Gradient of index and moduli is essential for optimal function of the eye lens

The human eye lens is a synchronized opto-mechanical structure designed to meet the dynamic focusing requirements of the visual system. It is held in place by a ring of suspensory ligaments that transmit force to control shape changes of the lens to view objects clearly over a range of distances, a process called accommodation. The major properties of the eye lens: transparency, refractive power and elasticity, determine the quality of images focused on retina. The eye lens is composed of layers of fiber cells filled with proteins called crystallins. The overall crystallin protein concentration of a human eye lens is around 65% of its weight. The relationship between protein concentrations and refractive index is linear over a wide range and this has been described by the Gladstone-Dale formula [1]. The link between biomechanical properties and the proteins, is not yet clear but a link between the refractive index and the biomechanical parameters is emerging.

The biomechanics of the human lens is neither fixed nor constant because of the inhomogeneous and variable nature of aging cells and tissues in the long term and the process of accommodation. The other complicating factor is that biomechanics can be described by a number of parameters and these have been investigated in the eye lens using a range of different techniques that have yielded a variety of results. The variations are caused by aging trends, spatial variations and assumptions inherent in the measurements. A recent study, using the only known non-invasive technique for studying biomechanics of the eye lens: Brillouin light scattering analysis, can measure mechanical modulus at a resolution of nearly 60 μm [2]. This study showed a gradient distribution of longitudinal elastic modulus along the central optical axis in *in vivo* human lenses aged from 19 to 63 years old [2]. Such a distribution resembles almost exactly the profiles of refractive index in *in vitro* human lenses of a similar age range that were measured by the X-ray Talbot interferometer located at beamline BL20B2 at SPring-8 [3]. The similarity in distributions of refractive index and mechanical modulus of elasticity (Fig. 1) suggests a potential link between biomechanics, optics and ultimately proteins in the eye lens.

Brillouin scattering analysis makes use of the interactions between optics and acoustics in two ways: a) acoustic periodical modulation of material density, which is related to refractive index, causes light scattering; b) incident light creates spatial and temporal variations in elastic strains of the material initiating acoustic waves. As a result, the incident light will either gain energy from existing acoustic waves in the medium with a frequency upshift or lose energy to induce new acoustic waves with a frequency downshift. The mechanical property of the material included in these frequency shifts can therefore be determined with a known density to refractive index ratio [4].

Experiments conducted at SPring-8 **BL20B2** use a monochromatic X-ray beam of 25 keV, which passes through the interferometer that consists of a tantalum phase grating G1 and a gold absorption grating G2 with pattern thicknesses of 2.1 μm and 16.6 μm, respectively. For both gratings the pitch is 10 μm and pattern size area is 25×25 mm^2. For phase retrieval, grating G2 is shifted using a 5-step fringe scan method with a Piezo stage. A scientific CMOS detector (ORCA Flash 4.0. Hamamatsu Photonics) is used to monitor the Moiré fringe patterns generated by X-ray beam after passing through the sample and the system. The phase shift image can be integrated using different images from the scan and the X-ray refractive index difference can be determined from the phase shifts per pixel. The protein concentration is calculated

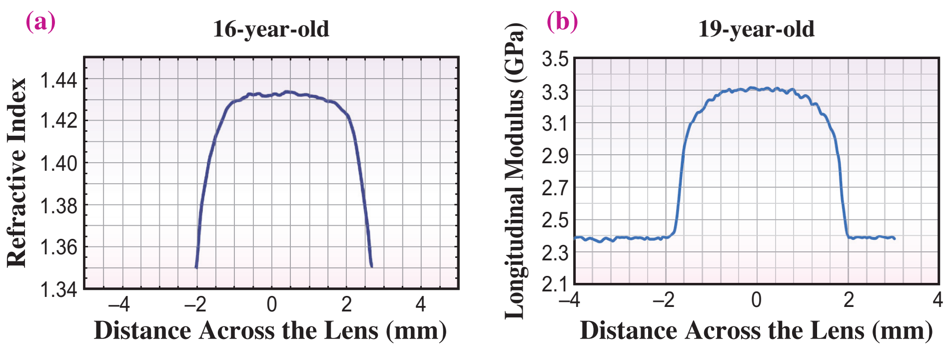

Fig. 1. **(a)** Gradient refractive index profile of a 16-year-old lens compared to **(b)** gradient profile of mechanical modulus of a 19-year-old lens.

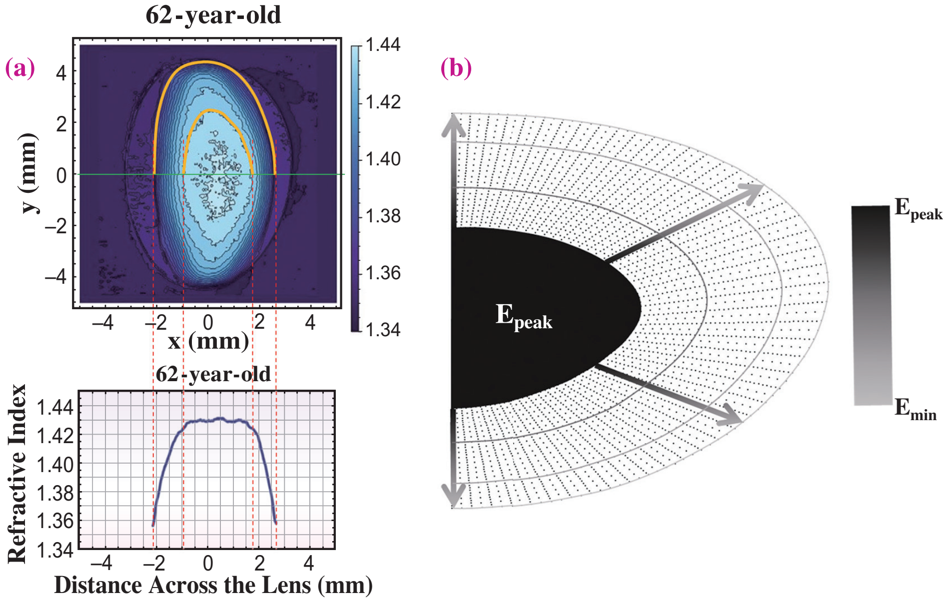

Fig. 2. **(a)** Geometry of lens nucleus and cortex from gradient refractive index profile of a 62-year-old lens measured at SPring-8 **(b)** discretized Finite Element model showing uniform Young's modulus in the nucleus and radial moduli in the cortex.

using the X-ray refractive index difference from which optical refractive index is determined according to the Gladstone-Dale formula [1].

To investigate the relationship between mechanical modulus obtained from Brillouin Light Scattering analysis and refractive index measured by X-ray Talbot interferometry, we developed computational models using a Finite Element method correlating these two parameters in lens models based on measurements made at SPring-8 at five different ages: 16, 35, 40, 57 and 62 years old (Fig. 2). Models with radial (gradient) cortical elastic moduli, following the distributions of both longitudinal elastic modulus and refractive index, were compared, in terms of deforming ability, optical power and internal stress patterns, with models that had a uniform cortical modulus [5]. Under stretching forces from zonular fibers over a range of physiologically acceptable directions, models with radial cortical elastic moduli showed similar amounts of optical power for a smaller change in thickness than did the models with a uniform cortical modulus indicating that the gradient provides a more efficient alteration of refractive power with shape change. Smooth stress changes, with no discontinuities, were found only in models with a gradient of moduli (Fig. 3) suggesting that the gradient is necessary for improved optical and mechanical function.

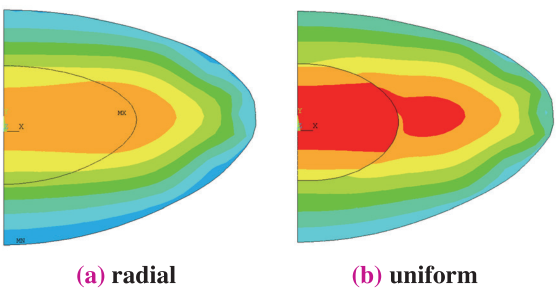

Fig. 3. **(a)** Uniform stress distribution in the 16-year-old model with radial cortical Young's moduli **(b)** discontinuities in stress distribution in the 16-year-old model with a uniform cortical Young's modulus (von Mises stress in MPa).

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