Open Journal of Ophthalmology, 2025, 15(3), 115-121
https://www.scirp.org/journal/ojoph
ISSN Online: 2165-7416
ISSN Print: 2165-7408

Linear Measurement of Corneal Density in Anterior Segment Spectral Optical Coherence Tomography (LM-SD-OCT)

Oscar Fernandez-Vizcaya, Luis R. Alvarez-Fernandez, Jonathan Quevedo-Martinez

Department of Cornea and Refractive Surgery, Fundacion Hospital Nuestra Señora de la Luz, Mexico City, Mexico
Email: droscarfdez@gmail.com

How to cite this paper: Fernandez-Vizcaya, O., Alvarez-Fernandez, L.R. and Quevedo-Martinez, J. (2025) Linear Measurement of Corneal Density in Anterior Segment Spectral Optical Coherence Tomography (LM-SD-OCT). *Open Journal of Ophthalmology*, **15**, 115-121.
https://doi.org/10.4236/ojoph.2025.153016

Received: July 18, 2025
Accepted: August 10, 2025
Published: August 13, 2025

Abstract

Purpose: assess the accuracy of linear corneal density measurements obtained using spectral domain optical coherence tomography (OCT) in various images of normal corneas with the Image J program. **Methods:** Descriptive, cross-sectional, and observational study. Corneal images were captured using OCT Heidelberg Engineering with an anterior segment lens to calculate the average density of each layer in healthy corneas. We included subjects without corneal pathologies or surgeries and categorized them based on their age: 30 to 50 years, 51 to 70 years, and older than 70 years. The program image J of the NIH was used to calibrate the OCT image at a standardized value of 200 µm. Densities were obtained for the central cornea using high-resolution 8-bit images with 256 gray levels. **Results:** Spectral corneal OCT images of 140 eyes (170 patients) were analyzed, comprising 90 males and 80 females. The average gray value density for each corneal layer was determined, as follows: Te ar layer (238.25 ± 17.49), epithelium interface (91.55 ± 17), basal membrane and Bowman's membrane (167.76 ± 16.19), anterior stroma (152.06 ± 9.09), posterior stroma (130.24 ± 8.32), and Descemet membrane (149.21 ± 7.80). Notably, optical densities significantly varied across different age groups and corneal layers ($p < 0.05$). **Conclusions:** The linear corneal layer densities obtained using the linear mode of spectral domain optical coherence tomography (SD-OCT) with Image J were a fast and practical method to complement the subjective descriptions of spectral anterior segment OCT images. These densities can then be compared across various pathologies.

Keywords

Anterior Segment Optical Coherence Tomography, Optical Density, Densitometry

1. Introduction

Optical coherence tomography (OCT), a contact, non-invasive, and high-resolution technique, was introduced to ophthalmology in 1991 by Huang et al. They pioneered the first eye registration based on the interferometry of Albert Abraham Michelson, who received the Nobel Prize in Physics in 1907 for demonstrating the non-existence of the ether and the effects of two or more waves in superposition, resulting in constructive interference (summatory effects) or elimination (destructive interference) when the waves are contrary.

AS-OCT, an imaging technique, revolutionized ophthalmology by capturing the structures of underlying tissue in vivo. It measures reflected light to provide high-resolution images [1].

Over time, technology has evolved, significantly improving image resolution. This advancement came from the shift from time domain to spectral domain imaging, which provides images with 10 times more resolution compared to ultrasound. However, this increased resolution comes at the cost of light being reflected or absorbed by various tissues.

Currently, OCT can perform quantitative analyses of various parameters in the anterior segment, including thicknesses, measurements, the camera angle, the anterior chamber, and distances between interfaces. It can also detect differences in planes. These applications have been extensively documented in cases of pathologies and surgeries [2] [3]. However, despite these advantages, there is currently no simple and fast tool that allows quantitative density measurements, similar to those found in corneal tomography or ultrabiomicroscopy. This limitation has led to a predominantly descriptive approach in most publications regarding pathologies, focusing solely on the reflectivity between different diagnoses.

The analysis of the images captured by OCT spectral domain today has primarily been used in the retina, where various methods have been validated in the progression of pathologies. Unlike conventional imaging methods, OCT can not only accurately quantify the thickness of retinal layers but also detect more subtle phenomena that reflect metabolic changes in the tissue. This capability could be highly beneficial if it could be extended to other eye structures, such as the cornea. Corneal opacification is one of the leading causes of preventable blindness globally [4] [5].

Image J, a platform for image processing developed by Wayne Rasband at the National Institute of Health (NIH) in 1997, has undergone significant transformations over the years to align with contemporary research practices [6]. The present study aims to utilize this tool, in conjunction with the OCT spectral domain (OCT Heidelberg Engineering GmbH), to calculate the average gray value for each corresponding layer and corneal interface in images of healthy corneas.

2. Materials and Methods

A cross-sectional, observational study was conducted at the third-level ophthalmology center, "Hospital de la Luz", in Mexico City. All procedures were approved by the local ethics committee of the Hospital de la Luz (2023-C1-ADS). The study adhered to the Association for Research in Vision and Ophthalmology statement on human subjects studies, complied with the World Medical Association's Declaration of Helsinki, and received prior approval from the Institutional Review Board. Written informed consent was obtained from all study participants.

The study included healthy adults, categorizing them according to age: 30 - 50 years, 51 - 70 years and over 71 years old. Patients with corneal pathology, contact lens users, corneal surgeries, infected or scared corneas, individuals with a history of ocular, trauma, or surgery, and those afflicted with systemic diseases, such as diabetes mellitus, hypertension, or rheumatologic disorders and inflammatory processes of the ocular surface were excluded from this study.

2.1. Clinical Examinations

All groups underwent comprehensive ophthalmologic examinations, including assessment, of best corrective visual acuity using the Snellen chart, anterior and posterior segment biomicroscopic examinations, intraocular pressure (IOP) measurements using the Goldman applanation tonometer, gonioscopy using the Goldman three mirror lens.

Routine slit lamp biomicroscopy was performed to examine the eyes for scars, clinical or subclinical inflammatory signs of the ocular surface and corneal opacities. Detailed demographic information, including age and gender, was collected from each subject. Additionally, corneal measurements were taken in the morning hours, and all images were captured by the same individual.

2.2. Method for Determining Linear Optical Density

For corneal B Scan images, the OCT-SD (Heidelberg Spectralis®) was equipped with the anterior segment lens in an 8.3 mm zoom.

The corneal reflectivity was measured using the Image J program from the National Institute of Health. The B Scan image obtained in a jpeg format was calibrated with the linear and set scale tool with the 200 μm standard value for OCT-SD provided in the lower part of the image translating pixels to microns.

Reflectivity was measured as intensity values in a gray scale of 256 shades of 8-bit gray for each image from 0 color black to 255 color white, the higher reflective values in a determined area or interface will result in a brighter image.

A vertical tracing was performed using the program's linear tool, starting from the tear film and ending at the Descemet-endothelium interface and represented in a plot chart (**Figure 1**).

To avoid the central hyper-reflective artifact caused by the passage of the interferometer's laser light, images were obtained from specific areas 1mm below the artifact. Only images with a quality greater than 20 and less than 35, as determined by the OCT, were included in the average density measurement of each corneal layer or interface.

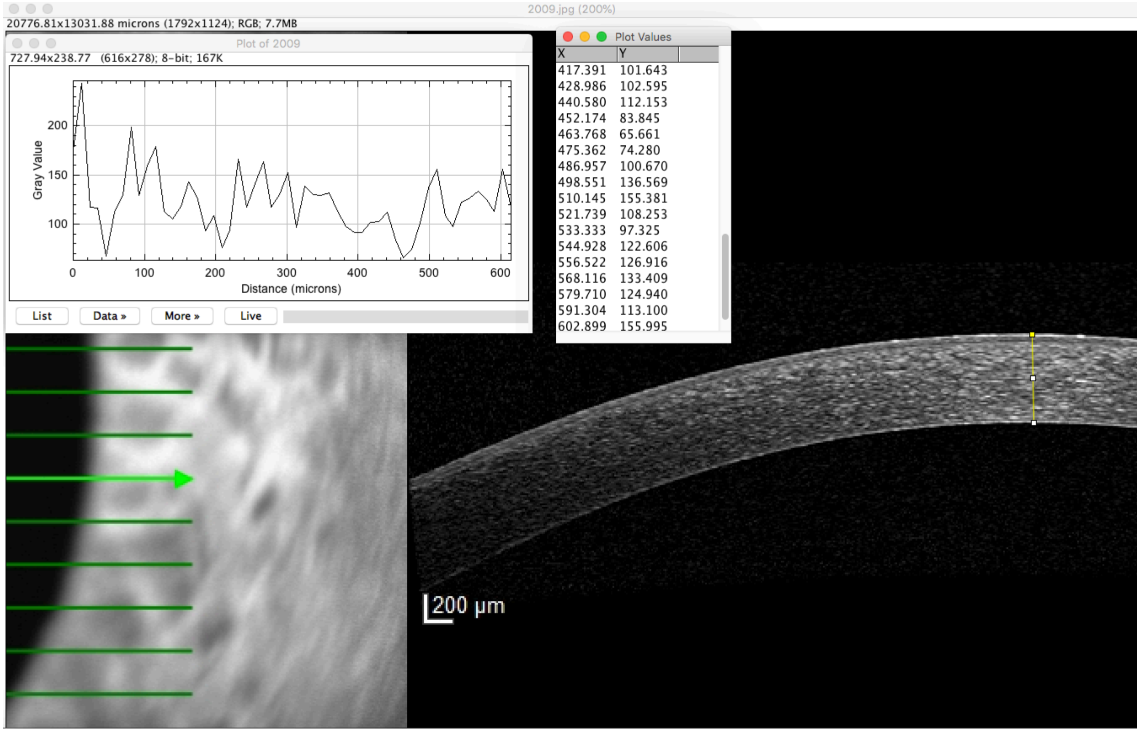

Figure 1. Calibration of Image J with known OCT image measurements establishes a scale for pixel measurement in micrometers.

Based on depth, the distinct interfaces were divided and described in relation to the spikes obtained for each density value. Tear film (~10 µm), epithelial interface (~50 µm), Bowman's layer (~12 µm), anterior stroma (~50 - 250 µm), posterior stroma (~250 - 500 µm), Descemet (~12 µm).

2.3. Statistical Analyses

All data were analyzed using IBM SPSS Statistics 21.0 Software. The patient's descriptive data for continuous variables were presented as mean ± standard deviation, while categorical variables were presented as counts and percentages. To assess the distribution of the studied variables, we used the Shapiro-Wilk test. Given the distribution of the data, comparative analysis between the different groups was performed using the independent samples t-test and analysis of variance (ANOVA). The accepted level of significance was set at $p < 0.005$ after Bonferroni correction.

3. Results

Spectral corneal OCT images of 140 eyes (90 patients), were analyzed from 50 males (75 eyes) and 40 females (65 eyes).

We found an average grey value density for tear layer 238.25 ± 17.49, epithelium interface 91.55 ± 3.13, basal membrane and Bowman 167.76 ± 16.19, anterior stroma 152.06 ± 9.09, posterior stroma 130.24 ± 8.32 and Descemet membrane 149.21 ± 7.80 (p =< 0.05) (**Figure 2**).

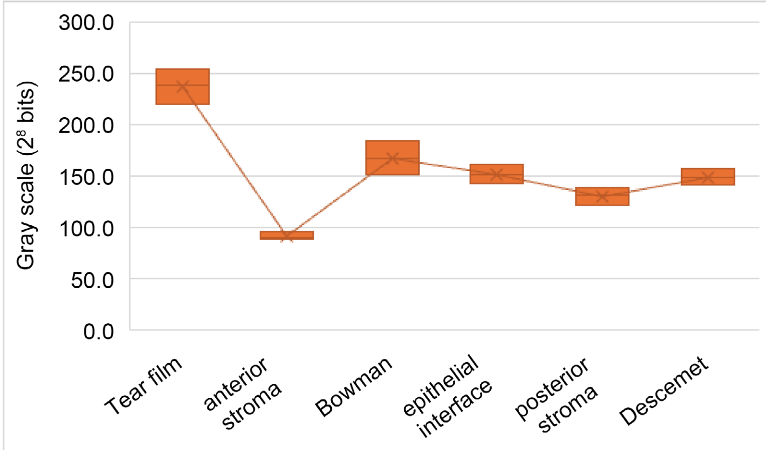

Figure 2. Mean gray scale density reflectivity values of each corneal layer and interface.

According to the patients from 30 - 50 years group (n = 48), the average grey value density for each interface was: tear film layer 255.06, epithelium interface 95.13, basal membrane and Bowman 167.40, anterior stroma 161.19, posterior stroma 131.08 and Descemet membrane 157.06. ($p =< 0.05$) For the 51 - 70 years group (n = 37) tear film layer 239.54, epithelium interface 90.22, basal membrane and Bowman 151.76, anterior stroma 152, posterior stroma 138.11 and Descemet membrane 149.08. ($p =< 0.05$). For the group over 71 years (n = 55) tear film layer 220.15, epithelium interface 89.31, basal membrane and Bowman 184.13, anterior stroma 143, posterior stroma 121.53 and Descemet membrane 141.47 ($p =< 0.05$).

The one-way ANOVA revealed a statistically significant difference in the optical densities among all the corneal layers across different age groups. However, the Bonferroni post-hoc analysis indicated that the comparison of Bowman with anterior stroma ($p = 0.84$), Bowman with Descemet ($p = 0.05$), and anterior stroma with Descemet ($p = 0.01$) was not significant for the 51 - 70 years group. Additionally, the comparison of anterior stroma with Descemet ($p = 0.06$) was not significant for the group over 71 years (**Table 1**).

Table 1. Mean corneal density reflectivity values by group of age and ANOVA test between groups.

Age	Tear film	epithelial interface	Bowman	anterior stroma	posterior stroma	Descemet
30 - 50 y (n = 48)	**255.06**	**95.13**	**167.40**	**161.19**	**131.08**	**157.06**
51 - 70 y (n = 37)	**239.54**	**90.22**	**151.76**	**152.00**	**138.11**	**149.08**
y ≥ 71 y (n = 55)	**220.15**	**89.31**	**184.13**	**143.00**	**121.53**	**141.47**
Mean value	238.25	91.55	167.76	152.06	130.24	149.21
SD	17.49	3.13	16.19	9.09	8.32	7.80
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	47097.2	5	9419.4	107.2	9.5024E-13	2.772
Within Groups	1581.2	18	87.8			
Total	48678.5	23				

4. Discussion

Corneal clarity is paramount for visual quality and acuity. Scheimpflug imaging systems have become the most widely utilized quantitative index for assessing corneal clarity due to their capacity to measure corneal density. Nevertheless, their resolution is inferior to that of spectral OCT in terms of tissue morphology, and they do not provide comprehensive information about each corneal interface. Furthermore, factors such as light scattering, age, contact lens wear, and corneal diseases can all influence these values [7].

The measurement of corneal optical clarity began in 1990 with Andrade et al. but was initially qualitative. Quantitative corneal density evaluation gained prominence with Scheimpflug systems, highlighting its significance in comparing optical values between healthy individuals and those with corneal diseases, as well as between people of different ages and regions.

In 2021, Wertheimer CM *et al*. [8] proposed a method to grade corneal stromal opacity using optical density measurements from OCT in Fuchs endothelial dystrophy. This method measured an area rather than a section, which could be more accurate but may also be more complex for daily analysis. Additionally, to our knowledge, ours is the first study to compare normality in linear density values for different age groups, which could serve as a reference for various pathologies.

While our results show significant differences in reflectivity measurements among all layers, the limited number of cases prevents us from establishing normality in the entire population. In contrast to our study, linear density studies using Scheimpflug and confocal systems have shown similar results for some layers [9]. However, parameters such as the backscattered light due to the corneal epithelium or interfaces of the tear film cannot be measured using OCT due to its resolution and measurement method, which are important factors to consider.

Comparing the corneal density values in the anterior and posterior layers for all the groups, we found a higher density for the tear film, which was importantly reduced at the epithelium with a rise at Bowman's layer that mantained a minimun diference with anterior stroma, but decreased at posterior stroma and had a last rise peak at Descemet membrane.

We acknowledge certain limitations in our study, since all measurements were captured and analyzed by the same individual and there's a possibility of some measurement artifacts. Two such artifacts are the corneal periphery (hyperreflectivity) and the zone of direct incidence of the laser source (hyperreflectivity). These reflectivity artifacts could potentially confound the measurement of density values.

Furthermore, differences in density values among individuals of different ages have been described for different instruments [9]. We found similarities in the 51 - 70 age group regarding certain interfaces, such as Bowman's membrane and the anterior stroma, and Descemet's membrane. These similarities might be attributed to cellular and collagen cross-linking changes that occur during these specific age periods and could influence some gray values. To confirm this analysis, a larger sample size would be beneficial for this particular group.

5. Conclusion

Linear corneal layer densities obtained using LM-SD-OCT with Image J were a fast and practical method to complement subjective descriptions of spectral anterior segment OCT images. These densities can then be later compared across different corneal pathologies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Elsawy, A., Abdel-Mottaleb, M., Sayed, I., Wen, D., Roongpoovapatr, V., Eleiwa, T., *et al*. (2019) Automatic Segmentation of Corneal Microlayers on Optical Coherence Tomography Images. *Translational Vision Science & Technology*, **8**, Article 39.

[2] Han, S.B., Liu, Y., Noriega, K.M. and Mehta, J.S. (2016) Applications of Anterior Segment Optical Coherence Tomography in Cornea and Ocular Surface Diseases. *Journal of Ophthalmology*, **2016**, Article ID: 4971572. https://doi.org/10.1155/2016/4971572

[3] Doors, M., Berendschot, T.T.J.M., de Brabander, J., Webers, C.A.B. and Nuijts, R.M.M.A. (2010) Value of Optical Coherence Tomography for Anterior Segment Surgery. *Journal of Cataract and Refractive Surgery*, **36**, 1213-1229. https://doi.org/10.1016/j.jcrs.2010.05.002

[4] Maidana, D.E., Tsoka, P., Tian, B., Dib, B., Matsumoto, H., Kataoka, K., *et al*. (2015) A Novel ImageJ Macro for Automated Cell Death Quantitation in the Retina. *Investigative Opthalmology & Visual Science*, **56**, 6701-6708.

[5] Butt, G., Brock, K., Ng, A., Vareechon, C., Pearlman, E., Hill, L.J., Wallace, G.R. and Rauz, S. (2018) Using ImageJ to Quantify Microbial Keratitis Related Corneal Opacification in a Mouse Model. *Investigative Ophthalmology & Visual Science*, **59**, Article 5851.

[6] Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., *et al*. (2017) ImageJ2: ImageJ for the Next Generation of Scientific Image Data. *BMC Bioinformatics*, **18**, Article No. 529. https://doi.org/10.1186/s12859-017-1934-z

[7] He, Y., Ma, B.S., Zeng, J.H. and Ma, D.J. (2023) Corneal Optical Density: Structural basis, Measurements, Influencing Factors, and Roles in Refractive Surgery. *Frontiers in Bioengineering and Biotechnology*, **11**, Article ID: 1144455. https://doi.org/10.3389/fbioe.2023.1144455

[8] Wertheimer, C.M., Elhardt, C., Wartak, A., Luft, N., Kassumeh, S., Dirisamer, M., *et al*. (2020) Corneal Optical Density in Fuchs Endothelial Dystrophy Determined by Anterior Segment Optical Coherence Tomography. *European Journal of Ophthalmology*, **31**, 1771-1778. https://doi.org/10.1177/1120672120944796

[9] Coskun, C., Çelik, G., Zeki Fikret, C., Çomçalı, S. and Evren Kemer, Ö. (2024) Evaluation of Corneal Densitometry Values with Pentacam in Cases of Ocular Hypertension and Pseudoexfoliative Glaucoma. *Photodiagnosis and Photodynamic Therapy*, **46**, Article 103988. https://doi.org/10.1016/j.pdpdt.2024.103988