Prof. dr hab. inż. Adam Liebert

Nalecz Institute of Biocybernetics and

Biomedical Engineering

Polish Academy of Sciences

Assessment of the dissertation by Ashish Gupta entitled: “In-vivo assessment of age-related changes in human crystalline lens using optical imaging systems” prepared for the Department of Biophysics, Nicolaus Copernicus University in Toruń

The dissertation addresses technological problems related to the imaging of the structure and age-related physiology of the human crystalline lens. It presents results of lens imaging and provides a thorough statistical analysis of factors that describe the lens's structure. This research, based on a cohort of healthy individuals, provides data important for understanding of the human eye anatomy and physiology.

The dissertation is well written and properly organized, in most parts concise but detailed enough. It comprises of two introductory chapters and three chapters containing description of the results of the research. The final chapter provides summary of the study. In an introduction the Candidate presented very clearly motivation of the study, properly formulated the hypothesis and goal of the study. Two aims of the research are related to the visualization of the age-dependent morphology and transparency of the human crystalline lens and the three-dimensional analysis of local optical inhomogeneities of the lens.

Chapter 1 provides a general overview of the anatomy and physiology of the human crystalline lens and presents processes which lead to degradation of the lens and cause serious eye diseases. Chapter 2 presents the eye imaging systems used in the research. Particularly, the Candidate described here the swept-source optical coherence tomography (SS-OCT) system - a very advanced optoelectronic technology which allows for acquisition of images of high resolution and contrast.

Three subsequent chapters presented three aspects of research concerning the aging process of the human crystalline lens.

1. Chapter 3 concentrates on evaluating the relationship between age and the transparency and shape of the human crystalline lens. A methodology of refraction correction was presented and several parameters describing morphology of the crystalline lens were proposed. For three of them (thickness, radius of curvature and optical density) an analysis of age-dependence was provided. Finally, the parameters extracted from OCT were correlated with the optical quality of the eye and quality of vision which were assessed with complementary techniques. These studies lead to a conclusion that with aging the lens becomes thicker and more convex and that the cortex is mostly responsible for the increase of the thickness of the lens.

2. Chapter 4 describes the study focused on assessment of age-dependent development of non-homogeneity of the human crystalline lens. The data gathered in a group of healthy volunteers was analyzed in context of so-called optical signal discontinuity (OSD) zones. Thickness of these zones and optical density in these areas were obtained and correlated with age of the subjects. A multivariate linear regression model was applied in order to determine the factors which influence

mostly the thickness of the lens and its optical density. This analysis suggests that the thickness of the lens is mostly influenced by the thickness of the nucleus and cortical layers, whereas the optical density is mostly dependent on the optical density of the nucleus and bright zone C3. This study demonstrated utility of SS-OCT technique in imaging of OSDs and presented that the most significant age-dependent changes in structure of the crystalline lens appear in C3 zone.

3. Chapter 5 is focused on analysis of a specific suture structure of the crystalline lens that is related to the organization of its fibers. The Candidate developed a method for extraction of the suture structure based on projection of the OCT volumetric data, its filtration and skeletonization. This automatic technique was validated by comparison of the number of extracted sutures branches with results of manual analysis. Imaging of the sutures showed interesting new feature – hypo-reflective structures within the sutures. The Candidate proposed several parameters describing the structure of sutures and assessed their dependence on age. The study is scientifically interesting and presents utility of SS-OCT in in-vivo assessment of the suture structure in the human crystalline lens.

In Chapter 6, the Candidate provided a summary of the dissertation, discussed the study's limitations, and outlined potential future research. This chapter is grounded in the study's findings, refers properly to the hypothesis and is articulated with an appropriate level of skepticism. The literature review is thorough, referencing 224 sources. However, only 7 of these sources were published after 2020.

The Ph.D. Candidate Ashish Gupta is an active researcher. He has authored two articles, with the primary authorship, in "Biomedical Optics Express," a journal of solid reputation within the biomedical optics community. Furthermore, the Candidate has disseminated his findings at multiple international conferences and has exhibited ability in securing financial support for his research activities.

The Candidate has exhibited expertise in the anatomy and physiology of the human eye, enabling him to define a very interesting line of research. Additionally, he has demonstrated high skills in the design of optical instrumentation, the in-vivo validation of that instrumentation, the formulation of data acquisition protocols, the development of biomedical signal and image analysis methodologies, and the statistical evaluation of the gathered data.

The Candidate carried out studies which may open new directions of research in human eye ball anatomy and physiology. Thus, the scientific impact of these studies is significant. Moreover, clinical ophthalmology could benefit from the techniques, which may provide tracking the progression and treatment of eye diseases. Specifically, the findings could influence the creation of advanced surgical methods for treating cataracts and restoring accommodation. Therefore, the research conducted by the Candidate have substantial societal relevance.

I have several critical remarks to the thesis:

General comments:

1. The dissertation presents a number of biomarkers which are of potential use in assessment of the human crystalline lens. The methodology presentation would have benefited from analysis of uncertainties of the estimation of these quantities.

2. The depiction of OSD zones in Figs. 10 and 23 is unclear. The figures lack units, the placement of arrows and zones within the densitogram is not clear, and the use of yellow color further complicates understanding of the typical OSD zones positioning in the lens.

3. While the Candidate acknowledged the limited number of subjects as a constraint of the study, it would be beneficial to determine the sample size necessary to draw statistically robust conclusions.

4. The validation of the automated suture extraction technique detailed in subchapter 5.4.2 relies on a comparison with a manual method. Typically, operator-dependent techniques can be influenced by the subjective judgment of a human expert. Implementing a multi-operator approach might help mitigate the impact of this human bias.

Minor remarks

5. Figure 10 is oriented incorrectly, making it challenging to interpret.

6. In chapter 2.2.2. when presenting the parameters of the wavelength swept-source system, it would be beneficial to include their typical values along with the respective units. The explanation of the wavelength swept laser on page 29 and as depicted in Fig.12 lacks clarity.

7. Figs. 14 and 15 need more detailed description.

8. Fig. 18 needs description of axes and units.

9. Page 48. The claim regarding the limited sensitivity and poor resolution of various eye imaging techniques requires a quantitative foundation. If possible, the parameters provided in Table 1 should be presented with their uncertainty.

10. Pages 57, 58. The subchapter 3.4.3 is very brief – the text repeats only the data provided in the table. Displaying the correlations from Table 5 graphically would enhance the clarity and informativeness of the presentation.

11. Fig. 28.c. is presented without coordinates, making it challenging to interpret. Fig. 29b lacks units.

12. Descriptions of some of the references are incomplete (positions 81, 82, 136, 137, 138, 158).

13. The dissertation would have been enhanced with the addition of a list detailing the acronyms and variables utilized.

Despite these remarks, I maintain a high regard for the quality of the doctoral dissertation presented. The work offers a novel approach to the significant scientific challenge of assessing the crystalline lens's structure and physiology of aging. The dissertation also presents the Candidate's deep understanding of medical physics, especially related to the ocular system imaging, and their capability to conduct independent research in this field. Based on these merits, I conclude that the presented dissertation meets the formal requirements for a Ph.D. thesis and I recommend admission of the Candidate to the subsequent steps of the procedures, including the public defense.