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OCT based corneal densitometry: the confounding effect of epithelial speckle

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Abstract: Corneal densitometry is a clinically validated method for objectively assessing the transparency of stroma. The technique is currently dominated by Scheimpflug technology. Still, optical coherence tomography (OCT), in which examination of the statistical properties of corneal speckle is undertaken, has also been considered to assess corneal densitometry. In-vivo, the stroma is observed via the epithelium. However, the effect of this external layer on stromal densitometry has not been considered as yet. This study aims to quantify the influence of epithelium integrity on corneal OCT densitometry. OCT images from eleven freshly enucleated porcine eyes before and after epithelial debridement were used. OCT densitometry was investigated at different stromal depths using four metrics of speckle statistics. Results indicate that there exist statistically significant differences in speckle statistics for a given stromal depth depending on the presence or absence of the epithelium. The estimation error in speckle statistics can reach over 20% depending on the stromal depth. The anterior stroma densitometry values are the ones most affected by epithelial integrity. In conclusion, if OCT densitometry stromal parameters are to be considered in absolute terms, it is essential to consider the confounding effect of the epithelial layer in the analysis.

1. Introduction

Objectively assessing corneal transparency is essential to evaluate ocular health [1,2]. In a clinical environment, there is a growing demand for objective and robust estimators of corneal transparency, cumulatively termed *corneal densitometry*. It is often used for assessing pathological corneas in cases such as keratoconus [2,3] and Fuchs endothelial dystrophy [4] but also corneas after certain surgical procedures such as, for example, cataract and refractive surgeries [5,6] and Bowman layer transplantation [7]. Any influence of the epithelial layer on densitometry measures has not been considered as yet, despite being known that the integrity of epithelium is often compromised in those pathological cases [8,9]. Corneal densitometry estimators are based on evaluating the light backscattered from the cornea (in short, corneal backscatter). Whenever light from the source reaches the tissue to be imaged (here, the cornea), part of it is backscattered toward the detector to form an image. The final image, consequently, depends on how light travels inside the cornea and how much of it is backscattered. As the cornea is not an isolated structure, besides corneal tissue integrity, corneal backscatter might also be influenced by confounding factors such as eye biometry [10] or eye tilt [11].

Currently, clinical assessment of corneal densitometry is dominated by Scheimpflug imaging technology, whereas Optical Coherence Tomography (OCT), despite having a better axial resolution, has been given much less attention for performing this task [12–14]. This imbalance is mostly driven by the commercial success of Scheimpflug-based corneal densitometry.

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OCT is based on interferometry, which produces speckle. Even though the speckle has been traditionally treated as noise that degrades the quality of OCT image, there exists experimental evidence of speckle being signal-carrying [15]. Therefore, speckle statistics can be applied to infer indirectly about the microstructure of the tissue. In corneal research, previous works have applied the statistical modeling of corneal speckle to investigate corneal integrity with pathological conditions [16,17] and corneal tissue alterations as a consequence of hydration deprivation [12,18]. Corneal speckle statistics (a term reserved for OCT images) and corneal densitometry (a general term commonly used clinically) refer to the same concept, i.e., corneal backscatter. To date, mimicking Scheimpflug-based corneal densitometry, only the mean speckle intensity has been considered for OCT-based corneal densitometry [13]. However, as shown earlier [16–19], other statistics of OCT speckle carry clinically useful information that could also be utilized for OCT densitometry.

The cornea is a multi-layered structure. The epithelium is the most anterior corneal layer. Underneath the epithelium rests the stroma, which makes up approximately 90% of the corneal thickness [20]. Corneal epithelium and stroma have different reflective properties. In regular corneal tomography, regardless of whether it is captured with Scheimpflug or OCT technology, the epithelium appears as a distinct thin layer on the top of the stroma. Hence, image pixels corresponding to the epithelium contain different signal information than that from pixels representing the stroma. In practice, corneal densitometry is limited to evaluating information from corneal stroma [1,13]. However, there exist validated computational models [21,22] and experimental evidence [23] on how the scattering properties are altered in multi-layered tissue structures in OCT images, suggesting that epithelium might play a substantial role in corneal densitometry estimation.

In a recent experiment based on corneal OCT imaging, performed on freshly enucleated porcine eyes, a statistically significant difference in stromal corneal speckle parameter (the contrast ratio) was found in eyes before and after epithelium removal, without modifying any experimental condition other than the removal of the epithelial layer [19]. This experimental result suggests that epithelium, a layer through which light necessarily travels to access the stroma, plays a key role in corneal backscatter.

The current work aims to quantify the influence of epithelium integrity on corneal densitometry estimates and assess whether this should be taken into consideration in clinical applications.

2. Methods

2.1. Dataset

Retrospective corneal OCT data of 11 porcine eyeballs, collected earlier in a study on crosslinking, were exported from OCT device (SOCT REVO, Optopol, Zawiercie, Poland). Eyeball preparation and data collection process are described in detail in [19]. To summarize, intact eyeballs with no damages, scars nor corneal edema were obtained from a certified abattoir. Each eyeball, regularly moistened with phosphate-buffered saline, was imaged three times before and three times immediately after the epithelial debridement (denoted hereinafter epi-on and epi-off mode, respectively). Porcine eyes were imaged *ex-vivo*, so there were no eye movements. Also, all samples were imaged within the depth-of-focus of the instrument. Each three OCT acquisitions were taken consecutively without re-aligning the instrument. The 5 mm single B-scan scanning protocol was used. It composes of 12,032 raw A-scans, from which an OCT image is formed with the fixed width of 1,538 pixels and a varying depth of approximately 733 pixels, corresponding to 5 mm and about 2 mm, respectively. Hence, the approximate resolution was 308 pixels/mm horizontally and 366 pixels/mm vertically.

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2.2. Image processing

The main purpose was to compare the corneal OCT densitometry parameters for the same stromal regions in epi-on and epi-off mode acquisitions and to assess how the corneal epithelium layer influences OCT densitometry in the stroma. Firstly, the image segmentation algorithm described earlier was employed [18]. In order to analyze corneal backscatter 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 Fig. 1). The maximum downward shift of ROI was adapted to the smallest central corneal thickness from the examined corneas (i.e., 526 µm) and fixed for all eyeballs. Such a setting resulted in 43 ROI positions in the range [0, 420] µm (further denoted as stromal depths). Sample axial position within the OCT image was noted by segmenting the anterior corneal surface and finding its highest position (i.e., that of the corneal apex). The epithelium layer in epi-on images was delineated manually using digital calipers and the first position of the ROI in epi-on samples was just under the measured epithelium. The mean ± standard deviation of measured epithelial thickness was 24 ± 2 pixels, corresponding to approximately 66.7 ± 5.9 µm. For epi-off samples, as there was no epithelium, the anterior edge of the first ROI was included in the anterior corneal edge (see Fig. 1). This allowed for the analysis of the same stromal regions for epi-on and epi-off modes. In the preprocessing stage, each B-scan underwent a normalization process [24] in which, first, the pixel values were divided by the maximum value of 255. Subsequently, the inverse-log transformation was applied. The transformed pixel values were further subjected to normalization to lead speckle values between 0 and 1.

2.3. Statistical analyses

To describe corneal OCT densitometry within each considered ROI, four measures of speckle statistics, further denoted as densitometry parameters, were calculated: Gamma distribution parameters (shape and scale), in a parametric approach, and mean (μ) and contrast ratio (CR, the ratio of the standard deviation to the mean value), in a non-parametric approach [24]. The probability density function of the Gamma distribution can be described as:

$$f_X(x;\alpha,\beta) = \frac{1}{\beta^{\alpha}\Gamma(\alpha)} x^{\alpha-1} e^{\frac{-x}{\beta}} \quad \text{for} \quad x \geq 0, \tag{1}$$

where α>0 is the shape parameter, β>0 is the scale parameter, and x denotes the normalized image pixel intensity values in a specified ROI. For further analyses, for every eyeball, the median of the parameter estimates from the three epi-on and a median from three epi-off acquisitions

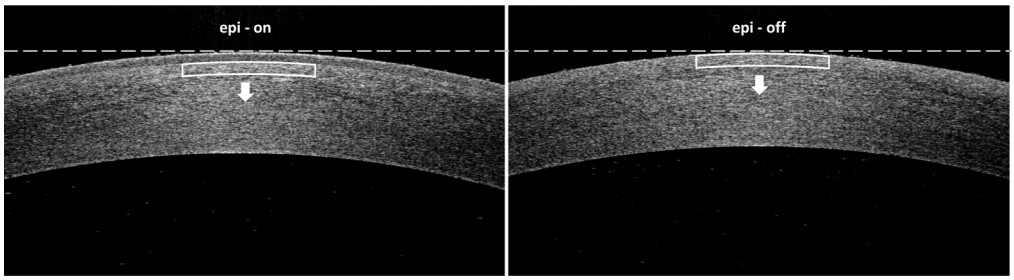

Fig. 1. Illustrative corneal OCT images for a porcine eye before (left, epi-on) and after (right, epi-off) epithelial debridement. White frames indicate the moving region of interest (ROI). Both ROIs for epi-on and epi-off mark out the same stromal regions set further for the OCT densitometry analysis. The horizontal dashed lines represent the position of the sample with respect to the imaging system.

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were gathered. Additionally, the coefficient of variation (CV) was evaluated for every three measurements. All OCT densitometry parameters were tested for the null hypothesis of Gaussian distribution using the Shapiro-Wilk test. The level of significance was set to 0.05 for all tests employed in the study. The rejection of null hypotheses of normality was included in further statistical analysis. To ascertain whether there are differences in $\alpha>0$, $\beta>0$, μ, and CR between epi-on and epi-off modes, Wilcoxon signed-rank test was used. Further, Spearman correlations were evaluated for epi-on versus epi-off parameters as well as partial correlations with controlling for 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.

To additionally assess the impact of the confounding effect of epithelial speckle on stroma densitometry, for each stromal depth the median error (expressed in percentages) was calculated as (e.g., for α):

$$\left(1 - m\left(\frac{\hat{\alpha}_{\text{epi-off}}}{\hat{\alpha}_{\text{epi-on}}}\right)\right) \times 100, \tag{2}$$

where m denotes the sample median, whereas as $\hat{\alpha}_{\text{epi-off}}$ and $\hat{\alpha}_{\text{epi-on}}$ are the estimators of the shape parameter α for the epi-off and epi-on modes at the particular stromal depth, respectively. The median errors for the scale parameter β, the sample mean μ, and CR were calculated in a similar fashion.

3. Results

The data for the analyses consisted of four densitometry parameters (α, β, μ, and CR) estimated for 11 eyeballs, for epi-on and epi-off modes, for 43 stromal depths. The mean coefficient of variation (CV) for three subsequent measurements, evaluated across eyeballs, modes and depths was less 5% (CV = 2.1%, 1.9%, 0.9% and 1.0% for α, β, μ and CR, respectively). Figure 2 shows the boxplots for the considered parameters for the epi-on mode juxtaposed with those for the epi-off mode. Asterisks indicate stromal depths where the Wilcoxon sign rank test indicated statistically significant differences between epi-on and epi-off modes. It is evident that stromal OCT densitometry parameters change after the removal of the epithelium. These changes are not straightforward and depend on the sample depth. For the shape parameter α, there are statistically significant differences at 270 and 280 µm and then at 340 µm and beyond. For the scale parameter β, there are statistically significant differences only at small sample depths (0–130 µm), where the stroma is close to the epithelial layer. For μ, statistically significant differences are observed at both small (0–120 µm) and larger stromal depths (190–420 µm). For CR, the differences seem to be stronger for small sample depths (10–40 µm) and then for 360–420 µm.

Figure 3 shows the scatter plots for the median values of α, β, μ, and CR with the stromal depth indicated by the saturation of blue marks. The correlations of the four considered parameters between the epi-on and epi-off modes are apparent. At the same time, the scatter plots indicate that the densitometry parameters of the corneal stroma are confounded when viewed by the intact layer of epithelium. Table 1 shows the corresponding results of Spearman's correlations and partial correlations of the four parameters in epi-on against epi-off modes. Despite incorporating stromal depths or the subject number as a controlling variable, the correlation is still maintained at the level of significance. Similarly, the difference in sample axial position that ranged between 0 and 17 pixels as well as the average intensity value in the epithelial region (mean ± standard deviation: 0.183 ± 0.029) did not influence those correlations. For each of the parameters, the differences in correlation coefficients evaluated without a control variable (the first column in Table 1) and those with a given control variable (columns 2 to 5 of Table 1) were not substantial, indicating that all control variables are not confounding factors.

Figure 4 shows the median error obtained for the four considered parameters for each stromal depth. An area of ±5% error, assumed here as clinically negligible, has been highlighted in light blue. Of particular note is the existence of over 20% median error for the parameters β and μ.

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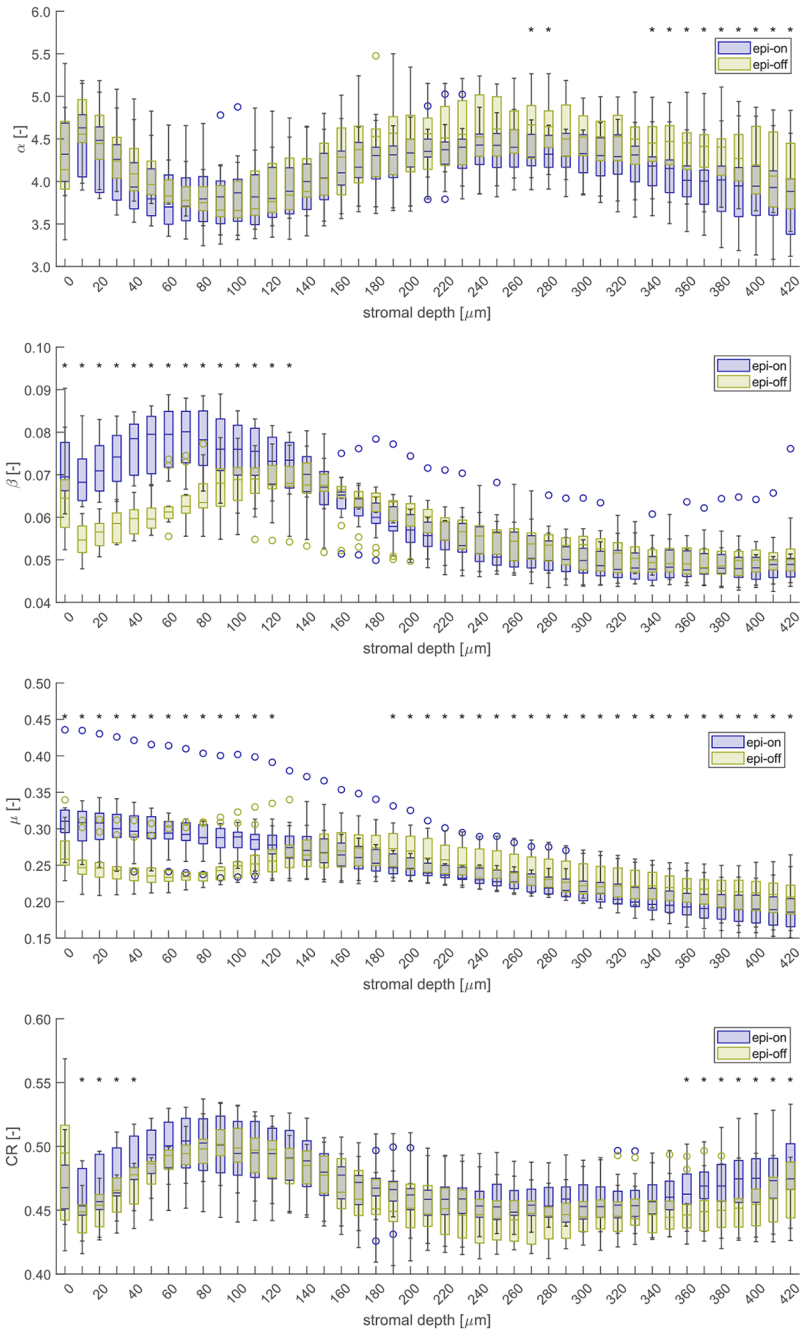

Fig. 2. Boxplots of the four considered OCT densitometry parameters calculated for pixel intensities within the ROI: α, β (shape and scale of Gamma distribution), μ (mean) and CR (contrast ratio) calculated for different stromal depths. The value for each eyeball is the median of three repeated measurements. 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.

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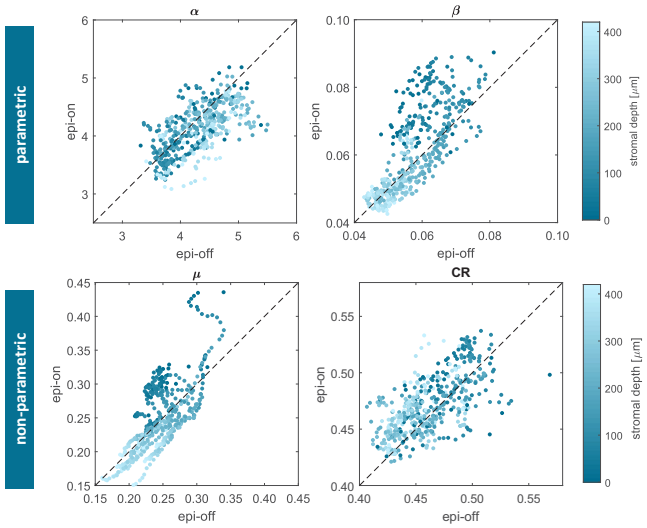

Fig. 3. Scatter plots of four considered OCT densitometry parameters calculated for pixel intensities within the ROI: α, β (shape and scale of Gamma distribution), μ (mean) and CR (contrast ratio) for epi-on and epi-off modes, where the stromal depth is indicated by the saturation of blue marks. Epi-on 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.

Table 1. The results of Spearman's correlations and partial correlations (Part. corr.) between the epi-on and epi-off modes, calculated for the four considered OCT densitometry parameters: α, β, μ and CR. 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.[a]

	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*
α	0.669	<0.001	0.679	<0.001	0.662	<0.001	0.661	<0.001	0.670	<0.001
β	0.804	<0.001	0.610	<0.001	0.802	<0.001	0.804	<0.001	0.789	<0.001
μ	0.696	<0.001	0.692	<0.001	0.695	<0.001	0.692	<0.001	0.672	<0.001
CR	0.646	<0.001	0.604	<0.001	0.635	<0.001	0.637	<0.001	0.646	<0.001

[a]SD – stromal depth,
[a]SN – subject number,
[a]AP – difference in sample axial position between epi-on and epi-off mode,
[a]MEI – mean epithelium intensity

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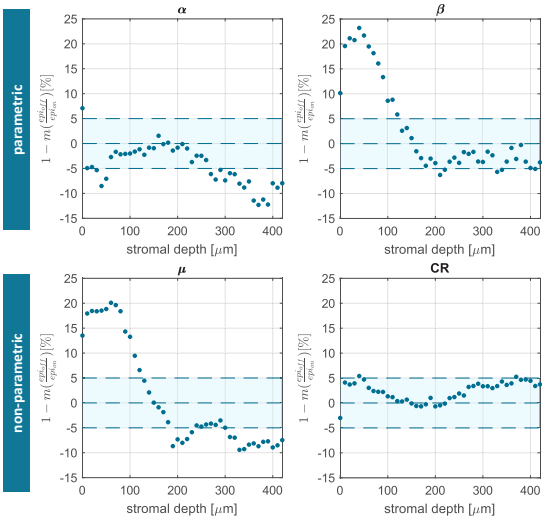

Fig. 4. Median errors between epi-on and epi-off mode of four considered OCT densitometry parameters calculated for pixel intensities within the ROI: α, β (shape and scale of Gamma distribution), μ (mean) and CR (contrast ratio), showing the confounding effect of epithelial speckle when evaluating the densitometry on different stromal depths. Each value is the median of three measurements taken for every eyeball. An area of ±5% error, assumed here as clinically negligible, has been highlighted in light blue.

4. Discussion

OCT corneal densitometry is in its infancy, despite the technology having a substantial potential for this task when compared to currently dominated field of Scheimpflug-based densitometry. This study investigated the confounding effect of epithelial speckle on stromal densitometry. The structure of epithelium greatly differs from that of the stroma. Consequently, light backscatter behaves differently in these two layers. This is meaningful, because the stromal densitometry is seen through the epithelium in standard clinical conditions. The results from the current work show that the corneal densitometry results are affected by epithelium integrity and that the epithelial layer tends, depending on the parameter considered, to over- or underestimate the stromal readings (see Fig. 3).

Corneal OCT densitometry was investigated at different stromal depths using four speckle measures, and parametric and non-parametric approaches were considered. The parametric approach, in agreement with previous literature on statistical modeling of corneal OCT speckle [18,25], consisted of Gamma distribution with shape (α) and scale (β) parameters. In the non-parametric approach, two parameters commonly used to describe OCT images were applied [19,24]: they are the mean pixel intensity (μ) and the contrast ratio (CR). Independently of the metric used, statistically significant differences in corneal stroma densitometry were found depending on the presence (epi-on) or absence (epi-off) of the epithelium (see Fig. 2) and those differences were depth dependent.

If the epithelium were not affecting corneal densitometry readings, one would expect the lack of statistical significance in Fig. 2 and the points in the scatter plots of Fig. 3 to fall on the 1:1 line. However, this is not observed. For example, when it comes to parameters β and μ, the points corresponding to small stromal depths (i.e., close to the epithelium, darker points in Fig. 3) fall over the line 1:1. In other words, the values of β and μ are overestimated in the epi-on mode. Contrarily, for α, stromal readings are underestimated in deeper stromal layers (i.e., far from the epithelium, brighter points in Fig. 3).

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Even though β and μ represent parametric and non-parametric approaches, respectively, they have a similar mathematical origin because the mean value of the Gamma distribution is the product of α and β. Consequently, the information they carry is analogous (see Fig. 3 and 4). Similarly, CR = $1/\sqrt{\alpha}$, so α tends to be underestimated whereas CR is overestimated (Fig. 3). Hence, the parametric and non-parametric approaches carry different, yet complementary information.

There is a clinical significance of the obtained results as the examination of corneal speckle is of interest in studies involving the debridement of the epithelium [26] or the healing processes occurring in cornea [27,28]. In fact, any change in the optical properties of the epithelium (e.g., caused by epithelial damage, hypoxia, or changes in permeability) may also change the stromal speckle parameters. If the presence of the epithelium is not considered, then anterior corneal stromal readings could be over- or underestimated. The more significant bias occurs in the anterior stroma, likely because of the attenuation of the OCT signal at deeper layers. However, there are minor but statistically significant differences also observed in posterior stroma layers.

Beyond clinical practice, there is an emerging promising field of study based on depth-resolved corneal images with full-field OCT (FF-OCT), which allows histology-like analysis of stromal features [29–31]. The results from the current study might also be of use for FF-OCT researchers, who are in the position to validate whether by increasing the axial resolution of OCT, similar confounding factor of epithelial layer on stromal densitometry parameters is evident.

The current study sheds a different light on the results of the experimental work performed earlier on crosslinking (CXL) using freshly enucleated porcine eyes [19]. In particular, this concerns the result of a CXL procedure corresponding to standard epi-off Dresden protocol, but without the exposure to UVA light. The statistically significant changes observed there in CR before the epithelium debridement and after the CXL (no UVA) procedure can be interpreted as changes between epi-on and epi-off modes, irrespective of CXL.

The study does not present strong limitations. Freshly enucleated porcine eyes with epithelial debridement were used in this research work. Nevertheless, it should be emphasized that the obtained results were achieved for the given set of porcine eyes and the particular type of imaging instrument with a given scanning protocol. The presented results highlight the problem of epithelium confounding densitometry results of the stroma. A similar effect of one tissue layer confounding the results of other tissue layer is expected to happen in the human eyes, but the extent of that effect is currently unknown. Another limitation of the study is that there were no standard Scheimpflug densitometry measures for comparison with those based on OCT images.

Concluding, in the case of estimating densitometry parameters of stroma, to avoid over- or underestimation, it is essential to consider the confounding effect of the epithelial layer in the analysis.

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Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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