Discontinuities in the gradient index structure of eye lenses

The eye has a gradient index lens to provide a better optical performance than would be possible with a homogenous index lens. The exact form or shape of this gradient requires further investigation because it is linked closely with the way that the lens grows: by accruing new cell layers over existing tissue, with no concomitant losses. Hence, every lens contains a chronological record of its development and growth and each layer of cells contributes to the shape of the gradient index profile. The index profile may therefore provide some insight into growth phases of the lens. This could eventually lead to a better understanding of the zones of discontinuity, lamellar-like features seen in the living human eye lens that may demarcate phases in lens development [1]. To date there has been no clear explanation of the physical nature of these features nor any confirmation of their purpose. If these 'zones' are signs of protein density changes at certain life stages, they should appear as fluctuations or discontinuities in the refractive index profile. Studies on the refractive index of the lens, thus far, have been unable to detect such fine, localized fluctuations.

Ideally, the refractive index should be measured on whole lenses, in any given plane. Measurements should be made with a level of accuracy that can detect any fluctuations or irregularities in the profile that may be meaningful physiologically. X-ray microtomography allows for quantitative measurement of sample density but does not permit recognition of structures within the eyeball [2]. An X-ray Talbot grating interferometer, that combines phase contrast imaging and microtomography, has been developed by Momose [3,4]. This interferometer makes use of the Moiré fringes generated by two gratings (an absorbance and phase grating). It also has the advantage, over interferometers such as the Bonse-Hart instrument, of being able to tolerate large density differences. This renders the technique applicable to the measurement of density changes within the eyeball. The aim of the study was to investigate whether this instrument could detect subtle changes in the refractive index gradient that could not be found with previously used techniques.

The X-ray grating interferometer, constructed at the bending magnet beamline **BL20B2**, utilizes a monochromatic X-ray beam that is passed through a Si(111) double crystal monochromator (Fig. 1). The X-ray energy was tuned to 15 keV, 25 keV or 35 keV and the photon flux at an energy of 15 keV was 6.5×10^9 (photons/sec/mm^2 @ 15 keV). The instrument has two transmission gratings: a phase (G1) and absorption grating (G2). Grating parameters and materials were varied depending on the size of the lens samples. For large lenses grating G1 was made of tantalum and G2 was made of gold with pattern thicknesses 2.1 μm and 16.6 μm, respectively. The grating pitch of both gratings was 10 μm and the pattern size area was 25 mm (H)×25 mm (V). G2 was inclined by 45° so as to increase the effective X-ray absorption at the grating. For smaller samples, both gratings were made of tantalum and the pattern thicknesses of G1 and G2 were equal to 0.96 μm and 4.75 μm, respectively. The pitch of both gratings was 5 μm and the pattern size area was 5 mm (H)×10 mm (V). The inclination angle of G2 was 60°. An appropriate X-ray imaging detector was selected to acquire an image of the whole eyeball with an adequate field of view.

Phase retrieval was achieved using a fringe-scan method [3,4]. Differential phase shift images were obtained and integrated to provide the phase shift image. Phase shifts are calibrated against solutions of known density and theoretically obtained values are compared to the experimentally derived phase shift values per pixel. The three-dimensional refractive index $\delta(x,y,z)$ is reconstructed from the phase shifts $\Phi(x,y)$ using the following integral:

$$\Phi(x,y) = 2\pi/\lambda \int \delta(x,y,z)\, dz \qquad (1)$$

Lenses from five species: pig (*Sus domestica*), fish (*Carassius auratus auratus*), mouse (*C57BL/6*), frog (*Rana catesbeiana*) and newt (*Cynops pyrrhogaster*) were examined within intact fresh eyeballs. Images of eyeballs from the five species are shown in Fig. 2.

The lens is the prominent structural feature in each image. The image intensity varies across each lens

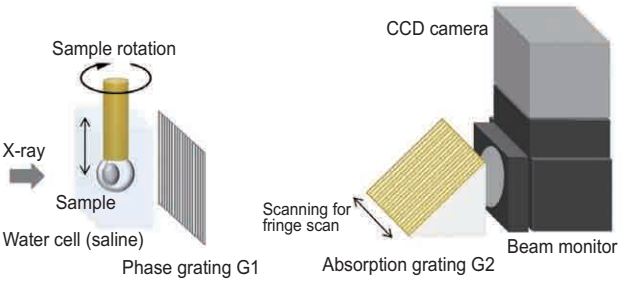

Fig. 1. Diagrammatic representation of the X-ray Talbot interferometer showing the sample cell with sample suspended on a rotatable rod; the phase and absorption gratings (G1 and G2 respectively) and the beam monitor. [2]

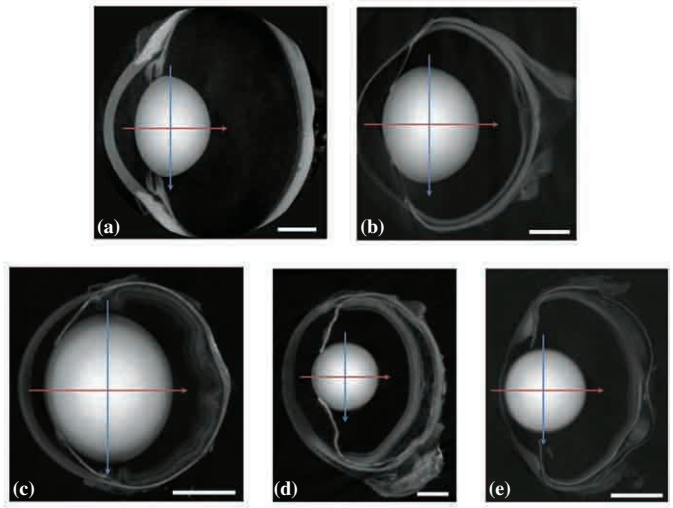

Fig. 2. Images of **(a)** pig; **(b)** frog; **(c)** mouse; **(d)** newt; **(e)** fish eyes in the sagittal plane. The position of the equatorial plane is marked with the blue arrow and the optic axis, along which the sagittal refractive index profiles were measured, is marked with a red arrow. The scale bars in the right hand lower corner are equal to **(a)** 4 mm (pig); **(b)** 2 mm (frog); **(c)** 1 mm (mouse); **(d)** 0.5 mm (newt); **(e)** 1 mm (fish) [2].

and it is possible to discern regions of different density. Refractive index variations were measured in the equatorial and sagittal planes: images for the equatorial plane are shown in Fig. 3. Whilst the profile shapes can be approximated to second order polynomials, there are 'discontinuities' in some of the functions where the curves deviate from second order polynomial fits. These kinks, seen in the peripheral sections of the profiles, are most prominent in the newt (Fig. 3(d)) and fish lenses (Fig. 3(e)) and least obvious in the frog lens (Fig. 3(b)).

The deviations from a smooth gradient may suggest some changes in the growth mode of the lens, in its rate or in the complement of proteins laid down in the cells in the region of these irregularities. It is still not clear what these features may manifest physiologically, but they may be the first optical evidence of the zones of discontinuity [1]. Thus far these have only been observed in human lenses in the living eye. These zones of discontinuity do not affect refraction nor impair vision. They may, however, be indicative of important stages in the growth and development of the lens [4] and require further investigation. As each lens contains a chronological record of its growth, these processes can be studied in single lenses.

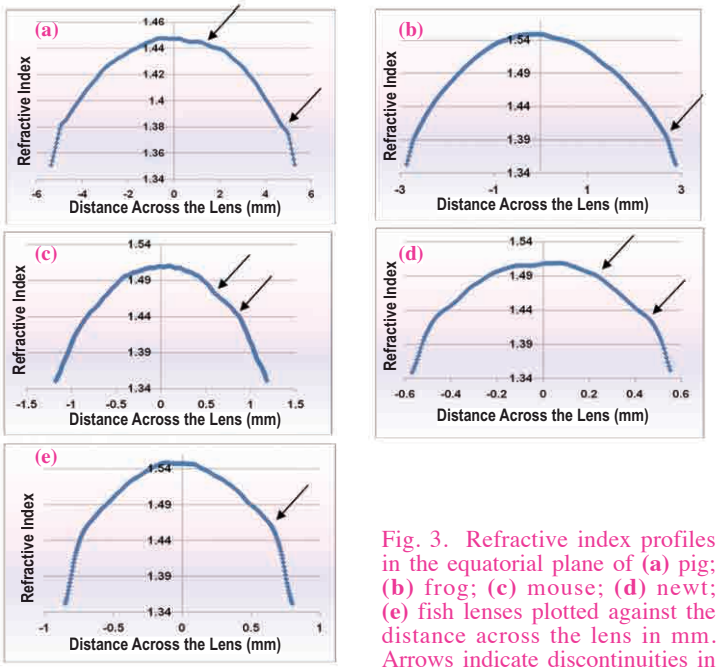

Fig. 3. Refractive index profiles in the equatorial plane of **(a)** pig; **(b)** frog; **(c)** mouse; **(d)** newt; **(e)** fish lenses plotted against the distance across the lens in mm. Arrows indicate discontinuities in the gradient index profiles. [2]

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