Accepted Manuscript

Isolated human crystalline lens three-dimensional shape: A comparison between Indian and European populations

Ashik Mohamed, Sushma Nandyala, Eduardo Martinez-Enriquez, Bianca Maceo Heilman, Robert C. Augusteyn, Alberto de Castro, Marco Ruggeri, Jean-Marie A. Parel, Susana Marcos, Fabrice Manns

PII: S0014-4835(21)00046-4
DOI: doi:[10.1016/j.exer.2021.108481](https://doi.org/10.1016/j.exer.2021.108481)
Reference: YEXER 108481

Published in: *Experimental Eye Research*

Received date: 9 November 2020
Revised date: 7 January 2021
Accepted date: 26 January 2021

Cite this article as: Mohamed A, Nandyala S, Martinez-Enriquez E, Heilman BM, Augusteyn RC, de Castro A, Ruggeri M, Parel J-MA, Marcos S, Manns F, Isolated human crystalline lens three-dimensional shape: A comparison between Indian and European populations, *Experimental Eye Research*, doi:[10.1016/j.exer.2021.108481](https://doi.org/10.1016/j.exer.2021.108481)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Title: Isolated human crystalline lens three-dimensional shape: a comparison between Indian and European populations

Authors: Ashik Mohamed (ఆషిక్ ముకమత్తు),^{a,b,*} Sushma Nandyala (సుష్మా నండ్యాల),^a Eduardo Martinez-Enriquez,^c Bianca Maceo Heilman,^{d,e} Robert C Augusteyn^{b,d}, Alberto de Castro,^c Marco Ruggeri,^{d,e} Jean-Marie A Parel,^{b,d,e,f} Susana Marcos,^c Fabrice Manns^{d,e}

Affiliations: ^aOphthalmic Biophysics, L V Prasad Eye Institute, Hyderabad, India; ^bBrien Holden Vision Institute Limited, Sydney, Australia; ^cVisual Optics and Biophotonics Lab, Institute of Optics, Consejo Superior de Investigaciones Científicas (IO-CSIC), Madrid, Spain; ^dOphthalmic Biophysics Center, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA; ^eDepartment of Biomedical Engineering, University of Miami College of Engineering, Coral Gables, FL, USA; ^fAnne Bates Leach Eye Center, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA.

***Corresponding author:**

Dr. Ashik Mohamed

Ophthalmic Biophysics, L V Prasad Eye Institute

L V Prasad Marg, Banjara Hills

Hyderabad – 500034, Telangana, India.

Phone: +91-40-68102121

Fax: +91-40-23548271

E-mail: ashikmohamed@lvpei.org

Funding: This work was supported by the National Institutes of Health/National Eye Institute [Grants 2R01EY021834 and P30EY14801 (Center Grant)]; the Hyderabad Eye Research Foundation; Ministerio de Educación y Ciencia, Spain (FIS2017- 84753-R), European Research Council (ERC-2018-AdG-833106) and IMCUSTOMEYE Ref. 779960 (H2020-ICT- 2017-1) to S. Marcos, and European Research Council (ERC) under European Union's Horizon 2020 research and innovation programme H2020-MSCA COFUND-2015 FP-713694, MULTIPLY(AdC), CSIC ICoop Program, Florida Lions Eye Bank and the Beauty of Sight Foundation, an unrestricted grant from Research to Prevent Blindness to the department of Ophthalmology, University of Miami and the Henri and Flore Lesieur Foundation (J-M. Parel). The funding sources had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Declarations of interest: Spanish patent: Procedure to calibrate and correct the scan distortion of an Optical Coherence Tomography system, P201130685 (Sergio Ortiz, Susana Marcos, Damian Siedlecki and Carlos Dorronsoro). USA patent application: Method of estimating a full shape of the crystalline lens from measurements taken by optic imaging techniques and method of estimating an intraocular lens position in a cataract surgery, US201662329392P (Eduardo Martínez-Enríquez, Susana Marcos, and Carlos Dorronsoro). None for all other authors.

Abstract

There have been many studies on lens properties in specific populations (e.g. in China, Europe, Singapore, etc.) some of which suggest there may be differences between populations. Differences could be caused by ethnic or environmental influences or experimental procedures. The purpose of this study is to evaluate if any differences exist between Indian and European populations in the central geometric and full shape properties of human lenses. Two custom-developed spectral domain optical coherence tomography systems were used to acquire the crystalline lens geometry: one in India (69 lenses from 59 donors) and the other in Spain (24 lenses from 19 donors). The steps for obtaining accurate 3-D models from optical coherence tomography raw images comprised of image segmentation, fan and optical distortion correction, tilt removal and registration. The outcome variables were lens equatorial diameter, lens thickness, anterior and posterior lens thicknesses and their ratio, central radius of curvature of the anterior and posterior lens surfaces, lens volume and lens surface area. A mixed effects model by maximum likelihood estimation was used to evaluate the effect of age, population and their interaction (age*population) on lens parameters. After adjusting for age, there were no population differences observed in anterior and posterior radii of curvature, equatorial diameter, lens thickness, anterior and posterior lens thicknesses and their ratio, volume and surface area (all $p \geq 0.08$). There was also no effect of the interaction term on anterior and posterior radii of curvature, equatorial diameter, lens thickness, anterior and posterior lens thicknesses and their ratio, volume and surface area (all $p \geq 0.06$). All central geometric and full shape parameters appeared to be comparable between the European and Indian populations. This is the first study

to compare geometric and full shape lens parameters between different populations *in vitro*.

Key words: 3-D model, crystalline lens, geometry, human lens, *in vitro*, optical coherence tomography, population, shape.

Journal Pre-proof

1. Introduction

It is well recognized that there are significant anatomical and performance differences between different ethnic groups. Differences in physical ability, skin colour and facial features are obvious, while numerous variations in bone and teeth have been documented (Brook et al., 2009; Hanihara and Ishida, 2005; Leslie, 2012; Zengin et al., 2015). Ocular structures also vary among different populations resulting in differences in the prevalence of many ocular diseases such as age-related macular degeneration, cataract and glaucoma (Blake et al., 2003; Bourne, 2011). Racial differences have been observed in the cornea, anterior chamber depth, retina, axial length and optic nerve (Aghaian et al., 2004; Knight et al., 2012; Sommer et al., 1991). However, very little is known about the lens. Understanding possible population differences in lens parameters will help understand the role of these differences on lens growth and optics as well possible effects on cataract and presbyopia.

The geometric and two-dimensional shape properties of the crystalline lens have been assessed using different techniques *in vitro* and *in vivo*, but little information is available on possible population differences. The only comparative study to date has been that of Augusteyn et al. (2011) who evaluated *ex vivo* lenses from India and USA and concluded that there were no differences in lens dimensions, optical power and stiffness. A limited number of *in vivo* studies have suggested that there could be differences in lens growth patterns due to ethnic, genetic or dietary variations (Hashemi et al., 2012; Wang et al., 2017).

The purpose of this study is to compare the central geometric and full shape properties of isolated human crystalline lenses from Indian and European populations.

2. Material and methods

2.1 Imaging system

Two custom-developed spectral domain optical coherence tomography (SD-OCT) systems were used to acquire 3D images of the crystalline lens:

2.1.1 Ophthalmic Biophysics Center SD-OCT system (Indian OCT)

The Ophthalmic Biophysics Center's imaging system (Indian OCT) that is combined with a laser ray tracing (LRT) system was built in Miami, USA and has been described in detail elsewhere (Martinez-Enriquez et al., 2020a; Ruggeri et al., 2018). Briefly, the system includes a commercial SD-OCT device (ENVISU R4400, Bioptigen Inc., Durham, NC, USA) that operates at a center wavelength of 880 nm with a modified beam delivery unit. The OCT system was coupled with a custom-built telecentric beam delivery system that creates a focused spot with a calculated diameter of approximately 60 μm in the lens. The axial range in air is 15.18 mm with a digital axial resolution (pixel size) of 7.4 μm and an optical axial resolution of 8.5 μm in air. The effective acquisition speed is 32,000 A-scans/s. Each 3-D volume was composed of 100 B scans each with 600 A-lines on a 15 mm x 15 mm lateral area.

2.1.2 VioBio Lab SD-OCT system (European OCT)

The VioBio Lab SD-OCT system (European OCT) has also been described in detail previously (Grulkowski et al., 2009). Essentially, the system operates at a center wavelength of 840 nm with an axial range of 7 mm in depth in air, a digital axial resolution (pixel size) of 4.7 μm and an optical axial resolution of 9.5 μm in air. The transverse resolution is 27 μm . The effective acquisition speed is 25,000 A-scans/s. In

these experiments, one 3-D volume was composed of 60 B scans each with 1668 A-lines on a 12 mm x 12 mm lateral area.

2.2 Experimental protocols

This research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Boards of L V Prasad Eye Institute (LVPEI), Hyderabad, India and Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain. The eye banks, in accordance with their practices and procedures, obtained the consent from the donor families to enucleate eyes for the purpose of transplantation, therapy, medical research, or education.

2.2.1 Ophthalmic Biophysics Lab, LVPEI

Human lenses were obtained from the Ramayamma International Eye Bank, LVPEI, and measured with the Indian OCT system. Damaged lenses or lenses with cataract, as judged by eye or OCT, were excluded. The tissue preparation was based on the protocol described previously (Martinez-Enriquez et al., 2020a; Mohamed et al., 2020). Essentially, the lens was isolated from the donor globe and immediately placed on a custom made lens holder (containing interrupted 10-0 nylon sutures to support the lens) within a tissue chamber filled with balanced salt solution (BSS, Alcon Laboratories Inc., Fort Worth, TX, USA) at room temperature.

In total, 69 lenses from 59 subjects (age range: 19-56 years; post-mortem time range: 0.5-4 days, median of 34 hours) were analyzed. The lens was first imaged with the anterior surface facing the OCT beam ("anterior-up" measurements) and then flipped over and imaged again with the posterior surface facing the OCT ("posterior-up"

measurements). This protocol enabled the acquisition of both anterior and posterior lens surface shapes without being affected by distortions due to the lens gradient refractive index or to interference by the nylon supporting sutures.

2.2.2 VioBio Lab, CSIC

Human eyes were obtained from Banc de Sang i Teixits (BST) Eye Bank, Barcelona, Spain. Full details of the shipping and handling protocol and geometrical parameters of the central area of this set of eyes had been reported previously (Birkenfeld et al., 2014; 2015). Briefly, the lens was carefully extracted from the eye and placed horizontally on a ring in a cuvette filled with Dulbecco's Modified Eagle Medium ([DMEM]/F-12, HEPES, no phenol red; GIBCO, Carlsbad, CA, USA). Damaged, excessively tilted, or lenses where the equatorial plane was not visible were identified from the OCT images and excluded from the study. A total of 24 crystalline lenses from 19 donors (age range: 19-60 years; post-mortem time range: 1-3 days, median of 33 hours) were analyzed. The lens was first imaged with the anterior surface facing the OCT beam and then flipped over and imaged again with the posterior surface facing the OCT.

2.3 OCT image processing for 3-D eye models construction and quantification

The steps for obtaining accurate 3-D models from OCT raw images were similar for both datasets (Indian OCT and European OCT) and all images from both populations were analyzed by the same individual using the same custom-built image processing software. As described earlier, the steps comprised image segmentation, fan and optical distortion correction, tilt removal and registration. In brief, the contour of the lens was first automatically detected from the edge in three different orientations using a custom-developed algorithm (Martinez-Enriquez et al., 2017; 2020a; 2020b). Both

anterior-up and posterior-up images were corrected for fan and optical distortions caused due to the preservation media (using $n = 1.345$ as the group refractive index of BSS at 880 nm and DMEM at 840 nm) (Pérez-Merino et al., 2015). Residual tilt in the surface was removed and axial and rotational registrations were performed (Martínez-Enríquez et al., 2020a).

All lens parameters were automatically quantified from OCT-based 3-D constructed lens models as explained previously (Martínez-Enríquez et al., 2020a). The outcome variables were the lens equatorial diameter (DIA), lens thickness (LT), anterior lens thickness (LTa), posterior lens thickness (LTp), the LTa/LTp ratio, the radius of curvature (central 6 mm) of the anterior (RAL) and posterior (RPL) lens surfaces, lens volume (VOL) and lens surface area (LSA).

2.4 Data analysis

The statistical analyses were performed using STATA v14.2 (StataCorp, College Station, TX, USA). The Mann-Whitney test was used to compare the ages between European and Indian lenses. A mixed effects model, using maximum likelihood estimation, was applied to evaluate the effect of age, population and their interaction (age*population) on lens parameters. A random effect (random intercept) was added to account for the correlation in outcomes between paired lenses of a donor, assuming that the intercept is the same for the two lenses of a donor (Ying et al., 2018), but different across different donors. A p-value of <0.05 was considered statistically significant.

There was a significant difference in age between the two populations ($p = 0.0002$).

Median donor age of European lenses was 47 years (inter-quartile range 37-53 years and range 19-60 years) and that of Indian lenses was 30 years (inter-quartile range 26-37 years and range 19-56 years).

3. Results

Figure 1 shows the representative reconstructed 3-D models in European and Indian lenses of three age groups: young (Figures 1A and 1B), middle (Figures 1C and 1D) and old-aged (Figures 1E and 1F). The age-dependence of lens parameters in European and Indian populations are presented in Figure 2 and the results of linear regression analyses are in Table 1. For both populations, lens equatorial diameter (DIA), thickness (LT), anterior and posterior thickness (LTa and LTp), anterior radius of curvature (RAL), volume (VOL) and surface area (LSA) exhibit a significant positive correlation with age ($p < 0.0001$ to 0.04). Increases were also observed in posterior radius of curvature (RPL) in both populations. The correlation with age was significant for the Indian lenses ($p = 0.02$), but not for the European lenses ($p = 0.18$). The LTa/LTp ratio was constant over the age range studied in both populations. All of the increases and the constant ratio are very similar to those variously reported in previous studies on *ex vivo* lenses (Augusteyn, 2007; Augusteyn et al., 2006; 2011; Birkenfeld et al., 2013; Manns et al., 2004; Martinez-Enriquez et al., 2020a; Marussich et al., 2015; Mohamed et al., 2012; 2020; Rosen et al., 2006; Urs et al., 2009) as well as those from *in vivo* measurements (Atchison et al., 2008; Dubbelman and Van der Heijde, 2001; Gamba et al., 2013; Garner and Yap, 1997; Hermans et al., 2009; Kasthurirangan et al., 2011; Martinez-Enriquez et al., 2016; 2017; 2018; Ortiz et al., 2012; Rosales et al., 2006].

The effect of population was assessed by having age, population and their interaction as independent variables in the mixed effects model. Table 2 summarizes the results of analysis between European and Indian lenses by age and population. After adjusting for age, there were no population differences observed in RAL, RPL, DIA, LT, LTa, LTp, LTa/LTp, VOL and LSA. There was also no effect of interaction (age*population) on RAL, RPL, DIA, LT, LTa, LTp, LTa/LTp, VOL and LSA.

4. Discussion

In the present study, we aimed to determine whether there are population differences in geometric and full shape parameters between European and Indian lenses *in vitro*. We found no differences in thickness and diameter between the two populations. A similar conclusion was reached on a smaller set of parameters from an *ex vivo* study which used a mechanical lens stretcher to evaluate the opto-mechanical properties of lenses. No differences were found in lens diameter, lens thickness and lens power between American and Indian populations (Augusteyn et al., 2011). Our data also indicate that central geometric parameters such as the radius of curvature of the anterior and posterior lens surfaces and full shape parameters such as lens volume and surface area were comparable between the populations (Table 2). Thus, based on *in vitro* observations, it would appear that there are no racial differences in lens properties.

By contrast, *in vivo* studies have suggested there are population differences in lens thickness and position (Hashemi et al., 2012; Wang et al., 2017). Hashemi et al (2012) found that their lens thickness measurements on Iranian adults were comparable to prior studies on American adults but lower than prior studies on Chinese adults. In a study on adult subjects who had cataract surgery at an academic institution in

California, Wang et al (2017) found that patients of Asian ethnicity had slightly thicker lenses than African American patients. However, these measurements on cataractous lenses may not necessarily be extrapolated to conditions where lenses are free of cataract. Hispanic, Asian and African-American subjects also appear to have a shorter lens position (anterior chamber depth + $\frac{1}{2}$ LT) than caucasians. These studies highlight the need to better understand differences in lens shapes among different populations.

Limitations of the present study include the unequal sample sizes of the two populations (24 from Europe vs 69 from India). Other limitations include the small sample size and the shortage of lenses from the pre-presbyopic age group (<40 years) in the European data. A potential difference between the two populations, even at the same age, may exist due to different antero-posterior globe or axial eye lengths that can influence lens biometry. Such differences have been observed (Mohamed, A., et al., 2020. Invest. Ophthalmol. Vis. Sci. 61, ARVO E-Abstract 537; Muralidharan et al., 2019), but information on this was not available in this *in vitro* study. Another possibility may be a difference in the peripheral geometry beyond the central 6 mm of lens surfaces. Another limitation of the study is that the eye banks do not provide any information on the ethnicity of the donors. Each of the two groups of lenses therefore represents the general population in each local area, rather than a specific ethnicity.

Although the OCT systems used in the two laboratories were different, both systems were well calibrated, distortion-corrected and the scanning pattern and image analysis are similar. The pixel size in the OCT systems was calibrated and measurements on gold standard optical surfaces were used to double check this calibration. The Spanish laboratory OCT calibration was validated with the Rowe whole model eye and spherical

and aspherical optical surfaces (Ortiz et al., 2011). The Indian laboratory OCT was calibrated with a specific commercial distortion target (Ruggeri et al., 2018) using the same procedure developed by Ortiz et al. (2011) to compensate for image distortions. The two laboratories used different immersion media: BSS in India and DMEM in Spain. The immersion medium, in particular one based on salts (e.g. BSS), can lead to swelling and capsular separation if lenses are immersed for a prolonged duration (>5 hours) (Augusteyn et al., 2006). In our experiments, the integrity of the capsule was monitored throughout the experiments using OCT imaging. No changes were found. In addition, all the lenses were imaged in both the anterior up and posterior up positions within two hours of immersion. We found no significant differences in LT between the two orientations. The lenses in the Indian laboratory were placed in a custom-designed lens holder with nylon sutures whereas lenses in the Spanish laboratory were placed on a ring. One could argue that this difference in the lens holding methods might have resulted in different lens shapes; however, the similarities in lens parameters between the two populations suggest that the two different systems used in the Indian and Spanish laboratories did not affect the measurements.

5. Conclusions

This is the first study to compare geometric and full shape lens parameters between different populations *in vitro*. All the central geometric parameters and the full shape parameters appeared to be comparable between the European and Indian populations. Knowledge of these population-based lens parameters becomes important in making population-specific formulas related to intraocular lens power calculations, development

of accommodating intraocular lenses and lens refilling methods and evaluation of biomechanical models of accommodation and presbyopia.

Journal Pre-proof

References

Aghaian, E., Choe, J.E., Lin, S., Stamper, R.L., 2004. Central corneal thickness of Caucasians, Chinese, Hispanics, Filipinos, African Americans, and Japanese in a glaucoma clinic. *Ophthalmology*. 111, 2211-2219.

<https://doi.org/10.1016/j.opthta.2004.06.013>.

Atchison, D.A., Markwell, E.L., Kasthurirangan, S., Pope, J.M., Smith, G., Swann, P.G., 2008. Age-related changes in optical and biometric characteristics of emmetropic eyes. *J. Vis.* 8,29.1-20. <https://doi.org/10.1167/8.4.29>.

Augusteyn, R.C., Rosen, A.M., Borja, D., Ziebarth, N.M., Parel, J.M., 2006. Biometry of primate lenses during immersion in preservation media. *Mol. Vis.* 12, 740-747.

Augusteyn, R.C., 2007. Growth of the human eye lens. *Mol. Vis.* 13,252-257.

Augusteyn, R.C., Mohamed, A., Nankivil, D., Veerendranath, P., Arrieta, E., Taneja, M., Manns, F., Ho, A., Parel, J.M., 2011. Age-dependence of the optomechanical responses of ex vivo human lenses from India and the USA, and the force required to produce these in a lens stretcher: the similarity to in vivo disaccommodation. *Vision. Res.* 51, 1667-1678.

<https://doi.org/10.1016/j.visres.2011.05.009>.

Birkenfeld, J., de Castro, A., Ortiz, S., Pascual, D., Marcos, S., 2013.

Contribution of the gradient refractive index and shape to the crystalline lens

spherical aberration and astigmatism. *Vision. Res.* 86, 27-34.

<https://doi.org/10.1016/j.visres.2013.04.004>.

Birkenfeld, J., de Castro, A., Marcos, S., 2014. Contribution of shape and gradient refractive index to the spherical aberration of isolated human lenses. *Invest. Ophthalmol. Vis. Sci.* 55, 2599-2607. <https://doi.org/10.1167/iov.14-14201>.

Birkenfeld, J., de Castro, A., Marcos, S., 2015. Astigmatism of the Ex Vivo Human Lens: Surface and Gradient Refractive Index Age-Dependent Contributions. *Invest. Ophthalmol. Vis. Sci.* 56, 5067-5073. <https://doi.org/10.1167/iov.15-16484>.

Blake, C.R., Lai, W.W., Edward, D.P., 2003. Racial and ethnic differences in ocular anatomy. *Int. Ophthalmol. Clin.* 43, 9-25. <https://doi.org/10.1097/00004397-200343040-00004>.

Bourne, R.R., 2011. Ethnicity and ocular imaging. *Eye (Lond)*. 25, 297-300. <https://doi.org/10.1038/eye.2010.187>.

Brook, A.H., Griffin, R.C., Townsend, G., Levisianos, Y., Russell, J., Smith, R.N., 2009. Variability and patterning in permanent tooth size of four human ethnic groups. *Arch. Oral. Biol.* 54, Suppl 1:S79-85. <https://doi.org/10.1016/j.archoralbio.2008.12.003>.

Dubbelman, M., Van der Heijde, G.L., 2001. The shape of the aging human lens: curvature, equivalent refractive index and the lens paradox. *Vision. Res.* 41, 1867-1877. [https://doi.org/10.1016/s0042-6989\(01\)00057-8](https://doi.org/10.1016/s0042-6989(01)00057-8).

Gambra, E., Ortiz, S., Perez-Merino, P., Gora, M., Wojtkowski, M., Marcos, S., 2013. Static and dynamic crystalline lens accommodation evaluated using quantitative 3-D OCT. *Biomed. Opt. Express.* 4, 1595-1609. <https://doi.org/10.1364/BOE.4.001595>.

Garner, L.F., Yap, M.K., 1997. Changes in ocular dimensions and refraction with accommodation. *Ophthalmic. Physiol. Opt.* 17, 12-17.

Grulkowski, I., Gora, M., Szkulmowski, M., Gorczynska, I., Szlag, D., Marcos, S., Kowalczyk, A., Wojtkowski, M., 2009. Anterior segment imaging with Spectral OCT system using a high-speed CMOS camera. *Opt. Express.* 17, 4842-4858. <https://doi.org/10.1364/oe.17.004842>.

Hanihara, T., Ishida, H., 2005. Metric dental variation of major human populations. *Am. J. Phys. Anthropol.* 128, 287-298. <https://doi.org/10.1002/ajpa.20080>.

Hashemi, H., Khabazkhoob, M., Miraftab, M., Emamian, M.H., Shariati, M., Abdolahinia, T., Fotouhi, A., 2012. The distribution of axial length, anterior chamber depth, lens thickness, and vitreous chamber depth in an adult population of Shahroud, Iran. *BMC. Ophthalmol.* 12, 50. <https://doi.org/10.1186/1471-2415-12-50>.

Hermans, E.A., Pouwels, P.J.W., Dubbelman, M., Kuijer, J.P.A., van der Heijde, R.G.L., Heethaar, R.M., 2009. Constant volume of the human lens and decrease in surface area of the capsular bag during accommodation: an MRI and Scheimpflug study. *Invest. Ophthalmol. Vis. Sci.* 50, 281-289.

<https://doi.org/10.1167/iov.08-2124>.

Kasthurirangan, S., Markwell, E.L., Atchison, D.A., Pope, J.M., 2011. MRI study of the changes in crystalline lens shape with accommodation and aging in humans. *J. Vis.* 11, 19. <https://doi.org/10.1167/11.3.19>.

Knight, O.J., Girkin, C.A., Budenz, D.L., Durbin, M.K., Feuer, W.J., Cirrus OCT Normative Database Study Group, 2012. Effect of race, age, and axial length on optic nerve head parameters and retinal nerve fiber layer thickness measured by Cirrus HD-OCT. *Arch. Ophthalmol.* 130, 312-318.

<https://doi.org/10.1001/archophthalmol.2011.1576>.

Leslie, W.D., 2012. Clinical review: Ethnic differences in bone mass--clinical implications. *J. Clin. Endocrinol. Metab.* 97, 4329-4340.

<https://doi.org/10.1210/jc.2012-2863>.

Manns, F., Fernandez, V., Zipper, S., Sandadi, S., Hamaoui, M., Ho, A., Parel, J.M., 2004. Radius of curvature and asphericity of the anterior and posterior surface of human cadaver crystalline lenses. *Exp. Eye. Res.* 78, 39-51.

<https://doi.org/10.1016/j.exer.2003.09.025>.

Martinez-Enriquez, E., Sun, M., Velasco-Ocana, M., Birkenfeld, J., Pérez-Merino, P., Marcos, S., 2016. Optical Coherence Tomography Based Estimates of Crystalline Lens Volume, Equatorial Diameter, and Plane Position. *Invest. Ophthalmol. Vis. Sci.* 57, OCT600-610. <https://doi.org/10.1167/iovs.15-18933>.

Martinez-Enriquez, E., Pérez-Merino, P., Velasco-Ocana, M., Marcos, S., 2017. OCT-based full crystalline lens shape change during accommodation in vivo. *Biomed. Opt. Express.* 8, 918-933. <https://doi.org/10.1364/BOE.8.000918>.

Martinez-Enriquez, E., Pérez-Merino, P., Durán-Poveda, S., Jiménez-Alfaro, I., Marcos, S., 2018. Estimation of intraocular lens position from full crystalline lens geometry: towards a new generation of intraocular lens power calculation formulas. *Sci. Rep.* 8, 9829. <https://doi.org/10.1038/s41598-018-28272-6>.

Martinez-Enriquez, E., de Castro, A., Mohamed, A., Sravani, N.G., Ruggeri, M., Manns, F., Marcos, S., 2020a. Age-Related Changes to the Three-Dimensional Full Shape of the Isolated Human Crystalline Lens. *Invest. Ophthalmol. Vis. Sci.* 61, 11. <https://doi.org/10.1167/iovs.61.4.11>.

Martinez-Enriquez, E., de Castro, A., Marcos, S., 2020b. Eigenlenses: a new model for full crystalline lens shape representation and its applications. *Biomed. Opt. Express.* 11, 5633-5649. <https://doi.org/10.1364/BOE.397695>.

Marussich, L., Manns, F., Nankivil, D., Heilman, B.M., Yao, Y., Arrieta-Quintero, E., Ho, A., Augusteyn, R., Parel, J.M., 2015. Measurement of Crystalline Lens

Volume During Accommodation in a Lens Stretcher. *Invest. Ophthalmol. Vis. Sci* 56, 4239-4248. <https://doi.org/10.1167/iovs.15-17050>.

Mohamed, A., Sangwan, V.S., Augusteyn, R.C., 2012. Growth of the human lens in the Indian adult population: preliminary observations. *Indian. J. Ophthalmol.* 60, 511-515. <https://doi.org/10.4103/0301-4738.103775>.

Mohamed, A., Durkee, H.A., Williams, S., Manns, F., Ho, A., Parel, J.M.A., Augusteyn, R.C., 2020. Morphometric analysis of in vitro human crystalline lenses using digital shadow photogrammetry. *Exp. Eye. Res.* 108334. <https://doi.org/10.1016/j.exer.2020.108334>.

Muralidharan, G., Martínez-Enríquez, E., Birkenfeld, J., Velasco-Ocana, M., Pérez-Merino, P., Marcos, S., 2019. Morphological changes of human crystalline lens in myopia. *Biomed. Opt. Express.* 10, 6084–6095. <https://doi.org/10.1364/BOE.10.006084>.

Ortiz, S., Siedlecki, D., Pérez-Merino, P., Chia, N., de Castro, A., Szkulmowski, M., Wojtkowski, M., Marcos, S., 2011. Corneal topography from spectral optical coherence tomography (sOCT). *Biomed. Opt. Express.* 2, 3232-3247. <https://doi.org/10.1364/BOE.2.003232>.

Ortiz, S., Pérez-Merino, P., Gamba, E., de Castro, A., Marcos, S., 2012. In vivo human crystalline lens topography. *Biomed. Opt. Express.* 3, 2471-2488. <https://doi.org/10.1364/BOE.3.002471>.

Pérez-Merino, P., Velasco-Ocana, M., Martinez-Enriquez, E., Marcos, S., 2015. OCT-based crystalline lens topography in accommodating eyes. *Biomed. Opt. Express*. 6, 5039-5054. <https://doi.org/10.1364/BOE.6.005039>.

Rosales, P., Dubbelman, M., Marcos, S., van der Heijde, R., 2006. Crystalline lens radii of curvature from Purkinje and Scheimpflug imaging. *J. Vis.* 6, 1057-1067. <https://doi.org/10.1167/6.10.5>.

Rosen, A.M., Denham, D.B., Fernandez, V., Borja, D., Ho, A., Manns, F., Parel, J.M., Augusteyn, R.C., 2006. In vitro dimensions and curvatures of human lenses. *Vision. Res.* 46, 1002-1009. <https://doi.org/10.1016/j.visres.2005.10.019>.

Ruggeri, M., Williams, S., Heilman, B.M., Yao, Y., Chang, Y.C., Mohamed, A., Sravani, N.G., Durkee, H., Rowaan, C., Gonzalez, A., Ho, A., Parel, J.M., Manns, F., 2018. System for on- and off-axis volumetric OCT imaging and ray tracing aberrometry of the crystalline lens. *Biomed. Opt. Express*. 9, 3834-3851. <https://doi.org/10.1364/BOE.9.003834>.

Sommer, A., Tielsch, J.M., Katz, J., Quigley, H.A., Gottsch, J.D., Javitt, J.C., Martone, J.F., Royall, R.M., Witt, K.A., Ezrine, S., 1991. Racial differences in the cause-specific prevalence of blindness in east Baltimore. *N. Engl. J. Med.* 325, 1412-1417. <https://doi.org/10.1056/NEJM199111143252004>.

Urs, R., Manns, F., Ho, A., Borja, D., Amelinckx, A., Smith, J., Jain, R., Augusteyn, R., Parel, J.M., 2009. Shape of the isolated ex-vivo human crystalline lens. *Vision. Res.* 49, 74-83. <https://doi.org/10.1016/j.visres.2008.09.028>.

Wang, D., Amoozgar, B., Porco, T., Wang, Z., Lin, S.C., 2017. Ethnic differences in lens parameters measured by ocular biometry in a cataract surgery population. PLoS. One. 12, e0179836. <https://doi.org/10.1371/journal.pone.0179836>.

Ying, G.S., Maguire, M.G., Glynn, R., Rosner, B., 2018. Tutorial on Biostatistics: Statistical Analysis for Correlated Binary Eye Data. Ophthalmic. Epidemiol. 25, 1-12. <https://doi.org/10.1080/09286586.2017.1320413>.

Zengin, A., Prentice, A., Ward, K.A., 2015. Ethnic differences in bone health. Front. Endocrinol (Lausanne). 6, 24. <https://doi.org/10.3389/fendo.2015.00024>.

Legends for figures

Figure 1: Construction of lens full shape model. This figure shows the representative reconstructed 3-D models in European and Indian lenses of three age groups: young (A and B), middle (C and D) and old-aged (E and F). Blue represents the equatorial plane that divides the lens (black) into anterior (bottom) and posterior (top) parts. The equatorial plane (blue central region) is defined as the lens cross-section parallel to x-y plane (i.e., normal to the optical axis 'z' of the lens) exhibiting maximum area. See (Martinez-Enriquez et al., 2016) for further details.

Figure 2: Central geometric and full shape lens parameters. This figure shows the changes with age in radius of curvature (central 6 mm) of the best fitting sphere of anterior (RAL) and posterior (RPL) lens surfaces (A and B), lens thickness (LT: C), lens equatorial diameter (DIA: D), anterior lens thickness (LTa: E), posterior lens thickness (LTp: F), lens volume (VOL: G) and lens surface area (LSA: H) in both European (red) and Indian (blue) lenses. Solid straight lines represent the line of best fit. Dashed straight lines represent no age-dependence.

Table 1: Age-dependence of lens parameters in European and Indian populations.

Parameter	European lenses (n = 24)	Indian lenses (n = 69)
RAL (mm)	$5.81 + 0.085 \cdot \text{age}$; $p < 0.0001$	$7.25 + 0.072 \cdot \text{age}$; $p < 0.0001$
RPL (mm)	$5.33 + 0.010 \cdot \text{age}$; $p = 0.18$	$5.39 + 0.018 \cdot \text{age}$; $p = 0.02$
LT (mm)	$3.54 + 0.019 \cdot \text{age}$; $p < 0.0001$	$3.67 + 0.015 \cdot \text{age}$; $p < 0.0001$
LTa (mm)	$1.67 + 0.005 \cdot \text{age}$; $p = 0.04$	$1.66 + 0.004 \cdot \text{age}$; $p = 0.04$
LTP (mm)	$1.88 + 0.014 \cdot \text{age}$; $p < 0.0001$	$2.01 + 0.011 \cdot \text{age}$; $p < 0.0001$
LTa/LTP	$0.87 - 0.002 \cdot \text{age}$; $p = 0.07$	$0.81 - 0.002 \cdot \text{age}$; $p = 0.06$
DIA (mm)	$8.76 + 0.012 \cdot \text{age}$; $p = 0.04$	$8.31 + 0.023 \cdot \text{age}$; $p < 0.0001$
VOL (mm ³)	$105.28 + 1.766 \cdot \text{age}$; $p < 0.0001$	$112.59 + 1.666 \cdot \text{age}$; $p < 0.0001$
LSA (mm ²)	$153.62 + 0.879 \cdot \text{age}$; $p = 0.0001$	$140.55 + 0.921 \cdot \text{age}$; $p < 0.0001$

DIA - Lens Diameter; LSA - Lens Surface Area; LT – Lens Thickness; LTa – Anterior Lens Thickness; LTP – Posterior Lens Thickness; RAL - Radius of curvature (central 6 mm) of the best fitting sphere of Anterior Lens surface; RPL - Radius of curvature (central 6 mm) of the best fitting sphere of Posterior Lens surface; VOL - Lens Volume.

Table 2: Effect of population on lens parameters – a comparison of European and Indian populations.

Dependent variable	p-value	Population term	Age term		Interaction term: Population*age	Constant term	
		p-value	p-value	Co-efficient	p-value	p-value	Co-efficient
RAL (mm)	<0.0001	0.21	<0.001	0.085 ± 0.023	0.64	<0.001	5.82 ± 1.05
RPL (mm)	0.02	0.91	0.39	0.010 ± 0.011	0.54	<0.001	5.33 ± 0.52
LT (mm)	<0.0001	0.50	<0.001	0.019 ± 0.004	0.35	<0.001	3.54 ± 0.18
LTa (mm)	0.0001	0.94	0.07	0.005 ± 0.003	0.62	<0.001	1.67 ± 0.13
LTp (mm)	<0.0001	0.38	<0.001	0.014 ± 0.003	0.48	<0.001	1.88 ± 0.14
LTa/LTp	0.05	0.43	0.15	-0.002 ± 0.002	0.81	<0.001	0.87 ± 0.07
DIA (mm)	<0.0001	0.08	0.02	0.012 ± 0.005	0.06	<0.001	8.76 ± 0.23
VOL (mm ³)	<0.0001	0.61	<0.001	1.767 ± 0.286	0.76	<0.001	105.25 ± 12.90
LSA (mm ²)	<0.0001	0.19	<0.001	0.882 ± 0.197	0.86	<0.001	153.42 ± 8.88

DIA - Lens Diameter; LSA - Lens Surface Area; LT – Lens Thickness; LTa – Anterior Lens Thickness; LTp – Posterior Lens Thickness; RAL - Radius of curvature (central 6 mm) of the best fitting sphere of Anterior Lens surface; RPL - Radius of curvature (central 6 mm) of the best fitting sphere of Posterior Lens surface; VOL - Lens Volume.

Journal Pre-proof

EUROPEAN LENSES

INDIAN LENSES

Highlights

- Little is known about differences between specific populations in lens properties.
- First of its kind study compared 3-D biometry between Indian and European lenses.
- Accurate 3-D lens models were obtained using optical coherence tomography *in vitro*.
- There are no population differences in lens central geometry and full shape.