcine corneas in-vitro, spectrally resolved in the range 300...1000 nm. These ellipsometric measurements have been performed at several angles of incidence at the apex and partially at the periphery of the corneas. The Mueller matrices have been decomposed into linear birefringence, circular birefringence (i.e. optical rotation), depolarization, and diattenuation. We found considerable circular birefringence, strongly increasing with decreasing wavelength, for most corneas. Furthermore, the decomposition revealed significant dependence of the linear retardance (in nm) on the wavelength below 500 nm. These findings suggest that uniaxial and biaxial crystals are insufficient models for a general description of the corneal birefringence, especially in the blue and in the UV spectral range. The implications on spectral-polarimetric approaches for glucose monitoring in the eye (for diabetics) are discussed.

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1. Introduction

Polarimetry is a promising approach to measuring the glucose level in the aqueous humor in order to realize a non-invasive glucose monitoring concept for diabetics [1]. However, the corneal birefringence is one of the most challenging issues for any approach to measuring glucose in the eye by polarimetric means. Sir Brewster discovered as early as 1815 that the corneas of human beings and animals change the polarization of light [2]. Structural properties of corneas were described in 1856 by His [3]. He analyzed corneas of numerous vertebrates, especially of humans, cows, sheep, pigs, rabbits, guinea pigs, doves, and crows, where he found great similarities regarding Bowman’s membrane (anterior lamina). At this time it was already well known that the cornea is composed of fibrils and lamellae. In 1912 Wiener derived mathematically that small transparent rods (fibrils) embedded in another transparent medium with different refractive index exhibit a so called form birefringence, even if neither the rods nor the embedding medium have intrinsic birefringence [4]. Maurice investigated the corneal birefringence based on Wiener’s theory and also experimental findings from microscopy and X-ray diffraction [5].

The mean thickness of the human cornea is about 0.53 mm at the apex and increases to 0.7 mm in the periphery. It consists of six layers dominated by the stroma (substantia propria) which contributes 90% of the thickness. The stroma is composed of hundreds of lamellae with a thickness of 1.5 to 2.5 µm and contributes most of the birefringence. The stromal lamellae are oriented parallel to the surface. They run from limbus to limbus and can be distinguished in histological sections by means of a polarizing microscope. Every lamella contains numerous parallel fibrils with a diameter between 19 nm in the anterior layers and 34 nm in the posterior layers in man. The orientation of fibrils varies by large angles between adjacent lamellae [6]. According to Wiener’s theory every lamella behaves as a positive uniaxial crystal with its optical axis parallel to the fibrils. If light is irradiated perpendicular to the fibrils such a lamella will act as a linear birefringent retarder. The form birefringence contributes only 2/3 of the entire birefringence, the rest is due to intrinsic birefringence of the fibrils [7].

In terms of total birefringence it can be expected that the cornea behaves at least approximately as a stack of hundreds of linear retarders with radially oriented fast axes, which are more or less randomly distributed. Even if a large part of the retardance effect of the linear retarders average out, some residual corneal retardance can be expected due to the limited number of lamellae and non-perfect randomness of the fast axis orientations. It has to be emphasized that the resulting retardance of a stack of retarders is not only linear but in general also has a circular component. At this point we refer to the experimental work published in 1869 by Reusch [8]. He investigated the polarization properties of stacked mica plates. Single mica plates behave as linear birefringent retarders. Reusch found that a pile of such mica plates exhibits circular birefringence if the plates are stacked in a manner that their fast/slow axes do not have the same directions. This circular retardance (also called optical rotation or optical activity) has to be added to the residual linear retardance. Also, Donohue showed in a theoretical calculation that a stack of planar lamellae, each approximated as a uniaxial birefringent layer, stacked one upon another with various angular orientations, exhibits optical rotation due to the arrangement of the lamellae [9].

The corneal birefringence in different species has been investigated with polarimetric methods by many researchers. Wang’s measurements show that the corneal birefringence in lambs, pigs, rabbits, carp, and humans may be different in detail, but generally exhibits similar behavior: In all species the areas of lowest birefringence were at the center of the cornea or slightly off center, whereas the birefringence increased towards the periphery of the cornea. Human corneas showed the smallest birefringence among the species he investigated [10]. Some authors assumed that the corneal birefringence behaves like an uniaxial crystal [11,12]. For this uniaxial model they presumed that the alignment of the fibrils in different lamellae is randomly distributed, and therefore they supposed that the retardance vanishes due to averaging effects as the angle of incidence approaches zero. The uniaxial model is
associated with a rotationally symmetric refractive index ellipsoid with two different axis lengths. Eventually most researchers preferred a biaxial crystal as a model for the corneal birefringence, because many experiments showed non-vanishing retardance for perpendicular irradiation onto the cornea surface [13–15] as well as two optical axes [16]. The biaxial birefringence model is associated with a triaxial refractive index ellipsoid with three different axis lengths. The triaxial index ellipsoid has two optical axes where the retardance vanishes. But for perpendicular incidence onto the cornea surface the expected retardance is non-zero for the biaxial model. The presence of an overlapping meshwork of orthogonal negative birefringent elements with spheroconic geometry has been proposed by Misson to explain the global positive biaxial behavior of corneal birefringence [17,18]. In order to investigate spatial variations of corneal birefringence on a microscopic level polarization-sensitive optical coherence tomography (PS-OCT) has been applied [19,20].

Measurements on human corneas showed a preferred direction of the slow axis. Knighton et al. reported that most of the investigated eyes exhibited corneal slow axes around 20° nasally downward, but with a broad distribution of about −10°…60° [21]. Here, the authors assumed that the cornea behaves as a linear retarder. Van Blokland et al. have applied Mueller matrix ellipsometry to human eyes in-vivo. They assessed the change in the state of polarization of a laser beam after two passes through the ocular media and scattering at the fundus of the eye [15]. An advantage of this method is that it provides results for the living eye. However, an unavoidable drawback is the fact that this is not a straight cornea measurement because the measurement light passes also the eye lens and is reflected at the retina. The aqueous humor, the crystalline lens, and the vitreous body may not contribute substantially to the total retardance [22]. But the nerve fiber layer of the retina is generally birefringent [23] and can adulterate the results. Furthermore, there is another fundamental issue: If the reflection takes place with perpendicular incidence onto the retina, at least part of the optical rotation is canceled out during the second passage of the light through the cornea due to the reversed direction of light propagation. Pierscionek et al. investigated human corneas in-vivo exploiting Purkinje images and mentioned that there may be some circular birefringence [24]. Corneas of cows and pigs were analyzed by Bueno et al. [25]. They used a Mueller matrix imaging polarimeter in transmission mode (using a 633 nm laser) and came to the conclusion that circular birefringence does not contribute much to the modification of the state of polarization and that corneas of porcines and bovines do not exhibit large effects of depolarization.

Most experiments on corneal birefringence have been performed by use of lasers, i.e. using single wavelengths, e.g., with an argon-krypton-ion laser at 488, 514, and 568 nm [15] or with a HeNe laser at 633 nm [25,26]. Only few investigations have been done for wavelength ranges of more than 100 nm, for example 440-700 nm [27] or 520-680 nm [16]. To our knowledge there are no investigations reported on corneal birefringence measurements in the UV range.

2. Aim and motivation of this study

Although corneal birefringence has been investigated by manifold means over the last 200 years, there is a lack of experimental information necessary to overcome the problems that occur during polarimetric glucose measurements in the aqueous humor of the eye. Such optical measurements could be a non-invasive and painless solution for diabetics to monitor their blood glucose level [1].

Polarimetric glucose measurements exploit a relatively specific physical property: the optical activity. Since the optical rotation by glucose in the aqueous humor is in the range of only millidegrees for physiological concentrations (typically 50 … 500 mg/dl), the corneal birefringence is a severe hurdle for all polarimetric approaches. On the other hand, glucose clearly dominates the optical activity in the aqueous humor [28]. Furthermore, the optical activity of glucose has a negligible temperature dependence [29], which is a very serious issue for other optical approaches like infrared or Raman spectroscopy.
A main issue for polarimetric approaches to glucose monitoring in the eye is the dependence of the corneal birefringence on the angle of incidence and also on the location where the light passes the cornea. The eye permanently executes small, fast movements (microsaccades) [30]. Additionally, there are slow movements due to heartbeat and breathing. There are also small changes of the angle of incidence and measurement location when a device is realigned to a test subject. It may be possible to significantly reduce the fast but small movements by use of a contact glass, as has been demonstrated *in vivo* with rabbits [31–33]. But it can be assumed that a contact glass will not be accepted by diabetics. Therefore, it is necessary to find a solution that is capable of differentiating between the optical activity of glucose and the birefringence of the cornea, preferably with a time resolution of 10 ms or less. Fortunately, the optical activity of glucose has a rather strong wavelength dependence and can be measured very accurately (see Fig. 8). This effect is called Optical Rotatory Dispersion (ORD). It is much stronger and more specific than the refractive index dispersion, since all dissolved salts in the aqueous humor exhibit no optical activity at all. The ORD effect can be exploited to distinguish between glucose and corneal birefringence by use of a spectrally resolved polarimetric measurement. But for such an approach it is indeed necessary to understand the spectral properties of corneal birefringence very well.

Consequently, the primary aim of this study was to explore the basic spectral properties of corneal birefringence down to the ultraviolet range. However, determining the spectral dependence of the cornea birefringence is a much more demanding task than measuring the glucose ORD, which is a completely isotropic effect, and therefore independent of the angle of incidence. In contrast, the corneal birefringence results from the lamellary structure of the cornea. Hence, dependencies on the angle of incidence can be expected. Moreover, there may be individual differences that have to be investigated.

3. Methods

![Cornea holder with two spherically shaped fused silica elements (Suprasil Q1, Schott AG). The cornea is placed between the glasses. The distance between the glasses can be adjusted according to the cornea thickness. Illumination light in the ellipsometer is incident from the direction opposite to the z-direction. For variation of the angle of incidence the holder can be rotated around the y-axis. The radii of the glass surfaces in contact with the cornea are 6.63 mm for the left glass and 7.61 mm for the right glass. The left glass has a thickness of 1.49 mm, the right glass of 1.58 mm.](image)

The best method to measure all corneal birefringence properties completely is probably Mueller matrix ellipsometry in single-pass transmission mode. In order to also get information about the depolarizing properties of the cornea 16-element Mueller matrix ellipsometry is necessary. This type of ellipsometry can be realized using two rotating retarders [34]. We used a commercial ellipsometer (Model RC2 from J.A. Woolam Co., Inc.) which covers the spectral range 211…1000 nm with spectral resolution of 1 nm. In our figures we displayed only the range 300…1000 nm because corneas are completely opaque below 300 nm. The accuracy of the ellipsometer in terms of the normalized Mueller matrix elements was specified to ± 0.001 by the manufacturer.
Nine porcine corneas from freshly slaughtered pigs were analyzed using the ellipsometer in transmission mode. The corneas were resected with surgical scissors and cleaned with a standard eye wash solution. All measurements were completed within a few hours after resection. This was necessary since the corneas showed noticeable clouding after more than six hours, especially in the UV spectral range. In order to bring the corneas back to their native shape as much as possible, a special holding device was manufactured (see Fig. 1). It contains two thin spherical meniscus lenses. The fused silica lens material (Lithosil Q0, Schott AG, Germany) is UV transparent and has very low birefringence. The radii of the menisci were adapted to the mean radius of human corneas, since pig and human corneas have similar sizes and shapes. All other parts of the holding device were made of stainless steel. The menisci were glued into the holding device to prevent drying-out of the corneas. The distance between the menisci was adjustable to take the variable cornea thicknesses into account. For every cornea the distance between the menisci was reduced until the cornea fitted closely to the glass surfaces and showed good transparency. As we had no information about the in-vivo position of the eyes, the azimuthal alignment of the corneas in the holder was arbitrary. The complete holding device could be moved in the x-direction and rotated around the y-axis. The nearly collimated measurement beam had a diameter of about 1 mm.

4. Theoretical description

Optical birefringence is a volume effect which can be described completely by 16-element Mueller matrices (for every wavelength) if the radiation is incoherent. In contrast to the Jones matrix formalism the Mueller matrix (MM) formalism is capable of taking depolarization into account. To reduce the influence of measurement errors, any measured Mueller matrix should first be checked for physical realizability. Cloude describes the conditions for physical realizability and also provides a matrix filtering concept to fulfill these conditions [35]. In most cases our measured Mueller matrices were modified only slightly by the filtering. In a next step the measured Mueller matrix can be separated into different Mueller matrices describing depolarization, retardance, and diattenuation [36]:

\[ MM_{\text{Measure}} = MM_{\text{Depolarisation}} \cdot MM_{\text{Retardance}} \cdot MM_{\text{Diattenuation}} \]  \hspace{1cm} (1)

The retardance cannot be explained as linear retardance alone as would be the case for a uniaxial or biaxial crystal model, but has to be further separated into linear retardance and rotation, i.e. circular birefringence:

\[ MM_{\text{Retardance}} = MM_{\text{LinRot}} \cdot MM_{\text{Rot}} \]  \hspace{1cm} (2)

The anatomical structure of the cornea, which is made up of numerous birefringent lamellae (see introduction), suggests that the total birefringence can be considered as a stack of linear retarders. Every linear retarder possesses a Mueller matrix of the following form:

\[
MM_{\text{Linear}}(\theta, \delta) = \begin{bmatrix}
1 & 0 & 0 & 0 \\
0 & \cos^2(2\theta) + \sin^2(2\theta) \cos \delta & \sin(2\theta) \cos(2\theta)(1 - \cos \delta) & -\sin(2\theta) \sin \delta \\
0 & \sin(2\theta) \cos(2\theta)(1 - \cos \delta) & \sin^2(2\theta) + \cos^2(2\theta) \cos \delta & \cos(2\theta) \sin \delta \\
0 & \sin(2\theta) \sin \delta & -\cos(2\theta) \sin \delta & \cos \delta
\end{bmatrix}
\]  \hspace{1cm} (3)

Here, $\theta$ denotes the angle of the fast axis and $\delta$ the linear retardance. A linear retarder generally transforms linearly polarized light into elliptically polarized light. Ellipticity and orientation of the polarization ellipse are coupled to each other. A stack of such linear retarders can be described mathematically by the product of their respective Mueller matrices. However, this product will only correspond to a resulting linear retarder for the special case of all fast or slow axes of the individual retarders being co-aligned. As mentioned earlier, this is not applicable for the cornea. The orientations of the fibrils of the individual lamellae can be
arbitrary distributed. This will generally lead to an additional rotation $\psi$, which has to be taken into account by a rotation Mueller matrix:

$$\text{MM}_{\text{Rot}}(\psi) = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(2\psi) & \sin(2\psi) & 0 \\ 0 & -\sin(2\psi) & \cos(2\psi) & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

(4)

If depolarization and diattenuation are tentatively neglected, the corneal birefringence can be described by following matrix product, with the three parameters $\theta$, $\delta$, $\psi$:

$$\text{MM}_{\text{Retardance}}(\theta, \delta, \psi) = \text{MM}_{\text{LinRet}}(\theta, \delta) \cdot \text{MM}_{\text{Rot}}(\psi)$$

(5)

The matrix elements of $\text{MM}_{\text{Retardance}}(\theta, \delta, \psi)$ in an analytical notation are as follows:

$$M_{11} = 1$$
$$M_{12} = M_{13} = M_{14} = M_{21} = M_{31} = M_{41} = 0$$
$$M_{22} = \left[ \cos \delta - 1 \right] \cos(2\theta) \sin(2\theta) \sin(2\psi) + \left[ \cos^2(2\theta) + \cos \delta \sin^2(2\theta) \right] \cos(2\psi)$$
$$M_{23} = \left[ 1 - \cos \delta \right] \cos(2\theta) \sin(2\theta) \sin(2\psi) + \left[ \cos^2(2\theta) + \cos \delta \sin^2(2\theta) \right] \sin(2\psi)$$
$$M_{24} = -\sin \delta \sin(2\theta)$$
$$M_{32} = \left[ 1 - \cos \delta \right] \cos(2\theta) \cos(2\theta) \cos(2\psi) - \left[ \cos \delta \cos^2(2\theta) + \sin^2(2\theta) \right] \sin(2\psi)$$
$$M_{33} = \left[ 1 - \cos \delta \right] \cos(2\theta) \sin(2\theta) \sin(2\psi) + \left[ \cos \delta \cos^2(2\theta) + \sin^2(2\theta) \right] \cos(2\psi)$$
$$M_{34} = \sin \delta \cos(2\theta)$$
$$M_{42} = \sin \delta \sin(2\theta + 2\psi)$$
$$M_{43} = -\sin \delta \cos(2\theta + 2\psi)$$
$$M_{44} = \cos \delta$$

(6)

By comparison of the matrix elements of $\text{MM}_{\text{Retardance}}(\theta, \delta, \psi)$ one obtains analytical expressions for the three angles $\theta$, $\delta$, $\psi$:

$$\delta = \arccos(M_{44})$$
$$\theta = \frac{1}{2} \arccos\left( \frac{M_{34}}{\sin \delta} \right)$$
$$\psi = \frac{1}{2} \arcsin\left( \frac{M_{23} - M_{32}}{1 + \cos \delta} \right) = \arctan\left( \frac{M_{23} - M_{32}}{\text{trace}(\text{MM}_{\text{Retardance}})} \right)$$

(7)

The description of the corneal birefringence by means of $\text{MM}_{\text{Retardance}}$ goes beyond the models of uniaxial or biaxial birefringent crystals because both models do not take an additional rotation $\psi$ into account. On the other hand, $\text{MM}_{\text{Retardance}}$ does not take into account any dependence on the angle of incidence, in contrast to the crystal models. For a general description one has to determine $\text{MM}_{\text{Retardance}}(\theta, \delta, \psi, \alpha, \vec{x})$ for every combination of angle of incidence $\alpha$ and location $\vec{x}$, due to the complex and individually variable structure of the cornea. If depolarization and diattenuation also have to be considered it may be necessary to fall back on the measured 16-element Mueller matrix $\text{MM}_{\text{Measure}}(\alpha, \vec{x})$. 

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In the Mueller-Stokes formalism it is rather simple to calculate the polarization state $\vec{S}_{OUT}$ after passage through the cornea if the Mueller matrix $MM_{Measure}$ and the incident Stokes vector $\vec{S}_{IN}$ are known:

$$\vec{S}_{OUT} = MM_{Measure} \cdot \vec{S}_{IN} \quad (8)$$

In this case it is also straightforward to calculate the degree of polarization $P$ after the cornea passage:

$$P = \frac{\sqrt{S_0^2 + S_2^2 + S_2^2}}{S_0} \quad \text{with} \quad \vec{S}_{OUT} = \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix}, \quad 0 \leq P \leq 1 \quad (9)$$

It is worth noting that the degree of polarization $P$ is not a property of the Mueller matrix describing the cornea but a property of the Stokes vector describing the light that has passed through the cornea.

5. Discussion of results

![Figure 2](image)

**Fig. 2.** Mueller matrix elements of cornea no. 2 as a typical example for results of a spectrally resolved ellipsometric measurement in transmission mode. The element M11 is normalized to 1 and hence not displayed. M44 is drawn bold because it is a direct measure for the linear retardance.

Figure 2 shows a typical spectrally resolved measurement of the 16-element Mueller matrix for a single passage through one of the porcine corneas. Since the Mueller matrices are normalized for each wavelength by dividing each matrix element by $M_{11}$, the element $M_{11}$ is always 1 and can be omitted in the diagrams. All matrix elements exhibit a continuous spectral behavior apart from some detector noise. Deviations from monotonic spectral behavior can be observed in the UV range (< 400 nm). The blue curve corresponding to element $M_{44}$ was drawn bold because this element is a direct measure for the retardance $\delta$ (as long as depolarization and diattenuation can be neglected), as indicated in Eq. (6).
Fig. 3. Birefringence parameters deduced from Mueller matrix measurements of nine porcine corneas in dependence on the wavelength. These measurements were taken at the apex of the corneas for a perpendicular angle of incidence. The three parameters (linear retardance, orientation of the fast axis, and optical rotation) are given in degrees [°].
The other measured matrix elements largely evade a direct physical interpretation. For this reason, it is useful to decompose the measured Mueller matrices, as described in the theoretical part.

In Fig. 3 the measured results in terms of the parameters δ (linear retardance), θ (orientation of fast axis), and ψ (optical rotation) are presented spectrally resolved for the nine porcine corneas. As can be expected from the measured matrix elements these parameters also show a continuous spectral behavior. The steps in the optical rotation curves for the corneas 6 and 9 are due to the limited degree range of the diagrams. In most cases the linear retardance curves do not have a monotonic behavior since the retardance drops again in the UV range.

In contrast, the curves corresponding to the orientation of the fast axis and to the optical rotation behave monotonic for all nine corneas. It is obvious that there are considerable individual differences between the corneas. This is to be expected for the orientation of the fast axis because the azimuthal orientation of the corneas in the holding device was arbitrary. It is remarkable that there is a considerable optical rotation for some corneas (no. 2, 3, 6, 9) but nearly no optical rotation for other corneas (no. 1, 7).

![Fig. 4. Linear retardance (here given in nanometers) of the nine porcine corneas, measured at the apex (x = 0 mm) for a perpendicular angle of incidence. For wavelengths below 500 nm the corneal retardance exhibits a significant spectral dependency.](image1)

![Fig. 5. Degree of polarization and diattenuation of the nine porcine corneas, measured at the apex (x = 0 mm) for a perpendicular angle of incidence. For wavelengths above 500 nm the degree of polarization is typically > 95%. Below 400 nm a strong decrease of the degree of polarization is observed. The degree of polarization was averaged for several polarization states of the incident beam. Compared to the other polarimetric properties of the corneas diattenuation is a negligible effect.](image2)
Figure 4 shows the linear retardance of all nine corneas in nanometers, instead of degrees. In case of a 1/λ dependency (of the retardance in degrees) constant values for the retardance in nanometers would be expected. But especially below 500 nm significant deviations from this expectation are revealed for all investigated corneas. This finding is not compatible with birefringence models based on a uniaxial or biaxial crystal.

In Fig. 5 the degree of polarization \(P\) and the diattenuation of the corneas are shown. Since the degree of polarization (after passing the cornea) depends on the polarization state (Stokes vector) of the incident light, \(P\) was calculated by use of four pure polarization states (horizontally linear, vertically linear, right circular, left circular) and then averaged. The degree of polarization drops monotonically with decreasing wavelength. In the infrared range (> 800 nm) \(P\) is larger than 97%, in the visible range (400…800 nm) it is typically between 88% and 97%, and in the ultraviolet range (< 400 nm) it drops rapidly below 90%.

Figure 6 depicts the linear retardance and the optical rotation as a function of the angle of incidence at the apex (\(x = 0\) mm) of cornea no. 2. It is conspicuous that both the retardance and the rotation are maximal for perpendicular incidence (0°). These findings are incompatible with the uniaxial crystal model for corneal birefringence. The maximum optical rotation for perpendicular incidence may be evidence for the assumption that the optical rotation does not come from isotropic optical activity (as is the case for glucose) but from structural properties of the cornea. For a more detailed analysis it has to be considered that...
there is a slight beam displacement due to the outer spherical glass of the cornea holder when rotating the holder around the y-axis. For the same reason the specified angles in the diagram are not identical to the angles of incidence.

Figure 7 shows the linear retardance and the optical rotation in dependence on the angle of incidence at the periphery (x = 3 mm) of cornea no. 2. Here, the spatial propagation of the measurement beam is even more complicated than at the apex. However, some qualitative statements can be made immediately. The comparison with Fig. 6 reveals a significantly different spectral behavior than at the apex. The linear retardance is much less constant at the periphery over the spectral range than at the apex of cornea no. 2. Another interesting finding is that the optical rotation is smaller at the periphery than at the apex, for all wavelengths. This is surprising because optical rotation is regarded as a volume effect and the cornea is usually thicker at the periphery than at the apex.

6. Implications on glucose monitoring

Beside the well-known fact that the corneal birefringence varies individually there are two fundamental hurdles which have to be considered for all polarimetric approaches to measuring glucose in the aqueous humor in-vivo.

6.1 Varying corneal birefringence

According to the results of previous studies as well as of this study, the corneal birefringence varies significantly for changing angles of incidence of the measurement beam. Comparable birefringence variations can also be expected for changes of the probed cornea area. Due to the complex shape of the cornea, usually both the angle of incidence and the probed area will change at the same time during movements of the eye or of the measurement device. Truly non-invasive optical methods (i.e. without contact glass) will always exhibit two relative movements which cannot be avoided completely. The first displacement occurs when the optical device is re-aligned to the head of a person for a new measurement. This effect can be reduced by sophisticated eye-tracking, yet not entirely.

![Fig. 8. Optical Rotatory Dispersion (ORD) of glucose for a concentration of 100 mg/dl and an interaction length of 10 mm. The ORD curve can be described adequately by a polynomial fit of 6th-degree. The polynomial function and the coefficients are given in the text.](image)

The second effect is due to movements of the eye itself. There are movements caused by the eye muscles (drifts, micro-tremor, micro-saccades) and in addition, there are movements caused by other parts of the body (heartbeat, breathing). The frequencies of these movements span a range of about 0.5…100 Hz. Furthermore, there might be changes of corneal
birefringence due to physiological changes (aging, intraocular pressure) on a long-term scale. Unfortunately, there are hardly any studies on these effects that have been published. Only Greenfield et al. noticed that in their experiments corneal polarization axis measurements showed a good 1-year stability [37], and in another study no significant age-related differences between children and adults were found in general [38].

All variations of corneal birefringence have to be measured in order to distinguish them from the optical activity of glucose in the aqueous humor. A potential approach may be to exploit the differences of the spectral signatures of corneal birefringence and optical activity of glucose. To this end, the corneal birefringence would have to be measured for every individual, comparable to the fitting of eyeglasses or intraocular lenses.

In contrast, the optical activity of glucose is independent of the individual and can be measured very precisely. The Optical Rotatory Dispersion (ORD) of glucose, i.e. the optical activity versus wavelength, is shown in Fig. 8. It was measured using the spectral ellipsometer RC2. For the range 400…1000 nm the ORD curve is consistent with the analytical form given by Ansari [39]. For the range 300…1000 nm a polynomial fit of 6th-degree can be used:

\[
\text{ORD}(\lambda)_{\text{corr}}[\circ] = p_0 + p_1 \cdot \lambda + p_2 \cdot \lambda^2 + p_3 \cdot \lambda^3 + p_4 \cdot \lambda^4 + p_5 \cdot \lambda^5 + p_6 \cdot \lambda^6 \quad \text{with } \lambda \text{ in [nm]} \tag{10}
\]

The polynomial coefficients are: \([p_0, p_1, p_2, p_3, p_4, p_5, p_6] = [2.8253e-18, -1.2192e-14, 2.1699e-11, -2.0447e-08, 1.0826e-05, -3.0941e-03, 3.8476e-01]\). The circular birefringence of the cornea (Fig. 6) and the ORD of glucose (Fig. 8) appear to have similar spectral dependencies but hugely different scales. However, it should be mentioned that the corneal circular birefringence never occurs separately from linear birefringence. Also, the dominant part of the corneal birefringence is always the linear retardance, with a clearly different spectral behavior compared to the glucose ORD.

Fig. 9. Simulated net rotation due to optical activity of glucose in the aqueous humor and eye lens reflectivity in dependence on the angle of incidence to the eye lens, for different incident (linear) polarizations. The exemplary curves are calculated at 400 nm wavelength for 100 mg/dl glucose concentration. The interaction length of the light with the aqueous humor is assumed to be 3.5 mm before and after reflection. At the Brewster angle (46.46°) the net rotation is half of the rotation expected for 90° angle of incidence since only the optical activity after reflection contributes to the net rotation in this case. A high portion of p-polarized light may yield high net rotation but also low reflectivity.

Although the intention of this publication is not to describe a specific experimental setup for non-invasive glucose monitoring, we would like to provide some ideas how to take the observed spectral birefringence properties of the cornea into consideration. Assuming direct illumination (i.e. without contact glass) reflection of the measurement light at the front surface of the eye lens is compelling. In this case we get five sequential interaction zones:

1. first cornea transition
2. first aqueous humor (a.h.) transition
3. eye lens reflection

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4. second a.h. transition
5. second cornea transition

It should be mentioned that the angle of incidence onto the lens surface must be larger than zero to avoid complete canceling of the optical activity after reflection, as illustrated in Fig. 9. Note that the simulated curves in Fig. 9 represent only the interaction zones 2, 3, and 4.

In terms of the Mueller matrix formalism the polarization properties of the eye can be described as a product of five corresponding Mueller matrices. The a.h. transitions correspond to simple rotation matrices that describe optical activity, mainly of glucose. For the reflection a corresponding theoretical Mueller matrix is also well known [40]. It depends only on the angle of incidence and the refractive indices of the a.h. and the eye lens. However, Mueller matrices for cornea transitions are much more complicated as shown in this paper. Note that all mentioned Mueller matrices are wavelength dependent.

One possibility for extracting the a.h. optical activity (or ORD) and therefore the a.h. glucose concentration may be a model based simulation of the complete measurement process. For such an approach polarimetric measurements with continuous spectral resolution promise much higher specificity than monochromatic measurements, as is well known from spectral ellipsometry for thin layer analysis.

As mentioned above, small changes of the light/cornea interaction zones due to eye movements have to be considered. An approach to address this issue could be a linear, multidimensional regression, also known as “spectral unmixing”. Here, one dimension is the spectral influence of the a.h. ORD on the polarimetric signal. The other dimensions correspond to disturbing effects like movement artifacts in x- or y-direction. When using this very powerful method it may be not necessary to know or to understand the microscopic structure of the individual cornea. Rather, it is important to know the spectral properties of the corneal birefringence at the interaction zone. In this context, a parametrization of the spectral dependency of the three most important corneal birefringence parameters ($\theta$, $\delta$, $\psi$) may be useful.

6.2 Temporal delay of glucose concentration in the aqueous humor

The glucose concentration in the anterior aqueous humor $G_{\text{AH}}$ corresponds to about 80% of the glucose concentration in the blood plasma $G_{\text{Blood}}$ [41]. Furthermore, it is known that the delay with which changes in the blood glucose level are reflected in the glucose concentration of the aqueous humor is about 3…5 minutes in rabbit eyes [31]. Regarding delay values it is important to consider the definition of the delay time. It would not be adequate to consider the delay in a “digital” sense, i.e. as a temporal shift of a step function in concentration. Instead, it seems more appropriate to assume a temporal behavior similar to analog low-pass filters. This can be easily understood if we consider a simple model for the glucose transport from the blood to the aqueous humor. The blood-aqueous humor barrier is a diffusion resistance which corresponds to the resistance $R$ of the low-pass. The aqueous humor volume corresponds to the capacity $C$ of the low-pass. The time constant of a low-pass is $\tau = RC$ and the step response is a simple exponential rise $(1 - e^{-t/\tau})$.

Assuming this model, the glucose concentration of the aqueous humor would rise by $(1 - 1/e) \cdot \Delta G_{\text{AH}} / \Delta G_{\text{Blood}} = 0.63 \cdot \Delta G_{\text{AH}} / \Delta G_{\text{Blood}}$ after the time $\tau$ if an instantaneous rise by $\Delta G_{\text{Blood}}$ took place in the blood at $t = 0$. Applying this low-pass behavior to a typical continuous blood glucose monitoring curve yields a modified curve which is essentially shifted by the time $\tau$. Therefore, it makes sense to define $\tau$ as the delay (or latency) time for the aqueous humor glucose concentration.

March et al. published that the glucose concentration in the aqueous humor of rabbits reaches 89% of the maximum level after 10 minutes [42]. Assuming a simple exponential rise to 63%, this corresponds to $\tau = 4.53$ minutes, which is in good agreement to the other mentioned measurements at rabbits [31].
Unfortunately, no valid measurement data of the glucose delay time in human eyes are available. But it is known that the eye length of rabbits is about 0.7 times the eye length of human beings [43]. The aqueous humor volume (corresponding to \( C \)) scales approximately with the third power of the eye length, while the surface (corresponding to \( 1/R \)) of the blood/aqueous humor barrier in the ciliary body should scale approximately with the second power of the eye length. Based on this estimation, the delay time \( \tau = RC \) should be \( 1/0.7 = 1.43 \) times larger for human eyes than for rabbit eyes. This yields \( \tau = 4..7 \) minutes for human beings. Such a delay time would be readily acceptable for glucose monitoring at diabetics. Cameron et al. came to a similar conclusion [44]. Nevertheless, there is clearly a need for direct measurements of the glucose delay in human beings to answer this question conclusively.

7. Conclusion

In this study complete 16-element Mueller matrices were measured for nine porcine corneas over a wide continuous spectral range of 300…1000 nm in single-pass transmission configuration. The benefit of the Mueller calculus is that depolarization is fully taken into account, which is important especially in the UV range. A surprise was the finding that the linear retardance (in nanometers) is far from constant for wavelengths below about 500 nm. And we found a significant optical rotation for most of the corneas.

Our results suggest that uniaxial or biaxial crystals are insufficient birefringence models for the corneal retardance since they do not consider optical rotation (circular birefringence). The dependence of the corneal optical rotation on the angle of incidence and the large differences in the properties of the examined porcine corneas support the assumption that corneal rotation is an effect caused by the structural corneal properties rather than by isotropic optical activity. In this context it is worth mentioning that the corneal rotation is smaller at the periphery than at the apex of the cornea, though the corneal thickness is smallest at the apex. An expected result was the almost negligible diattenuation of all investigated corneas. Depolarization is not negligible, particularly below 500 nm. It should be taken into account by using the depolarization Mueller matrix separated from the measured cornea Mueller matrix. Large individual variations of the birefringence strength confirm the observations of other researchers. For polarimetric glucose monitoring at the eye this means that the corneal birefringence properties will have to be measured very accurately and furthermore individually, comparable to the procedures for eyeglasses or intraocular lenses. The two most serious issues for non-invasive glucose measurements in the aqueous humor, movement artefacts and time delay of glucose concentration, have been discussed. In this context, it has been argued that there is a research need regarding the long-term stability of the corneal birefringence and also regarding the glucose concentration time delay in human eyes.

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