Linear Birefringence of the Central Human Cornea

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PURPOSE. To determine the polarization properties of the central cornea at perpendicular incidence in a normal human population on the assumption that the cornea behaves as a linear retarder.

METHODS. A corneal polarimeter provided a view of the fourth Purkinje image of a yellow (585 nm) light-emitting diode through crossed polarizers and a variable retarder. The Purkinje image was extinguished by adjusting the fast axis and retardance of the retarder to match the slow axis and double-pass retardance of the cornea. Both eyes of 73 normal subjects (49 women, 24 men; ages, 21–71 years) were measured. Correlations were expressed as Pearson’s r.

RESULTS. In most corneas the slow axis pointed nasally downward, with the peak of the axis distribution falling between 10° and 20° nasally downward. Double-pass corneal retardance varied widely (range, 0–250 nm); 80% of retardance values were uniformly distributed from 40 to 140 nm. Retardance was moderately correlated with axis (r = 0.5), such that weaker retardance was associated with axes that were more nasally downward. Corneal birefringence was well correlated between the two eyes of a subject in both axis (r = 0.77) and retardance (r = 0.75).

CONCLUSIONS. The variation of corneal birefringence among individuals is substantial enough to produce large, uncontrolled differences in the polarization state of a measuring beam, differences that can introduce variability in newer technologies for ophthalmic diagnosis. The interocular similarity of corneal birefringence suggests deterministic control of corneal development.

Several newer technologies for ophthalmic imaging and diagnostics use polarized light, making the polarization properties of the eye an important consideration. For example, retinal tomography performed with a confocal scanning laser ophthalmoscope uses polarizers to emphasize the reflectance from the retinal surface and reduce the signal from multiply scattered light; optical coherence tomography uses an interferometer to resolve the depth of reflecting structures and requires similar polarization states in the reference and sample beams to maximize interference; scanning laser polarimetry (SLP) measures the change in polarization state between the incident and reflected beams to sense the birefringence of the retinal nerve fiber layer (RNFL); retinal birefringence scanning senses the radially oriented macular birefringence to detect foveal fixation; and double-pass estimates of retinal image quality are sensitive to polarization effects. In all these technologies, uncontrolled changes in polarization state can reduce performance or act as a confounding variable.

The cornea is the ocular structure most likely to cause a change in polarization state because, as shown in numerous studies (reviewed by Bour), the cornea is birefringent; on passage through the cornea linearly or circularly polarized light generally becomes elliptically polarized. Lens birefringence, although present, is usually weak. For many technologies the light beam is approximately perpendicular to the corneal surface, and the cornea is well described as a linear retarder that is, an optical element characterized by two orthogonal linear polarizations, such that light of one polarization propagates through the material more slowly than light of the other and thus becomes retarded in phase. The orientation of the more slowly propagating polarization defines the slow axis of the retarder; the axis at 90° is called the fast axis. Retardance can be expressed as a phase shift (in either degrees or fractions of a wavelength) or, as is done in this article, as a difference in optical path length, that is, nanometers (nm) that a beam must travel in free space to undergo the observed phase shift. Understanding the effect of corneal birefringence on a particular technology requires knowledge of the range of values that might be encountered in a population. Until recently the literature contained information on only a limited number of subjects, although it is generally agreed that the slow axis of corneal birefringence points nasally downward (ND) in most people. Now Greenfield et al. have shown in 112 eyes of 63 subjects that the distribution of axes is highly skewed; 34% of axes (the mode of the distribution) lie between 10° and 20° ND, 17% of axes lie above the mode, and 49% of axes lie below the mode. Although their instrument could not measure retardance, in six eyes the retardance was too weak to determine an axis. This suggests that corneal retardance may vary over a wide range, a suggestion supported by the few values that appear in the literature. For example, double-pass corneal retardance in nine eyes of six subjects ranged from 70 to 171 nm, in three eyes of three subjects was 78, 81, and 113 nm, and in four eyes of four subjects was 64, 96, 106, and 114 nm.

The purpose of this study was to increase our understanding of corneal birefringence and its potential role in ophthalmic imaging technologies. To this end, we constructed a corneal polarimeter that measured both axis and retardance and used it to measure the central corneas in both eyes of normal subjects.

MATERIALS AND METHODS

Corneal birefringence was measured with the corneal polarimeter shown schematically in Figure 1. A yellow (peak wavelength, 585 nm) light-emitting diode (LED) illuminated the cornea and lens from 7.1° below the optic axis. Light reflected from the posterior surface of the lens formed the so-called fourth Purkinje image (P4) in a small, inverted image of the LED that was observed at 10 × magnification with an optical system composed of identical achromatic collimating lenses L1, L2 (focal length, 100 mm) and eyepiece L3. Rotating linear polarizers (P, A) linked to a common shaft were oriented with their axes perpendicular to one another; thus, light from the LED polarized by P was blocked by A unless it had undergone a change in its polarization state. For most subjects at most polarizer orientations, image P4 was generally not resolved.

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visible because the birefringent cornea converted the linearly polarized light from P to elliptical polarization. A Berek variable retarder (BVR; New Focus, Santa Clara, CA), a tiltable, rotatable plate of MgF₂ located in the collimated beam, could be adjusted in azimuth and retardance to cancel the effect of a double-pass through the corneal birefringence. Formulas provided by the manufacturer relate retarder dial readings to nominal retardance and include the dispersion of MgF₂. Calibration of the retarder was performed with a 633-nm HeNe laser, linear polarizers, and a waveplate with known retardance; null conditions at several different settings of the retardance dial yielded a scale factor that converted nominal to actual retardance values. The birefringence of BVR varied substantially with incident angle over the 8° field-of-view of the instrument. The eyepiece, therefore, was provided with a crosshair reticle, and all measurements were made with PIV located at the center of the crosshair.

An estimate of the measured corneal area was obtained by ray tracing (OSLO Light 6.05; Lambda Research Corp., Littleton, MA) the polarimeter aligned with a LeGrand theoretical eye. The measuring beam entered the eye through a 0.5-mm-diameter area centered 0.5 mm below the corneal apex and exited through a 0.6-mm-diameter area centered 0.35 mm above the apex. These estimates did not vary significantly when the parameters of the theoretical eye were changed to account for accommodation.

According to the curved biaxial crystal model of the cornea (see Discussion), deviations of the measuring beam from perpendicular incidence could introduce retardance from the perpendicular fast axis. Ray tracing showed that no portion of the beam exceeded an angle of incidence of 4.5°, an angle calculated to produce a retardance of approximately 5 nm. Thus, the retardance values reported here were all attributed to the tangential axes of the cornea. The subject fixated on a small green LED located 5.4° nasally at 120 mm from the cornea. Accommodation was not controlled. A measurement proceeded in two steps. First, BVR was set to zero retardance, and the polarizer axes were rotated until PIV disappeared, a null that occurred when the axis of P was aligned with one of the corneal axes. This step was the same in principle as the method used by Greenfield et al. and is attributed to Bone. The null was found three times, and the corneal axis was taken as the average of the three P settings. The average SD (repeatability) of the axis measurements was 2.2°. Second, the axes of P and A were set at 45° to the corneal axis to maximize the intensity of PIV. The fast axis of BVR was set parallel to the corneal axis, and the retardance of BVR was increased from zero while looking for the first null in PIV. If the corneal axis found in the first step was the slow axis, the retardance of BVR canceled the corneal retardance, and the first null occurred when these retardances were equal. If, however, the fast axes of BVR and the cornea were parallel, their retardances were additive, and the first null occurred when the total retardance was equal to a full wavelength of the yellow LED (585 nm). The two types of null were distinctly different in character and could be distinguished easily. At the cancellation null, PIV remained yellow while its intensity smoothly declined in intensity. At the full-wave null, PIV changed color from reddish to greenish as the total retardance successively canceled different wavelengths in the extended spectrum of the LED. The full-wave null was further distinguished because, as will be seen in Results, double-pass corneal retardances never exceeded 250 nm and the full-wave null always occurred at higher settings than the cancellation null. If the first null encountered was a full-wave null, BVR was rotated 90° and the cancellation null was found. The SD of the retardance measurements increased with retardance value and was estimated to be 10% of the value.

Corneal birefringence was measured in both eyes of 73 subjects (49 women, 24 men) aged 21 to 71 years, recruited from the staff and students at the Bascom Palmer Eye Institute. The research study followed the tenets of the Declaration of Helsinki. The only selection criteria were a subject’s willingness to participate and denial of corneal abnormalities. Subjects who wore contact lenses removed them for the measurements. Correlations were calculated with Pearson’s r.

**Results**

The age of the subject was not correlated with either the corneal axis or retardance (each eye analyzed separately; r < 0.2, P > 0.09 in all cases).

The slow axes and retardances of all corneas are shown as scatterplots in Figure 2 and histograms in Figure 3. In Figure 2 the data for the left eyes (LE) are displayed in a rectangular format. The data for the right eyes (RE) were similar, but are displayed as a polar plot. The polar format de-emphasizes the axes of corneas with weak retardance, and the data appear more clustered than in the rectangular format. As is more apparent in the rectangular format, corneal retardance and axis were moderately correlated (RE, r = 0.47; LE, r = 0.59; P < 0.002), such that weaker retardance was associated with axes that were more ND.

The distribution of corneal axes (Fig. 3A) agreed with that presented by Greenfield et al. Thirty-two of the 73 subjects had participated in that earlier study, and the axes determined by the two methods correlated well (RE, r = 0.85; LE, r = 0.93; P < 0.0001). Axis values ranged from 13° nasally upward (NU) to 72° ND and were skewed toward ND. The mode of the distribution lay between 10° and 20° ND.

Corneal retardance varied widely (Fig. 3B), with double-pass values ranging from 0 to 190 nm. Taking both eyes together, approximately 81% of retardance values were uniformly distributed between 40 and 140 nm, 7% were >140 nm, and 12% were <40 nm.

In eyes with the lowest retardance (below 40 nm), the PIV intensity viewed through crossed polarizers was very weak, and the null required special care to measure. Three corneas (2 RE, 1 LE) had PIV intensities too weak to allow measurements and were arbitrarily set to 0 nm retardance and 0° axis (filled circles in Fig. 2). These axis values were not included in the axis histograms or correlations.

The retardances shown in Figures 2 and 3 do not include the highest values measured with the corneal polarimeter; retardance ranging as high as 250 nm was found in subjects previously identified as having unusual RNFL patterns in SLP images. Their data were not included in this study to avoid biasing an otherwise unselected population.

Corneal birefringence was well correlated between the two eyes of each subject (Fig. 4): r = 0.77 for slow axis and r = 0.75 for retardance (P < 0.0001). The apparent asymmetry in axis distributions between the two eyes (Figs. 3A, 4A) is discussed below.
DISCUSSION

This study was undertaken to expand our knowledge of the polarization properties of the central corneas of a normal population. The chief result was that corneal birefringence varied widely among subjects (Figs. 2 and 3). We confirmed the relatively narrow, ND distribution of corneal axes previously reported and discovered that double-pass retardance ranged from minimum values not perceptibly different from zero to maximum values of at least 250 nm. These measurements were made at a wavelength of 585 nm but should also apply at the longer wavelengths used by some diagnostic instruments. Corneal retardance, therefore, can be large enough to affect the polarization state of a measuring beam and should be regarded as a significant confounding variable for any polarization sensitive technology. Importantly, Greenfield et al. showed that several SLP parameters are strongly correlated with the corneal axis.

Although the corneal axes were well correlated between the two eyes, the data of Figure 4A did not fall exactly along the line of equality; the LE axes on average fell 5.4° more nasally downward than the RE axes. The cause of this asymmetry is unknown, but it could have been due to an undetected bias in the tilt of the subjects’ heads relative to the horizontal axis of the corneal polarimeter. A 2.7° correction (ND in RE, NU in LE) brought the data of Figure 4A to the line of equality and made the two histograms in Figure 3A look more similar. Because torsional eye movements compensate only 25% of a small head tilt, a bias in head tilt of 3.6° would have rotated the corneal axes the required amount. Small head tilts may be an additional source of measurement variability for technologies that are sensitive to corneal rotation.

Although we have assumed that all the measured retardance came from the cornea, it is possible that weak lens birefringence made a contribution to the lowest values. Bueno and Campbell recently used an Imaging Mueller matrix polarimeter to measure the birefringence of lenses in vitro and found that axis varied with position and that single-pass retardance averaged 8° at an unspecified wavelength. Assuming a wavelength of 633 nm as previously published, such a lens would act as a 28-nm retarder (double-pass) in series with the two passes through the cornea with an effect that would depend on the relative axes of the two structures. This effect was not noticed in vivo. Often when making a measurement we could see the third Purkinje image (reflection from the front of the lens), and it appeared to reach a null at the same dial readings.

![Figure 2. The corneal birefringence of 73 subjects plotted in two formats. Retardance values in this and the following figures are for a double-pass through the cornea. Filled circles represent corneas with retardance too weak to measure; the axis is undefined but was set arbitrarily to 0°. LE, data from left eyes plotted in rectangular coordinates. RE, data from right eyes plotted in polar coordinates. In polar coordinates a line connecting a point and the origin has the same orientation as the corneal slow axis and a length equal to the corneal retardance. NU, nasally upward; ND, nasally downward.](image)

![Figure 3. (A) Distributions of the corneal slow axes in 71 right and 72 left eyes of 73 subjects. (B) Distributions of the corneal retardance in both eyes of the 73 subjects.](image)
as PIV, implying that the lens did not contribute much to the measured retardance. We did not systematically study this phenomenon, however, and it deserves more quantitative exploration.

Accommodation was not controlled in this study and, with the fixation LED at 120 mm, should have varied significantly with the subject’s age. Lens birefringence, however, does not change much with accommodation, and the birefringence measured here showed no age dependence. Lack of age dependence indirectly supports the hypothesis that corneal birefringence is stable with time.

Measurements of corneal birefringence provide insight into corneal structure. Corneal birefringence arises because the corneal stroma is anisotropic, that is, its index of refraction depends on direction. Although corneal collagen is anisotropic, the birefringence in the living cornea is thought to be due mainly to the orderly arrangement of collagen into parallel fibrils embedded in a stromal matrix of lower refractive index (form birefringence). The parallel fibrils are arranged further into a stack of lamellae, with the fibrils of adjacent lamellae lying at large angles to one another. Each lamella may be considered as a thin linear retarder with its slow axis oriented along the fibril direction; corneal birefringence represents the cumulative action of the entire stack. Current understanding holds that the cornea behaves like a curved biaxial crystal with its fastest principal axis perpendicular to the surface. In many diagnostically important situations light is incident nearly perpendicular to the corneal surface, the perpendicular axis has little effect on the polarization state, and corneal birefringence is governed by the two principal axes that lie tangential to the surface. The presence of tangential axes is taken as evidence for one or more preferred orientations of the lamellae.

The nature of the preferred lamellar orientation remains unclear. X-ray diffraction studies reveal a population of fibrils (perhaps as many as one half to two thirds) that are oriented in the horizontal and vertical directions. The rest of the fibrils are randomly oriented. Neither of these fibril populations can explain the ND axis. If the retardance of each lamella is small, the birefringence of two perpendicular but otherwise identical lamellae should cancel; a stack of perpendicular lamellae should exhibit no retardance determined by the excess of one orientation over the other, and a slow axis aligned with the orientation in excess (i.e., horizontal or vertical but not ND). For lamellae with random orientations, numerical modeling demonstrates that statistical deviations of a finite number of lamellae cause retardance values to range upward from zero but with a distribution that has a smaller range and a different shape than that shown in Figure 3B. Furthermore, corneal birefringence due to statistical deviations from a random orientation should have a broad distribution of axes, not the ND distribution shown in Figure 3A and found by many others. The available evidence suggests that in most corneas there must be an excess of ND-oriented lamellae that have not yet been convincingly demonstrated. Importantly, as was observed in a few corneas in this study, ensembles of both perpendicular and randomly oriented lamellae can produce zero net retardance.

The broad distribution of corneal retardance (Fig. 3B) suggests that the number of ND-oriented lamellae varies greatly among individuals. The similarity of corneal birefringence between the two eyes of a subject (Fig. 4) suggests, in addition, that specific mechanisms control the number and orientation of these lamellae during corneal development and that within an individual these developmental mechanisms operate symmetrically.

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