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Anterior segment optical coherence tomography scanning protocols and corneal thickness repeatability

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ABSTRACT

Purpose: To examine the influence of anterior segment optical coherence tomography imaging protocols on the intraobserver and intrasession repeatability of epithelial, stromal, and total corneal thickness measurements.
Methods: Repeated anterior segment optical coherence tomography (AS-OCT) images (Spectralis, Heidelberg) were obtained from 15 adults using single 8.3 mm wide horizontal line scans with an average of 2, 10, 20, 30, 50 and 100 B-scans. Volumetric scans consisting of nine 8.3 mm horizontal line scans encompassing a 1.3 mm vertical region were also captured (with 20 B-scans per line scan). Single point thickness measures (at the normal to the tangent of the anterior corneal surface) were compared with thickness measures averaged over the central 6 mm. The impact of B-scan averaging and intraobserver variability were examined for single line scans. For volumetric scans, the impact of the number of line scans upon intraobserver and intrasession variability were calculated.
Results: Intraobserver repeatability did not vary significantly as a function of the number of averaged B-scans per line scan, but was lowest for 20–30 averaged B scans. For volumetric scans, increasing the number of line scans did increase scan duration ($p < 0.001$), with minimal impact upon the average scan quality index ($p = 0.06$). Averaging more than 3 line scans did not significantly improve intraobserver or intrasession repeatability for either single point or average thickness measurements.
Conclusion: AS-OCT volumetric scans with 3 lines each consisting of 20 B-scans with measurements averaged over a central 6 mm of the cornea provide highly repeatable measures of epithelial, stromal and total corneal thickness (95 % LoA ≤ ± 3.2 μm for intraobserver repeatability and ≤ ± 3.7 μm for intrasession repeatability). This scanning protocol can provide reliable information when monitoring subtle changes in corneal thickness.

1. Introduction

Anterior segment optical coherence tomography (AS-OCT) is a non-invasive imaging technique with a range of potential clinical applications [1,2], including; the early detection of subtle corneal pathology (e.g. keratoconus) [3–5], monitoring and predicting potential disease progression (e.g. corneal hydrops) [6], pre and post intervention corneal profiling (e.g. morphological or optical changes following corneal collagen cross-linking or surgery) [5] and scleral contact lens fitting [1]. Scleral lenses have been successfully fitted for over a century using trial lenses, patient feedback, and careful slit lamp observation. However in recent years AS-OCT has also been used to further the understanding of scleral lens thickness profiles [7], variations in lens centration [8] and central [9–12], mid-peripheral [13] or limbal corneal clearance over time [14], scleral topography and elevation [15,16], and the interaction between the haptic landing zone with the underlying conjunctival and scleral tissue [17,18]. Consequently, in modern scleral lens practice, many practitioners utilise AS-OCT to aid initial trial lens selection, assess the initial fit, and monitor changes over time.

An OCT creates a 2- or 3- dimensional cross-sectional image by measuring the echo time delay of light backscattered from tissue structures [2]. A B-scan is a lateral beam of light and is composed of multiple axial scans (A-scan) [4,5]. Instruments vary with respect to default settings but can range from 1 to 100 B-scans. Studies examining corneal thickness have used various scan protocols ranging from 4 to 25 radial scans [19–22], thickness maps up to 9 mm in diameter [23,24], corneal cross lines [25,26] and multiple horizontal or volumetric scans [27].

Several recent studies have also used high resolution AS-OCT (typically $3-5$ μm axial resolution) to reliably quantify the nature and

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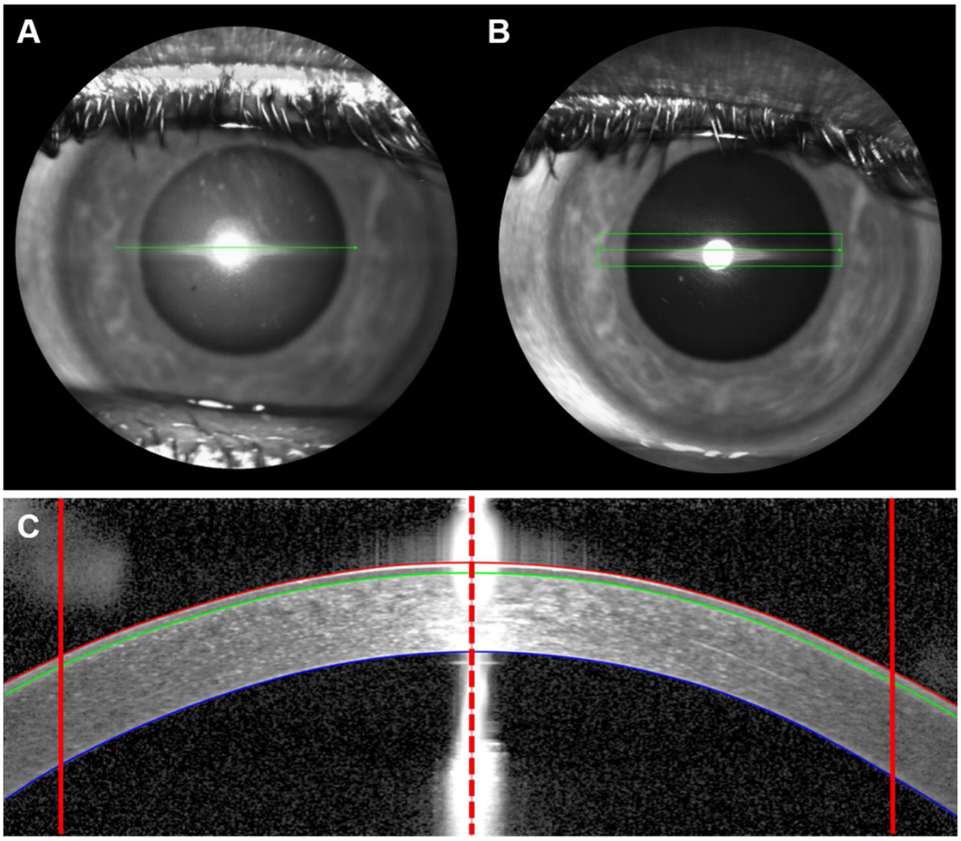

Fig. 1. (A) En-face AS-OCT image depicting the single line scan protocol. (B) En-face AS-OCT image depicting the volumetric scan protocol. (C) An example of a manually segmented cross sectional AS-OCT image; anterior epithelium (curved red line), posterior epithelium/anterior stromal border (green line) and the endothelium (blue line). A single point measurement was taken at the normal to the tangent of the corneal apex (vertical red dashed line). The solid vertical red lines show the 6 mm region analysed for the average thickness metric.

time course of scleral lens induced corneal oedema in healthy eyes [28,29], and the influence of altering modifiable lens parameters upon corneal swelling (e.g. corneal clearance, material Dk/t) [30,31]. Other studies have utilised Pentacam imaging [32,33] to measure corneal thickness, however this required lens removal prior to image capture and will underestimate the true magnitude of oedema, as deswelling commences immediately following lens removal.

These repeated measure experiments often utilise different imaging protocols to derive corneal thickness, and therefore, corneal oedema values; for example, a single point estimate from a radial line scan centred on the corneal apex [28,31], or a central thickness value averaged across the central 4 mm of the cornea centred on the pupil derived from 12 radial line scans [29]. Despite substantial differences between the imaging protocols outlined in the above examples, these two studies [28,29] yielded remarkably similar results of 1.65 % [28] and 1.18 % [29] total corneal oedema, when averaged across the study participants. However, no study has systematically investigated the influence of AS-OCT imaging protocols (e.g. the optimum number of line scans, B-scans per line scan, or width of the line scan) upon the reliability of corneal thickness measurements. Optimising AS-OCT imaging parameters is critical for repeated measure experimental studies of corneal thickness (since the measurement error or variability directly influences the required sample size) and also in clinical contact lens practice (e.g. to reliably quantify the short-term response to hypoxic stress over a few hours during scleral lens wear, or to monitor longer-term changes in corneal thickness over a number of years).

Averaging multiple B-scan images improves the signal-to-noise ratio in OCT images [34], and in theory, averaging the maximum possible number of B-scans (limited by instrument parameters) should reduce measurement noise and optimise tissue visibility. However, increasing the number of B-scans that are averaged also extends the overall scan duration, and therefore may increase subject fatigue and eye movements during image acquisition. While a small number of studies have examined the influence of B-Scan averaging on OCT images of the posterior eye (retina and choroid) [35–37], currently no studies have investigated B-Scan optimisation for corneal imaging. Therefore, the aim of this study was to determine the influence of OCT B-Scan averaging, line scan averaging, and line scan width (a single central point compared to an average across the central 6 mm) on epithelial, stromal, and total corneal thickness intraobserver and intrasession repeatability.

2. Methods

2.1. Participants

This study was approved by the Queensland University of Technology human research ethics committee and followed the tenets of the Declaration of Helsinki. All participants provided informed consent. Fifteen young healthy participants aged between 20–37 years with no ocular pathology and visual acuity of 0.00 logMAR or better in both eyes were recruited. Participants with any history of ocular injury, surgery, regular rigid contact lens wear or current use of topical medications were excluded. Soft contact lens wearers were included but ceased lens wear for at least 24 h prior to any experimental measurements.

2.2. OCT imaging and analysis

The Spectralis OCT (Heidelberg) has an axial resolution of 3.9 μm and was used to measure central corneal thickness. The default imaging values are 60 B-scans for a single line scan and 9–16 B-scans depending on the type of volumetric scan [38]. Unlike OCT imaging of the posterior eye, gaze tracking and anatomical landmark registration is not currently possible during AS-OCT imaging with commercially available instruments. Therefore, participants were instructed to maintain steady fixation of the instrument's internal fixation target during image acquisition. If an image was affected by a blink artefact or poor fixation, the scan was retaken.

2.2.1. B-scan averaging protocol

A series of 6 repeated measures were performed, consisting of an 8.274 mm single horizontal line scan centred on the pupil, with a range in the number of B-scans averaged within each line scan (2, 10, 20, 30, 50 and 100 averaged B-scans per line scan captured in a randomised order) (Fig. 1A). The participant was required to remove their head from the chin and head rest between each measurement and the single examiner re-aligned the instrument to be centred on the pupil using the scanning laser ophthalmoscope image for each scan captured.

2.2.2. Volumetric line scan protocol

Two sets of volumetric line scans encompassing an 8.274 mm horizontal x 1.251 mm vertical section of the central cornea were captured using a scanning protocol of 9 horizontal line scans (each separated by 139 μm and consisting of 20 averaged B-scans) centred on the pupil (Fig. 1B). The participant was required to remove their head from the chin and head rest between each OCT measurement and the single examiner re-aligned the instrument to be centred on the pupil using the scanning laser ophthalmoscope image for each scan captured.

2.2.3. OCT image analysis

Custom written software was used to analyse each individual OCT line scan. Each image was exported in high-resolution format and imported into Matlab. A single observer manually segmented the anterior corneal epithelial boundary, the anterior stromal boundary, and the endothelium (Fig. 1C). 20 points were selected to fit a spline curve to each layer. The normal to the tangent of the corneal apex was also manually marked in each line scan as a reference point, similar to previous studies [8,29]. Two corneal thickness metrics were then extracted from the manually segmented data; a single point thickness measurement (*single point metric*) derived at the normal to the tangent of the central corneal apex, and an average thickness derived from the thickness values sampled across the central 6 mm of the cornea (*average metric*), centred at the normal to the tangent of the corneal apex (an average of 1551 thickness measurements, spaced 3.87 μm apart over the central 6 mm of the cornea) (Fig. 1C). The single point and average thickness values were calculated for the epithelial, stromal, and total corneal thickness. For the volumetric line scan protocols, thickness values were generated from an average of all 9 line scans, and also resampled 8, 7, 6, 5, 4, 3, 2, and 1 randomly selected line scans from within the volumetric scan. The acquisition time and image quality for each OCT scanning protocol for each participant were retrieved from the *.xml file exported from the instrument. On average, the duration of single horizontal lines scans varied between 0.9 ± 1.3 s (2 averaged B-scans) and 9.1 ± 14.0 s (100 averaged B-scans).

2.3. Statistical analyses

2.3.1. B-scan averaging protocol

A series of repeated measures analysis of variance (ANOVA) were conducted with a within-subject factor of the number of B-scans for each thickness measurement (epithelial, stromal, and total thickness using the single point and average metric). Bland-Altman analyses were carried out to examine the mean difference, the 95 % limits of agreement (LOA) and their exact confidence intervals [39] for repeated corneal thickness measurements derived from an assumed gold standard (the line scan consisting of 100 averaged B-scans) and the other OCT line scan protocols (2, 10, 20, 30, and 50 averaged B-scans). The exact 95 % confidence intervals provide a more accurate representation of the population variation, particularly for small sample sizes, as the LOA's provide only an estimate of the population LOA [39]. The F-test was used to examine whether the 95 % LOA's varied significantly between the different scanning protocols. The intraobserver repeatability (the same OCT scan manually segmented twice by the same observer) was also examined as a function of the number of B-scans for measures of epithelial, stromal, and total corneal thickness (for both the single point and average metric) using a series of Bland-Altman analyses and F-tests.

2.3.2. Volumetric line scan protocol

A series of Bland-Altman analyses (as outlined above) were conducted to examine the intraobserver and intrasession repeatability (for the epithelial, stroma, and total corneal thickness derived from both the single point and average metric) as a function of the number of line scans included to generate the thickness value. Repeated measures ANOVA and linear regression were also used to analyse the effect of number of line scans upon image quality and scan duration.

3. Results

3.1. B-scan averaging protocol

3.1.1. Central corneal thickness

As expected, corneal thickness varied significantly based on the metric used. For the epithelium, the single point metric overestimated thickness by 1.8 ± 0.4 μm (2.9 %) compared to the average metric ($p < 0.001$). Conversely, the single point metric underestimated the thickness value compared to average metric for both the stromal (23 ± 1 μm, 4.9 %) and total corneal thickness (21 ± 1 μm, 4.0 %) (both $p < 0.001$). Epithelial and stromal thickness values did not vary significantly with the number of averaged B-scans per line scan (maximum variation across the B-scan conditions was 1.2 ± 0.9 μm for the epithelium and 2.5 ± 0.8 μm for the stroma) (both $p > 0.05$). For the total cornea, the thickness did vary significantly with the number of B-scans (maximum variation across B-scan conditions 3.6 ± 1.1 μm, $p < 0.01$), with the thickness values derived from the 2 averaged B-scan condition typically slightly less than those derived from the other B-scan conditions, for both the single point and average metric.

3.1.2. Agreement analysis (comparison to 100 averaged B-scans)

Fig. 2 displays the condensed Bland-Altman plots for the agreement of corneal thickness measurements using a different number of B-scans compared to an assumed gold standard of 100 B-scans, where the error bars represent the exact 95 % confidence intervals for the upper and lower limits of agreement. While the mean difference does not vary substantially across the different B-scan conditions, for epithelial, stromal and total thickness measurements, the limits of agreement and the confidence intervals for the thickness difference are reduced for the average metric compared to the single point metric. Examination of Fig. 2 suggests that the agreement with the assumed gold standard of 100 averaged B-scans does not improve beyond averaging ~20 B-scans. Statistically, for both the single point and the average metric, the level of agreement did not vary significantly with the number of B-scans averaged (F test, $p > 0.05$).

3.1.3. Intraobserver repeatability and B-scan averaging

Fig. 3 displays the condensed Bland-Altman plots for the intraobserver repeatability as a function of the number of averaged B-scans per line scan (Supplementary Table 1). The mean difference in corneal thickness between repeated manual segmentation of the same image by the same observer was very close to zero (< 0.02 μm across all measurement conditions), with the 95 % limits of agreement ≤ ± 0.2 μm. Measurements derived from the average metric were typically more repeatable than those derived using the single point metric, as anticipated, particularly for the epithelium, most likely due to the less uniform anterior epithelial surface compared to the anterior and posterior stromal boundaries. For measures of stromal and total corneal thickness, there were no significant differences in intraobserver repeatability with respect to the number of B-scans averaged or the thickness metric used (both $p > 0.05$). Visual inspection of panels B and C in Fig. 3 (total corneal thickness data provided in Supplementary Table 2) suggests an improvement in repeatability from 2 to 20–30

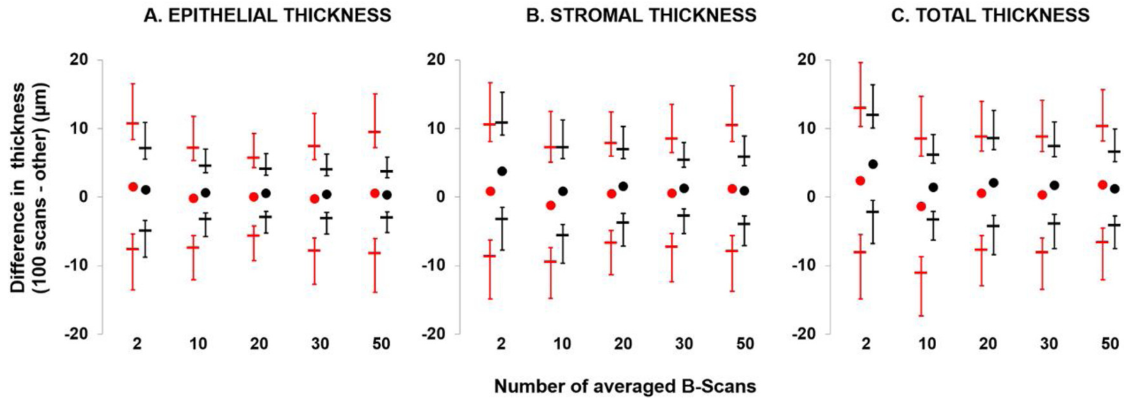

Fig. 2. Condensed Bland-Altman plots displaying the agreement between corneal thickness from single line scans derived from 2, 10, 20, 30 and 50 averaged B-scans compared to an assumed gold standard of 100 averaged B-scans. Circles represent the mean difference, horizontal bars represent the limits of agreement, and the error bars represent the exact 95 % confidence intervals of the limits of agreement. Red denotes data derived from the single point thickness metric, and black denotes data derived from the average thickness metric (across the central 6 mm of the cornea centred on the normal to tangent of the corneal apex).

averaged B-scans and then a reduction in repeatability from 20–30 to 100 B-scans (i.e. the approximate turning point of optimum repeatability is around 20–30 averaged B-scans).

3.2. Volumetric scan protocol

3.2.1. Scan duration and image quality

Fig. 4 displays the relationship between the number of line scans included in a volumetric scan and the duration of imaging and the image quality (averaged across all line scans included in the analysis). Scan duration increased significantly with an increasing number of line scans ($p < 0.001$), by ~1.8 s per additional line scan, with greater variability observed for a greater number of line scans. No significant change in the average scan quality index was observed as a function of the number of line scans ($p = 0.06$). None of the OCT images displayed a quality index (QI) less than 15 dB (the manufacturer's recommended QI threshold) and only three average QI values were between 15 − 20 dB (out of 135 combinations of volumetric scans), from volumetric scans consisting of 1 or 6 averaged line scans.

3.2.2. Intraobserver repeatability

The average metric displayed slightly better repeatability values compared to the single point metric, particularly when fewer line scans were averaged (1–2). While the F-test revealed there were no statistically significant differences in the limits of agreement with respect to the number of averaged line scans, visual inspection of Fig. 5 suggests that a plateau occurs around ~3 averaged line scans, with no major improvement in intraobserver repeatability beyond this for either metric (Supplementary Table 3). Using 3 line scans compared to 1 line scan resulted in a 42, 50, and 40 % reduction in the 95 % LOA for the epithelial, stromal, and total thickness respectively using the average metric (Table 1).

3.2.3. Intrasession repeatability

Similar to the intraobserver repeatability data, the average metric displayed slightly better repeatability values compared to the single point metric, particularly when fewer line scans were averaged (1–2). While the F-test revealed there were no statistically significant differences in the limits of agreement with respect to the number of averaged line scans, visual inspection of Fig. 6 suggests that a plateau occurs around ~3 averaged line scans, with no substantial improvement in intrasession repeatability beyond this for either metric (total corneal thickness data provided in Supplementary Table 4). Using 3 line scans compared to 1 line scan resulted in a 5, 46, and 41 % reduction in the 95 % LOA for the epithelial, stromal, and total thickness respectively using the average metric (Table 1).

4. Discussion

The key finding from this study examining AS-OCT images of healthy eyes was that intraobserver and intrasession repeatability can be improved by averaging 20 B-scans per line scan, and by averaging three line scans within a volumetric scan, but any further reduction in measurement error by increasing the number of B-scans or line scans

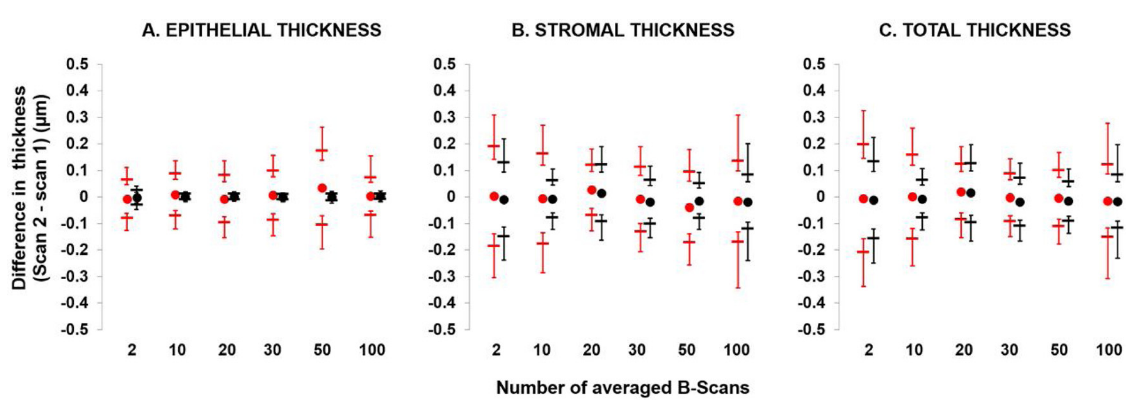

Fig. 3. Condensed Bland-Altman plots highlighting the intraobserver repeatability for corneal thickness measurements derived from 2, 10, 20, 30, 50 and 100 averaged B-scans. Circles represent the mean difference, horizontal bars represent the limits of agreement, and the error bars represent the exact 95 % confidence intervals of the limits of agreement. Red denotes data derived from the single point thickness metric, and black denotes data derived from the average thickness metric (across the central 6 mm of the cornea centred on the normal to tangent of the corneal apex).

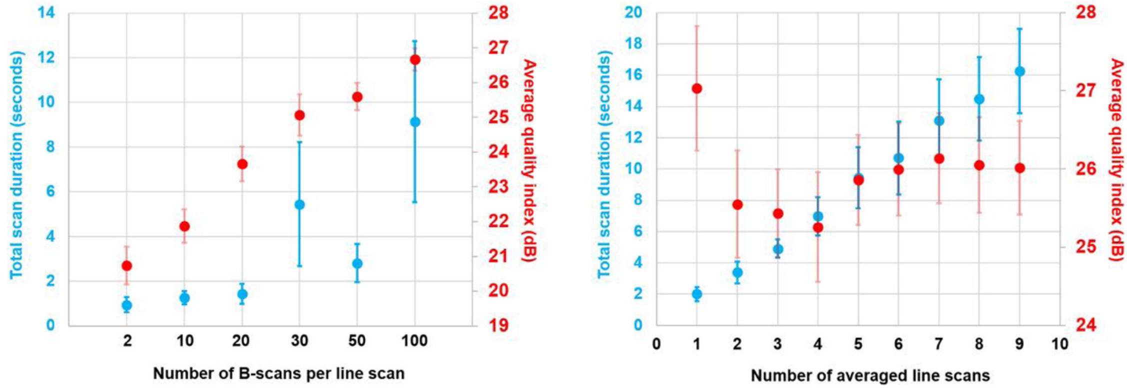

Fig. 4. Left: The mean AS-OCT single line scan duration (averaged across all participants) (blue) and the mean scan quality index (red) as a function of the number of B-scans averaged per line scan. Right: The mean AS-OCT volumetric scan duration (seconds, based on the sum of the duration of each randomly selected individual line scan) (blue) and the mean scan quality index (averaged across all line scans) (red) as a function of the number of line scans included in the volumetric scan. Error bars represent the standard error of the mean.

averaged is negligible. For the single line scan analysis (B-scan optimisation), the intraobserver repeatability did not vary significantly with respect to the number of B-scans averaged. However, for the stromal and total corneal thickness measurements in particular (Fig. 3B and C), measurement variability was clearly lower for 20–30 averaged B-scans, and greater for 2, 10, and 100 averaged B-scans based on visual inspection of the 95 % confidence intervals of the limits of agreement. This was most apparent when using the single point thickness metric, rather than averaging thickness values across the central 6 mm of the cornea.

While no other study has examined the influence of B-scan averaging for AS-OCT measurements, optimal values of 20–30 averaged B-scans have been reported for posterior OCT imaging to minimise retinal or choroidal measurement error [35,36]. Poorer repeatability for a lower number of averaged B-scans is likely due to a reduced signal to noise ratio, and the increased variability associated with a larger number of averaged B-scans may be associated with increased eye movements during a longer period of image acquisition (the average scan duration for a single line scan consisting of 2 averaged B-scans was 0.95 ± 1.32 s, compared to 9.14 ± 13.98 s for 100 averaged B-scans). Although image tracking during AS-OCT capture using anatomical landmarks is currently not available in commercial instruments, motion compensation using pupil tracking may further reduce measurement error by correcting for motion artefacts in real time [40].

Based on the volumetric line scan analysis, as expected, scan duration significantly increased with an increasing number of lines scans (each consisting of 20 averaged B-scans), but not at the expense of image quality (Fig. 4). Using the average metric and calculating the thickness from an average of 2–3 line scans gave the best intrasession and intraobserver repeatability (Figs. 5 and 6). Averaging across a larger number of line scans provided no statistically significant reduction in measurement error for measures of epithelial, stromal or total thickness measures using either metric. Sin and Simpson [27], also examined the repeatability of corneal epithelial thickness using a modified Zeiss retinal OCT device with a 1.13 mm central line scan and observed that averaging the epithelial thickness from three repeated measures improved the repeatability by ∼50 % compared to a single measurement. This is consistent with the volumetric analysis from the current study; a 42 % and 52 % reduction in the 95 % intraobserver LOA when averaging across 3 line scans compared to 1, for the average and single point metrics respectively (Table 1). A reduction in the limits of agreement was observed for all corneal thickness measures when averaging across 3 line scans compared to 1, but this improvement was almost always greater for the single point metric compared to the average metric (46 % compared to 37 % on average). This finding is particularly relevant for future studies examining small changes in corneal thickness over time, since improved repeatability (reduced limits of agreement) through image optimisation or averaging reduces the required sample size to reach a desired statistical power.

Only one previous study has investigated the repeatability of the Spectralis OCT device used in this study. López de la Fuente et al. [41] calculated the intraobserver repeatability for measures of corneal thickness obtained at the pupil centre using the in-built callipers of the manufacturer's software, however, details of the scanning protocol were not reported. The mean difference and standard deviation for relevant corneal layers were; epithelial 0.82 ± 5.12 µm, stromal 0.00 ± 4.34 µm, and total corneal thickness 0.62 ± 5.01 µm. These results are similar to the intraobserver repeatability data in the current

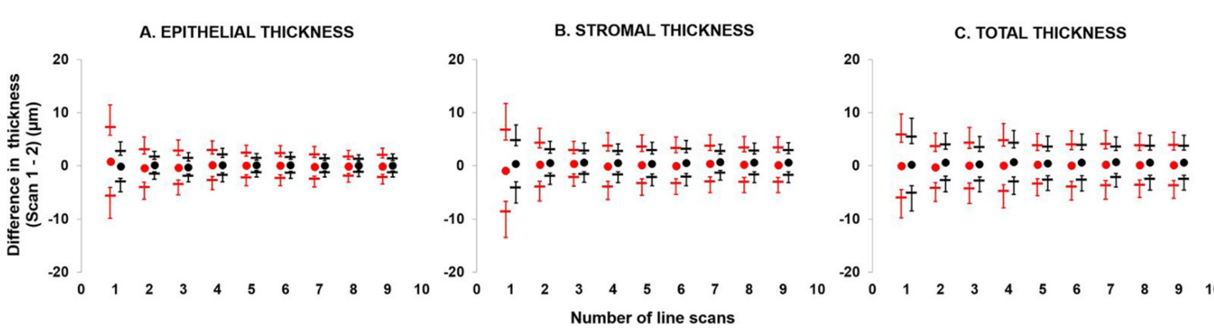

Fig. 5. Condensed Bland-Altman plots highlighting the intraobserver repeatability for corneal thickness measurements derived from 1 to 9 randomly selected line scans from a 9 line scan volumetric protocol (consisting of 20 averaged B-scans). Circles represent the mean difference, horizontal bars represent the limits of agreement, and the error bars represent the exact 95 % confidence intervals of the limits of agreement. Red denotes data derived from the single point thickness metric, and black denotes data derived from the average thickness metric (across the central 6 mm of the cornea centred on the normal to tangent of the corneal apex).

Table 1
The 95 % limits of agreement (LOA) of the mean difference for intraobserver and intrasession repeatability calculated from 1 or an average of 2 or 3 line scans using both the single point and average metric. The reduction in the limits of agreement when using an average of 2 or 3 line scans compared to 1 is presented as a percentage.

Number of scans		Thickness metric									
		Single point					Average (6 mm)				
		1	2		3		1	2		3	
Layer	Repeatability	LOA (μm)	LOA (μm)	Reduction (%)	LOA (μm)	Reduction (%)	LOA (μm)	LOA (μm)	Reduction (%)	LOA (μm)	Reduction (%)
Epithelium	Intraobserver	−5.7, 7.4	−4.0, 3.1	45	−3.5, 2.8	52	−3.0, 2.7	−1.5, 1.7	44	−2.0, 1.4	42
	Intrasession	−6.4, 4.4	−6.6, 4.3	0	−4.2, 3.0	33	−3.5, 2.6	−3.7, 2.5	−2	−3.6, 2.1	5
Stroma	Intraobserver	−8.6, 6.8	−4.0, 4.4	46	−2.2, 3.0	67	−4.1, 4.9	−1.9, 3.0	45	−1.6, 2.8	50
	Intrasession	−7.5, 9.2	−4.8, 5.9	36	−4.0, 3.3	56	−4.9, 6.6	−3.1, 4.1	37	−3.0, 3.2	46
Total	Intraobserver	−6.0, 5.9	−4.2, 3.7	34	−4.3, 4.4	27	−5.1, 5.5	−2.7, 4.0	37	−2.9, 3.5	40
	Intrasession	−8.1, 8.5	−6.2, 5.0	33	−5.7, 3.8	43	−5.9, 6.7	−5.8, 5.5	12	−4.4, 3.0	41

study obtained from a single line scan randomly selected from a volumetric line scan using the single point metric; mean difference and standard deviation of 0.84 ± 3.33 μm for the epithelium, −0.88 ± 3.93 μm for the stroma, and −0.04 ± 3.04 μm for the total cornea. The Pentacam has coefficients of repeatability of 14.18 and 13.5 μm for measures of central corneal thickness [42,43]. This is approximately 2.5-3x greater than the coefficients of repeatability for OCT imaging in the current study; 4.5 μm and 5.2 μm for intraobserver and intrasession repeatability respectively, for total corneal thickness averaged across the central 6 mm from 3 line scans. Comparisons of corneal thickness with Pentacam and OCT have shown little difference, ranging from 0.9 to 7.2 μm [22,44]. However, measurements of the Pentacam can be influenced by pupil decentration [22,42].

Although gaze tracking and anatomical landmark registration is not currently possible using commercially available AS-OCT, the results from the current study suggest that measurements taken by a single observer carefully aligning the OCT instrument with the pupil centre and extracting measurements from the central cornea (the subjectively determined normal to the tangent of the corneal apex) are repeatable, but can be improved by ~40-50 % if three line scans are averaged. One previous study averaged 12 measurements from a radial line scan protocol at each time point [29], however most other studies have utilised a single point measure [28,31], while others have not reported the details of their scanning protocol or number of measurements captured [30,45]. However, the method of measurement averaging is particularly important in a clinical or research setting when quantifying corneal oedema. For example, typical levels of oedema observed in healthy eyes during modern scleral lens daily wear typically peaks around 1–2 % or 5−10 μm [28–33], and is less for highly oxygen permeable soft contact lenses [46].

The results indicate that the default value of 60 B-scans for a single line scan protocol could be reduced to 20–30, as there is no significant benefit in terms of repeatability beyond these values. This is consistent with the default values of the Zeiss Cirrus HD-OCT of 20 B-scans [47]. When applying this value (i.e. 20 B scans) to a volumetric scanning protocol, which is greater than the default of 9–16, analysis from the current study suggests that a minimum of three lines will give a highly repeatable measure. However, this may not be true for an average of the default values of 9–16 B-scans, as there was greater variability observed with 10 B-scans in the singles line scan analysis. This is an important consideration when trying to detect small changes in corneal thickness.

A limitation of the current study is that only healthy eyes with normal corneal curvature were examined. Consequently, the repeatability data presented cannot be directly applied across a range of ocular conditions, particularly for corneal pathologies in which the corneal epithelium is affected [48–50], or the stroma is significantly structurally altered [51,52] (e.g. corneal scarring in keratoconus). Additionally, AS-OCT images were obtained without a contact lens on the eye, which may affect image quality and the repeatability of thickness metrics. While it is known that imaging through a contact lens alters the assumed refractive index of the tissue being imaged, and therefore directly affects the derived thickness measurement [1,7,53], currently no studies have examined the impact of scleral contact lens wear upon the repeatability of AS-OCT derived corneal thickness. Repeatability may vary during lens wear due to subtle changes in the optics of the anterior surface of the scleral lens [54] or the post lens tear layer due to lens settling or decentration [8,55] between measurements that are separated by a period of time. Repeatability will also vary with the particular AS-OCT device used (e.g. instruments of lower axial resolution [56]), and possibly radial compared to the horizontally oriented volumetric protocols used in this study.

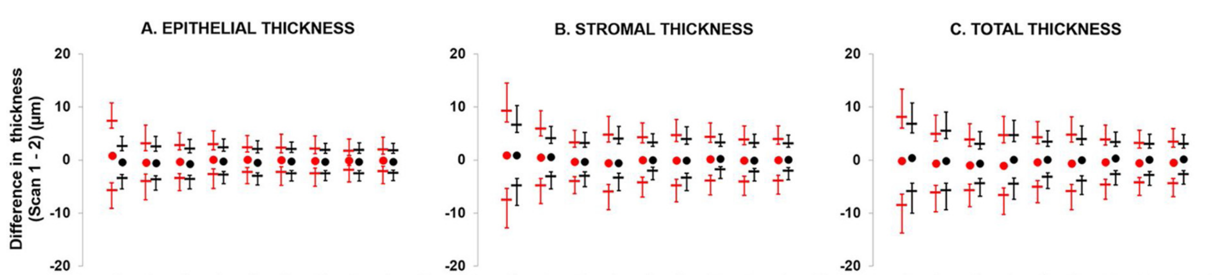

Fig. 6. Condensed Bland-Altman plots highlighting the intrasession repeatability for corneal thickness measurements derived from 1 to 9 randomly selected line scans from a 9 line scan volumetric protocol (consisting of 20 averaged B-scans). Circles represent the mean difference, horizontal bars represent the limits of agreement, and the error bars represent the exact 95 % confidence intervals of the limits of agreement. Red denotes data derived from the single point thickness metric, and black denotes data derived from the average thickness metric (across the central 6 mm of the cornea centred on the normal to tangent of the corneal apex).

5. Conclusion

For measures of epithelial, stromal, and total corneal thickness, intraobserver or intrasession repeatability did not vary significantly with the number of B-scans averaged per single line scan, or in relation to the metric used to derive the thickness value (single point or average). However, since the intraobserver repeatability turning point (best intraobserver agreement) appears to occur for approximately 20–30 averaged B-scans, at least 20 B-scans should be averaged per line scan to optimise the repeatability of measurements of corneal thickness when using a single line scan. For measures of central corneal thickness using a volumetric imaging protocol, at least three line scans (or three repeated measures for single line scan protocols) should be averaged across a wide corneal area instead of a single point estimate in order to minimise intraobserver and intrasession variability.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

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