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A review of the doctoral thesis by Ashish Gupta, MSc:

In-vivo assessment of age-related changes in the human crystalline lens using optical imaging systems

In his thesis, Ashish Gupta examines the age-related changes in the human eye's crystalline lens by quantifying forward and backward light scattering assessed using two optical modalities: swept-source optical coherence tomography (SS-OCT) and double-pass imaging of the retinal point spread function (PSF). The motivation for this work is that age-related changes of the eye's crystalline lens is associated with various eye diseases, including presbyopia and cataract. So, quantifying the structural crystalline lens changes in time can help to treat selected eye diseases. The research was performed in the world-recognized OCT lab using state-of-the-art swept-source OCT for imaging the human eye anterior.

In the first Chapter of the thesis, the Author describes the crystalline lens's anatomy and explains the forward and backward light scattering in the human eye using the power law, parameterized by the attenuation coefficient, μ_t. Then, he takes the Reader through the scattering phase functions, crystalline lens development and its metabolism, age-related eye diseases, and explains the lens's optical discontinuity zones (OSDs). These are from the refractive index discontinuities.

Chapter 2 describes the imaging modalities used by the Author to perform the study. The major focus is on swept-source optical coherence tomography (SS-OCT). The Author provides a theoretical introduction to various OCT methods, including time-domain, spectral domain, and swept-source OCT. He explains the essential operation of the tunable laers, athe nd parameters of those lasers affecting the imaging quality and range (for instance, tuning bandwidth, instantaneous linewidth, relative intensity noise). Then, the Author discusses the lateral resolution and imagin range, and explains how those parameters are adjusted to the specific experiments of crystalline lens imaging. Subsequently, the Author explains OCT acquisition protocols and the processing pipeline. Chapter 2 also describes the double-pass system, adaptive optics visual stimulator, and contrast sensitivity function.

Afterward, Chapter 3 investigates the age-related changes in morphometry and optical density of the crystalline lens *in vivo* using SS-OCT. The results of this Chapter are already published in Biomedical Optics Express. I found this Chapter very interesting. Specifically, Tables 1 and 2 present a good summary of the age-related changes in the lens thickness and transparency assessed using various methods, including slit-lamp Scheimpflug photography, Scheimpflug camera, MRI, and OCT. This Chapter also demonstrates the post-processing methods for correcting the SS-OCT data: motion artifacts correction and refraction correction. Then, the OCT-derived morphometry and densitometry of the crystalline lens are presented, along with the description of the statistical analysis. Finally, Chapter 3 describes the results, including age-related changes in the morphometry and optical density of the lens and its components and the impact of those changes on the eye's optical quality and visual function. The results are compelling, well-presented, and clearly show age-related alterations of morphology and transparency of the human lens. Moreover, the Author confirmed that those changes are correlated with vision degradation. Hence, this Chapter demonstrates the ability of an Author to perform research projects independently.

Chapter 4 analyzes optical signal discontinuity (OSD) zones. To this end, Author uses Oxford clinical cataract classification and grading system. The author manually extracts those OSD zones from densitograms derived from the cross-sectional images from the OCT system. He uses the statistical analysis based on the multivariate linear regression model to find the relation between OSD zones and aging. The author identified the C3 OSD zone to be the most sensitive to age-related changes. Specifically, the Author demonstrated that the thickening and opaqueness of the C3 zone is the fastest among analyzed OSD zones.

In Chapter 5, Author demonstrates his results on the analysis of the images of the lenticular sutures and how they change with age. The data for this study were acquired in collaboration with the University of Murcia. The author shows developed image processing algorithms (average intensity projection, band-pass filtering, binarization, and skeletonization) and then applies them to the OCT data to extract lenticular sutures. He then used these algorithms to render sutures in 3D. The results are impressive. Subsequently, Author extracted various suture features and then analyzed their age changes. This work is the first demonstration of the age-related human suture organization.

Finally, Chapter 6 summarizes the thesis, identifies the limitations of the presented study, and draws plans.

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I found the thesis to be well-written and scientifically sound. However, I found several issues that, after addressing would make the thesis even stronger:

1. A discussion on OCT systems could be supplemented by line- and full-field approaches and adaptive optics OCT. Notably, the latter provides cellular-level resolution over an extended depth of field. As the Author indicated, the present study uses low NA objectives to attain the depth of field at the cost of reduced transverse resolution. Hence, line- and full-field and AO-OCT can inspire further study continuation.
2. Figure 14 is significant to explain the selection of imaging parameters. However, the figure could be improved by overlaying axial and lateral PSFs over the sketch of the actual crystalline lens and keeping the proper aspect ratio between the sample and PSFs (a good example can be found in the Theory of OCT by Izatt and Choma).
3. Equations (2.2) and (2.3) should also be rewritten to explicitly use numerical aperture. This will help the Reader to understand why low NA objectives were used (to attain the depth of field).
4. The definition of N_s (the number of samples per sweep) is not correctly defined below Eq. (2.4). However, Author provides the correct definition under the section "k-space linearization."
5. The expression for the raw SS-OCT signal [Eq. (2.8)] indicates that SS-OCT measures the depth-integrated signal (the right-hand side of this equation contains an integral over z). This is not true as the signal is depth-resolved (as adequately explained in Fig. 11). Moreover, the Author cites Ref. 136, after Eq. (2.8). However, the pointed reference does not contain such an equation.
6. For the above reason, the term "raw OCT data" used in Chapter 4 can be confusing, where Author explains the generation of densitograms, I would understand the "raw OCT data" to mean the signal after k-linearization, FFT, and axial motion/dispersion correction (the output of the block diagram shown in Figure 16). However, "raw data" is also shown as the input of the block diagram in Fig. 16. This should be clarified to avoid any confusion.
7. A caption of Figure 17 could briefly describe symbols used in a diagram. Also, it would be nice to describe the purpose of an aperture A2 when explaining the double-pass system.
8. The plots in the right part of Figure 18 should contain axes labels. This would help to relate those plots to the theoretical equations introduced earlier in Chapter 1.

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9. A caption of Figure 19 should also be expanded. Moreover, using 1-2 paragraphs Author could describe a wavefront sensor and deformable mirror used in the adaptive optics visual stimulator would be good.
10. Concerning refraction correction, Chapter 4 could reference papers on computational aberration correction.
11. The reference list is not well-prepared. Many citations are missing a year—for example, 136, 137, 138, 143.

In summary, Ashish Gupta demonstrated his ability to perform scientific work independently. He proposed an original solution to a scientific problem: quantifying the age-related changes of the crystalline lens by analyzing data generated by advanced noninvasive optical imaging modalities. He conducted experiments with human subjects, developed and applied advanced image processing algorithms, employed statistical analysis of the data, provided explicit confirmation of the research hypothesis, and accurately listed limitations and future directions of the study.

Parts of the thesis (Chapter 3) were already published in scientific journals (Biomedical Optics Express) and presented by the author at international scientific conferences as oral presentations.

The submitted dissertation meets the requirements in Articles 186 and 187 of the Law on Higher Education and Science of 20 July 2018 (Journal of Laws of 2021, item 478, as amended). Specifically, as listed above, the Author confirmed his general theoretical knowledge in physics (biophysics). More specifically, Author understands the light scattering in biological tissue, the foundations of optical coherence tomography, low-coherence interferometry, the operation of rapidly tunable lasers, double-pass imaging of retinal point spread function, and a complex crystalline lens structure.

To conclude, I recommend the thesis for public defense.

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