

# Comparison of central corneal thickness measurements using ultrasound pachymetry, ultrasound biomicroscopy, and the Artemis-2 VHF scanner in normal eyes

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**Purpose:** To compare the precision of central corneal thickness (CCT) measurements taken with the handheld ultrasound pachymeter (USP), ultrasound biomicroscopy (UBM), and the Artemis-2 very high frequency ultrasound scanner (VHFUS) on normal subjects.

**Design:** Prospective study.

**Methods:** One eye from each of 61 normal subjects was randomly selected for this study. The measurements of the CCT were taken with the USP, VHFUS, and UBM. Results were compared statistically using repeated-measures analysis of variance (ANOVA), Pearson's correlation coefficient, and limits of agreement.

**Results:** The average CCT ( $\pm$  standard deviation) was  $530.1 \pm 30.5 \mu\text{m}$ ,  $554.9 \pm 31.7 \mu\text{m}$ , and  $559.5 \pm 30.7 \mu\text{m}$  for UBM, VHFUS, and USP respectively. The intraobserver repeatability analyses of variance are not significant for USP, UBM, and VHFUS.  $P$ -values were 0.17, 0.19, and 0.37 respectively. Repeated-measures ANOVA showed a significant difference between the three different methods of measuring CCT ( $P = 0.0001$ ). The ANOVA test revealed no statistically significant difference between USP and VHFUS ( $P > 0.05$ ), yet statistical significant differences with UBM versus USP and UBM versus VHFUS ( $P < 0.001$ ). There were high correlations between the three instruments ( $P < 0.0001$ ). The mean differences (and upper/lower limits of agreement) for CCT measurements were  $29.4 \pm 14.3$  (2.7/56),  $4.6 \pm 8.6$  (-14.7/23.8), and  $-24.8 \pm 13.1$  (-50.4/0.8) for USP versus UBM, USP versus VHFUS, and UBM versus VHFUS, respectively.

**Conclusion:** The UBM produces CCT measurements that vary significantly from those returned by the USP and the VHFUS, suggesting that the UBM may not be used interchangeably with either equipment for monitoring the CCT in the clinical setting.

**Keywords:** central corneal thickness, ultrasound pachymetry, ultrasound biomicroscopy, Artemis-2 VHFUS, Artemis, normal eyes

## Introduction

Accurate measurements of the central corneal thickness (CCT) are an essential tool in management of glaucoma, since the accuracy of intraocular pressure measurements by applanation tonometry are affected by corneal thickness.<sup>1-5</sup> Accurate CCT measurements are also useful in refractive surgery because CCT is an important parameter pre- and postoperatively.<sup>6-12</sup> The measurement of CCT is also vital in the diagnosis and management of certain corneal diseases such as keratoconus, keratoglobus, and pellucid marginal degeneration,<sup>13-16</sup> as the CCT is a direct correlate of the physiologic condition of the corneal endothelium.<sup>6</sup> Furthermore, Ambrósio et al<sup>17</sup> and Ambrósio

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and Wilson<sup>18</sup> reported that CCT measurements were used as the standard methods for screening refractive surgery candidates for a risk of developing ectasia.

Recently several devices have been introduced to measure the CCT such as optical coherence tomography,<sup>19</sup> confocal microscopy,<sup>20</sup> ultrasound biomicroscopy,<sup>6,21</sup> and the Artemis-2 very high frequency ultrasound scanner (VHFUS).<sup>22</sup>

The handheld ultrasound pachymeter (USP) is the most commonly used instrument for measuring CCT. It operates at frequencies of 20 to 50 MHz, emits short acoustic pulses, and detects reflections from the anterior and posterior surfaces of the cornea. Corneal thickness is then calculated from the measured time-of-flight between these reflections.<sup>1</sup> Ultrasound pachymetry measurements have demonstrated high intraobserver reproducibility.<sup>24–27</sup> However, Salz et al<sup>23</sup> and Bechman et al<sup>19</sup> both reported that the CCT measured with the USP varied significantly between observers. There are potential sources of error for the handheld pachymeter such as inaccurate placement of the probe and placing the probe obliquely to the corneal surface.<sup>18,23,26–28</sup> These potential errors would both lead to thicker measures of CCT.

The ultrasound biomicroscope (UBM) uses a high-frequency (50 MHz) ultrasound beam to measure various parameters in the eye. Urbak et al<sup>29</sup> and Tam and Rootman<sup>6</sup> both reported that the intraobserver reproducibility was high for all measurements of CCT. In addition, Dada et al<sup>30</sup> reported that there was no statistically significant difference between the mean CCT measured with the anterior segment optical coherence tomograph (AS-OCT) and the UBM. Tello et al<sup>31</sup> showed that intraobserver reproducibility was high with the UBM. They also reported that the interobserver reproducibility for the measured parameters varied considerably and was affected by the subjective interpretation of visualized anatomic landmarks.

The Artemis-2 VHFUS is a very high frequency (VHF) digital ultrasound device. It is an arc-scanner tool capable of imaging and measuring the whole anterior segment, or the whole cornea in one scan sweep. The measurement zone of CCT was 3 mm diameter of the cornea. It was designed particularly for refractive, cataract, and presbyopic surgery, to improve anatomical diagnosis for surgical planning and postoperative diagnostic monitoring. The Artemis-2 uses a broad-band 50 MHz VHF ultrasound transducer (bandwidth approximately 10 to 60 MHz). The cornea is swept by a reverse arc high-precision mechanism to acquire B-scans as arcs that follow the surface contour of anterior or posterior segment structures of interest. The Artemis-2 is used to obtain

scanning for different curvatures within the globe such as cornea, iris plane, and retina. Ultrasound data is first digitized and stored. The digitized ultrasound data is then transformed, using Cornell digital signal processing technology, which statically significantly reduces noise and enhances signal-to-noise ratio.<sup>32–34</sup>

A number of studies have compared the CCT measurements of the handheld USP, and the UBM.<sup>35,36</sup> Other studies compared the handheld USP with the Artemis-2 VHFUS.<sup>20</sup> To the best of the authors' knowledge, this is the first study to compare the CCT measurements with UBM and Artemis-2 VHFUS devices.

The purpose of this study was to compare the precision of CCT measurements taken with the handheld USP, ultrasound biomicroscopy (UBM), and the Artemis-2 VHFUS.

## Subjects and methods

This prospective, cross-sectional study enrolled 61 consecutive, healthy, exclusively normal subjects (30 women). Their ages ranged from 19 to 30 years ( $21.7 \pm 2.3$  mean  $\pm$  standard deviation [SD]). Comprehensive anterior segment examinations of all subjects were performed using slit lamp. The exclusion criteria included a positive history (or observable signs) of systemic disease affecting the anterior segment; pregnancy; spherical equivalent refractive error  $\geq \pm 4.00$  D and/or corneal astigmatism  $\geq 3.00$  D, and corneal curvature  $\geq 48$  D. The corneal curvature was determined by auto-refractometer (Auto Kerato-Refracto-Tonometer TRK-1P; Topcon Corporation, Tokyo, Japan). All measurements of the CCT were conducted by a single investigator at the same location. One eye of each subject was selected randomly using a table of random numbers generated in Microsoft Excel (Microsoft Corporation, Redmond, WA). The CCT has been shown to increase overnight and return to baseline within 1 to 2 hours of awakening.<sup>36,37</sup> Thus, all the measurements were collected in the afternoon and between 12 pm and 2 pm. The CCT was first assessed using the USP, then the Artemis-2 VHFUS, and finally the UBM. There was an interval of 30 minutes between techniques to minimize possible confounding factors caused by prior CCT measurement with another technique. The purpose of the study was explained to all subjects and informed consent was obtained from each subject before beginning the examination. The study was conducted in conformance with the ethical considerations laid out in the 2008 Declaration of Helsinki, and the study protocol was approved by the research ethics review board of the College of Applied Medicine Science at King Saud University.

Three measurements were taken for each subject with the USP (PacScan300p; Sonomed Escalon, New Hyde Park, NY) following the instillation of one drop of a topical anesthetic (benoxinate hydrochloride 0.4%) in the eye. The probe was disinfected with an alcohol swab. The subject sat on the chair and was asked to fixate on a distant target, while the ultrasound probe was aligned perpendicular to the center of the cornea and placed gently in contact with the cornea.

For the Artemis-2 VHFUS (Scott Phillips Engineering, Victoria, Canada) measurements, the patient sat and positioned his or her face on a three-point forehead and chin rest while placing the eye into a soft-rimmed eye-cup akin to a swimming goggle. The sterile coupling fluid filled the compartment in front of the eye and the scanning was performed via an ultrasonically transparent (sterile) membrane, without the need for a speculum. As such, there was no contact of the scanner probe with the eye. Performing a 3-D scan set with the Artemis-2 VHFUS required 2 to 3 minutes for each eye. The CCT values were obtained from the pachymetry map, which was derived from the four-scan set. Three readings were obtained for each eye and the average was calculated.

For the VuMAX™ USB (Sonomed) measurements, the subject was asked to look at a fixation target on the ceiling. One drop of topical anesthesia (benoxinate hydrochloride 0.4%) was instilled. The cup was disinfected with an alcohol swab. The transducer head was immersed in methylcellulose 1% within an eye-cup, which was placed on the sclera. Centrality was ensured by acquiring an image in which the pupil diameter was greatest. Perpendicularity was ensured by adjusting the transducer head until the brightest reflection lines from the various corneal layers were observed in real time. Three separate images of each eye, meeting the above criteria, were captured and stored on the instrument system.

## Statistical methods

The demographic data of all subjects were analyzed using Microsoft Excel 2007. InStat statistical software version 3.06 (GraphPad Software Inc, La Jolla, CA) was used for further statistical analyses. First, repeated-measures ANOVA was conducted to compare the mean CCT values for the three instruments. Second, Bland–Altman analysis was performed to determine the repeatability of measurements for each instrument used and to assess the limits of agreement between different pairings of the three devices. Finally, a Pearson correlation coefficient test was performed to compare the mean CCT values for the three instruments. The level of statistical significance for this study was set at 0.05.

## Results

The study included 61 normal subjects and consisted of 33 right eyes and 28 left eyes (total 61 eyes; one per subject). Three subjects dropped out of the study as they were apprehensive about being examined with the UBM.

### Intraobserver repeatability of central corneal thickness measurements

The intraobserver repeatability ANOVA *P*-values were considered not significant for ultrasound pachymetry, ultrasound biomicroscopy, and the Artemis-2 VHFUS, and had values of 0.17, 0.19, and 0.37, respectively. The difference between the ultrasound pachymetry and the Artemis-2 values was not statistically significant ( $P > 0.05$ ).

### Comparison of mean CCT

The mean and SD of the CCT measurements for the USP, UBM, and Artemis-2 VHFUS are summarized in Table 1.

Repeated-measures ANOVA showed that the mean values of CCT measurements differed significantly between the three instruments ( $P < 0.0001$ ). There were significant differences between the USP and the UBM and between the UBM and the Artemis-2 VHFUS ( $P < 0.001$ ). There was no significant difference between the USP and the Artemis-2 VHFUS ( $P > 0.05$ ).

### Agreement between the three instruments

The CCT measurements' mean differences, SD, and limits of agreement of the repeated measurements of the CCT for the USP, UBM, and Artemis-2 VHFUS are summarized in Table 2. The highest mean differences were found in the CCT measurements between the USP and UBM and the UBM and Artemis-2 VHFUS, yet lowest between the USP and Artemis-2. The mean difference between the USP and Artemis-2 VHFUS was 4.55  $\mu\text{m}$ , which is very small compared with the differences between the UBM and USP and between the UBM and Artemis-2 VHFUS. Figure 1 is a Bland–Altman plot of agreement; it shows the mean difference was least between the USP and Artemis-2 VHFUS when

**Table 1** The means and standard deviations of CCT measurements for the three instruments

Techniques	Mean CCT $\pm$ SD ( $\mu\text{m}$ )
Ultrasound pachymetry	559.5 $\pm$ 30.7
Ultrasound biomicroscopy	530.1 $\pm$ 30.5
Artemis-2	554.9 $\pm$ 31.7

**Abbreviations:** CCT, central corneal thickness, SD, standard deviation.

**Table 2** The mean differences, standard deviations, and LOA of CCT measurements

Techniques	Mean difference ( $\mu\text{m}$ )	LOA ( $\mu\text{m}$ )
Ultrasound pachymetry vs ultrasound biomicroscopy	$29.4 \pm 14.3$	2.7–56
Ultrasound pachymetry vs Artemis-2	$4.6 \pm 8.6$	–14.7–23.8
Ultrasound biomicroscopy vs Artemis-2	$-24.8 \pm 13.1$	–50.4–0.8

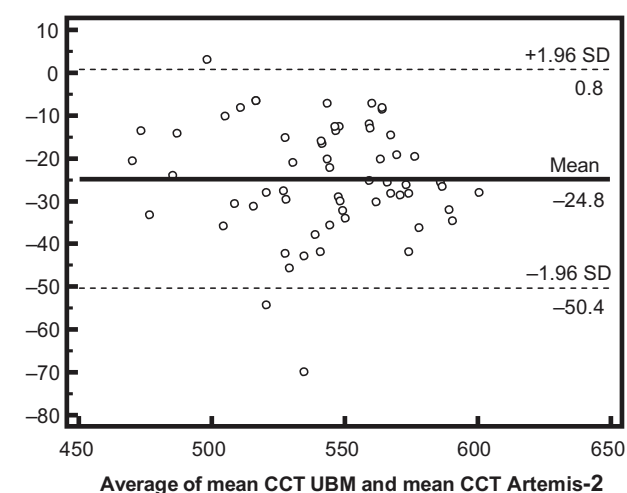
**Abbreviations:** LOA, limits of agreement; CCT, central corneal thickness.

compared to UBM with USP and Artemis-2 VHFUS mean difference. Figures 2 and 3 are Bland–Altman plots of agreement; they show that the highest mean differences were found in the CCT measurements between the UBM and Artemis-2 VHFUS and between the USP and UBM, respectively.

## Discussion

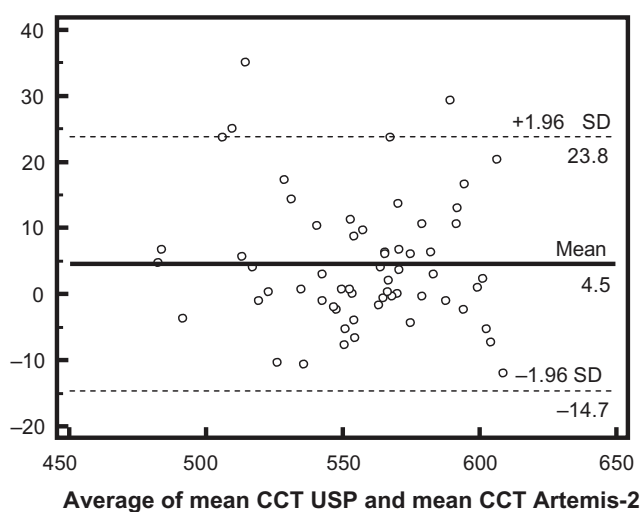
The central corneal thickness is one of the crucial parameters for patient inclusion criteria for keratorefractive surgery. The handheld USP is the gold standard technique used to measure CCT. Several studies have reported that there are various sources of variation in handheld pachymetry due to instillation of topical anesthesia, wrong positional placement of the probe, compression of the cornea by the probe, displacement of the tear film by the probe, and not placing the probe perpendicularly on the cornea.<sup>11,24,27,36,37</sup>

Our results indicate that the intraobserver repeatability of the CCT measurements is highly significant, and demonstrates very high and comparable inter-repeatability. The USP, UBM, and Artemis-2 VHFUS measurements showed strong

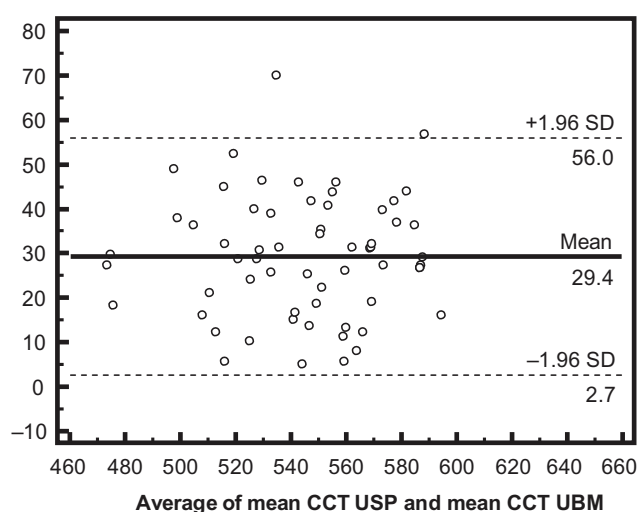
**Figure 2** Bland–Altman plot of agreement showing the mean difference between UBM and Artemis-2 VHFUS.

**Abbreviations:** CCT, central corneal thickness; UBM, ultrasound biomicroscopy; VHFUS, very high frequency ultrasound.

positive correlation. The agreement between the instruments, as proposed by Bland and Altman, better illustrates the clinical relevance of differences between two instruments. The Bland–Altman analysis showed a high level of agreement between the USP and Artemis-2 VHFUS. The measurements differed by a mean of  $4.55 \mu\text{m}$ . The 95% limits of agreement were between  $-14.7 \mu\text{m}$  and  $23.8 \mu\text{m}$ . This means that the difference is clinically acceptable, and the two instruments can be used interchangeably.<sup>12</sup> The agreement was worse between both the UBM and the USP and between the UBM and Artemis-2 VHFUS, with mean differences of  $29.4 \mu\text{m}$ , and  $24.8 \mu\text{m}$  respectively. The ANOVA of the CCT mean

**Figure 1** Bland–Altman plot of agreement showing the lowest mean difference was between USP and Artemis-2 VHFUS.

**Abbreviations:** CCT, central corneal thickness; USP, ultrasound pachymeter; VHFUS, very high frequency ultrasound; UBM, ultrasound biomicroscopy.

**Figure 3** Bland–Altman plot of agreement showing the greatest mean difference was between USP and UBM.

**Abbreviations:** CCT, central corneal thickness; USP, ultrasound pachymeter; UBM, ultrasound biomicroscopy.

values for the three instruments show that there were no statistically significant differences between the USP and Artemis-2 VHFUS. However, there were statistically significant differences between the USP and UBM and between the UBM and Artemis-2 VHFUS.

In the present study, Bland–Altman plots showed the compared corneal measurements taken with the USP, the UBM, and the Artemis-2 VHFUS. The USP consistently measured CCT thicker than the Artemis-2 VHFUS and the UBM. However, the UBM CCT measurements showed high variability and a trend towards underestimation of CCT compared to the USP and Artemis-2 VHFUS. The mean difference and limits of agreement for the UBM versus the USP was  $29.4 \pm 14.3$  (2.7/56), and the UBM versus the Artemis-2 VHFUS was  $29.4 \pm 14.3$  (2.7/56) and  $-24.8 \pm 13.1$  (-50.4/0.8). In 2008, Paul et al<sup>22</sup> reported that the CCT measurements with the Artemis-2 VHFUS correlated highly with ultrasound pachymetry with a mean difference of 11  $\mu\text{m}$ . In their study, the subjects' ages ranged from 25–60 (average 31.5 years), and the refractive state, or keratometry, of the subjects was not mentioned, whereas, in this study, the subjects' ages ranged from 19 to 30 years ( $21.7 \pm 2.3$ ). The difference between their results and ours might be explained by the use of older subjects in their study.<sup>12,38</sup>

Comparing repeatability of the instruments is important, because a poor repeatability of an instrument limits the amount of agreement that is possible between instruments.<sup>39</sup> However, the inter-repeatability and intra-repeatability results of CCT measurements as affected by different instruments is due to the different methodologies of these instruments.<sup>6,39</sup> Some studies have reported that the USP has a high degree of interobserver and inter-instrument reproducibility,<sup>27,40,41</sup> but other studies have shown that the USP measurements of CCT results between observers vary significantly.<sup>2,40</sup> A number of studies indicate that accurate measurement of corneal thickness with the USP depends on the technique. It requires direct contact and precise placement of the probe relative to the center of the cornea, which is often uncomfortable for the patient, depends on the reflection of ultrasound from the anterior and posterior corneal surfaces, and does not measure the tear film that is displaced by the technique used in the probe.<sup>11,18,23,24,26–28,36,37,40</sup> In this study, the CCT results using the USP demonstrated a good intraobserver repeatability, a result which is supported by previous studies.<sup>27,40,41</sup> The measurement of CCT using the UBM showed high intraobserver reproducibility, but the interobserver reproducibility was low,<sup>29</sup> which could be explained by the technique used in the UBM. The UBM uses a transducer, is

immersed in transduction fluid, and is not in contact with the cornea. Thus the level of perturbation of the pre-corneal tear film and epithelium is different from that with USP. The examiner must manually adjust the transducer head to maximize centrality and perpendicularity of the images that require more time to perform. The analog-based UBM is not able to image the interface consistently because analog processing does not produce a high enough signal-to-noise ratio between the interface echo complex and the surrounding tissue.<sup>6</sup> This could explain the underestimation of corneal thickness measurement with the UBM relative to the USP and the Artemis-2 VHFUS. The ANOVA test results were significantly different between the UBM and the USP and between the UBM and the Artemis-2 VHFUS in our study.

Several studies have revealed that the Artemis-2 VHFUS technique is accurate, repeatable, and reproducible.<sup>42,43</sup> In this technique, the cornea is offset from the probe by a normal-saline immersion medium. During scanning, the probe is moved in an arc-shape trajectory that is matched approximately to the corneal curvature, enabling near-normal incidence at all positions. The device incorporates a fixation light and optical camera for visualization of the eye to assure centration. It contains a unique scan-arc adjustment mechanism to enable maximum perpendicularity (and signal-to-noise ratio) to be obtained for scanning any of the different curvatures within the globe such as the cornea. Ultrasound data are first digitized and stored. The digitized ultrasound data are then transformed using Cornell digital signal processing technology. Digital signal processing significantly reduces noise and enhances signal-to-noise ratio.<sup>32,34,35,42,43</sup> This could explain our finding that the Artemis-2 VHFUS demonstrated good repeatability of the CCT measurements compared to the other instruments in this study.

Other studies on noncontact equipment for measuring CCT, such as the Visante AS-OCT (Carl Zeiss; Medtec, Dublin, CA) and Pentacam Scheimpflug (Pentacam; Oculus Inc, Lynnwood, WA) system, suggest that these methods provide repeatable measurements of the CCT.<sup>39,44,45,47</sup> These studies imply that the limited agreement between these instruments is due to the distinct measuring techniques of the instruments. For example, the Visante AS-OCT is a noncontact technique that uses signals at interfaces as a result of reflection of infrared waves of 1310 nm wavelength from anterior to posterior corneal surfaces.<sup>38</sup> In contrast, the Pentacam Scheimpflug is a noncontact optical system. It has a rotating Scheimpflug camera that takes up to 50 slit images of the anterior segment in less than 2 seconds. Software is then used to construct a three-dimensional image. A second camera captures eye movements and makes appropriate corrections.<sup>46</sup>



In addition, several studies compared ultrasound contact and non-contact techniques with non-contact optical pachymetry systems such as the Visante AS-OCT and the Pentacam-Scheimpflug on normal and keratoconus subjects. These studies reported that the Artemis-2 VHFUS and the Visante AS-OCT systems provide equivalent and repeatable measurements of the CCT, and can be used interchangeably.<sup>43</sup> Chen et al<sup>47</sup> reported that the USP CCT measurements were significantly different from the Visante AS-OCT. Further, Li et al<sup>39</sup> and Zhao et al<sup>48</sup> reported that the measurements of CCT on normal subjects revealed that the Visante AS-OCT CCT measurements were consistently less than those made with the USP. Furthermore, studies that conducted comparisons of these techniques on keratoconus subjects reported that the USP consistently produced thicker CCT measurements than the Visante AS-OCT.<sup>45</sup> Other studies have shown that CCT measurements made with the Scheimpflug were thinner than those made with the USP in normal and keratoconus subjects.<sup>44,46,49</sup> Moreover, the results of a comparison between the UBM and the Visante AS-OCT revealed comparable measurements of the CCT.<sup>30</sup>

There are fluctuations in the differences between the mean measured CCT between techniques in previous studies as mentioned above. Our results for the USP versus the Artemis-2 VHFUS and versus the UBM are comparable with those of previous studies conducted on non-contact optical pachymetry systems in that the measurements of the CCT by the USP tend to be thicker. However, the mean difference values and the significance of the mean differences are not similar between techniques.<sup>39,43,48,50,51</sup> The UBM measurements of CCT are likely thinner than the Visante AS-OCT measurements.<sup>30</sup> Our results show no statistically significant difference between the USP and Artemis-2 VHFUS. This difference of CCT indicates that the Artemis-2 VHFUS measurements are thinner than the USP. In the case of the Artemis-2 VHFUS, Chen et al<sup>47</sup> reported that there was no statistically significant difference between the measurements of the CCT made by the Artemis 2 VHFUS and the Visante AS-OCT.

## Conclusion

The UBM produces CCT measurements that vary significantly from those returned by the USP and the Artemis-2 VHFUS, suggesting that the UBM may not be used interchangeably with either equipment for monitoring the CCT in the clinical setting.

## Disclosure

The authors report no conflicts of interest in this work.

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