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Optical properties of the lens: An explanation for the zones of discontinuity

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A B S T R A C T

The structural basis of zones of discontinuity in the living human eye lens has not been elucidated, and there is no conclusive explanation for what relevance they may have to the structure and function of the lens. Newly developed synchrotron radiation based X-ray Talbot interferometry has enabled the detection of subtle fluctuations in the human eye lens which, when used in mathematical modelling to simulate reflected and scattered light, can recreate the image of the lens seen in the living human eye. The results of this study show that the zones of discontinuity may be caused by subtle fluctuations in the refractive index gradient as well as from random scattering in the central regions. As the refractive index contours are created by cell layers with progressively varying protein concentrations, the zones are linked to growth and will contain information about ageing and development. The index gradient is important for image quality and fluctuations in this gradient may add to quality optimisation and serve as models for designs of new generation implant lenses.

1. Introduction

The growth of the lens, by accrual of new fibre cells over existing tissue with no concomitant tissue losses, creates a concentric structure of layers varying in age and in protein content. When observed in vivo, using slit lamp biomicroscopy, distinct regions are seen in the lens and these have been labelled to accord with the certain stages of life at which the tissue within any given region was synthesised (Vogt, 1919, 1931, Mann, 1925; Goldmann, 1937). Their presence is a manifestation of growth processes and collectively, the regions have been called zones of discontinuity (Vogt, 1931). They have been considered to manifest sharp demarcations in the refractive index (Mann, 1925; Huggert, 1948) or to indicate scatter (Yaroslavsky et al., 1994). Subsequent studies have found that the refractive index does not alter as abruptly as previously suggested (Mann, 1925; Huggert, 1948) but increases relatively gradually from periphery to centre (reviewed in Pierscionek and Regini, 2012).

A biomicroscopic image of the lens seen in the living eye is formed by light that is back scattered and reflected from internal structures. In a healthy eye, the light scatter that contributes to the zones of discontinuity does not impair vision. Any further interpretation of the biological characteristics of the zones of discontinuity requires a better understanding of the structural features which form the scattering sources and the optical characteristics of the lens.

The lens has a gradient refractive index and the proportion and direction of scattered light depends on the steepness of the refractive index gradient as well as on how smoothly varying it is (Born and Wolf, 1970). Recently, small fluctuations or kinks in the refractive index gradient of animal lenses have been found along the optic axis and in the equatorial plane (Hoshino et al., 2011) and it has been hypothesised that these fluctuations may account, at least in part, for the zones of discontinuity (Pierscionek and Regini, 2012).

Mathematical models can be used to reconstruct the refractive index gradient and fluctuations within it as seen in the axial region and to extend these paraxially, adhering to the concentric lamellar structure of the lens that gives rise to iso-indicial contours. Measurements taken along the optic axes of human lenses using X-ray synchrotron radiation were used to model and simulate biomicroscopic images. The findings suggest a direct relationship between zones of discontinuity and the refractive index of the lens.

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2. Methods

2.1. X-ray Talbot interferometry

Human eyeball samples (16, 35 and 48 years old), from which corneal discs were removed for transplantation, were obtained from the Bristol Eye Bank (UK) and transported frozen to the SPring-8 synchrotron radiation facility in Japan. Ethical approval was granted by the National Health Service (NHS) committee (Oxford, UK). Samples were thawed within 24 h of measurement and lenses removed from eyeballs and set in a physiologically-balanced agarose gel within a specially designed sample holder. Refractive index of lenses was measured using the X-ray Talbot grating interferometer (Momose, 2005; Momose et al., 2003) as described in Hoshino et al. (2011) constructed at the bending magnet beamline BL20B2 at Spring-8. Briefly, the instrument utilises a monochromatic X-ray beam, tuned to 25 keV that passes through a Si(111) double crystal monochromator and two transmission gratings: a phase grating (G1) made of tantalum and an absorption grating (G2) made of gold with pattern thicknesses 2.1 μm and 16.6 μm respectively and pitch of 10 μm. Moiré fringes generated by G1 and G2 were detected by the beam monitor and a scientific CMOS detector (ORCA Flash 4.0. Hamamatsu Photonics). For phase retrieval, G2 was shifted with a Piezo stage and 5-step 'on-the-fly' fringe-scan method used. The measurement time per sample took 82 min and around 900 scans were made for each lens.

2.2. Modelling geometric scatter

Geometric scattering in a discrete medium can be seen as reflections at interfaces where there is a change in the refractive index. The intensity of this reflection is described by the Fresnel reflection equation:

$$R=\frac{1}{2}\left(\left|\frac{n1\cos\theta-n2\sqrt{1-\left(\frac{n1}{n2}\sin\theta\right)^2}}{n1\cos\theta+n2\sqrt{1-\left(\frac{n1}{n2}\sin\theta\right)^2}}\right|^2+\left|\frac{n1\sqrt{1-\left(\frac{n1}{n2}\sin\theta\right)^2}-n2\cos\theta}{n1\sqrt{1-\left(\frac{n1}{n2}\sin\theta\right)^2}+n2\cos\theta}\right|^2\right) \quad (1)$$

where R is the reflection coefficient, θ is the angle between the incident ray and the normal vector of the surface, and $n1$ and $n2$ are the refractive indices of two neighbouring points.

In a gradient refractive index (GRIN) medium, $n1$ and $n2$ can be described as $n(x1,y1,z1) = n1$ and $n(x2,y2,z2) = n2$, where $n(x,y,z)$ is the refractive index function of the medium at each point (x,y,z). If $(x2,y2,z2) = (x1,y1,z1) + \overrightarrow{\Delta}$, where $|\overrightarrow{\Delta}|$ approaches 0, the incremental difference between $n1$ and $n2$ can closely approximate a continuous function. The gradient of the refractive index function $n(x,y,z)$ can therefore be simulated by very small increments and equation (1) applied iteratively for each sequential increment in refractive index along the gradient. Equation (1) was applied to spherical lenses with different radially varying refractive index profiles: linear, quadratic, cubic and quartic to determine the pattern of reflected light and to see how these vary with different refractive index forms.

Equation (1) was also applied to experimentally measured refractive index gradients in three human lenses, the surface shapes of which were extracted from the X-ray images. The number of iterative steps varied depending on lens size and ranged between 550 and 700 for on axis calculations; for calculations in 2-dimensions this ranged from 6.05×10^5 to 9.8×10^5. Image processing libraries of Mathematica Computational Software v8 were used to detect lens edges. The coordinates of detected points were fitted with higher order aspheric polynomials (as in Bahrami and Goncharov, 2012).

In addition to simulating reflected light a linear congruential generator algorithm (Park and Miller, 1988) was used to generate fine random fluctuations in the central lens to simulate scatter. The algorithm produces random vectors $\overrightarrow{\delta}$, where $0 \le |\overrightarrow{\delta}| \le 0.1$ mm that add a minor fluctuation to the coordinates of the function $R(x,y,z)$. The fluctuations were higher in the axial regions and declined with distance away from the axis, simulating decreased scatter with distance from the centre of the lens based on morphological findings (Taylor et al., 1996; Al-Ghoul and Costello, 1997; Costello et al., 2007; Costello et al., 2008; Shestopalov and Bassnett, 2000).

3. Results

When the GRIN profile for a spherical radially symmetric structure is altered from a linear GRIN profile to a series of profiles, each an integral function of the previous one (Fig. 1A), the images of light reflection obtained from applying Fresnel's equations to the gradient index profiles appear as shown in Fig. 1B. These patterns are seen as long as the GRIN profile is relatively smooth with incremental changes that are negligible in comparison to the slope of the profile.

The bisecting dark line is visible in all patterns with only the linear profile showing a break in the line at the central point. This dark band is a result of the spacing between the iso-indicial contours. As collimated incident light travels from the left side of the lens to its right side (parallel to the optical axis z), the rays experience a slower gradient at the equatorial zone. This refractive index profile can be described as:

$$n(r) = n_c + (n_s - n_c)r^p \quad (2)$$

where n_c is the refractive index of the centre of the lens, n_s is the refractive index of its surface, r is the normalised radial distance from the centre, and p is an exponent which adjusts for the curvature of the profile.

As p increases from 1 to 4, the refractive index profile takes on a progressively flatter central region (Fig. 1A). When $p = 1$, the lens shows a defined point of high reflectance at its centre with a relatively fine dark bow region emanating from this central point (Fig. 1B). With increasing value of the exponent p, this point is no longer seen and the bow becomes a straight bisecting line with the shadowing gradually decreasing towards the periphery. This gradual decrease occurs along a perpendicular to the dark bisecting line for $p = 2$ and becomes more radially varying for higher values of p (Fig. 1B). The central dark line continues to be visible for $p = 3$ and $p = 4$ and the light reflection is greatest in the peripheral regions; the variation in the amount of reflectance takes on an oval-shaped form (Fig. 1B).

Applying equation (1) to a lens model with well-defined iso-indicial contours that follow a lenticular surface shape (Fig. 2A) gives a reflectance pattern shown in Fig. 2B. The value of $p = 4$, $n_C = 1.415$, and $n_S = 1.37$ (Pierscionek, 2009; Bahrami and Goncharov, 2012). The oval-shaped darker region, which indicates minimal reflection, is evident and here the dark bisecting feature is not a straight line but is slightly curved (Fig. 2B).