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PhD Thesis entitled
"Anatomical and structural changes of anterior eye with age"
by Danilo Andrade de Jesus

The anterior segment of the human eye forms a basic optical system directly impacting our vision. Traumatic or pathological changes in the anterior segment may lead to deterioration of vision and, in some cases to blindness. Such changes are especially severely affecting the vison in aging process. Optical instrumentation and specific measurement methods for imaging of the anterior segment have been developing since eighteenth century, helping in modern ophthalmology either for diagnosis or for treatment of ocular diseases.

Present-day ophthalmic clinics widely use devices such as slit lamp microscopes, gonioscopes, keratometers, and anterior eye topographers. Additionally, imaging systems, like rotating Scheimpflug, scanning slit topography, ultrasound biomicroscopy or Optical Coherence Tomography are commonly used to obtain quantitative information on the corneal thickness and topography. This "arsenal" of imaging methods is capable to provide qualitative and quantitative information about the morphology and sometimes function of the anterior segment of the human eye, which may be especially useful for disease diagnosis, precise localization of lesions, and planning of medical and surgical treatments (especially refractive surgery). However, comprehensive understating of eye morphology and its changes related to aging is still limited.

Presented thesis reports on development of new data analysis methods based on information provided by well-known and broadly used imaging techniques including Eye Surface Profiler, Optical Coherence Tomography, Low Coherence Interferometry and Applanation Tonometry applied to quantify age dependent structural changes occurring in anterior eye and to characterize objectively various parameters like: scleral radius, demarcation of corneo-scleral limbus and intraocular pressure.

Scientific problems in this thesis are dominated by engineering questions more than that of physical, phenomenological or theoretical nature. Yet the thesis fulfills the condition of scientific originality and significance. The candidate introduces new methods based on physical processes, which require deep understanding and mathematical modeling of light interacting with human cells and biophysical / anatomical properties of the human anterior segment. The project is also interdisciplinary in nature combining optical engineering, informatics and vision sciences.

The thesis consists of seven chapters including two introductory parts and five chapters dedicated to description of research tasks realized during the PhD project. There are additionally: abstract, list of papers, references, list of figures and list of acronyms. The technical quality of the thesis does not rise any objection – layout, figures and tables were made with high attention and are convenient to read. Surpassingly each chapter has own quotes sometimes not necessarily very relevant to the merit of the chapter.

Chapter_1 "Introduction" presents motivation and main objectives of the thesis. I found this part of the thesis unsatisfactory since it does not define clearly the inspiration of the work and does not present one consistent hypothesis of the PhD project. Instead of creating unnecessary complexity of the thesis organization presented in paragraph 1.4 it would be better to enclose all activities presented in the PhD project as one coherent subject with one leading scientific question that should be defended. Current version of the "Introduction" may generate an impression that candidate's activities were too much scattered. The "Introduction" includes also not relevant information about European Network Aging Eye project. Taking part in the European consortium cannot be treated as a main objective for conducting research. This information should be moved to "Preface" section.

Chapter_2 presents very brief overview of the general knowledge about anatomy and biomechanics of the anterior segment of the human eye put in the context of the aging process. The candidate chooses those aspects of the knowledge that are critical for understanding research described in the following chapters of the thesis.

Chapter_3 describes a sub-project (or scientific task), which is closely related to the main subject of the thesis. The scientific problem brought here is to check whether the speckle signal existing in corneal OCT images can provide useful information about the structural changes related to aging. The idea is original and it is feasible since the optical inhomogeneity of the corneal structure is relatively small (at least when compared to other tissue present in human body). Therefore, speckle signals look quite similar for numerous OCT measurements performed on different corneas. That's why there is a chance that the individual variability in a given indicator (statistical parameters describing the speckle field) could be significantly small across the population imaged with the same OCT instrument. At the other hand speckles should be extremely sensitive to changes of the internal structure of corneal stroma, which scattering properties depend on how collagen fibers are organized. Age related changes of this organization could then be considered as a factor influencing measured OCT signals and could be validated based on OCT images. The first step in described research work was to

identify physical parameters of OCT corneal signals ensuring low individual variability for groups of healthy volunteers of the same age. The author proposed using various models describing speckle field formation including Generalized Gamma, Weinbull, Nakagami, Rayleigh, K-distribution, Rician and Lognormal distribution. In order to choose the optimal statistical distribution describing the speckle field the author used one randomly selected set of OCT data and performed speckle analysis with known statistical selection criteria. The optimal statistical distribution of Generalized Gamma (GG) distribution was indicated as the best model for studying OCT Corneal signal variation as a function of age. Actually this choice was made based on the smallest number of parameters that is required even if the repeatability of GG approach was worse than other techniques. Unfortunately, this part is described in very laconic manner. It is very hard to follow all mathematical procedures undertook by the candidate. There are missing details of fitting procedures, for example it is not known how many and what kind of parameters were used for fitting with different models. What is physical interpretation of fitting parameters? Even in case of GG the fitting parameters are given only in the "Discussion" section introducing confusion to the reader. What are a physical and numerical limitations of fitting procedures? What accuracy and repeatability was achieved for each procedure versus variable region of interest (ROI)? Are the speckles fully evolved in the experimental system used by author? What is the influence of SNR and defocus of the imaging system on the speckle modeling? Also the speckle field in OCT images may be non-isotropic in XY and XZ planes due to different mechanisms responsible for the speckle field formation – XY depends mostly on the numerical aperture of the imaging system while speckles in XZ plane depends on the spectral range of illuminating light. That will definitely affect the distribution describing the speckle field giving it additional specificity. It would be very interesting to analyze this problem and propose alternative speckle modeling.

As a next step in the project long term and short term changes of corneal structure were analyzed by looking at differences between average probability density functions of Generalized Gamma distribution calculated for various age groups. Particular parameters chosen for analyses were GG_a, GG_p and GG_v.

- Long term changes were analyzed statistically by choosing various age groups. Results of this studies are very interesting – giving realistic indication that this way of data analysis can be used in practice. The methodology does not rise any objection. The missing point is a follow-up of these studies including a proposal of how to optimize the method – maybe only one parameter would be enough to get significant read-outs. If not then multidimensional space of parameters easily accessible from OCT images could be used to make the method more robust (for example corneal thickness, corneal curvature, intraocular pressure etc). It would be also a good way of making the thesis more consistent.
- Short term corneal changes were related to observation of corneal swelling introduced by using contact lens and patch covering one eye for three hours. Author claims that presented results indicate statistically significant difference between all parameters for various age groups and also for cases with corneal swelling. This part of experiment I found incomplete. First of all, the experiment is not well controlled – method of

introduction of the corneal swelling is rather simple and it may depend on many parameters including individual variability. This is confirmed by author in discussion section: "…it is unknown how the microstructure is affected [by the swelling]". Also data analysis is not described in detail. There is no information if each measurement were analyzed against reference data from IOL Master: Central Corneal Thickness values are displayed in table 3.2 but they seem to be not used in statistical analysis. It is also not clear if CCT is the only parameter characterizing the corneal swelling. I have a feeling that there is a missing next step in the studies including more reliable and controlled experiment with induced short term corneal changes. Maybe it should be done on fresh animal corneas *in vitro*.

Summarizing this part of the thesis I believe that it would be much better if the entire PhD project is dedicated to this particular subject and all questions that I rose above would be addressed in the thesis.

Chapter_4 is dedicated to a description of another sub-project related to accurate estimation of the scleral radius. The hypothesis of this part of the PhD work was whether it is possible to improve ocular biometry by introducing measurement of scleral radius. The proposal was to use data from commercially available high definition projection based, fluorescence profiler (Eye Surface Profiler, ESP, Eagle-Eye). Author anticipated that using the simplest dual spherical approximation based on least squares fitting procedure should provide information about the scleral radius. He tested repeatability of the fit procedure based on ten measurements performed on the same volunteer's eye and to test accuracy he used artificial eye phantom. This simple repeatability study shows that the standard deviation is less than 3% and the accuracy is better than 1%. It is not clear how the reference curvature of the phantom is estimated or specified. In the next step author performed biometrical studies to find out if the scleral radius can be correlated with other parameters including axial eye length, anterior chamber depth, corneal radius, central corneal thickness, white to white corneal diameter. Measurements of those parameters were performed by IOL Master to get independent instrumentation and analysis method. Strong correlation was achieved only for axial length, which was not entirely independent since the scleral radius estimation was also based on IOL Master readout. Other parameters correlated weakly. In order to get rid of the second instrument for scleral radius estimation author decided to introduce wide-open palpebral aperture measurements only with ESP. The results have proven that the spherical fit can be performed with similar accuracy when more of the sclera is imaged by ESP.

Chapter_5. As a follow up to the corneal radius project the candidate proposed to study an improvement of corneo-scleral demarcation methods. He planned to use corneal and scleral data obtained by ESP to fit bilinear Zernike polynomial function corresponding to corneal and scleral regions. The description of fitting procedure is very vague and quite chaotic mixing methods with theory and lacking in substantial information. For example, it is very hard to understand what information is exactly delivered by ESP that can be further processed with Zernike polynomial fit. Is there any pre-assessed demarcation line between two segments? How the Zernike polynomial models are fit separately for outer and inner segments? There is also a missing part describing the methods and exact algorithms for undertaken procedures.

In the next step the proposed method is compared statistically with other existing modalities including imaged enhanced topography and image-only based methods. The results indicate that Zernike polynomial technique works in similar way to other methods but there is more error probably due to artifacts generated by ESP (related to dynamic tear film built-up after blinking).

Chapter _6 describes another sub-project related to development of new techniques measuring Intraocular Pressure (IOP) in human eyes in more reliable way than currently available instruments. One of the most popular techniques being currently in clinical use for IOP measurement is applanation tonometry. This technique uses corneal deformation to calculate IOP and has many limitations due to lack of information and huge individual variability of biomechanical properties of the cornea. For last ten years' various research groups are trying to find out how to modify applanation tonometry to get reliable IOP readouts. One of the current trends is to modify OCT technique to either combine it with air-puff systems or to introduce optical elastography techniques. Also the literature on this subject is very reach – especially focusing on the aspect of mechanical modeling of the cornea. It would be very beneficial to add more background to this chapter – that would give the reader more understanding of the problem. In this work author proposes using the speckle modeling to analyze corneal microstructure and correlate it with IOP. This is very interesting approach but it has to tackle very complex problem – that is why the project itself extends beyond the frames of short subproject presented in this chapter. Similarly to chapter _3 the candidate used generalized gamma distribution to characterize the speckle field in corneal OCT images and correlate the GG parameters with measured IOP. He did impressive prospective studies with the group of 56 healthy volunteers measuring not only OCT and IOP but also other parameters provided by IOL Master. In my opinion the concept of these studies is very interesting but the final realization is very unsatisfactory. All demonstrated correlation plots show very weak dependence of analyzed parameters. The speckle statistics does not work at all. Probably the reason is that the dependence between the microstructure and IOP is not straightforward and may depend on more parameters. Additionally, the Generalized Gamma distribution maybe not optimal and the anisotropy of the speckle field in XZ plane can also play a role.

Chapter _7 is dedicated to ray-tracing algorithm introduced by the candidate as a modification of existing and published technique. This is very instrumental chapter and it is a little bit off the main subject of the thesis. I found this chapter as artificially and unnecessarily added to the main text since the algorithm has not been used in other experiments.

Concluding, I have to admit that the variety of subjects undertook in the thesis and the outcome measured in publications and conferences is impressive. However, the same diversity of topics makes the thesis very inconsistent and hard to follow - even for the expert in that field. Also information about the state of the art, theoretical background and methodology - all are described very superficially giving other researchers no chance to repeat experiments and verify formulated scientific hypotheses. From this perspective it is hard to understand why the candidate chose the form of full thesis once there is also possibility to present a set of original publications to finish the PhD process. In my opinion such choice

would match better to his project. The presented thesis has a hybrid form - between those two well accepted solutions. At the other hand the work presented in corresponding publications convincingly demonstrates an ability of Danilo Andrade de Jesus to design experiments including statistical analysis and complex signal processing based on optical set-ups. Author was capable to obtain reasonable results from *in vivo* studies. The thesis demonstrates his significant skills in signal and image processing and high level of knowledge in the field of biomedical optics. It definitely provides an evidence of the candidate's independent accomplishment of a scientific problems. Despite the doubts I expressed above I accept this thesis and recommend the candidate to be awarded the PhD degree.

Yours Sincerely,