Epithelial Thickness Mapping in Keratoconic Corneas: Repeatability and Agreement Between CSO MS-39, Heidelberg Anterion, and Optovue Avanti OCT Devices

Yue Feng, MD, MSc; Dan Z. Reinstein, MD, DABO, FRCOphth; Tore Nitter, MD, PhD; Timothy J. Archer, MA(Oxon) DipCompSci (Cantab), PhD; Colm McAlinden, MD, PhD, Geir Bertelsen, MD, PhD; Aleksandar Stojanovic, MD, PhD

ABSTRACT

PURPOSE: To assess repeatability and agreement of corneal epithelial thickness mapping in eyes with keratoconus using three optical coherence tomography (OCT) devices featuring different technologies: spectral-domain (SD) OCT combined with Placido disk corneal topography (MS-39), swept-source OCT (Anterion), and SD-OCT (Avanti).

METHODS: Three consecutive measurements were acquired with the three devices in 60 eyes with keratoconus. The mean epithelial thickness was calculated in the central 2-mm zone and in 2- to 5-mm and 5- to 7-mm diameter rings. The repeatability was calculated using pooled within-subject standard deviation (S_w). The agreement was assessed by paired *t* tests and Bland-Altman plots.

RESULTS: The repeatability (S_w) of the epithelial thickness for the central 2-mm zone was 0.91, 0.71, and 0.93 µm for the MS-39, Anterion, and Avanti, respectively. All thicknesses with the MS-39 were greater than those of the Anterion and Avanti, with mean differences of 4.11 ± 1.34 µm ($P < .001$) and 0.52 ± 1.30 µm ($P = .003$), respectively. The 95% limits of agreement were 1.484 to 6.736 µm for the MS-39 and Anterion, -3.068 to 2.028 µm for the Avanti and MS-39, and 1.258 to 5.922 µm for for the Avanti and Anterion.

CONCLUSIONS: Epithelial thickness mapping results were most repeatable with the Anterion, followed by the MS-39 and Avanti. The MS-39 gave the thickest values, followed by the Avanti and Anterion. The differences were significant, making the devices not interchangeable for epithelial thickness mapping in eyes with keratoconus.

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As its first cellular layer and refractive medium, the corneal epithelium has an important role in smoothing the ocular surface by actively growing thicker over stromal divots and becoming thinner over bumps, a phenomenon described as epithelial remodeling.[1] In this way, the epithelium regularizes the corneal refractive properties and, in most cases, leads to less corneal astigmatism, less prolate asphericity, and fewer higher order aberrations[2,3] compared to the same parameters measured on the stromal surface. The epithelium also decreases the eye's refractive power by simply increasing the corneal radius of curvature by the amount of its thickness.[4] Due to eyelid blinking mechanics, a slightly non-uniform epithelial thickness profile is induced in normal virgin eyes.[5]

Keratoconus is a progressive ectatic disease with localized biomechanical failure, which leads to a local protrusion and can lead to significant visual impairment due to irregular corneal optics.[6,7] The local protrusion causes intense epithelial remodeling, resulting in a donut-shaped epithelial pattern consisting of a compensatory thinning over the protruding part (cone) and a surrounding annulus of thicker epithelium.[1] One study also found that an overall epithelial thickness increase may be an early sign of keratoconus.[8] Epithelial thickness mapping (ETM) has become an important tool for the early diagnosis of keratoconus,[1,8-12] revealing the specific epithelial thickness pattern that hides the underlying stromal changes. Hence, the ETM may be thought of as an imprint of the protruding stromal surface from underneath the moldable epithelial tissue. ETM was pioneered by Reinstein et al in 1994,[1,5] and they have described "epithelial remodeling" across the whole cornea in eyes with keratoconus[1,9] using very high-frequency (VHF) digital ultrasound scanning (Artemis Insight 100; ArcScan, Inc). Since then, ETM has also become indispensable in therapeutic refractive surgery, assisting in analyzing irregular corneal optics[13] and identifying alternative treatment options. The epithelial thinning level over the cone has been used as a follow-up parameter to determine the progression of keratoconus before considering corneal cross-linking (CXL) and in evaluating its effect.

Optical coherence tomography (OCT)–based ETM appeared much later (2011),[10] without surpassing VHF digital ultrasound scanning in terms of repeatability, but it has become the most prevalent technology in current clinical practice due to its speed and ease of use. The first commercially available OCT-based instrument was the Optovue RT-100 (Optovue, Inc), featuring spectral-domain (SD) OCT technology, providing 6-mm diameter ETM, later replaced by a 9-mm ETM device (Avanti), which became the most commonly used ETM device.[14]

The more recently introduced swept-source (SS) OCT technology has a light source of longer wavelength than SD-OCT, allowing greater image depth and high-contrast imaging of the entire anterior segment.[15] The Anterion (Heidelberg Engineering) and Casia 2 (Tomey Corporation) are high-resolution anterior segment OCT devices featuring SS-OCT technology.[16] We recently reported the repeatability of ETM with the Anterion and its agreement with the Avanti for three different diagnostic groups (virgin, post-laser vision correction, and keratoconic eyes).[17]

The MS-39 (CSO) employs hybrid technology, combining SD-OCT with Placido disk imaging. It was released in 2018 and has been used for anterior segment imaging including ETM. So far, three studies[18-20] have investigated its repeatability for ETM, but no comparison with other devices was done. In the current study, we compared the repeatability and agreement between the MS-39 SD-OCT, Anterion SS-OCT, and Avanti SD-OCT. For data obtained by the MS-39, we used the manufacturer's recommended scanning mode (12 × 5 @ 10 mm), which has not been validated previously.

PATIENTS AND METHODS

Seventy-eight consecutive patients with keratoconus were examined at the eye department of the University Hospital of North Norway. All examinations were performed between March and December 2021. Inclusion criteria were: age 16 years or older; diagnosis of keratoconus; and spherical equivalent of myopia of 8.00 diopters (D) or less. Exclusion criteria were: history of previous ocular surgery (except for CXL); patients with conjunctival, limbal, or corneal disease (except for keratoconus); poor fixation or inability to complete the examination; and use of rigid gas permeable contact lens within 2 weeks of the examination day. One of each patient's eyes was randomly selected in patients where both eyes met the inclusion criteria; in cases where only one eye met the inclusion criteria, this eye was selected. Only one eye of each patient was used to avoid statistical bias, and, finally, 60 eyes of 60 patients were included.

From the Institute of Community Medicine, Faculty of Health Sciences, University in Tromsø, Norway (YF, GB); Reinstein Vision, London, United Kingdom (DZR, TJA); London Vision Clinic, EuroEyes Group, London, United Kingdom (DZR, TJA); Columbia University Medical Center, New York, New York (DZR); Sorbonne Université, Paris, France (DZR); and Biomedical Science Research Institute, Ulster University, Coleraine, United Kingdom (DZR); private practice (TN); the Department of Ophthalmology, Royal Gwent Hospital, Aneurin Bevan University Health Board, Newport, United Kingdom (CM); the Department of Ophthalmology, University Hospital North Norway, Tromsø, Norway (GB, AS); and Institute of Clinical Medicine, Faculty of Health Sciences, University in Tromsø, Norway (AS).

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Correspondence: Aleksandar Stojanovic, MD, PhD, Eye Department, University Hospital North Norway, Sykehusveien 38, 9019 Tromsø, Norway. Email: aleks@online.no

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Age, sex, and personal and family history of eye diseases were registered. Refraction, visual acuity, standard ophthalmological examination with slit-lamp examination, and funduscopy were performed before ETM measurements. This prospective study was approved by the Norwegian Regional Committee for Medical & Health Research Ethics (REK Nord 72084) and complied with the tenets of the Declaration of Helsinki. All patients provided informed consent for the anonymous use of their data in scientific analyses and publications.

Epithelial Thickness Measurements

The measurements with the three devices were obtained in a random order according to a randomized list generated by Microsoft Excel 2016 (Microsoft Corporation). Three consecutive measurements were taken with each device. All measurements with all three devices were acquired within 10 minutes between 10 AM and 2 PM.

Patients were asked to fixate on the fixation target while the examiner centered the OCT scan on the corneal vertex. Patients were instructed to blink immediately before each measurement and to keep their eyes wide open during the measurement. No eye drops were applied during testing.

The MS-39 (Phoenix software, v.4.1.1.5) uses a super luminescent diode at 845 nm as the illumination source for SD-OCT and a super luminescent diode at 635 nm for Placido disk. The "Corneal topography" mode "12 × 5 @10 mm" was used in this study because it provides a higher resolution than the "25 × 1 @16 mm" mode, which has been used in previous studies.[18-20] Data for the anterior surface from the Placido image and the elevation data of the anterior surface from OCT data are merged, using a proprietary method. After the acquisition, the MS-39 calculates the epithelial thickness within the 8-mm diameter and provides ETM, divided into a total of 25 sections.

The Anterion SS-OCT (software version 2.5.2) generates images using a laser light source of 1,300 nm wavelength and an active eye-tracker. It performs 65 radial scans with 256 A-scan lines centered on the corneal vertex within a 7-mm diameter.

The Avanti SD-OCT (software version 6.11.0.12) generates images using a SLed light source of 840 nm. ETM and the corneal pachymetry maps are divided into a total of 25 sections over a 9-mm diameter. The mean epithelial thickness of each section is presented.

For all three devices, the user may measure the epithelium thickness at any point on the map by mouse pointing. To compare the three devices, the mean values of the same 17 sections (including the central 2-mm zone) within a 7-mm diameter, as well as for the whole 2- to 5-mm and 5- to 7-mm rings, were calculated (**Figure A** available in the online version of this article). The technical specifications of the devices are summarized in **Table A** (available in the online version of this article)

Statistical Analysis

We used vertically mirrored symmetry superimposition so that nasal/temporal characteristics could be combined.[21] To assess the repeatability, we calculated the pooled within-subject standard deviation (S_w) (lower values of S_w indicate better repeatability).[22] The repeatability limit (r), defined as $1.96 \sqrt{2} \times S_w$ (= $2.77 \times S_w$), gives the value below which the absolute difference between two measurements of S_w would lie with 0.95 probability.[22]

To assess the agreement, we calculated the following parameters: difference in thickness readings; the 95% limits of agreement (LoA), defined as the mean difference in thickness ± 1.96 × standard deviation; and paired two-tailed *t* tests. Bland-Altman plots were generated to visualize the agreement between any two devices.

Data were entered into Microsoft Excel 2016 and then imported into statistical software (SPSS v25; IBM Corporation). A *P* value of less than .05 was considered to be statistically significant.

Sample Size Estimation

To achieve a 15% confidence in the estimate,[17-19] the required sample size is 43.[23] The current study comprised 60 eyes (n = 60), which gives 12% confidence.

RESULTS

This study evaluated 60 eyes (34 right eyes and 26 left eyes) of 60 patients (mean ± standard deviation age: 30.04 ± 9.50 years; range: 16 to 57 years; 50 men and 10 women) for both repeatability and agreement analyses.

Repeatability

The repeatability of the measurements was calculated for the 17 sections, as well as for the 2- to 5-mm, and 5- to 7-mm diameter rings (**Table B**, available in the online version of this article). Within the central 2-mm zone, S_w was 0.91 µm for the MS-39, 0.71 µm for the Anterion, and 0.93 µm for the Avanti. For the 2- to 5-mm diameter ring, the S_w range was 0.53 to 1.62 µm for the MS-39, 0.81 to 0.99 µm for the Anterion, and 1.04 to 1.68 µm for the Avanti. For the 5- to 7-mm diameter ring, the S_w range was 0.73 to 1.79 µm for the MS-39, 0.86 to 1.59 µm for the Anterion, and 0.75 to 1.79) µm for the Avanti.

Agreement Between MS-39, Anterion, and Avanti

The mean difference in epithelial thickness, the 95% LoA values, and paired, two-tailed *t* test *P* values were calculated for the 17 sections, the 2- to 5-mm and 5- to 7-mm diameter rings, and the total measured area (**Table C**, available in the online version of this article).

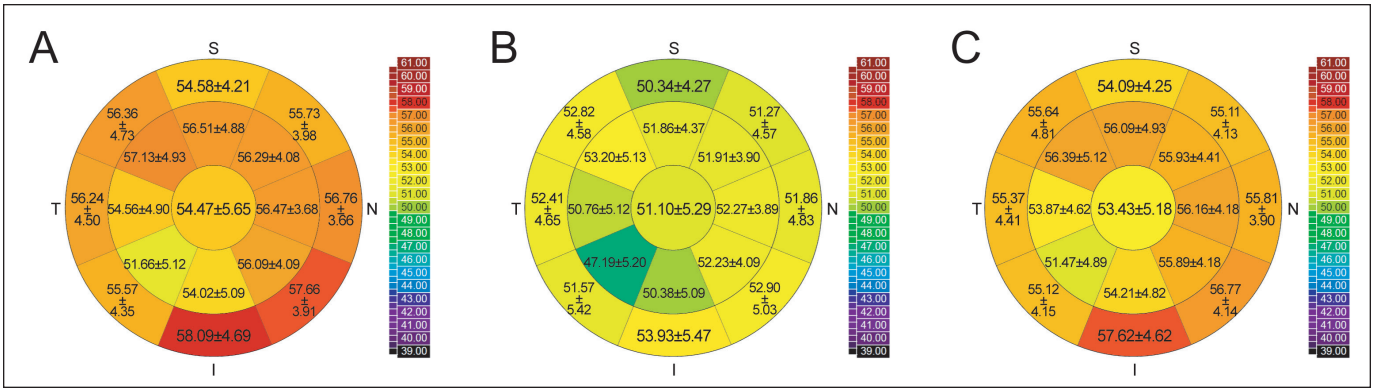

Figure 1. Average epithelial thickness mapping for the (A) MS-39 (CSO), (B) Anterion (Heidelberg Engineering), and (C) Avanti (Optovue, Inc) within the central 7-mm diameter. I = inferior; N = nasal; S = superior; T = temporal; unit: µm

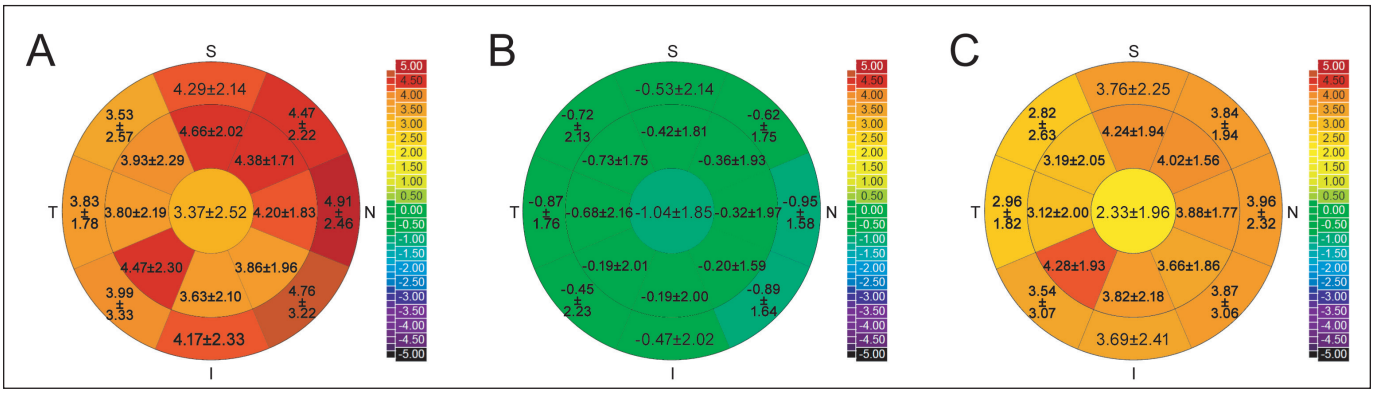

Figure 2. Differential mappings between each two devices within the central 7-mm diameter. (A) MS-39 – Anterion; (B) Avanti – MS-39; (C) Avanti – Anterion. The Avanti is manufactured by Optovue, Inc, the Anterion is manufactured by Heidelberg Engineering, and the MS-39 is manufactured by CSO. I = inferior; N = nasal; S = superior; T = temporal; unit: µm

The MS-39 measured significantly thicker epithelium than the Anterion in all sections, with a mean difference ranging from 3.37 to 4.91 µm ($P < .001$ for all). It also measured slightly thicker epithelium than the Avanti in all 17 sections except in the inferior section within the 2- to 5-mm ring, with a mean difference of 0.52 ± 1.30 µm ($P = .003$). Overall, the Anterion measured the thinnest, MS-39 the thickest, and the Avanti was in between.

The 95% LoAs were 1.484 and 6.736 µm for the MS-39 and Anterion, -3.068 and 2.028 µm for the Avanti and MS-39, and 1.258 and 5.922 µm for the Avanti and Anterion. The mean ETMs for the three devices are shown in **Figure 1**, and maps of the difference between the devices are shown in **Figure 2**. Bland-Altman plots for the agreement for the three pairs of devices are shown in **Figures B-D** (available in the online version of this article). For all three pairs of devices, the difference between the upper and lower 95% LoA was greater in the central 2-mm zone than in the 2- to 5-mm and 5- to 7-mm rings.

DISCUSSION

We compared repeatability of the MS 39 hybrid (SD-OCT + Placido disk) device with the SS-OCT Anterion and the SD-OCT Avanti in a group of 60 eyes with keratoconus. We also assessed the agreement between the three devices. The repeatability (S_w) values in the central 2-mm zone and 2- to 5-mm ring (**Table B**) were 0.91 and 1.06 µm for the MS-39, which was slightly worse than 0.71 and 0.91 µm for the Anterion but slightly better compared to 0.93 and 1.28 µm for the Avanti.

Repeatable corneal epithelial thickness measurements are important for the management of keratoconus,[1,9,12] as well as for safe corneal refractive surgery. The first ETM measurements by Reinstein et al with the Artemis VHF digital ultrasound showed S_w: 0.43 to 1.36 µm in 90% of the locations within the central 6-mm diameter after five consecutive measurements of 10 eyes 1 year after LASIK.[24] The repeatability should also be considered in the context of the measurement resolution of the device; VHF digital ultrasound can measure the epithelial thickness with less than 1 µm resolution, whereas OCT devices have a resolution of closer to 3.6 µm for the MS-39, 8 µm for the Anterion, and 5 µm for the Avanti.

Introduced 17 years later than the Artemis, the SD-OCT Optovue RT-100 has shown good repeatability and reproducibility of ETM in normal and not normal eyes

(dry eye syndrome, contact lens wear, post-laser refractive surgery, and keratoconic).[10,25,26] Ma et al[27] reported that the Avanti produced excellent repeatability and reproducibility for ETM measurements up to a 9-mm zone in normal eyes and eyes with different corneal conditions, showing S_w: 1.4 to 2.3 µm in 14 eyes with keratoconus. Lu et al[28] measured ETM with the Avanti within a 7-mm diameter, showing S_w: 1.31 to 2.43 µm in eyes with mild keratoconus and S_w: 1.90 to 3.89 µm in eyes with advanced keratoconus, whereas the current study found better repeatability of ETM measured with the Avanti, S_w: 0.75 to 1.68 µm, and Anterion, S_w: 0.71 to 1.59 µm within a 7-mm diameter in our group of 60 eyes with keratoconus. These results were consistent with the repeatability that we recently reported for the Avanti, S_w: 0.75 to 1.96 µm, and Anterion, S_w: 0.60 to 1.36 µm in eyes with keratoconus.[17]

Using the MS-39 in eyes with keratoconus, Vega-Estrada et al[18]found an S_w of 1.24 µm in the central 3-mm zone and ranges of 1.16 to 1.69 µm in the 3- to 6-mm ring and 1.42 to 2.70 µm in the 6- to 8-mm ring. Schiano-Lomoriello et al[19] reported an S_w of 1.57 µm in the central 3-mm zone in eyes with keratoconus with the MS-39. The current study showed better repeatability with the MS-39 with an S_w of 0.91 µm in the central 2-mm zone, and S_w ranges of 0.53 to 1.62 µm in the 2- to 5-mm and 0.73 to 1.79 µm in the 5- to 7-mm ring, which were better than the results from the two mentioned studies. **Table D** (available in the online version of this article) summarizes the literature findings on the repeatability of ETM studies.

We assume that 65 radial scans used by the Anterion versus the 12 radial scans used by the MS-39 and 8 radial scans used by the Avanti (both repeated five times for each meridian), as well as the Anterion's eye-tracking ability, are the likely factors explaining the better repeatability with the Anterion. We hypothesize that the repeated, wider-spread radial scans used by both the MS-39 and Avanti do not compensate for the denser coverage of the Anterion (**Table A**).

Concerning the agreement between any two devices, the epithelium measured by the MS-39 was significantly thicker than with the Anterion and slightly thicker than with the Avanti, whereas the measurements with the Anterion were significantly thinner than with the Avanti (**Table D**, **Figures 1-2**). The agreement between the Anterion and Avanti was consistent with the results we recently reported, with the Anterion measuring significantly thinner epithelium than the Avanti, with a mean difference of 3.68 ± 2.51 µm ($P < .001$).[17]

The three OCT devices use their own proprietary methods for their respective segmentation algorithms, and they treat differently the inclusion of the tear film in their ETM measurements. Corneal epithelial thickness measured by VHF ultrasound excludes the pre-corneal tear film thickness.[11] According to their respective manufacturers, the MS-39 measures the distance between the tear film layer and Bowman's layer, whereas the Anterion is "looking for the highest intensity of the anterior surface, which can provide the ability to reliably find the underlying structure in a repeatable way." The Avanti's manufacturer claims that its ETM measurements include the tear film.[29]

The CSO designers deemed it necessary to equip their SD-OCT device with a Placido disk and combine high-quality anterior curvature data with OCT-derived elevation data, to achieve the best possible resolution of the anterior surface morphology. However, conversion of curvature to elevation may be subject to errors inherent to the arc-step method.

All three devices use Fourier-domain detection, but they feature different imaging wavelengths and bandwidths. Although the Anterion uses a tunable swept laser light source (wavelength: 1,300 nm),[30] the Avanti and MS-39 use a broadband near-infrared SLed as their light source (wavelength: 840 nm and 845 nm, respectively). This results in different transversal and axial resolutions (3.6 × 35 µm for MS-39, 10 × 45 µm for Anterion, and 5 × 15 µm for Avanti), which presumably leads to different performance. Both SS-OCT and SD-OCT technologies record an interference spectrum that carries the information of the sample, but SS-OCT features a light source that sweeps the wavelength in time, whereas SD-OCT uses a spectrometer for wavelength separation. SS-OCT imaging features a denser scan pattern, due to its higher acquisition speed, as well as a larger scan depth, due to the use of a longer wavelength and reduced sensitivity roll-off. Hence, SS-OCT may quickly acquire the images of the whole anterior segment,[30] whereas SD-OCT provides higher contrast and resolution within a shorter depth range. In contrast to the other two devices, the Anterion features real-time eye-tracking during the acquisition of multiple B-scans, which allows precise alignment and enhanced detailed imaging.[16] For a given cornea, it appears that multiple factors may influence the repeatability of a device, such as axial resolution, image contrast, penetration rate, tracking, scanning speed, and scanning density. So, just by looking at the technical specifications, one cannot decide which device is superior, which emphasizes the importance of clinical evaluations.

As shown in **Table A**, the Anterion takes 0.33 second, followed by the Avanti with 0.58 second and the MS-39 with 1 second for acquisition. Concerning patient comfort, the red Placido rings lighting during the acquisition with the MS-39 may often cause the patient to blink. Overall, the Anterion has the advan-

tage of a shorter acquisition time and no issue with eye blinking, making it a more patient-friendly option.

Data within a 7-mm diameter were analyzed because all three devices covered that area (the maximum for Anterion, 1 mm off the maximum for MS-39, and 2 mm off the maximum for Avanti). Different coverage of the devices should be considered when comparing their clinical applicability, but there is no indication that this could affect our repeatability results within 7 mm.

A clinician will definitely recognize the same patterns on the ETMs of the three devices, which may be sufficient in most cases when used for diagnostics, but if used for surgical planning or scientific work, then the measurements of the three devices cannot be interchanged. Due to the complex relationship between the measurements, using a simple conversion factor is not advisable.

We found that the repeatability of the ETM measurements in eyes with keratoconus was high for all three devices, but the agreement between them was low. The repeatability with the MS-39 was slightly worse than with the Anterion, but better than with the Avanti. The mean epithelium thicknesses measured with the three devices were significantly different, making them not interchangeable for ETM in eyes with keratoconus.

AUTHOR CONTRIBUTIONS

Study concept and design (GB, AS); data collection (YF); analysis and interpretation of data (YF, DZR, TN, TJA, CM, AS); writing the manuscript (YF); critical revision of the manuscript (DZR, TN, TJA, CM, GB, AS); statistical expertise (CM); administrative, technical, or material support (TJA); supervision (DZR, TN, GB, AS)

REFERENCES

1. Reinstein DZ, Archer TJ, Gobbe M. Corneal epithelial thickness profile in the diagnosis of keratoconus. *J Refractive Surg*. 2009;25:604-610. https://doi.org/10.3928/1081597X-20090610-06
2. Zhou W, Reinstein DZ, Archer TJ, Chen X, Utheim TP, Feng Y, Stojanovic A. Intraoperative swept-source OCT-based corneal topography for measurement and analysis of stromal surface after epithelial removal. *J Refractive Surg*. 2021;37:484-492.
3. Reinstein DZ, Silverman RH, Sutton HF, Coleman DJ. Very high-frequency ultrasound corneal analysis identifies anatomic correlates of optical complications of lamellar refractive surgery: anatomic diagnosis in lamellar surgery. *Ophthalmology*. 1999;106(3):474-482. https://doi.org/10.1016/S0161-6420(99)90105-7 PMID:10080202
4. Simon G, Ren Q, Kervick GN, Parel JM. Optics of the corneal epithelium. *Refract Corneal Surg*. 1993;9(1):42-50. https://doi.org/10.3928/1081-597X-19930101-10 PMID:8481372
5. Reinstein DZ, Silverman RH, Coleman DJ. High-frequency ultrasound measurement of the thickness of the corneal epithelium. *Refract Corneal Surg*. 1993;9(5):385-387. https://doi.org/10.3928/1081-597X-19930901-12 PMID:8241045
6. Rabinowitz YS. Keratoconus. *Surv Ophthalmol*. 1998;42(4):297-319. https://doi.org/10.1016/S0039-6257(97)00119-7 PMID:9493273
7. Padmanabhan P, Lopes BT, Eliasy A, Abass A, Elsheikh A. In vivo biomechanical changes associated with Keratoconus progression. *Curr Eye Res*. 2022;47(7):982-986. https://doi.org/10.1080/02713683.2022.2058020 PMID:35385372
8. Kanellopoulos AJ, Aslanides IM, Asimellis G. Correlation between epithelial thickness in normal corneas, untreated ectatic corneas, and ectatic corneas previously treated with CXL: is overall epithelial thickness a very early ectasia prognostic factor? *Clin Ophthalmol*. 2012;6:789-800. https://doi.org/10.2147/OPTH.S31524 PMID:22701079
9. Silverman RH, Urs R, Roychoudhury A, Archer TJ, Gobbe M, Reinstein DZ. Epithelial remodeling as basis for machine-based identification of keratoconus. *Invest Ophthalmol Vis Sci*. 2014;55(3):1580-1587. https://doi.org/10.1167/iovs.13-12578 PMID:24557351
10. Li Y, Tan O, Brass R, Weiss JL, Huang D. Corneal epithelial thickness mapping by Fourier-domain optical coherence tomography in normal and keratoconic eyes. *Ophthalmology*. 2012;119(12):2425-2433. https://doi.org/10.1016/j.ophtha.2012.06.023 PMID:22917888
11. Reinstein DZ, Gobbe M, Archer TJ, Silverman RH, Coleman DJ. Epithelial, stromal, and total corneal thickness in keratoconus: three-dimensional display with Artemis very-high frequency digital ultrasound. *J Refractive Surg*. 2010;26:259-271. https://doi.org/10.3928/1081597X-20100218-01
12. Haque S, Jones L, Simpson T. Thickness mapping of the cornea and epithelium using optical coherence tomography. *Optom Vis Sci*. 2008;85:E963-E976. https://doi.org/10.1097/OPX.0b013e318188892c
13. Reinstein DZ, Archer TJ, Dickeson ZI, Gobbe M. Transepithelial phototherapeutic keratectomy protocol for treating irregular astigmatism based on population epithelial thickness measurements by Artemis very high-frequency digital ultrasound. *J Refractive Surg*. 2014;30:380-387. https://doi.org/10.3928/1081597X-20140508-01
14. El Wardani M, Hashemi K, Aliferis K, Kymionis G. Topographic changes simulating keratoconus in patients with irregular inferior epithelial thickening documented by anterior segment optical coherence tomography. *Clin Ophthalmol*. 2019;13:2103-2110. https://doi.org/10.2147/OPTH.S208101 PMID:31802839
15. Shoji T, Kato N, Ishikawa S, et al. In vivo crystalline lens measurements with novel swept-source optical coherent tomography: an investigation on variability of measurement. *BMJ Open Ophthalmol*. 2017;1(1):e000058. https://doi.org/10.1136/bmjophth-2016-000058 PMID:29354706
16. Asam JS, Polzer M, Tafreshi A, Hirnschall N, Findl O. Anterior segment OCT. In: Bille JF, ed. *High Resolution Imaging in Microscopy and Ophthalmology: New Frontiers in Biomedical Optics*. Springer; 2019:285-299. https://doi.org/10.1007/978-3-030-16638-0_13
17. Feng Y, Reinstein DZ, Nitter T, Archer TJ, McAlinden C, Chen X, Bertelsen G, Utheim TP, Stojanovic A. Heidelberg Anterion swept-source OCT corneal epithelial thickness mapping: repeatability and agreement with Optovue Avanti. *J Refractive Surg*. 2022;38:356-363.
18. Vega-Estrada A, Mimouni M, Espla E, Alió Del Barrio J, Alió JL. Corneal epithelial thickness intrasubject repeatability and its relation with visual limitation in keratoconus. *Am J Ophthalmol*. 2019;200:255-262. https://doi.org/10.1016/j.ajo.2019.01.015 PMID:30689987
19. Schiano-Lomoriello D, Bono V, Abicca I, Savini G. Repeatability of anterior segment measurements by optical coherence tomography combined with Placido disk corneal topography in eyes with keratoconus. *Sci Rep*. 2020;10(1):1124. https://doi.org/10.1038/s41598-020-57926-7 PMID:31980662
20. Savini G, Schiano-Lomoriello D, Hoffer KJ. Repeatability of automatic measurements by a new anterior segment optical coherence tomographer combined with Placido topography and agreement with 2 Scheimpflug cameras. *J Cataract Refract Surg*.

2018;44(4):471-478. https://doi.org/10.1016/j.jcrs.2018.02.015 PMID:29705008

21. Zhou W, Stojanovic A. Comparison of corneal epithelial and stromal thickness distributions between eyes with keratoconus and healthy eyes with corneal astigmatism ≥ 2.0 D. *PLoS One*. 2014;9(1):e85994. https://doi.org/10.1371/journal.pone.0085994 PMID:24489687

22. Bland JM, Altman DG. Measurement error. *BMJ*. 1996;313(7059):744. https://doi.org/10.1136/bmj.313.7059.744 PMID:8819450

23. McAlinden C, Khadka J, Pesudovs K. Precision (repeatability and reproducibility) studies and sample-size calculation. *J Cataract Refract Surg*. 2015;41(12):2598-2604. https://doi.org/10.1016/j.jcrs.2015.06.029 PMID:26796439

24. Reinstein DZ, Archer TJ, Gobbe M, Silverman RH, Coleman DJ. Repeatability of layered corneal pachymetry with the Artemis very high-frequency digital ultrasound arc-scanner. *J Refractive Surg*. 2010;26:646-659. https://doi.org/10.3928/1081597X-20091105-01

25. Ma XJ, Wang L, Koch DD. Repeatability of corneal epithelial thickness measurements using Fourier-domain optical coherence tomography in normal and post-LASIK eyes. *Cornea*. 2013;32(12):1544-1548. https://doi.org/10.1097/ICO.0b013e3182a7f39d PMID:24145634

26. Sella R, Zangwill LM, Weinreb RN, Afshari NA. Repeatability and reproducibility of corneal epithelial thickness mapping with spectral-domain optical coherence tomography in normal and diseased cornea eyes. *Am J Ophthalmol*. 2019;197:88-97. https://doi.org/10.1016/j.ajo.2018.09.008 PMID:30240724

27. Ma JX, Wang L, Weikert MP, Montes de Oca I, Koch DD. Evaluation of the repeatability and reproducibility of corneal epithelial thickness mapping for a 9-mm zone using optical coherence tomography. *Cornea*. 2019;38(1):67-73. https://doi.org/10.1097/ICO.0000000000001806 PMID:30379719

28. Lu NJ, Chen D, Cui LL, Wang L, Chen SH, Wang QM. Repeatability of cornea and sublayer thickness measurements using optical coherence tomography in corneas of anomalous refractive status. *J Refract Surg*. 2019;35:600-605. https://doi.org/10.3928/1081597X-20190806-03

29. Azartash K, Kwan J, Paugh JR, Nguyen AL, Jester JV, Gratton E. Pre-corneal tear film thickness in humans measured with a novel technique. *Mol Vis*. 2011;17:756-767. PMID:21527997

30. Aumann S, Donner S, Fischer J, Müller F. Optical coherence tomography (OCT): principle and technical realization. In: Bille JF ed, *High Resolution Imaging in Microscopy and Ophthalmology: New Frontiers in Biomedical Optics*. Springer; 2019:59-85.

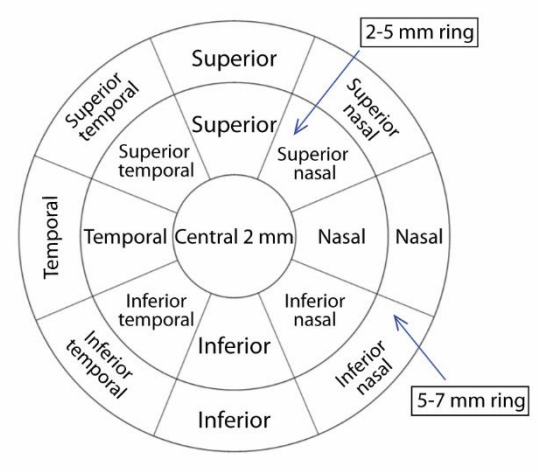

Figure A. Epithelial thickness mapping 17 sections and two rings used in the analysis of the measurements.

Table A
Technical Specifications of the Three Devices for ETM

Parameter	MS-39	Anterion	Avanti
Light source wavelength (nm)	OCT: 845 Placido: 635	1300	840
A-scan speed	102,400	50000	70000
Axial resolution (μm)	3.6	<10	5
Transverse resolution (μm)	35	<45	15
A-scan depth (mm)	7.5	14	3
Maximum Scan width (mm)	16	16.5	12
B scan	10 × 5[a]	65 × 1	8 × 5
Number of A-scans per B-scan	1024[b]	256	1024
Acquisition time (s)	1	0.33	0.58

ETM = epithelial thickness mapping; OCT = optical coherence tomography
[a]*Customized in this study as recommended by the manufacturer.*
[b]*1600 A-scan on 16 mm and 800 A-scan on 8 mm.*

Table B
Repeatability of ETM (μm) Measurements of the Three Devices

Repeatability, S_w (Repeatability Limit, r)[a]	**MS-39**	**Anterion**	**Avanti**
Zone 0 to 2 mm			
Central	0.91 (2.53)	0.71 (1.97)	0.93 (2.59)
Ring 2- to 5-mm			
Nasal	0.53 (1.46)	0.81 (2.24)	1.11 (3.08)
Superior nasal	0.97 (2.68)	0.90 (2.48)	1.22 (3.39)
Superior	0.90 (2.49)	0.99 (2.73)	1.15 (3.18)
Superior temporal	1.13 (3.12)	0.98 (2.72)	1.04 (2.89)
Temporal	1.27 (3.52)	0.94 (2.59)	1.68 (4.65)
Inferior temporal	1.62 (4.48)	0.92 (2.56)	1.45 (4.00)
Inferior	1.39 (3.84)	0.91 (2.53)	1.33 (3.69)
Inferior nasal	0.72 (1.98)	0.82 (2.29)	1.28 (3.55)
Ring 2-5 mm total	1.06 (2.95)	0.91 (2.52)	1.28 (3.55)
Ring 5- to 7-mm			
Nasal	0.80 (2.22)	0.86 (2.39)	1.11 (3.07)
Superior nasal	1.36 (3.76)	1.13 (3.12)	1.07 (2.97)
Superior	1.41 (3.92)	1.28 (3.54)	1.19 (3.28)
Superior temporal	1.22 (3.38)	1.34 (3.71)	1.34 (3.70)
Temporal	0.94 (2.61)	1.21 (3.35)	1.44 (3.99)
Inferior temporal	1.79 (4.95)	1.17 (3.25)	1.79 (4.97)
Inferior	1.37 (3.80)	1.59 (4.40)	0.99 (2.75)
Inferior nasal	0.73 (2.02)	1.21 (3.36)	0.75 (2.07)
Ring 5- to 7-mm total	1.20 (3.33)	1.22 (3.39)	1.21 (3.35)

ETM = epithelial thickness mapping; S_w = pooled within-subject standard deviation
[a]Repeatability limit= 2.77 × S_w
The Anterion is manufactured by Heidelberg Engineering, the Avanti is manufactured by Optovue, Inc, and the MS-39 is manufactured by CSO.

TABLE C

Agreement of ETM Measurements Between Two Devices

Parameter	Epithelial Thickness, Mean ± SD (μm)			MS-39 – Anterion Difference			Avanti - MS-39 Difference			Avanti - Anterion Difference		
	MS-39	Anterion	Avanti	Mean ± SD (μm)	*P*	95% LoA Range (μm)	Mean ± SD (μm)	*P*	95% LoA Range (μm)	Mean ± SD (μm)	*P*	95% LoA Range (μm)
Zone 0 to 2 mm												
Central	54.47 ± 5.65	51.10 ± 5.29	53.43 ± 5.18	3.37 ± 2.52	< .001	-1.569 to 8.309	-1.04 ± 1.85	< .001	-4.666 to 2.586	2.33 ± 1.96	< .001	-1.512 to 6.172
Ring 2-5 mm												
Nasal	56.47 ± 3.68	52.27 ± 3.89	56.16 ± 4.18	4.20 ± 1.83	< .001	0.613 to 7.787	-0.32 ± 1.97	.219	-4.181 to 3.541	3.88 ± 1.77	< .001	0.411 to 7.349
Superior nasal	56.29 ± 4.08	51.91 ± 3.90	55.93 ± 4.41	4.38 ± 1.71	< .001	1.028 to 7.732	-0.36 ± 1.93	.153	-4.143 to 3.423	4.02 ± 1.56	< .001	0.962 to 7.078
Superior	56.51 ± 4.88	51.86 ± 4.37	56.09 ± 4.93	4.66 ± 2.02	< .001	0.701 to 8.619	-0.42 ± 1.81	.079	-3.968 to 3.128	4.24 ± 1.94	< .001	0.438 to 8.042
Superior temporal	57.13 ± 4.93	53.20 ± 5.13	56.39 ± 5.12	3.93 ± 2.29	< .001	-0.558 to 8.418	-0.73 ± 1.75	.002	-4.160 to 2.700	3.19 ± 2.05	< .001	-0.828 to 7.208
Temporal	54.56 ± 4.90	50.76 ± 5.12	53.87 ± 4.62	3.80 ± 2.19	< .001	-0.492 to 8.092	-0.68 ± 2.16	.017	-4.914 to 3.554	3.12 ± 2.00	< .001	-0.800 to 7.040
Inferior temporal	51.66 ± 5.12	47.19 ± 5.20	51.47 ± 4.89	4.47 ± 2.30	< .001	-0.038 to 8.978	-0.19 ± 2.01	.457	-4.130 to 3.750	4.28 ± 1.93	< .001	0.497 to 8.063
Inferior	54.02 ± 5.09	50.38 ± 5.09	54.21 ± 4.82	3.63 ± 2.10	< .001	-0.486 to 7.746	0.19 ± 2.00	.466	-3.730 to 4.110	3.82 ± 2.18	< .001	-0.453 to 8.093
Inferior nasal	56.09 ± 4.09	52.23 ± 4.09	55.89 ± 4.18	3.86 ± 1.96	< .001	0.018 to 7.702	-0.20 ± 1.59	.334	-3.316 to 2.916	3.66 ± 1.86	< .001	0.014 to 7.306
Ring 2- to 5-mm total	55.34 ± 3.51	51.22 ± 3.31	55.00 ± 3.52	4.12 ± 1.54	< .001	1.102 to 7.138	-0.34 ± 1.43	.071	-3.143 to 2.463	3.78 ± 1.30	< .001	1.232 to 6.328
Ring 5- to 7-mm												
Nasal	56.76 ± 3.66	51.86 ± 4.83	55.81 ± 3.90	4.91 ± 2.46	< .001	0.088 to 9.732	-0.95 ± 1.58	< .001	-4.047 to 2.147	3.96 ± 2.32	< .001	-0.587 to 8.507
Superior nasal	55.73 ± 3.98	51.27 ± 4.57	55.11 ± 4.13	4.47 ± 2.22	< .001	0.119 to 8.821	-0.62 ± 1.75	.008	-4.050 to 2.810	3.84 ± 1.94	< .001	0.038 to 7.642
Superior	54.58 ± 4.21	50.34 ± 4.27	54.09 ± 4.25	4.29 ± 2.14	< .001	0.096 to 8.484	-0.53 ± 2.14	.061	-4.724 to 3.664	3.76 ± 2.25	< .001	-0.650 to 8.170
Superior temporal	56.36 ± 4.73	52.82 ± 4.58	55.64 ± 4.81	3.53 ± 2.57	< .001	-1.507 to 8.567	-0.72 ± 2.13	.011	-4.895 to 3.455	2.82 ± 2.63	< .001	-2.335 to 7.975
Temporal	56.24 ± 4.50	52.41 ± 4.65	55.37 ± 4.41	3.83 ± 1.78	< .001	0.341 to 7.319	-0.87 ± 1.76	< .001	-4.320 to 2.580	2.96 ± 1.82	< .001	-0.607 to 6.527
Inferior temporal	55.57 ± 4.35	51.57 ± 5.42	55.12 ± 4.15	3.99 ± 3.33	< .001	-2.537 to 10.517	-0.45 ± 2.23	.123	-4.821 to 3.921	3.54 ± 3.07	< .001	-2.477 to 9.557
Inferior	58.09 ± 4.69	53.93 ± 5.47	57.62 ± 4.62	4.17 ± 2.33	< .001	-0.397 to 8.737	-0.47 ± 2.02	.075	-4.429 to 3.489	3.69 ± 2.41	< .001	-1.034 to 8.414
Inferior nasal	57.66 ± 3.91	52.90 ± 5.03	56.77 ± 4.14	4.76 ± 3.22	< .001	-1.551 to 11.071	-0.89 ± 1.64	< .001	-4.104 to 2.324	3.87 ± 3.06	< .001	-2.128 to 9.868
Ring 5- to 7-mm total	56.40 ± 3.69	52.18 ± 3.70	55.73 ± 3.64	4.22 ± 1.30	< .001	1.672 to 6.768	-0.67 ± 1.44	.001	-3.492 to 2.152	3.56 ± 1.25	< .001	1.110 to 6.010
Total 17 sections	55.81 ± 3.27	51.70 ± 3.16	55.29 ± 3.26	4.11 ± 1.34	< .001	1.484 to 6.736	-0.52 ± 1.30	.003	-3.068 to 2.028	3.59 ± 1.19	< .001	1.258 to 5.922

ETM = epithelial thickness mapping; LoA = limits of agreement; SD = standard deviation

[a]*According to the paired t test.*

The Anterion is manufactured by Heidelberg Engineering, the Avanti is manufactured by Optovue, Inc, and the MS-39 is manufactured by CSO.

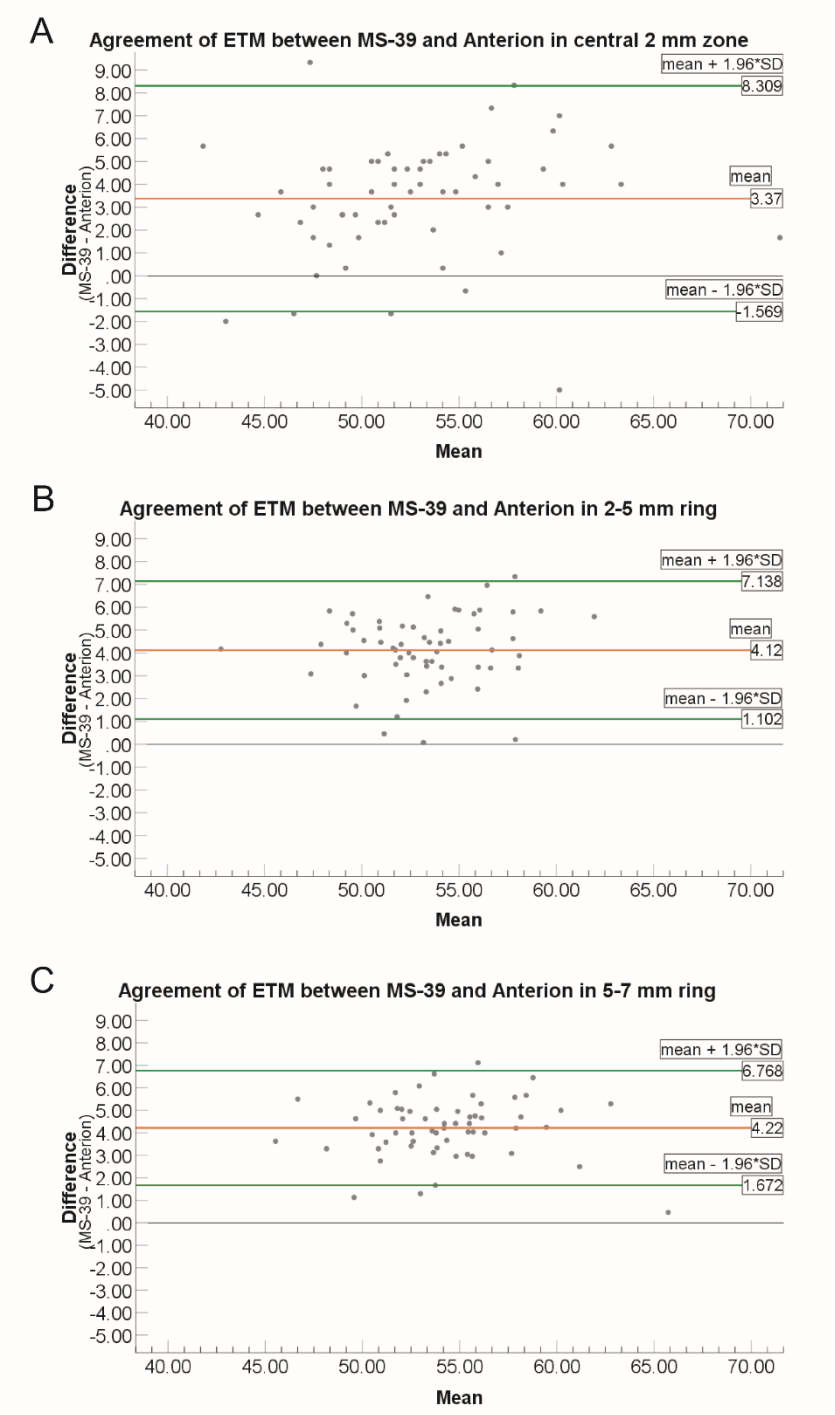

Figure B. Bland-Altman plots showing the difference in epithelial thickness measurements (MS-39 – Anterion) as a function of the mean epithelial thickness of the two devices in the (A) central 2-mm zone, (B) 2- to 5-mm diameter rings, and (C) 5- to 7-mm diameter rings, respectively. The red lines represent the mean difference and green lines represent the 95% limits of agreement. The Anterion is manufactured by Heidelberg Engineering and the MS-39 is manufactured by CSO. Unit: μm

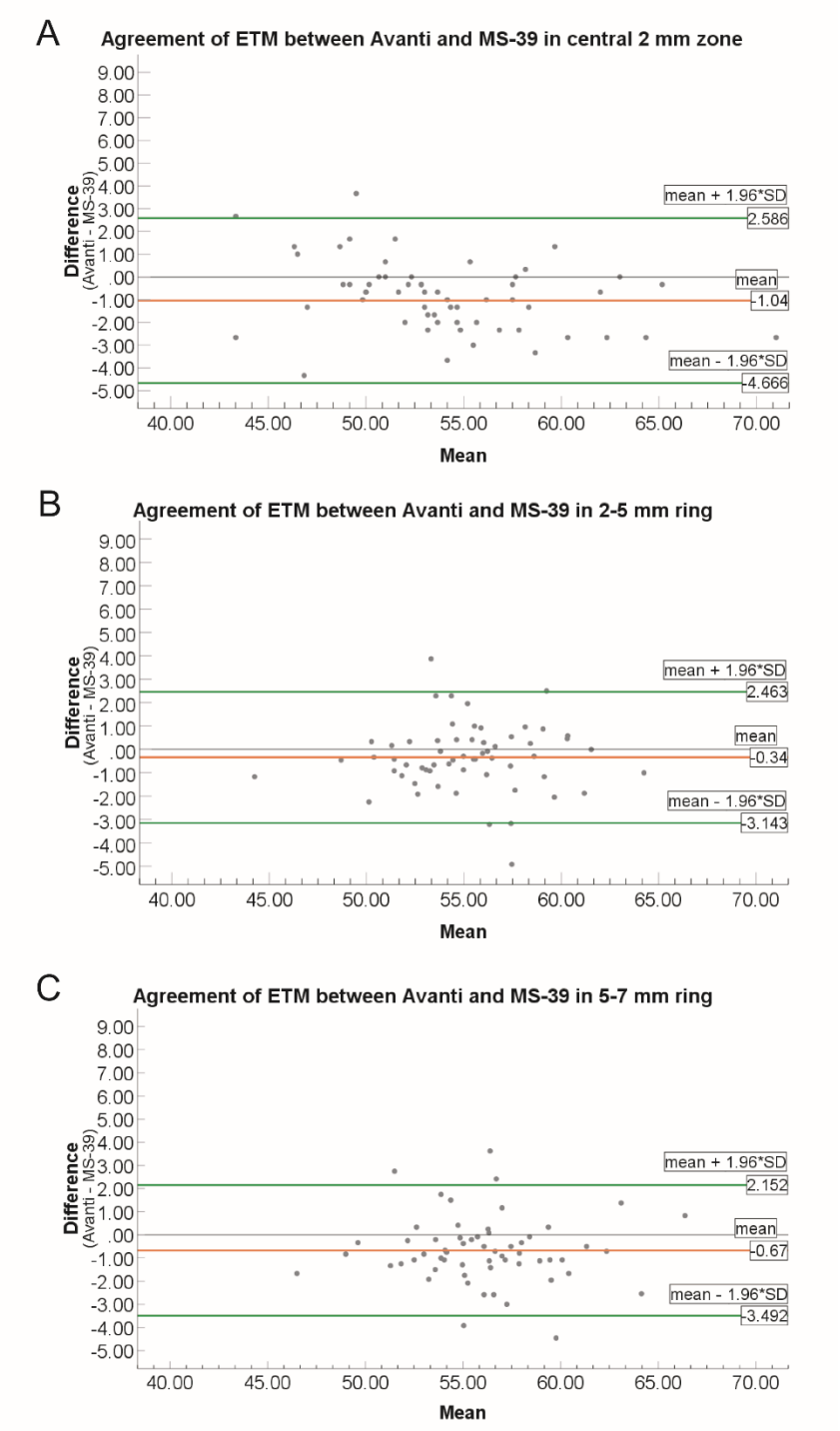

Figure C. Bland-Altman plots showing the difference in epithelial thickness measurements (Avanti – MS-39) as a function of the mean epithelial thickness of the two devices in the (A) central 2-mm zone, (B) 2- to 5-mm diameter rings, and (C) 5- to 7-mm diameter rings, respectively. The red lines represent the mean difference and green lines represent the 95% limits of agreement. The Avanti is manufactured by Optovue, Inc and the MS-39 is manufactured by CSO. Unit: μm

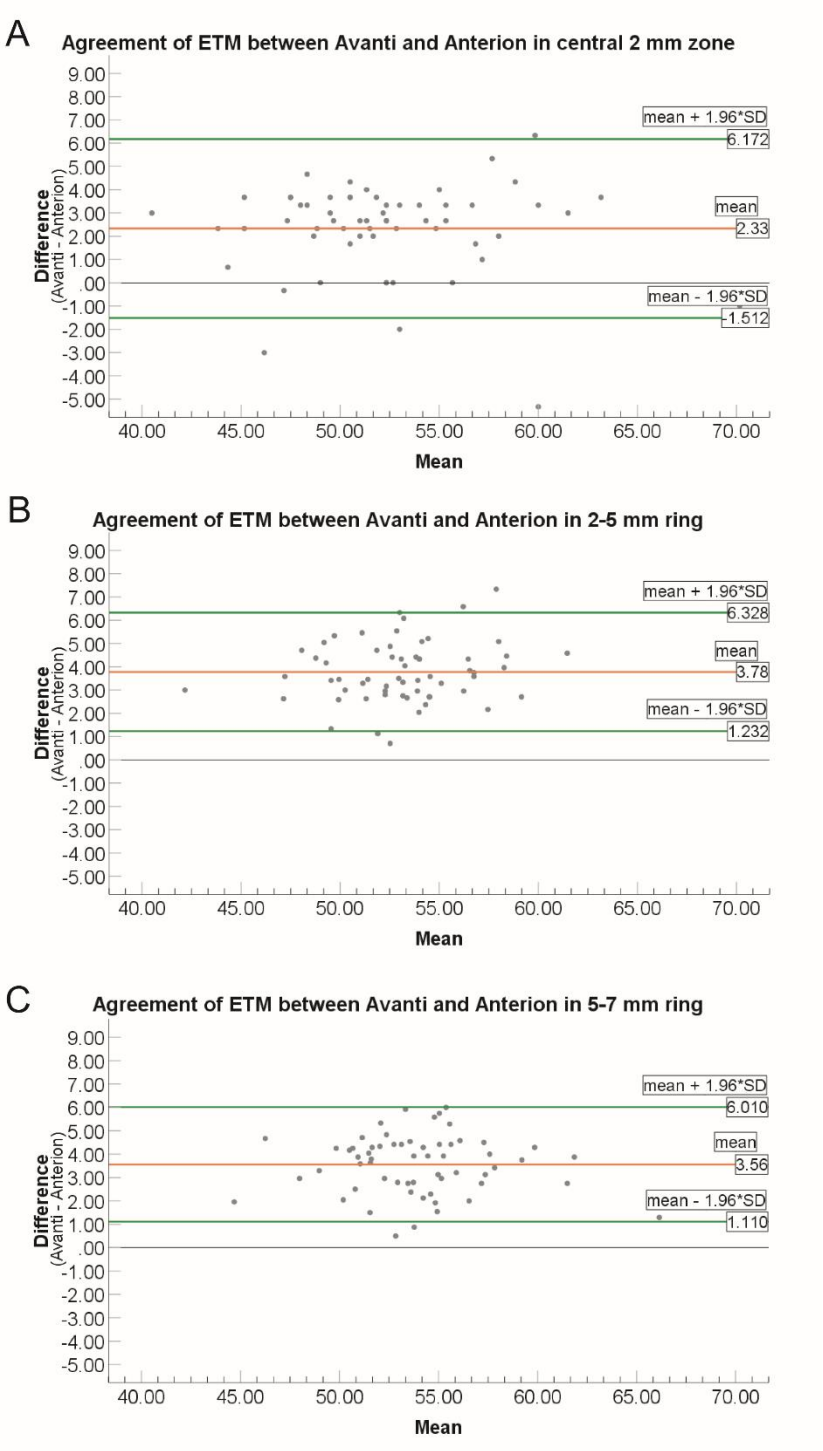

Figure D. Bland-Altman plots showing the difference in epithelial thickness measurements (Avanti – Anterion) as a function of the mean epithelial thickness of the two devices in the (A) central 2-mm zone, (B) 2- to 5-mm diameter rings, and (C) 5- to 7-mm diameter rings, respectively. The red lines represent the mean difference and green lines represent the 95% limits of agreement. The Avanti is manufactured by Optovue and the Anterion is manufactured by Heidelberg Engineering. Unit: μm

Table D
Repeatability of ETM Measurements Reported by Previous Investigators

		Repeatability, S_w				
Authors/ Year	**Eyes (n)**	**Normal**	**PLRS**	**KC**	**Areas**	**Instrument used**
Reinstein and colleagues (2010)	KC: 10		0.58		vertex	Very-high frequency ultrasound
			0.43-1.36		central 6 mm	
Ma and colleagues (2013)	Normal: 35 PLRS: 45	0.7	0.7		central 2 mm	Optovue RT-100 SD-OCT
		0.6-0.9	0.8-1.7		2-5 mm	
		0.8-1.2	1.4-2.2		5-6 mm	
Sella and colleagues (2019)	Normal: 12 PLRS: 48	0.9	1.2		central 2 mm	
		0.9-1.3	1.3-1.5		2-5 mm	
		1.0-1.4	1.5-1.9		5-6 mm	
Lu and colleagues (2019)	Normal: 75 PLRS: 204 KC:73	0.89	1.35-2.34	1.41-2.42	central 2 mm	Optovue Avanti SD-OCT
		0.99-1.24	1.2-3.56	1.36-3.89	2-5 mm	
		1-1.26	1.42-3.04	1.31-3.83	5-7 mm	
		0.92-1.62	1.57-2.94	1.02-4.01	7-9 mm	
Savini and colleagues (2018)	Normal: 96 PLRS: 43	0.99	1.84		central 3 mm	MS-39 SD OCT
		1.06-1.57	1.50-2.10		3-6 mm	
Vega-Estrada and colleagues (2019)	Normal: 60 KC:170	2.03		1.24	central 3 mm	
		0.84-1.18		1.16-1.69	3-6 mm	
		0.99-2.72		1.42-2.70	6-8 mm	
Schiano-Lomoriello and colleagues (2020)	KC: 43			1.57	central 3 mm	
Feng and colleagues (2022)	Normal: 90 PLRS: 46 KC:122	0.98	0.75	1.15	central 2 mm	Optovue Avanti SD-OCT
		1.08-1.19	1.07-1.49	1.17-1.52	2-5 mm	
		0.94-1.27	1.70-2.40	1.29-1.72	5-7 mm	
		0.64	0.6	0.91	central 2 mm	Anterion SS- OCT
		0.69-0.89	0.79-0.96	0.91-1.09	2-5 mm	
		0.86-1.11	1.07-2.05	1.10-1.47	5-7 mm	
Current study	KC: 60			0.93	central 2 mm	Optovue Avanti SD-OCT
				1.04-1.68	2-5 mm	
				0.75-1.79	5-7 mm	
				0.71	central 2 mm	Anterion SS- OCT
				0.81-0.98	2-5 mm	
				0.86-1.59	5-7 mm	
				0.91	central 2 mm	MS-39 SD OCT
				0.53-1.62	2-5 mm	
				0.73-1.79	5-7 mm	

ETM = epithelial thickness mapping; KC = keratoconus; OCT = optical coherence tomographer; PLRS = post-laser refractive surgery; SD = spectral-domain; SS = swept-source; S_w = pooled within-subject standard deviation
The Avanti is manufactured by Optovue and the Anterion is manufactured by Heidelberg Engineering.