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**Review of Ashish Gupta's doctoral dissertation**
**„In-vivo assessment of age-related changes in the human crystalline lens using optical imaging systems"**

The doctoral dissertation of Ashish Gupta presented to me for evaluation was prepared under the supervision of Ireneusz Grulkowski, PhD, DSc, from the Department of Biophysics of the Nicolaus Copernicus University in Toruń. The dissertation is carried out in the field of Physical Sciences.

## The purpose of the work and the legitimacy of taking up the topic

The dissertation concerns research and interpretation of optical parameters of the eye lens. It is devoted mainly to the quantitative and qualitative determination of changes in the physical and structural properties of the eye lens with age and their impact on the quality of vision. This topic is important from the point of view of predicting age-related changes in the ophthalmic lens, and the results of the work will certainly contribute to the understanding of eye development processes related to aging, such as cataracts and presbyopia.

The author put forward the following hypothesis in the dissertation: **"In-vivo optical imaging enables objective measurement of age-related changes in the crystalline lens, which in turn affects vision"** and the related goal of the work **"The main goal of the study was to demonstrate and quantify characteristic age-related alterations in the crystalline lens by the measurement of forward- and back-scattering in healthy subjects and to associate those changes with vision degradation"**. In the introductory chapter, **Motivation, hypothesis and aims**, he comprehensively presented and justified the legitimacy of taking up the topic and the research measures used to justify the hypothesis.

## Dissertation structure

The doctoral dissertation submitted for review, consisting of about one hundred pages and written in English, is divided into six chapters. The chapter sizes are as follows: chapter 1 **Introduction** has 22 pages, chapter 2 **Optical imaging systems** - 24 pages, chapter 3 **Age-related changes...** - 16 pages, chapter 4 **Changes of...** - 12 pages, chapter 5 **In-vivo imaging...** - 17 pages,



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chapter 6 **Summary, limitations...** - 6 pages. Chapters 1 and 2 (a total of 46 pages) present the phenomena that are the subject of the dissertation and the tools that the author used to achieve the objectives of the work, while chapters 3-6 (51 pages) - the results of the author's research (chapters 3-5) with conclusions ( chapter 6).

The structure of the work is correct and clear. Navigating the text is easy, one can return to the explanation suggested by the author at any time, although sometimes it is difficult to find definitions of the basic concepts and terms used by him. It should be noted that each of the experimental chapters 3-5 is preceded by a very informative and explanatory introduction, **Motivation and aim of the study**. Doubts, however, may be raised by the too extensive introductory part (chapters 1-2), which has about the same number of pages as the part related to the author's results (chapters 3-6). In my opinion, this is a bit too much, especially the second chapter, which is too numerous, where almost 16 pages are devoted to the OCT measurement system, and only 7 pages to the other two research tools (the OQAS double pass system and the VAO system). I am aware that the presented measurement systems are the authorship (in the sense of compilation, testing, calibration, etc.) of the scientific team in which the author worked, which in turn is a great merit of this dissertation, however, it was possible to reduce the amount of unnecessary, in my opinion, information , or move them to the chapters on experimental methods.

**Substantive remarks**

The dissertation analyzes the results of the examination of the physical properties of the eye lens depending on the age of the patients. These studies were divided into three categories:

1. Shape and transparency of the human crystalline lens (Chapter 3).
2. Non-homogeneity of the crystalline lens (Chapter 4).
3. Architecture of lenticular sutures (Chapter 5).

In Chapter 3, it was shown that the geometric parameters describing the eye lens (thickness and curvature of various elements of the eye lens structure) change with age, but also the transmission parameters, as well as those describing the quality of vision (Table 4 is key here). Moreover, significant correlations were found (Table 5) between the results of the studies conducted with the use of three different tools (OCT, OQAS, VAO) described in Chapter 2. I consider this a very valuable result of this work.

Chapter 4 presents the results of studies on the homogeneity of the eye lens with the age of the subjects, performed using OCT. The MLR (Multivariate Linear Regression) statistical model was used. It has been shown that the features of almost all zones of the ophthalmic lens change with age (Table 7),

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with the strongest changes occurring in the C3 zone (geometry) and in the nucleus and C2-C4 zones (transparency). These results provide important information about the structure of age-related changes in the ophthalmic lens.

In Chapter 5, using 3D imaging with OCT, the features of sutures present in the ophthalmic lens were analyzed. A qualitative description of lens suture patterns was proposed and selected parameters potentially variable with the patient's age were analyzed. The advantage of the approach used by the Author is, as he himself writes, and I quote the Author: "A comprehensive in-vivo analysis of human lens suture organization in the aging eye has not been reported before although anterior segment swept-source OCT was widely used for crystalline lens imaging". Nothing more nothing less.

It is somewhat unsatisfactory that the chapters in which the results of the work are presented are, in a sense, independent of each other. There is no separate chapter linking these three categories of phenomena, information about the interconnections of phenomena is scattered throughout the texts of chapters 3-5, or, for example, there is no information whether there is a relationship between the quality of vision and lenticular sutures architecture. However, this does not significantly diminish the value of the experimental part of the dissertation.

The humility with which the author approaches his work deserves to be emphasized. It manifests itself in a separate subchapter 6.2 **Limitations**, which lists six reasons, the inclusion of which in further work related to this topic would allow to obtain more complete information about the aging phenomena occurring in the eye lens. And what is satisfying: In chapter 6.3 **Future scope**, the author presents further directions of development of this research.

**Editorial and nomenclature notes**

The work is written in the correct language, but there are stylistic errors, which, however, do not affect the comfort of reading it. Unfortunately, it is not very well edited. Some figures (Fig. 12, 14, 15, 17, 19) are definitely too large for the substantive content. On the other hand, most of the graphs are too small for their substantive content (eg Fig. 22). Others of the drawings are poorly made, especially illustrative Figure 5, where it is not known how the image is formed in the case of the emmetropic non-accommodating eye. In addition, with respect to part a of this Figure (Fig. 22a), light propagation in wave terms (pupil diffraction) and geometric terms (image formation) are confused. Figure 17 of



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the optical schematic of the OQAS system begs to be supplemented with the Badal system (not explained in the text, by the way). The VAO (adaptive optics visual simulator) system is described very briefly, without naming the elements present in it (what is the HS wavefront sensor? Hartman-Schack sensor? what is the DM? deformable mirror?) In addition, with regard to the diagram shown in Fig. 19, the upper mirror of the BS is incorrectly oriented, so that the beam from the test object would never have a chance to fall on the subject's eye. The definitions of some of the key parameters for the dissertation are not sufficiently exposed in the dissertation, for example, the AULCSF parameter is defined in the caption under the figure (Fig.20; it appears there for the first time), and the abbreviations defining the parameters come from words that do not start with capital letters (I quote "The area under logarithmic contrast sensitivity function (AULCSF)"). In Table 3, each parameter has the same number N of tested eyes. What for, if it is in the caption under the drawing? The same is true of Table 6. In addition, the Author states that the optical density is in arbitrary units, so what is the $10^3$ multiplier for? To sum up: understanding the content of the dissertation is a bit problematic, you have to look for definitions, meanings, units, parameters, etc. in too many places.

**Summary**

The above critical remarks, however, do not change my positive opinion of the dissertation presented to me for review. In my opinion Mr Ashish Gupta correctly implemented the assumed main research goals, and thus confirmed the hypotheses. I highly appreciate the achievements in the field of research on the optical properties of the eye lens. On this basis, I conclude that the doctoral thesis of Ashish Gupta, MSc, entitled "In-vivo assessment of age-related changes in the human crystalline lens using optical imaging systems" meets all the requirements for doctoral dissertations in accordance with the "Act on Academic Degrees and Academic Titles and on degrees and title in the field of art" of March 14, 2003 and the "Law on Higher Education" of July 20, 2018. Therefore, I am applying for admission of Mr. Ashish Gupta to further stages of the doctoral procedure.

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