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Polarization properties of the *in vitro* old human crystalline lens

Juan M. Bueno[1,2] and Melanie C. W. Campbell[2,3]

[1]Laboratorio de Óptica, Universidad de Murcia, Campus de Espinardo (Edificio C), 30071, Murcia, Spain; [2]School of Optometry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1 and [3]Guelph-Waterloo Physics Institute, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

Abstract

We have studied the spatially resolved polarization properties of the *in vitro* intact old human crystalline lens (from 56 to 88 years old) by using Mueller-matrix imaging polarimetry. Analysis was performed within an average of 54 h of death. Results show that the overall retardation is small (7° on average) and decreases from the centre of the lens to the periphery. Lenticular birefringence is linear but has a spatial dependence, reducing outwards along the radius. The distribution of azimuthal angle of the birefringent structure of the crystalline lens changes depending on each individual lens. Diattenuation and polarizance were found to be small, however, depolarization was about 35% for the set of lenses studied here.

Keywords: birefringence, crystalline lens, depolarization, Mueller matrix, retardation

Introduction

The presence of birefringent structures in the eye has been a useful tool used by many researchers to investigate ocular optical properties and to develop clinically oriented experimental and commercial devices (Bour, 1991; Dreher *et al*., 1992; Hunter *et al*., 1999).

Changes produced in the polarization state of a light beam passing through an optical system depend on both the interfaces and the internal structures. The polarization state is described by a four-element column vector known as Stokes vector. Any change will be expressed as a 4×4 real-valued matrix called the Mueller matrix (Shurcliff, 1962). Properties such as diattenuation, retardation caused by birefringence, depolarization and polarizance are responsible for changes in the polarization state (Chipman, 1995).

Corneal polarization properties have been studied and discussed extensively [see Bour (1991) as a general review], perhaps because of its easy accessibility. However, polarimetric studies of the crystalline lens are less numerous than those of the cornea and its polarization properties are not as well understood. Moreover, investigations of the polarization properties of the lens other than its birefringence, as well as a spatially resolved study, are lacking in the literature.

The lens is the refractive component of the eye responsible for accommodation and it grows, increasing its weight and thickness, throughout life. Its power is based on both the gradient of refractive index and its curved surfaces (Nakao *et al*., 1968; Campbell, 1984; Pierscionek, 1989). The outer part of the lens (cortex) has a lower refractive index than its core and consists of fibres arranged rather like the layers of an onion of micrometer dimensions (Charman, 1991). Transparency of the lens is a result of short range ordering of the fibres. In the nucleus, the fibres are densely packed and the membranes among them break down. In the cortex, the typical thickness of the fibres (8 μm) is larger than the wavelength of visible light, and a certain amount of form birefringence is expected (Bour, 1991). This form birefringence as well as intrinsic birefringence, based on the internal distribution of molecules in the fibres, gives rise to the optical anisotropy of the lens.

Boehm (1940) compared the mean retardation of normal and aphakic eyes and did not find differences. Takeguchi and Nakagary (1968) and Bettelheim (1975) observed little birefringence in the *in vitro* bovine lens.

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Correspondence and reprint requests to: Juan M. Bueno.
Tel.: 34 968 228335; fax: 34 968 363528.
E-mail address: bueno@um.es

Values of birefringence of *in vitro* human lenses reported by Weale (1979) were one order of magnitude higher than those for bovines measured by Bettelheim. klein Brink (1991) used Mueller-matrix polarimetry to analyse the birefringence of both accommodated and unaccommodated *in vivo* human eyes. More recently, the analysis of Purkinje images has shown that the lens may be optically active (Pierscionek and Weale, 1998). In general, previous experiments indicate that the lens, unlike the cornea, does not contribute substantially to the total optical retardation of the eye (Bour and Lopes Cardozo, 1981; van Blokland and Verhelst, 1987; Pelz *et al*., 1996).

On the other hand, various authors have shown the usefulness of imaging (spatial resolution) polarimetry to assess spatially resolved polarization properties of the eye (Dreher *et al*., 1992; Pelz *et al*., 1996; Bueno and Artal, 1999; Bueno, 2000). Knowledge of the spatially resolved polarization properties of the crystalline lens is as necessary as its refractive index distribution to define the optical properties of the eye. In this paper we study the spatially resolved polarization properties of the *in vitro* isolated older human lens [including the degree of polarization (DOP), diattenuation, birefringence and retardation]. These properties have been calculated from the elements of the spatially resolved Mueller matrix measured using a rotating imaging polarimeter in transmission mode. A complete description of the polarization properties of the lens is then available for the first time.

Methods

Experimental system

The experimental setup used to study the spatially resolved polarization properties of *in vitro* samples has been previously described (Bueno and Jaronski, 2001). The system is a Mueller-matrix imaging polarimeter using rotating retarders. A schematic diagram is depicted in *Figure 1*. Briefly, a He–Ne laser (633 nm) is used as light source. The beam passes a neutral density filter (NDF) and a collimator before entering the generator unit composed of a dichroic sheet polarizer (P1, transmission axis in horizontal position) and a rotating quarter-wave plate (QWP1). Aperture AP controls the size of the beam, which always fits the entire lens. After passing through the sample under study (an *in vitro* human lens), the light enters the analyser unit. In this unit the rotating quarter-wave plate (QWP2) is placed in front of a second linear polarizer (P2, with its transmission axis parallel to P1). A photographic objective makes the equatorial plane of the human lens conjugate with the CCD plane of a camera. Measurements were carried out in nine lenses from six donors, ranging in age from 56 to 88 years (mean 76.0, standard deviation ± 11.8). Each lens was measured twice. Although lenses of this age range are expected not to have residual accommodative ability (Glasser and Campbell, 1998), the dissected specimens are assumed to be in an accommodated state.

Two series of 16 images for each lens (500 ms exposure time and 256×256 pixels with eight bits/pixel) were recorded, each corresponding to independent combinations of polarization states of the generator and analyser units. Independent polarization states are generated by orienting the fast axes of QWP1 and QWP2 at different angles (-45, 0, 30 and 60°). From each set of 16 images the spatially resolved elements of the four Stokes vectors associated with the light emerging from the sample were reconstructed. As the four incident polarization states are known, once these emergent vectors have been calculated, the Mueller matrix $[M_{ij}(i, j = 0, 1, 2, 3)]$ can be computed. This procedure is composed of a set of matrix inversions broadly explained elsewhere (Bueno and Jaronski, 2001).

Calculation of the parameters of polarization

Although the Mueller matrix contains information on all the polarization properties of a system, the parameters characterizing the effects of polarization do not appear explicitly in the matrix. In particular, the DOP, the total diattenuation (D) and the polarizance (P) can be directly obtained from the elements of the matrix (Chipman, 1995):

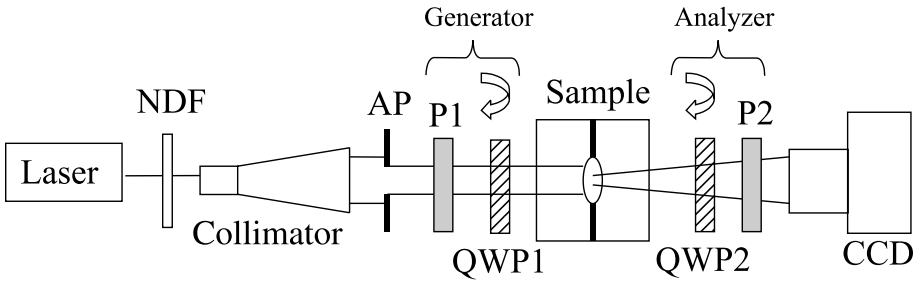

Figure 1. Schematic diagram of the Mueller-matrix imaging polarimeter. NDF, neutral density filter; AP, aperture; P1 and P2, horizontal linear polarizers; QWP1 and QWP2, rotating quarter-wave plates.

$$\mathrm{DOP} = \frac{\sqrt{\left(\sum_{i,j=0}^{3} M_{ij}^2\right) - M_{00}^2}}{\sqrt{3} \cdot M_{00}} \quad D = \frac{\sqrt{M_{01}^2 + M_{02}^2 + M_{03}^2}}{M_{00}}$$
$$P = \frac{\sqrt{M_{10}^2 + M_{20}^2 + M_{30}^2}}{M_{00}} \tag{1}$$

Briefly, DOP describes the coupling by an optical system of incident polarized light into depolarized light in the exiting beam, D refers to the intensity transmittance as a function of the incident polarization state and P represents the DOP of the transmitted light when unpolarized light is incident. All these parameters range from 0 to 1. A higher value of DOP indicates a lower depolarization; higher values of D and P correspond to larger dichroism and polarizing power, respectively. The remaining polarization parameters (retardation, azimuth and ellipticity angle) are associated with the birefringent structures of the sample and their calculation requires the application of a theorem of polar decomposition (Lu and Chipman, 1996). The relationship between the retardation (δ) and the physical properties of a medium with spatially invariant refractive index along the incident direction of the beam is:

$$\delta = \frac{2\pi}{\lambda} \Delta n\, t \tag{2}$$

where Δn is the birefringence (difference between ordinary and extraordinary indices), t is the thickness and λ is the wavelength of the incident light (Born and Wolf, 1980).

Data of lenticular thickness (used to compute the birefringence) were extracted from previous unpublished measurements by Glasser and Campbell for 75-year-old lenses [see Glasser and Campbell (1999) for further information on the method used to calculate this thickness].

The lenticular refractive power makes an incident collimated beam (perpendicular to the plane containing the lens) focus at its focal point, which is out-of-focus on the CCD camera. As a result of this a bright spot corresponding to that defocused focal point appears in the centre of the images. As the refractive power of the lens does not depend on the incident polarization state, the size of these spots is similar for the 16 images of each series. Moreover, spots were always smaller than 2 mm for all specimens and combinations of polarization states. Although pixels corresponding to this area are sometimes saturated, they were included in the calculation of both spatially resolved Mueller matrix and parameters of polarization to save computation time and to get a faster procedure (see maps in Results). However, grey levels corresponding to this central part are misleading and should not been taken into account for this particular experimental configuration (Pezzaniti and Chipman, 1995). For that reason, when numerical values (radial averages) are given for better understanding, data corresponding to that area were deleted from the plots.

Preparation of samples

Human eyes were obtained from the Eye Bank of Canada in Toronto. The eyes had been enucleated within an average of 6 h after death. After corneal removal, the eyes were kept on moist gauze in sealed bottles in chilled coolers during transit. Upon receipt, the eyes were placed in a dissecting dish filled with a human isotonic, pH balanced, saline solution with an osmolarity close to that of the vitreous. Dissections to extract the lenses (at the School of Optometry, University of Waterloo) began within an average of 54 h of death (a minimum of 20 h and a maximum of 96 h). No cataracts (opacities) were observed when lenses were inspected under the dissecting microscope. Details about the preparation of specimens, process of dissection and tissue handling procedures have been described elsewhere (Glasser and Campbell, 1998, 1999). Briefly, the lens, zonules, ciliary body and a ring of sclera were dissected as a unit and placed in a stretching apparatus (Glasser and Campbell, 1998) that holds and stretches the tissue. This apparatus was used because when the lens is dissected from the eye, the zonules relax and the lens takes on an accommodated shape. In the future, we wish to extend this technique to younger lenses with residual accommodation. A rectangular Plexiglas chamber filled with the solution described above holds the stretching apparatus. The chamber has two clear glass windows and the laser beam passes through them.

Results

Prior to recording images of the lenses under study, a calibration of the experimental system was undertaken to determine the performance of the instrument. For this operation the chamber was empty and the camera was focused on the second surface of this chamber. This is not expected to change the polarization state of the incident beam. The Mueller matrix of the chamber was calculated (a 4×4 identity matrix was expected) and the systematic errors in the elements were always smaller than 2.5%, values which are similar to other results reported in previous literature (Bernabeu and Gil, 1985; Pelz *et al.*, 1996; Bueno and Artal, 1999; Bueno, 2000). The DOP was computed from the matrix (Equation 1). *Figure 2* shows the distribution for this parameter. The average value across the image was 0.95 ± 0.04, close to the ideal value of 1.0. A quarter-wave plate was also measured: values of 89.0° for retardation and 44.2° for

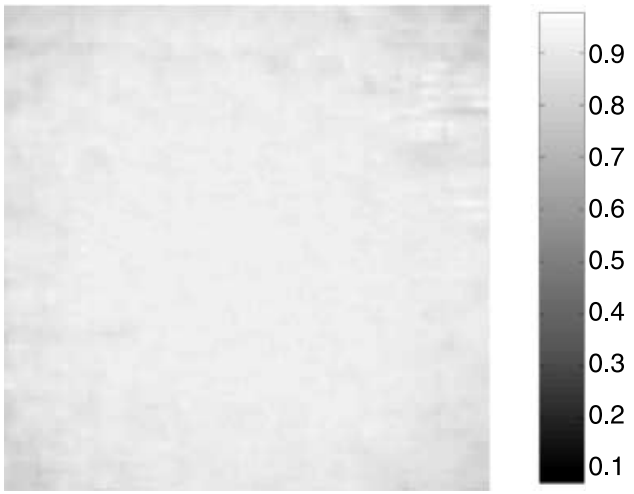

Figure 2. Spatially resolved DOP for the empty chamber. The image subtends 5.6 × 5.6 mm.

azimuth (nominal values, 90 and 45°, respectively) were obtained.

After 16 images for each human lens were recorded, the corresponding Mueller matrix was obtained at every pixel of the image. The lenticular cross (Cogan, 1941), did not appear in any image. The cross only appeared when the lens was placed between perpendicularly oriented linear polarizers. As an example, *Figure 3* displays the spatially resolved Mueller matrix for an 80-year-old intact non-accommodated lens. Each matrix element is given as a two-dimensional image 9 × 9 mm in size. This matrix contains information on all the polarization properties of this human lens, which was analysed 30 h after enucleation.

Once the spatially resolved Mueller matrix is known, parameters of polarization such as DOP, D and P were computed at each pixel of the image using Equations (1). For the calculation of retardation, azimuth and ellipticity angle of the lens a polar decomposition theorem (Lu and Chipman, 1996) was used.

Figure 4a presents the spatially resolved DOP for the same unaccommodated *in vitro* lens as in *Figure 3*. The mean value for this image averaged across all pixels was 0.69 ± 0.03 (mean ± standard deviation). A further analysis of this parameter (averaged radial profile without central values as explained in 'Calculation of the parameters of polarization') is given in *Figure 4b*. To obtain this one-dimensional function, values of the image for a fixed radial distance to centre were integrated and then averaged in all directions. The average DOP across all lenses was 0.64 ± 0.04.

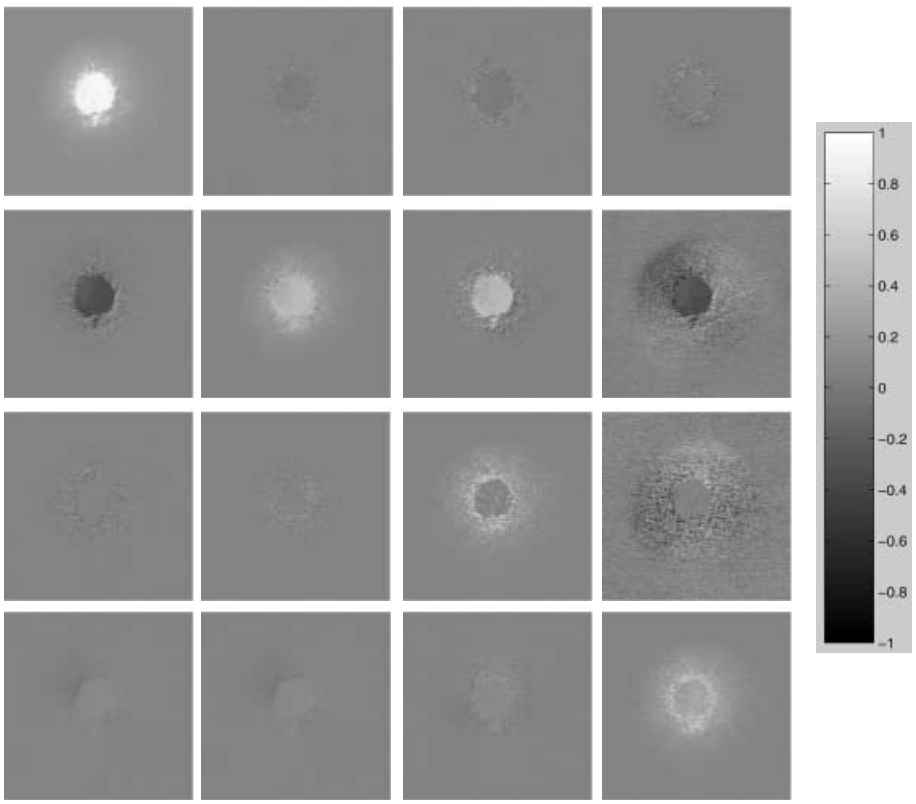

Figure 3. Images corresponding to the elements of the spatially resolved Mueller matrix for an *in vitro* human lens (aged 80 years). Each image represents an element of the matrix, laid out in rows and columns. For a better visualization all 16 elements are normalized to the maximum of element M_{00} so that the amplitude ranges from −1 to +1.

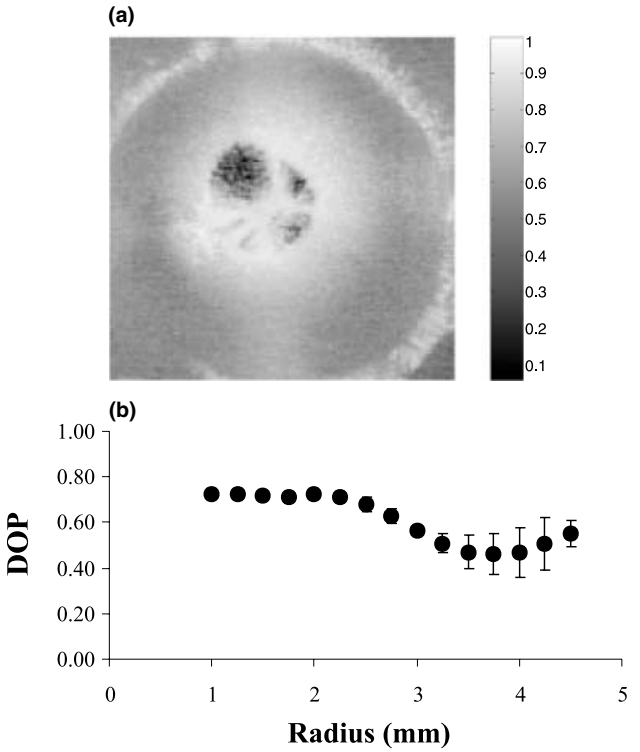

Figure 4. (a) Map for the DOP of the same *in vitro* human lens. Each image has a full size of 9 × 9 mm. (b) Averaged DOP radial profile. Error bars indicate standard deviation. 0-mm position corresponds to the centre of the image. Central values (1 mm in radius) have been deleted from the plot as indicated in 'Methods'.

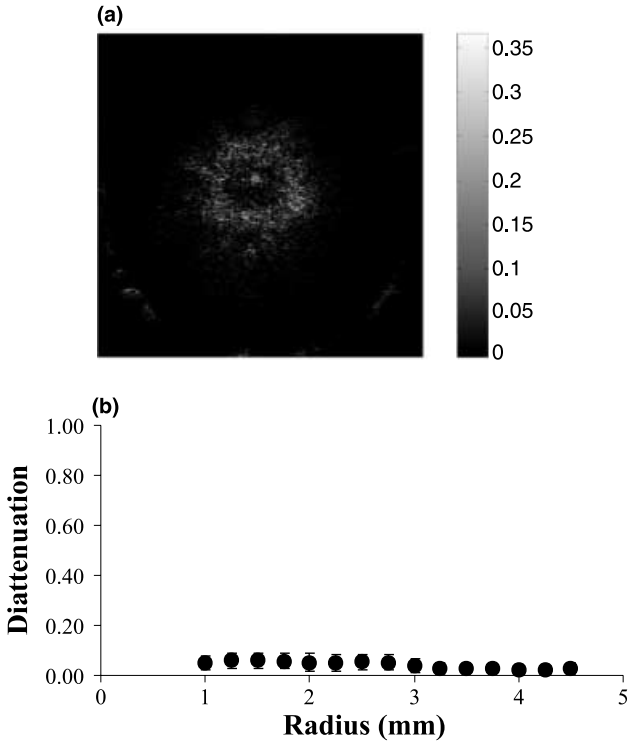

Figure 5. (a) Distribution of lenticular diattenuation for the same lens as in previous figures. (b) Averaged *D* radial profile for all the set of lenses. Error bars indicate standard deviation.

The map for the diattenuation in the same lens is shown in *Figure 5a*. This parameter is close to zero (0.03 ± 0.02), which means that lenticular dichroism is low (as described above, the maximum is 1 and corresponds to a total linear polarizer). The averaged diattenuation for all the lenses was 0.051 ± 0.032. In *Figure 5b*, the radial averaged profile (all lenses) has been plotted. Error bars overlap and differences between centre and periphery are not significant.

The distribution corresponding to the polarizance in this specimen is shown in *Figure 6a* (mean = 0.029 ± 0.014). The average polarizance across the whole set of lenses was 0.054 ± 0.016. Similar to the situation with diattenuation, values are small which indicates that the human lens hardly increases the DOP of non-polarized incident light. *Figure 6b* presents the averaged radial profile for the nine lenses studied here. A non-significant reduction of polarizance along the radius was also found.

The birefringent structure of an optical system is described by the magnitude of retardation, and the azimuth and ellipticity angles of the eigenvector associated with the equivalent retarder. As an example, *Figure 7a* shows the distribution of ellipticity angle for the same lens as in previous figures. The averaged

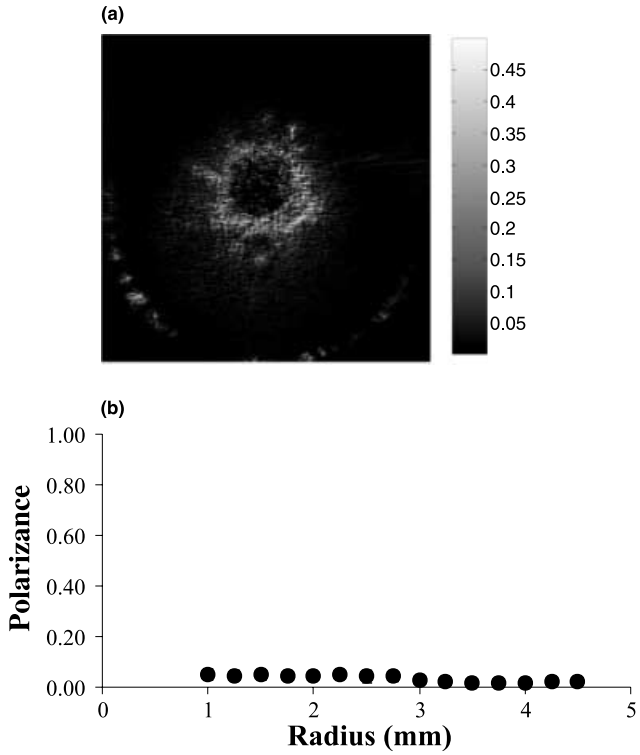

Figure 6. (a) Spatially resolved polarizance for the same *in vitro* non-accommodated lens (9 × 9 mm). (b) Averaged *P* radial profile across all the specimens.

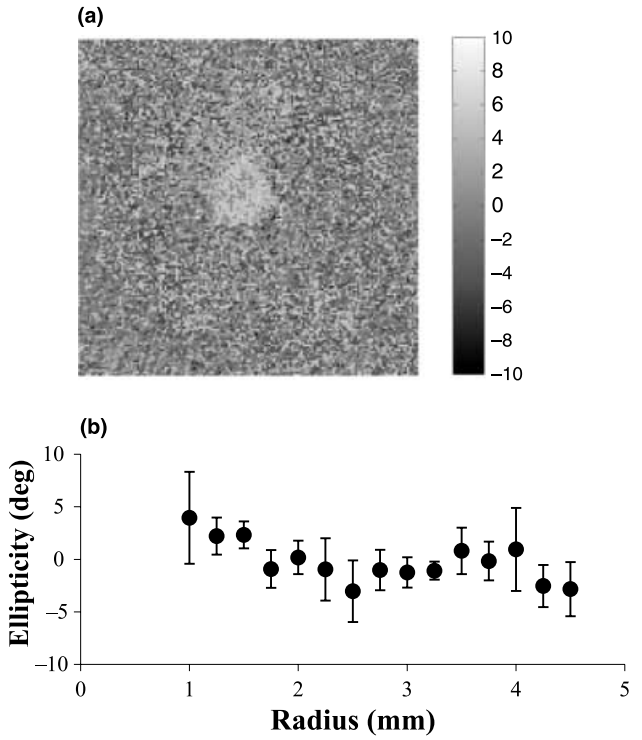

Figure 7. (a) Map of ellipticity angle (°) for the previous human lens. The size of the image is the same as in above figures. (b) Averaged ellipticity radial profile for all the lenses. Error bars represent standard deviation.

ellipticity radial profile for all the specimens is given in *Figure 7b*. The mean of this parameter for all lenses was 0.86 ± 2.39°.

The spatially resolved azimuthal angle for two of the analysed lenses (aged 80 and 75 years) is presented in *Figure 8*. Unlike the ellipticity, maps for azimuth are not uniform across the image and differ for each lens. Values ranged from −25 to +60°.

In *Figure 9* we present the distribution of retardation for the same lenses as in *Figure 8* (6.96 ± 2.01 and 5.59 ± 1.70° on average, respectively). Retardation is approximately symmetric around the centre of the lens. The mean retardation for all the lenses and across images was 6.97 ± 1.79°. *Figure 10* shows the averaged retardation radial profile for the whole set of lenses. There is a significant decrease in retardation with distance from the centre of the lens ($R = 0.95$, $p < 0.0001$). In the same plot, the averaged radial profile of birefringence (calculated by using Equation 2) is also given. Birefringence also reduces towards the periphery ($R = 0.95$, $p < 0.0001$).

Discussion and conclusions

We have used an imaging polarimeter to measure spatially resolved Mueller matrices of *in vitro* older, non-accommodated crystalline human lenses. Parameters of polarization associated with the lens have been calculated from these matrices. Repeatability within each lens was high. For instance, values of 0.68 (0.041) and 0.67 (0.043) for the DOP (D) were obtained in two consecutive series of the same lens. This means that our system is reliable and appropriate for these kinds of measurements.

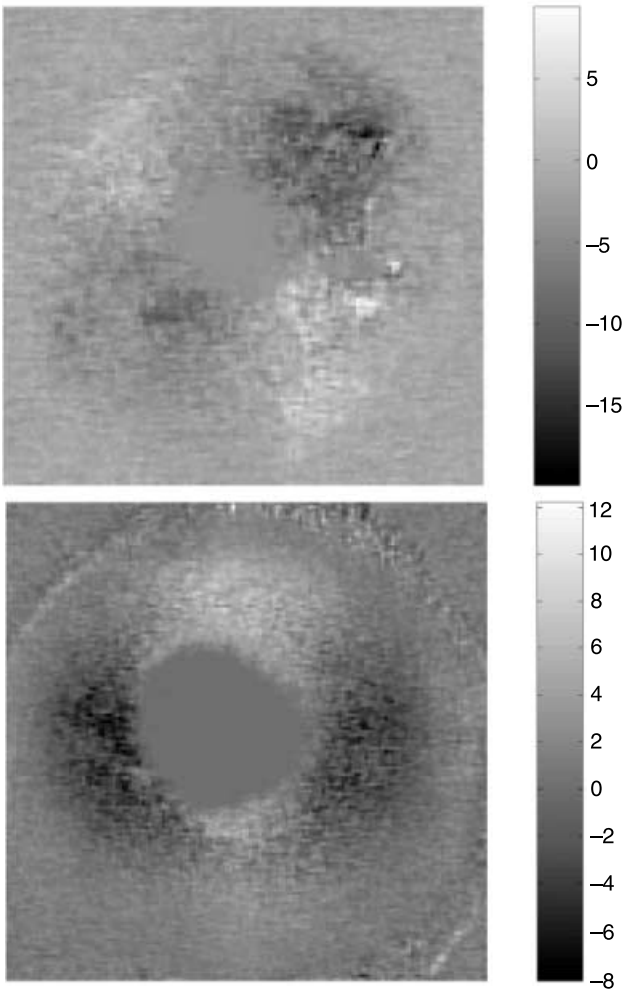

Figure 8. Distribution of azimuth for two lenses, aged 80 (same as previous figures) and 75 years. Scale represents degrees. An azimuth of 0° corresponds to the horizontal direction and angles increase anticlockwise.

Results for the DOP show that the *in vitro* old lens produces a depolarization of about 35%. This indicates that depolarizing effects of old lenses are important and they should be considered when assessing ocular parameters (i.e. retinal thickness) obtained by using polarization properties of the light passing twice through the ocular media.

Depolarization is a natural property intrinsically associated with the scattering and loss of coherence in the polarization state (Chipman, 1995). This is a process which couples polarized light into unpolarized light and characterizes averaged and random polarizations

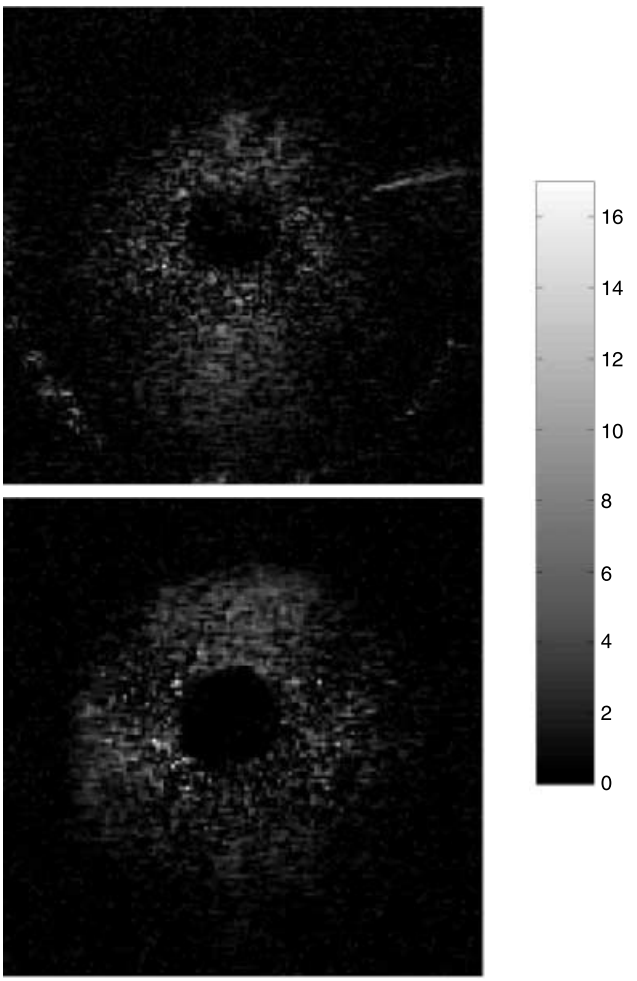

Figure 9. Spatially resolved retardation (°) for the two *in vitro* non-accommodated human lenses of *Figure 8*.

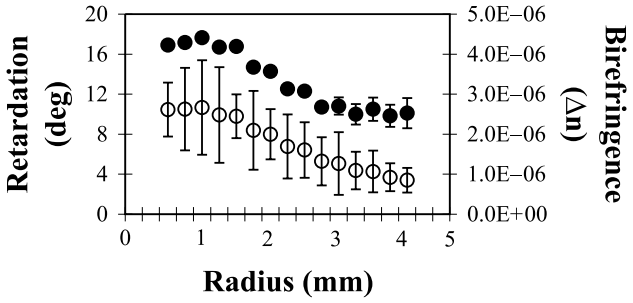

Figure 10. Averaged radial profiles of retardation (white symbols) and birefringence (black symbols) for all the samples as a function of the distance to the centre of the lens. Data were computed using Equation (2) as explained in the text. Error bars indicate standard deviation.

generated by reflection, transmission or scattering of light by a medium or optical components (Nee, 1999). In our case, a reduction in the DOP of the light passing through the lens will be associated with lenticular sources of scattering and/or diffusion. As the loss of lenticular transparency with age is well known (Tripathi and Tripathi, 1983), the age of the lenses used in this study could explain this depolarizing effect. In addition, the amount of light scattered from *in vitro* lenses increases with the time after death (van den Berg and Ijspeert, 1995). Although one of the lenses was analysed 96 h after death, the rest were studied within 72 h. However, in this group of lenses we did not observe a significant dependence of the DOP as a function of the time to enucleation or time after death. On the other hand, it has been reported that retinal image quality decreases and intraocular scattering increases with age (Guirao *et al*., 1999). Probably the latter is associated with the loss of transparency in the lens.

There are a number of results in the literature referring to the DOP of light after a double-pass through the ocular media. Although some studies found a complete depolarization of the light reflected at the retina [see Bour (1991) as a general reference], others reported preservation of the DOP (van Blokland, 1985; van Blokland and van Norren, 1986; Pelz, 1997; Bueno, 2001). Whereas the DOP for the cornea itself was about 0.90 (Bueno and Jaronski, 2001), for *in vitro* retinaes the parameter ranged from 0.50 to 0.98 (Dreher *et al*., 1992; Huang and Knighton, 2000). Results in older adults are scarce. Although these previous studies reported a decrease in the DOP located at the retinal fundus, our results indicate that the lens (particularly in older adults) could be an additional and important source of depolarization. This depolarization is likely associated with increased scatter post-mortem and lens changes associated with ageing and sub-clinical cataract.

Parameter D in *Figure 5* shows that the transmittance of the lens is almost independent of the incident polarization state which indicates that dichroic properties are not important in the older human lens. Transmittance of the lens was measured as a function of the wavelength (Takeguchi and Nakagary, 1968), but no previous data relating lenticular transmittance to polarization states of the incident light have been found in the literature. Values of D between 0.05 and 0.15 for the whole eye have been previously reported (Bone, 1980; Pelz, 1997; Bueno, 1999). This property depends on the incident wavelength and has always been attributed to the retina. Studies of *in vitro* samples found values ranging from 0 to 0.2 for retinas (Dreher *et al*., 1992) and about 0.03 for corneas (Bueno and Jaronski, 2001). Results of diattenuation obtained in this study are consistent with the retina being the only important source for dichroism of the eye.

The DOP of the emergent light only depends on the elements of the first column when non-polarized light is incident on the system. Emergent light is depolarized if these elements are zero. Results of P measured here show that if initially non-polarized light passes through the lens, it will hardly increase its DOP. Corneal

polarizance has been reported to be negligible (Bueno and Jaronski, 2001), but a polarizing effect attributed to the retina was previously found (Röhler and Schmielau, 1976). In addition, Bueno (2001) has recently suggested the presence of a retinal polarizing power in the eye mainly because of the existence of both circular birefringence and dichroic properties. Here we found that the polarizing power of the *in vitro* older human lens does not contribute much (*Figure 6*).

Results for D and P indicate that the main change in the polarization state of the light passing through the lens will be due to depolarization and the retardation introduced by any birefringent structure. In particular, the ellipticity of the equivalent retarder associated with the birefringent structure of the crystalline lens is close to zero (*Figure 7*). This means that the lenticular birefringence is linear and optical activity hardly modifies the state of polarization.

Most previous experiments reported linear birefringence for *in vitro* lenses (Takeguchi and Nakagary, 1968) and corneas (Stanworth and Naylor, 1950; Naylor, 1953; Bueno and Jaronski, 2001), and for the whole human eye (van Blokland, 1985; Bueno, 2000). However, other studies found properties of optical activity in a variety of *in vitro* animal lenses (Kirschenbaum, 1962) and more recently in the living human lens (Pierscionek and Weale, 1998). This property has been thought to have its origin in the protein composition of the lenses. Our spatially resolved Mueller matrices show only a contribution because of linear birefringence.

The distribution of azimuth varies depending on both the lens and the selected area of the lens (*Figure 8*). Whereas in some areas the angle is positive, in others it is clearly negative, with a variable pattern. The azimuth for the whole eye, and the retina and the cornea themselves has been measured by different authors (Kaplan and Bettelheim, 1972; Bour and Lopes Cardozo, 1981; van Blokland and Verhelst, 1987; klein Brink and van Blokland, 1988; Pelz *et al*., 1996; Jaronski and Kasprzak, 1999; Bueno, 2000; Bueno and Jaronski, 2001). To our knowledge this paper presents the first results of a spatially resolved lenticular azimuth. Similar to the cornea, where the distribution of azimuth is related to the orientation of the corneal lamellae (Bour, 1991; Donohue *et al*., 1995), the azimuth of the crystalline lens is probably related to the organization of fibres inside the lens. Bueno (2000) found changes in the azimuth of the whole eye when the diameter of the incident beam increased. A similar non-uniformity in the azimuth of the lens may exist. However, as we did not control for the orientation of the specimens, a direct comparison cannot be made.

Figure 9 shows that the lenticular retardation for different lenses has a common behaviour. In *Figure 10*, a direct comparison between the retardation introduced at each location and the corresponding birefringence can be made. Both retardation and birefringence are maximum near the central area and decrease along the radius.

Previous studies discussing polarization properties in the lens have been focused on averaged measurements of birefringence and retardation at the central lens, but they did not measure spatial resolution. Early experiments in chicken lenses reported birefringence values of about 10^{-6} for the core and 1.56×10^{-5} for the cortex (von Lenhard, 1934). Bettelheim (1975) examined the birefringence of sections of bovine lenses 1-mm thick and found values varying from 10^{-7} to 10^{-6}. This was thought to be due to the fact that form and intrinsic birefringence cancel each other, that is, they have approximately the same magnitude but the opposite sign. Weale (1979) studied the lenticular birefringence in humans of both sexes and different ages. Although values ranged from -0.5×10^{-6} to -3.5×10^{-5}, no significant dependence on either sex or age was reported. klein Brink (1991) used *in vivo* polarimetry (no spatial resolution) to calculate the ocular polarization parameters for both accommodated (5 diopters) and unaccommodated eyes (0 diopters). Results directly extracted from the Mueller matrices showed that ellipticity angle and azimuth for the whole eye seem to be unaffected by accommodation and they were essentially determined by the corneal birefringence. In general klein Brink supported the previous hypothesis of a cancellation between form and intrinsic birefringence within the lens. Recent experiments involving Purkinje images (Pierscionek and Weale, 1998) have also reported that most of the retardation of the light coming back from the second surface of the lens is due to the cornea.

Our results agree with previous studies reporting that the overall contribution of the lens to the total ocular retardation is not as important as that from the cornea. The lenticular retardation (7° on average) we found for a single passage through the lens is still small (about 10 times less) than the retardation of the central cornea [see for instance Bour (1991)].

The results presented in *Figure 10* show a spatial dependence of the total birefringence across the lens, ranging from 4.3×10^{-6} in the central part to 2.5×10^{-6} near the margins. This indicates that the spatial variation of retardation is likely due in part to a spatial variation of the birefringence and in part to the thickness of the lens. Furthermore a change from well defined lens fibres in the cortex to closer packing and a breakdown of the fibres near the centre of the lens does not appear to reduce the birefringence. This suggests that the primary contribution to the birefringence may be intrinsic rather than form birefringence. A possible alternate explanation is that Equation (2) is too simplistic. This equation assumes that the birefringence

of the lens is constant along the optical axis and that the path length of a ray through the lens approximates the local lens thickness. In a lens with a gradient of refractive index along both the optical and equatorial axes, the optical path of rays are more complex. Refractive index increase over that of water is proportional to protein concentration (Barer and Joseph, 1954). If birefringence varies with protein type or concentration (Schachar and Solin, 1975), then the variation would be along both equatorial and optical axes, in proportion to the protein concentration and the resulting refractive index increment integrated along the path length. Although this statement cannot be directly checked until we know the refractive index variation for the older lens, our measurements are the first step in understanding this variation.

To summarize, we have analysed the spatially resolved polarization properties of a group of older post-mortem human lenses. These spatial distributions allow a more complete description of changes in the polarization state of the light passing through the lens. Results show that effects of depolarization by these old lenses are significant and probably associated with precataractous changes. They may also need to be considered particularly in clinical instruments that use the light coming back from the ocular fundus. Polarizing power and dichroic properties are smaller. Maps of azimuth are not uniform and depend on the lens. The lens does not contribute substantially to the total ocular retardation of the eye. Total birefringence, although linear, has a spatial dependence across the lens.

The polarization properties of younger *in vitro* lenses, and *in vivo* lenses, particularly depolarization, may differ from the older lenses measured here and this remains to be determined. Studies in a small group of animal lenses at selected time intervals could help to complete this study and to better understand different phenomena. An implementation of this technique using a scanning system or a polarimeter in reflection mode instead of a CCD imaging apparatus might also be very useful in avoiding the central spot and obtaining data from the central part.

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