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Review of the doctoral dissertation by Maria Miażdżyk, MSc

The doctoral dissertation entitled *"Estimation of corneal light scattering parameters, which are independent of external factors and anterior eye geometry"* submitted by Ms. Maria Miażdżyk, MSc to the Faculty of Fundamental Problems of Technology at Wrocław University of Science and Technology addresses an important and timely problem in contemporary ophthalmic imaging and vision science. The work was carried out under the supervision of Prof. Daoud Robert Iskander and Dr. Alejandra Consejo Vaquero and focuses on the development of objective methods for assessing corneal light scattering and transparency using non-invasive optical imaging techniques. Given the growing clinical importance of quantitative corneal assessment for the diagnosis, monitoring, and treatment of ocular diseases, the topic of the dissertation is both scientifically relevant and of significant practical value.

The scientific achievements forming the basis of the dissertation are documented in three peer-reviewed journal articles published in *Biomedical Optics Express* between 2022 and 2026, all of which are directly related to the research topics addressed in the thesis. In addition, the doctoral candidate actively disseminated her results through presentations at five national and international scientific conferences and successfully obtained a PRELUDIUM grant from the National Science Centre. The dissertation has the form of a comprehensive monography written in English. The thesis is carefully structured and consists of an Abstract, Polish Summary, Scientific Output, Acknowledgements, List of Figures, List of Tables, Acronyms, List of Symbols, Motivation and Research Hypothesis, Scope of the Thesis, Author's Contribution, seven substantive research chapters, and a bibliography. The manuscript is logically organized, progressing from the formulation of the research problem and methodological foundations through experimental investigations and numerical modelling to a coherent presentation of conclusions and future research directions.

The main objective of the dissertation is to develop estimators of corneal light scattering parameters that are robust against confounding factors arising from measurement conditions, anterior eye geometry, and the multilayered structure of the cornea. To verify the research hypothesis that eliminating the influence of such confounding factors would improve the diagnostic value of corneal scattering metrics, the candidate conducted quite broad investigation comprising three interconnected research projects. These projects addressed: (i) the identification and compensation of biometric factors affecting Scheimpflug-based corneal densitometry, (ii) the influence of the corneal epithelium on OCT - derived stromal scattering parameters, and (iii) the development of OCT corneal phantom and a novel methodology for estimating corneal optical properties from OCT speckle statistics.

The assessment of corneal light scattering has become an important area of research in ophthalmology and vision science because corneal transparency is one of the key determinants of visual performance. In healthy, young individuals, the cornea is nearly perfectly transparent; however, microscopic irregularities within the corneal tissue - including alterations in the organization of collagen fibrils - cause slight light scattering. This effect can be amplified by pathological changes that result in alterations in keratocyte density, extracellular matrix remodeling, edema, inflammation, wound healing processes, and age-related structural modifications. Consequently, quantitative evaluation of corneal scattering provides information that extends beyond simple morphological measurements and may serve as a sensitive biomarker of tissue health, disease progression, and therapeutic outcomes. Modern in vivo imaging techniques have enabled non-invasive investigation of these phenomena with unprecedented detail. Scanning Laser Ophthalmoscopy (SLO) and Scheimpflug imaging have been widely used to assess corneal backscatter and densitometry, providing clinically accessible measures of transparency that have been applied in studies of keratoconus, corneal dystrophies, refractive surgery outcomes, corneal transplantation, and aging. However, these techniques typically provide integrated measures of scattering and are often susceptible to imaging conditions, ocular geometry, and instrument-specific factors. The introduction of Optical Coherence Tomography (OCT) has significantly expanded the possibilities for studying corneal scattering *in vivo*. Beyond its established role as a high-resolution tomographic imaging modality, OCT provides access to the statistical properties of the backscattered optical field through speckle analysis. Numerous studies have demonstrated that OCT speckle contains information related to tissue microstructure and optical properties, potentially allowing the estimation of parameters such as the scattering coefficient, anisotropy factor, and structural heterogeneity of the tissue. Furthermore, OCT enables layer-specific analysis of the cornea, making it possible to distinguish contributions from the epithelium, Bowman's layer, stroma, and endothelium, thereby overcoming some limitations of conventional densitometry. Despite substantial progress in this field, the interpretation of scattering-related metrics remains challenging due to the influence of confounding factors such as imaging geometry, signal attenuation, multiple scattering, speckle statistics, and the multilayered nature of the cornea. Therefore, the development of objective, robust, and biologically meaningful estimators of corneal light scattering remains an active and highly relevant research topic, with significant implications for both clinical diagnostics and our understanding of corneal physiology and pathology.

The introductory chapter begins with a clear explanation of light scattering in biological tissues, defining the fundamental optical parameters such as the scattering coefficient, absorption coefficient, anisotropy factor, and reduced scattering coefficient. Ms Miażdżyk then places these concepts in the specific context of the cornea, emphasizing that its high transparency results from the highly ordered, short-range arrangement of stromal collagen fibrils, the specific distribution of keratocytes, and the optical matching of corneal components. At the same time, the chapter correctly indicates that even subtle structural disturbances caused by aging, edema, keratoconus, dystrophies, inflammation, contact lens wear, or wound healing may increase backscattering and thus provide diagnostically useful information. The introduction also presents the main in vivo imaging modalities relevant to the dissertation, namely Scheimpflug imaging and optical coherence tomography. Scheimpflug-based densitometry is described as a clinically accessible method for assessing corneal optical density, although one that remains dependent on image intensity, geometry, and device-specific factors. OCT is introduced not only as a high-resolution tomographic technique but also as a coherent imaging modality in which speckle, traditionally treated as noise, may carry information about sub-resolution tissue microstructure. By discussing OCT parameters, speckle statistics, and their relationship to stromal microstructure, the author tried to justify the central idea of the dissertation: that quantitative analysis of corneal backscatter and OCT speckle can provide objective information about corneal optical properties and tissue health, provided that confounding factors are properly identified and compensated. Although, the introductory chapter provides a well-structured and comprehensive overview of the current state of knowledge necessary to understand the scientific context of the work I would like to rise two points:

1. The author introduces the concept of *"coherence volume"* as a useful framework for relating OCT speckle statistics to the underlying corneal microstructure. While this concept is known in the broader fields of optical coherence theory and statistical optics, it is less commonly employed in mainstream OCT literature, where related notions such

as three-dimensional resolution, imaging voxel, or coherent detection volume are more frequently used. In my opinion, the use of the term *coherence volume* should be accompanied by a rigorous discussion of both temporal and spatial coherence properties of the illuminating field. However, the OCT systems utilized in this work are based primarily on partial temporal coherence, while spatial coherence is not explicitly addressed. Consequently, the introduction of the term *coherence volume* may be somewhat misleading, as it suggests a more complete coherence characterization of the optical field than is actually considered in the subsequent analysis. Furthermore, I was unable to identify any direct use of the coherence volume concept in the presentation or interpretation of the experimental results. Its introduction therefore appears to have limited relevance for understanding the investigations reported in the dissertation. At the same time, the introductory chapter lacks methodological background on several statistical models that play a central role in the subsequent analyses, including the Weibull, Rician, Gamma, and related distributions. A discussion of these statistical frameworks would arguably be more beneficial for the reader and would better support the interpretation of the results presented in the later chapters.

2. The estimation of the number of collagen fibrils within the OCT coherence volume provides an interesting order-of-magnitude assessment of the scattering environment. However, it should be noted that collagen fibrils in the corneal stroma cannot be regarded as independent scatterers. Their spatial arrangement exhibits a significant degree of short-range order, which is known to play a fundamental role in maintaining corneal transparency through interference effects. Consequently, the estimated number of fibrils should be interpreted only as a geometrical approximation rather than the actual number of statistically independent scattering centers contributing to the OCT speckle pattern.

The first research task, described in Chapter 2, focuses on the identification and compensation of biometric factors influencing Scheimpflug-based corneal densitometry. In this part of the work, the author addresses a significant and often underestimated challenge associated with corneal densitometry, namely the susceptibility of measured scattering parameters to variations in anterior eye geometry and imaging conditions, which may compromise their clinical interpretation. Using Scheimpflug images acquired from cohorts of young and older subjects, the Author demonstrated that commonly used densitometric metrics are significantly affected by factors such as anterior chamber depth, pupil size, and corneal thickness, which complicates their clinical interpretation. To overcome this problem, novel image normalization and histogram-equalization approaches were developed and validated. Ms Miażdżyk claims that the proposed methods substantially reduced or eliminated correlations between densitometry parameters and confounding biometric variables, leading to more robust and objective measures of corneal transparency. This work represents a valuable methodological contribution to the field of corneal imaging and provides a solid foundation for the subsequent investigations presented in the dissertation. This section is based on the results and description of the method used in the publication by Miażdżyk, M. et al., *Biomedical Optics Express* 13, 6258–6272 (2022) and, as such, does not require further review on my part; however, I would like to ask two methodological questions:

1. Is there an independent reference measure of light scattering by the cornea that could serve as a universal reference point for densitometric parameters and justify the correlation between changes in these parameters and the condition of the cornea?
2. Another question regarding this section concerns the extent of variability in the eyes of ocular pathologies compared to healthy control groups with variable age, as measured in the experiments presented.

The second research task, described in Chapters 3 and 4, addresses an important methodological issue associated with OCT-based assessment of corneal scattering. The Candidate investigates whether the speckle pattern originating from the corneal epithelium influences the estimation of stromal scattering properties, an assumption that is frequently neglected in OCT densitometry studies. Using ex vivo porcine corneas imaged before and immediately after epithelial removal, the Author quantitatively evaluated changes in several statistical descriptors of stromal OCT speckle, including Gamma distribution parameters, mean signal intensity, and contrast ratio. The study convincingly demonstrates that the presence of the epithelial layer significantly alters the measured stromal speckle statistics and may introduce errors of up to approximately 20% in the estimation of stromal scattering parameters, with the magnitude of the effect depending on imaging depth. An interesting extension of this work is presented in Chapter 4, where the Author conducted a pilot in vivo study investigating the influence of hypoxia-induced corneal swelling on OCT speckle statistics. By imaging two human subjects before and after controlled corneal hypoxia induced by contact lens wear, the Author demonstrated measurable changes in both epithelial and stromal densitometric parameters, with the strongest effects observed in the epithelial layer and posterior stroma. Although the study is limited by its exploratory nature and very small sample size, it provides preliminary evidence that OCT speckle statistics are sensitive to physiological and microstructural changes occurring in the living cornea. Importantly, the results further support the central hypothesis of the dissertation that scattering-related parameters derived from OCT are influenced by multiple interacting tissue layers and therefore require careful interpretation when used as biomarkers of corneal structure and health. These findings highlight the importance of considering the multilayered structure of the cornea when interpreting OCT-based densitometric measurements and provide a valuable contribution toward the development of more reliable and physiologically meaningful corneal scattering biomarkers. Again, this work is based on the results and description of the method used in the publication by Miażdżyk, M. et al Biomedical Optics Express 14, 3871–3880 (2023). This part of Ms. Miażdzyk's doctoral project is very interesting and sheds light on a completely overlooked aspect of the evolution of speckle fields in systems with spatially modulated phase induced by the object itself. In general, speckle fields in such systems become unquantifiable because information is lacking regarding boundary conditions - given uncontrolled changes in the refractive index at the boundaries, variations in aberrations introduced by the object, signal attenuation, and beam focusing conditions. Although an experimental design based on imaging the same cornea before and after removal of the epithelium is elegant and provides valuable information on the influence of surface layers on stroma OCT densitometry, interpreting the results is not entirely straightforward. This raises the following questions:

1. What are the temporal changes in biological structure following enucleation and epithelial removal?
2. Was corneal hydration taken into account?
3. Does the removal of the epithelium accelerate osmotic changes that could affect the optical properties of the cornea?

The biological interpretation of the changes observed in the gamma distribution parameters remains largely phenomenological and - in future - it would benefit from verification based on independent structural or optical measurements.

The third research project, presented in Chapters 5 and 6 and it addresses one of the most ambitious objectives of the dissertation: the estimation of intrinsic optical properties of corneal tissue directly from OCT speckle statistics. To achieve this goal, the Candidate developed a model of light propagation through of the cornea by combining Monte Carlo simulations of photon transport with a model of OCT signal formation adapted to the optical characteristics of corneal tissue. Based on this framework, a novel method was proposed for estimating the scattering coefficient (μs) and anisotropy factor (g) through statistical matching between simulated and experimental OCT data. The study demonstrates that OCT speckle statistics can be used not only as empirical descriptors of tissue appearance but also as a source of quantitative information about tissue optical properties. The estimated values obtained for porcine corneal stroma were found to be consistent with the limited data available in the literature. This work represents a significant methodological contribution toward the development of quantitative OCT-based tissue characterization and provides an original framework for linking OCT image statistics with the underlying optical and microstructural properties of biological tissue.

I have some reservations about referring to a partially phenomenological mathematical model as a "digital phantom" - it is misleading for the experimentalist. However, since the term is defined in the paper, it can be considered the technical name for this particular model. Furthermore, I would like to know:

1. How unique is the proposed solution? Could different combinations of μ_s and g produce similar OCT speckle statistics and therefore lead to non-unique parameter estimates?
2. In the absence of independent reference measurements of the optical parameters for individual corneas, how can the accuracy of the estimated optical parameters provided by the model be assessed?
3. To what extent does multiple scattering contribute to the observed speckle statistics and how is it accounted for in the proposed model?
4. The model works well without the additional light modulation introduced by the epithelial layer - isn't this too much of a limitation of the method?

Furthermore, I believe that Chapter 5 could be presented as an appendix to improve the readability of the dissertation.

In conclusion, I find that Ms. Maria Miażdżyk's doctoral dissertation **fully satisfies the requirements for doctoral theses set forth in the Act on Higher Education and Science (Article 187 of the Act of 20 July 2018, Journal of Laws of 2023, item 742, as amended), and I therefore recommend that she be admitted to the public defense of her dissertation.** I consider the thesis to be above average in quality due to its thorough and independent analysis of the complex problem of the influence of corneal stromal structure on light propagation, which constitutes a valuable contribution to the development of reliable and physiologically meaningful biomarkers. Subject to the quality of the presentation and responses to questions discussed during the dissertation defense, **I conditionally recommend that the dissertation be awarded a distinction.**

Respectfully submitted,

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