

ESTIMATION OF CORNEAL LIGHT SCATTERING
PARAMETERS WHICH ARE INDEPENDENT OF
EXTERNAL FACTORS AND ANTERIOR EYE
GEOMETRY

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PHD thesis

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Maria Miazdzyk: *Estimation of corneal light scattering parameters which are independent of external factors and anterior eye geometry*, PHD thesis, © January 2026

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to my husband and daughter



ABSTRACT

This dissertation provides a comprehensive investigation on corneal light scattering parameters and the ways of their assessment. Currently used measures seem to be confounded by factors of various nature. Therefore, the superior aim of the work is to create estimators of corneal light scattering parameters that would be independent of confounding factors and will improve diagnostic value of these parameters. As corneal scattering properties are addressed in this work in different points of view, it was necessary to divide the main research part into three projects.

The first project raises the problem of confounding factors observed in Scheimpflug-based corneal densitometry, a clinically used quantity to assess corneal backscatter and, subsequently, health. The research is performed on Scheimpflug images of 141 young and 73 old subjects. Three densitometry parameters were analyzed, which were the mean pixel intensity and the Weibull scale and shape parameters, calculated for the chosen region of interest (ROI). The main confounders have been identified as the eye biometry parameters, namely, anterior chamber depth, pupil size, and central corneal thickness. Also, extensive elaboration on image preprocessing has been performed. The result is an algorithm which successfully compensates densitometry measures for the observed confounding factors.

The second project considers the matter of multilayered structure of the cornea. The purpose was to assess and quantify the influence of the corneal epithelium on the scattering signal corresponding to the corneal stroma. In this case, another imaging technique, optical coherence tomography (OCT) has been utilized. A retrospective data set of 11 porcine OCT images acquired before and after epithelial debridement was analyzed to compare the OCT speckle statistics in the stroma for these two measurement modes. Four measures were considered, which were the shape and scale parameters of the Gamma distribution, the mean pixel intensity and contrast ratio for the given ROI, delineating corneal stroma. The investigation confirmed the significant differences in speckle statistics depending on whether the stroma is measured with or without epithelium. The estimation error of the stromal parameters caused by the presence of epithelium was shown to reach over 20%, depending on the stromal depth.

The last project introduces the concept of creating a digital OCT phantom for the cornea. The primary aim was to simulate corneal stroma OCT B-scan, with close to reality speckle statistics. First, a commonly used Monte Carlo algorithm for simulating photon propagation through tissue and an algorithm for calculating the OCT signal were implemented. Both were adapted for the particular OCT device and adjusted to the optical properties of the cornea. Further, a novel method for in-vivo OCT-based estimation of optical properties (that is, scattering and anisotropy coefficients), currently unresolved for the cornea, was developed. For that, a set of multi-reference B-scans was generated for various values of the input optical parameters, selected on the basis of the information available in the literature on the dissected corneal tissue. Then the estimation method based on statistical matching between simulated and experimental data was designed. The group mean of the scattering coefficient and the scattering anisotropy factor for the porcine stroma were $\bar{\mu}_s = 0.146 \pm 0.020 \text{ mm}^{-1}$ and $\bar{g} = 0.893 \pm 0.021$, respectively.

STRESZCZENIE

Niniejsza rozprawa doktorska przedstawia kompleksowe badania nad parametrami rozpraszania promieniowania w rogówce oraz nad sposobami ich oceny. Obecne, klinicznie stosowane parametry okazują się być zakłócone czynnikami o różnej naturze i pochodzeniu. Nadrzędnym celem było więc stworzenie takich estymatorów parametrów rozpraszania, które będą niezależne od czynników zakłócających i poprawią wartość diagnostyczną. Praca rozpatruje właściwości rozpraszające rogówki na wielu płaszczyznach, więc główną część badawczą można podzielić na trzy projekty.

Pierwszy projekt ujawnia problem czynników zakłócających obserwowanych w densytometrii rogówki obrazowanej metodą Scheimpfluga. Densytometria jest kliniczną miarą, wykorzystywaną do oceny rozpraszania w rogówce, a co za tym idzie, oceny stanu zdrowia. Badania przeprowadzono na obrazach rogówki rejestrowanych metodą Scheimpfluga u 141 młodych i 73 starszych osób, estymując trzy miary densytometryczne: średnią wartość pikseli oraz parametry skali i kształtu rozkładu Weibulla. Głównymi czynnikami zakłócającymi były parametry biometrii oka: głębokość komory przedniej, rozmiar źrenicy i grubość centralnej części rogówki. Obszernie przeanalizowano różne metody normalizacji obrazów oraz zaproponowano skuteczny algorytm, który eliminuje wpływ wspomnianych czynników na densytometrię.

Drugi projekt porusza kwestię wielowarstwowej struktury rogówki. Celem była ocena i ilościowe określenie wpływu nabłonka rogówki na sygnał pochodzący ze zrębu w obrazach zarejestrowanych przy pomocy optycznej tomografii koherencyjnej (OCT). Porównywano statystykę szumu plamkowego OCT ze zrębu świńskiej rogówki obrazowanej z nabłonkiem i natychmiast po jego usunięciu. Przeanalizowano cztery parametry: parametry kształtu i skali rozkładu Gamma, średnią intensywność pikseli i współczynnik kontrastu, dla obszaru zainteresowania (ROI) obejmującego zrąb rogówki. Potwierdzono istotne różnice w statystyce szumu plamkowego w zależności od tego, czy zrąb obrazowano z nabłonkiem czy bez. Wykazano, że błąd estymacji parametrów zrębu spowodowany obecnością nabłonka sięga 20% i jest zależny od głębokości próbki.

Ostatni projekt podejmuje koncepcję stworzenia cyfrowego fantomu OCT dla rogówki. Podstawowym celem była symulacja B-skanu OCT zrębu rogówki, o statystyce szumu plamkowego zbliżonej do rzeczywistych obrazów. W pierwszej kolejności zaimplementowano powszechnie używany algorytm Monte Carlo symulujący propagację fotonu przez tkankę oraz algorytm pozwalający na wyliczenie sygnału OCT. Obie metody zostały zaadaptowane dla konkretnego urządzenia OCT oraz dostosowane do właściwości rozpraszających rogówki. Dodatkowo, opracowano nowatorską metodę estymacji właściwości optycznych rogówki (współczynnika rozpraszania i anizotropii: μ_s i g). W tym celu stworzono zestaw wieloreferencyjnych B-skanów, wygenerowanych przy różnych wartościach wejściowych parametrów optycznych, wybranych na podstawie istniejących, choć ograniczonych, danych literaturowych dotyczących rogówek zwierzęcych. Metodę estymacji oparto na statystycznym dopasowaniu między danymi symulowanymi a eksperymentalnymi. Średnia grupowa współczynnika rozpraszania i współczynnika anizotropii dla zrębu świńskiej rogówki wyniosła odpowiednio $\bar{\mu}_s = 0.146 \pm 0.020 \text{ mm}^{-1}$ i $\bar{g} = 0.893 \pm 0.021$.

SCIENTIFIC OUTPUT

JOURNAL ARTICLES

1. Miażdżyk, M., Consejo, A. & Iskander, D. R. Assessing and compensating for the confounding factors in Scheimpflug-based corneal densitometry. *Biomedical Optics Express* **13**, 6258–6272 (2022).
2. Miażdżyk, M., Consejo, A. & Iskander, D. R. OCT based corneal densitometry: the confounding effect of epithelial speckle. *Biomedical Optics Express* **14**, 3871–3880 (2023).
3. Miażdżyk, M., Consejo, A. & Iskander, D. R. Estimating the optical properties of corneal tissue from the OCT speckle. *Biomedical Optics Express* **17**, 544–554 (2026).

CONFERENCES

1. Miażdżyk, M. *Tracking the local backscatter changes in cornea Scheimpflug images during tonometry measurement with OCULUS Corvis ST* in *IEEE Engineering in Medicine & Biology Society (EMBC) International Student Conference*. Kraków, Poland (Dec. 2020).
2. Miażdżyk, M. & Iskander, D. R. *Age-related changes in dynamic corneal backscatter observed in Scheimpflug imaging in 2022 44th Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)*. Glasgow, Scotland (July 2022).
3. Miażdżyk, M., Consejo, A. & Iskander, D. R. *Corneal epithelium influences OCT based densitometry of stroma in Association for Research in Vision & Ophthalmology Annual Meeting (ARVO)*. New Orleans, United States (Apr. 2023).
4. Miażdżyk, M. & Iskander, D. R. *Validation of OCT B-scan Monte Carlo simulation algorithm for cornea. Work in progress in 12th Meeting on Visual and Biological Optics (VPO)*. Wrocław, Poland (Aug. 2024).
5. Maria Miażdżyk, A. C. & Iskander, D. R. *Digital phantoms for improved OCT based corneal densitometry in 28th European Association for Vision and Eye Research (EVER) Congress*. Florence, Italy (Oct. 2025).

GRANT APPLICATIONS

1. accepted: *Advanced techniques for a holistic analysis of corneal OCT speckle*. PRELUDIUM-22. National Science Centre. 2023.
2. rejected: *Modeling speckle evolution in optical coherence tomography images for multilayered samples*. PRELUDIUM-21. National Science Centre. 2022.

Nauka wszakże to tylko jeden ze sposobów poznawania rzeczywistości. Wiedział o tym Platon. I gdy docierał do kresu poznania naukowego, sięgał po poezję by przekroczyć granicę, przed którą musiał cofnąć się rozum. Poezją, sztuką łowił prawdy dla nauki niedostępne. Lecz czymże jest prawda? “Prawda jest ruchomą armią metafor”, odpowiada F. Nietzsche. Jeśli ta prawda o prawdzie jest prawdziwa, wówczas krąg się domyka, sztuka spotyka się z nauką [...].

— Andrzej Szczeklik [1]

At the beginning, I would like to express my deep gratitude to my first supervisor, Robert Iskander. Thank you for being not only the supervisor but also a friend. Thank you for introducing me to scientific thinking and for your mindful guidance over these years. Thank you for the useful tips, such as: “worth seeing film: *The Bucket List*” or “try l’Ortolan cheese with truffles” (I have them all written down in the margins of my PhD notebook). Also, thank you for supporting my passion for music. Seeing you with your family in the audience during my concerts meant a lot to me. Finally, I will never forget that during your opening speech at VPO 2024, I was singing “Waltzing Matilda” *a vista*, without even realizing what I was singing.

Secondly, I am truly grateful to Alejandra Consejo, my second supervisor. Thank you for helping me mature scientifically. Thank you for asking inconvenient scientific questions and for your spot-on observations, and for bringing my ideas back down to earth to move things forward. I may not speak Spanish, but “¡Vale!” has stuck in my mind. It has been great fun to work together as a team of three over these years.

I would also like to mention here the entire BSPG team. Thank you for the great atmosphere you create in the lab. It was a pleasure to share a desk with all of you. Special thanks go to Mela, a fellow “first-time mum” during the PhD. Thank you for your advice and help.

This thesis would not have been written without my family. Thank you for every “how is it going?”. Thank you for being the best babysitters for Łucja — from aunties Faustyna and Karolina and uncle Szymon, to grandmas Aneta and Gosia and grandpas Andrzej and Tomek. For the dinners in jars. Your help and support were invaluable.

Additionally, I would like to express special thanks to my parents. Thank you for teaching me that every piece of work should be done with passion and quality. Thank you for teaching me to be inquisitive and for your mindful presence throughout my life.

I also cannot forget my friends from the music world. Thank you for your support. Special thanks to Karolina and Karina, who took care of Łucja. Thank you for the music projects we worked on together — they kept me from going crazy.

Finally, I would especially like to thank my husband. Thank you for being my safe haven and for enduring my ups and downs. Thank you for your patience and peace of mind during this crazy time, and for taking over all the responsibilities during my all-nighters.

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ACRONYMS

AC	anterior chamber
ACD	anterior chamber depth
ACF	autocorrelation function
BCG	image background
C	central
CCT	central corneal thickness
CL	contact lens
CLAHE	contrast-limited adaptive histogram equalization
CXL	crosslinking
CV	coefficient of variation
EHF	Extended Huygens Fresnel
EXP	experimental
FD-OCT	Fourier-domain optical coherence tomography
FWHM	full width half maximum
GoF	goodness of fit
GSU	grayscale units
IAD	inverse adding-doubling
IMC	inverse Monte Carlo
KM	Kubelka-Munk
N	nasal
NCT	nasal corneal thickness
NIR	near infrared region
O	older subject or group of older subjects
OCT	optical coherence tomography
PBS	Phosphate Buffer Saline
PUP	pupil size
RMS	root mean square
ROI	region of interest
SIM	simulated
SD-OCT	spectral-domain optical coherence tomography
SNR	signal-to-noise ratio
SS-OCT	swept-source optical coherence tomography
STD	standard deviation
T	temporal

TCT	temporal corneal thickness
UVA	ultraviolet A
Y	young subject or group of young subjects

LIST OF SYMBOLS

A	amplitude of OCT signal
BCG	image background
CR	contrast ratio
d	mean pixel value (“densitometry”)
$d\Omega$	differential solid angle
D_{CR}	difference in CR between two samples
D_{KL}	Kullback–Leibler divergence
D_{KS}	Kolmogorov–Smirnov distance
D_{RMS}	root-mean-square distance between two kernel density estimators
e	Euler’s number
$\exp(\cdot)$	exponential function
g	scattering anisotropy coefficient
I	radiance intensity
k	shape parameter of Weibull distribution
l_c	coherence length
$m(\cdot)$	ensemble median
n	sample size
n_1, n_2, n_3	refractive indices of media 1, 2 and 3
n_i, n_t	refractive index of the preceding (incident) and following media (transmission) at the interface
NA	numerical aperture
N_{sc}	number of scattering particles within coherence volume
$p(\cos \theta)$	phase function
p	p -value
r	Pearson or Spearman correlation coefficient
r_{sphere}	radius of a spherical particle
R_{sp}	coefficient of specular reflection
$R(\theta)$	Fresnel reflection coefficient
S	total path of the photon packet
r_x	horizontal resolution of the Scheimpflug image
r_y	vertical resolution of the Scheimpflug image
w	beam radius
w_0	beam radius at the beam waist
v	shape parameter of K-distribution
v_{sphere}	volume of a spherical particle
V_c	volume of coherence
Z_α, Z_β	standard normal deviates
α	scale parameter of Weibull or Burr 2 distribution
β	shape parameter of Weibull or Burr 2 distribution
$\Gamma(\cdot)$	Gamma function
δz	vertical resolution of OCT system
δx	transversal resolution of OCT system
θ	scale parameter of Gamma distribution
ϑ	scattering (deflection) angle (also, normal angle in spherical coordinate system)

ϑ_i	angle of the incident beam
ϑ_t	angle of the transmitted beam
λ	wavelength
λ_0	central wavelength of the beam
$\Delta\lambda$	bandwidth of the lightsource
$\Delta\lambda_{FWHM}$	bandwidth of the lightsource at half maximum (half band-width)
μ_a	absorption coefficient
μ_s	scattering coefficient
μ_t	attenuation coefficient
μ_x, μ_y, μ_z	directional cosines in Cartesian coordinate system
ξ	Monte Carlo random number distributed uniformly in range $[0, 1]$
ρ_a	density of absorbing particles
ρ_s	density of scattering particles
σ	scale parameter of Rayleigh distribution
$\sigma_x, \sigma_y, \sigma_z$	standard deviations for 3-D Gaussian distribution
ϕ_j	phase of j th phasor
ϕ	phase of the complex amplitude of speckle field
φ	azimuthal angle in spherical coordinate system
ω_z	frequency of phasor at given sample depth z
$\langle \cdot \rangle$	ensemble mean value
$\hat{\cdot}$	estimate

MOTIVATION AND RESEARCH HYPOTHESIS

Cornea — its radius of a few millimeters and usual thickness of approximately half of a millimeter make it one of the smallest tissues in the body. Being differentiated from the ectoderm in embryonic evolution [3], similarly as nerve tissues and the epidermis, only the cornea has developed such a sophisticated structure. The remarkable arrangement of its micro- and nano-components, allows the cornea to be a highly transparent window for the eye. Different factors may influence this fine corneal microstructure, causing decrease in its transparency and finally lead to the vision deterioration or even vision loss. Assessing corneal scattering is therefore of interest, for prescreening, early disease detection and also healing or disease progression observation. Since, mentioned, the small tissue volume, *in vivo* non-invasive assessment of corneal health is not only the likely approach, but also the desired one, while biopsy is limited.

At the heart of the topic of this doctoral dissertation lies the observation of the corneal Scheimpflug images of older and younger subjects (see Chapter 2, Figure 2.5), where the strong impact of the eye biometry on corneal appearance in the image was noticed. It was recognized that assessing corneal transparency *in vivo* with the use of different imaging methods is distorted with confounding factors. Following this supposition and further observations, the research hypothesis was formulated:

Creating objective estimators of corneal light scattering parameters that will be independent of confounding factors will improve the diagnostic value of these parameters. The confounding factors are the external conditions under which the measurement is taken, the anterior segment geometry, and the multilayered character of the cornea.

The aim of this work was to perform a comprehensive investigation on corneal backscatter, using different imaging modalities and different methods of corneal transparency assessment. A significant part of this thesis is devoted to one of the measures of corneal backscatter: corneal densitometry. Currently, in clinical applications densitometry is bonded with one Scheimpflug-imaging-based device, one layer — the corneal stroma, and with one simple measure — the mean pixel intensity. This work contributes to the currently emerging *paradigm shift* regarding corneal densitometry, by widening this term to other imaging techniques such as OCT, other corneal layers, that is, corneal epithelium, and other measures, such as distributional parameters. Also, an effort is put on quantification and compensation for factors confounding objective densitometry assessment. For that, creating an OCT corneal digital phantom with close to reality backscatter signal seems to have potential in analyzing confounding factors arising from multilayered character of the cornea. Finally, speckle representing corneal backscatter in OCT are indirectly correlated with corneal microstructure. Nevertheless, there is no validated method, for *corneal* optical properties (i.e., the scattering and anisotropy coefficient) estimation based on OCT signal. This work aimed to elaborate on the original method for evaluating the optical properties of the cornea from OCT corneal images. OCT device is now a basic clinical equipment in ophthalmology and any effort on improvement of its diagnostic power and scope is of value. Thus, this work contributes to the concept of “all-in-one” device, exploring OCT as a complex platform for diagnostics and screening.

SCOPE OF THE THESIS

Chapter 1 gives an overview of the concepts related to the research carried out in this work. Chapters 2–6 present the main, research part of this dissertation. The author performed a multi-directional study taking the corneal scattering properties from different points of view. Hence, the subsequent chapters can be distinguished into three main projects. Chapter 2 addresses the problem of confounding factors observed in corneal Scheimpflug-based corneal densitometry. Chapters 3 and 4 concentrate on OCT speckle statistics from the corneal stroma and the confounding effect of the epithelial layer. Chapters 5 and 6 introduce more theoretical approach in order to correlate optical properties of the cornea with the OCT speckle statistics, by simulation of OCT B-scans of corneal stroma. Each chapter ends with a discussion and a comment on future work. Finally, Chapter 7 provides final remarks and major conclusions.

AUTHOR'S CONTRIBUTION

All algorithms, including the ones for statistical analysis were written in Matlab R2024b (The MathWorks Inc., Natick, Massachusetts) by the author. Most of them were devised by the author, some of them were also supported with the literature (always mentioned as reference). The exception is the Monte Carlo algorithm for photon transport through the scattering medium (see Chapter 5), which was manually implemented by the author based on the ready, detailed description of implementation in other programming language.

The analyzed corneal images are mostly retrospective data, except those analyzed in Chapter 4, where the author was also responsible for the experiment and thus image acquisition.

INTRODUCTION

The following chapter collects and introduces all key topics relevant to this dissertation. Therefore, first, the phenomenon of scattering is explained and the optical properties of tissues are defined (Section 1.1). Subsequently, Section 1.2 provides a description of the micro- and nano-structure of the cornea with emphasis on its transparency and scattering. Further, imaging techniques related to this work are described (Section 1.3 and Section 1.4). Finally, in Section 1.5 the author summarizes and organizes parameters related to corneal scattering described in the preceding sections.

1.1 SCATTERING IN TISSUES

All media, except a pure vacuum scatter light. Even those that are considered to be homogeneous (such as water or gases). Scattering phenomena may be shortened to two words: excitation and reradiation. An electromagnetic wave that encounters an obstacle (e.g., a particle, molecule or electron) first induces its oscillatory motion and is subsequently reradiated in all directions [4]. Thus, under the name of scattering there are also: refraction, specular and diffuse reflection and diffraction. Additionally, part of the incident electromagnetic wave may also be transformed into other forms (e.g., thermal energy), meaning absorption [4]. All of these phenomena depend on the wavelength of the radiation.

Assessing the scattering properties of tissue can potentially provide information about its structure and health, while bypassing the sample staining process or the addition of chemical markers needed, for example, for microscopic imaging or spectroscopic examinations [5]. Biological tissues are usually composed of a range of micro- to nano-components, such as cell organelles, collagen fibers and other protein molecules, and membranes (listed in decreasing size order). Therefore, light scattering is a collective phenomenon with contributions from all structures. As the range of their sizes is close to the usually considered wavelength (visible and near infrared range), both Mie scattering theory and the Rayleigh approximation (commonly called Rayleigh scattering) should be here accounted¹. The Mie theory was introduced by Gustav Mie in 1908 (and independently by Peter Debye and Ludvig Lorenz). It provides a solution to Maxwell's equations, which describes absorption and elastic scattering of an electromagnetic wave by a sphere. Mie theory is applicable to the particles of any size [4, 7], however it is usually applied for spheres of size comparable to the wavelength. The strength of Mie scattering is proportional to $1/\lambda^b$, where b is the factor dependent on particle size and for soft tissues $b \approx 1$ [7, 8]. For larger structures, such as cells (10–30 μm) or cell arrays it may be more convenient to describe them using classical optics as microlenses or microlens arrays [5]. Rayleigh scattering is actually the name for the limiting case of Mie theory, where the particle size is much smaller than the wavelength (conventionally, the particle size is $< \frac{1}{10}\lambda$).

¹ Here, it is also valuable to refer the reader to Young (1982) [6], who highlights the problem of conflicting uses of the term “Rayleigh scattering” and makes the excursion through the history and physics of scattering by gas molecules.

Due to approximation, the strength of scattering for small spheres is proportional to $1/\lambda^4$. It was also proved numerically that for the Rayleigh particle size range, the dependence of the scattering strength on the scattering angle is negligible and it can be assumed that the scattering is isotropic in all directions [8, 9]. Above this range, forward scattering starts to dominate and increases with particle size [9]. The optical properties of the tissue are usually described by the following parameters [4, 5, 7, 10, 11], which are defined in subsequent subsections.

Scattering and absorption coefficients

Mie theory provides the theoretical basis and derivation of equations for scattering and absorption coefficients, respectively:

$$\mu_s = \rho_s \sigma_s \quad (1.1)$$

$$\mu_a = \rho_a \sigma_a, \quad (1.2)$$

where ρ_s and ρ_a are the volume densities of scattering and absorbing particles, respectively, σ_s and σ_a are the effective cross sections of scattering and absorption in the Mie theory [12]. Particles were originally assumed to be spherical, but derivations also exist for other specific particle shapes, e.g., for cylinders or ellipsoids. The above quantities can be calculated from the Mie theory and are well described by Bohren & Huffman in [4], however this is a complicated process. Notably, freeware computer programs for calculations of Mie quantities for spheres also exist, allowing the user to avoid delving deeply into the Mie equations². On the other hand, the absorption and scattering coefficients can also be described in a more experimental way, as explained below.

The intensity of the input radiance I transported through biological tissue is depleted over an infinitesimal length dx due to the phenomena of absorption and scattering, resulting in an output radiance of $I - dI$ (see Figure 1.1 a)). The quantity dI is the amount of radiance lost to absorption and scattering.

$$dI = I\mu_a dx + I\mu_s dx = I(\mu_a + \mu_s)dx. \quad (1.3)$$

The absorption and scattering coefficients (μ_a and μ_s) describe the probability of absorption and scattering in the following way [10]:

$\mu_a dx$ is the probability of absorption in an infinitesimal distance dx ,

$\mu_s dx$ is the probability of scattering in an infinitesimal distance dx ,

Consequently, the mean free path that the wave will travel between the scattering events is given by:

$$\langle s \rangle = \frac{1}{\mu_s}. \quad (1.4)$$

Absorption and scattering are often inseparable phenomena together causing attenuation of the radiance, therefore, they are usually combined into the attenuation coefficient:

$$\mu_t = \mu_a + \mu_s. \quad (1.5)$$

² Two of them are listed below:

Laven, P. *MiePlot* <http://www.philiplaven.com/mieplot.htm> (2021),

Prahl, S. *Mie Scattering Calculator* https://omlc.org/calc/mie_calc.html (2023).

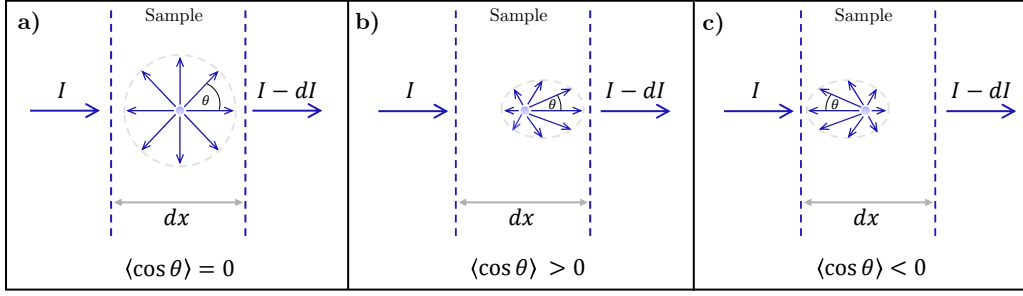


Figure 1.1: A simplified illustration of a single scattering event on a spherical particle over an unit length dx . The input radiance intensity (I) is depleted to $I - dI$. The angle of scattering is denoted by ϑ , whereas $\langle \cos \vartheta \rangle$ is the average value of the cosine of the scattering angle, hereafter referred to as scattering anisotropy coefficient g . Three cases are shown: a) isotropic scattering, b) anisotropic scattering with dominant forward scattering, c) anisotropic scattering with dominant backward scattering [own work based on [17]].

Equation 1.3 can be transformed (by integration and exponential transform) to the well known Beer-Lambert law, which is presented in Equation 1.10. The following Equations 1.6–1.9 guide the reader through the derivation of this law. By reshaping the Equation 1.3, one obtains:

$$\frac{dI}{I} = (\mu_a + \mu_s)dx, \quad (1.6)$$

and then integrating both sides:

$$\int \frac{1}{I} dI = \int (\mu_a + \mu_s) dx \quad (1.7)$$

$$\ln I = -(\mu_a + \mu_s)x + c, \quad (1.8)$$

where c is a constant. By exponentially transforming both sides, one gets:

$$I = Ce^{-(\mu_a + \mu_s)x}, \quad (1.9)$$

which describes the intensity of the output beam after traveling a distance x through tissue. The coefficient C is a rescaling coefficient, which can be substituted with I_0 , denoting the incident intensity. If no scattering and absorption occur, the expression $e^{-(\mu_a + \mu_s)x}$ equals 1, meaning that the output beam intensity being is equal to the incident intensity.

$$I = I_0 e^{-(\mu_a + \mu_s)x} \quad (1.10)$$

Equation 1.10 presents the Beer–Lambert law (also referred to as the Beer–Bouguer–Lambert law)³.

³ Bouguer first formulated the law in 1729 for astronomy [14], then it was popularized in Lambert’s publication in 1760 [15]. Beer independently derived the same relationship in chemical analysis and published it in 1852 [16].

Scattering anisotropy coefficient

The probability distribution for the cosine of the scattering angle (also referred to as the deflection angle) can be described by the Heyney-Greenstein phase function (also referred to as a scattering diagram [4]⁴) originally designed for galactic scattering:

$$p(\cos \vartheta) = \frac{1 - g^2}{2} \frac{1}{(1 + g^2 - 2g \cos(\vartheta))^{\frac{3}{2}}}, \quad (1.11)$$

where g is the *scattering anisotropy coefficient* defined as the average of the cosine of the scattering angle ϑ , and is also equivalent to the integral of $p(\cos \vartheta)$ over the full angular space (4π) using the differential solid angle of $d\Omega$ [4, 10]⁵:

$$g = \langle \cos \vartheta \rangle = \int_{4\pi} p(\cos \vartheta) d\Omega. \quad (1.12)$$

Reduced scattering coefficient

The input radiance intensity I will be less strongly depleted in Figure 1.1 b), where forward scattering dominates, compared to Figure 1.1 a) — the case of isotropic scattering. In most tissues, anisotropic forward scattering dominates, and the coefficient g takes relatively high values [10]⁶. The primary scattering coefficient μ_s , defined previously, does not include information about the scattering angle and assumes isotropic scattering in its definition. Therefore, the reduced scattering coefficient allows for a more accurate calculation of radiance depletion:

$$\mu'_s = \mu_s(1 - g). \quad (1.13)$$

If $g > 0$, forward scattering dominates, thus $\mu'_s < \mu_s$, and the resultant radiation depletion due to scattering will be smaller than that in isotropic scattering (i.e., $I\mu'_s dx < I\mu_s dx$). For $g < 0$ backward scattering dominates, thus $\mu'_s > \mu_s$, and the resultant radiation depletion due to scattering will be greater than that in isotropic scattering ($I\mu'_s dx > I\mu_s dx$).

In practice, particularly for biological tissues, it is common to measure the reduced scattering coefficient by measuring beam depletion [7].

1.2 CORNEAL ANATOMY AND CHARACTERISTICS

Serving as a shield protecting the eye from outside, withstanding the intraocular pressure, and at the same time keeping transparency at the level of 90% sounds like a mission impossible for a tissue. Despite other organisms having developed surprising abilities for vision, to mention just a few, nictitating membrane (birds) or infrared vision (snakes), the mammalian cornea still holds a high position due to its complex composition and functions. The following sections will describe the structural and optical properties, particularly of the *human* cornea.

⁴ See page 72 in [4].

⁵ See page 38 in [10], page 72 in [4].

⁶ See page 38 in [10].

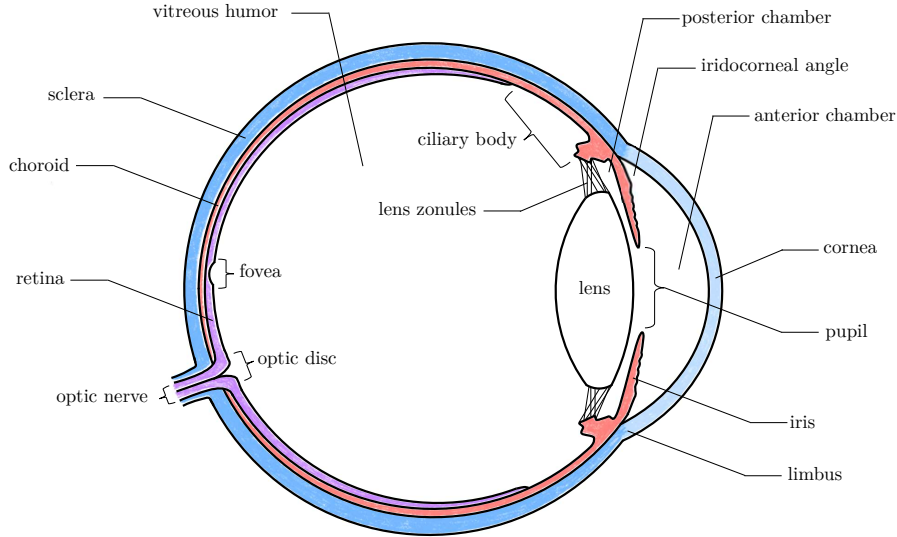


Figure 1.2: Schematic of the eye anatomy with the three tunics coloured: fibrous (blue), uvea (red) and neural (purple) [own work based on Forrester *et al.* [2]].

1.2.1 Brief anatomy of the eye

The eye is a spherical organ with a volume of approximately 6.5 ml. It is composed of three coats, also called tunics: the fibrous (cornea and sclera), the uvea (iris, choroid, ciliary body) and the neural layer (retina). These coats, together with the lens, limit the spaces in the eye, which are the anterior chamber (AC) filled with aqueous humor, the posterior chamber, and the vitreous body (Figure 1.2).

The spherical shape and eye protection are ensured by two partial spheres: the sclera, with an average radius of 11.5 mm [2], which constitutes the majority — five-sixth of the globe, and the cornea with anterior and posterior radii of approximately 7.8 mm and 6.4 mm, respectively [18–20]. Hence, the corneal thickness increases from the center to the periphery, from an average of 0.52 mm to 0.67 mm [2]. The spherical shape makes the cornea the major converging lens of the eye, providing about two thirds of the total refractive power (approximately 40 diopters [20]). In frontal perspective, the cornea appears as an ellipsoid of radius 10.6 vertically and 11.5 mm horizontally [2]. The border between the cornea and sclera is called the limbus.

1.2.2 Composition of the cornea

The cornea is a soft connective tissue built up of six layers: the epithelium (50–60 μm), Bowman’s layer (8–12 μm), the stroma (90% of corneal thickness), Descemet’s membrane (50–60 μm), and the endothelium (5–6 μm). Figure 1.3 a) presents an illustrative cross section of the corneal tissue. The most responsible for corneal transparency is the corneal stroma being the major layer, whereas the epithelium also contributes as the second layer in thickness. Therefore, a more detailed description of these two layers is provided.

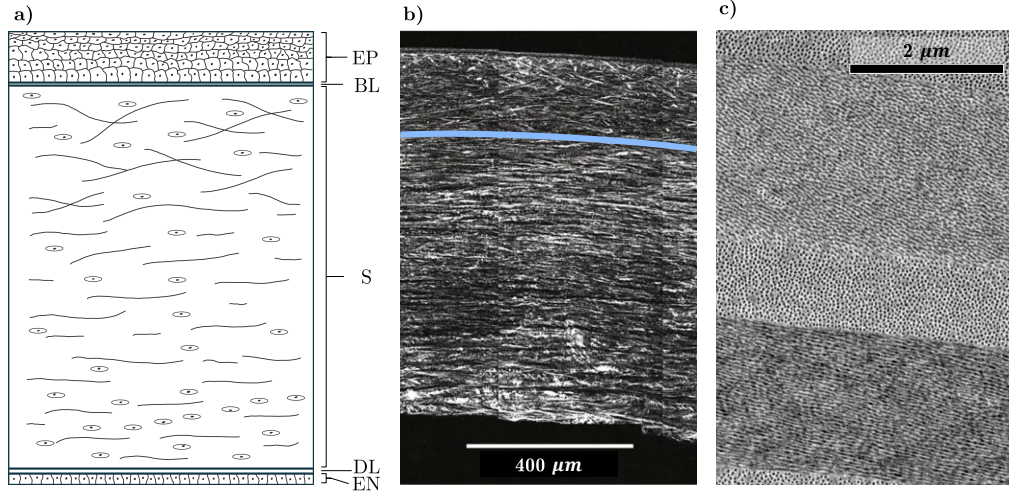


Figure 1.3: a) Schematic of the corneal tissue composition. EP — epithelium, BL — Bowman's layer, S — stroma, DL — Descemet's membrane, EN — endothelium [own work based on [21, 22]]. b) Second harmonic generation nonlinear microscopic image of the corneal stroma (epithelium removed), showing the intertwining of collagen lamellae in the anterior part (outlined with the blue line). The image is used under [CC BY 4.0¹](#): cropped from the original. The author: Keith M. Meek. Source [here²](#). The detailed description of the imaging method can be found in [23]. c) Electron microscopy image of the corneal stroma, showing high organization of collagen fibrils within lamellae. The image is used under [CC BY 4.0¹](#): cropped from the original. The author: Keith M. Meek. Source [here³](#).

The epithelium (50–60 μm) consists of 5–6 layers of cells [2, 21]. Superficial cells are flattened compared to the underlying layers. Between the cells, there appear nociceptors (7000 nociceptors per mm^2 [2]). In addition to providing protection and nutrition of the underlying stroma, epithelium also interacts and stabilizes the tear film on the corneal surface [2].

The corneal stroma (substantia propria) is the thickest layer, constituting approximately 90% of the thickness of the entire cornea. It is composed of lamellae formed by bundles of collagen fibers, predominantly collagen type I of an average diameter of 30 nm, with additional presence of other types (V, VI, XII, XIII, XIV, and XXIV [24, 25]). The fibers are arranged with remarkable regularity: a constant interfibrillar spacing with an average of 67 nm [26] is ensured with glycosaminoglycans and proteoglycans, which form bridges between fibers [2]. The spacing is also referred to as “D-periodicity” (specific to collagen I organization in tissues) and means the center-to-center separation [26, 27]. This composition is unique for the cornea among other tissues. Each fiber is further built of small fibril collagen molecules of approximate diameter of 4 nm [22, 26]. The lamellae are arranged parallel to the corneal surface. The stroma is estimated to consist of 200–250 lamellae, each approximately 2 μm thick and 0.2 mm wide. Their organization is different in the anterior one third of the stroma than in the remaining posterior part. In the posterior stroma, the lamellae lay on each other. Recently, it was also reported that they appear predominantly in the superior-inferior and nasal-temporal directions along the corneal curvature [22].

1 <https://creativecommons.org/licenses/by/4.0/>

2 <https://www.nature.com/articles/s41433-024-02969-7/figures/6>

3 <https://www.nature.com/articles/s41433-024-02969-7/figures/4>

Table 1.1: Refractive indices of corneal components reported in the literature. The average value for the cornea provided in the first row is well-established, and can be found in many sources, for example in [34].

		Refractive index	Reference
	Cornea (average)	1.376	
Stroma	Anterior stroma	1.380 ± 0.005	Patel et al. 1995 [35]
	Mid stroma	1.369 ± 0.007	Patel et al. 2008 [36]
	Posterior stroma	1.373 ± 0.001	Patel et al. 1995 [35]
	Collagen fibrilis	1.411 ± 0.004	Leonard & Meek 1997 [37]
	Extracollagen substance	1.365 ± 0.009	Leonard & Meek 1997 [37]
	Keratocytes	1.381 ± 0.004	Gardner et al. 2015 [38]
Other layers	Epithelium	1.410 ± 0.005	Patel et al. 1995 [35]
	Bowman's layer	1.380 ± 0.011	Patel et al. 2008 [36]

For the anterior part, the general arrangement is parallel to the corneal curvature, however, the fibers intertwine and have been compared to the fibers in a bird nest [22, 28]. Figure 1.3 b) illustrates the alignment of the corneal lamellae, whereas Figure 1.3 c) shows the high organization of collagen fibers within each lamella.

Between the collagen lamellae, there appear fibroblasts specific to the cornea, called keratocytes. Their density differs along the stromal depth ranging from 10% in the anterior (20 000–24 000 cells/mm² [2]), decreasing in the central stroma to 9% and increasing to 17% in the posterior stroma [29]. Other components of the stroma are the nerves, which travel from the sclera in parallel to the corneal curvature just below the anterior one-third of the cornea. In its central part (approximately 1 mm from the limbus), they lose their perineurium and myelin sheaths, divide into small branches and start changing direction to pierce the Bowman's layer and reach the epithelium.

1.2.3 Corneal transparency

Despite its complex composition, the cornea appears transparent in the visible and near-infrared (NIR) spectral range, maintaining the transmittance level above 90% for the wavelength range 500–1300 nm [26, 30]. The first theories from the nineteenth century deliberated about its optical homogeneity, or the uniformity of the refractive index [26, 31], but measurements of refractive indices of corneal components have excluded these approaches. The refractive index appeared to be significantly different for particular structures [26] (see Table 1.1). Further, Maurice (1957) [32] suggested that the corneal stroma is ordered in an ideal hexagonal crystalline lattice (exhibiting long-range order). He stated that the scattering occurs, but there is constructive interference in the forward direction and destructive interference in the backward direction (analogous to crystals). However, electron microscopy and X-ray diffraction imaging techniques revealed that collagen fibers are ordered only in the short-range, and that no hexagonal lattice was observed [24, 33]. Short-range order is understood here as a quasi-random arrangement of collagen fibers with local order, meaning a uniform but not identical spacing [24, 26, 33].

Currently, short-range order was shown to be sufficient for appearance of constructive and destructive interference in the forward and backward directions, respectively [24]. As such, the concept of Maurice, although modified, has survived. Additionally, it was demonstrated that introducing irregularities within a theoretical model of highly organized collagen fibers leads to increased backscatter [26].

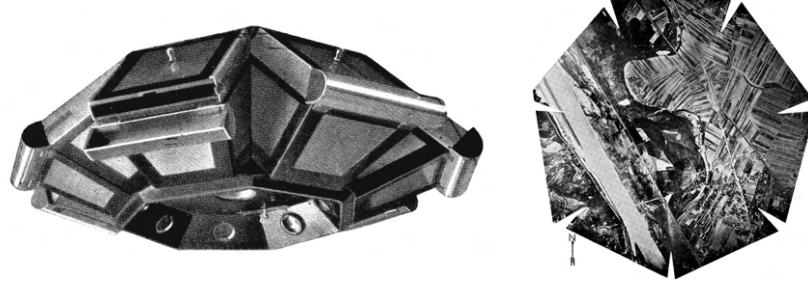


Figure 1.4: Left: a photograph of Scheimpflug camera designed to enable terrestrial photography from a balloon. Right: one of the first photos of the area taken from a balloon with Scheimpflug camera [Scheimpflug 1911 [48]].

Healthy corneal stroma naturally contains irregularities, such as transitions between lamellae or keratocytes (including organelles and lipid membranes). These structures contribute to the creation of backscattered light observed in OCT or Scheimpflug imaging. Also, pathological irregularities may appear with conditions that distort fibril arrangement, such as keratoconus, some types of Ehlers-Danlos syndrome [39] and aging [40]), as well as corneal swelling after contact lens wear [41] or Fuchs' dystrophy [42, 43]). In such cases, the backscatter signal, altered relative to the healthy cornea, may be utilized for diagnostics.

Another issue is the internal transparency of stromal keratocytes. Jester et al. [44, 45] presented evidence for the presence of so-called corneal crystallins. Their work reports that, similarly to the human lens, crystallins are water-soluble proteins expressed by keratocytes to match their refractive index to that of the surrounding stroma. It is also noteworthy that, as mentioned in Section 1.2.2, corneal nerves appear only in the periphery and are devoid of a myelin sheath, which also contributes to transparency [2].

1.3 CORNEAL IMAGING: SCHEIMPFLUG TECHNOLOGY

The principle currently known under name “Scheimpflug principle” was first formulated in 1901, in a patent by Jules Carpentier — a French engineer and designer of cameras [46]. Three years later, Theodor Scheimpflug, an Austrian naval officer annoyed with the poor quality of nautical charts [47], published his own patent based on a rule analogous to that proposed by Carpentier. He presented a design of a camera that allowed one to take panoramic photographs from a balloon (see Figure 1.4) [48]. The work attributed much to cartography, introducing photographic maps of the area, but also to photography and ophthalmology.

1.3.1 Principle

In classical photography, the detector plane (plane of sharp reproduction), object plane and the planes of the optical system are set perpendicular to the optical axis. This allows the observation of objects equidistant (within a depth of focus) from the optical system. Jules Carpentier and Theodor Scheimpflug proposed a configuration that will allow the observation of objects of unequal distances. The detector, optical

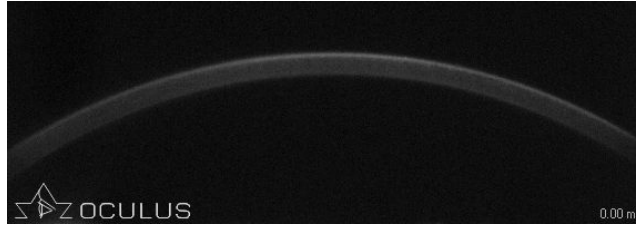


Figure 1.5: Exemplary Scheimpflug image of the human cornea registered with Corvis ST (Oculus Optikgeräte, GmbH, Wetzlar, Germany).

system and observed object are set at appropriate angles so that their planes intersect in a common straight line, called the Scheimpflug line [49].

1.3.2 Anterior eye imaging

In ophthalmology, “Scheimpflug principle” was first introduced in years 1960–1970 by Drews, Niesel, Brown and Dragomirescu & Hockwin, while the latter two created the first commercially available camera, Topcon SL-45 (Topcon, Tokyo, Japan) [49]. Currently the market is dominated by Pentacam HR (Oculus Optikgeräte, GmbH, Wetzlar, Germany). However, other devices are also available, such as Corvis ST (also by Oculus), GALILEI (Ziemer Ophthalmic Systems AG, Port, Schweiz), SIRIUS (Costruzione Strumenti Oftalmici, Firenze, Italy) or Optical Biometer AL-Scan (NIDEK CO., LTD.) [50]. One of the challenges of Scheimpflug-based imaging devices is image distortion caused by the oblique angles of the object and image planes [51]. To correct for the distortion, a series of geometrical transformations is applied. After these corrections, Scheimpflug imaging allows not only for observation but also for quantitative evaluation of anterior eye biometry or corneal topography (with rotating camera) [52]. Figure 1.5 presents an exemplary Scheimpflug image of the human cornea.

1.3.3 Corneal densitometry

The first attempts at quantitatively describing and measuring corneal scattering date back to the early 1980s [53]. Corneal densitometry can be intuitively understood as the measure of optical density of the cornea, associated with the brightness of the corneal appearance in the image. Early studies using Scheimpflug images of the anterior eye have already shown the potential of this measure both for the cornea and the crystalline lens [54, 55]. The clinical assessment of corneal densitometry has become widely accessible through the commercial Scheimpflug-based system: Pentacam HR [49, 56, 57]. It has demonstrated significant utility, serving as an early biomarker for subclinical keratoconus [58], an indicator of corneal health during and after bacterial keratitis [59], a method for tracking changes after laser surgery [60]. Conventionally, densitometry in Pentacam HR is expressed in grayscale units (GSU) of a digital Scheimpflug image. It is known to be calculated as the average intensity of pixels in a given region of interest (ROI), although information on standardization or calibration procedures, if any, is not publicly available. As such, corneal densitometry is essentially calculated as the average pixel intensity of the different layers and zones of the cornea. Recently, several studies have introduced alternative devices

and measures for corneal densitometry [56, 57, 61, 62], however, clinically it remains restricted to Pentacam HR.

1.4 CORNEAL IMAGING: OPTICAL COHERENCE TOMOGRAPHY

1.4.1 Principle

In principle, an OCT system is based on a Michelson interferometer [63, 64] and nowadays is being almost exclusively designed as one that explores the frequency (spectral) domain of the signal. In this way, Fourier-domain optical coherence tomography (FD-OCT) does not have a moving reference mirror and is equipped with either a spectrometer (usually named spectral-domain optical coherence tomography (SD-OCT) or SOCT) or a light source, which sweeps over a broad range of wavelengths (swept-source optical coherence tomography (SS-OCT)) [63].

The interferometric signal resulting from the recombination of the sample and reference beams is complex, however, in clinical devices, it is usually restricted to the amplitude or intensity [65]. The B-scan, i.e., a cross-sectional OCT image recorded for a sample is a set of in-depth amplitude or intensity profiles, named A-scans. Every single A-scan is a radiation echo of the reflective components of the sample [66]. Therefore, OCT requires quite a large penetration depth, that is, low absorption and, consequently, low signal attenuation. For biological tissues, it is achieved by setting the central wavelength of the broadband source in the red and near infrared region (NIR) [67]. The first biological application of OCT was reported by Fercher in 1988 [68] and considered measuring of eye axial length. Ophthalmic applications constitute the majority of current research in OCT [66] until now. This is particularly because the high transparency of the eye components ensures penetration to the retina, showing the subsequent components with satisfactory resolution and allowing the distinction of the main layers [66]. Figure 1.6 presents an exemplary OCT B-scan recorded for human cornea.

1.4.2 Volume of coherence

One of the parameters that describe OCT is the volume of coherence (also referred to as the coherence volume, the scattering volume [69], the coherent probe volume [67] or the probed optical resolution element [70]). Different definitions can be found in the literature for this quantity. Nevertheless, all of them use the coherence length of the light source l_c and the beam radius (“spot size”) w in the sample, which are also specific to the medium (they will be different in the air than in the sample due to the difference in refractive indices). These two measures are directly correlated with axial δx and transversal δz resolutions of OCT device, which will be explained in following paragraphs.

OCT light sources use the Gaussian beam, whereas the beam diameter $2w$ is usually calculated as the full width of the beam at $\{1/e \cdot \text{amplitude of OCT signal}\}$. Its radius w , can be equal to the radius at beam waist (w_0) if the beam is focused at particular depth for which the coherence volume is calculated. The equation connecting beam radius with the transversal (horizontal) resolution δx of OCT in the sample [64] is:

$$\delta x = \sqrt{2 \ln 2} w_0 = \sqrt{2 \ln 2} \frac{\lambda_0}{\pi NA}, \quad (1.14)$$

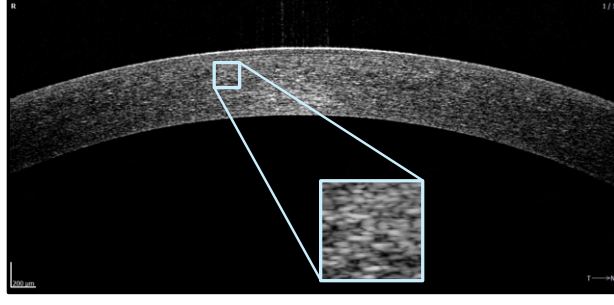


Figure 1.6: OCT B-scan of the human cornea registered with SOCT REVO 80 (Optopol, Zwiercie, Poland). The outlined region zooms-in the OCT speckle pattern.

where the beam w_0 is the beam radius at the beam waist (assuming the beam being focused), λ_0 is the central wavelength, NA is the numerical aperture. The expression $\sqrt{2 \ln 2}$ is used to transform w_0 from full width at $\{1/e \cdot \text{amplitude of OCT signal}\}$ to full width at $\{0.5 \cdot \text{amplitude of OCT signal}\}$, conventionally called full width half maximum (FWHM). This transformation can be applied for the Gaussian beam. Coherence length l_c is correlated with the spectral bandwidth of the light source and thus it represents the temporal coherence. The spectral shape of the light source for OCT is assumed Gaussian [67]. The bandwidth $\Delta\lambda_{FWHM}$ is described as the wavelength range at FWHM. Then, the coherence length equals the axial (vertical) resolution δz of OCT in the sample [64, 67]:

$$l_c = \delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta\lambda_{FWHM}}. \quad (1.15)$$

Volume of coherence can be understood as the volume of space within which the radiation is coherent (spatially and temporally). Mandel & Wolf [71] and Zardecki et al. [72] define coherence volume as:

“The right-angled cylinder, whose base is the area of coherence in a plane normal to the direction of propagation and whose height is the coherence length. It is also the volume corresponding to one cell of phase space of the photons [72].”

The area of coherence is defined here as the area of the beam cross section, calculated as πw^2 . Consequently, the formula for coherence volume is:

$$V_c = \pi w^2 l_c. \quad (1.16)$$

Weatherbee et al. [69] defines the scattering volume as:

$$V_c = \pi^{\frac{3}{2}} w^2 \frac{l_c}{2}. \quad (1.17)$$

The above equation is derived as an integral over 3-D Gaussian beam point spread function (being the fusion of 2-D point spread function and the light source spectrum). This 3-D Gaussian beam point spread function can be presented as the three-variate Gaussian function, with mean equal to 0 for all dimensions and the following standard deviations in Cartesian coordinates:

$$(\sigma_x, \sigma_y, \sigma_z) = \left(w, w, \frac{l_c}{2} \right), \quad (1.18)$$

giving the resultant equation for Gaussian function:

$$f(x, y, z) = \frac{1}{w^2 \frac{l_c}{2} (2\pi)^{\frac{3}{2}}} \exp \left(- \left[\frac{x^2}{w^2} + \frac{y^2}{w^2} + \frac{z^2}{(\frac{l_c}{2})^2} \right] \right). \quad (1.19)$$

There is some inconsistency in this approach, as both l_c and w are defined at [FWHM](#), whereas σ parameter of the Gaussian function is the standard deviation. Therefore, probably instead directly using l_c and w the standard deviations of the light source spectrum and the Gaussian beam function should be calculated, based on l_c and w respectively.

Sugita et al. [70] present the equation for V_c as the volume of ellipsoid, including also the correction for beam dimensions in a particular medium:

$$V_c = \frac{4}{3} \pi w^2 \frac{l_c}{2} \frac{1}{n}, \quad (1.20)$$

where n is the refractive index of the medium (sample). Almasian et al. [65] give another version of this formula:

$$V_c = \frac{4}{3} \pi w_0^2 \sqrt{2 \ln 2} \frac{l_c}{2}, \quad (1.21)$$

where w_0 is the radius at beam waist.

1.4.3 Speckle

Speckle is a characteristic and inherent feature of coherent imaging, and thus [OCT](#). In a single [OCT](#) B-scan, it appears as a specific spot pattern, blurring the structural details in the image (see Figure 1.6). Therefore, it is also referred as [OCT](#) speckle noise. Speckle phenomena is well studied and has strong theoretical basis. It stems from voxel-to-voxel phase and amplitude fluctuations of the radiation reflected by small tissue components. The detailed description of speckle formation can be found in Goodman [73]. Here, only its most important statistical characteristics are provided. Depending on the number of scatterers in the volume of coherence (defined in previous subsection: 1.4.2), the speckle evolution can be distinguished into two cases:

1. Fully developed speckle field

For the large number of scatterers, tending to infinity, the real and imaginary parts of the complex amplitude (phasor) of the speckle field of amplitude A and phase ϕ , defined as:

$$A \exp(j\phi) = A \cos \phi + iA \sin \phi, \quad (1.22)$$

follow the 2-D Gaussian distribution in compliance to the central limit theorem.

Consequently, it can be derived that amplitude (i.e, the length A of the phasor) is governed with the Rayleigh distribution with the following probability density function [73]:

$$p(A) = \frac{A}{\sigma^2} \exp \left(-\frac{A}{2\sigma^2} \right), \quad A > 0, \quad (1.23)$$

where A is the OCT signal amplitude and σ is the scale parameter of Rayleigh distribution. The contrast ratio, i.e, the ratio of the standard deviation to the mean for Rayleigh distribution is equal to [74]:

$$CR = \sqrt{4/\pi - 1} \approx 0.5227. \quad (1.24)$$

When speckle follows the above statistics it is recognized as “fully developed speckle field”. In practice, the amplitude is usually normalized by its RMS value:

$$A/\sqrt{\langle A^2 \rangle}. \quad (1.25)$$

Such a normalization leads the speckle statistics to follow the Rayleigh distribution with scale parameter σ fixed to $\sqrt{2}/2$ [75].

2. Small number of scatterers

For small number of scatterers the central limit theorem is not applicable. Then, the speckle field amplitude was shown to follow the K-distribution [76]. The probability density function for K-distribution is then defined as follows:

$$p(A) = \frac{4}{\Gamma(v)} \sqrt{\frac{v}{\langle A^2 \rangle}} \left(\frac{vA^2}{\langle A^2 \rangle} \right)^{v/2} K_{v-1} \left(2\sqrt{\frac{vA^2}{\langle A^2 \rangle}} \right), \quad (1.26)$$

where v is the shape parameter of K-distribution, $\Gamma(\cdot)$ is the gamma function, $\langle \cdot \rangle$ is the average value, $K_v(\cdot)$ is a modified Bessel function of the second kind of v -th order [76].

A substantial amount of studies were devoted to the problem of denoising OCT images, as speckle noise disrupts the clear observation of the tissue [77]. However, another approach focuses on the correlation of speckle statistics with the microstructure of the material [77–79]. For example, OCT was utilized for tissue segmentation [80], assessing its mechanics [81], measuring intraocular pressure from static OCT images [82] or estimating optical parameters of the tissue [83]. It is now well established that OCT speckle of corneal stroma carries useful information about its microstructural components much smaller than the resolution of OCT [75, 84, 85]. This has recently been further confirmed by examining changes in collagen fibers, induced by a cross-linking procedure, with spectroscopy [86].

1.4.4 Calculations for SOCT REVO 80 and cornea

It was of interest, what is the volume of coherence (V_c) for the particular OCT device used in this work. Therefore, all necessary technological specifications were collected from SOCT REVO 80 manual (Optopol, Zawiercie, Poland, see Table 1.2). Despite the transversal resolution δx is mentioned to be “12 μm , typical 18 μm ” [87] the actual resolution can vary depending on the scanning protocol. Therefore, the transverse resolution was estimated to 11 μm for anterior eye scanning protocol⁷.

Usually, current OCT light sources use super luminescent diodes (SLD), and this is also the case of SOCT REVO 80. The shape of the spectrum for such sources

⁷ Thanks to OPTOPOL company, upon request, the transversal resolution was tested with 1951 USAP Test Target (Thorlabs Inc., Newton, New Jersey, United States) with a diameter of 1".

Table 1.2: Specification of SOCT REVO 80 (Optopol, Zawiercie, Poland) device [87].

Name	Symbol	Value
Central wavelength	λ_0	850 nm
Half bandwidth	$\Delta\lambda_{FWHM}$	50 nm
Axial resolution in tissue	δz	5 μm
Transversal resolution	δx	12 μm , typical 18 μm

is usually non-Gaussian, however, for the matter of rough estimation of coherence volume it was here assumed to be Gaussian. Although the coherence length l_c is equal to transversal resolution δz [64], for control, it was also calculated with Equation 1.15:

$$l_c = \frac{2 \ln 2}{\pi} \frac{0.850^2}{0.050} \approx 6.38 \text{ } [\mu\text{m}]. \quad (1.27)$$

The resultant value slightly differs with the value of δz given by the producer (see Table 1.2), therefore the one calculated was further utilized. Both w_0 and l_c were assumed to be the beam parameters in the sample. Subsequently, three V_c values were calculated using Equations 1.16, 1.17 and 1.21, resulting in 1.75, 1.55 and 1.37 μm^3 , respectively. Figure 1.7 illustrates the volume of coherence calculated with Equation 1.17. The values obtained were close to each other, therefore further only the one calculated with Equation 1.21 was used.

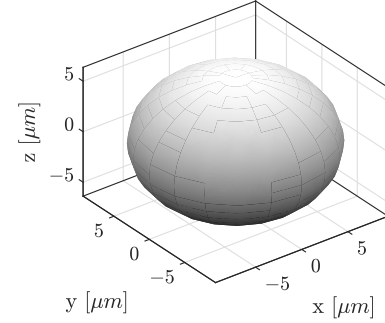


Figure 1.7: Volume of coherence calculated for SOCT REVO 80 (Optopol, Zawiercie, Poland) using the Equation 1.21.

Corneal stroma is composed of structures of different sizes and shapes. Hence, estimating the number of particles within the volume of coherence is a complex task which should be considered as a separate investigation. Here, it was aimed to calculate only a rough estimate, to have an overview on how distinct structures relate in size to V_c . The problem was subdivided to account separately for different tissue components: (1) collagen fibers, (2) collagen lamellae and (3) keratocytes. Firstly, (1) collagen fibers were simplified as infinite cylinders, which are aligned in parallel and “pierce” horizontally the volume of coherence. This approach reduced the calculations to 2-D problem. The center-to-center spacing between collagen fibers is 67 nm (see Section 1.2.2). Hence, every cross section of collagen fiber with the margin around it was simplified to be a circle of radius $r_{fibril} = 67/2$ nm, and cross-sectional area of $a_{fibril} = \pi r_{fibril}^2$. The number of scattering particles was calculated by dividing cross-sectional area of volume of coherence (A_c), approximated with ellipse ($A_c = \pi w_0 \frac{l_c}{2}$) by a_{sphere} . This worked out to number 2.65×10^4 , understood here as a number of collagen fibers within volume of coherence.

Subsequently, collagen lamellae (2) were simplified to be horizontal layers of thickness of 2 μm , stack on each other. As their width of approximately 0.2 cm and length of a few millimeters significantly exceed the size of volume of coherence (see Section 1.2.2) it was assumed that one lamella fills the whole horizontal space of volume of coherence. Thus, calculations were reduced to 1-D problem. The coherence

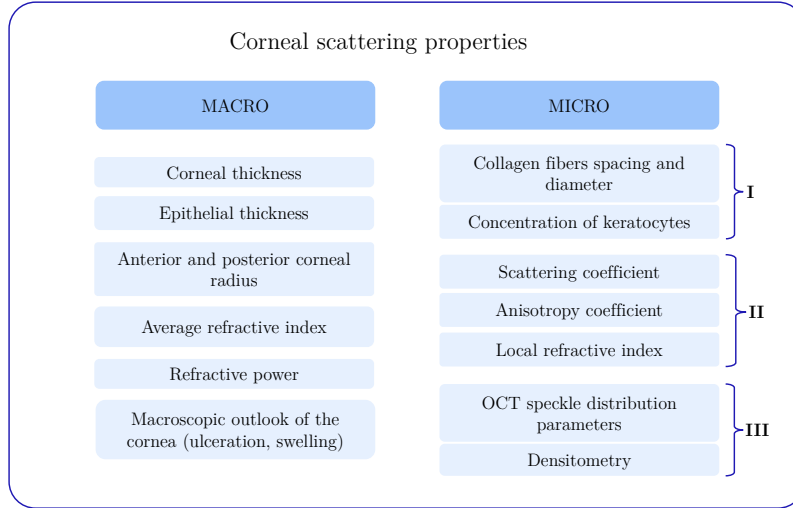


Figure 1.8: Illustrative macroscopic and microscopic parameters related to corneal scattering. The microscopic parameters can be divided into three groups: I. Anatomical parameters (collagen spacing and diameter, keratocytes concentration); II. Optical parameters directly correlated with physical parameters (scattering, anisotropy coefficients and local refractive index); III. Parameters indirectly correlated with corneal anatomical parameters (OCT speckle statistics and densitometry) [own work].

length (l_c) was divided by the layer thickness, giving the number of lamellae within the volume of coherence, which was $3.18 \approx 3$.

Finally, having the density of keratocytes (3) of 20 000 per mm^3 (see Section 1.2.2), allowed for rescaling this number to the volume of coherence obtained from Equation 1.21). The resultant value was 0.035, indicating volume of coherence can include only parts of keratocytes.

1.5 SUMMARY OF MACRO AND MICRO PARAMETERS DESCRIBING CORNEA

The preceding sections describe the main concepts for corneal scattering phenomena and present imaging techniques for its assessment. In summary, the parameters describing corneal scattering are depicted in Figure 1.8 and divided to those related to macroscopic and microscopic corneal characteristics.

CONFOUNDING FACTORS IN SCHEIMPFLUG-BASED DENSITOMETRY

Partially based on:

Miażdżyk, M. *et al.* Assessing and compensating for the confounding factors in Scheimpflug-based corneal densitometry. *Biomedical Optics Express* **13**, 6258–6272 (2022).

The clinically used corneal densitometry is in its infancy and appears to be confounded by several factors. In this chapter, those resulting from eye biometry are evaluated and compensated using image preprocessing. Getting to the final methodology (also presented in [88]) was not straightforward. A comprehensive investigation on image preprocessing methods was needed, as common methods simply taken off-the-shelf appeared to be insufficient in the context of this study. The author finds value in sharing with the reader not only the successful methodology, but also in showing, for future reference, which considered methods were found to be unsuitable. Therefore, this chapter will be kept chronologically. Firstly, preliminary observation, which was the motivation for writing this thesis, is introduced and expanded to more thorough research (Section 2.1). Further, Section 2.2 presents the dissertation process to find the satisfactory compensation method for the recognized confounding factors. The final algorithm and results are presented in Section 2.3. In the end, the summary, including the discussion of the results and further work is provided (Section 2.4).

2.1 INTRODUCING THE OBSERVED ISSUE

2.1.1 Initial dataset

A pilot study on corneal backscatter was performed in two age groups. The purpose of the study was to check the differences in the pixel values of the cornea in Scheimpflug images for older and younger subjects, hoping to assess the effect of microstructural aging using corneal densitometry. The retrospective data set consisted of measurements from the Corvis ST air puff tonometer (Oculus Optikgeräte, GmBH, Wetzlar, Germany), which records corneal images with Scheimpflug technology at a sampling rate of 4330 frames per second. The age groups consisted of 68 younger subjects and 71 older objects and after manual outlier removal 61 young, aged 20–30 years (mean $\pm$ standard deviation (STD): 23.7 ± 2.2) and 62 older, aged 50–75 years (mean $\pm$ STD: 61.3 ± 6.6) subjects remained. The criteria for removing the outliers were artifacts visible in images. Also, one cornea with CCT = 410 μm was removed. The groups of subjects are denoted further as group Y (young) and group O (older). For all subjects, both eyes were examined, but for further analysis, images of only one eye (right or left) were chosen randomly. For each subject, Corvis ST data include a sequence of 140 Scheimpflug images (approx. 32 ms recording) showing the corneal dynamic response to an air-puff. Each cross-sectional image (cross section along transversal plane) from the Scheimpflug camera covers 8.5 mm [89] of the central cornea (see Figure 2.1). The exported grayscale images were of size 200×576 pixels.

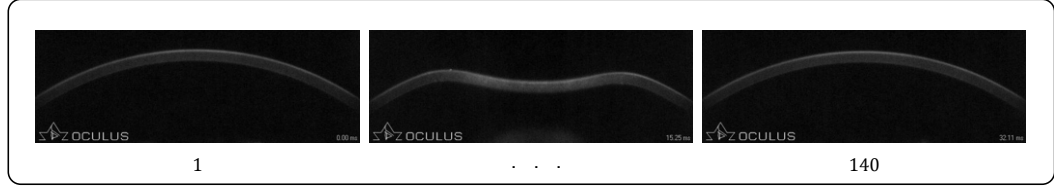


Figure 2.1: Illustrative three out of 140 frames imaging corneal response for an air puff recorded by Corvis ST tonometer for one subject.

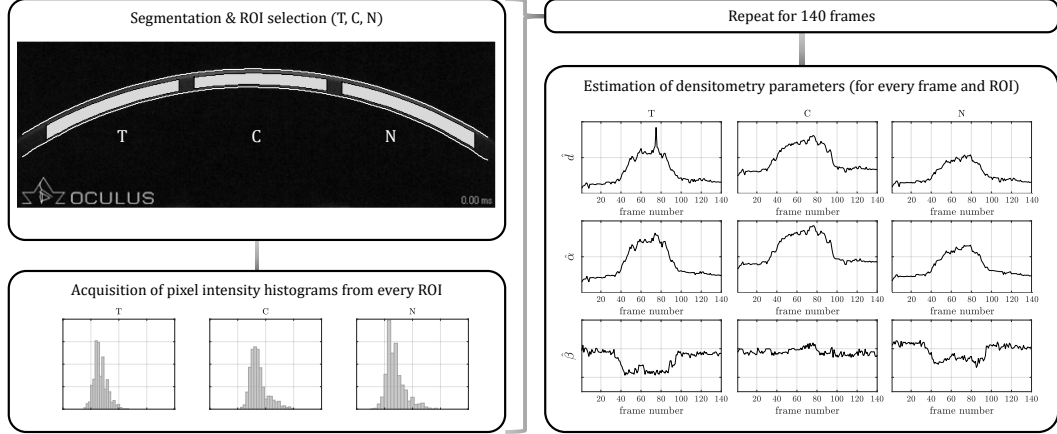


Figure 2.2: Flowchart of the algorithm introduced earlier in [90] and improved in [91]. **T**, **C**, **N**: temporal, central and nasal ROIs used for pixel value analysis. α, β, d : parameters estimated from pixel values in ROI: scale and shape parameters of Weibull distribution and the mean, respectively [own work based on [90]].

The resolution of the image was estimated to $r_x = 14.8 \mu\text{m}/\text{pixel}$ horizontally, and $r_y = 24 \mu\text{m}/\text{pixel}$ vertically. The horizontal resolution was calculated by dividing the horizontal measuring range of Corvis ST ($8500 \mu\text{m}$) and the horizontal size of the image (576 pixels). The vertical resolution was estimated using the CCT of the subject, measured by Corvis ST software and dividing it by the CCT in pixels, measured in images as the vertical distance between corneal edges (the measurement method will be described later in Section 2.2.3). The mean vertical resolution for the whole group of 123 subjects was $23.9961 \pm 0.2125 \mu\text{m}/\text{px}$ (mean $\pm$ STD), and was rounded to $24 \mu\text{m}/\text{px}$.

2.1.2 Primary densitometry algorithm

The algorithm for corneal Scheimpflug image segmentation and pixel analysis introduced earlier by Consejo et al. [90] was adapted and improved. The algorithm segments the corneal stroma by performing edge detection in every frame, defines the regions of interest (ROI), and then estimates pixel parameters from ROI data, collectively named in this work as densitometry parameters (see the flowchart in Figure 2.2). The improvement included adaptation for the analysis of regions other than the central corneal region and robustness for the dynamic corneal response, which was part of the previous work [91]. Pixels including the company logo were replaced by a set of Gaussian random variables with mean and standard deviation corresponding to the sample mean and sample standard deviation of the logo sur-

roundings. A part of this work was also extending the algorithm to collect a number of different densitometry parameters (instead of estimating only Weibull distribution parameters):

1. *Parametric (distributional) approach* with 2-parameter Weibull and 2-parameter Gamma distribution fitting. Both distributions are characterized with scale and shape parameters, estimated with the method of maximum likelihood (`wblfit()` and `gamfit()` Matlab functions). The probability density function of Weibull distribution is defined as:

$$f_X(x | \alpha, \beta) = \frac{\beta}{\alpha} \left(\frac{x}{\alpha} \right)^{\beta-1} e^{-(x/\alpha)^\beta} \quad \text{for } x \geq 0, \quad (2.1)$$

where $\alpha > 0$ is the scale parameter, $\beta > 0$ is the shape parameter, x denotes the values of image pixel intensity in a specified region of interest (ROI). The probability density function for Gamma distribution is:

$$f_X(x | \theta, k) = \frac{1}{\theta^k \Gamma(k)} x^{k-1} e^{-\frac{x}{\theta}} \quad \text{for } x \geq 0, \quad (2.2)$$

where $\theta > 0$ is the scale parameter, $k > 0$ is the shape parameter, x denotes the values of image pixel intensity in a specified region of interest (ROI) and $\Gamma(\cdot)$ is the Gamma function. As to the author's knowledge, there is no theoretical model available describing the pixel intensity distribution in corneal Scheimpflug images, the choice of the distribution in this case is the trade-off between GoF and having possibly a small number of parameters to avoid over-parameterization. The application of these particular distributions is justified because both have already been used successfully in the corneal Scheimpflug image analysis [90, 92].

2. *Non-parametric approach*, which included taking the mean pixel value of the ROI data, called further simply densitometry, $\hat{d}$, as this parameter is used clinically in commercial devices [52]. Two more parameters for non-parametric approach were attempted, since these two are successfully used in OCT speckle analysis [75]: the contrast ratio (CR), calculated as the standard deviation of the ROI data divided by its mean, and the root mean square (RMS) distance (D_{RMS}) between the kernel density estimator of ROI data and the probability density function of standardized Rayleigh distribution:

$$D_{\text{RMS}} = \sqrt{\int \left(f_X(x | \frac{\sqrt{2}}{2}) - \hat{f}_{\text{ROI}}(x) \right)^2 dx}, \quad (2.3)$$

where $\hat{f}_{\text{ROI}}(x)$ is the kernel density estimator of ROI data, calculated with the `ksdensity()` Matlab function, and $f_X(x | \frac{\sqrt{2}}{2})$ is the probability density function of standardized Rayleigh distribution, fixing its parameter to $\sigma = \frac{\sqrt{2}}{2}$. However, in the final methodology, both CR and D_{RMS} were not considered because, unlike in the case of OCT speckle, there is no physical interpretation linking the pixel values in corneal Scheimpflug images and these two parameters.

The idea of analyzing pixel values using parametric and non-parametric approach was introduced in [75]. Utilizing a non-parametric approach allows to avoid distribution fitting procedure and tackling with the goodness-of-fit. However, when choosing the appropriate distribution for the data, the parametric approach provides the results with additional information.

2.1.3 Considerations on the ROI size

Three ROIs, that is, temporal, central and nasal, further denoted as T, C, N were chosen, because other studies also consider corneal segmentation into central and non-central parts [93]. In addition, a preliminary study, in which a horizontally moving ROI was implemented (ROI size: 200 pixels, step: 1 pixel, left and right margin: 30 pixels), showed that the shape parameter of the Weibull distribution has a greater dynamic range in the lateral parts than in the central part (see Figure 2.3). The images of the left eyes were flipped to correspond to those of the right eyes. During the analysis, different ROI widths were tested. Starting from horizontal size of 200 pixels, in the final methodology it was set to 120 pixels, to avoid the overlap of the ROIs. In other units, the central ROI covered about 1.8 mm of the horizontal corneal section. Symmetrically situated temporal and nasal ROI covered the corneal diameter section at a range from about 2 mm to 4 mm. The corneal image at the boundaries has lower contrast and the spillover from the iris and sclera is stronger. Therefore, to exclude the analysis of peripheral corneal parts and avoid these boundary effects, the left and right margins were empirically determined to 90 pixels (approximately 1.5 mm). Due to the low resolution of the Scheimpflug images, it was difficult to identify specific corneal layers. Therefore, the analysis was limited to the stroma and endothelium, as these two layers have similar light-scattering properties. To avoid including the tear film and corneal epithelium, a 120 μm (5-pixel) margin was established. One may debate whether the eye size could affect the predefined temporal (T), central (C), and nasal (N) ROIs. By modeling the cornea as a circular segment with the smallest and largest radii of 7.2 mm and 8.5 mm, respectively, the difference in arc length was less than 0.2 mm. This minimal variation justified the decision to use fixed lateral positions for the ROIs in this study. The final setup for ROI selection also finds confirmation in commercial devices, such as Pentacam HR, where similar regions are chosen for densitometry [93]. The ROI depth was initially set to fit the detected edges of the corneal stroma, and depended on the subject CCT.

2.1.4 First results: confounding factors alert

The observation of the results: dynamic plots of estimated Weibull scale $\hat{\alpha}$ and shape $\hat{\beta}$ parameters (see Figure 2.4) for three ROIs and 140 frames plotted as the mean group with standard error bars was the inspiration for the topic of this doctoral dissertation. The differences between Y and O groups in given densitometry parameters were so strong, that it would be irrelevant to conclude from that about corneal tissue microstructure variance. It may be deduced that the observed divergence rather stems from confounding factors. The observation of raw corneal images when examining the age groups separately confirms this supposition (see Figure 2.5). The main difference between images of older subjects compared to those of young subjects is a stronger appearance of iris backscatter caused by the shallower anterior chamber depth (ACD).

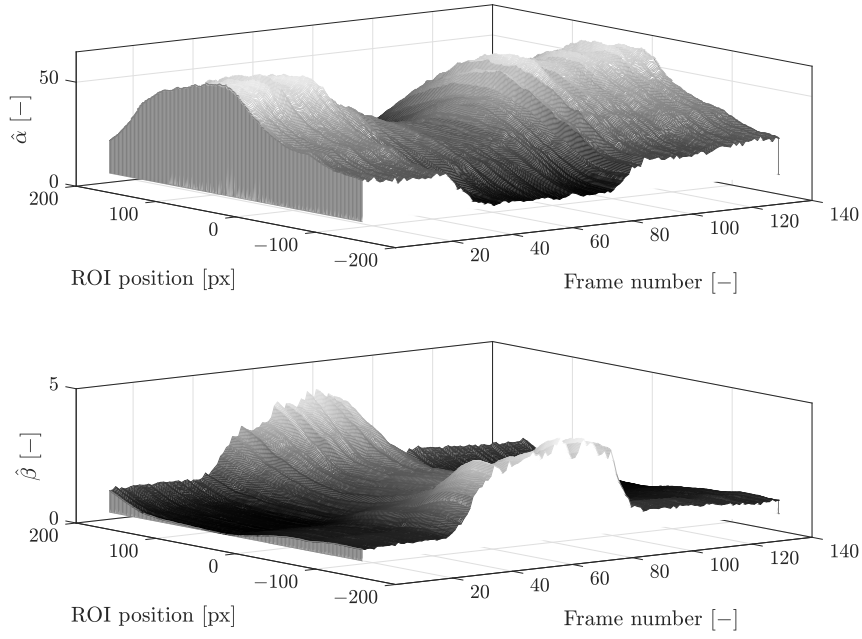


Figure 2.3: Illustrative result from preliminary study. The ROI of size 200 pixels, moving horizontally along the cornea with the step of 1 pixel, was applied to every of 140 frames acquired with Corvis ST device. The plot shows the estimated scale ($\hat{\alpha}$, top) and shape ($\hat{\beta}$, bottom) parameters of Weibull distribution for every ROI position and for every frame.

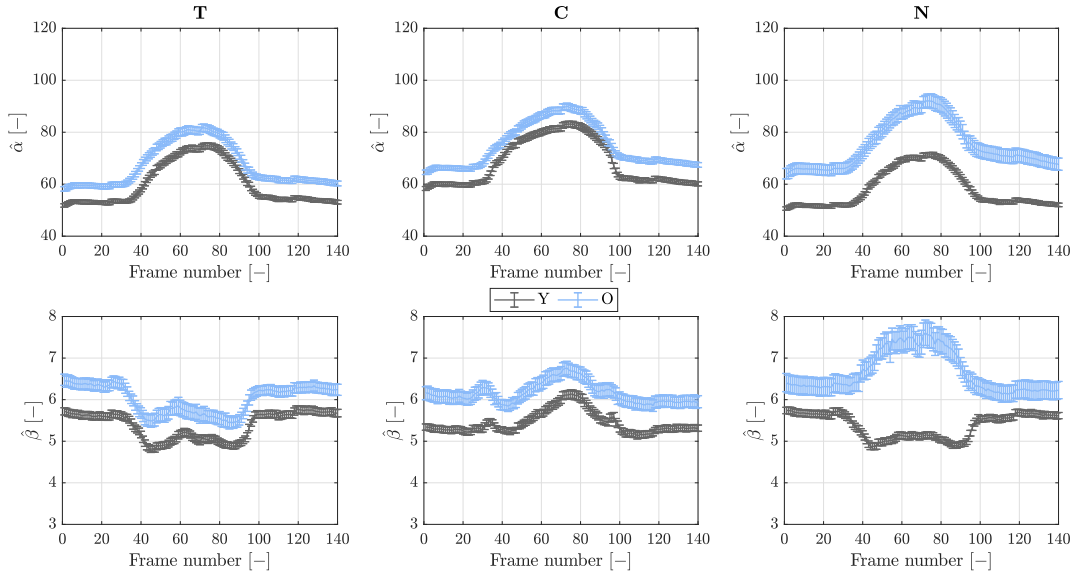


Figure 2.4: Group mean calculated separately for Y and O with standard error bars of Weibull scale ($\hat{\alpha}$) and shape ($\hat{\beta}$) parameter time series for three ROIs: temporal, central and nasal (T, C and N, respectively).

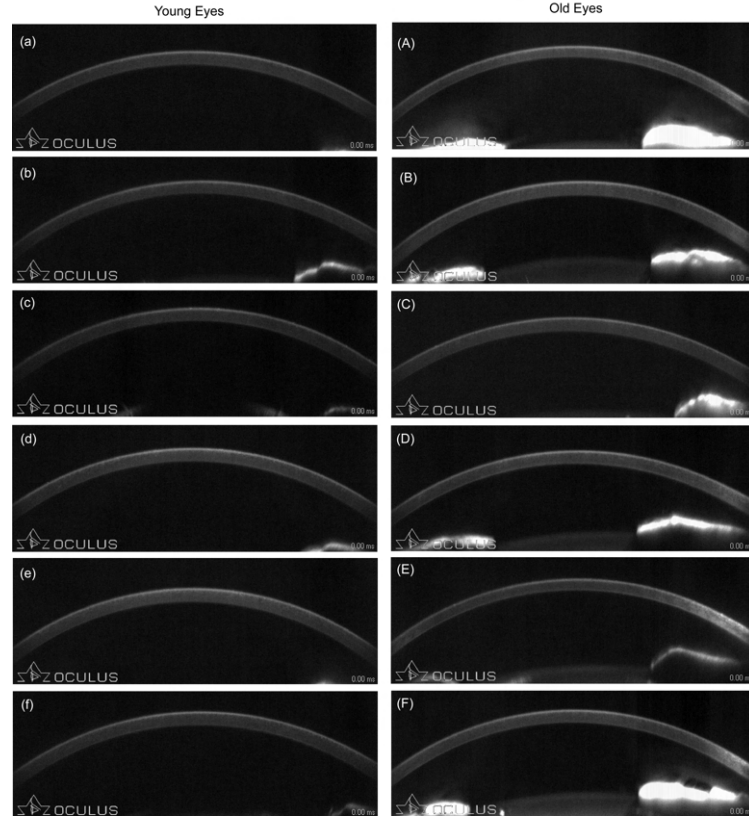


Figure 2.5: Illustrative examples of Scheimpflug corneal images acquired with Corvis ST. Left column contains young (Y) subjects, whereas the right is for older subjects (O).

Furthermore, in some images of older subjects, the outline of the pupil size (PUP) is apparent (see Figure 2.5 A, B, D, F). Consequently, the measurement from Corvis ST was supplemented with biometry parameters, that is ACD, PUP and CCT measured with IOLMaster 700 (Carl Zeiss Meditec AG, Jena, Germany). Based on the pilot study described above, it was concluded that densitometry is confounded by several factors. Relying on the first-order statistics, such as the mean of the ROI, which is currently used clinically leads to ambiguous or unexpected results. It was assumed that creating estimators of corneal densitometry parameters that will be independent of confounding factors will improve the diagnostic value of these parameters. The confounders identified in Scheimpflug imaging technology were eye biometry parameters: ACD, PUP and potentially CCT.

2.1.5 Pupil size

It was of interest if the pupil size (PUP), measured with IOLMaster 700 with a frontal camera corresponds to that observed in Scheimpflug images from Corvis ST, although all measurements were performed in the same room in mesopic lighting conditions. Of the whole Scheimpflug image dataset, only these subjects were chosen, which had the outline of the pupil visible in the first frame (49 subjects were found). Then the pupil was measured with “digital calipers”, localizing the edges of the iris (see

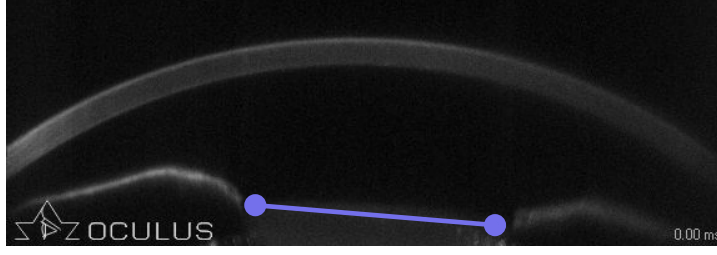


Figure 2.6: Illustrative example of pupil size (PUP) measurement in Scheimpflug image with “digital calipers”.

Figure 2.6) and calculating the distance between them, including the vertical and horizontal resolution of the image:

$$\text{PUP} = \sqrt{((x_1 - x_2)r_x)^2 + ((y_1 - y_2)r_y)^2}, \quad (2.4)$$

where (x_1, y_1) and (x_2, y_2) denote the location of the two edge points of the iris in pixels, whereas r_x , r_y are the horizontal and vertical resolutions of the image, respectively.

The manually extracted pupil size was strongly correlated with that measured with IOLMaster 700 (Pearson correlation coefficient, $r = 0.788$) and the correlation was highly statistically significant ($p < 0.001$). The linear regression indicated that the slope of the linear dependence between these two variables differs from identity function ($y = 0.695x + 0.799$), showing that the pupil size measured from Scheimpflug images is not the real pupil size. This may result, among others, from the rotation of optical planes in Scheimpflug technology. However, as the dependence is linear, and the correlation was high, the PUP from IOLMaster 700 could be successfully used as a representation of this confounding factor. The image showing PUP measured with IOLMaster 700 versus that measured manually from Scheimpflug images is shown in Figure 2.7. By the way the PUP manual measurement from Scheimpflug image was conducted, also the “eye tilt” was measured simply as the angle between the line connecting iris edges (as shown in Figure 2.6) and the horizontal line. No correlation of the “eye tilt” with subject age was found (Pearson correlation coefficient $r = 0.168$, $p = 0.249$).

2.1.6 First ideas on assessing the problem

Firstly, the idea of normalizing image data appeared naturally with observation of raw corneal images. Two primary image preprocessing methods were taken of the shelf: the normalization by the mean of the image and normalization of the ROI by its root-mean-square (RMS) value which is successfully used in OCT data normalization and has there a physical explanation [75, 79]. Figure 2.8 shows the resultant time-series plots of estimated Weibull ($\hat{\alpha}$ and $\hat{\beta}$) for the images normalized by its mean. At first sight, it may be noticed that this normalization method changed the values of $\hat{\alpha}$ parameter, also making groups more distinguishable in comparison to the analogical plot created for raw, non-normalized data, shown earlier (see Figure 2.4). The $\hat{\beta}$ parameter remained unchanged. Simple observation of time-series does not give straightforward reasoning on confounding factors, the median from first 10 values

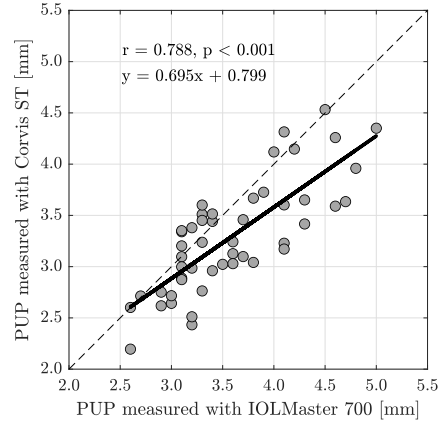


Figure 2.7: Pupil size (PUP) measured with Corvis ST against the PUP measured with IOLMaster 700. The scatter plot is created for 49 subjects from the older group (O), which had the outline of the pupil visible in Scheimpflug images. The dashed line indicates 1:1 line.

(before corneal deformation) and last 10 values (after return to the natural shape) of $\hat{\alpha}$ and $\hat{\beta}$ were taken, representing the natural, convex corneal shape. The parameters were denoted further as $\hat{\alpha}_M$ and $\hat{\beta}_M$. Then, the pairwise Pearson correlations between $\hat{\alpha}_M$, $\hat{\beta}_M$, biometry and age were tested before and after normalization for the whole cohort of subjects (Y and O together). The normalization of the image by its mean resulted in increase of correlation $\hat{\alpha}_M$ with age, ACD and PUP for temporal (T) and central (C) ROIs, whereas at the nasal side (N) the inverse effect was observed. Correlations with $\hat{\beta}_M$ remained unaffected for all of the ROIs. Figures 2.10 and 2.11 show the scatter plots of $\hat{\alpha}_M$ against age, ACD and PUP respectively before and after normalization. Due to changes in $\hat{\alpha}_M$, this method of image processing was rejected, as it was identified to incorporate the confounding factors into the densitometry parameter estimators. The reason for this is that images of subjects with shallower ACD were generally brighter (according to the flash from the iris), causing the mean pixel value of the whole image being higher. The correlation of mean of the image with ACD was $r = -0.515, p < 0.001$ and $r = -0.340, p = 0.003$, for Y and O group, respectively. Regarding the normalization of the ROI by its RMS value, in the resulting time series (see Figure 2.9) it can be observed that the estimated $\hat{\alpha}$ became the “mirror” of $\hat{\beta}$, suggesting some bias in estimation of α . That is why short investigation on the Weibull and Gamma estimation bias was performed.

Investigation on bias of the estimators

A set of vectors with Weibull and Gamma distributed random values was generated with `wblrnd()` and `gamrnd()` functions, respectively. The scale parameter (α for Weibull and θ for Gamma) was fixed to the value of 60. The shape parameter (β for Weibull and k for Gamma) took values of range $[1, 20]$ with the step of 0.05. This resulted in 381 vectors for Weibull distributed data and 381 vectors for Gamma distributed data. All vectors were normalized with the mean value and, separately, with the RMS value. Then `wblfit()` and `gamfit()` functions were used to estimate the $\hat{\alpha}$, $\hat{\beta}$ and $\hat{\theta}$, $\hat{k}$, respectively, for raw data and for data normalized in two ways. Figure 2.12 show the estimated scale $\hat{\alpha}$ against the β used to generate the Weibull

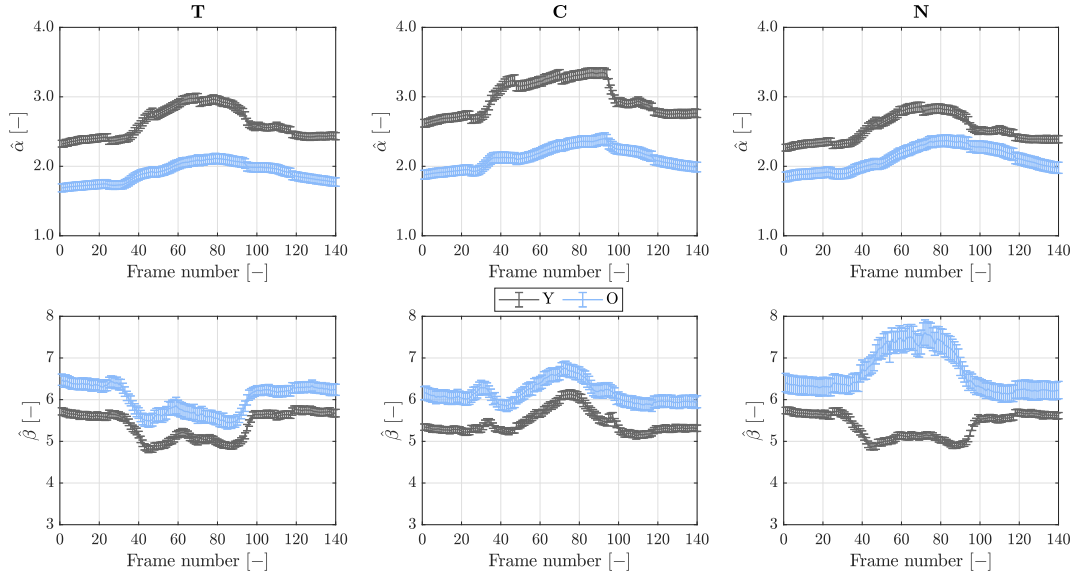


Figure 2.8: Group mean calculated separately for **Y** and **O** with standard error bars of Weibull scale ($\hat{\alpha}$) and shape ($\hat{\beta}$) parameter time series for three ROIs: temporal, central and nasal (**T**, **C** and **N**, respectively). Pixel values in the image were normalized by the mean calculated from whole image.

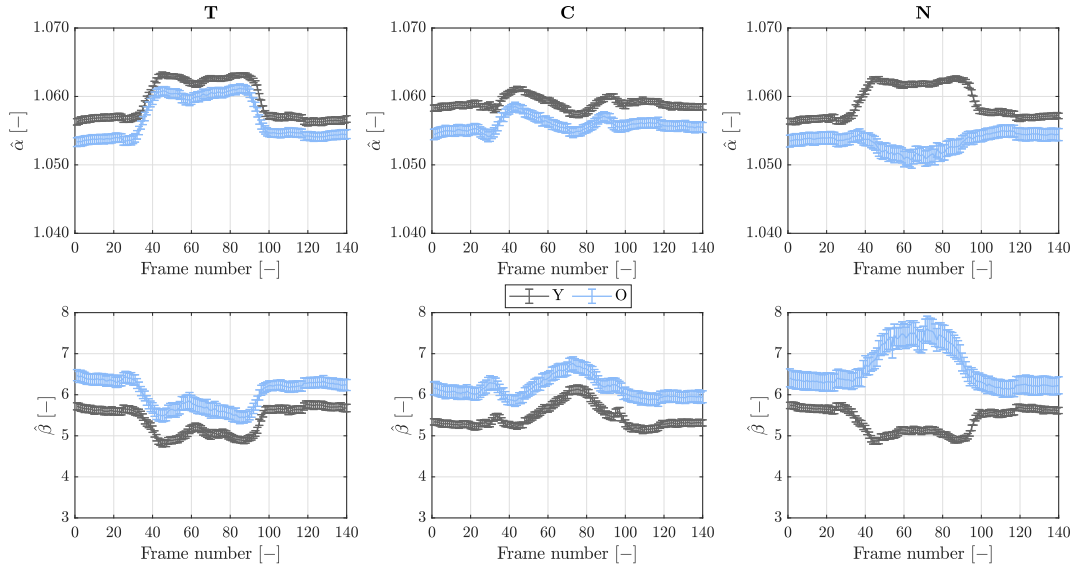


Figure 2.9: Group mean calculated separately for **Y** and **O** with standard error bars of Weibull scale ($\hat{\alpha}$) and shape ($\hat{\beta}$) parameter time series for three ROIs: temporal, central and nasal (**T**, **C** and **N**, respectively). Pixel values within each ROI were normalized by the root-mean-square (RMS) of this ROI.

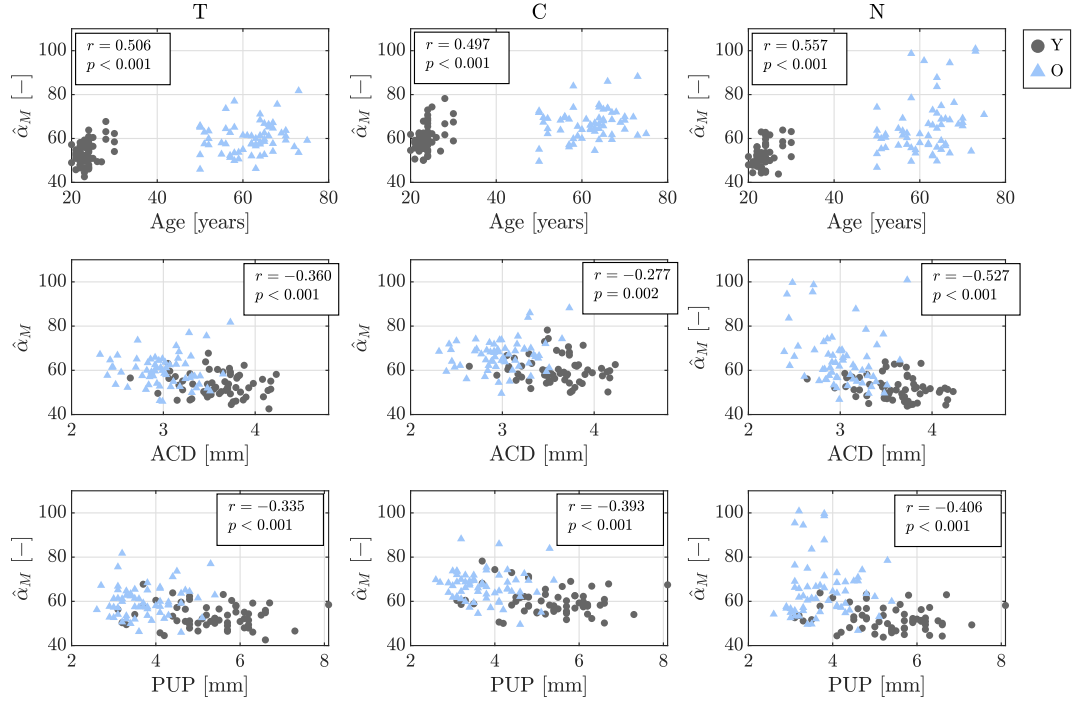


Figure 2.10: Scatter plots of Weibull scale parameter $\hat{\alpha}_M$ against age (first row), **ACD** (second row) and **PUP** (third row), respectively created for raw, unprocessed **ROI** data. r and p in the boxes indicate Pearson correlation coefficient and the corresponding p -value calculated for **Y**&**O**.

distributed data. Figure 2.13 presents accordingly $\hat{\theta}$ against k for Gamma distribution. It is apparent that $\hat{\alpha}$ and $\hat{\theta}$ estimators become biased on β and k respectively, after both normalization methods, both for Weibull and Gamma distributions. Also, for raw, Weibull distributed data, the variability of $\hat{\alpha}$ is higher for lower β . The shape parameter estimators — $\hat{\beta}$ and k — were not biased, and the estimated parameters corresponded well to β and k . For control, analogical experiment was done with fixing the shape β , k parameter to 5 and α , θ ranging from 10 to 100 with step of 1. Neither for $\hat{\alpha}$, $\hat{\theta}$ nor $\hat{\beta}$, $\hat{k}$ estimation, for raw and normalized data no bias was detected.

2.1.7 Further elaboration on preprocessing method

Although both Weibull and Gamma distributions were tested along the investigation, the following results will be shown only for Weibull distribution, as they performed similar in terms of **GoF**. **GoF** was calculated, similarly as in Equation 2.3, as the root-mean-square distance between the kernel density estimator of pixels in the **ROI** and the probability density function of the given distribution with the estimated Weibull $\hat{\alpha}_M$, $\hat{\beta}_M$ and Gamma $\hat{\theta}_M$, $\hat{k}_M$. Its mean values calculated along the time series were of order 10^{-3} for **Y** and **O**, both for Weibull and Gamma distributions. Also, the estimated Gamma parameters did not provide any new information compared to the Weibull parameters. The next idea to solve the problem was to find a multilinear model for eye biometry (**ACD**, **PUP**, **CCT**), age, and densitometry parameters. The differences in corneal pixel values (expressed as densitometry parameters) between **Y** and **O** groups are already measurable in natural, convex state of the cornea: two

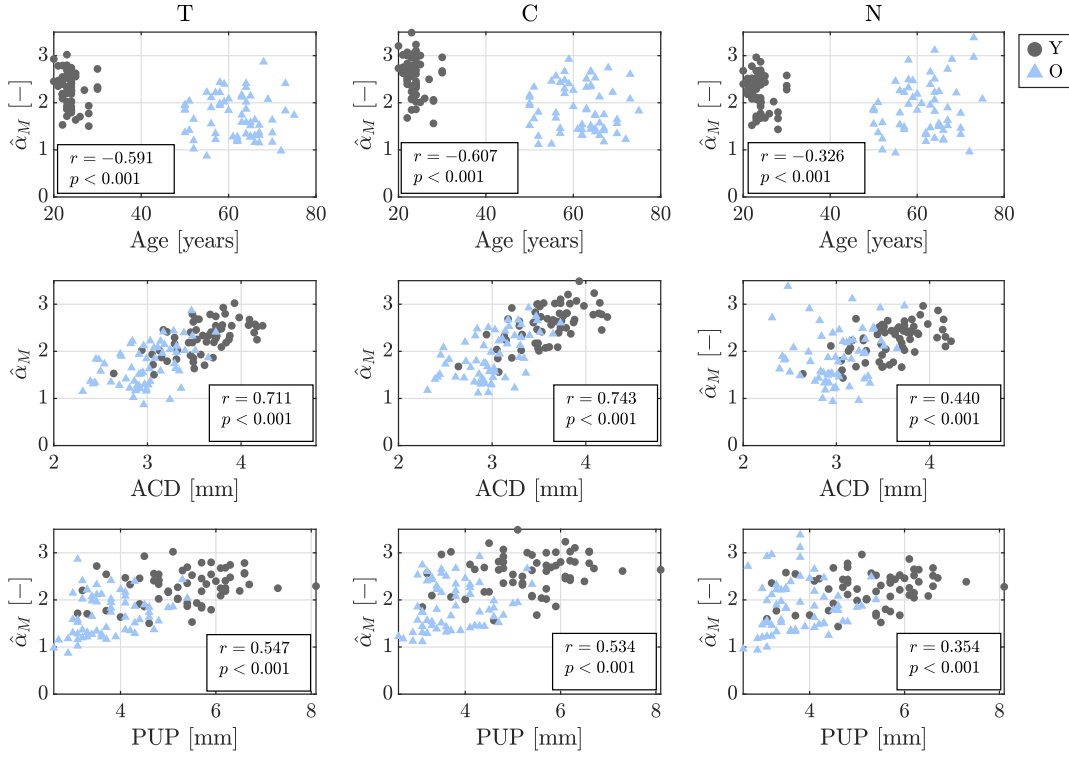


Figure 2.11: Scatter plots of Weibull scale parameter $\hat{\alpha}_M$ against age (first row), **ACD** (second row) and **PUP** (third row), respectively created for **ROI** data normalized by the mean pixel intensity of the image. r and p in the boxes indicate Pearson correlation coefficient and the corresponding p -value calculated for **Y&O** (young and older subjects taken together).

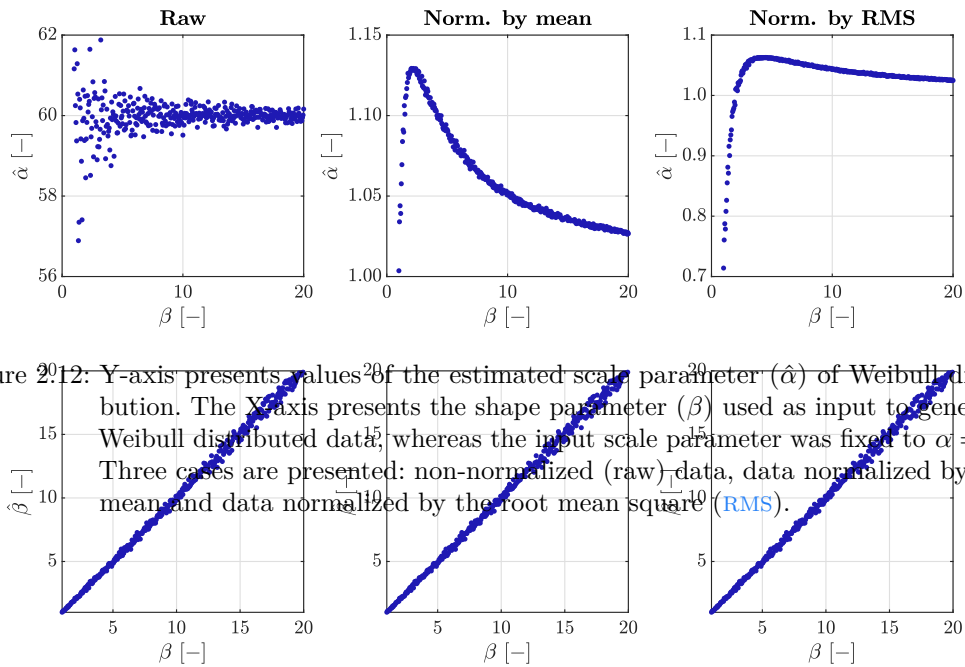


Figure 2.12: Y-axis presents values of the estimated scale parameter ($\hat{\alpha}$) of Weibull distribution. The X-axis presents the shape parameter (β) used as input to generate Weibull distributed data, whereas the input scale parameter was fixed to $\alpha = 60$. Three cases are presented: non-normalized (raw) data, data normalized by the mean and data normalized by the root mean square (RMS).

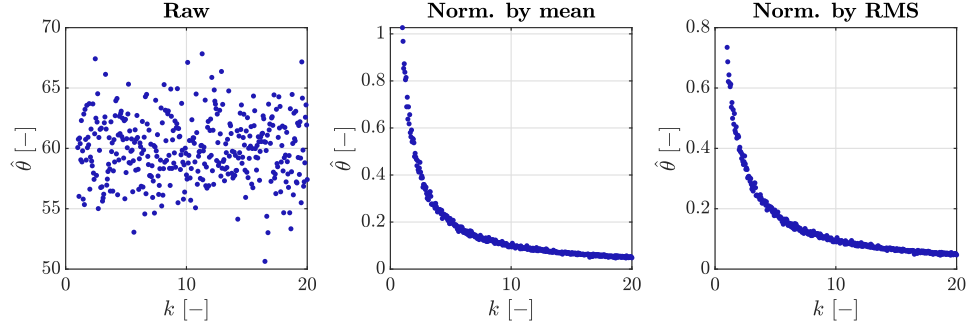


Figure 2.13: Y-axis presents values of the estimated scale parameter ($\hat{\theta}$) of Gamma distribution. The X-axis presents the shape parameter (k) used as input to generate Gamma distributed data, whereas the input scale parameter was fixed to $\theta = 60$. Three cases are presented: non-normalized (raw) data, data normalized by the mean and data normalized by the root mean square (RMS).

sample t-test comparing $\hat{\alpha}_M$ between Y and O groups revealed statistically significant differences for non-central, i.e., T and N ROIs ($p < 0.001$), whereas for $\hat{\beta}_M$ it revealed $p < 0.005$ for of the considered ROIs. Therefore, $\hat{\alpha}_M$ and $\hat{\beta}_M$ were taken for further analysis instead of the parameter time series.

For the same range of frames (i.e., first 10 and last 10 frames), the median of densitometry ($\hat{d}$) was taken and denoted further as $\hat{d}_M$. In some of the images, pixels lying beneath the cornea include iris or “spurious brightness” [94] from the iris, which influences the brightness of image background (BCG). Therefore, the mean value of the pixels laying under each ROI (i.e., T, C and N) was calculated for every subject (BCG). Analogically to other parameters, the median from first 10 and last 10 frames was calculated and the parameter was named $\overline{\text{BCG}}_M$. Several different approaches were unsuccessfully attempted to combine the above variables and model their dependencies: ANCOVA, multilinear regression, stepwise regression, partial correlations (`anovan()`, `stepwiselm()`, `regress()`, `partialcorr()` Matlab functions, respectively), with treating the densitometry parameters as dependent variables and background, biometry and age as independent variables. Each approach was also tested with different combinations of parameters and compositions such as $\text{age} \times \text{ACD}$, $\text{age} \times \text{PUP}$, $\frac{\text{ACD}}{\text{PUP}}$ or $\text{age} \times \frac{\text{ACD}}{\text{PUP}}$. In addition, power transformations ($Y^{\frac{1}{4}}$ and Y^4) of combinations of variables (i.e., $\hat{\alpha}_M/\overline{\text{BCG}}_M^{\frac{1}{4}}$, $\hat{\alpha}_M/\overline{\text{BCG}}_M^4$) were considered in the simple pairwise Pearson correlation test, as such transformations (particularly $Y^{\frac{1}{4}}$) are used to achieve data linearity or normality [95–98]. However, every attempt to collect all the confounding factors in one model ended with no statistical significance and tangling the problem even more, showing the indirect relationship of the variables. Finally, because of this complicated multicollinearity, a set of simple pairwise Pearson correlations was recognized as the best way of analyzing dependencies.

2.1.8 The dataset for the final methodology

The data set was extended with more retrospective data to 151 young (Y) and 82 older (O) subjects and after the manual outliers removal 141 and 73 subjects remained, respectively. The age and biometry metrics for the groups is presented in Table 2.1 supported also with the two sample t-test p -values.

Table 2.1: Mean $\pm$ one standard deviation and range [.,.] of age and eye biometry parameters (ACD, PUP, CCT) for three groups: Y&O, young (Y), and older (O) subjects. The last column presents p -values of the two sample t -test performed for Y versus (vs.) O.

	Y & O ($n = 214$)	Y ($n = 141$)	O ($n = 73$)	Y vs. O p
Age [years]	36.7 ± 18.6 [20, 75]	23.6 ± 2.3 [20, 30]	61.7 ± 6.3 [50, 75]	—
ACD [mm]	3.37 ± 0.40 [2.31, 4.25]	3.56 ± 0.29 [2.64, 4.25]	3.00 ± 0.31 [2.31, 3.73]	< 0.001

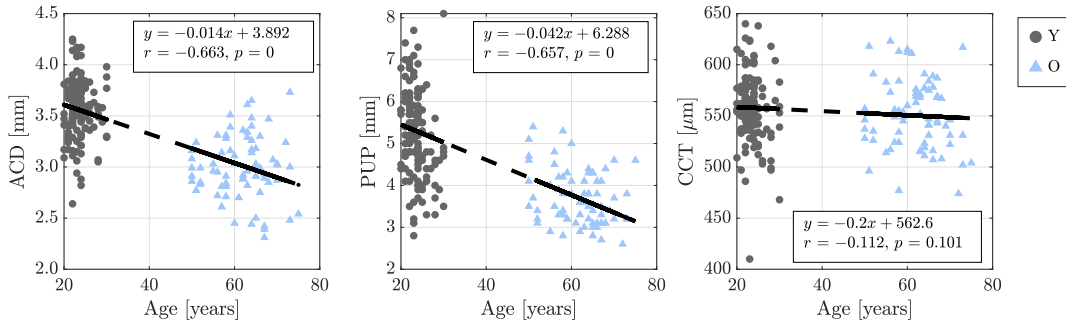


Figure 2.14: Plots of biometry parameters ACD, PUP, and CCT as functions of age, for young (Y) and older (O) subjects. Black lines show the general (calculated for Y&O) trends of these parameters with age, together with Pearson correlation coefficient and p -value (see also Table 2.1).

Throughout the analysis it was reviewed, whether the groups Y and O should be analyzed separately or together as one group of subjects. It is well understood that the aging is not a simple, linear process, and this is particularly true for the human cornea. The dynamics of a given corneal parameter can be different when comparing a younger individual to an older one. While a person ages, it may become difficult to distinguish specific changes caused purely by time from those that have been influenced by environmental exposure or underlying genetic factors [99]. In younger individuals, a parameter may have minimal variability, allowing for a clear and strong correlation with age. However, in an older population, the same parameter can exhibit a high individual variability, making its correlation with age statistically insignificant.

When considering groups together, arriving at statistically significant correlations of corneal parameters with age (or with ACD and PUP), the dataset consisted of two clusters of points (see Figure 2.14). However, the observed general trends in biometry with age closely corresponded to those obtained for other populations (see Figure 2.1.8). That is why in this study, every analysis was done both for the Y&O group together, and Y and O group separately. The biometry parameters for all of the considered groups (Y, O, Y&O) were tested for normality with Jarque-Bera test, retaining the null hypothesis of normality for all of the variables at 0.05 level of significance, except for the PUP for Y&O, where $p = 0.03$ and the null hypothesis was

rejected. However, as it was mentioned earlier, considering the group Y&O together caused the data division into clusters, and since the PUP is dependent on age, it could influence its distribution. However, despite this result, still PUP for Y&O was taken as a variable in the pairwise Pearson correlations.

The values of the Pearson correlation coefficient (r) should be considered as statistically significant depending on the sample size. Therefore, calculation for sample size, following [100], was performed. The null hypothesis was assumed as $r = 0$ — no correlation. Firstly a range of 1000 Pearson coefficients ($r \in [0, 1]$) was chosen and Fisher's Z transform was applied:

$$C_i = \frac{1}{2} \ln \left(\frac{1 + r_i}{1 - r_i} \right) \quad (2.5)$$

Then, probability of type I error (level of significance) was set to 0.05 and probability of type II error was set to 0.2 (meaning 80% power), as these numbers are commonly used in biomedical sciences. Using Matlab function `norminv()` the standard normal deviates, Z_α and Z_β , for two-sided alternative hypothesis were received. Employing

$$n_i = \frac{Z_\alpha + Z_\beta}{C_i} + 3, \quad (2.6)$$

where n_i is the minimal sample size for the given r_i , results in the range of minimal sample sizes for corresponding range of r values. Having already the groups with sample sizes of $n = 214$ for Y&O, $n = 141$ for Y, and $n = 73$ for O, three r threshold values were read: $r \geq 0.191$, $r \geq 0.234$, and $r \geq 0.323$, respectively. Hence, the correlation was classified as statistically significant if the p-value was less than 0.05 and, at the same time, the Pearson correlation coefficient exceeded the threshold value. The plot in Figure 2.15 shows the relation between n and r with three marked thresholds.

Since densitometry is known to be age-dependent [93], and also the biometry data seemed to confound the densitometry parameters in this study, it was first necessary to determine the correlation between biometry and age for considered subject group. Table 2.2 presents the Pearson's correlation coefficients and corresponding p -values for the biometry parameters. Figure 2.14 illustrates the general trends of biometry parameters with age with Pearson's correlation coefficients and linear function fitted. The results correspond to those obtained for other populations, indicating that the selected cohort of subjects is adequate for this study. In [101] the slope for ACD decrease with age was estimated to -0.011 mm/year, whereas in [102] the slope and the intercept were estimated to -0.0145 mm/year and 3.5 mm, respectively, which is close to the received here -0.014 mm/year and 3.802 mm. The pupil size is not as well comparable as it depends not only on age but also on lightning conditions, and also [103] indicates the relation of PUP and age to be non-linear. However, Kiel et al. [104] shows the results on a big cohort of healthy subjects in mesopic (likewise in this study) light conditions, a linear model was fitted and the slope was estimated to -0.02 mm/year (comparable to here received -0.042 mm/year). Finally, there were not statistically significant differences between CCT in Y and O groups and no correlation of it with age.

2.2 DECORRELATION OF DENSITOMETRY PARAMETERS FROM IMAGE BACKGROUND

Table 2.3 shows two sets of pairwise Pearson correlations for the corneal convex state:

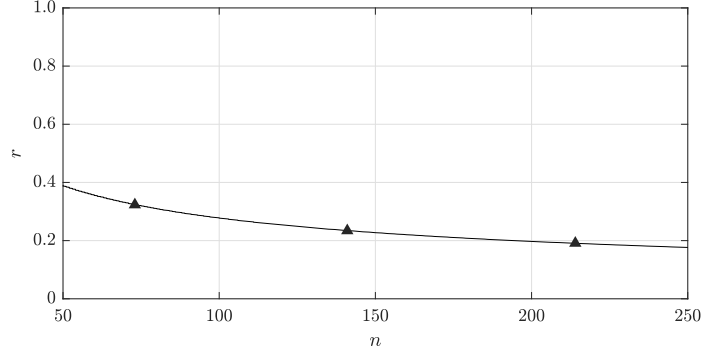


Figure 2.15: The relation of threshold value of Pearson correlation coefficient (r) indicating statistical significance of correlation for different sample sizes n . The threshold value is calculated for 0.05 level of significance and 80% of power. Because for low n , r coefficient takes very high values, the range for X-axis was limited to $[50, 250]$, to zoom-in the r threshold values for sample sizes from this study, i.e., 73, 141 and 214, respectively (marked with triangles).

Table 2.2: Pearson correlation coefficient (r) and the corresponding p -value for correlation of biometry parameters (ACD, PUP, CCT) with age, for three groups: Y&O, young (Y), and older (O). Significant non-zero correlations at 5% level of significance and 80% power for the given sample sizes, n are indicated with bold font.

		Y & O ($n = 214$)	Y ($n = 141$)	O ($n = 73$)
ACD	r	-0.663	-0.006	-0.022
	p	< 0.001	0.947	0.857
PUP	r	-0.657	-0.284	-0.277
	p	< 0.001	0.001	0.018
CCT	r	-0.112	-0.137	-0.134
	p	0.101	0.106	0.258

1. Between corneal densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$) and age, ACD, PUP, and CCT.
2. Between the mean background ($\overline{\text{BCG}}_M$) and densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$), age, ACD, PUP, and CCT.

The results show statistically significant correlations of $\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$ with ACD, PUP and age for Y&O. The background for Y&O is correlated both with the corneal densitometry parameters, ACD, PUP, and age. Notable is that for the Y group considered separately $\overline{\text{BCG}}_M$ is still correlated with ACD for three ROIs. Also for Y group, $\overline{\text{BCG}}_M$ in the temporal and central ROIs is correlated with all of the densitometry parameters, despite there is no iris visible in images for this group. It may be then deduced, that even if no confounders are visible in Scheimpflug image, the spillover from the iris still influences the pixels in the cornea and thus the densitometry. No such correlation in the older group may stem from high variability in the sample. The CCT remains uncorrelated in all of the combinations, despite the correlation with $\hat{\beta}_M$ in the central ROI. Accordingly to the above observations, $\overline{\text{BCG}}_M$ was recognized as the best representation of the ACD and PUP in Scheimpflug images, as its pixels include the backscatter from the iris.

Several methods of normalization and different combinations of them were attempted. Each method was justified by performing pairwise Pearson correlation tests

Table 2.3: Pearson correlation coefficients (r) and corresponding p -values for estimated densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$), age, eye biometry parameters (ACD, PUP, CCT), and the mean image background $\overline{\text{BCG}}_M$ for unprocessed images. The calculations were performed for three groups (Y&O), young (Y), and older (O). Significant non-zero correlations at 5% level of significance and 80% power are indicated with bold font.

		Y&O			Y			O		
		T	C	N	T	C	N	T	C	N
$\hat{d}_M$										
Age	r	0.322	0.265	0.499	0.310	0.303	0.306	0.226	0.204	0.341
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.055	0.084	0.003
ACD	r	-0.251	-0.121	-0.436	-0.178	-0.129	-0.150	0.071	0.310	-0.277
	p	< 0.001	0.077	< 0.001	0.034	0.127	0.077	0.548	0.008	0.018
PUP	r	-0.273	-0.266	-0.374	-0.175	-0.189	-0.137	-0.005	-0.122	-0.205
	p	< 0.001	< 0.001	< 0.001	0.038	0.024	0.106	0.966	0.304	0.083
CCT	r	0.077	0.022	0.005	0.133	0.101	0.070	0.059	-0.050	0.043
	p	0.261	0.749	0.941	0.117	0.232	0.411	0.621	0.673	0.719
$\hat{\alpha}_M$										
Age	r	0.302	0.239	0.480	0.316	0.304	0.308	0.211	0.196	0.334
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.073	0.097	0.004
ACD	r	-0.232	-0.109	-0.426	-0.167	-0.123	-0.149	0.074	0.287	-0.285
	p	< 0.001	0.111	< 0.001	0.048	0.145	0.077	0.535	0.014	0.015
PUP	r	-0.255	-0.241	-0.361	-0.170	-0.178	-0.137	0.014	-0.101	-0.203
	p	< 0.001	< 0.001	< 0.001	0.044	0.034	0.104	0.906	0.397	0.085
CCT	r	0.084	0.035	0.009	0.132	0.104	0.067	0.072	-0.028	0.050
	p	0.224	0.607	0.896	0.118	0.221	0.430	0.546	0.813	0.675
$\hat{\beta}_M$										
Age	r	0.422	0.438	0.247	-0.106	-0.076	-0.097	0.118	0.189	-0.008
	p	< 0.001	< 0.001	< 0.001	0.211	0.372	0.251	0.320	0.110	0.944
ACD	r	-0.332	-0.185	-0.088	-0.191	-0.026	0.013	0.047	0.332	0.217
	p	< 0.001	0.007	0.199	0.023	0.760	0.883	0.694	0.004	0.065
PUP	r	-0.369	-0.405	-0.177	-0.123	-0.163	0.023	-0.237	-0.323	-0.072
	p	< 0.001	< 0.001	0.010	0.147	0.054	0.784	0.044	0.005	0.544
CCT	r	-0.186	-0.197	-0.155	-0.081	-0.076	-0.050	-0.264	-0.287	-0.222
	p	0.006	0.004	0.023	0.342	0.368	0.553	0.024	0.014	0.058
$\overline{\text{BCG}}_M$										
Age	r	0.568	0.537	0.623	0.239	0.195	0.244	0.078	0.159	0.090
	p	< 0.001	< 0.001	< 0.001	0.004	0.020	0.004	0.513	0.179	0.450
ACD	r	-0.557	-0.612	-0.530	-0.354	-0.454	-0.331	-0.287	-0.487	-0.159
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.014	< 0.001	0.180
PUP	r	-0.517	-0.368	-0.501	-0.252	-0.084	-0.169	-0.389	-0.073	-0.313
	p	< 0.001	< 0.001	< 0.001	0.003	0.323	0.045	0.001	0.538	0.007
CCT	r	-0.049	-0.003	-0.018	-0.047	0.097	-0.063	0.034	0.035	0.120
	p	0.473	0.966	0.796	0.584	0.253	0.460	0.773	0.770	0.312
$\hat{d}_M$	r	0.257	0.234	0.396	0.392	0.339	0.164	-0.056	0.067	0.175
	p	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.052	0.635	0.573	0.138
$\hat{\alpha}_M$	r	0.231	0.218	0.379	0.371	0.314	0.155	-0.079	0.071	0.167
	p	0.001	0.001	< 0.001	< 0.001	< 0.001	0.067	0.509	0.550	0.159
$\hat{\beta}_M$	r	0.520	0.289	0.389	0.397	0.299	0.178	0.375	0.028	0.330
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.034	0.001	0.812	0.004

such as those presented in Table 2.3. The purpose of normalizing the data was to achieve decorrelation of $\overline{\text{BCG}}_M$ from densitometry parameters $\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$, and thus achieve also decorrelation of densitometry parameters with age and biometry. The attempted methods are described below.

2.2.1 Normalizing with image background

Different ways of normalizing with the background of the image were tested, due to the recognized relationship of the background with the biometry of the eye:

1. *Normalize with the mean of the whole background of the image.* The term “whole background” is defined here as taking the pixels from whole image (cropped from both sides with 90 pixels margins, accordingly to 2.1.3), except of the pixels included in the cornea. Then, pixel values within ROI were divided by the mean of the pixel values from the background.
2. *Normalize with the mean of the whole background & normalize with the RMS of the ROI.* Firstly pixel values within ROI were normalized as in number 1. of this listing, and then normalized with RMS value (as described in 2.1.6).
3. *Normalize with the mean background under each ROI.* ROI data from each ROI (T, C, N) was divided by corresponding BCG values.
4. *Normalize with the mean of the upper quartile of the background under each ROI.* The upper quartile values were chosen firstly by sorting ascending pixel values from ROI into one vector (V), then taking the range of indexes from 1 to $0.75 \cdot \text{length}(V)$, and then calculating the mean of the vector for this range. Then, pixels within each ROI (T, C, N) were divided with such calculated value for corresponding backgrounds.
5. *Column-based normalization with the mean of the background under cornea.* Every pixel column in the image was divided by the mean value of the vertical line of pixels lying under the cornea.
6. *Column-based normalization with the mean of the upper quartile of the background under the cornea.* Every pixel column in the image was divided by the mean value of the upper quartile of column of pixels lying under the cornea. The upper quartile of values was selected with the same method as described in number 4. of this listing.

2.2.2 Normalizing with corneal shoulders

An in-depth literature review has revealed that early works on anterior segment densitometry based on Scheimpflug photography already identified several standardization techniques to compensate for confounding factors [49, 105] such as the parameters of photographic film. As it was suggested by Wegener in 2009 [49]:

“Densitometric analysis of the images is influenced by a variety of factors, such as pupil size, flash light intensity and constancy of the recording medium. The effect of variation in these parameters on the accuracy of the density data over time can be detrimental such that standardization is necessary. Among the various standardization options, setting the peak height of the second main corneal shoulder to a predetermined value proved to be the most reliable method to compensate for most of the influencing factors.”

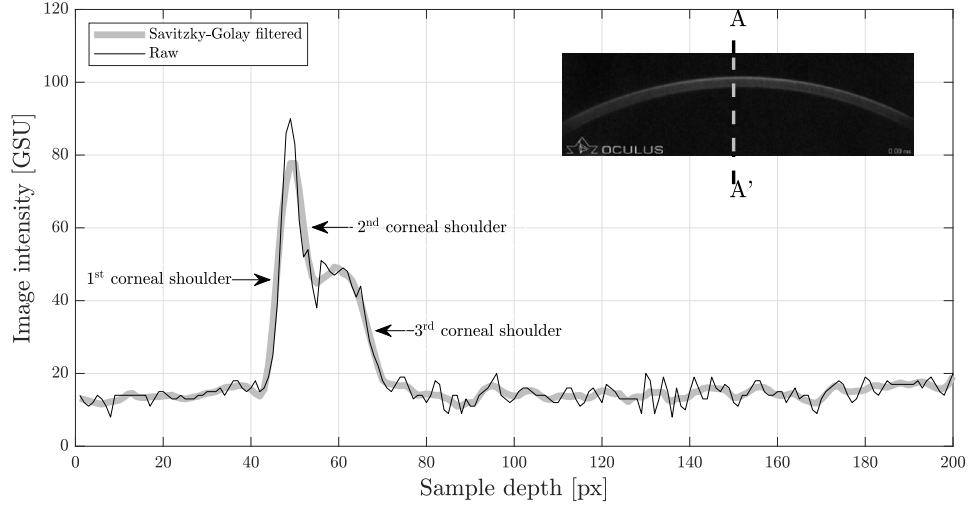


Figure 2.16: Illustrative pixel value profile (AA') of central column of a corneal image. Three distinct corneal shoulders are indicated by arrows. Only normalization by the pixel value at 2nd shoulder provided successful decorrelation results. GSU: gray scale units.

The shoulders are recognized here as the inflection points of the column pixel value profile of the cornea from the corneal Scheimpflug image (see Figure 2.16). The shoulders correspond to the transitions between the subsequent corneal layers. Due to the image resolution, three corneal shoulders can be detected in Scheimpflug image, corresponding to three interfaces: air/epithelium, epithelium/stroma and endothelium/anterior chamber. In order to localize the corneal shoulders, 4 central pixel columns of the Scheimpflug image were chosen. Then, column pixel value profiles were smoothed using the Savitzky-Golay infinite impulse response filter of polynomial order of 3 and the window size of 11. Calculating the first derivative of this smoothed profile and identifying where it achieves local extrema within the segmented earlier corneal sector allowed localizing of the 3 highest peaks, which corresponded to three corneal shoulders (see Figure 2.16). As 4 pixel columns were used, the mean from 4 peaks was set as the final location of given corneal shoulder. The recognized location of three corneal shoulders in the centre of the image was also applied along the whole cornea, accordingly to the detected edges, as the image contrast impeded running the above algorithm for all of the image pixel columns. Below the attempted normalization methods based on the corneal shoulder pixel values are depicted:

1. *Column-based normalization with the 1st corneal shoulder.* Every pixel column in the image was divided by the pixel value localized (as described above) at the 1st corneal shoulder.
2. *Column-based normalization with the 2nd corneal shoulder.* Every pixel column in the image was divided by the pixel value localized (as described above) at the 2nd corneal shoulder.
3. *Column-based normalization with the 3rd corneal shoulder.* Every pixel column in the image was divided by the pixel value localized (as described above) at the 3rd corneal shoulder.

Table 2.4: Pearson correlation coefficients (r) and corresponding p -values for estimated densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$), age, eye biometry parameters (ACD, PUP, CCT), and the mean image background $\overline{\text{BCG}}_M$ for column-based processed images (normalization with the 2nd corneal shoulder). The calculations were performed for three groups (Y&O), young (Y), and older (O). Significant, non-zero correlations at 5% level of significance and 80% power are indicated with bold font. The results shown here are already for ROI width fixed to 13 pixels, which is explained later in 2.2.3.

		Y&O ($n = 214$)			Y ($n = 141$)			O ($n = 73$)		
		T	C	N	T	C	N	T	C	N
$\hat{d}_M$	r	0.049	-0.018	-0.177	0.002	-0.016	0.012	0.067	-0.003	-0.183
Age	p	0.480	0.794	0.009	0.984	0.853	0.892	0.573	0.981	0.121
ACD	r	0.002	0.069	0.059	-0.028	0.002	-0.097	0.144	0.174	-0.020
	p	0.973	0.316	0.387	0.743	0.984	0.250	0.223	0.142	0.868
PUP	r	-0.101	0.003	0.115	-0.081	0.027	0.014	-0.156	-0.089	0.034
	p	0.140	0.963	0.094	0.340	0.752	0.873	0.188	0.456	0.777
CCT	r	-0.063	0.032	-0.001	0.071	0.070	0.006	-0.261	-0.017	-0.047
	p	0.357	0.637	0.989	0.405	0.406	0.948	0.026	0.887	0.695
$\hat{\alpha}_M$	r	-0.163	-0.203	-0.413	0.009	0.024	0.024	-0.006	-0.081	-0.365
Age	p	0.017	0.003	<0.001	0.920	0.780	0.779	0.959	0.497	0.002
ACD	r	0.151	0.167	0.253	0.049	0.033	-0.052	0.057	0.060	0.060
	p	0.027	0.015	<0.001	0.562	0.695	0.542	0.631	0.613	0.614
PUP	r	0.092	0.178	0.297	-0.010	0.089	0.048	-0.044	0.037	0.171
	p	0.182	0.009	<0.001	0.906	0.294	0.571	0.714	0.757	0.149
CCT	r	0.012	0.105	0.079	0.099	0.082	0.038	-0.160	0.103	0.068
	p	0.863	0.124	0.248	0.244	0.333	0.656	0.177	0.387	0.565
		Y&O			Y			O		
		T	C	N	T	C	N	T	C	N
$\overline{\text{BCG}}_M$	r	0.097	0.037	-0.063	-0.064	-0.022	0.117	0.199	0.090	0.017
$\hat{d}_M$	p	0.158	0.587	0.363	0.447	0.795	0.165	0.091	0.451	0.887
$\hat{\alpha}_M$	r	-0.114	-0.091	-0.325	-0.177	-0.151	0.029	0.073	0.088	-0.216
	p	0.097	0.185	<0.001	0.035	0.075	0.730	0.538	0.459	0.066

4. *Column-based normalization with the 1st corneal shoulder & normalization with the RMS of the ROI.* Firstly pixel values were normalized as in number 1. of this listing, and subsequently normalized with RMS value (as described in 2.1.6).
5. *Column-based normalization with the 2nd corneal shoulder & normalization with the RMS of the ROI.* Firstly pixel values were normalized as in number 2. of this listing, and subsequently normalized with RMS value (as described in 2.1.6).
6. *Column-based normalization with the 3rd corneal shoulder & normalization with the RMS of the ROI.* Firstly pixel values were normalized as in number 3. of this listing, and subsequently normalized with RMS value (as described in 2.1.6).
7. *Normalize with the mean of the whole background & column-based normalization with the 1st corneal shoulder.* Firstly, pixel values were normalized as in 2.2.1, and subsequently normalized as in number 1. of this listing.

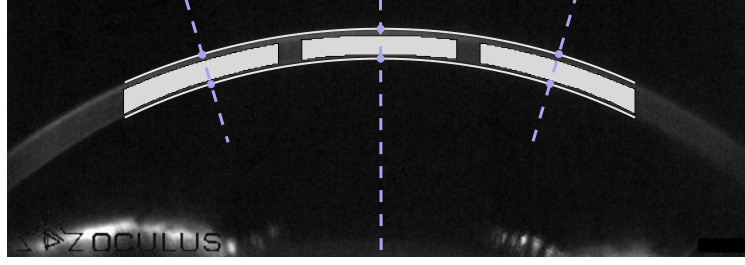


Figure 2.17: Illustration of the measurement of corneal thickness for each of the ROI. The dashed lines indicate the lines normal to the anterior edge in the centre of each ROI, the scatter points in each ROI limit the sections used to calculate corneal thicknesses.

From all of the above methods, the one of normalization with the 2nd corneal shoulder (number 2. from the above listing) appeared to successfully decorrelate $\overline{BCG}_M$ from $\hat{d}_M$, $\hat{\alpha}_M$ (see Table 2.4), whereas $\hat{\beta}_M$ remained correlated. That is why further methods were elaborated to achieve the decorrelation also for $\hat{\beta}_M$ (see Section 2.2.4).

2.2.3 Reconsidering ROI depth and recognizing CCT as confounding factor

To control whether the differences of densitometry parameters between the groups do not stem from differences in the corneal thickness of the ROIs, the corneal thickness in the center of each ROI for the first frame was measured and converted to micrometers. The measurement protocol included fitting 4th polynomial to the detected anterior edge, subsequently finding the normal line to the anterior edge in center of each ROIs, and then solving equation for crossing points of these normal lines to the posterior edge, finding real, positive roots lying in a range of the image size. Figure 2.17 illustrates the calculated normal lines to the edges. Then the distance between the ROI centre in anterior edge and the crossing point was measured and converted to micrometers analogically to the calculations for PUP (see Equation 2.4), i.e., with accounting for non equality in vertical and horizontal resolutions.

The t-student test for independent samples performed on temporal corneal thickness (TCT), CCT, and nasal corneal thickness (NCT) data did not show statistically significant differences between groups Y and O at the level of 0.05 significance. Also t-test performed on CCT data acquired with IOLMaster 700 biometer revealed no statistically significant differences at $\alpha = 0.05$ (see Table 2.1). However, the normalization with the 2nd corneal shoulder resulted in increase of correlation between densitometry parameters and CCT up to statistical significance (see the Table 2.5). That is why, the ROI depth was finally fixed to 13 pixels (to fit the thinnest cornea) instead of making it CCT-dependent. After such a correction, as it can be observed in the table, the correlations with CCT have fallen to non significant, except the correlation of $\hat{\beta}_M$ for Y&O group in the central ROI.

2.2.4 Contrast-limited adaptive histogram equalization

Adaptive histogram equalization methods are preprocessing techniques which can be used, for example to normalize images of differently illuminated objects [106, 107].

Table 2.5: Pearson correlation coefficients (r) and corresponding p -values for CCT and densitometry parameters ($\hat{\beta}_M$, $\hat{d}_M$, $\hat{\alpha}_M$). Three cases are shown: results for raw data, data normalized with 2nd corneal shoulder, where the ROI depth is dependent on the CCT of the subject, and data normalized with 2nd corneal shoulder but with fixing the ROI depth to 13 pixels. The calculations were performed for three groups (Y&O), young (Y), and older (O). Significant non-zero correlations at 5% level of significance and 80% power are indicated with bold font.

		Y&O			Y			O			
		T	C	N	T	C	N	T	C	N	
raw, ROI depth $\propto$ CCT	CCT										
	$\hat{d}_M$	r	0.015	-0.049	-0.022	0.055	0.013	0.026	0.022	-0.095	0.022
		p	0.823	0.475	0.749	0.517	0.879	0.763	0.853	0.426	0.853
	$\hat{\alpha}_M$	r	0.024	-0.038	-0.018	0.065	0.021	0.019	0.024	-0.087	0.031
		p	0.728	0.576	0.799	0.444	0.808	0.822	0.840	0.464	0.792
	$\hat{\beta}_M$	r	-0.214	-0.207	-0.124	-0.178	-0.137	-0.019	-0.228	-0.254	-0.180
		p	0.002	0.002	0.071	0.034	0.105	0.825	0.052	0.030	0.128
2 nd shoulder, ROI depth $\propto$ CCT	CCT										
	$\hat{d}_M$	r	-0.437	-0.400	-0.224	-0.425	-0.520	-0.271	-0.446	-0.260	-0.161
		p	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.027	0.172
	$\hat{\alpha}_M$	r	-0.384	-0.358	-0.177	-0.396	-0.507	-0.266	-0.387	-0.198	-0.098
		p	0.000	0.000	0.009	0.000	0.000	0.001	0.001	0.093	0.408
	$\hat{\beta}_M$	r	-0.221	-0.209	-0.161	-0.232	-0.143	-0.077	-0.198	-0.253	-0.200
		p	0.001	0.002	0.019	0.006	0.091	0.362	0.094	0.031	0.090
2 nd shoulder, ROI depth = 13 px	CCT										
	$\hat{d}_M$	r	0.074	0.020	0.006	0.125	0.096	0.072	0.061	-0.046	0.043
		p	0.284	0.771	0.932	0.139	0.258	0.394	0.609	0.697	0.719
	$\hat{\alpha}_M$	r	0.084	0.035	0.009	0.132	0.104	0.067	0.072	-0.028	0.050
		p	0.224	0.607	0.896	0.118	0.221	0.430	0.546	0.813	0.675
	$\hat{\beta}_M$	r	-0.186	-0.197	-0.155	-0.081	-0.076	-0.050	-0.264	-0.287	-0.222
		p	0.006	0.004	0.023	0.342	0.368	0.553	0.024	0.014	0.058

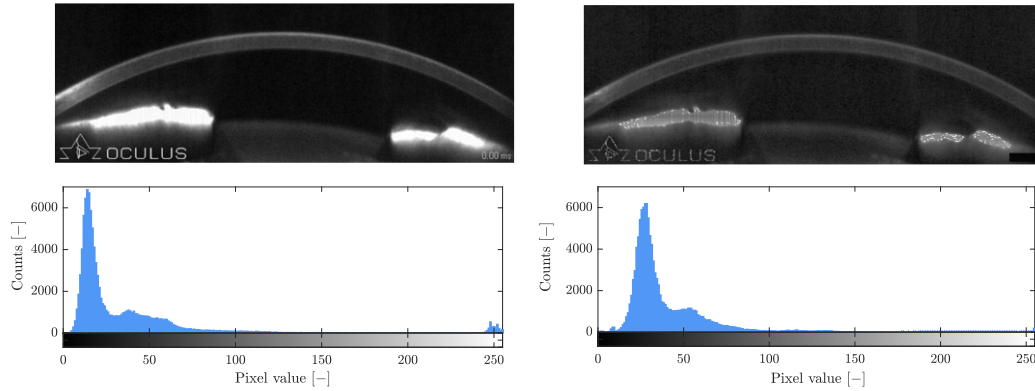


Figure 2.18: Illustrative OCT B-scans with corresponding histograms, showing the effect of application of contrast-limited adaptive histogram equalization (CLAHE) governed with Rayleigh distribution of its scale parameter set to 0.2 (Alpha = 0.2). The left column presents unprocessed images, the right column presents images after CLAHE.

Among them, contrast-limited adaptive histogram equalization ([CLAHE](#)) is a region-based method, recommended to enhance image quality, but at the same time avoid image saturation and increase in background noise [108]. The image is divided into rectangular contextual regions (called also tiles), then histogram equalization is performed separately on every region, governed with the chosen distribution, so that the resultant histogram of every region fits approximately to this distribution. Subsequently, bilinear interpolation is applied to combine the tiles and eliminate artificially induced boundaries. Here, the Matlab function `adapthisteq()` was employed to perform [CLAHE](#) on corneal Scheimpflug images. Number of regions (`NumTiles` parameter) was fixed to the recommended value of 64 [108], as well as the clip limit was set to 0.01, which was the default value. Different combinations of other input parameters, i.e. `Distribution` and `Alpha`, were tested to experimentally choose the one that would achieve satisfying results in decorrelation of variables:

1. [CLAHE](#) with `Distribution` = "uniform"
2. [CLAHE](#) with `Distribution` = "Rayleigh", `Alpha` = 0.4
3. [CLAHE](#) with `Distribution` = "exponential", `Alpha` = 0.4
4. [CLAHE](#) with `Distribution` = "Rayleigh", `Alpha` = 0.1
5. [CLAHE](#) with `Distribution` = "Rayleigh", `Alpha` = 0.2
6. [CLAHE](#) with `Distribution` = "Rayleigh", `Alpha` = 0.8

Finally, the use of [CLAHE](#) with the Rayleigh distribution and $\sigma = 0.2$ appeared to successfully decorrelate $\overline{\text{BCG}}_M$ and $\hat{\beta}_M$ (see Table 2.6), however $\hat{d}_M$, $\hat{\alpha}_M$ remained correlated. Figure 2.18 shows the image histogram before and after [CLAHE](#) governed with Rayleigh distribution with its parameter set to 0.2. The original image is characterized with bimodal distribution — there is a peak in bright pixels range, around the value of 250. After image processing, the amount of these pixels is minimized.

2.3 FINAL ALGORITHM FOR COMPENSATION FOR CONFOUNDING FACTORS

Among all of the normalization methods tested, there was no single image processing procedure, that would successfully decorrelate all of the considered densitometry parameters from the image background. However, the column-based normalization with 2nd corneal shoulder appeared to be efficient in decorrelation from background $\overline{\text{BCG}}_M$ for $\hat{\alpha}_M$ and $\hat{d}_M$, whereas the region-based, [CLAHE](#), was efficient for $\hat{\beta}_M$. The Table 2.7 provides a general overview of the correlations of densitometry parameters and background before and after application of decorrelation method. ✓ denotes successful decorrelation and ✗ denotes statistically significant correlation (unsuccessful). The numbers supporting this general overview can be found in previous tables: Table 2.3 for raw, non-normalized images, Table 2.4 for normalization with 2nd corneal shoulder and Table 2.6 for [CLAHE](#). An algorithm that takes the above two methods in parallel was devised to estimate the given densitometry parameters from separately preprocessed images, with appropriate compensation for confounding factors for every parameter. The flowchart for this final algorithm is shown in Figure 2.19. A summary

Table 2.6: Pearson correlation coefficients (r) and corresponding p -values for the estimated densitometry parameter $\hat{\beta}_M$, age, eye biometry parameters (**ACD**, **PUP**, **CCT**), and the mean image background $\overline{\text{BCG}}$ for region-based processed images (histogram equalization). The calculations were performed for three groups (**Y&O**), young (**Y**), and older (**O**). Significant non-zero correlations at 5% level of significance and 80% power are indicated with bold font.

		Y&O ($n = 214$)			Y ($n = 141$)			O ($n = 73$)		
		T	C	N	T	C	N	T	C	N
$\hat{\beta}_M$										
Age	r	-0.003	-0.017	-0.288	-0.090	-0.230	-0.168	-0.138	-0.004	-0.150
	p	0.968	0.805	<0.001	0.290	0.006	0.047	0.244	0.973	0.207
ACD	r	-0.026	0.022	0.304	-0.028	-0.015	0.096	0.004	0.117	0.273
	p	0.705	0.747	<0.001	0.746	0.864	0.257	0.976	0.323	0.019
PUP	r	-0.084	-0.033	0.204	-0.119	-0.075	0.071	-0.051	0.062	0.037
	p	0.221	0.632	0.003	0.158	0.376	0.405	0.670	0.600	0.755
CCT	r	-0.188	-0.171	-0.138	-0.178	-0.119	-0.121	-0.205	-0.269	-0.227
	p	0.006	0.012	0.043	0.035	0.158	0.151	0.082	0.022	0.053
		Y&O			Y			O		
		T	C	N	T	C	N	T	C	N
$\overline{\text{BCG}}_M$										
$\hat{\beta}_M$	r	0.174	-0.063	-0.062	0.228	0.035	-0.034	0.184	-0.161	0.191
	p	0.011	0.361	0.370	0.006	0.679	0.692	0.119	0.174	0.106

Table 2.7: Indication of decorrelation (✓) or statistically significant correlation (✗) between estimated densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$) and image background $\overline{\text{BCG}}_M$ for **Y&O**. Results for three cases are shown: unprocessed images (“None”), images normalized with 2nd corneal shoulder (see Section 2.2.2) and with contrast-limited adaptive histogram equalization (**CLAHE**, see Section 2.2.4). Blue color denotes the successful decorrelation received for the final methodology presented in Figure 2.19.

Decorrelation method		Y&O		
		T	C	N
None	$\hat{d}_M$	✗	✗	✗
	$\hat{\alpha}_M$	✗	✗	✗
	$\hat{\beta}_M$	✗	✗	✗
2 nd shoulder	$\hat{d}_M$	✓	✓	✓
	$\hat{\alpha}_M$	✓	✓	✗
	$\hat{\beta}_M$	✗	✗	✗
CLAHE	$\hat{d}_M$	✗	✗	✗
	$\hat{\alpha}_M$	✗	✗	✗
	$\hat{\beta}_M$	✓	✓	✓

of correlations of densitometry parameters with age and biometry parameters before and after image normalization are depicted in Figure 2.20.

The study results are presented without correcting for multiple comparisons, which some may view as a limitation [109]. Applying an adjustment, such as the Bonferroni correction, would not alter our conclusions. Each densitometric parameter involved four comparisons (against age and three biometry parameters), while the mean

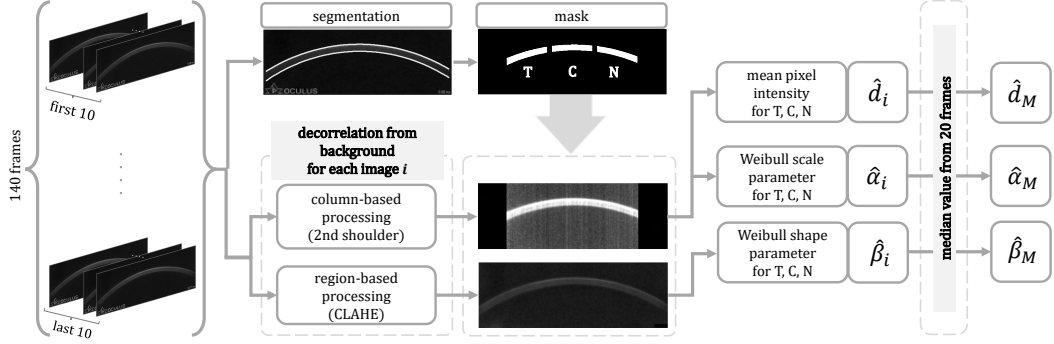


Figure 2.19: The final methodology developed to compensate corneal densitometry for confounding factors related to age and biometry. The methods of decorrelation, i.e., “2nd corneal shoulder” and **CLAHE** are described in Sections 2.2.2 and 2.2.4, respectively.

background involved seven, reducing significance level to 0.007. Nevertheless, all reported statistically significant p -values were ≤ 0.005 , remaining significant even under stricter conditions.

2.4 DISCUSSION

Scheimpflug-based corneal densitometry is a validated ophthalmic method for evaluating corneal transparency [59, 110, 111]. It is particularly robust for following treatment outcomes [112–114] by before-and-after comparisons, as this approach isolates treatment effect from patient specific variables, i.e., the age (until the short period is under observation) or anterior eye biometry, until it is not influenced by the treatment, unlike in implantation of intrastromal corneal ring segment [115]. Unfortunately, it is difficult to assess the value of other, continually increasing number of studies on the Scheimpflug-based corneal densitometry, as most of these studies do not consider confounding factors, nor image standardization techniques [49, 116].

It is particularly important when studying the corneal backscatter parameters for populations. Several recent works confirm a significant correlation of densitometry with age for large cohorts [93, 117–120], reporting the “normative” densitometry values for the age population. It should be considered which part of this dependence is determined by confounding factors, e.g., stemming from the relationship between age and biometry, and which part originates from aging of the corneal microstructure.

The literature widely documents the impact of aging on corneal tissue. Aging leads to a reduction in both the density of stromal keratocytes and endothelial cells [121]. The density of small nerve fibers in the epithelium and Bowman’s layer was reported to decrease with age [122], while at a nano-structural level, the stromal collagen fiber diameter and axial period increase with age [40]. The age-related changes appeared also to be detectable in **OCT**. Studies have shown statistically significant differences in corneal speckle within the stroma between various age groups [78]. Additionally, in vivo confocal microscopy study, which seems to be less influenced by eye biometry than Scheimpflug images, have reported age-related differences specifically within the anterior stroma [123].

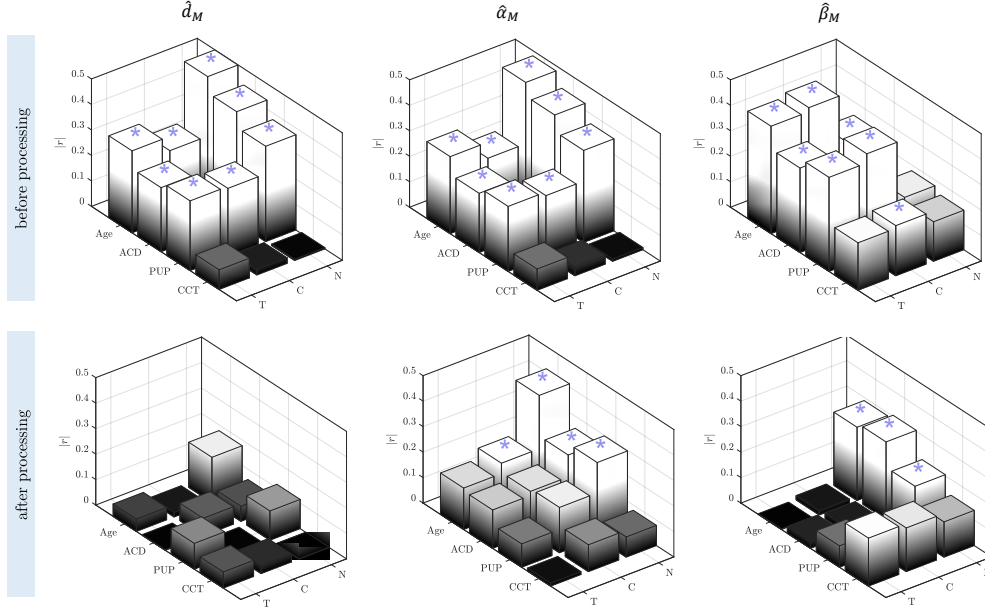


Figure 2.20: A graphic summary (created only for Y&O) of Pearson correlation absolute values $|r|$ of densitometry parameters ($\hat{d}_M$, $\hat{\alpha}_M$, $\hat{\beta}_M$) with age and biometry parameters (ACD, PUP, CCT) before (top) and after (bottom) image processing (summary of the method in Figure 2.19). Asterisks denote statistically significant correlations.

After correction for confounding factors, age correlation persisted only for $\hat{\alpha}_M$ and $\hat{\beta}_M$ in the nasal ROI, and for $\hat{\alpha}_M$ in the central ROI. Notably, $\hat{d}_M$ showed to be fully decorrelated from age and biometry. The lack of correlation between age and the objectively evaluated corneal densitometry parameters does not mean absence of aging microstructural changes in the examined corneas. Instead, it rather indicates a limitation of the utilized imaging technique. The resolution of analyzed here Scheimpflug images seems to be low to effectively detect any possible corneal tissue reorganization based on densitometry parameters.

It must be acknowledged that the image standardization technique devised here have only been verified for the Corvis ST Scheimpflug device. Analogous analysis should be conducted using higher resolution devices, such as the Pentacam HR, as it is difficult now to compare the obtained here results to other studies performed in major on Pentacam HR. Additionally, such study would provide the necessary data to evaluate the clinical efficacy of the proposed methods for compensating for confounding factors.

Results showed asymmetry between the nasal and temporal ROIs. Particularly, it was not possible to achieve decorrelation of $\hat{\alpha}_M$ from the mean background specifically in the nasal region. This difference between nasal and temporal areas could arise from actual anatomical changes [124], the influence of natural UV radiation [125, 126], geometrical distortion inherent to the Scheimpflug imaging method [127], or because of non-central position of the cornea during image capture.

The reported image processing techniques have lead most correlations to statistical insignificance. However, they do not intervene in the physical process of Scheimpflug

imaging. Therefore, the methods could not fully resolve the problem of decoupling biometry parameters from corneal backscatter.

Nevertheless, the present study addresses the overlooked issue of corneal densitometry parameters being correlated with the image background. Adequate assessment of corneal transparency certainly requires independence from underlying structures (such as the anterior chamber, crystalline lens, and iris).

Although this research had been performed and published in 2022 — at the halfway of the author’s doctoral studies, some new works have already been published disputing on corneal densitometry. Alanazi et al. in 2024 published a work elaborating on Pentacam HR corneal densitometry artifacts and their influence on keratoconus detection [128]. They clarified that the Pentacam HR densitometry grayscale units are calculated by rescaling the usual image pixel values grayscale range of 0–255 (0 meaning black and 255 meaning white) to the range of 0–100, however, no standardization technique was mentioned. The artifact identified by the authors was the shadow of the eyelid, which affects densitometry in the superior-nasal region of the cornea (which may partially explain the asymmetry between the temporal and nasal side obtained in this study). The recent works of Consejo et al. and Arizcuren et al. [94, 129, 130] contribute significantly to the knowledge of the factors confounding Scheimpflug-based densitometry, giving evidence that eye tilt, iris color, as well as brightness and reflections from the iris, sclera, and lens, correlate with corneal densitometry, disturbing its objective assessment.

Finally, the necessity of compensating for confounding factors is, remarkably, not a new topic. It was recognized already in late 1980s while first applications of Scheimpflug photography for the cornea (see the review by Wegener et al. [49]). Unfortunately, these early findings and methods seem to have stagnated. The current study addresses and fills this apparent gap in research concerning Scheimpflug-based corneal densitometry.

EPITHELIUM CONFOUNDS THE OCT SPECKLE OF THE STROMA

Partially based on:

Miażdżyk, M. *et al.* OCT based corneal densitometry: the confounding effect of epithelial speckle. *Biomedical Optics Express* **14**, 3871–3880 (2023).

As mentioned in Chapter 2, densitometry based on Scheimpflug imaging, due to its limited resolution, may not reliably represent microstructural differences. Moreover, unlike in OCT, there is no physical model, to the author’s knowledge, relating the pixel values and the physical properties of the sample. Therefore, in recent studies OCT has become an emerging platform for corneal densitometry [57, 61, 128], and the present work contributes to this research direction.

A preliminary investigation (see Section 3.1) has revealed another confounding factor. The epithelial layer appears to influence the signal from the corneal stroma in OCT-based densitometry. This observation motivated a more detailed investigation aimed at quantifying this effect. This chapter is divided into three parts. Section 3.1 reports the preliminary observations and motivation for the study. Sections 3.2–3.6 present the methodology and investigation process in chronological order. In addition, Section 3.6 describes the final methodology for quantifying the influence of the epithelium on stromal speckle statistics and presents the corresponding results. Section 3.7 provides the discussion.

3.1 PRELIMINARY OBSERVATION

Since the cornea is a multilayered tissue, the OCT signal that returns to the detector summarizes the backscattering components originating from all its layers. It is therefore of interest to understand how the signal from the epithelial layer influences the stromal speckle. As noted already in 1970s [131], light scattering by the epithelium is often undetected or underestimated, while that of the stroma is often overestimated. In practice, corneal densitometry is limited to corneal stroma [59, 61]. The corneal epithelium and stroma exhibit different scattering properties. Regardless of whether the cornea is captured with Scheimpflug or OCT technology, the epithelium is an easily distinguishable layer. A preliminary observation performed on the human cornea imaged in vivo with OCT has shown differences in the speckle statistics between the epithelium and the stroma. Figure 3.1 shows scaled histograms of speckle amplitudes assessed within a ROI covering the corneal epithelium and a ROI located in the corneal stroma, together with the corresponding Burr 2 distribution fit [79]. In other words, pixels representing the epithelium carry information distinct from that corresponding to the stroma. Additionally, the literature on multilayered tissues provides computational models [132, 133] and experimental evidence [134] suggesting that the scattering properties vary between the tissue layers. This suggests that the epithelium, as the superficial layer covering the stroma and the second thickest corneal layer, could play a substantial role in estimating corneal densitometry.

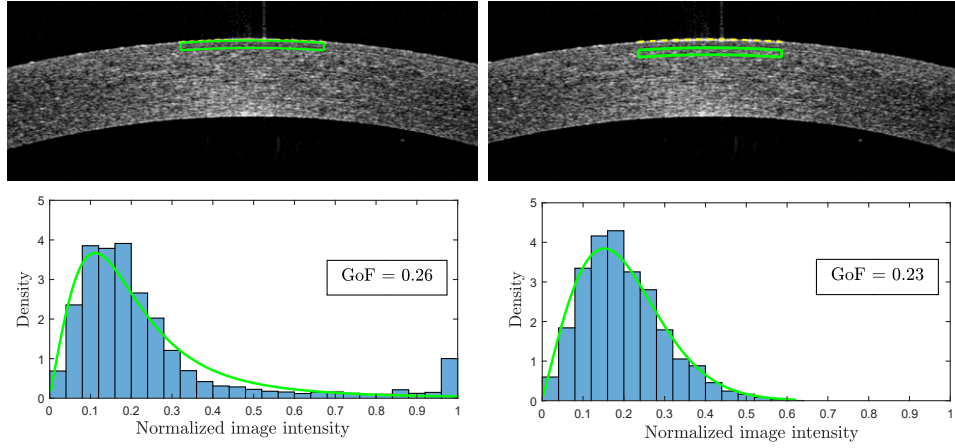


Figure 3.1: OCT B-scans (top row) of a human cornea imaged in vivo with corresponding (bottom row) scaled histograms and the Burr 2 distribution fitted to the data within the ROI, outlined in the OCT scans in green. The left column refers to epithelial, right to the stromal (i.e., a part of the stroma) speckle. The scale and shape parameters for the Burr 2 distribution are $\alpha = 0.21$, $\beta = 1.27$ and $\alpha = 0.86$, $\beta = 15.40$ for the epithelium and stroma, respectively. GoF denotes the goodness-of-fit (the mean square error between the fitted distribution and the kernel density estimator).

A recent experiment presented in [86] on porcine corneas imaged with OCT before and immediately after epithelial debridement, without modifying any other experimental conditions, revealed statistically significant differences in contrast ratio between the stroma observed through the epithelium and that observed without it. Referring to the above mentioned work, another brief study was performed. Figure 3.2 shows two OCT scans of the same porcine cornea obtained before and after epithelial removal, together with scaled histograms of the speckle amplitudes assessed in a given ROI, and with the corresponding the Burr 2 distribution fit [79]. Based on this example it is evident that despite imaging the same stromal sample in both scenarios, the distribution parameters differ. Furthermore, the fit of the Burr 2 distribution is several times worse for the scenario without epithelium compared to that with epithelium.

The above observations clearly demonstrate the limitations of current OCT speckle characterization when imaging multilayered samples and identify the epithelium as a *confounding factor* in the stromal speckle. Therefore, a thorough investigation was conducted to quantify the influence of the epithelial layer in OCT-based corneal densitometry and judge whether this should be taken into consideration in clinical applications.

3.2 DATASET: OCT IMAGES OF PORCINE CORNEAS

Retrospective OCT data from 11 intact porcine eyeballs were used for this study. The eyes were obtained from a certified abattoir, came from animals of the same breed and age, and were carefully screened for any corneal damage, scars, or edema. Throughout the process, including transport and preparation for the experiment, each eyeball was regularly moistened with Phosphate Buffer Saline (PBS) and refrigerated at 4°C, until 20 minutes before imaging (room temperature). Each eye was mounted in

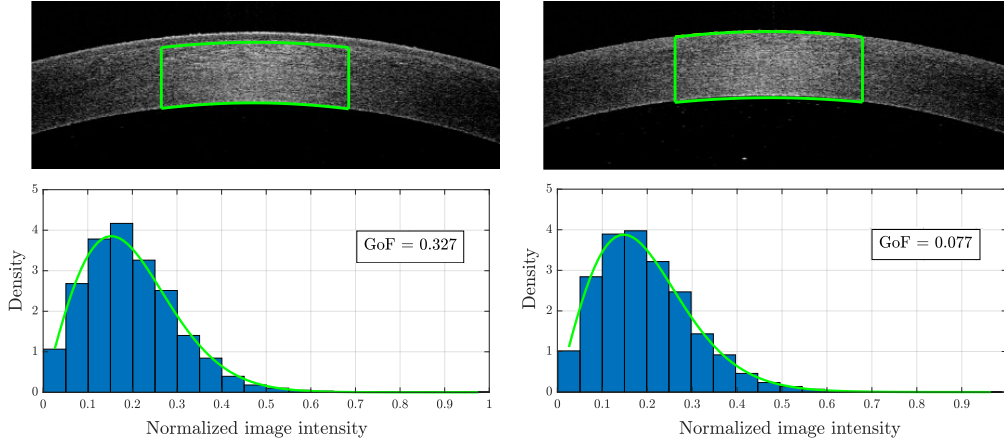


Figure 3.2: OCT scans (top row) of a porcine cornea before (left column) and after (right column) removal of the epithelium with corresponding (bottom row) scaled histograms and the Burr 2 distribution fitted to the data within the ROI, outlined in the OCT scans in green. The scale and shape parameters for the Burr 2 distribution are $\alpha = 0.70$, $\beta = 10.59$ and $\alpha = 0.98$, $\beta = 20.09$ for the epi-on and epi-off mode, respectively. GoF denotes the goodness-of-fit (the mean square error between the fitted distribution and the kernel density estimator).

a custom-designed setup that limited movement and allowed setting and maintaining intraocular pressure (IOP) of 20 mmHg in the anterior chamber. The OCT images were collected using the SOCT REVO 80 device (Optopol Technology, Zawiercie, Poland). Three central, horizontal B-scans were acquired consecutively without realigning the instrument, and all samples were imaged within the depth-of-focus of the instrument. The triple acquisition was subsequently repeated immediately after epithelial debridement. Images recorded for intact corneas will be referred to as the epi-on mode, whereas those after removing the epithelium will be referred to as the epi-off mode. The eyeballs were prepared within 3 hours post-mortem, and the time of the main stage of the experiment was 15 minutes. The imaging protocol was set to a 5 mm single (non-averaged) B-scan, which consisted of 12,032 raw A-scans. The resulting OCT image has fixed width of 1,538 pixels and varying depth of approximately 733 pixels, corresponding to approximate physical dimensions of 5 mm and 2 mm, respectively. The approximate resolution was estimated to 308 pixels/mm horizontally and 366 pixels/mm vertically. A more detailed description of the eyeball preparation and data collection process can be found in [85, 86].

3.3 IMAGE SEGMENTATION

Firstly, the algorithm for the analysis of the corneal stroma OCT speckle, already used in several publications [75, 78, 135] was employed for hereby data set. Its main workflow is as follows: performing the sample edge detection, image preprocessing (i.e., reverse log10-transform and normalizing pixels by value of 255), ROI selection and estimation of the OCT speckle parameters. The purpose was to compare the stromal densitometry parameters for the corresponding regions in epi-on and epi-off mode acquisitions. In order to analyze pixel values for different stromal depths, a moving region of interest (ROI) with a width of 1 mm, depth of 100 μm , and a downward step of 10 μm , corresponding to 90% overlap, was applied (see Figure 3.3). As the

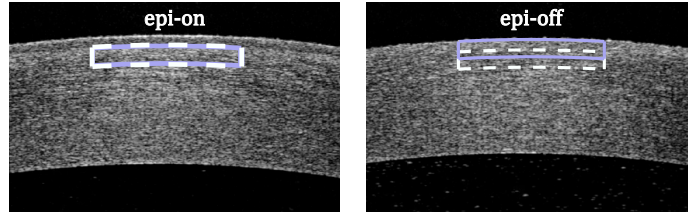


Figure 3.3: Illustration of the moving ROI applied to correlate pixel values of porcine corneal imaged before and after epithelial debridement (epi-on and epi-off, respectively). The purple ROIs represent approach 1 (fixed stromal region), while the white ROIs represent approach 2 (fixed image region).

vertical resolution of the images slightly varied between the subjects depending on the sample positioning (pixel size ranged from 2.70 to 2.76 μm), it was decided to fix the ROI positions in pixels independently on the vertical resolution, to make them the same for every porcine corneal image. Firstly, two approaches were proposed (presented in Figure 3.3):

1. *fixed stromal region*: to compare the ROIs in epi-on and epi-off mode at the same stromal depth.

The first position of the ROI for the epi-on mode was just below the epithelium, while for epi-off mode it was at the corneal apex, allowing comparison of the same stromal regions. The interface between epithelium layer and stroma was localized manually. From that also the epithelial thickness was measured as the vertical distance between automatically detected corneal apex and the pointed interface. The mean $\pm$ STD of measured epithelial thickness was 24 ± 2 pixels, corresponding to approximately $66.7 \pm 5.9 \mu\text{m}$.

2. *fixed image region*: to compare the ROIs in epi-on and epi-off mode at the same stromal depth.

For the second approach, the first ROI position for epi-on mode was the same as in the first approach (just below the measured epithelium). The distance in pixels from the top of the image to the anterior edge of the first ROI position in epi-on mode was recorded and subsequently used as ROI position for epi-off mode in order to compare the same image regions. The justification for the second approach is that sample positioning during acquisition may significantly influence the speckle statistics due to the roll-off effect and beam focusing [64, 79, 136].

The average corneal thickness of the porcine corneas imaged in this study was $624.5 \pm 42.8 \mu\text{m}$ (mean $\pm$ STD). The maximum downward shift of ROI was adapted to the thinnest cornea (i.e., 526 μm) and fixed for all eyeballs. Therefore, speckle statistics were acquired from 43 ROI positions (also denoted as sample depths) in the range of $[0, 420] \mu\text{m}$.

3.4 DENSITOMETRY PARAMETERS

Four densitometry parameters were chosen in this experiment to analyze the speckle statistics: shape (k) and scale (θ) parameters of 2-parameter Gamma distribution

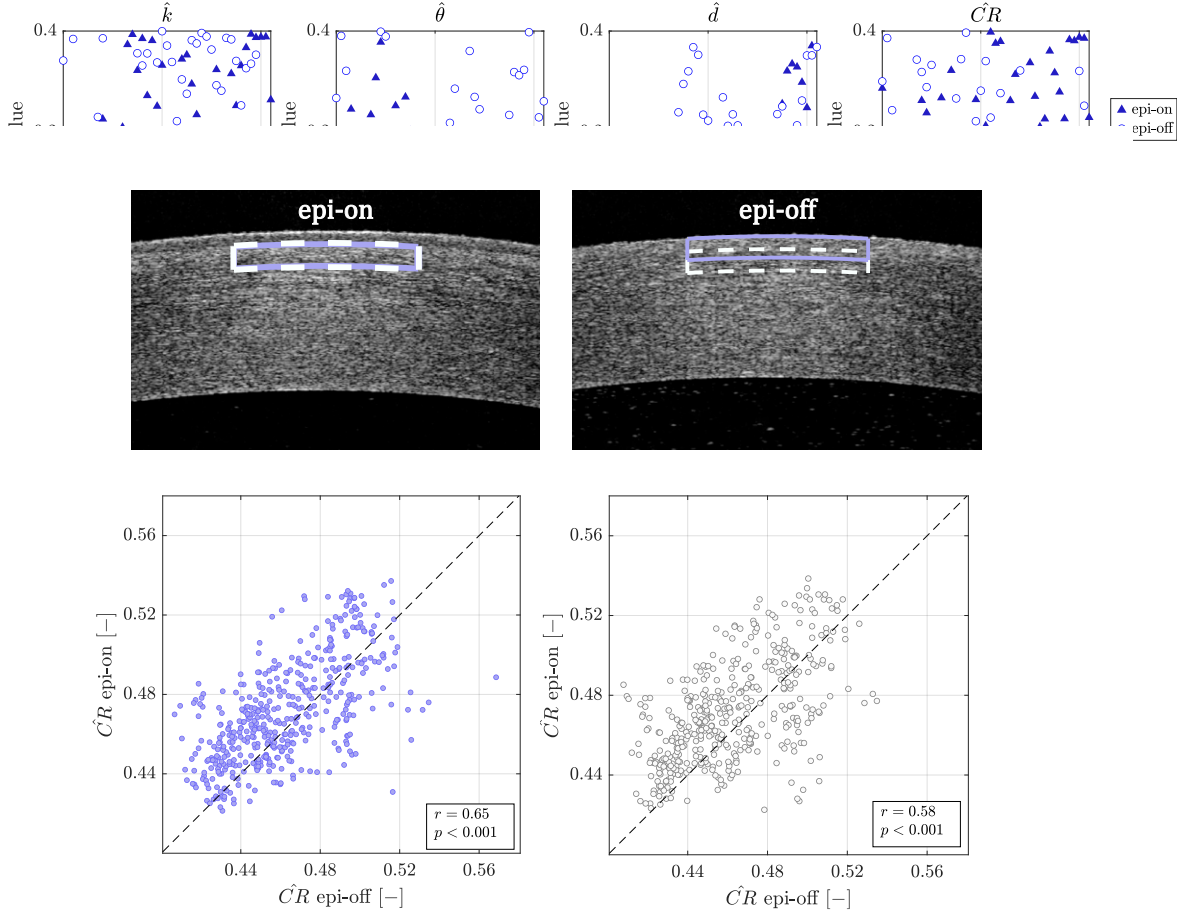


Figure 3.5: Left: approach 1 (*fixed stromal region*), right: approach 2 (*fixed image region*). Values of contrast ratio ($\hat{C}R$) estimated for porcine eyeball before and after epithelial debridement (epi-on and epi-off, respectively). The scatter plots collect $\hat{C}R$ received for all sample depths tested and for all 11 eyeballs. The dashed line indicates the 1:1 line.

(see Equation 2.2) as a parametric approach, and mean (d) and contrast ratio (CR) as a non-parametric approach. Gamma distribution has already shown good fit to the OCT speckle [75, 78, 79], CR is commonly used measure for OCT speckle, while d is currently clinically used densitometry parameter in Scheimpflug imaging, therefore it was included also in introduced here OCT-based densitometry. As every eyeball was measured three times in the epi-on and three times in epi-off mode, the medians of densitometry parameter estimates were collected from every three acquisitions. Also, the mean coefficient of variation (CV) for these three subsequent measurements, evaluated across eyeballs, modes, and depths, was calculated and it achieved less than 5% ($CV = 2.1\%$, 1.9% , 0.9% and 1.0% for $\hat{k}$, $\hat{\theta}$, $\hat{d}$ and $\hat{C}R$, respectively). Every densitometry parameter in a given mode, collected as a vector of densitometry parameter values for all ROI positions, was tested for normality using the Shapiro-Wilk test. As it was expected, p -values were small (for all parameters and modes p was less than 0.001, except $p = 0.01$ for $\hat{k}$ in epi-on mode), so the null hypothesis assuming the Gaussian distribution was rejected. Additionally, the test was also performed for particular ROI positions (every tested sample contained 11 values for 11 eyeballs). Although, p -values were not consistent along the sample depth, and for some of the

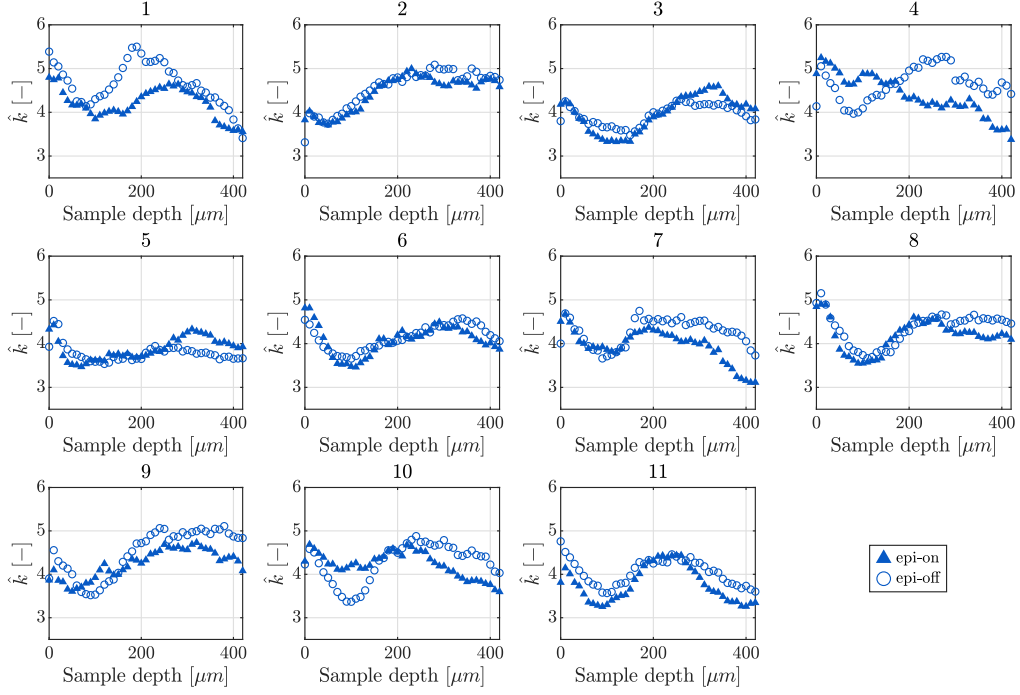


Figure 3.6: Values of Gamma shape parameter ($\hat{k}$) against the ROI position (sample depth). Every subplot is created for one porcine cornea (numbered in the title). Two series are presented for every eyeball: values received for intact cornea (epi-on) and after epithelial debridement (epi-off).

depths were less than 0.05 (see Figure 3.4). For the above reasons, in further analysis, the statistical tests for non-Gaussian samples were chosen.

To briefly justify, whether to analyze the densitometry parameters received from approach 1 (*fixed stromal region*) or 2 (*fixed image region*), simply the Spearman correlations between epi-on and epi-off for both approaches were calculated. Figure 3.5 shows the Spearman correlation coefficient r and p -value for $\hat{C}R$ in the epi-on and corresponding $\hat{C}R$ in the epi-off mode for all ROI positions, for the two approaches. Although the correlation appears high and statistically significant for both, the approach 1 (*fixed stromal region*) performs higher correlation. Under this criterion, this approach was considered in further analysis.

Figures 3.6–3.9 show densitometry parameters estimated for epi-on and epi-off modes, against the ROI position (stromal depth), plotted for 11 porcine corneas. It can be observed that in all of the assessed here densitometry parameters, the presence of epithelial layer influences the speckle signal from stroma. The mechanism is not straightforward and different behavior is observed for different ROI depths and different parameters. For example, $\hat{k}$ and $\hat{d}$ seem to be affected mostly in the anterior stroma, while $\hat{\theta}$ and $\hat{C}R$ seem to be affected at the top of the stroma, then the differences between the modes decrease and increase again in posterior stroma. Also, it can be observed that $\hat{\theta}$ and $\hat{C}R$ signal look quasi sinusoidal along the stromal depth. It was of consideration whether these fluctuations stem from microstructural changes along the stroma, from artifact of the optical system, or maybe from applying moving ROI with 0.9 overlap and non-stationarity of the densitometry parameters.

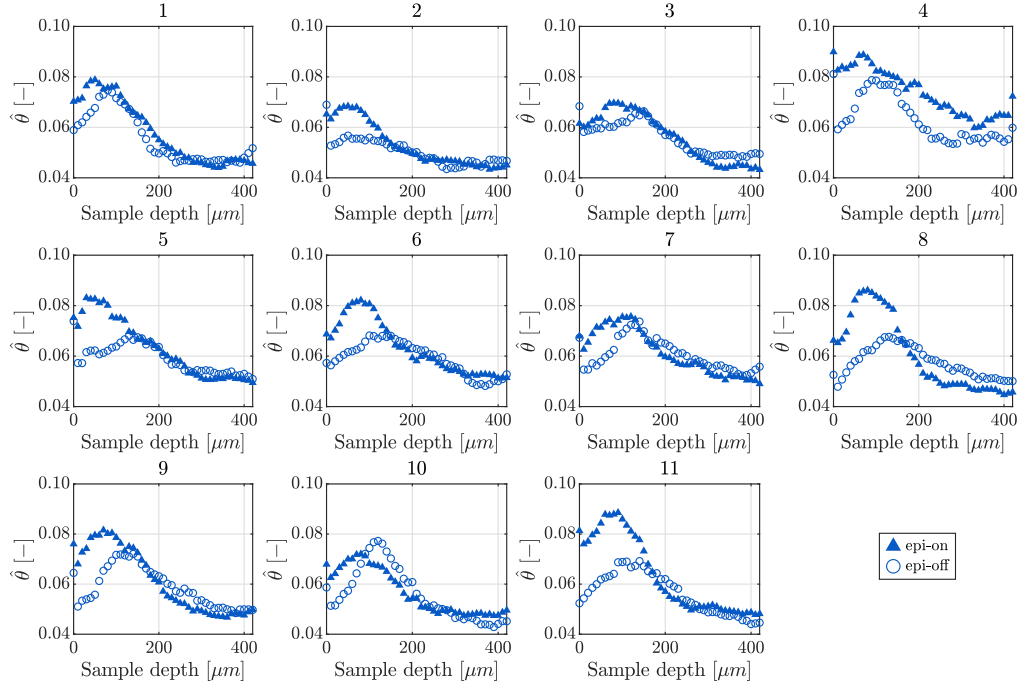


Figure 3.7: Values of Gamma scale parameter ($\hat{\theta}$) against the ROI position (sample depth). Every subplot is created for one porcine cornea (numbered in the title). Two series are presented for every eyeball: values received for intact cornea (epi-on) and after epithelial debridement (epi-off).

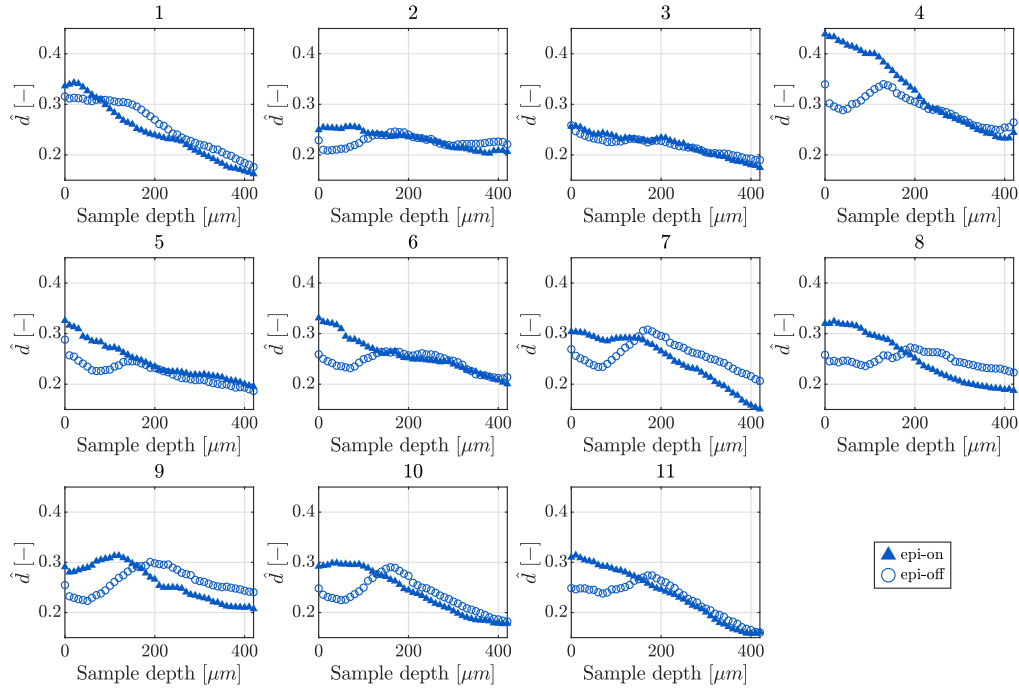


Figure 3.8: Values of mean ($\hat{d}$) against the ROI position (sample depth). Every subplot is created for one porcine cornea (numbered in the title). Two series are presented for every eyeball: values received for intact cornea (epi-on) and after epithelial debridement (epi-off).

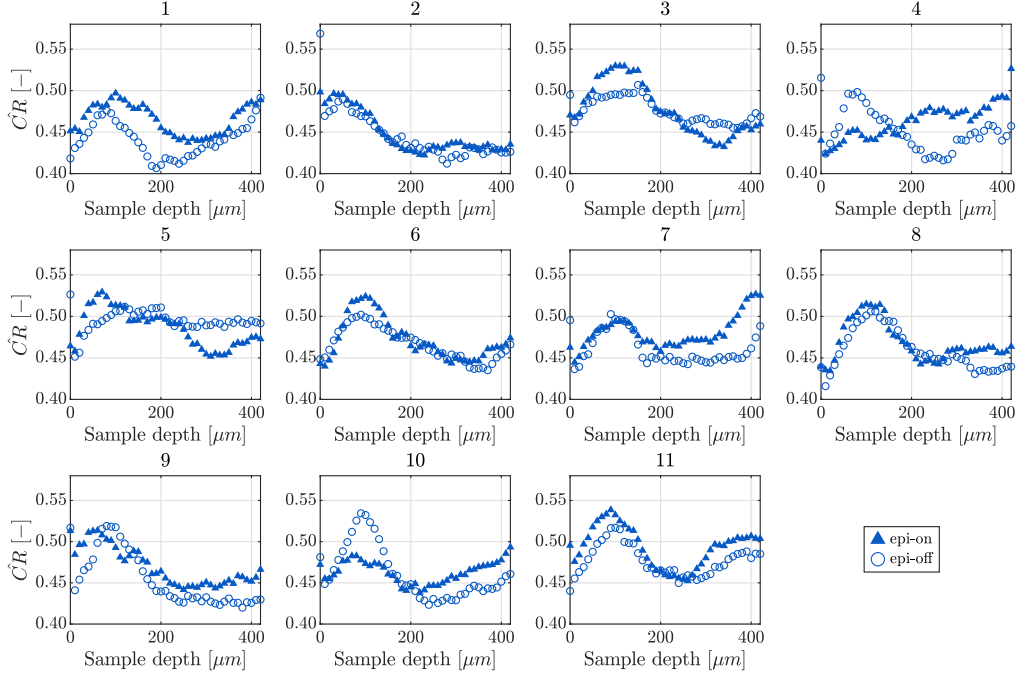


Figure 3.9: Values of contrast ratio ($\hat{C}R$) against the ROI position (sample depth). Every subplot is created for one porcine cornea (numbered in the title). Two series are presented for every eyeball: values received for intact cornea (epi-on) and after epithelial debridement (epi-off).

Attenuation could also have an influence on the pixel intensity profile, however it should have a minimal effect on the cornea for the NIR range, due to the low scattering and absorption coefficients [22, 137, 138]. Therefore, additional investigation on phantoms and simulated B-scan was performed, and will be described in the following section.

3.5 PHANTOMS AND SIMULATION: MEANWHILE INVESTIGATION

Retrospective data set of OCT images of 3 phantoms, recorded with SOCT REVO 80 were employed. The detailed description of phantom production can be found in [75]. In summary, the phantoms consisted of transparent epoxy resin L-285 (Havel composited, Cieszyn, Poland) mixed with a blue dye powder and poured onto microscope slides. They were in shape of convex discs with approximate diameter and thickness of 10 mm and 1 mm, respectively. The blue dye particles were uniformly distributed in the resin and varied in size from a few to tens of micrometers. The stroma was simplified here to a mixture of spheres suspended in a medium, as such approach is commonly used for creating corneal and other tissue phantoms for OCT [65, 69, 139]. Three phantoms with three different concentrations of blue dye were produced by adding approximately 10, 30 and 90 mm³ of blue dye to 1000 mm³ of epoxy resin. Figure 3.10 presents photos of three phantoms with corresponding OCT images.

The imaging protocol was set to a 5 mm single (unaveraged) B-scan, which consisted of 12,032 raw A-scans. Similarly as in porcine data, moving ROI of depth 100 μm, width 1 mm and forward step of 10 μm was introduced. The number of ROI positions was adapted to the thinnest phantom sample (700 μm), resulting in 61 sample depths

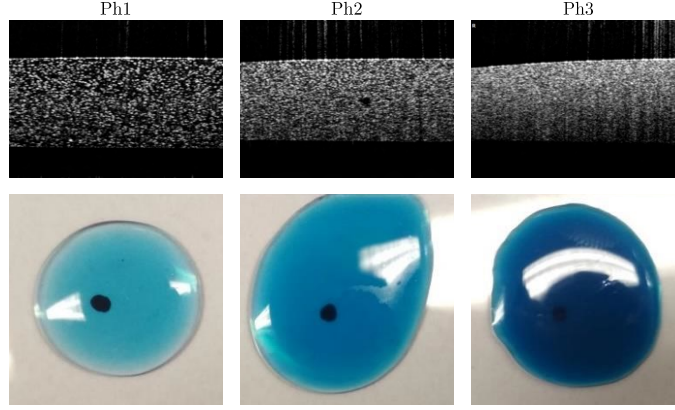


Figure 3.10: Three phantoms comprised of the mixture of blue dye particles and epoxy resin (bottom row) of three different concentrations with corresponding OCT images (top row).

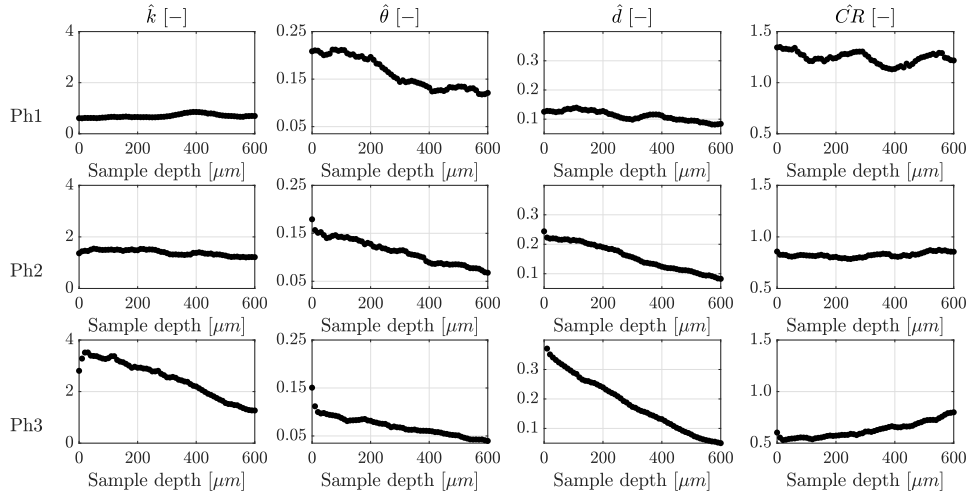


Figure 3.11: Plots of four densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$, in columns) against sample depth, produced for 3 phantoms (named subsequently Ph1, Ph2, and Ph3).

($N_D = 61$). The images were processed as porcine images, which was described in the previous section 3.4. Figure 3.11 shows plots of four densitometry parameters against sample depth, produced for 3 phantoms (named subsequently Ph1, Ph2, and Ph3). It can be observed that the intensity profiles for Ph2 and Ph3 are affected with attenuation of the signal (visible drop of the mean $\hat{d}$ and $\hat{\theta}$ with the ROI depth). At the same time, the earlier observed fluctuations of the $\hat{CR}$ and $\hat{k}$ parameters are almost not visible for Ph2 and Ph3. However, in Ph1 the attenuation is small, and it can be observed that despite uniform distribution of scattering particles in the sample, there still exist the fluctuations of $\hat{CR}$ and $\hat{k}$.

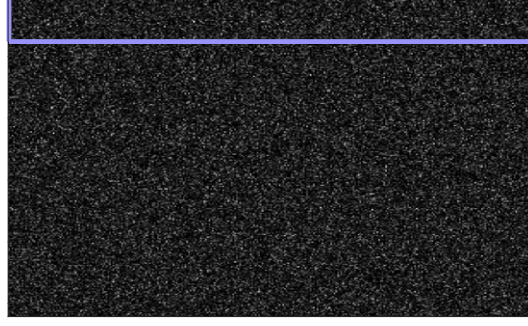


Figure 3.12: Simulated image, with pixel values governed by the Gamma distribution of shape parameter $k = 1$ and scale parameter $\theta = 0.15$. Image size: 308×260 pixels. Purple rectangle delineates the moving ROI of size 308×37 pixels and 1-pixel transversal step.

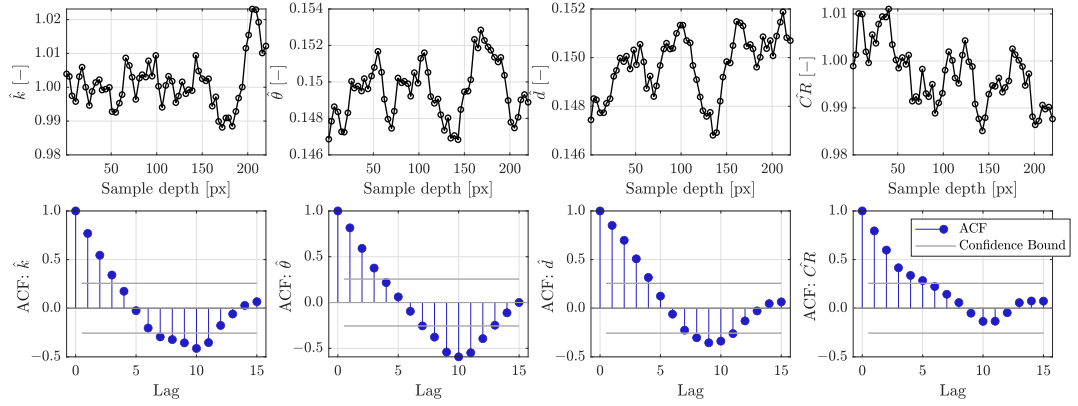


Figure 3.13: Plots of four densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$, in columns) against sample depth, calculated for simulated image. The second row presents the autocorrelation functions for every parameter.

Therefore, to check whether densitometry parameter estimators are stationary along the image depth and to exclude other factors such as device artifacts or sample irregularity, a simulated image was analyzed.

A matrix with random values governed with Gamma distribution of shape parameter $k = 1$ and scale parameter $\theta = 0.15$ was created. The size of the image was 308×260 pixels (according to the image size in phantom OCT data), and the moving ROI was of size 308×37 pixels with 61 ROI positions corresponding to the above described phantom study (see Figure 3.12). Figure 3.13 shows plots of four considered densitometry parameters against sample depth.

Consequently, since the sample size was large enough (61 ROI positions), also the autocorrelation function (ACF) was calculated and observed for 15 Lags (i.e., shifts of the examined series). The second row of the Figure 3.13 presents ACF with confidence bound of $[-0.25, 0.25]$. The ACF values drop to zero at Lag = 5 ($\hat{k}$, $\hat{\theta}$, $\hat{d}$) and at Lag = 10 for $\hat{CR}$. Based on Figure 3.13, densitometry parameters were assumed to be stationary for the simulated B-scan and not rigorously tested.

In conclusion, the analysis of the simulated data revealed that quasi-sinusoidal fluctuations of the parameters appear only when the real OCT data are processed. Therefore, the fluctuations were attributed to the imaging method (roll-off effect, beam focusing, and apex position in reference to the detector) and partially to

Table 3.1: The results of Spearman’s correlations and partial correlations (Part. corr.) between the epi-on and epi-off modes, calculated for the four estimated OCT densitometry parameter: $\hat{k}$, $\hat{\theta}$, $\hat{d}$ and $\hat{C}R$. Partial correlations were performed with stromal depth (SD), the subject number (SN), difference in sample axial position between epi-on and epi-off mode (AP) and mean epithelium intensity (MEI) as the controlling variable (Ctrl). r : correlation coefficient, p : the p -value.

	Correlation		Part. corr. Ctrl: SD		Part. corr. Ctrl: SN		Part. corr. Ctrl: AP		Part. corr. Ctrl: MEI	
	r	p	r	p	r	p	r	p	r	p
$\hat{k}$	0.669	<0.001	0.679	<0.001	0.662	<0.001	0.661	<0.001	0.670	<0.001
$\hat{\theta}$	0.804	<0.001	0.610	<0.001	0.802	<0.001	0.804	<0.001	0.789	<0.001
$\hat{d}$	0.696	<0.001	0.692	<0.001	0.695	<0.001	0.692	<0.001	0.672	<0.001
$\hat{C}R$	0.646	<0.001	0.604	<0.001	0.635	<0.001	0.637	<0.001	0.646	<0.001

SD – stromal depth,

SN – subject number,

AP – difference in sample axial position between epi-on and epi-off mode,

MEI – mean epithelium intensity

the weak but present sample attenuation. Excluding data non-stationarity for the underlying stochastic process in the moving ROI approach allowed to continue the analysis without preprocessing the data.

3.6 COMING BACK TO PORCINE EYES: QUANTIFY INFLUENCE OF EPITHELIAL LAYER

To determine whether there exist statistically significant differences in $\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{C}R$ and between the epi-on and epi-off modes, the Wilcoxon signed-rank test was used. Figure 3.14 presents the boxplots of the group densitometry parameters across the sample depth for epi-on and epi-off modes juxtaposed. The test showed statistically significant (at the level of significance set to 0.05) differences in stromal densitometry between epi-on and epi-off modes at specific depths, which are indicated in the figure with asterisks. Removal of the epithelium changes the stromal speckle parameters in a depth-dependent manner. For the shape parameter ($\hat{k}$), significant differences were found at 270–280 μm and again at 340 μm and deeper. The scale parameter ($\hat{\theta}$) showed significant differences only in the anterior stroma (0–130 μm), close to the epithelial layer. The mean ($\hat{d}$) had significant differences at both shallow (0–120 μm) and deeper (190–420 μm) stromal depths. For $\hat{C}R$, the differences were mostly notable at shallow depths (10–40 μm) and then again at 360–420 μm .

Further, Spearman correlations were evaluated for epi-on versus epi-of modes, as well as partial correlations. The following control variables were chosen: stromal depths, subject number (eyeballs), difference in sample axial position between epi-on and epi-off modes, and the mean epithelial intensity in the epi-on mode. Notable, the difference in the axial position of the sample ranged between 0 and 17 pixels, while the mean pixel value in the epithelial region was 0.183 ± 0.029 (mean $\pm$ standard deviation). Figure 3.15 shows the scatter plots for $\hat{k}$, $\hat{\theta}$, $\hat{d}$, and $\hat{C}R$ with the sample depth, indicated by the color saturation of markers. Table 3.1 supplements the corresponding results of Spearman’s correlations and partial correlations. All densitometry parameters perform statistically significant correlation between the epi-on and epi-off modes.

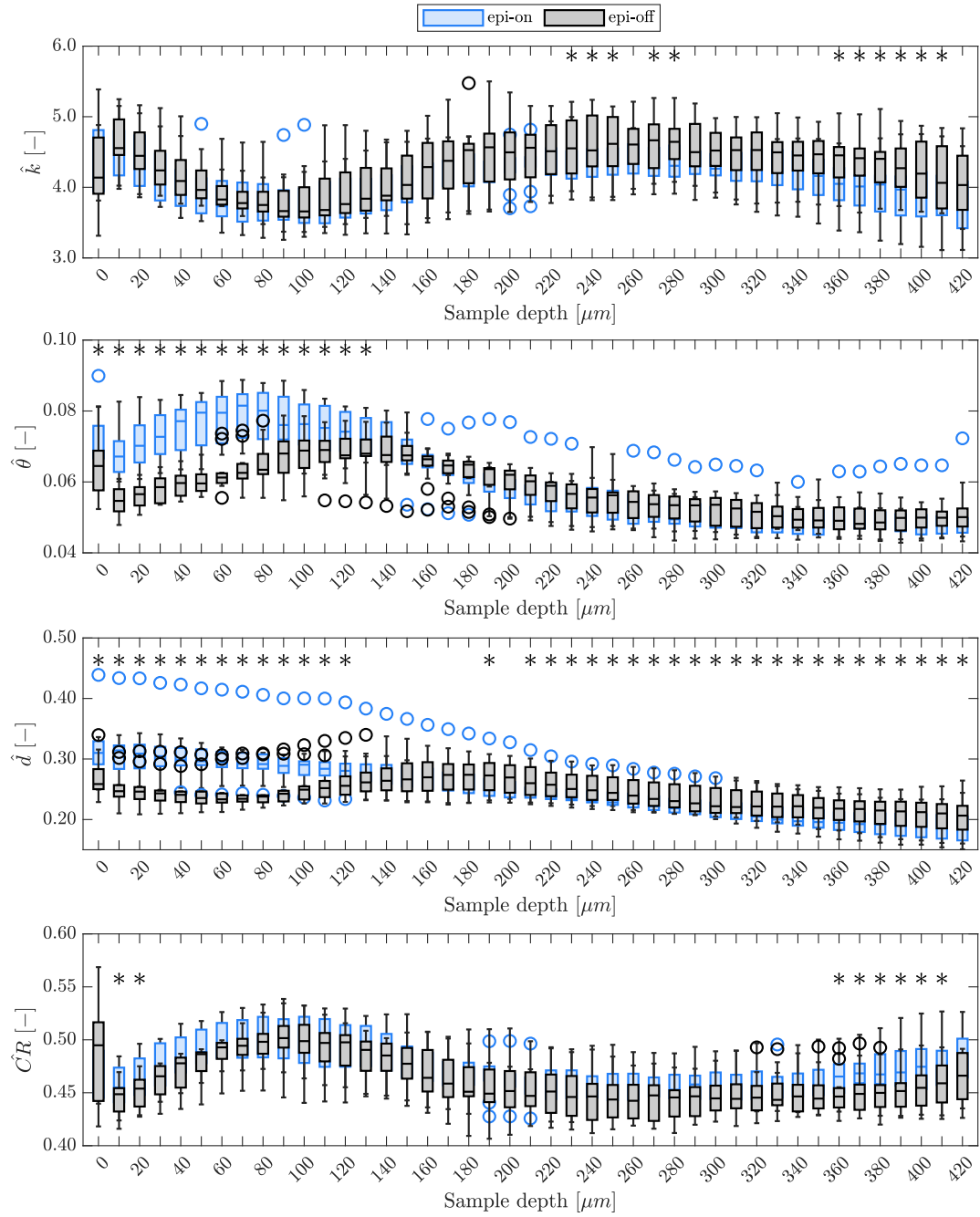


Figure 3.14: Boxplots of the four considered OCT densitometry parameters calculated for pixel intensities within the ROI: $\hat{k}$, $\hat{\theta}$ (shape and scale of Gamma distribution), $\hat{d}$ (mean) and $\hat{CR}$ (contrast ratio) calculated for different stromal depths. The value for each eyeball is the median of three repeated measurements. Each box is built of 11 values (for 11 porcine eyeballs). Circles denote outliers (Tukey's fences with $k = 1.5$). Asterisks indicate stromal depths at which there are statistically significant differences between epi-on and epi-off mode. Epi-on and epi-off mode means the measurement of a porcine eyeball before and after epithelial debridement, respectively.

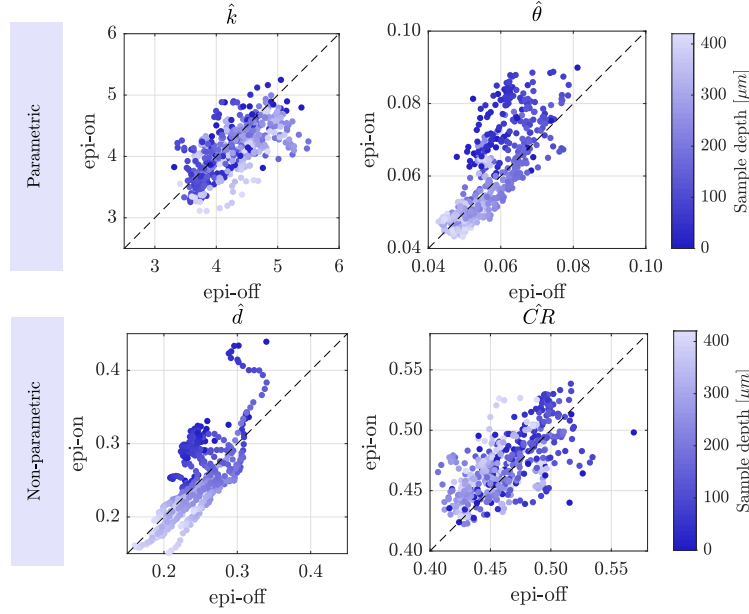


Figure 3.15: Scatter plots of four considered [OBT](#) densitometry parameters calculated for pixel intensities within the [ROI](#): $\hat{k}$, $\hat{\theta}$ (shape and scale of Gamma distribution), $\hat{d}$ (mean) and $\hat{CR}$ (contrast ratio) for epi-on and epi-off modes, where the stromal depth is indicated by the saturation of purple marks. Epi-on and epi-off mode means the measurement of a porcine eyeball before and after epithelial debridement, respectively. Each value is the median of three measurements taken for every eyeball. The dashed line indicates the 1:1 line.

In addition, the scatter plots indicate that the densitometry parameters of the corneal stroma are confounded when viewed through the intact epithelium layer, as part of points lies beneath or above the identity line, depending on the densitometry parameter and sample depth. Despite incorporating different control variables, the correlation is still maintained at the level of significance. For each of the parameters, the differences in correlation coefficients evaluated with (the first column in [Table 3.1](#)) and without a control variable (columns 2 to 5 of [Table 3.1](#)) were not substantial, indicating that all chosen control variables do not confound the results.

As the values of densitometry parameters estimated for epi-on are generally correlated to these for epi-off mode, but at the same time the differences between them depend on the sample depth, it was of interest how these parameters correlate at particular sample depths. Therefore, Spearman correlation tests between densitometry parameters in epi-on mode versus epi-off, were performed at every [ROI](#) position. [Figure 3.16](#) shows Spearman correlation coefficient. Purple points indicate $p < 0.05$, whereas the dashed line indicates the threshold value of 0.7578 for r , calculated for given sample size $n = 11$ (see [Equation 2.6](#)). The threshold value indicates the minimum r value at which the null hypothesis of no correlation can be rejected at level of significance 0.05 and 80% power. It can be observed that, unless a high correlation exists for the mean d along the entire sample depth, for other parameters, the value r strongly depends on the sample depth. Moreover, specific trends can be observed. For example, $\hat{CR}$ in epi-on and epi-off seems to be highly correlated in the anterior stroma, just beneath the epithelium, then at the depth of 200 μm and then again at the depth of 400 μm , while remaining uncorrelated for other depths.

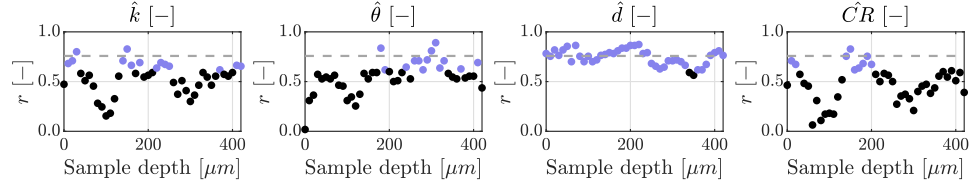


Figure 3.16: Spearman correlation coefficients calculated for 11 porcine subjects at particular sample depths. Purple points indicate $p < 0.05$, whereas the dashed line indicates the threshold value of 0.7578 for r , calculated for given sample size $n = 11$ (see Equation 2.6). The threshold value indicates the minimum r value at which the null hypothesis of no correlation can be rejected at level of significance 0.05 and 80% power.

Similar reasoning can be performed for the Gamma parameters. This observation may suggest the most affected are those depths for which the correlation is low. It should be further investigated whether epithelial brightness or thickness influences this result.

To fairly quantify the influence of the epithelial layer on the stromal speckle, another measure was attempted: the median error (expressed in percentages) calculated for each stromal depth. For example, for $\hat{k}$, shape parameter:

$$\left(1 - m\left(\frac{\hat{k}_{\text{epi-off}}}{\hat{k}_{\text{epi-on}}}\right)\right) \times 100, \quad (3.1)$$

where m denotes the sample median, where $\hat{k}_{\text{epi-off}}$ and $\hat{k}_{\text{epi-on}}$ are the estimators of the shape parameter k for the epi-off and epi-on modes at the particular sample depth, respectively. The median errors for other parameters: scale $\hat{\theta}$, the sample mean $\hat{d}$, and $\hat{CR}$ were calculated analogously. The values of median error are presented in Figure 3.17. The region highlighted with light purple is an area of $\pm 5\%$ error, assumed here as clinically negligible. Particularly noteworthy is the median error of over 20% for $\hat{\theta}$ and $\hat{d}$.

3.7 DISCUSSION

In standard clinical conditions, the stromal layer is always seen through the epithelium. The current work shows that OCT-based corneal densitometry is affected by the epithelial layer and that the epithelium tends, depending on the parameter considered, to over- or underestimate stromal readings (see Figure 3.15). In this study, four speckle measures for corneal densitometry were investigated: k , θ for the parametric approach, and d , CR for the non-parametric approach. Independently of the metric used, corneal stroma densitometry differed in a statistically significant manner depending on whether the epithelium was present (epi-on) or absent (epi-off) and on sample depth (see Figure 3.14).

If the epithelium did not affect the stromal densitometry, no statistical significance would be expected in Figure 3.14 and the points in the scatter plots of Figure 3.15 would fall on the 1:1 line. However, the results show the opposite. For example, the parameters $\hat{\theta}$ and $\hat{d}$ exhibit similar behavior: at small stromal depths (close to the epithelium, darker points in Figure 3.15), their values lie above the 1:1 line, signifying overestimation in the epi-on setting. In contrast, $\hat{k}$ shows the opposite trend, as its values are underestimated in deeper stromal layers (further from the

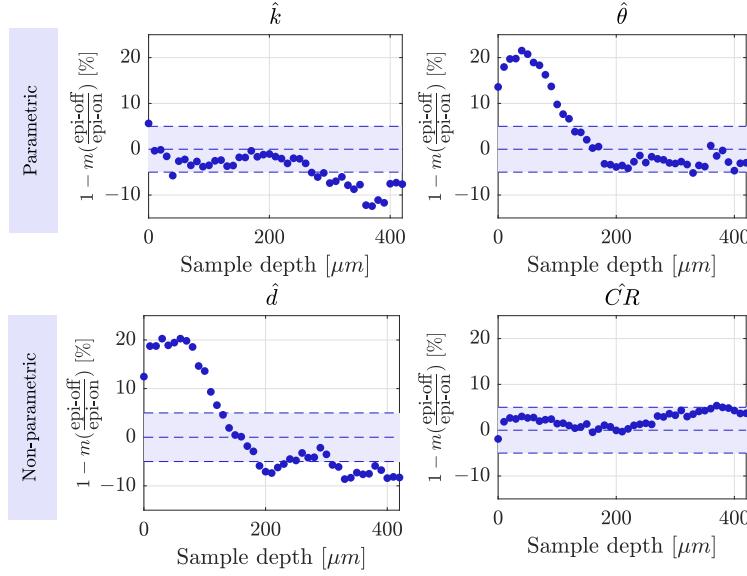


Figure 3.17: Median errors between epi-on and epi-off mode of four considered OCT densitometry parameters calculated for pixel intensities within the ROI: $\hat{k}$, $\hat{\theta}$ (shape and scale of Gamma distribution), $\hat{d}$ (mean) and $\hat{CR}$ (contrast ratio), showing the confounding effect of epithelial speckle when evaluating the densitometry on different stromal depths. Each value is the median of three measurements taken for every eyeball. An area of $\pm 5\%$ error, assumed here as clinically negligible, has been highlighted in light purple.

epithelium, brighter points in Figure 3.15). The most pronounced bias occurs in the anterior stroma, likely because of the attenuation of the OCT signal in deeper layers. Nevertheless, minor but still statistically significant differences were also observed in the posterior stroma region.

Despite θ being a parametric approach and d being non-parametric, they carry analogous information (see Figures 3.15 and 3.17). This similarity arises from their common mathematical origin, as the mean value of the Gamma distribution is defined as the product of k and θ . Similarly, the mathematical relation of CR and k can be described: $CR = 1/\sqrt{k}$. Consequently, $\hat{k}$ tends to be underestimated, while $\hat{CR}$ is overestimated (Figure 3.15). Hence, the parametric and non-parametric approaches carry different, complementary information, and both should be considered for corneal densitometry.

One may be uncertain as to whether the changes in stromal speckle stem truly from the absence of epithelial layer or singly from the change in the sample surface reflectivity due to the debridement process. Possibly, epithelium removal could increase the surface roughness, causing increased diffuse reflectance, instead of specular reflectance, and thus altering the OCT signal from the stroma. The eyeballs were constantly moistened with PBS, ensuring the same immersion conditions both for epi-on and epi-off acquisitions, therefore the reflectivity of the sample surface was assumed to remain unchanged. In addition, no notable changes in the reflectivity between epi-on and epi-off acquisitions were observed in OCT B-scans. Nevertheless, additional analysis should be performed, taking pixels values from the interface region between the sample and the medium as a control variable.

Also, the current study sheds a new light on the results of the experimental work performed earlier by Danielewska et al. crosslinking (CXL) using freshly enucleated porcine eyes [86]. In particular, this concerns the result of a CXL procedure corresponding to standard epi-off Dresden protocol, but without the exposure to ultraviolet A (UVA) radiation. The statistically significant changes observed there in $\hat{C}R$ before epithelial debridement and after the CXL (without UVA) procedure can be interpreted as differences between epi-on and epi-off modes, irrespective of CXL.

The study has no strong limitations. It should be acknowledged that the results obtained were achieved for the given set of porcine eyes and a particular OCT device with a given scanning protocol. The presented results highlight the problem of the epithelium confounding stromal densitometry measurements. A similar effect of one tissue layer confounding the results of the underlying layer is expected to occur in human eyes, but the extent of that effect is currently unknown.

In the end, the clinical significance of the results obtained should be considered. The examination of corneal speckle is of interest in studies involving the debridement of the epithelium [140] or the corneal healing processes [141, 142]. In fact, any change in the optical properties of the epithelium can inherently alter the stromal speckle statistics. For example, this can occur after epithelial damage, corneal hypoxia, or epithelial structural change, as reported in diabetes [143]. If the influence of the epithelium is not considered, then particularly anterior corneal stromal readings could be over- or underestimated.

INDUCED HYPOXIA IN HUMAN CORNEA: A PILOT STUDY

In Chapter 3 it was proved that the epithelium significantly influences the speckle statistics of the corneal stroma. Therefore, as discussed in that chapter, any microstructural change in the corneal epithelium may also affect the stromal speckle. A preliminary study was considered, in which microstructural changes in the corneal epithelium would be induced in vivo in the human eye. A commonly used approach for inducing changes in the eye is the induction of corneal hypoxia through contact lens application. This has been shown to result in microstructural changes in both the corneal epithelium and the stroma [41, 144], due to corneal swelling. It has also been documented that the posterior stroma swells faster than the anterior [145–147], possibly due to the more interwoven arrangement of collagen lamellae in the anterior part [144, 148].

4.1 DATASET AND IMAGING PROTOCOL

Two participants, one of age < 30 (Y) and the second of age > 50 (O), underwent the following procedure: the right cornea was imaged (three consecutive measurements without re-aligning the instrument) with the SOCT REVO 80 device (Optopol Technology, Zawiercie, Poland). The imaging protocol was set to a 5 mm single (non-averaged) B-scan, with a number of A-scans of 12,032. Subsequently, contact lens (CL) were applied on the cornea and the OCT imaging was repeated immediately. Then, the eye with CL applied was patched for 3 hours, thereby inducing hypoxia. After this time, the eye was uncovered and the cornea was imaged again with CL and immediately after CL removal. Two more subsequent measurements were taken: 1 hour and 2 hours after eye uncovering. The measurement mode (and all parameters calculated from the images) with CL applied will be further denoted as CL-on mode, whereas the one without CL will be denoted as CL-off mode. The workflow with illustrative corneal OCT images is presented in Figure 4.1. For this study a low-water-content silicone-hydrogel lens was chosen to amplify the effect of corneal swelling and, at the same time, to ensure a visible speckle appearance in OCT images. An illustrative corneal image of the older subject, with CL applied is shown in Figure 4.2.

4.2 IMAGE SEGMENTATION, ROI SELECTION AND DENSITOMETRY PARAMETERS

According to the previous analysis described in Chapter 3, the algorithm for OCT image speckle analysis was employed. CL and the epithelium were manually segmented by automatically identifying the location of the apex within the anterior edge of the sample and by manually identifying the interfaces of CL-epithelium and epithelium-stroma. The thicknesses of the CL and epithelium were measured and applied along the anterior edge (assuming that, for the given horizontal imaging range, the thickness of the CL and epithelium are constant along the cornea). The width of the region

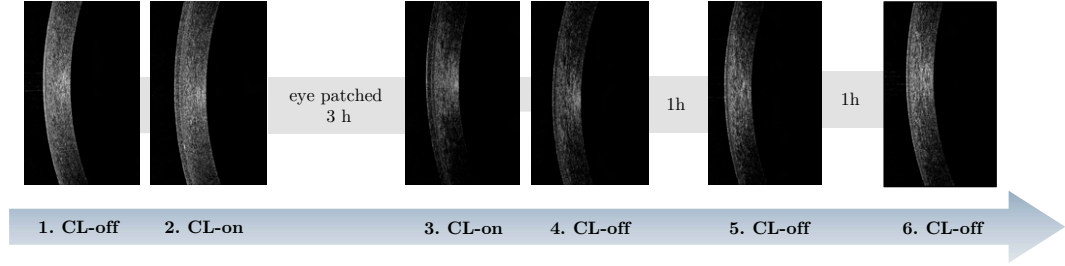


Figure 4.1: Sequence of acquisitions presented on a timeline of the experiment with additional comments. **CL-on** denotes the measurement with contact lens applied, **CL-off** is the measurement without contact lens. Eye patching the eye with **CL** applied induced the corneal hypoxia.

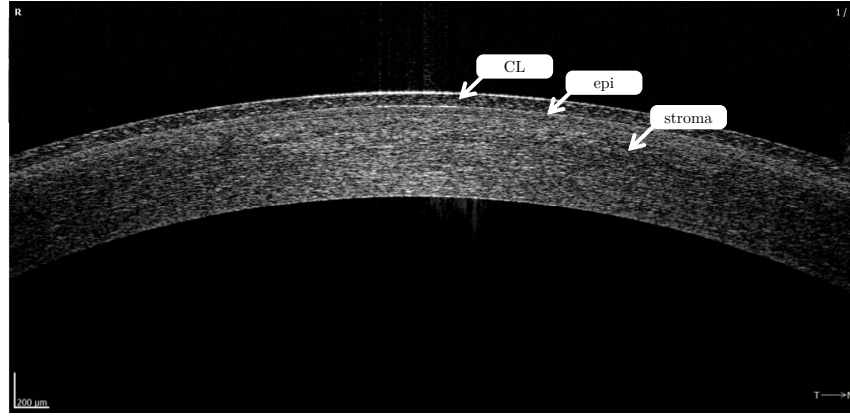


Figure 4.2: **OCT** image presenting the eye of the older subject with contact lens applied. Particular layers are tagged: contact lens (**CL**), epithelium (**epi**) and the stroma.

of interest (**ROI**) was set to 1 mm, while its depth was equal to the thicknesses of the **CL** and epithelium, respectively, and for the stroma, a moving **ROI** of depth 100 μm and forward step of 10 μm was applied. In addition, the margin of 2 pixels for the tear film was set for all images and these pixels were excluded from further analysis. Four densitometry parameters: Gamma shape (k) and scale (θ), the mean (d) and the contrast ratio (CR) were estimated and collected for **CL**, epithelium and subsequent depths of the stroma. At every **ROI** position, the median of three consecutive measurements (taken at every stage of experiment) was calculated and used for further analysis.

4.3 RESULTS

Firstly, Figure 4.3 presents densitometry parameters collected for measurements taken before (in black) and just after (in blue) hypoxia induction (see acquisition numbers 1–4 in Figure 4.1 for reference). Additionally, densitometry parameters for the **CL** and the epithelium are provided. The triangles indicate the **CL-on** acquisitions, whereas the circles indicate **CL-off** acquisitions. It is apparent that $\hat{k}$ and $\hat{d}$ are reduced after induced corneal hypoxia, while $\hat{\theta}$ and $\hat{CR}$ behave inversely. It can also be observed, that for the younger subject (**Y**), the differences between before and after hypoxia induction are larger than those for older subject (**O**). The epithelium

seems to be much more affected than the stroma (see the distance between the black and blue circle for “epi”). Notably, when the epithelium is observed via CL, its speckle statistics differ from those obtained without it. The text boxes in each plot present the densitometry parameters assessed for the CL itself. Interestingly, $\hat{k}$ and $\hat{\theta}$ parameters of the Gamma distribution estimated for CL differ before and after hypoxia induction. The mean pixel value, $\hat{d}$, does not change and $\hat{CR}$ changes only slightly. Furthermore, a non-straightforward effect of CL presence on the subsequent sample layers can be observed. For example, for $\hat{k}$ and $\hat{d}$: before induced hypoxia, CL presence reduces the epithelial value, while after — the opposite effect is observed, i.e., the epithelial values are higher when recorded in CL-on than in CL-off mode.

Figure 4.4 presents the obtained values of densitometry parameters as a function of sample depth, for measurements 1–6 along the timeline of the experiment, denoted as subsequent series (1)–(6) in every subplot (see Figure 4.1 for reference). For the Y subject, the recovery process can be observed, as $\hat{k}$ and $\hat{\theta}$ values obtained from acquisitions after 1 hour (yellow in Figure 4.4) and 2 hours (purple) after hypoxia induction lie between the values for the acquisitions before and just after hypoxia induction. It can further be interpreted that the posterior stroma recovers more slowly than the anterior part, as the distance between purple and yellow points relative to the black points (before hypoxia) increases with sample depth. Finally, Figure 4.5 presents the percentage difference between corneal speckle statistics assessed before and just after hypoxia induction. The percentage difference was calculated separately for CL-on and CL-off acquisitions. The method of calculation was analogous to the one described in Equation 3.1. For example, for the k parameter:

$$\left(1 - \frac{\hat{k}_h}{\hat{k}_c}\right) \times 100, \quad (4.1)$$

where $\hat{k}_h$ and $\hat{k}_c$ are the estimators of the shape parameter k for the cornea under induced hypoxia (h) and the cornea in its normal state (c) at a given sample depth, respectively. The metrics for other parameters: scale θ , sample mean d , and CR were calculated analogically. The axes limits for Figure 4.5 were chosen for convenient visualization of the difference values and for comparison between the subjects. Therefore, the values at the beginning of the plot for the Y subject and at the end of the plot for the O subject were considered as outliers and excluded from the view. Notably, the differences between measurements before and after hypoxia induction are much higher for the Gamma parameter estimators ($\hat{k}$, $\hat{\theta}$) than for $\hat{d}$ and $\hat{CR}$. A clear trend can be observed for O subject, across all densitometry parameters, showing the highest differences at the sample depth of approximately 150 μm . The trend is visible regardless of whether the corneal stroma is observed via CL or not. The plots for Y subject are more noisy, and no such clear interpretation can be made, however $\hat{k}$ and $\hat{\theta}$ appear to follow the trend of these estimates for O subject.

4.4 DISCUSSION

Corneal swelling is a non-uniform process across the corneal depth. The pilot study performed here has shown that the most affected layer is the corneal epithelium (see Figure 4.3). Also, the differences in densitometry parameters between the corneal state before and after induced hypoxia were uneven across the corneal stroma. The most affected part, particularly for the older (O) subject, seems to be in the

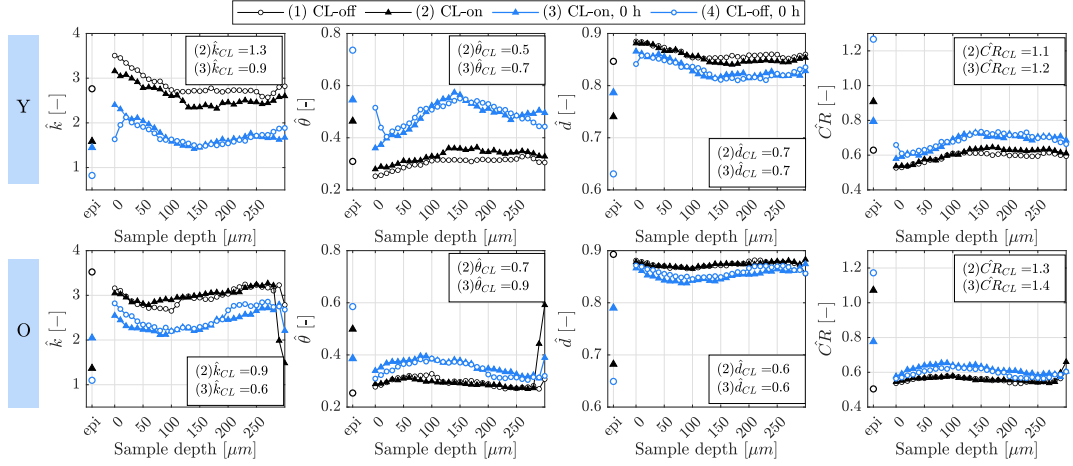


Figure 4.3: Plots of four densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$, in columns) against sample depth, i.e., **CL**: contact lens (in text boxes), epi: epithelium and further stromal depths. These produced for the younger (**Y**) subject are placed in the top row, whereas results for the older (**O**) subject are presented in bottom row. Only 4 acquisitions are here presented: those taken before eye patching (black) and those taken just after eye patching, inducing hypoxia (blue). Subsequent series numbered in the legend as (1)–(4) and denoted with different colors and shapes refer to the experiment timeline (see Figure 4.1). The triangles indicate the acquisitions with contact lens (**CL-on**) whereas the circles are for acquisitions without lens (**CL-off**).

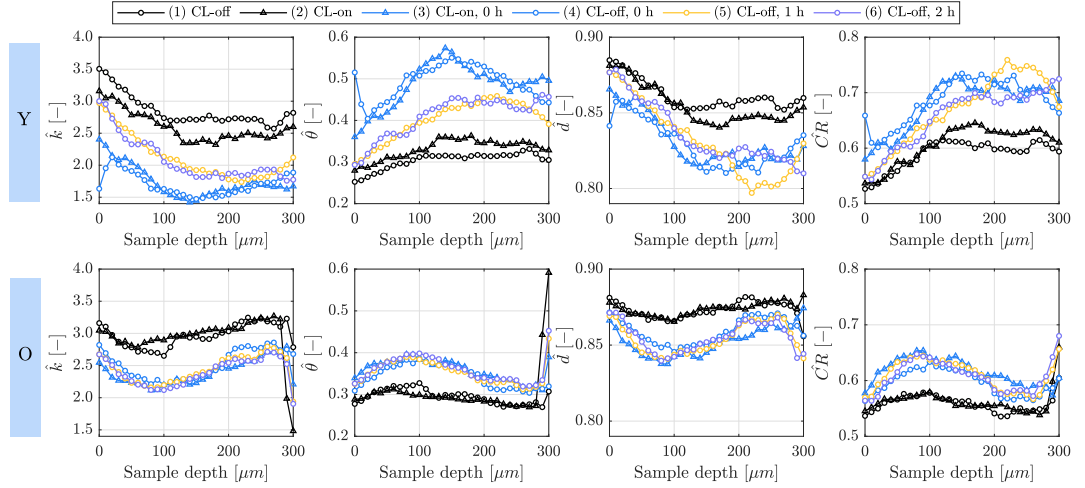


Figure 4.4: Plots of four densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$, in columns) against sample depth (here only the stroma is presented). These produced for the younger (**Y**) subject are placed in the top row, whereas results for the older (**O**) subject are presented in bottom row. Subsequent series numbered in the legend as (1)–(6) and denoted with different colors and shapes refer to the experiment timeline (see Figure 4.1). The triangles indicate the acquisitions with contact lens (**CL-on**) whereas the circles are for acquisitions without lens (**CL-off**).

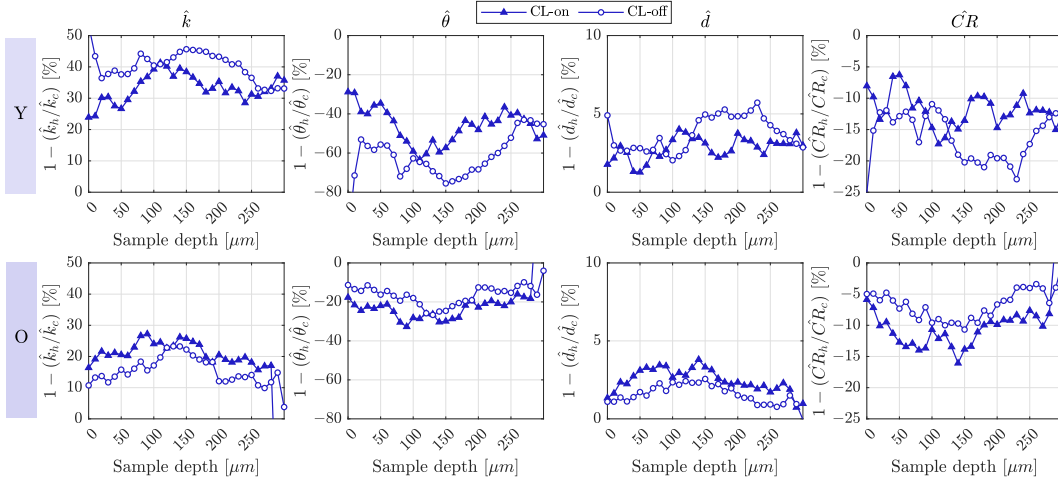


Figure 4.5: Percentage difference calculated for four densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$) for acquisitions before and just after induced hypoxia, and against stromal depth. These produced for the younger (Y) subject are placed in the top row, whereas results for the older (O) subject are presented in bottom row. The triangles indicate the acquisitions with contact lens (CL-on), i.e., the difference was calculated between acquisition number 2. and 3. from the experiment timeline (see Figure 4.1). Circles are for acquisitions without lens (CL-off), i.e., the difference was calculated between acquisition number 1. and 4. from the experiment timeline.

posterior part, at the sample depth of 150 μm , whereas deeper stromal depths seem to be less affected. Therefore, the obtained values are in partial compliance with the literature, as the posterior stroma was reported to swell faster [145–147]. This work also confirms the parametric (distributional) approach as a valuable method for assessing corneal densitometry, as it supports the non-parametric results with complementary information. For example, Gamma distribution parameters $\hat{k}$ and $\hat{\theta}$ have been shown to be more sensitive to differences between acquisitions before and after induced hypoxia (indicated by higher values of the percentage difference in Figure 4.5 compared to $\hat{d}$ and $\hat{CR}$).

The purpose of the study was to induce changes in the epithelium to investigate how these changes affects the stromal speckle in the human cornea in vivo, since in the previous chapter it was demonstrated that the epithelium confounds the OCT signal from the stroma in porcine eyes. However, since hypoxia induces corneal swelling in both the epithelium and the stroma, it is not easy to separate the effect of epithelial changes from the effect of change in the stroma itself. Therefore, for such a purpose another experiment should be designed in which only epithelial changes would be induced. In reality, such conditions do not exist, as even diseases that were reported to affect mainly the epithelium, e.g. diabetes [143, 149–151], influence also the stroma due to epithelial-stromal interactions [143, 152, 153]. Therefore, only an OCT B-scan simulation or a multi-layered phantom study could be used to explore the problem and to model the conditional nature of the speckle statistics. Nevertheless, it is worth noting that introducing additional speckle-generative layer on the eye also appears to change the speckle statistics of the underlying cornea, however, the direction of this change, i.e, whether it increases or decreases the speckle parameters was not consistent in this study. Including a larger number of subjects could possibly clarify this dependence. At the end, it needs to be acknowledged that the results

reported here for the younger and older subjects are single-case studies. In addition, acquisitions for these two subjects were collected by two researchers, as they were both examined subjects and experimenters. Therefore, no strong conclusions can be drawn regarding the reported speckle statistic values.

OCT DIGITAL CORNEAL PHANTOM FOR OCT SPECKLE ANALYSIS

Funding:

National Science Center (Poland), PRELUDIUM 2023/49/N/ST7/04071 (Title: *Advanced techniques for a holistic analysis of corneal OCT speckle*).

Throughout this thesis it was demonstrated that the assessment of corneal scattering parameters from corneal images is confounded by multiple factors (Chapter 2). Moreover, its multilayered nature was underlined, and the confounding effect of subsequent layers on corneal backscatter (Chapter 3) was visualized. The OCT imaging technique has higher resolution than Scheimpflug imaging. Furthermore, the signal received in OCT, namely speckle, has a physical explanation and well-described statistics [65, 73, 75, 78], while Scheimpflug imaging does not. As such, OCT is a promising platform for corneal densitometry. Additionally, a gap in the current state-of-the-art was recognized: stromal OCT speckle is currently under investigation [75, 78, 79, 154], while that of the epithelium has been largely ignored for a long time [131]. Nowadays, OCT imaging resolution allows for the easy distinction of this particular layer in the cornea and, subsequently, the analysis of its scattering properties. As a further step in this scientific journey, the concept of creating a holistic model for corneal OCT backscatter that would account for all corneal layers and their interactions naturally emerged. This idea was also approved for funding by the National Science Center (Poland) and the following chapter is supported under the grant PRELUDIUM 2023/49/N/ST7/04071. One of the stages of this project is to create an OCT digital phantom for the cornea including its two major layers: epithelium and stroma. Different methods exist in the literature for OCT signal simulations and different scenarios can be considered: the one assuming single scattering and another assuming multiple scattering. The latter is likely to happen in highly forward scattering tissues [83]. It can be divided into a group of analytical models [74, 136, 155, 156], particularly those based on the Extended Huygens Fresnel (EHF) principle [157, 158] and probabilistic models based on Monte Carlo simulations of photon transport through the sample [159–161]. However, to date, there has been only one attempt to simulate the corneal OCT signal [162], utilizing the Monte Carlo approach. Therefore, the purpose of this work was to adapt the existing Monte Carlo simulation methods of photon transport [132] and the OCT signal [159] to the cornea and to ophthalmic OCT, and to justify the utility of the method for speckle analysis and corneal densitometry. The whole process of creating a corneal OCT digital phantom was manually implemented in Matlab by the author, based on the aforementioned works. Understanding the theory and implementation of these methods was an important part of the implementation and adds value to the interpretation of the results and to adapting the algorithm for ophthalmic OCT. Therefore, firstly, Section 5.1 provides a detailed explanation of the Monte Carlo algorithm implemented in this work. Subsequently, in Section 5.2 the equation for OCT signal calculation is presented, and the equation for the signal envelope is derived. Sections 5.3–5.5 present and justify the input parameters chosen for the particular OCT device and the cornea. Section 5.6 presents the first results obtained, while Section 5.7 shows the methodology and results of the comparison

between simulated and experimental images acquired for porcine corneas. The chapter ends with a discussion (Section 5.8).

5.1 MONTE CARLO SIMULATION OF PHOTON TRANSPORT

The Monte Carlo method dates back to 1945 — the time when wartime demands set the pace for scientific development. One of the major achievements of that period was the construction of the first electronic computer, ENIAC, which brought a renaissance to statistical sampling. The authors, Stanislaw Ulam, Nicholas Metropolis, and John von Neumann introduced an alternative to the analytical way of modeling phenomena or processes, based on the use of random numbers. The heart of this method is the generation of uniformly distributed pseudo-random numbers. Every step and every decision is governed by a random number transformed to a desired distribution, appropriate for the simulated process. The name of the method is humorously connected with the uncle of Stanislaw Ulam¹, who used to borrow money from relatives, explaining himself that “he had to go to Monte Carlo”, a district in Monaco known at that time as a sophisticated and extravagant gambling destination. As Monte Carlo was designed to simulate processes it was also adapted to simulate photon transport through biological tissue.

The algorithm utilized in this work was designed in the 1990s by Scott Prahl [132, 163], based on the Monte Carlo simulations of scattering in reflection nebulae² [164], and is commonly used for biomedical purposes. To mention just a few applications, it was applied to the simulation of magnetic resonance images of the head [165] or to the prediction of heat diffusion and tissue damage [166]. Its core is Mie scattering theory. One may debate whether the Mie scattering theory is adequate for simulating photon transport through corneal tissue. Corneal stroma consists of collagen fibers and keratocytes, which constitute up to 17% of the tissue volume [22, 29]. According to this composition, as in most of biological tissues, two types of light scattering should be considered: Mie and Rayleigh scattering [5, 22]. However, the Rayleigh scattering is actually a special case of Mie theory. Therefore, the latter can be applied exclusively [4, 7].

The general workflow of the algorithm is as follows: a photon packet is launched at the sample surface and propagated through it. The path of the photon packet is governed by the optical properties of the tissue and its surroundings (see Figure 5.1), i.e., the scattering coefficient μ_s , the absorption coefficient μ_a , the anisotropy coefficient g and the refractive index n , until it leaves the sample, being transmitted, backscattered, or absorbed. At the end, quantities such as the total photon packet path or its weight are collected. According to the Monte Carlo idea, multiple photon packets are launched and tracked. This number should be carefully chosen and will be explained later in this chapter. Figure 5.2 introduces the primary flowchart of this algorithm for a one-layer, semi-infinite tissue. The term semi-infinite means that the sample is limited only by vertical planes representing the anterior and posterior sample surfaces. The subsequent steps presented in the flowchart will be addressed in the following subsections. The explanation will be kept in the context

¹ Stanislaw Ulam was a well-known Polish mathematician of Jewish origin, who was born in Lwów, and later moved to the USA. He is recognized not only for the Monte Carlo method, but also for his contribution to the Teller-Ulam design of thermonuclear weapons (while working on Oppenheimer’s Manhattan Project).

² A reflection nebula is a cloud of interstellar dust which scatters the radiation from neighboring stars.

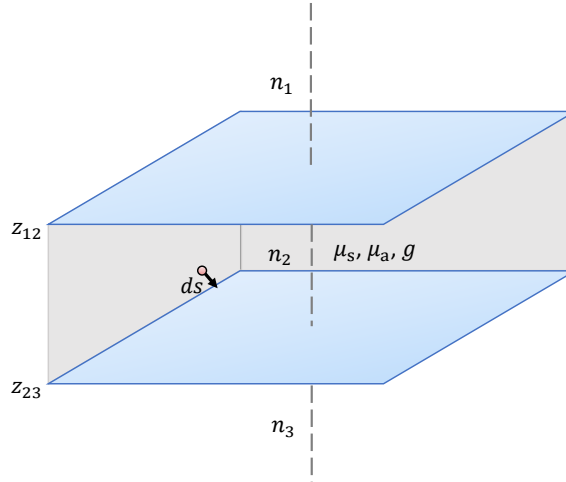


Figure 5.1: Illustration of the tissue parameters taken as input for Monte Carlo simulation of photon transport. Blue surfaces limit the semi-infinite tissue sample, orange point illustrates the photon packet transported. Symbols: n_2 is the refractive index of the sample, n_1 , n_3 are refractive indices of surrounding media, μ_s , μ_a , g : scattering, absorption and anisotropy coefficients of the sample, respectively, z_{12} , z_{23} : sample borders, ds : a single step randomly generated for a photon packet in Monte Carlo algorithm.

of the application presented here (that is, ophthalmic OCT), so some variants of the simulation, such as diffuse irradiance or placing the sample under the glass slab, will be left out.

5.1.1 Random variable in Monte Carlo method

As it was mentioned, Monte Carlo algorithms are based on sampling pseudo-random uniformly distributed numbers at every step of simulation. In this work the random variable is generated with Matlab (`rand()`) and will be denoted further as:

$$\xi \in [0, 1] \quad (5.1)$$

and will be transformed at different step-processes due to the scattering theory.

5.1.2 Launch photon packet of weight of 1

The term “photon packet” is understood here as a packet of photons that travel through the tissue together, having exactly the same path. The weight W equivalent here to the beam “intensity” has assigned a value of 1 to the packet at the beginning and drops at every absorption event, meaning the part of the photons being absorbed (See Section 5.1.6). Also in specular reflection, a part of the photons enters the sample, while the rest is reflected. Each photon packet is described with its weight W , total path traveled through the sample S , location in Cartesian coordinates (x, y, z) , and direction using directional cosines (μ_x, μ_y, μ_z) . The directional cosine is defined as the

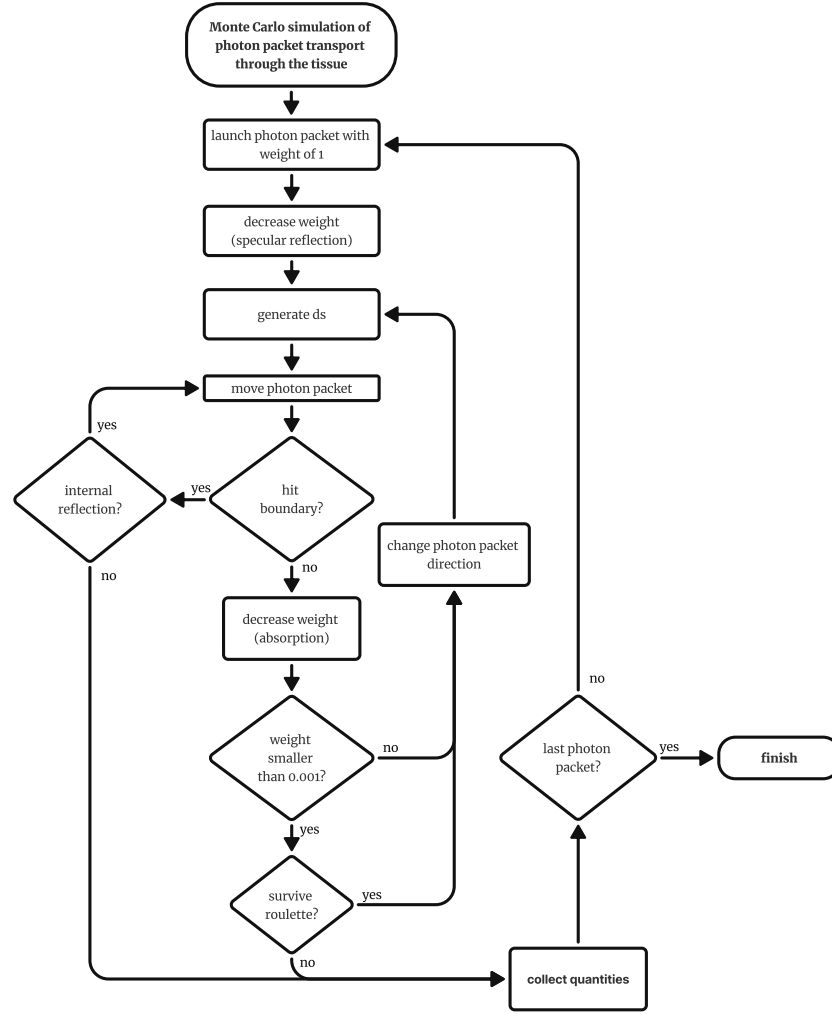


Figure 5.2: Simplified schematic for the Monte Carlo algorithm for photon packet transport through the one-layered scattering tissue [own work based on [132, 163]]

cosines of the angle between the particular Cartesian coordinate and the Euclidean vector of the beginning in $(0, 0, 0)$. Therefore, they may be calculated as follows:

$$\begin{aligned}
 \mu_x &= \frac{x}{\sqrt{x^2 + y^2 + z^2}} \\
 \mu_y &= \frac{y}{\sqrt{x^2 + y^2 + z^2}} \\
 \mu_z &= \frac{z}{\sqrt{x^2 + y^2 + z^2}}.
 \end{aligned} \tag{5.2}$$

At the moment of launch of photon packet, its starting location is set at the sample surface, which is the beginning of the Cartesian coordinate system. The direction

is set downward, perpendicular to the sample surface. The total photon path S is initially set to 0. Hence, these initial photon parameters take the following values:

$$\begin{aligned} W &= 1, \\ (x, y, z) &= (0, 0, 0) \\ (\mu_x, \mu_y, \mu_z) &= (0, 0, 1) \\ S &= 0. \end{aligned} \tag{5.3}$$

Also, four flags are assigned to the photon packet, with initial values:

```
transmitted = false
absorbed = false
backscattered = false
at_detector = false,
```

where the last flag is the information on whether the backscattered photon hit the detector.

5.1.3 Specular reflection

The beam projected on the sample surface is reflected at the medium-sample interface and only a part of photon packets enters the tissue. The fraction of photons that continue to propagate in the tissue is calculated using the equation for the reflection coefficient (Equation 5.4)[167], and accordingly the weight W of the photon packet is reduced (Equation 5.5):

$$R_{sp} = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2}, \tag{5.4}$$

where n_1 and n_2 are the refractive indices of the medium preceding the sample and of the sample, respectively (see Figure 5.1).

$$W = 1 - R_{sp}. \tag{5.5}$$

5.1.4 Generate step and move photon packet

Starting at the sample surface, the photon packet is subjected to subsequent, stochastic scattering events depending on the attenuation coefficient $\mu_t = \mu_s + \mu_a$ and the mean free path which is equal to $\frac{1}{\mu_t}$. It is executed by generating the small steps ds with the use of Monte Carlo uniformly distributed variable (see Section 5.1), transformed with the use of Lamberts-Beer law. This step is understood as the distance that a photon packet will travel before interaction (absorption or scattering).

$$ds = -\frac{\ln(\xi)}{\mu_t}. \tag{5.6}$$

After generating ds , photon packet is moved (i. e. its location is updated), accordingly to the ds and directional cosines:

$$\begin{aligned} x' &= x + \mu_x ds, \\ y' &= y + \mu_y ds, \\ z' &= z + \mu_z ds. \end{aligned} \tag{5.7}$$

Also, the total path of the photon packet is updated:

$$S' = S + ds. \quad (5.8)$$

Then, it is verified if the photon have crossed the boundary of the tissue. If the interaction happened, the photon packet is moved back with the partial step, ds_b to the boundary (and the total path S is reduced accordingly). This partial step is the distance of the current photon position to the sample boundary, calculated as:

$$ds_b = \frac{z_b - z}{-\mu_z}, \quad (5.9)$$

where z and μ_z is the current position and directional cosine of the photon packet and z_b is the position of the sample boundary. Then the decision is made whether there occurs internal reflection or not.

5.1.5 Internal reflection

The probability of internal reflection is defined with the Fresnel reflection coefficient and depends on the incident angle of the beam:

$$R(\theta_i) = \frac{1}{2} \left[\frac{\sin^2(\theta_i - \theta_t)}{\sin^2(\theta_i + \theta_t)} + \frac{\tan^2(\theta_i - \theta_t)}{\tan^2(\theta_i + \theta_t)} \right], \quad (5.10)$$

where $\theta_i = \cos^{-1}(\mu_z)$ is the incident angle and θ_t is the transmission angle (after refraction at the media interface) and is calculated with Snell's law:

$$n_i \sin(\theta_i) = n_t \sin(\theta_t), \quad (5.11)$$

so after transforming the equation one obtains:

$$\theta_t = \sin^{-1} \left(\frac{n_i \sin(\theta_i)}{n_t} \right). \quad (5.12)$$

In this case, n_i and n_t are the refractive indices of the tissue and of the medium surrounding the tissue sample, respectively. The decision about internal reflection is taken here depending on the value of the random variable ξ , since both $R(\theta_i)$ and ξ take values from 0 to 1. If $\xi \leq R(\theta_i)$ then photon packet is internally reflected and continues propagation with the remaining path ds_b in the sample with new direction cosines:

$$(\mu'_x, \mu'_y, \mu'_z) = (\mu_x, \mu_y, -\mu_z). \quad (5.13)$$

Otherwise it is transmitted through the sample-medium interface, and its direction and the remaining path ds_b is updated due to the refraction and propagation in new medium. Hence, the new directional cosines are calculated:

$$\begin{aligned} \mu'_x &= \mu_x \frac{n_t}{n_i} \\ \mu'_y &= \mu_y \frac{n_t}{n_i} \\ \mu'_z &= \frac{\mu_z}{|\mu_z|} \cos(\theta_t). \end{aligned} \quad (5.14)$$

The remaining path could be then transformed to (for the case of multi-layered sample):

$$ds'_b = ds_b \frac{\mu_{tt}}{\mu_{ti}}, \quad (5.15)$$

where ds'_b is the updated remaining path, μ_{ti} is the attenuation coefficient of the preceding medium and μ_{tt} the attenuation coefficient of the following medium. Then, the photon packet can be moved with its remaining path ds'_b which is also updated in total path S . However, in the case of one-layer sample, if photon packet crosses the tissue-medium interface, its travel ends at the sample border and no more distances are accounted for the total path. The completion of the process with the collection of the quantities will be addressed in 5.1.8.

5.1.6 Absorption

It is assumed that at every small step of the travel of a photon packet, besides the scattering, absorption occurs. Therefore, the weight of the photon packet is reduced accordingly:

$$W' = W \frac{\mu_a}{\mu_t}. \quad (5.16)$$

The weight of the photon packet is controlled, and if it drops below the given threshold (in the description of this algorithm the authors suggest the value of 0.001) the decision is taken whether it should be killed or survive. This decision is based on roulette. Using the roulette technique, a photon packet with weight W is subjected to a random selection: if the generated Monte Carlo random variable ξ is less than $1/10$, the photon packet survives, and its weight is increased to $10W$. Otherwise, the photon packet is terminated, and its weight is set to 0.

5.1.7 Change direction

For the purpose of changing the photon packet direction, a transformation to the spherical coordinate system is performed to simplify the calculation of scattering angles. The spherical coordinate system is characterized with radial distance r , azimuthal angle (φ) and normal angle (ϑ), which is denoted here, in terms of scattering, as the deflection angle (deviation from the forward direction). At every scattering event, these two angles are generated using the Monte Carlo random variable ξ . The azimuthal angle is uniformly distributed between 0 and 2π , meaning that no direction is favored. Since ξ is uniformly distributed φ is calculated as:

$$\varphi = 2\pi\xi. \quad (5.17)$$

For generating the deflection angle when $g \neq 0$, the Heyney-Greenstein phase function is utilized (see Section 1.1). Based on Equation 1.11, the deflection angle for $g \neq 0$ in Monte Carlo algorithm is generated as:

$$\cos \vartheta = \frac{1}{2g} \left(1 + g^2 - \left[\frac{1 - g^2}{1 - g + 2g\xi} \right]^2 \right) \quad \text{for } g \neq 0 \quad (5.18)$$

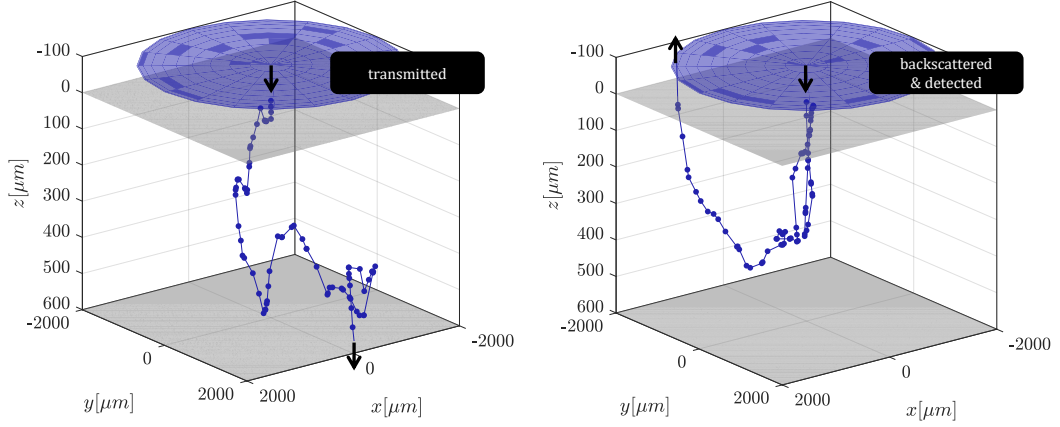


Figure 5.3: Illustrative example of the Monte Carlo simulation of the photon packet transport through the tissue of depth of 600 μm . Grey surfaces indicate tissue borders. Blue circle indicates the detector of radius 0.2 cm located 100 μm above the sample (for explanation see Section 5.3). Two cases are shown: photon packet transmitted and photon packet backscattered & detected, so it is accounted in Equation 5.22.

When $g = 0$, this corresponds to isotropic scattering in all directions, so the deflection angle is uniformly distributed between 0 and 2π , and $\cos \vartheta$ takes values from -1 to 1 :

$$\cos \vartheta = 2\xi - 1 \quad \text{for} \quad g = 0. \quad (5.19)$$

5.1.8 Collect quantities

When the photon exits the tissue the following steps are performed:

1. Flags are updated: if the photon left the tissue sample by crossing the posterior border of the tissue sample, then the flag **transmitted** is set to *true*, otherwise, the flag **backscattered** is updated to *true*.
2. The quantities, i.e., weight W , the total path S , the flags are saved for the given photon packet.

In the descriptions of this Monte Carlo algorithm [132, 163] there is also explained the method for recording the discrete transmission and reflection (backscatter) at the tissue anterior and posterior surfaces, respectively and discrete absorption in the tissue volume in the specially designed grid system. However, for the purpose of generating OCT signal, these quantities are here not used, and thus, they are not considered and not collected.

5.1.9 Photon detection

The work of Kirillin et al. [159] utilizes the Monte Carlo simulation for OCT A-scan calculations. It appends the algorithm with setting the detector radius and its position above the sample. Therefore, if the photon packet is backscattered, its directional cosines are used to calculate whether it hits the detector or not, by first transporting

the photon to $z = z_d$, which is the vertical position of the detector. The distance to the detector can be calculated as:

$$s_d = z_d / \mu_z, \quad (5.20)$$

where s_d is the distance to the detector, z_d is the vertical position of the detector, μ_z is the directional cosine after the photon leaves the sample. Secondly, the inequality is verified (for example, for round detector):

$$x_d^2 + y_d^2 \leq r_d^2, \quad (5.21)$$

where (x_d, y_d, z_d) , is the position of the photon packet at the plane common to the detector plane and r_d is the detector radius. It is assumed that after exiting the sample, the photon packets travels freely, without scattering. The distance to the detector is not included in the total path.

5.2 OCT SIGNAL CALCULATION

Kirillin et al. propose the following equation for the time domain [OCT](#) A-scan depending on the depth of the sample (z):

$$A(z) = A_0 \sum_{j=1}^{N_{ph}} \sqrt{W_j} \cos \left(\frac{2\pi}{\lambda_0} (2z - S_j) \right) \exp \left[- \left(\frac{2z - S_j}{l_c} \right)^2 \right], \quad (5.22)$$

where N_{ph} is the number of photon packets launched, W_j is the weight of the j -th photon packet, λ_0 is the central wavelength of the [OCT](#) lightsource, S_j is the total path of the j -th photon packet, z is the sample depth, which takes discrete values (equivalent to subsequent transversal steps of the reference mirror in time domain [OCT](#)), l_c is the coherence length of the [OCT](#) light source and A_0 is the constant defined by OCT system characteristics and in this study it was assumed to be equal to 1. The total path S_j is here analogical to the path of the sample beam, while $2z$ is equivalent to the path of reference beam. The difference between these two ($2z - S_j$) is directly transformable to the difference in phases via multiplication by $2\pi/\lambda$. Therefore, the cosine component in Equation 5.22 introduces the speckle pattern, dependent on difference in phases. Also, the exponential component ensures that those photon packets, which traveled large distances in comparison to the “reference beam”, meaning that $2z - S_j \gg l_c$ will minimally contribute to the signal.

As stated in [159] [OCT](#) A-scan resulting from Equation 5.22 includes the carrier frequency, which results in pixel values oscillating around zero and thus taking negative values. It makes them not comparable with the experimental data. As suggested in [159], the carrier frequency should be removed, which means calculating the signal envelope of $A(z)$. However, no hint was ensured what should be the way of calculation. Therefore, the author proposes own solution, by treating the sum from Equation 5.22 as a sum of phasors of the same frequency. The Equation 5.22 can be rewritten as:

$$A(z) = \sum_{j=1}^{N_{ph}} \sqrt{W_j} \exp \left[- \left(\frac{2z - S_j}{l_c} \right)^2 \right] \cos \left(\frac{2\pi}{\lambda_0} 2z - \frac{2\pi}{\lambda_0} S_j \right). \quad (5.23)$$

In such form, this expression can be generalized to:

$$A(z) = \sum_{j=1}^{N_{ph}} A_j(z) \cos[\omega(z)t + \Phi_j] \quad (5.24)$$

and for the particular sample depth z it will take:

$$A_z = \sum_{j=1}^{N_{ph}} A_{j,z} \cos[\omega_z t + \Phi_j]. \quad (5.25)$$

The above can also be expressed with Euler's formula:

$$A_z = \sum_{j=1}^{N_{ph}} \Re \{ A_{j,z} \exp(i[\omega_z t + \Phi_j]) \}. \quad (5.26)$$

Now, lets separate the sum under the exponential expression, and change the order of its elements:

$$A_z = \sum_{j=1}^{N_{ph}} \Re \{ A_{j,z} \exp(i\Phi_j) \exp(i\omega_z t) \}. \quad (5.27)$$

The j th phasor is then defined as:

$$A_{j,z} \exp(i\Phi_j). \quad (5.28)$$

Hence, at a particular sample depth z , the sum can be interpreted as the sum of phasors of the same frequency $\omega_z t$ ("carrier frequency"), where the amplitude of j th phasor is:

$$A_{j,z} = \sqrt{W_j} \exp \left[- \left(\frac{2z - S_j}{l_c} \right)^2 \right], \quad (5.29)$$

the carrier frequency is:

$$\omega_z t = \frac{2\pi}{\lambda_0} 2z \quad (5.30)$$

and the phase is:

$$\Phi_j = \frac{-2\pi}{\lambda_0} S_j. \quad (5.31)$$

Since the carrier frequency remains constant over j , and takes real values, it can be moved outside the sum and outside $\Re$. Also, the sum of the real parts is equal to the real part of the sum, so $\Re$ was also moved outside:

$$A_z = \exp(i\omega_z t) \Re \left\{ \sum_{j=1}^{N_{ph}} A_{j,z} \exp(i\Phi_j) \right\} \quad (5.32)$$

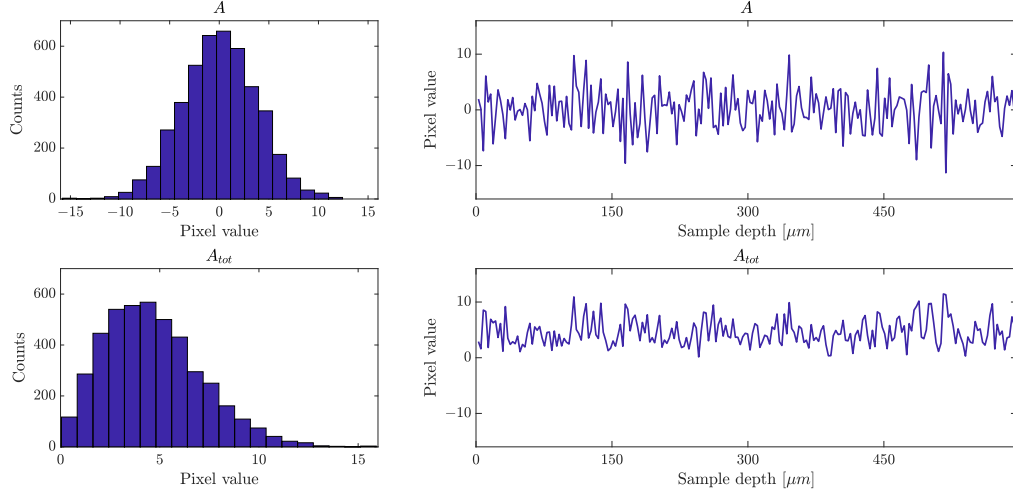


Figure 5.4: Illustrative histograms of the image pixel values with corresponding exemplary column (A-scan) for the original image A , calculated with Equation 5.22 and image A_{tot} after removing the carrier frequency (see Equation 5.33).

As the purpose of this reasoning was to remove the carrier frequency $\omega_z t$, the expression $\exp(i\omega_z t)$ was removed from the final equation. The expression under the sum allows for geometric addition of N_{ph} phasors as vectors in the complex plane, working out to the resultant amplitude $A_{tot,z}$ and resultant phase Φ_{tot} :

$$A_z^{(*)} = \Re \{ A_{tot,z} \exp(i\Phi_{tot}) \} = A_{tot,z} \cos \Phi_{tot}. \quad (5.33)$$

For the final A-scan A_{tot} values were used. Figure 5.4 presents the histogram of the received B-scan before (A) and after (A_{tot}) removing the carrier frequency. In the second column an illustrative A-scans are presented to show the pixel values along the sample depth z .

Originally, in equation from [159] (here as Equation 5.22), the OCT signal of an A-scan was denoted by $I(z)$. The symbol I is conventionally associated with the intensity of the pixels. However, there exist works that also denote in the same way the pixel amplitude. Therefore, it was considered whether the signal in the simulated OCT A-scan is the pixel amplitude or intensity. The right-skewed shape of the histogram of image pixel values (after removing carrier frequency) in Figure 5.4 allowed to interpret the pixel values to be the speckle amplitude, similarly as it is observed in SOCT REVO 80 device.

5.3 CHOOSING THE SETUP FOR ANTERIOR EYE OPHTHALMIC OCT

The OCT setup for the simulation was adapted to that of SOCT REVO 80 (Optopol, Zawiercie, Poland), which was used for acquiring OCT experimental data in this thesis. Therefore, the central wavelength was set to $\lambda_0 = 850$ nm and bandwidth to $\Delta\lambda_{FWHM} = 50$ nm. Having these two parameters allowed for calculation of coherence length l_c of the light source (assuming the Gaussian spectrum) [64, 67]:

$$l_c = 2 \ln 2 \frac{\lambda_0^2}{\pi \Delta\lambda_{FWHM}}. \quad (5.34)$$

The transverse pixel size was set to 2.7 μm , to follow the estimated vertical resolution of SOCT REVO 80 (see Section 3.3). Since the beam was assumed to be infinitely thin, the lateral resolution was not considered. For each B-scan, 40 A-scans were simulated. This choice was made ad-hoc as a balance between computing time and having enough sample size. The sample depth was set to 600 μm , to mimic the porcine corneas, as it was planned to compare simulation with described earlier porcine OCT data set (see Chapter 5, Section 3.2). Consequently, the simulated B-scan was of size 40×223 pixels. The Equation 5.22 accounts only for the backscattered and detected photon packets. Hence, it was of interest where to position the round detector and what should be its radius, to ensure sufficient signal.

In real OCT systems, the numerical aperture (NA) is low to keep higher depth of focus and thus the axial range. Equation 5.35 [64] for the lateral resolution of OCT allowed to calculate the NA for the SOCT REVO 80.

$$\delta x = \frac{\sqrt{2 \ln 2} \lambda_0}{\pi NA}. \quad (5.35)$$

For this OCT device, the resolution was estimated to $\delta x = 11 \mu\text{m}$ (see Section 1.4.4) and central wavelength was $\lambda_0 = 850 \text{ nm}$, which worked out to a numerical aperture of $NA = 0.0290$. Using the exact OCT detector geometry in the simulated setup would necessitate an unfeasible number of photon packets for the Monte Carlo simulation. Consequently, to maintain a reasonable computational cost, detector of radius of 0.2 cm was positioned 100 μm above the sample (as illustrated in Fig. 5.3).

5.4 CHOOSING THE OPTICAL PARAMETERS TO SIMULATE CORNEAL OCT IMAGES

To investigate how OCT speckle statistics relate to optical parameters (μ_s , μ_a , and g), but at the same time remain close to the corneal OCT simulation, a range of values was selected based on existing, however limited, literature for corneal tissue. The scattering coefficient (μ_s) range was set from 0.1 to 0.2 mm^{-1} (11 values) with a step of 0.01 mm^{-1} . This range is supported by ex-vivo animal studies. Yust et al. [137] used the integrating spheres setup to measure bovine corneal transmittance and then calculate μ_s with Kubelka-Munk (KM), inverse adding-doubling (IAD) and inverse Monte Carlo (IMC) methods, for the range of NIR. For $\lambda = 850 \text{ nm}$, for the first cornea they reported 0.1466, 0.1347 and 0.1348 mm^{-1} , with KM, IAD and IMC respectively. For second cornea, they obtained: 0.1809, 0.1727 and 0.1648 mm^{-1} , respectively. Regal et al. [138] applied the same calculation methods to spectroscopy measurements of tissues of six porcine eyes. For the cornea and $\lambda = 810 \text{ nm}$ they received the average values of $\mu_s = 0.126 \pm 0.049 \text{ mm}^{-1}$ and $\mu_s = 0.106 \pm 0.037 \text{ mm}^{-1}$ for KM and IAD, respectively. The absorption coefficient (μ_a) was set to zero, simplifying analysis given the cornea's weak absorption (of order 0.01 mm^{-1} [137]) at 850 nm. The scattering anisotropy factor (g) ranged from 0.85 to 0.99 (15 values), with increments of 0.01. Such high values correspond to strong forward scattering, which is characteristic for biological tissues in general [7, 10, 168, 169] and particularly for the cornea [22, 137]. To simulate an OCT measurement, the following refractive indices were used: $n_1 = 1$ (air), $n_2 = 1.376$ (cornea) and $n_3 = 1.33$ (aqueous humor) [34]. Having range of 11 values of μ_s and 15 values of g resulted in 165 unique pairs of μ_s and g , and 165 simulated B-scans.

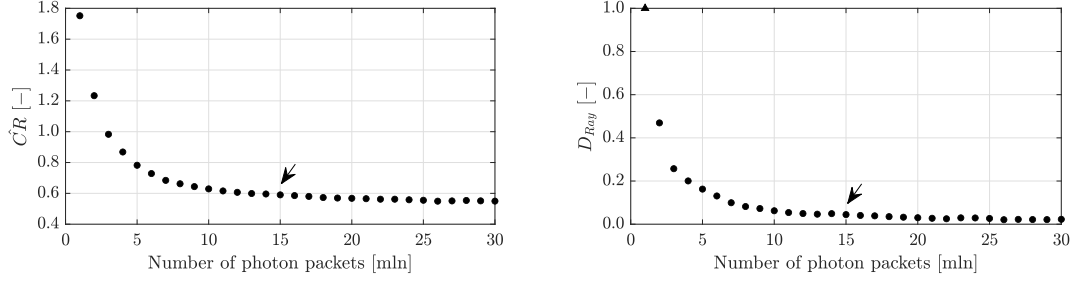


Figure 5.6: Contrast ratio ($\hat{C}R$) and root-mean-square distance (D_{Ray}) to the benchmark Rayleigh distribution for 30 B-scans simulated with 1:30 millions photon packet launched. All B-scans were simulated for $(\mu_s, g) = (0.1 \text{ mm}^{-1}, 0.99)$, that is, the “worst” case in terms of strength of backscattered signal. The triangle in D_{Ray} plot at $(x = 1, y = 6)$ indicates the value received for 1 million of photon packets, which was of order of 10^4 . Black arrows indicate the number of launched photon packets chosen ad-hoc for further analysis.

where x are the pixel values, $\hat{f}_{\text{SIM}}(x)$ is the kernel density estimator of the simulated image, $f_{\text{Ray}}\left(x|\sigma = \frac{\sqrt{2}}{2}\right)$ is the probability density function of the standardized Rayleigh distribution. Also, contrast ratio ($\hat{C}R$) of every image was calculated. Figure 5.6 presents $\hat{C}R$ and D_{ray} against the number of photon packets launched.

Notably, $\hat{C}R$ converges to approximately 0.56 (the mean $\pm$ STD for the range of 15 to 30 millions of photon packets is 0.5641 ± 0.0066) and from 15 millions it changes minimally. This point corresponds to 4595 photon packets caught at the detector, which was assumed to be the minimal number to receive a reliable B-scan. That is why for further analysis the number of launched photon packets was set ad hoc to 15 millions. D_{Ray} values also settle at 15 millions of photon packets to 0.0292 ± 0.0073 (mean $\pm$ STD) for the range of 15 to 30 millions).

The fraction of backscattered photon packets was calculated for every A-scan, for 30 B-scans:

$$fr_{back} = \frac{N_{back}}{N_{ph}} 100\%. \quad (5.37)$$

For this pair of μ_s and g the mean fraction (over all A-scans and B-scans) was 0.0311% and minimally depended on N_{ph} . Analogically calculated, the mean fraction of detected photons was 0.0306%.

5.6 RESULTS

Figure 5.7 b) and c) presents two illustrative B-scans: the one simulated for $(\mu_s, g) = (0.2 \text{ mm}^{-1}, 0.85)$ and the second simulated for $(\mu_s, g) = (0.1 \text{ mm}^{-1}, 0.99)$. These two pairs correspond respectively to “the best” and “the worst” scenarios in terms of the strength of backscattered signal, which is illustrated in Figure 5.7 a), where the mean number of detected photons is shown for all of the μ_s and g pairs.

Notable, in the simulated B-scans, the last three lines exhibit high pixel values, probably because of the internal reflection of photon packets on the stroma-aqueous humor interface. Possibly, these values would be smaller with setting non-zero absorption coefficient μ_a . Consequently, three last lines of the image were excluded from image analysis (and were also not taken into account in image normalization).

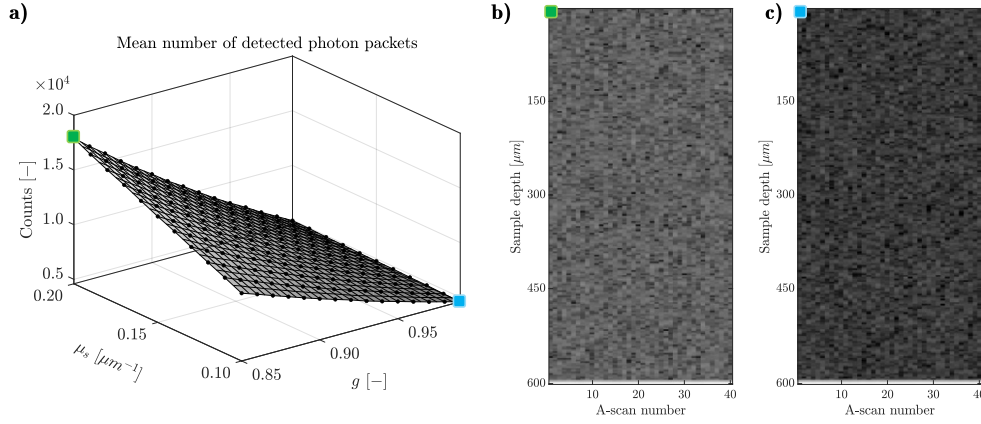


Figure 5.7: a): Mean number of photon packets caught at the detector, calculated from whole B-scan (40 A-scans). x and y axes indicate g and μ_s input parameters for the simulated images. b) and c): Illustrative examples of the simulated B-scan, for 2 pairs of parameters: $[\mu_s = 0.2 \text{ mm}^{-1}, g = 0.85]$ and $[\mu_s = 0.1 \text{ mm}^{-1}, g = 0.99]$, respectively. Green and blue squares indicate the cases shown in b) and c). Images are shown after $\log_{10}$ transformation, as it is visualized in SOCT Revo 80.

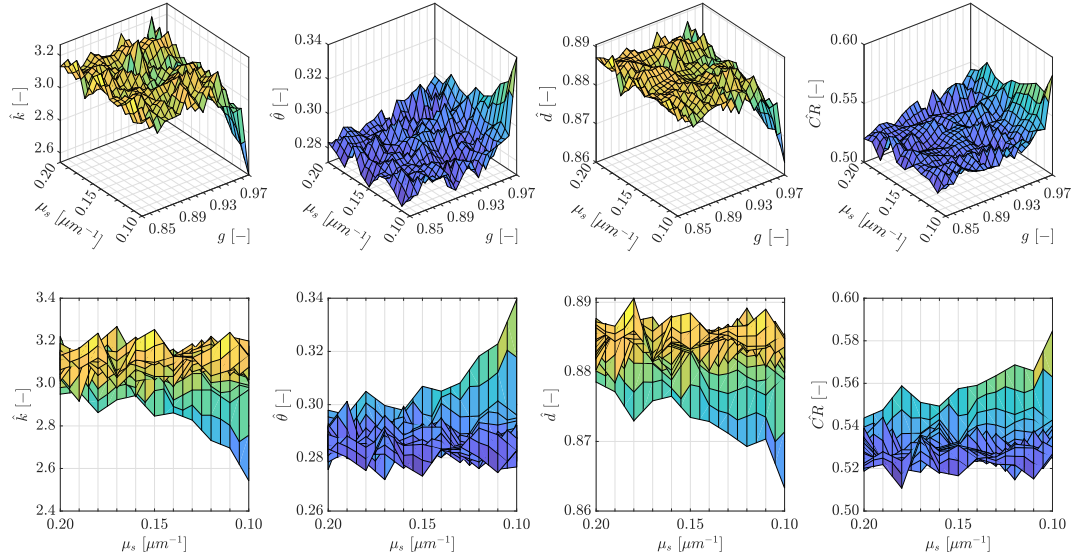


Figure 5.8: 3-D plots of densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$) estimated from 165 OCT B-scans, simulated for 165 unique (μ_s, g) pairs (X, Y-axis). The second row of plots presents the same data, but the view is fixed to observe the dependence of densitometry parameters on the anisotropy coefficient g .

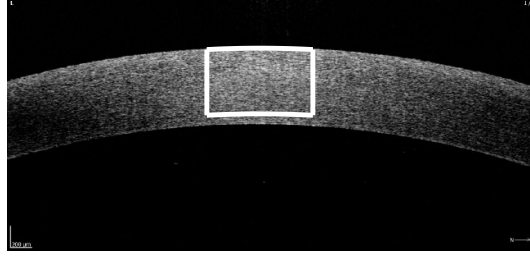


Figure 5.9: Illustrative OCT B-scan of a porcine cornea (after epithelial removal) imaged with SOCT REVO 80 device. Size of the image: 1,538 pixels horizontally and approximately 733 pixels vertically, which corresponds to the estimated pixel size of $3.25\ \mu\text{m}$ and $2.70\ \mu\text{m}$, respectively. ROI of size $520 \times 1000\ \mu\text{m}$ chosen for analysis is delineated in white.

Subsequently, the ROI of size 20×193 pixels was delineated and normalized using the RMS value. Its width was set to include all 40 simulated A-scans. The depth was adapted for further comparison with porcine data, according to the thinnest porcine cornea (see Chapter 3, Section 3.2). To analyze the simulated OCT quasi-speckle pattern, four densitometry parameters were estimated: Gamma shape k and scale θ , the mean d and the contrast ratio CR . The width was set to include all 40 simulated A-scans. Figure 5.8 presents the densitometry parameters for all (μ_s, g) pairs. The second row of subplots shows the same figures as in the first row, but with a fixed 2-D view of the $z - x$ axis. The parameters exhibit exponential growth ($\hat{\theta}$, $\hat{CR}$) or exponential decrease ($\hat{k}$, $\hat{d}$) with g . The increasing ($\hat{\theta}$, $\hat{CR}$) or decreasing tendencies ($\hat{k}$, $\hat{d}$) are also observed with increasing μ_s , and their slopes depend on g . Also, GoF of Gamma distribution was calculated as the RMS distance between the kernel density estimator of the ROI data and probability density function of the fitted Gamma distribution. The mean $\pm$ STD over 165 simulated B-scans was 0.0481 ± 0.0056 and no dependence on the input μ_s or g was observed.

5.7 COMPARISON OF SIMULATED B-SCANS AND EXPERIMENTAL DATA

The OCT image dataset from 11 porcine eyeballs, described in the previous chapter (Chapter 3) was taken for the analysis. From the images, only those without epithelium were used, to ensure single-layer samples (to exclude the confounding effect of an additional layer), since the images simulated here were also single-layer corneal digital phantoms. The central ROI of 308 pixels in width (approximately $1000\ \mu\text{m}$) around the corneal apex was used to derive the speckle statistics. Its depth (193 pixels, approximately $520\ \mu\text{m}$) was limited by the central corneal thickness of the thinnest sample.

Figure 5.9 shows an illustrative example of an OCT B-scan of a porcine cornea without epithelium with the ROI outlined in white. Figure 5.10 presents a box diagram comparing densitometry parameters estimated from experimental (EXP) and simulated (SIM) OCT images. The boxes representing simulation illustrate the range of received parameter values, while the box representing experimental data illustrates the statistics of the parameters received for 11-subject group. All parameters are in agreement to an order of magnitude. However, a shift between them can be observed: $\hat{k}$ and $\hat{d}$ obtained from experimental images are higher, while $\hat{\theta}$ and $\hat{CR}$ are lower

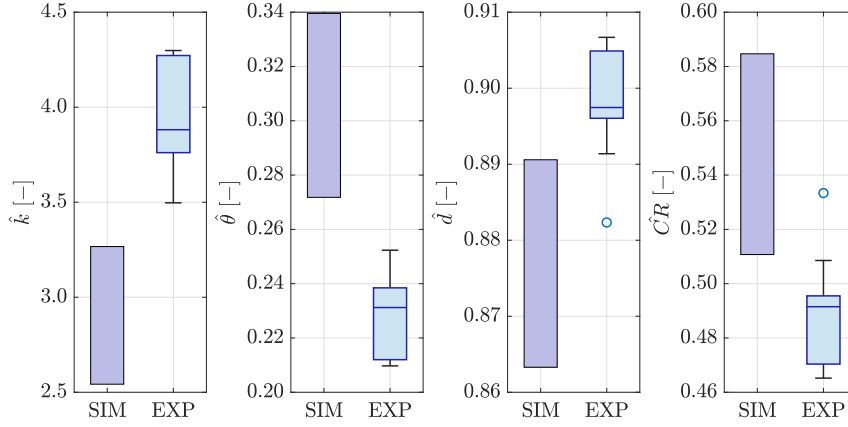


Figure 5.10: Box diagram comparing densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{C}R$) estimated from experimental (EXP) and simulated simulated (SIM) OCT images. The purple boxes represent the simulation and the *range* of received speckle parameter values. The blue boxes represent experimental data and illustrate the *group statistics* of the parameters received for 11-subject group, i.e. the median, the lower and upper quartiles (box), the non-outlier minimum and maximum (whiskers). Circles denote outliers ($IQR = 1.5$).

than those obtained from the simulation. Further work relating the experimental OCT images to the simulated ones will be presented in Chapter 6.

5.8 DISCUSSION

A commonly used Monte Carlo algorithm was adapted here for ophthalmic OCT and corneal imaging, with validated OCT system parameters and optical tissue parameters based on the literature. The results indicate that this algorithm adequately simulates synthetic OCT data, which can successfully serve as digital phantoms. The equation for OCT signal proposed by Kirillin et al. [159] was utilized, because it has already been previously applied to simulate corneal OCT images [162]. The produced OCT B-scans exhibited speckle statistics that were well matched to those from real images, however their accuracy was not compared with that of other models. It needs to be acknowledged that the experimental corneal images were recorded with the SOCT REVO 80, which is an SD-OCT device, while the equation used in this study is dedicated to time-domain OCT. Therefore, other equations should also be considered, such as the one proposed by Lu et al. [170] or the spectral-domain OCT model from Mao et al. [161], and their performance compared to the current method. Also, another algorithmic proposal performing Monte Carlo simulation of photon packet transport, but with fixed positions of scattering particles [171], could be tested.

The Monte Carlo simulation algorithm employed here also makes several simplifications. The light source beam is assumed to be infinitely thin, and the next step will be to apply a Gaussian beam with a defined radius [172]. This would introduce lateral resolution into the simulated OCT images. Furthermore, including a non-zero absorption coefficient could alter the intensity profile of the A-scan and affect the internal reflection at the last three lines of the simulated image. Corneal curvature was also omitted at this stage, and the digital sample was assumed to be flat, which likely affects the number of photons reaching the detector and their pathways. These

simplifications can be considered limitations, but they also allow for the isolation of various factors influencing the signal. This allows for the refinement of the algorithm while ensuring strict control of each factor’s impact on speckle statistics.

In the end, it is worth noting that light scattering is fundamentally a wave process, and OCT signal formation is the result of wave interference. Monte Carlo algorithms, by definition, are probabilistic methods used to simulate complex phenomena through the repeated sampling of properly transformed random variable. Therefore, a relatively large number of repetitions is required, which should be adjusted to the particular purpose of the simulation. In this simulation, the scattering phenomenon is simplified, by treating photons as “packets” traveling through the sample. Moreover, the input optical parameters are the quantities averaged over the sample volume, which precludes defining the spatial distribution of scatterers. Although the equation utilized here from [159] incorporates a component for the speckle pattern depending on the phase difference between the sample and reference “beams”, it needs to be taken into account that this is not an analytical simulation of the wave propagation and interference within a well-defined scatterer network. Alternatively, analytical models could be tested for comparison, such as those based on the EHF principle [83].

In addition, future work may extend the simulation to multilayered tissue, allowing for an analysis of the relationship between the speckle signals of different layers. Ultimately, a holistic analysis of OCT speckle, based on synthetic corneal data, will help characterize the main corneal layers and their interactions.

ESTIMATION OF SCATTERING AND ANISOTROPY COEFFICIENTS OF PORCINE CORNEAL STROMA WITH USE OF OCT DIGITAL PHANTOMS

Partially based on:

Miażdżyk, M. *et al.* Estimating the optical properties of corneal tissue from the OCT speckle. *Biomedical Optics Express* **17**, 544–554 (2026).

Observing the behavior of densitometry parameters calculated for simulated OCT images in relation to input parameters μ_s and g led to an idea to estimate these parameters from experimental data. The simulated 165 B-scans for 165 different (μ_s, g) pairs in this context were assumed to form a multi-reference look-up map of digital OCT phantoms. The proposal for the estimation method was to design a statistical tool that would allow identifying the best statistical match in the look-up map for the given corneal experimental image.

Currently used methods for tissue optical properties assessment are mainly those using a double integrating sphere setup [137, 174, 175] or those using a spectroscopic technique [138]. They allow for ex vivo measurement of sample transmittance, and then three methods can be applied for the scattering and absorption coefficient calculation, that is, inverse adding-doubling (IAD), Kubelka-Munk (KM) and inverse Monte Carlo (IMC) methods. The above approach was utilized to measure the scattering coefficient of bovine and porcine corneas. The measurement of the anisotropy coefficient has not been reported so far for corneal tissue.

OCT imaging has the potential for in vivo assessment of these optical properties. For most tissues, including the cornea, in the NIR spectrum, the scattering coefficient μ_s dominates the absorption coefficient μ_a , leading the total attenuation coefficient μ_t to approximate μ_s . This approximation allows μ_s to be estimated by evaluating the slope of the OCT signal on a logarithmic scale. Meanwhile, the scattering anisotropy factor (g) can be measured by fitting the depth-resolved OCT signal to a theoretical framework, such as one based on the Extended Huygens-Fresnel principle [134, 176]. Both measurements rely on a good signal-to-noise ratio (SNR) [177]. Another method was proposed by Vermeer et al., enabling depth-resolved μ_s estimation, however it assumes that most of the incident light is attenuated within the imaging depth range, which is not applicable to the anterior eye [177]. In short, the current state-of-the-art in the estimation of tissue optical properties from OCT images indicates that this is still a challenge, and particularly highlights the gap for methods applicable to the anterior eye segment.

This chapter is organized in chronological order, first showing unsuccessful attempts at estimation (Section 6.1) and subsequently presenting Section 6.2, which describes the finally devised method together with the μ_s and g values estimated for 11 porcine eyes. The chapter ends with Section 6.3, which provides a discussion.

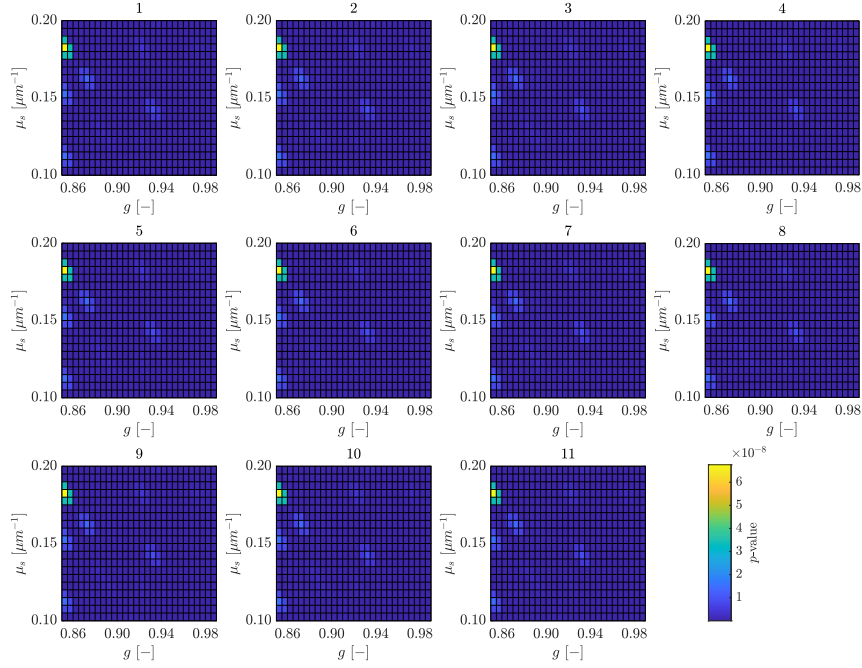


Figure 6.1: Every subplot contains the resultant p -values of two sample Kolmogorov-Smirnov test performed for comparison of particular porcine corneal OCT data (number of porcine eyeball is given in the subplot tile) and look-up map of 165 simulated OCT images. Color bars indicate the p -value.

6.1 FIRST, UNSUCCESSFUL ATTEMPTS AND THE PROBLEM OF LOW P -VALUES

The initial idea, which was to fit a non-linear, two-variable model to the densitometry parameters ($\hat{k}$, $\hat{\theta}$, $\hat{d}$, $\hat{CR}$), was rejected. Despite the fact that tendencies in $\hat{k}$, $\hat{\theta}$, $\hat{d}$, and $\hat{CR}$ in relation to μ_s and g are clearly visible in the plots (see Figure 5.8), there is also substantial internal variability, which disrupts the clarity of general trends. Additionally, the Gamma parameters were rejected due to their low goodness-of-fit, which in this case may cause a loss of sensitivity in distinguishing slight changes between the speckle statistics. Instead analyzing densitometry parameters, it was decided to directly analyze the histograms of pixel values from the ROI. Therefore, the histogram obtained for a given porcine subject was compared to each histogram received from the simulated multi-reference look-up map. This time, unlike in the previous chapters, only the first of three consecutive B-scans acquired in the experimental data was chosen, as taking the median of the pixel values to create the histogram would average the images and reduce the speckle signal. The first method used for histogram comparison was the two-sample Kolmogorov-Smirnov test (Matlab: `kstest2()`). The null hypothesis of this test is that the data in two samples are from the same, continuous distribution. As a result, a map of p -values was obtained for every porcine subject (see Figure 6.1).

In the obtained maps, all of the p -values were of order 10^{-8} and for every porcine cornea there appeared one peak at $\{\mu_s = 0.18 \text{ mm}^{-1}, g = 0.85\}$, however its p -value was still of order 10^{-8} . This could be interpreted as indicating that, for all of the 165 histograms received for simulated B-scans, the null hypothesis is rejected, but the particular histogram is slightly closer to the histogram received for the porcine B-scans.

Hence, the peaks could be the estimates of μ_s and g for the given subject. However, drawing conclusions based on such small p -values is a fundamental mistake [178]. It was also figured out that performing statistical tests with such large sample sizes ($40 \times 221 = 884$ pixels for simulation and over 50000 pixels for experimental data) generally results in very small p -values in general [178]. Therefore, data pruning was considered by taking the first half of the B-scan, i.e., 20 A-scan columns instead of 40. The p -values increased to the order of 10^{-4} . However, the location of the peaks changed remarkably, and changed again after taking the second half of the B-scan (the remaining 20 A-scan columns), yielding inconsistent results. Finally, it was decided to look for other methods of estimation.

6.2 THE SUCCESSFUL ALGORITHM AND RESULTS

The successful method appeared to be the evaluation of the distance between the speckle statistics of a given sample, and the speckle statistics of the simulated multi-reference look-up map. For every ROI the contrast ratio ($\hat{C}R$), the kernel density estimator of the probability density function and the cumulative distribution function were calculated (`ksdensity()`). Further, a set of four distances was considered, one based on $\hat{C}R$ values, two based on probability density function and the last on cumulative distribution function:

$$D_{CR}(k) = |\hat{C}R_{EXP} - \hat{C}R_{k,SIM}|, \quad (6.1)$$

$$D_{RMS}(k) = \sqrt{\int (\hat{f}_{EXP}(x) - \hat{f}_{k,SIM}(x))^2 dx}, \quad (6.2)$$

$$D_{KL}(k) = \sum_x \hat{f}_{EXP}(x) \log \frac{\hat{f}_{EXP}(x)}{\hat{f}_{k,SIM}(x)}, \text{ and} \quad (6.3)$$

$$D_{KS}(k) = \sup |\hat{F}_{EXP}(x) - \hat{F}_{k,EXP}(x)|, \quad (6.4)$$

as functions of the simulated reference $k \in \{1, 2, \dots, N_{SIM}\}$, and k corresponds to a specific (μ_s, g) pair. $\hat{f}(x)$ and $\hat{F}(x)$ are the kernel density estimators of the probability density function (pdf) and cumulative distribution function (cdf) of ROI data, respectively. D_{CR} is the difference between the contrast ratios of the simulated (SIM) and experimental (EXP) ROI data, D_{RMS} is the RMS error between pdf kernel density estimators, D_{KL} corresponds to the Kullback-Leibler divergence and D_{KS} is the Kolmogorov-Smirnov distance. To statistically match the ROI from a porcine stroma to one of the $N_{SIM} = 165$ simulated multi-references from the look-up map, the minima of $D_{CR}(k)$, $D_{RMS}(k)$, $D_{KL}(k)$ and $D_{KS}(k)$ were calculated, leading to four μ_s and g pairs.

Figure 6.2 presents an illustrative example of a 3-D plot of the four distances created for one subject. $D_{KS}(k)$ was found to be more noisy, that is, high variability was observed in its 3-D plot (Figure 6.2). Moreover, the (μ_s, g) pairs designated by the minimum of this distance measure, were different (for every eyeball) from

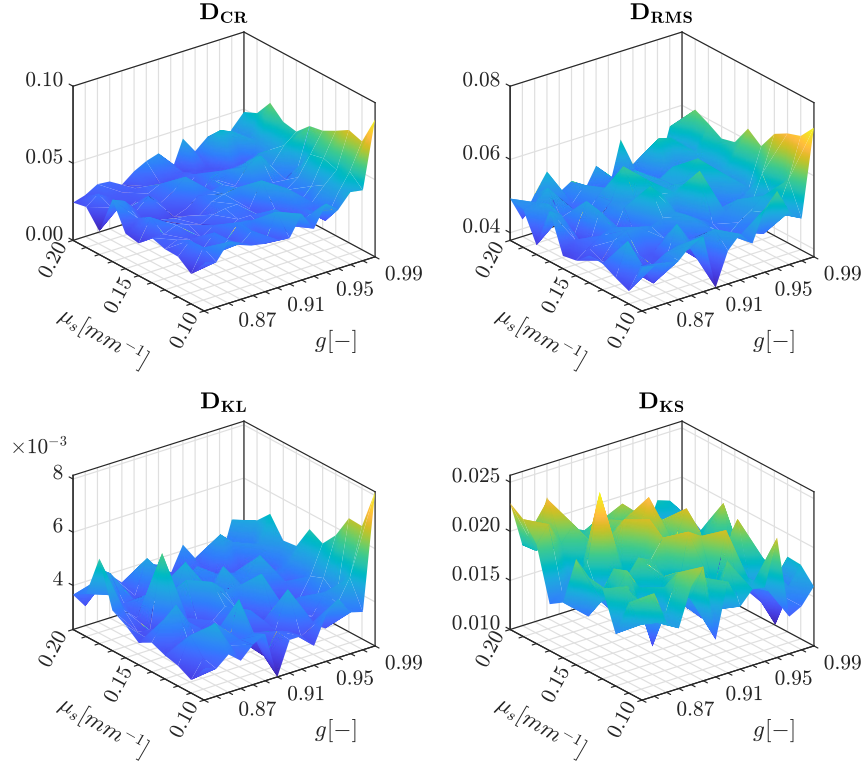


Figure 6.2: Four distance measures calculated for exemplary porcine **OCT** data and look-up map of 165 simulated **OCT** images. The distances are plotted against μ_s and g used as input parameters for the simulated images.

the remaining three pairs. Therefore, results from $D_{KS}(k)$ were rejected in the final methodology.

Finally, the estimated optical properties ($\hat{\mu}_s$ and $\hat{g}$) for particular subject were taken as the average over the results obtained from the three remaining distance measures. The choice of using three distances and their minima, instead of a single measure, helps to overcome the bias present in individual distance metrics. The schematic summarizing the subsequent steps in the final methodology is presented in Figure 6.3. Table 6.1 presents the minima and designated μ_s and g pairs. The last two columns of the table show the final (mean) estimates of $\hat{\mu}_s$ and $\hat{g}$ obtained from the three distance measures considered. The average of these parameters for the 11 eyeballs amounted to $\bar{\mu}_s = 0.146 \pm 0.020 \text{ mm}^{-1}$ and $\bar{g} = 0.893 \pm 0.021$.

6.3 DISCUSSION

In vivo estimation of the corneal optical parameters is challenging. While **OCT** technology is the most promising tool for this task, accurately determining these characteristics from the **OCT** signal attenuation profile requires two conditions: a high signal-to-noise ratio (**SNR**) and a sufficiently strong decay of the signal amplitude as it penetrates the tissue depth. For this reason, until now, the **OCT**-based techniques for determining tissue parameters have rarely been applied to the cornea [177].

The proposed statistical approach overcomes these previous barriers, because it does not require either of the above restrictive conditions. By matching the tissue sample speckle statistics to a look-up map of simulated multi-reference phantom

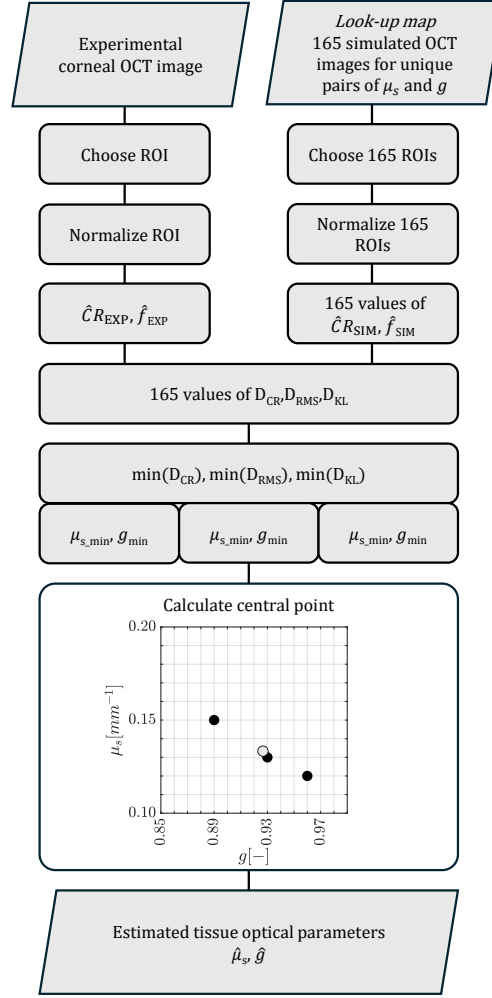


Figure 6.3: Subsequent steps of the algorithm for estimating the scattering coefficient μ_s and the scattering anisotropy factor g .

OCT B-scans, the method enables accurate analysis using a single, non-averaged corneal OCT image. Although the proposed technique was applied here to ex vivo porcine eyes, its extension to in vivo measurements is feasible. In principle, this can be achieved by simulating multi-reference surfaces (such as those proposed here with D_{CR} , D_{RMS} and D_{KL}), taking into account the two main layers of the cornea and the influence of the epithelium on the stromal backscatter statistics [62].

The components of the anterior eye are highly transparent at the wavelengths used in OCT. Therefore, the speckle pattern can be observed almost uninterrupted by signal attenuation. The signal attenuation correlates with speckle statistics [65] and, consequently, could be a confounding factor for this estimation method. The algorithm designed here for estimation of optical properties was not verified for highly scattering and absorbing samples, for example skin. Nevertheless, successful methods for such conditions already exist, however not applicable to the cornea [177]. Therefore, this method fills the gap for OCT-based estimation of optical parameters for corneal tissue.

Table 6.1: The μ_s and g pairs designated by the minima (provided left to every μ_s and g pair) of three distance measures: $D_{CR}(k)$ in columns 3–5, $D_{RMS}(k)$ in columns 6–8, $D_{KL}(k)$ in columns 9–11. The last column are the ensemble averages across the results for the three distances. m#: measurement number.

eyeball	m#	D_{CR}			D_{RMS}			D_{KL}			final estimates	
		min($\cdot$)	μ_s	g	min($\cdot$)	μ_s	g	min($\cdot$)	μ_s	g	$\hat{\mu}_s$	$\hat{g}$
1	1	0.0200	0.18	0.85	0.0403	0.10	0.91	0.0027	0.10	0.91	0.127	0.890
	2	0.0167	0.18	0.85	0.0380	0.14	0.93	0.0023	0.10	0.91	0.140	0.897
	3	0.0151	0.18	0.85	0.0379	0.14	0.93	0.0023	0.10	0.91	0.140	0.897
2	1	0.0437	0.18	0.85	0.0609	0.18	0.85	0.0078	0.19	0.89	0.183	0.863
	2	0.0407	0.18	0.85	0.0626	0.18	0.85	0.0082	0.19	0.89	0.183	0.863
	3	0.0439	0.18	0.85	0.0605	0.18	0.85	0.0077	0.19	0.89	0.183	0.863
3	1	0.0076	0.18	0.85	0.0314	0.10	0.91	0.0022	0.12	0.92	0.133	0.893
	2	0.0194	0.18	0.85	0.0346	0.17	0.92	0.0024	0.12	0.92	0.157	0.897
	3	0.0177	0.18	0.85	0.0360	0.17	0.92	0.0025	0.12	0.92	0.157	0.897
4	1	0.0264	0.18	0.85	0.0649	0.10	0.91	0.0080	0.10	0.91	0.127	0.890
	2	0.0275	0.18	0.85	0.0668	0.10	0.91	0.0081	0.10	0.91	0.127	0.890
	3	0.0231	0.18	0.85	0.0633	0.10	0.91	0.0071	0.10	0.91	0.127	0.890
5	1	0.0005	0.10	0.96	0.0349	0.10	0.91	0.0044	0.14	0.97	0.113	0.947
	2	0.0000	0.12	0.95	0.0353	0.10	0.91	0.0043	0.14	0.97	0.120	0.943
	3	0.0001	0.12	0.97	0.0322	0.10	0.91	0.0040	0.14	0.97	0.120	0.950
6	1	0.0431	0.18	0.85	0.0488	0.18	0.85	0.0040	0.17	0.92	0.177	0.873
	2	0.0349	0.18	0.85	0.0434	0.18	0.85	0.0032	0.17	0.92	0.177	0.873
	3	0.0386	0.18	0.85	0.0461	0.18	0.85	0.0037	0.17	0.92	0.177	0.873
7	1	0.0256	0.18	0.85	0.0419	0.14	0.93	0.0030	0.12	0.92	0.147	0.900
	2	0.0192	0.18	0.85	0.0398	0.17	0.92	0.0032	0.12	0.92	0.157	0.897
	3	0.0189	0.18	0.85	0.0377	0.10	0.91	0.0027	0.12	0.92	0.133	0.893
8	1	0.0455	0.18	0.85	0.0577	0.18	0.85	0.0054	0.12	0.92	0.160	0.873
	2	0.0467	0.18	0.85	0.0565	0.18	0.85	0.0051	0.12	0.92	0.160	0.873
	3	0.0453	0.18	0.85	0.0545	0.18	0.85	0.0051	0.12	0.92	0.160	0.873
9	1	0.0409	0.18	0.85	0.0572	0.14	0.93	0.0054	0.12	0.92	0.147	0.900
	2	0.0413	0.18	0.85	0.0577	0.14	0.93	0.0054	0.12	0.92	0.147	0.900
	3	0.0352	0.18	0.85	0.0528	0.14	0.93	0.0047	0.12	0.92	0.147	0.900
10	1	0.0140	0.18	0.85	0.0405	0.10	0.91	0.0034	0.12	0.92	0.133	0.893
	2	0.0147	0.18	0.85	0.0416	0.10	0.91	0.0032	0.12	0.92	0.133	0.893
	3	0.0156	0.18	0.85	0.0438	0.10	0.91	0.0034	0.12	0.92	0.133	0.893
11	1	0.0034	0.18	0.85	0.0299	0.10	0.91	0.0021	0.12	0.92	0.133	0.893
	2	0.0021	0.18	0.85	0.0294	0.10	0.91	0.0023	0.12	0.92	0.133	0.893
	3	0.0019	0.18	0.85	0.0254	0.10	0.91	0.0018	0.12	0.92	0.143	0.893

The estimated group mean values of the scattering coefficient and scattering anisotropy factor correspond well to those previously measured ex vivo with integrating spheres (Yust et al. [137]) and spectroscopy (Regal et al. [138]) already mentioned in Section 5.4. In addition, there are two works reporting attempts to estimate corneal μ_s from OCT signal attenuation analysis, one based on fitting the exponential function to the A-scan [179] and one using method from Vermeer et al. [177] for depth-resolved estimation of attenuation coefficient [79]. However, these results differ significantly from the reference literature reported by Yust et al. and Regal et al. and from the values obtained in this study. In particular, Bocheux et al. reported the value of photon mean free path for the healthy and pathological human corneal stroma to be $108 \pm 2.5 \mu\text{m}$ and $69 \pm 2.8 \mu\text{m}$, respectively [179]. The mean free path can be easily transformed to the attenuation coefficient (see Equation 1.4 in Chapter 1). The calculated values of the attenuation coefficient expressed in mm^{-1} are then 9.25 and 14.49 mm^{-1} which is for a healthy cornea almost two orders of magnitude higher

than the results of the above study and the reference literature on bovine and porcine corneas. Similarly, Ge et al. reported $\mu_s = 4.91 \text{ mm}^{-1}$ [79] obtained for porcine cornea, however it was calculated with method from Vermeer et al. [177] which the author defines as *not applicable to the anterior eye segment*. This comparative summary indicates, that the statistical matching method designed here seems to be the first one resulting in μ_s values close (in terms of order of magnitude) to those measured by Yust et al. [137] and Regal et al. [138]. Fundamentally, this implies that even though speckle is a random variable, its statistical properties are linked to the tissue's inherent optical parameters and microstructure, thereby containing physically useful information.

The noteworthy repeatability of $\hat{\mu}_s$ and $\hat{g}$ obtained here for porcine corneal stroma needs a comment. It should be emphasized that in this study only intact eyeballs with no scars or damage were recorded with OCT. The porcine eyes originated from an abattoir certified for animal studies, from animals of the same age and breed. More importantly, they were measured in semi-physiological conditions. The imaging process was performed using a specially designed platform to ensure constant intraocular pressure of 20 mmHg in the anterior chamber and the eyeballs were kept moist with PBS throughout the measurements, done shortly after enucleation. This resulted in a very consistent set of corneal OCT acquisitions. In contrast, other works [137, 138] that lack such a setup and dissect the corneas are characterized with large variability. Notably, despite the μ_s and g estimates establish high consistency, the particular values of the minima (i. e. $\min(\cdot)$ values in Table 6.1) differ between each other, which excludes any systematic or methodological error. In conclusion, the remarkable consistency obtained points to the rigor in the study design and subsequent data acquisition.

This work presents the first attempt on assessing optical properties of the sample from OCT with statistical matching to the multi-reference set of digital phantoms, and the task is certainly not finished. Different directions have yet to be verified or improved. Specifically, when matching sample statistics to the multi-reference look-up map, three distinct measures were utilized. Due to the relatively small available sample size, the individual performance and potential biases of these three measures were not examined. It is important to recognize that these chosen measures are not validated as superior ones, and the possibility remains that a better measure could supplement or replace them. Future work should consider alternative measures, such as those based on the speckle distribution function or the speckle characteristic function, as considered in [75]. In addition, the step for both μ_s and g was set to 0.01, due to computational capability. Future work should verify the estimates with higher resolution of the look-up map and broader range of μ_s and g .

In summary, this innovative approach, which involves matching the speckle statistics of a sample with a digital phantom of known optical parameters, expands the utility of OCT, allowing for in vivo clinical evaluation of biological tissues that was previously impossible for the cornea.

FINAL CONCLUSIONS

This dissertation presents multidirectional research on corneal scattering properties. The first project focusing on the clinically used corneal densitometry revealed its immaturity and lack of coherence. To improve its diagnostic power, the image preprocessing algorithm compensating for confounding factors related to eye biometry was proposed. The study was conducted using Corvis ST data, which is an alternative device to Pentacam HR — the current gold standard for Scheimpflug-based clinical densitometry. Hence, the software devised here needs to be re-evaluated for the Pentacam HR.

OCT imaging used in the second project appears to be a superior modality for densitometry assessment, as it not only has better resolution, but also, through the well-described mechanism of speckle pattern evolution, provides a theoretical foundation for linking corneal transparency to pixel values in the images. As the fundamentals of the interferometric OCT signal formation lie, similarly to Scheimpflug-based imaging, in backscatter from the anterior eye, the OCT-based densitometry is also potentially confounded by different factors. This work provides evidence that the pixel values of OCT B-scans corresponding to the corneal stroma are confounded by the signal from the preceding epithelial layer. Future work on OCT densitometry should include testing for other confounding factors, such as eye biometry.

A main conclusion from the two studies addressed above can be stated: regardless of the imaging technique considered here, the final signal received as the pixel values from the cornea in the corneal image is a composition of corneal backscatter together with other reflections from different structures surrounding the cornea, which should be accounted for as confounding factors.

The third project emerged as a natural consequence of the preceding work. The well-known Monte Carlo algorithm employed together with the equation for OCT signal calculation resulted in a reliable speckle pattern, statistically matching the real OCT B-scans. In addition, the author devised a method capable of estimating the optical properties of the cornea, that is, the scattering and anisotropy coefficients, which cannot be measured in vivo. The author plans to extend the algorithm to simulate a two-layered cornea comprising the epithelium and the stroma. The simulated speckle pattern can be tested separately for the two layers, but what is more tempting, it will allow for the analysis of the influence of one layer on another. Possibly, this can result in a complex model for the cornea as a multi-layered or at least bilayered structure incorporating the conditional character of the OCT speckle statistics. Going forward, a new research on epithelial-stromal interactions can be considered, as it is already known that such interactions exist physiologically in the cornea. This latter project being more theoretical than experimental, connects to basic sciences, allowing one to inquire the physics underlying the scattering phenomena.

OCT imaging is a common clinical modality, however, its potential has not yet been fully exploited. The future outlines the OCT device as a complex “all-in-one” platform for human health assessment.

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