Spatially resolved Brillouin spectroscopy to determine the rheological properties of the eye lens

Stephan Reiß, 1,2,* Gerolf Burau, 1 Oliver Stachs, 2 Rudolf Guthoff, 2 and Heinrich Stolz 1

1 Institute for Physics, Semiconductor Optics Group, University of Rostock, D-18055 Rostock, Germany
2 Faculty of Medicine, Ophthalmology Clinic, University of Rostock, D-18055 Rostock, Germany
*stephan.reiss@uni-rostock.de

Abstract: Presbyopia is closely associated with the loss of accommodation, and hence with a decline in the viscoelastic properties of the human eye lens. In this article we describe a method for obtaining spatially resolved in vivo measurements of the rheological properties of the eye lens, based on the spectroscopic analysis of spontaneous Brillouin scattering using a virtually imaged phased array (VIPA). The multi-pass configuration enhances resolution to the extent that measurements are possible in elastic biological tissue characterized by intense scattering. We also present spatially resolved measurements obtained in extracted animal eyes and lenses. The results yield entirely new insights into the aging process of the eye lens.

© 2011 Optical Society of America

OCIS codes: (170.0110) Imaging system; (170.4470) Ophthalmology; (290.5830) Scattering, Brillouin; (330.7327) Visual optics, ophthalmic instrumentation

References and links

1. H. von Helmholtz, “Ueber die Accommodation des Auges,” Arch. Ophthalmol. 1, 1–74 (1855).
2. H. A. Weeber, G. Eckert, W. Pechhold, and R. G. L. van der Heijde, “Stiffness gradient in the crystalline lens,” Graefes Arch. Clin. Exp. Ophthalmol. 245(9), 1357–1366 (2007).
3. R. F. Fisher, “The elastic constants of the human lens,” J. Physiol. 212(1), 147–180 (1971).
4. R. F. Fisher, “The force of contraction of the human ciliary muscle during accommodation,” J. Physiol. 270(1), 51–74 (1977).
5. R. F. Fisher, “Elastic constants of the human lens capsule,” J. Physiol. 201(1), 1–19 (1969).
6. S. Krag and T. T. Andreassen, “Mechanical properties of the human posterior lens capsule,” Invest. Ophthalmol. Vis. Sci. 44(2), 691–696 (2003).
7. R. A. Schachar, R. W. Chan, and M. Fu, “Viscoelastic shear properties of the fresh porcine lens,” Br. J. Ophthalmol. 91(3), 366–368 (2007).
8. S. T. Bailey, M. D. Twa, J. C. Gump, M. Venkiteshwar, M. A. Bullimore, and R. Sooryakumar, “Light-scattering study of the normal human eye lens: elastic properties and age dependence,” IEEE Trans. Biomed. Eng. 57(12), 2910–2917 (2010).
9. J. Randall and J. M. Vaughan, “The measurement and interpretation of Brillouin scattering in the lens of the eye,” Proc. R. Soc. Lond. B Biol. Sci. 214(1197), 449–470 (1982).
10. G. Scarcelli and S. H. Yun, “Confocal Brillouin microscopy for three-dimensional mechanical imaging,” Nat. Photonics 2(1), 39–43 (2008).
11. K. Wileke, “Morphologische und physiologische Untersuchungen an transparenten und kataraktösen Linsen von Farm und Wildlachsen,” Dissertation (Freie Universität Berlin, 2008).
12. M. Dubbelman and G. L. Van der Heijde, “The shape of the aging human lens: curvature, equivalent refractive index and the lens paradox,” Vision Res. 41(14), 1867–1877 (2001).
13. Robert-Bosch-Stiftung Regierungspräsidium Karlsruhe, “Einführung in die Rheologie von Emulsionen,” www.nat.nrw-bw.de/./RBS_Grundlagen_Rheologie.pdf.
14. A. S. Dukhin and P. J. Goetz, “Bulk viscosity and compressibility measurement using acoustic spectroscopy,” J. Chem. Phys. 130(12), 124519 (2009).
15. J. M. Vaughan and J. T. Randall, “Brillouin scattering, density and elastic properties of the lens and cornea of the eye,” Nature 284(5755), 489–491 (1980).
16. N. Berovic, N. Thomas, R. A. Thornhill, and J. M. Vaughan, “Observation of Brillouin scattering from single muscle fibres,” Eur. Biophys. J. 17(2), 69–74 (1989).
17. J. Randall, J. M. Vaughan, and S. Cusak, “Brillouin scattering in systems of biological significance,” Philos. Trans. R. Soc. Lond. A 293(1402), 341–348 (1979).
1. Introduction

The process of accommodation enables the human eye to perceive a sharp retinal image of objects at different distances. According to von Helmholtz [1], accommodation is achieved by a change in the shape of the eye lens and thus by a change in refractive power. This process depends to a major extent on the rheological properties of the eye lens and it is subject to age-related changes. A number of in vitro measurements have been conducted invasively to...
determine these elastic properties as a function of the age of the lens tissue [2–7]. A new and important advance has been the development and application of laser-based non-invasive techniques [8–10]. The advantages of in vitro measurements include the precise alignment of the tissue to be examined within the test configuration and the potential to carry out measurements over longer periods. However, due to the interrupted metabolism between the aqueous humor of the eye and the cells of the lens epithelium, post-mortem changes in lens structure and shape exert a significant influence on the properties of lens tissue [11,12]. In order to describe accurately the biomechanical character of the crystalline lens—in particular, to clarify whether its viscosity is Newtonian or non-Newtonian—and to avoid temperature-dependent changes in viscosity [13], in situ and in vivo studies are necessary [14].

The earliest investigations of lens elasticity using Brillouin scattering were undertaken by Vaughan & Randall in 1980 [15] using a Fabry-Perot interferometer, which entailed very long measuring times. A decisive landmark in the development of techniques permitting in vivo measurement of the elastic properties of the eye lens was reported by Scarcelli & Yun in 2007 [10]. They described a confocal Brillouin spectrometer based on the use of highly dispersive elements, which also opened the way in principle for in vivo measurements of the eye. This imaging method forms the basis for the investigations reported here.

Acoustically induced inelastic Brillouin light-scattering utilizing the advantages offered by confocal microscopy represents a non-invasive approach to determining the spatially resolved viscoelastic properties of biological tissue [16,17].

The multiple scattering of an optical beam, which occurs inevitably in many natural objects, can be used to obtain information about their material properties. Three important scattering processes are distinguished: elastic Rayleigh scattering, and inelastic Raman and Brillouin scattering. In 1922 Brillouin described for the first time the effect in which a beam of coherent light that is scattered by thermally excited acoustic waves undergoes a frequency shift that is equal to the frequency of the scattering sound wave [18]. A background of thermally induced, randomly distributed acoustic waves is always present in all media over a wide frequency range. These are known as acoustic lattice vibrations or, in the quantum interpretation, as acoustic phonons. The light scattered on them is usually extinguished by destructive interference, but it may also overlap constructively. It is then referred to as spontaneous Brillouin scattering (Fig. 1). These acoustic lattice vibrations can also be interpreted as material density variations and they are based on the strong coupling of adjacent molecules or other structural elements. They are measured using Brillouin spectroscopy and this provides a direct correlation with the elastic properties of the material in question. This technique determines the spectral change of the scattered light in response to the incident monochromatic laser light. Since incident light scattering is the result of the vibration of the molecules around their equilibrium position, then according to the Doppler principle, Brillouin-scattered light is shifted by the same amount to the higher and lower frequency in relation to the incident light frequency. This phenomenon is known as the Stokes and anti-Stokes Brillouin frequency shift. It follows directly from the conservation of energy and momentum and can be formulated as follows:

\[ \nu_{B} = \pm \frac{2n}{\lambda} V \cos(\theta/2) \]  

\( \nu_{B} \)—Brillouin frequency shift  
\( n \)—refractive index of the material  
\( \lambda \)—wavelength of the incident laser  
\( V \)—acoustic velocity in the material  
\( \theta \)—scattering angle
The challenge in Brillouin spectroscopy is to separate inelastically scattered light from the additional phenomenon of elastically scattered Rayleigh light. Firstly, the elastically scattered light is orders of magnitude more intense than the inelastic Brillouin-scattered light, and secondly, the frequency shift between these scatter fractions is very low—only a few GHz. In implementing Brillouin spectroscopy for in vivo measurements a further disadvantage arises from the direct relationship of the incident wavelength to the wavelength shift of the inelastically scattered light (Fig. 2). In order to minimize any stress to the biological tissue, it is necessary to use a laser with high wavelength. However, increasing the wavelength of the laser employed reduces the frequency shift of the inelastically scattered light relative to the dominant elastic scatter fraction.

![Fig. 1. Schematic representation of Brillouin scattering.](image1)

![Fig. 2. Increasing the wavelength ($\lambda$) of the laser lessens the wavelength shift ($\nu_B$) of inelastically scattered light.](image2)

This situation therefore places high demands on the spectral resolution capability of the measurement configuration, and such demands have been met by using a virtually imaged phased array (VIPA) [10,19,20]. This takes the form of a high-resolution multiple-beam interferometer, which has up to 20-fold higher angular dispersion compared with an optical lattice [21] and an incident beam intensity loss of less than 20% (Fig. 3). The VIPA consists of an approx. 2.0 mm thick glass plate which has three coatings with different reflectivity. Apart from a small window which is anti-reflection coated ($R \approx 0\%$), the light input side is almost 100% reflective. The light output side is coated with a partially (95%) reflective layer.

The scattered light from the lens tissue to be examined is line-focused into the VIPA with a cylindrical lens at a small incident angle ($\delta$). The resulting multitude of reflections produces an array of exit beams. Due to the tilt of the VIPA phase shift occurs between the reflecting beams, which ultimately interfere with each other. This interference has the effect that,
depending on the incident wavelength, the light exits the VIPA at different angles and thus is spatially imaged separately on a detector.

Conclusions about the rheological properties of the measured point in the sample can be drawn from the spatial shift of the inelastically scattered Brillouin wavelength relative to the elastically scattered Rayleigh wavelength. However, these properties can be determined to only a limited extent due to the measurement of backscatter because only interactions of photons with longitudinal acoustic phonons are detected. The amplitudes of scattering on transverse acoustic phonons cannot be determined in back-scattering [8,10]. Therefore, Young’s elasticity modulus (E) or the shear modulus (G) cannot be determined from the measurement of the longitudinal Brillouin frequency shift. And it is precisely these moduli that provide a measure of the stiffness of a material [22]. The greater the values for Young’s modulus (E) and the shear modulus (G), the greater the resistance offered by a material to deformation by tensile or shear forces.

However, with the method presented here it is possible to make a direct statement about volume elasticity (also known as bulk modulus (K)), and thus to provide an assessment of the rheological behavior of the eye lens during the accommodation process. The bulk modulus indicates the ratio of all-round pressure change to volume change. Bulk modulus, Young’s modulus and shear modulus are interlinked via Poisson’s ratio (μ):

\[ \mu = \frac{E}{2G} - 1 = \frac{3K - E}{6K} = \frac{3K - 2G}{6K + 2G} \]  

Poisson’s ratio expresses the relationship of the relative thickness change to the relative length change of a material under the action of an external force. For the eye lens this ratio is reported in the literature as ranging from 0.47 to 0.5 [3,23–25]. The eye lens consists of about 66% water, 28% water-soluble proteins, 5% water-insoluble proteins and 1% lipids. Thus, 94% of the lens material is made up of water and water-soluble proteins [11,26,27]. Liquids lack the retrograde elastic shear forces required for the propagation of transverse oscillations. Therefore, sound waves propagate in liquids as compression waves, i.e. as longitudinal waves [28]. Thus, the shear modulus (G) and, consequently, Young’s elasticity modulus (E) too are equal to zero. Because only 6% of the eye lens consists of solid substance, the shear modulus is about 9 orders of magnitude smaller than the bulk modulus [24]. Conversion of the bulk modulus into the shear or elasticity modulus is therefore not appropriate. The bulk modulus (volume elasticity) corresponds to the real part (storage modulus, M’) of the complex longitudinal modulus (M *) [14,29], which describes the viscoelastic properties of a material, including the eye lens:

\[ M' + iM'' = \left(\frac{1}{2n\cos(\theta/2)}\right)^2 \rho + i\rho \frac{\Delta V_g}{V_g} \]  

#146703 - $15.00 USD  
Received 2 May 2011; revised 29 Jun 2011; accepted 2 Jul 2011; published 5 Jul 2011  
(C) 2011 OSA
Here $\rho$ denotes the density of the material in which the longitudinal sound wave propagates and $\Delta \nu_B$ the line width of the Brillouin spectrum, which characterizes the attenuation of the sound wave and corresponds to the reciprocal of the lifetime of acoustic phonons [30]. The imaginary part (loss modulus, $M''$) is a measure of the energy consumed during compression and hence lost for return deformation. This energy is either consumed to change the sample structure or is dissipated as heat due to molecular friction. Therefore, the bulk viscosity of the sample is represented by the loss modulus [14]. In this context it is worth noting the distinction between viscosity in Newtonian and non-Newtonian fluids. Newtonian fluids are characterized by constant viscosity when external forces change. In non-Newtonian fluids viscosity does not remain constant.

In terms of understanding the accommodation of the eye lens and in view of the studies conducted by Urs [31], the properties of the lens substance need to be investigated and characterized in greater detail using in vivo measurements.

(In the rest of this article the terms ‘compression modulus’, ‘storage modulus’ and ‘volume elasticity’ will be used synonymously. The terms ‘bulk viscosity’ and ‘loss modulus’ are also used to refer to the same process—namely, the loss of energy introduced into the sample to be tested.)

2. Measurement configuration

Two identical VIPAs were used in a multi-pass configuration for spectral resolution of the low Brillouin shift. This tandem VIPA configuration has already been proposed by Scarcelli & Yun and used to measure the properties of biological tissue [10,20]. To achieve high spatial resolution the configuration was designed as a form of confocal imaging (Fig. 4).

A semiconductor laser specially designed for this measurement delivers a coherent beam source with a wavelength of 780 nm. This wavelength represents an acceptable compromise between beam exposure of the biological tissue to be examined and the achievable spectral separation of the inelastic and elastic scattered light components. Due to the Littrow configuration the line width of the laser light is 3.7 MHz and may be regarded as sufficiently small.

Via an objective lens (LO) with a focal length of 40 mm the laser beam (Ø = 5.0 mm) is focused into the eye to be examined. Due to the decentralized beam path through the objective
lens, the incident beam at the eye lens is at an angle of $\theta/2 = 12^\circ$. This dual-axis configuration [32] was selected in order to avoid the strong reflections that occur at the individual optic components and on the eye being measured when a vertical beam path ($\theta/2 = 0^\circ$) is used. The back-scattered light portion is collected again through the same lens and imaged via a collector lens (L1) onto a pinhole (P). The configuration therefore utilizes the advantages offered by confocal microscopy [33]: namely that aberration demands on the objective and collector lenses are low and ‘optical sections’ can be performed. In other words, only that region in a three-dimensional object is represented that is located close to or in the focal plane of the objective lens (LO). With a pinhole diameter of 100 μm, scattered light from a sample volume of about 0.008 mm (x) × 0.008 mm (y) × 0.20 mm (z) = 1.3 × $10^{-4}$ mm$^3$ is line-focused onward through the positive cylindrical lens (CP) into the first VIPA (V1). This customized VIPA is tilted to the beam path at an angle ($\delta$) of 2°, thus achieving an angular dispersion of $\approx 3^\circ$/nm [21]. Via another lens (L3) and a negative cylindrical lens (CM) the light that is already dispersed into its frequencies is line-focused into a second, identical VIPA (V2) arranged perpendicularly to the first. This tandem set-up corresponds in principle to the multistage VIPA configuration published by Scarcelli & Yun [20]. However, in order to minimize any loss of intensity due to reflection at the optic components, use of a negative cylindrical lens (CP) allowed us to dispense with an additional collector lens between the two VIPAs (V1 and V2). In theory, this means an approx. 5% higher light yield. It was only the multi-pass set-up also proposed by Scarcelli & Yun [10,20] that enabled us to perform measurements in biological tissue because the elastic scattering there is markedly more pronounced than in synthetic materials (e.g. acrylic) or water. The back-scattered Rayleigh light obscures the actual Brillouin signal, thus reducing the resolution of the single VIPA configuration (Fig. 5a). Resolution in this context should be understood to mean the Brillouin signal that can still be separated from the Rayleigh signal [28]. The tandem VIPA construction enabled the Brillouin peaks to be moved out of the intensive Rayleigh range (Fig. 5b), resulting in improved spectral resolution. In practice it was found that a Brillouin shift of 3.5 GHz in lens tissue can just still be separated from the intensive Rayleigh signal. According to Stroppe [28], the performance and hence the resolution (A) of a spectrometer is calculated as

$$A = \frac{\lambda}{\Delta \lambda}.$$  

Fig. 5. Typically detected scattering signal: (a) after first VIPA (V1); (b) after second VIPA (V2).
In this equation λ is the wavelength of the Rayleigh signal and Δλ is the Brillouin shift in nm. The practical outcome is therefore a resolution of 1x10^5. This means that our spectrometer can provide spectral resolution for a wavelength difference of approx. 0.0071 nm. The second VIPA reduced the intensity of the detected Brillouin signal by a factor of 2, causing a deterioration in the signal-to-noise ratio. This does admittedly influence the magnitude of the relative error, as can be seen from the presentation of the measured data (error bars), but it may be regarded as acceptable.

As the detector of the measured signals, the CCD camera is the key component in the configuration for achieving in vivo measurements. With a −30°C Peltier-cooled CCD camera from Starlight Xpress Ltd (Holyport, Berkshire, UK) and a laser power of 12mW, measuring times of 90 seconds were required for biological tissue structures, such as the crystalline lens. In contrast, with an iXon DU888, EMCCD camera (Andor Technology plc, South Windsor, CT, USA) at the same laser power, it was possible to reduce the exposure time to 0.3 seconds.

3. Measurements and results

Various measurements of Brillouin-scattering were performed to permit subsequent determination of the rheological properties of the eye lens. Spatially resolved in vitro measurements were obtained in an extracted rabbit eye and a porcine eye as well as in one removed eye lens each from a rabbit, a pig and a human subject.

In vitro measurement of the rabbit eye was performed about 3 hours after the 6-month-old animal had been sacrificed. During this period the eye was stored in BSSplus solution at approx. 15°C. 100 ml of this liquid contains 0.49 g NaCl, 0.75 g KCl, 0.0715 g CaCl₂, 0.03 g MgCl₂, 0.39 g NaAc, and 0.17 g C₆H₅Na₃O₇ at a pH of 7.2. For measurement the eye was placed in a cuvette containing 0.9% physiological saline solution to prevent interim drying of the cornea. The eye was positioned in the cuvette in such a way that the cornea was applanated slightly against the cuvette edge. This was done with the intention of avoiding reflection at the anterior corneal surface.

Brillouin measurement took the form of an axial depth scan along the optical axis of the cornea—aqueous humor—eye lens. In this process the eye was moved in 500 μm steps, starting from the cuvette edge using a linear motor with a positioning accuracy of 0.1 μm. Analysis of the measured data was performed using mathematics software. Figure 6 shows the CCD image of a typical spectrum. The result was impaired by the Rayleigh peak, which was some orders of magnitude more intense. The Brillouin line (black dashes) was imaged in the form of an arc because of the negative cylindrical lens (CM in Fig. 4). This arc was read by the analytical software and shown as a Brillouin spectral curve (yellow). A spline function

![Fig. 6. Typical Brillouin spectrum of biological lens tissue.](#146703 - $15.00 USD)
was used to fit the raw data (white), and the Brillouin frequency shift was calculated from the average distance between the two Brillouin peaks. The line width (FWHM) was determined by separately fitting a Lorentz function on the Brillouin peaks [34].

The results measured for the Brillouin frequency shift are shown in Fig. 7 as black diamonds. The red diamonds show the calculated storage and bulk modulus according to Eq. (3), while the green diamonds represent the loss modulus as a measure of volume viscosity. The results are related to the real position of the focal point of the laser source after passing through the various optical refractive media. The following assumptions in terms of density and refractive index were made for calculating the bulk moduli [35]:

- aqueous humor: density: 1000 kg/m$^3$,
  refractive index: 1.33
- eye lens: density: 1098 kg/m$^3$,
  refractive index: 1.60

A significant change in the Brillouin frequency shift and hence in the bulk modulus was observed over the entire range of the axial scan. The highest values for storage and loss moduli were detected in the central area of the lens. The aqueous humor (AH), the lens (L) and the vitreous humor (VH) are readily distinguishable on the basis of the measured data. This distinction was already possible while measurement was in progress in terms of increased scattering which appeared as an intense Rayleigh peak. These scatters, caused by interface reflections, also explain the larger relative errors in the regions of the anterior and posterior lens capsule. The relative discrepancies of the calculated bulk modulus relative to the measured Brillouin frequency shift in the anterior and posterior region of the lens (L), and in the vitreous humor (VH) are based on the assumption of a uniform refractive index and a uniform density for the entire eye lens. It is known that the eye lens has a gradient refractive index pattern [36,37]. If this parameter were to be adjusted accordingly, then the correlation between the two results could be improved. It is evident that the bulk modulus in the cortical lens region is lower than in the lens nucleus, suggesting increased volume elasticity in the center of the lens. Since the bulk viscosity in the lens nucleus also shows a maximum, greater toughness may therefore be assumed in the nucleus than in the cortex.

![Fig. 7. Brillouin frequency shift (black), storage modulus (red) and loss modulus (green) through a rabbit eye (in vitro) in relation to the real position of the measuring point; AH—aqueous humor, L—lens; VH—vitreous humor.](image-url)

After the axial scan had been completed, the eye lens was extracted from the eye and then positioned in the measurement configuration in a cuvette containing 0.9% physiological saline solution. An axial depth scan in a lateral direction over the central region of the lens was performed to achieve two-dimensional spatially resolved determinations of the storage modulus and the loss modulus. In this process the lens was moved laterally to 4 positions each...
separated by a distance of 1.5 mm. In each lateral location, the lens was measured axially at 10 equidistant points each separated by a distance of 500 μm.

This spatially resolved determination of the storage modulus and loss modulus in a lateral and axial direction through the eye lens yields interesting new insights. While there is still a higher bulk modulus in the lens nucleus than in the cortical region of the lens, this displays a locally fluctuating pattern. Figure 8 shows the calculated storage moduli (green) and the

---

**Fig. 8.** Storage modulus (green) and loss modulus (orange) of an axial depth scan at various lateral measurement points in an extracted rabbit lens (*in vitro*).
calculated loss moduli (orange) of all axial measurement points for the 4 lateral measuring positions. It further reveals an increased compression modulus in the lens nucleus compared with the cortical region. However, local fluctuation of the maximal compression modulus is discernible in relation to the lateral measuring positions. Similarly, the loss modulus is significantly greater in the lens nucleus than in the cortical region. In this case too the local fluctuation of the volume viscosity maxima is evident between the lateral positions.

*In vitro* measurements were also performed on the eye of a 2.5-year-old pig approximately 24 hours after the animal was sacrificed. During the intervening period the eye was stored at 5°C in BSSplus solution. For measurement the cornea of the eye was applanated in 0.9% physiological saline solution against the cuvette edge. Here too the axial depth scan was performed along the optical axis in 500 μm steps.

As noted previously with the rabbit eye, a significant difference in the Brillouin frequency shift was also detected here. This was greatest in the lens nucleus and decreased in an anterior and posterior direction. The lowest values were found in the aqueous humor and vitreous humor (Fig. 9). The storage moduli were calculated from the frequency shifts. The following values were assumed for the densities and refractive indexes [38]:

- **aqueous humor:** density: 1000 kg/m³,
  refractive index: 1.33
- **eye lens:** density: 1098 kg/m³,
  refractive index: 1.443

It should be noted at this point that the literature contains varying data with regard to the refractive index of the porcine lens. Dillingham et al. [39] gave the refractive index as 1.51, whereas De Castro et al. [38], who used optical coherence tomography (OCT) measurements, reported a refractive index range from 1.362 for the cortex to 1.443 for the lens nucleus.

This porcine eye showed the same volume elasticity and bulk viscosity pattern as the rabbit eye. The relative discrepancies of the calculated bulk moduli relative to the measured Brillouin frequency shifts in the eye lens and vitreous humor were also apparent here, again due to the assumption of a single uniform value for density and refractive index. Bulk viscosity was greatest in the lens nucleus, suggesting increased toughness and strength.

![Fig. 9. Brillouin frequency shift (black), storage modulus (red) and loss modulus (green) through a porcine eye (in vitro) in relation to the real position of the measuring point; AH—aqueous humor, L—lens; VH—vitreous humor.](image-url)

As with the measurements performed on the rabbit lens, the porcine lens was next extracted from the eye and measured in a cuvette containing 0.9% saline solution. In a lateral direction 11 axial measuring points were recorded at each of 5 positions 1.75 mm apart, thus
Fig. 10. Storage modulus (green) and loss modulus (orange) of an axial depth scan at various lateral measurement points in an extracted porcine lens (*in vitro*).
Fig. 11. Storage modulus (green) and loss modulus (orange) of an axial depth scan at various lateral measurement points in an extracted human lens (in vitro).
giving a field of 55 measurements. The compression moduli and loss moduli were calculated from these measurements (Fig. 10). Once again it was noted that the bulk modulus in the lens nucleus was greater than in the cortical region, but here too local fluctuations of the compression modulus maxima of the lateral measuring positions were again seen relative to each other. The pattern of the loss modulus distribution of the lateral positions appears to correlate well with the curve shapes of the calculated compression moduli. Again the highest values were found in the lens nucleus, although local fluctuation of the loss modulus was noteworthy.

Further measurements were performed on a 70-year-old human lens. Five measuring points in a lateral direction were recorded at each of 7 axial measuring positions 1.75 mm apart. The calculated storage moduli and loss moduli are shown in Fig. 11. For the calculation, the following assumptions were made for density and refractive index [8]:

- density: 1085 kg/m$^3$,
- refractive index: 1.42

Each measuring curve of the 5 lateral measuring positions has a maximum value at different axial locations. This local fluctuation of rheological properties becomes especially clear in the presentation of the loss moduli. In this case the bulk viscosity maxima show marked distribution within the lens.

4. Discussion and conclusions

Using the tandem VIPA configuration described here it is possible to measure the Brillouin scattering of biological tissue, specifically of the eye lens. The measured results yield evidence of a location-dependent change in the Brillouin frequency shift and hence in the bulk modulus. All axial measurements in the eye reveal higher values for the bulk modulus in the lens nucleus than in the lens cortex. Results measured in the lens differ significantly from those in the aqueous humor and vitreous humor. The discrepancy of the calculated bulk modulus relative to the measured Brillouin frequency shift in the lens cortex and vitreous humor is due to the assumption of a uniform refractive index and a uniform density. If it is recalled that the eye lens has a gradient refractive index structure, then the measured results become consistent with the calculated values. The values for refractive index (1.60) and density (1098 kg/cm$^3$) known from the literature [35] were assigned to the measured maximum Brillouin shift. The minimum Brillouin shift was assigned to the known values for aqueous humor (refractive index 1.33; density 1000 kg/cm$^3$). For each measurement point therefore the Brillouin data could be used to find corresponding values for refractive index and density [40]. The calculated compression modulus thus corresponds to the measured Brillouin shift for each measurement point. Figure 12 provides an illustrative example based on the results measured in the rabbit eye.

De Castro et al. [38] and Borja et al. [41] have developed a new and interesting method based on OCT measurements for determining the gradient index (GRIN) distribution of porcine lenses and human lenses (in vitro). If the algorithm presented above is applied to the Brillouin measurements of the porcine lens (lateral measuring position 0 mm in Fig. 10) and if a refractive index of 1.443 [38] is assigned to the maximum measured Brillouin shift, the resulting refractive index for the cortex is 1.361. This value agrees very well with the refractive index of 1.362 determined by de Castro et al. [38] for the cortical region of porcine lenses.

The loss moduli of the two eye measurements correlate with the calculated storage moduli (Fig. 7 and Fig. 9). Bulk viscosity and volume elasticity are therefore higher in the lens nucleus than in the lens cortex. Vaughan & Randall [15], Bailey et al. [8], and Scarcelli & Yun [10] also noted an—albeit continuous—increase in the Brillouin shift from the lens cortex to the lens nucleus. The reasons for the differences between the cortex and nucleus are to be found in the composition of the lens substance. As mentioned above, the eye lens
Fig. 12. Adjusting the storage modulus based on the refractive index and density. (Black curve: measured Brillouin shift data; red curve: storage modulus assuming uniform refractive index and uniform density for the eye lens; green curve: storage modulus adjustment using the refractive index and density within the lens of the eye)

consists of 66% water on average. Its water concentration decreases from the cortex toward the nucleus, and with increasing age [42]. Since the eye lens is the most protein-rich organ in the human body [43,44], the role of proteins in relation to lens consistency, and their distribution within the lens, also needs to be considered in greater detail. Writing in 1990, Ulrich [44] already noted that a correlation exists between the protein content of the lens and its consistency, and between protein content and refractive index. Studies in animal lenses have shown for example that fish lenses with the highest protein concentration display great lens hardness and a high refractive index. In contrast, bird lenses are characterized by low protein content and are consequently soft with a low refractive index [11]. Furthermore, the type of protein (water-soluble or water-insoluble) has an influence on the rheological properties of the lens substance. Water-soluble proteins account for some 85% of the total protein content of the eye lens, and they are located in higher concentrations within the cortex. However, the highest concentration of water-insoluble proteins is found in the nucleus. With increasing age, water-soluble proteins are converted into water-insoluble proteins, leading to an increase in the concentration of water-insoluble proteins in the cortex [43].

If we now return to the observation by Vaughan & Randall [15], according to which a correlation exists between the Brillouin frequency shift value, the protein concentration level, and water content, then the results obtained from the axial eye scan become understandable. It provides an explanation firstly for the greater frequency shift and secondly for the increased attenuation of the sound waves propagating by lattice vibrations in the area of the water-insoluble proteins. This means that the lifetime of phonons is shorter in the nucleus than in the cortex. The lens possesses greater strength in the nucleus than in the cortex.

These insights into biochemical composition and into aging-related change can also help to explain the measured results obtained in the lateral / axial scans of the rabbit lens, porcine lens and human lens. Storage modulus and loss modulus were subject to pronounced location-dependent fluctuation in eye lenses from all three species. It seems that the degree of fluctuation correlates with the age of the lens, i.e. the older the eye lens, the greater the location-dependent fluctuation. If this suspicion were to be confirmed it would open up a new way of assessing aging-related change in the eye lens. To the best of our knowledge these local fluctuations in the rheological properties of the eye lens have not previously been described in this way. Measurements obtained in animal and human lenses reported by other
research groups have not shown these location-dependent changes in terms of sound velocity or refractive index [10,37,45]. A pointer to the likely correctness of our suspicion is provided by measurements conducted by Kasthurirangan et al. [36], which revealed slight non-uniform fluctuations in the refractive index distribution of the human lens with age.

The aim of further research therefore should not be limited exclusively to investigating the dependence of volume elasticity or bulk viscosity on age. This question has also already been addressed by Bailey et al. [8] who found that the propagation velocity of a sound wave within the eye lens did not depend significantly on age. However, these measurements are not entirely reliable because they were performed in vitro.

On the other hand, investigations into the degree of local fluctuation of storage and loss moduli in relation to the age of the eye lens are promising and necessary. However, such studies should be conducted in vivo because with in vitro measurements it cannot be ruled out that a change in the metabolic processes inside the lens potentially influences protein structure. It is therefore not impossible that the local rheological fluctuations detected might be linked with post-mortem changes in the lens tissue. This aspect requires further systematic investigation.

One further advantage of in vivo measurement is the possibility offered to measure the storage modulus and loss modulus during accommodation. The results would permit inferences to be drawn concerning the viscosity properties of the lens substance. To this end, Urs [31] used ultrasound biomicroscopy to perform measurements on the accommodating lens and found different sound velocities in various accommodative states. This could be an indication of non-Newtonian viscosity behavior of the lens matrix.

The measurement method developed here permits the non-destructive determination of the rheological behavior of the eye lens. It opens up hitherto unexpected possibilities in terms of improving our understanding of the aging process of the eye lens. Plans are in place to further improve spectral resolution (by suppressing the Rayleigh signal) as well as spatial resolution. As a result it will be readily possible to use this technique in real time for a very broad range of in vivo investigations in ophthalmology. The use of an electron multiplying (EM) CCD camera allows measurement times to be kept very short, thereby ensuring adherence to the maximum permissible exposure (MPE) levels for the human eye, as set out in European standard DIN EN 60825-1. In vivo measurements in the human eye will be possible in the not-too-distant future.

Acknowledgments

The authors are grateful to the Department ‘Science and Technology of Life, Light and Matter’ of the University of Rostock, the Deutsche Forschungsgemeinschaft (Transregio 37, ‘Micro- and Nanosystems in Medicine—Reconstruction of Biological Functions’), and REMIDIS for their support. They also wish to acknowledge the editorial assistance of Mr. David Beattie (UK) in preparing this manuscript for publication.