

# Relationships between Central Tear Film Thickness and Tear Menisci of the Upper and Lower Eyelids

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**PURPOSE.** To investigate the relationship between central tear film thickness (TFT) and tear menisci of the upper and lower eyelids using real-time optical coherence tomography (OCT).

**METHODS.** Both eyes of healthy subjects were imaged with a real-time OCT to obtain height, curvature, and area of upper and lower tear menisci simultaneously. Central TFT was indirectly measured by calculating the difference between baseline measurements of the central corneal thickness plus tear film and the true corneal thickness obtained after instillation of artificial tears. Results from two normal blinks were obtained from one eye at each visit and repeated the next day.

**RESULTS.** The average central TFT was  $3.4 \pm 2.6 \mu\text{m}$ . The upper tear meniscus curvature, height, and area were  $239 \pm 112 \mu\text{m}$ ,  $268 \pm 68 \mu\text{m}$ , and  $22,732 \pm 11,974 \mu\text{m}^2$  respectively. There were no significant differences in curvatures, heights, or areas between upper and lower tear menisci, nor were there any differences in measured variables between the two blinks at each visit or between the two repeated visits in the right and left eye groups ( $P > 0.05$ ). The upper and lower tear menisci in each eye group on each day correlated strongly with curvature, height, and area (all  $P \leq 0.03$ ). However, no tear meniscus variable was a significant predictor of TFT (all  $P > 0.44$ ).

**CONCLUSIONS.** OCT is a promising tool in the measurements of TFT and dimensional variables of tear menisci. Upper and lower tear menisci have nearly identical dimensions. (*Invest Ophthalmol Vis Sci.* 2006;47:4349–4355) DOI:10.1167/iov.05-1654

Maintenance of a normal tear film is essential both for the health and the optical quality of the eye. Unlike other functional structures of the eye, the tear film must be renewed with each blink. Structural failure results in symptoms of “dry eye syndrome,” which include burning, foreign body sensation, reflex tearing, and visual distortion. In its most severe forms, dry eye may lead to painful recurrent corneal erosion or secondary infection causing severe visual loss.<sup>1,2</sup> There are 3.2 to 4.7 million people suffering from dry eye syndrome in the United States.<sup>3</sup> One of the currently accepted causes is an imbalance between tear secretion and loss.<sup>4</sup>

Approximately 4.5  $\mu\text{L}$  of the tears secreted by the lacrimal gland are distributed into the cul-de-sac, and approximately 2.9

and 1.1  $\mu\text{L}$  enter the tear menisci and preocular tear film, respectively.<sup>5</sup> Through drainage into the puncta or evaporation, these volumes are maintained in a dynamic balance throughout the tear cycle. The tears are collected at the edge of the eyelids and cul-de-sac for deposition on the ocular surface to form the tear film. A malfunction or disturbance of any aspect of the tear cycle will compromise the integrity of the tear film and potentially cause ocular discomfort and disease.<sup>6–8</sup> Attempts to research the fundamental properties of the tear film cycle have included the quantitative analysis of the tear film structure,<sup>9</sup> the deposition of the tear film by blinking,<sup>10</sup> redistribution after blinking,<sup>11</sup> and the formation of dry spots.<sup>12</sup>

Despite the critical importance of tears, the relation between the tear film and tear menisci between blinks remains untested. The menisci supply the microscopically thin tear film layer as it spreads over the cornea with each blink. The film rapidly changes during the blink interval due to redistribution or tear loss. The tear menisci around both upper and lower eyelids also change during and between blinks. Because it is extremely difficult to measure these tear variables individually at the same time and because these factors may vary widely from person to person based on physiological conditions, attempts to characterize the tear system using any traditional methods have proved elusive. In this study, we used optical coherence tomography (OCT) for simultaneous measurement of dimensional variables of the tear menisci and indirect measurement of the central tear film thickness. We then determined the relationship among these tear variables over the ocular surface.

## METHODS

The study was approved by the research review board of the University of Rochester. Twenty healthy participants (13 women and 7 men; mean age:  $40.5 \pm 14.1$  years) with no history of contact lens wear and no current ocular or systemic disease were enrolled. Informed consent was obtained from each subject in accordance with the tenets of the Declaration of Helsinki. In a small consulting room, central air conditioning was used to control the temperature within a range of 15°C to 25°C, and two humidifiers (HM7306; Holmes, Milford, MA) maintained the humidity at 30% to 50%. A temperature and humidity meter (TM121; Dickson, Addison, IL) monitored these variables during the study period.

At each subject's first visit, two blinks within 1 minute from one eye were imaged by real-time OCT with continuous recording. The procedure was repeated at the same time of day on the next day. Each subject had four visits to complete the study so that both eyes were tested. The subject was asked to blink normally during OCT imaging, described in detail later. No visual light except for room light was shined on the eye, and the OCT light was invisible. After baseline OCT imaging, a drop of artificial tears (Refresh Liquigel; Allergan, Irvine, CA) was instilled into the eye to highlight by OCT the interface between the corneal surface and the tears. This facilitated the measurement of true corneal thickness (Fig. 1) required for the calculation of tear film thickness.<sup>9</sup> The OCT imaging took place immediately (within less than 1 minute) after the instillation.

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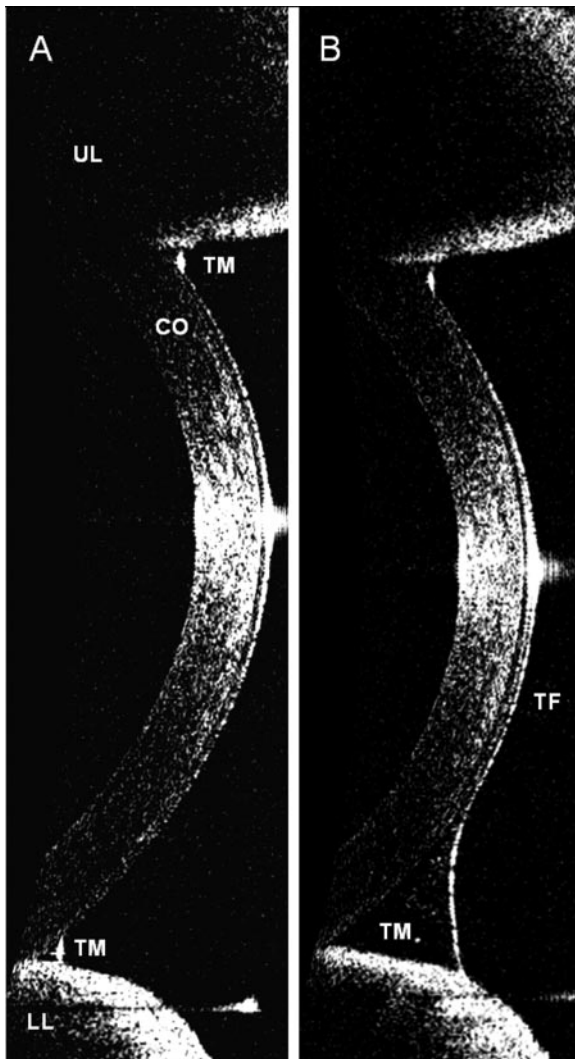
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**FIGURE 1.** A vertical 12-mm OCT scan was performed. (A) The condition immediately after a full blink, before the instillation of a drop of artificial tears. (B) After the instillation of one drop of the artificial tears (Refresh Liquigel; Allergan, Irvine, CA). The interface between the tears and cornea from which the corneal thickness was obtained. Cornea (CO), upper eyelid (UL), lower eyelid (LL), tear film (TF), and tear meniscus (TM).

Real-time OCT was used, with up to a 15-mm scanning width. With a light source at a 1310- and a 60-nm bandwidth, the OCT was connected to a telecentric optical probe. The optical resolution was less than 10  $\mu\text{m}$  in the cornea. The telecentric design used light that was parallel at any scan spot for a wide scan and was specifically designed to capture the real spatial relationship of the tears over the ocular surface. The OCT scanning probe, with a computer-controlled galvanometer, was mounted on a standard slit lamp, which also incorporated a digital video system through the viewing system. This combination was used to facilitate the imaging of the tears when the subject was looking at an external central target.

The vertical optical section crossing the central cornea and eyelids was taken continuously at eight frames per second when a specular reflex was visualized through the video system. The specular reflex of the OCT image confirmed the perpendicularity of the OCT beam to the central cornea. The entire scanned image was set to a 960-pixel/12-mm width and 384-pixel/2.0-mm depth in air. The axial interval between two image pixels was 3.7  $\mu\text{m}$  when 1.389 was used as the group index of the cornea under 1310-nm light according to Lin et al.<sup>13</sup> These settings were suitable for imaging the entire cornea and tear meniscus

areas around both the upper and lower eyelids. The tear film thickness was then calculated by using a factor of 1.03 because of the differences between the indices of the cornea (1.389) and the tears (1.343).<sup>13</sup>

A series of custom software was used to process OCT images to yield all variables. To avoid the distortion of central specular reflex of each image, the central 30 pixels (0.39 mm width) where the hyper-reflective specular reflex was located were removed. After that, the central 21 axial scans of eight consecutive images taken in 1 second immediately after blinking were averaged to yield corneal thickness plus tear film thickness at baseline, and true corneal thickness when applying the artificial tears. The thickness of the tear film (TFT) was obtained by subtracting the corneal thickness without tear film measured after the instillation of artificial tears (Fig. 1B[b]) from the total thickness with tear film measured at baseline (Fig. 1A). The peak location of the OCT longitudinal reflectivity profile was used to locate the inner and outer borders, similar to that used in many previous studies by us and other investigators.<sup>14,15</sup> Averaged reflectivity profiles were processed from 168 axial scans (21 axial scans with 8 images) as detailed in the legend of Figure 2.

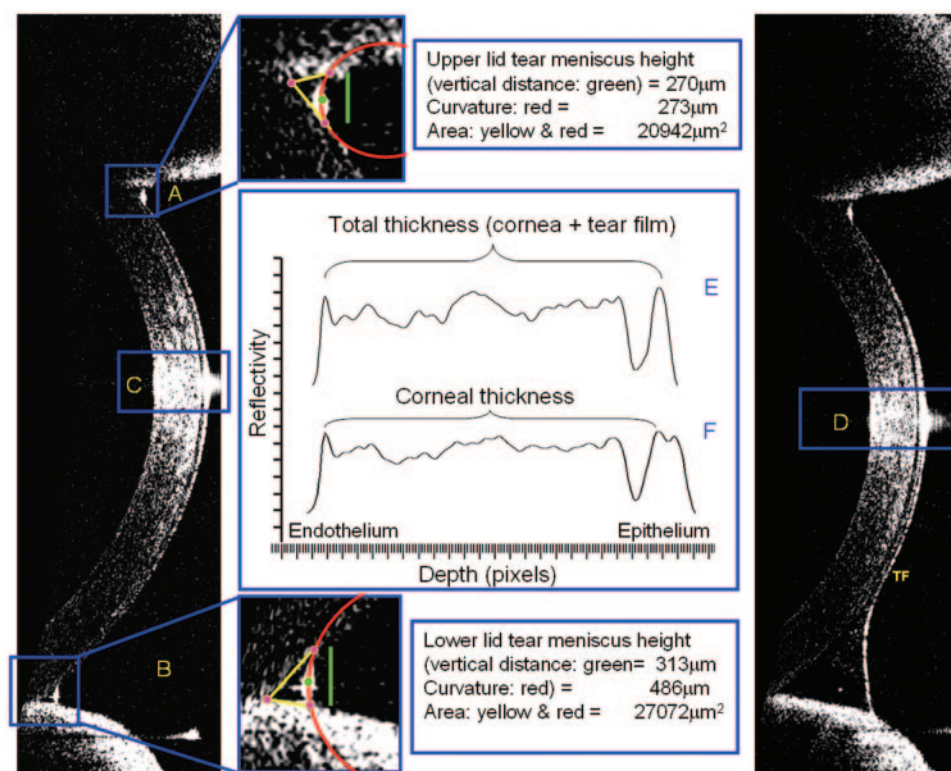
The first good image showing both upper and lower tear menisci of the first eight images taken immediately after blinking was processed to obtain the variables of the tear menisci. These included upper tear meniscus curvature (UTMC), height (UTMH), and area (UTMA) and lower tear meniscus curvature (LTMC), height (LTMH), and area (LTMA). Custom software was used to process all meniscus variables with operator inputs obtained by identifying touch points between two of these elements (eyelid, cornea, and tear meniscus) and the middle point of the tear meniscus front edge, as detailed in the legend of Figure 2. After that, the software processed the image and yielded the results. The three-point method was used to fit a circle that showed the tear meniscus curvature. These three points included points where the tear surface touches the cornea and the lids and the middle point on the surface. Infinite curvature (straight line lined up by these three points) was unacceptable and repeated measurement was conducted if it occurred.

The repeatability of thickness measurement was determined in vitro with a set of eight PMMA lenses having central thicknesses of 100.6 to 691.2  $\mu\text{m}$  and front surface curvatures of 7.15 to 7.52 mm. The central thickness was measured at the apex of each lens, and the images were processed as described earlier. The measurements were taken twice within a day, and the repeatability was calculated as the SD of the differences between the two measurements. We also tested the short-term repeatability in the measurement of corneal thickness by measuring corneal thickness twice within 10 minutes in 20 eyes of 10 healthy subjects. The repeatability of the lens measurements was 0.94  $\mu\text{m}$  and that of the corneal thickness was 1.5  $\mu\text{m}$ . The repeatability of the measurements of tear menisci were estimated in this study between blinks at baseline and reported in Table 1 as the SD of the differences between blinks.

Data are presented as mean  $\pm$  SD. Data analysis was performed on computer (SAS Institute Inc., Cary, NC). Paired *t*-tests were used to determine whether there were any significant changes ( $P < 0.05$ ) between blinks and between days in each eye group at each visit. The averaged data were analyzed with paired *t*-tests to determine the difference between eyes. Pearson correlation was used to test correlation between upper and lower tear menisci, and a regression of TFT on meniscus variables by linear mixed effects model was used to test any significant predictor of TFT in each eye group at each visit.

## RESULTS

The average central TFT from repeated measurements of 40 eyes was  $3.4 \pm 2.6 \mu\text{m}$  (Table 1). The UTMC was  $239 \pm 112 \mu\text{m}$  and was not significantly different from the LTMC ( $P > 0.05$  in right and left eye groups). The UTMH was  $268 \pm 68 \mu\text{m}$  and was not significantly different from the LTMH ( $P > 0.05$ ). The UTMA was  $22,732 \pm 11,974 \mu\text{m}^2$  and was not significantly



**FIGURE 2.** Image processing of tear film thickness and tear meniscus height. All meniscus variables of upper (A) and lower (C) tear menisci were measured with a custom software program with operator inputs. Touch points (pink dots) between two of these elements (eyelid, cornea, and tear meniscus) and a middle point (green dot) of the front edge of the tear meniscus were marked. After that, the software processed the image, to yield the results. The three-point method was used to fit a circle to yield tear the meniscus curvature. After central unusable 30 axial scans with specular reflex were removed from 8 consecutive images, the central 21 axial scans of these images from the cornea at baseline (C) and after instillation of artificial tears (Refresh Liquigel; Allergan, Irvine, CA) (D) were processed to create averaged reflectivity profiles of the baseline (E) and postinstillation image (F). Note that the tear film was too thin to visualize at baseline. From the baseline image (left), the calculated thickness of the cornea plus the tear film was  $487.5 \mu\text{m}$ . After the instillation, the image (right) showed the interface between the tears and cornea, enabling the determination of corneal thickness. The thickness of the tear film at baseline image was obtained by subtracting the corneal thickness (without tear film) of the right image from the total thickness (with tear film) of left image. The thickness of the cornea of the right image was  $480 \mu\text{m}$ . Therefore, the thickness of the tear film before the instillation of the eye drop was  $7.5 \mu\text{m}$ .

different from the LTMA ( $P > 0.05$ ). There were no significant differences between left and right eyes except for UTMA ( $P = 0.02$ ). There were no significant differences in any of the measured variables between the two blinks obtained during each visit or between the two mean results measured on two consecutive days in both right and left eye groups ( $P > 0.05$ ).

Among the individuals, there were very strong linear correlations ( $r = 0.47$ - $0.87$ ) between upper and lower tear menisci in each eye group on each day in the curvature, height, and area (all  $P \leq 0.03$ ; Figs. 3, 4, and 5, respectively). However, none of the tear meniscus variables was a significant predictor of TFT when a random coefficient for each participant was assumed (all  $P > 0.44$ ; Figs. 6, 7, and 8, respectively).

## DISCUSSION

OCT is an imaging modality based on the magnitude of back-scattered light reflected from target tissues.<sup>16-20</sup> This quick, noncontact and noninvasive method has been intensively used in vivo and in vitro for quantitative and qualitative analyses of the posterior segment of the eye, including thickness measurements of the retina and nerve fiber layers in various conditions.<sup>21-24</sup> OCT has also been used to measure the thickness of

the cornea and epithelium,<sup>25-27</sup> corneal flap thickness during LASIK,<sup>28,29</sup> anterior chamber angle,<sup>30</sup> and iris thickness.<sup>30</sup> The OCT with approximately  $10\text{-}\mu\text{m}$  optical resolution has good repeatability, with variations from approximately  $1 \mu\text{m}^{15}$  to  $4 \mu\text{m}^{27,31}$  in the measurement of corneal thickness. In other words, the location of an interface would be as precise as  $1 \mu\text{m}$  if many scans or measurements are averaged. In this study, we developed a real-time OCT to image the tear meniscus around the upper and lower eyelids simultaneously. At the same time, corneal thickness with tear film was imaged for further calculation to obtain central TFT from eight images obtained within 1 second. Our OCT and software, which processes multiple axial scans of multiple images, has approximately  $1\text{-}\mu\text{m}$  repeatability in the measurements of the thickness of PMMA lenses and  $1.5 \mu\text{m}$  in the measurements of corneal thickness. With a sample size of 20% and 90% statistical power, the system is able to detect  $3 \mu\text{m}$  as the true difference. The TFT was found to be  $3.4 \mu\text{m}$ , which is unlikely, due to a random measurement error. The result is in good agreement with the finding of King-Smith et al.<sup>32</sup> and our previous results, using a commercially available OCT in another country (Canada).<sup>33</sup> A similar method was developed in the previous study based on the fact that all OCT measurements of corneal thickness include the precorneal tear

TABLE 1. Tear Film and Menisci Variables\*

	TFT ( $\mu\text{m}$ )	UTMC ( $\mu\text{m}$ )	UTMH ( $\mu\text{m}$ )	UTMA ( $\mu\text{m}^2$ )	LTMC ( $\mu\text{m}$ )	LTMH ( $\mu\text{m}$ )	LTMA ( $\mu\text{m}^2$ )
Total Mean	3.4	239.2	268.1	22,731.5	259.7	258.8	23,999.5
SD	2.6	112.2	68.4	11,974.4	153.0	77.2	15,179.5
Difference between days							
Day 1 – day 2	0.5	-3.5	0.5	-1,402.7	-19.2	11.3	1,759.0
SD	3.8	144.3	70.3	11,044.9	168.9	87.2	12,009.7
$P$ †	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Differences between eyes							
Right – left	-0.5	4.3	33.1	7,880.9	75.1	25.0	6,960.9
SD	2.8	145.0	81.9	13,246.1	172.9	79.1	16,862.0
$P$ (paired $t$ -test)	0.45	0.90	0.09	0.02	0.07	0.17	0.08
Difference between blinks							
Blink 1 – blink 2	0.3	10.7	-0.7	532.3	-7.3	4.7	-761.2
SD	3.6	112.2	68.6	11,790.9	168.6	40.7	8,305.2
$P$ †	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

\*  $n = 40$  eyes of 20 subjects.

† Paired  $t$ -tests in both right and left eye groups.

film.<sup>34</sup> OCT appears to be a useful tool in studying the distribution of the tears on ocular surface. The results between blinks and between two consecutive days indicated that the measurements of these variables can be repeated with small differences. This method appears to be capable of detecting changes in the tears after treatment, such as instillation of artificial tears, punctual occlusion, and so on. It may also be useful in tracking dynamic changes in the tears and study retention time of artificial tears.

The thickness of tear film is regarded as a key variable in the study of the formation and maintenance of a normal tear film layer. However, due to the difficulty of measuring a changing fluid layer, estimates of even the static human TFT have varied from approximately 3<sup>32</sup> to 40  $\mu\text{m}$ ,<sup>35</sup> with no consensus. The first measurements of TFT used invasive methods that potentially disturbed the tear film. Both Mishima<sup>36</sup> and Benedetto et al.<sup>37</sup> measured fluorescence after instilling fluorescein and reported a TFT of 4  $\mu\text{m}$ ; however, the tear film may have been diluted by the saline-fluorescein instillation. In addition, the saline-fluorescein layer thickness may not have been the same as that of the normal tear film. Prydal et al.<sup>35</sup> reported thickness values of 41 to 46  $\mu\text{m}$  using confocal microscopy. Mishima,<sup>36</sup>

using invasive methods with fine glass filaments and fluorometry, found an average thickness of 7  $\mu\text{m}$  in the rabbit eye. This thickness has been widely cited in the literature, although whether the rabbit's tear film differs from the human's is unknown. By applying an absorbent paper disc to the cornea,<sup>38</sup> human TFT was found to be approximately 8  $\mu\text{m}$ , which could be an overestimate if reflex tears were generated and/or if the fluid was drawn from the tear film surrounding the disc or epithelium. Chen et al.<sup>39</sup> examining the tear film in rat corneas using an in vivo cryofixation method, found that the tear film varied from 2 to 6  $\mu\text{m}$  in thickness on the corneal surface. Using modified OCT, Wang et al.<sup>33</sup> studied 80 human eyes and found the thickness of the tear film to be 3.3  $\mu\text{m}$ , which is in agreement with King-Smith et al.,<sup>32</sup> who used an interferometric method in vivo. These results are in very good agreement with the results of the study presented here. In the study by Wang et al.,<sup>33</sup> a steep contact lens was used to reveal the interface between tear film and the cornea so that the tear film thickness could be calculated. We used a similar approach in this study where artificial tears increased the TFT and enabled the determination of the corneal thickness for calculation of the tear film at baseline. Once the true corneal thickness is obtained, the changes of TFT can be studied by measuring total corneal thickness between blinks over time. Investigation of the drying of tear film before and after instillation of differ-

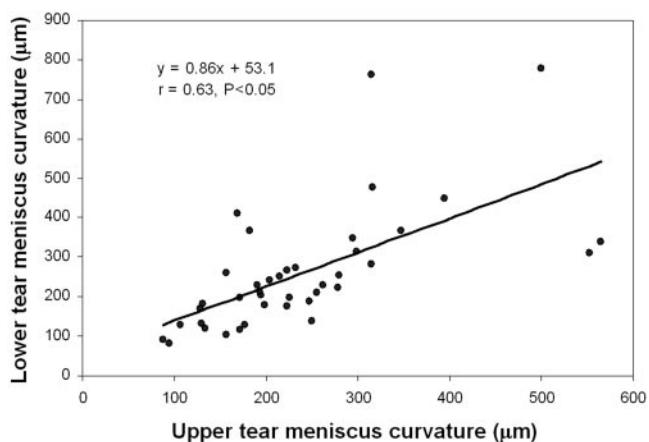


FIGURE 3. Tear meniscus curvatures around the upper and lower eyelids. There was a good correlation between upper and lower tear menisci with no significant differences in the mean values. Each dot represents the mean of four measurements taken after two blinks repeated on two consecutive days in 40 eyes of 20 subjects. The same population and treatment provided the data for Figures 3 through 8.

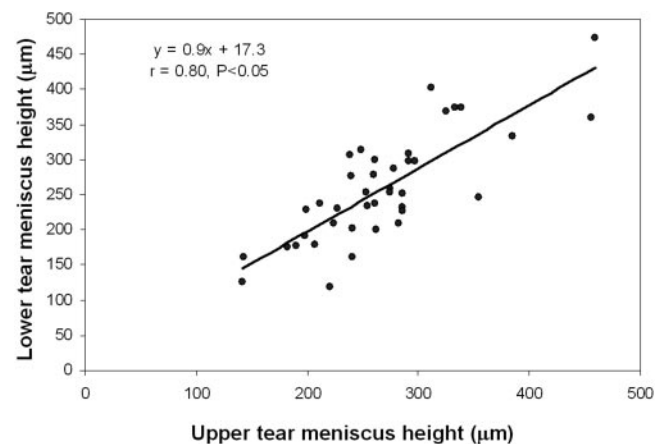
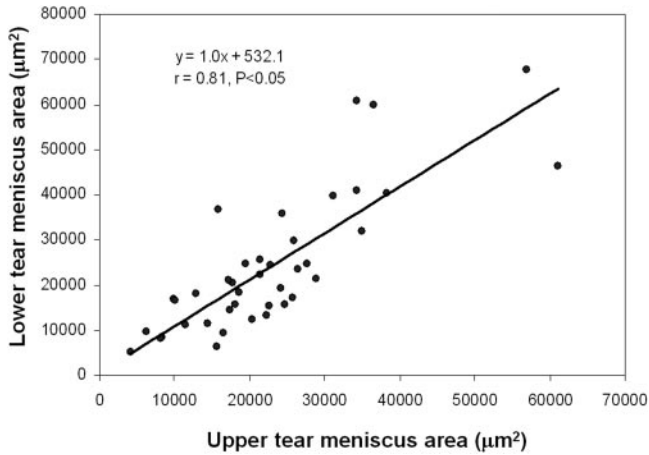


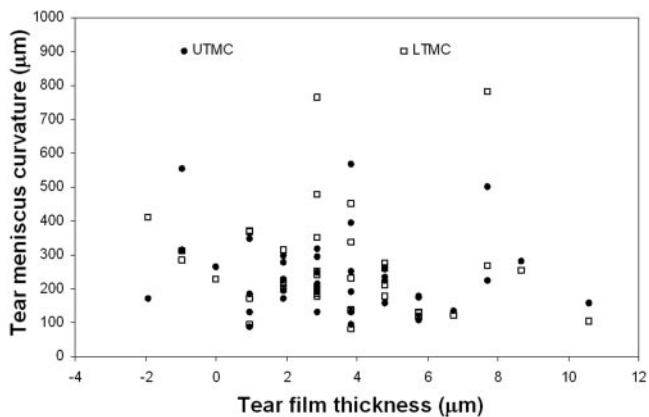
FIGURE 4. Tear meniscus heights around the upper and lower eyelids. There was a good correlation between upper and lower tear menisci, with no significant differences in the mean values.



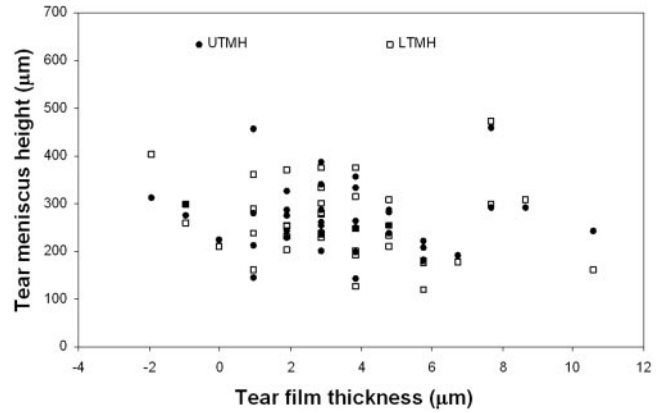
**FIGURE 5.** Tear meniscus area around the upper and lower eyelids. There was a good correlation between upper and lower tear menisci, with no significant differences in the mean values.

ent artificial tears can also be conducted with this method. A most recent study demonstrating the thinning of the tear film used an interferometric method<sup>40</sup> and indicated the dynamic changes of the tear film during blinking. To study the whole system of the tears, a real-time imaging modality would be useful in simultaneous imaging of upper and lower lid margins and central cornea.

The dimensional values of the upper and lower tear menisci were obtained simultaneously for the first time to the best of our knowledge. Previously, individual aspects of tear menisci were measured with different approaches.<sup>10,41-44</sup> Using a video meniscometer,<sup>42,43</sup> photography,<sup>41</sup> and video assessment, LTMH was measured and yielded different results ranging from 0.17 mm in elderly,<sup>44</sup> 0.19 to 0.24 mm in dry eyes,<sup>41,42</sup> and 0.46 mm in normal human eyes. In this study, LTMH was 0.259 mm, which was lower than that found by Mainstone et al.<sup>41</sup> using fluorescein. The use of fluorescein by Mainstone et al. might have added extra fluid onto the tear film or caused some reflex tearing that could have resulted in an increase of tear meniscus as was evident in a study by Oguz et al.<sup>42</sup> Yokoi et al.<sup>43</sup> and Oguz et al.<sup>42</sup> measured LTMC by video meniscometry in dry eye patients and found that it was 0.12 to 0.24 mm, which is less than the 0.26 mm that we found in normal subjects. In contrast, Mainstone et al.,<sup>41</sup> using fluorescein,



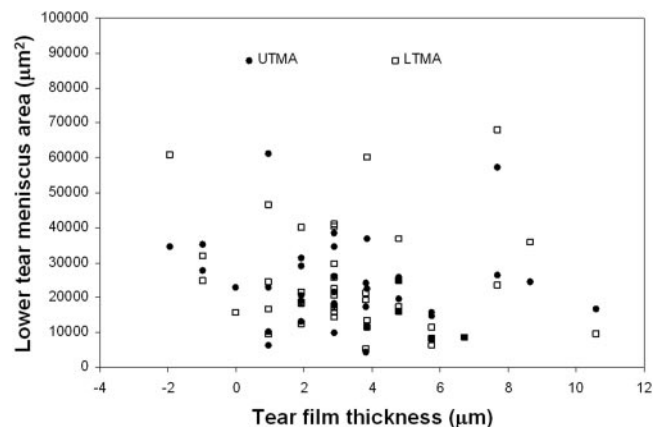
**FIGURE 6.** Central TFT and tear meniscus curvatures. There was no significant correlation ( $P > 0.05$ ) between TFT and tear meniscus curvatures. Negative TFT values were due calculations from two separate measurements. Corneal thickening may also have contributed to the negative results.



**FIGURE 7.** Central TFT and tear meniscus heights. There was no significant correlation ( $P > 0.05$ ) between TFT and tear meniscus heights.

cein, reported a mean LTMC of 0.31 mm in patients with dry eye and 0.55 mm in normal control subjects. Both the light or flashlight needed with the tear meniscometer and the use of fluorescein might cause some tearing and result in overestimation of the tear meniscus. In contrast, no visible light is required for the OCT system, and room light is sufficient for monitoring the eye position. It appears that the OCT method may be very suitable for studying the tear meniscus. In our results, the UTMH of 0.24 mm is consistent with that obtained by Creech in 1995 using photographic methods and cited as a personal communication by Wong et al.<sup>10</sup>

The relationships among dimensional variables may be the key in understanding the tear system and predicting problematic compartments in ocular diseases. In this study, there were no significant correlations between TFT and any of the measured variables of tear menisci in normal healthy subjects. Correlations have been predicted from mathematical models by Creech et al.<sup>8</sup> and Wong et al.<sup>10</sup> They predicted that the tear film formation is based on a coating process of the upper lid meniscus and suggested that at a critical central TFT, the tear breaks up over the cornea. The tear-thinning process occurring between the blink and the tear breakup was thought to be related to the initial meniscus radius and initial TFT. However, their predictions have never been verified by precise and repeatable measurements of these variables due to the extreme difficulty of measuring the TFT and tear meniscus in real time. Creech et al.<sup>8</sup> used theoretical predictions of fluid mechanics



**FIGURE 8.** Central TFT and tear meniscus areas. There was no significant correlation ( $P > 0.05$ ) between TFT and tear meniscus areas.

to estimate TFT, yielding a mean of 10.4  $\mu\text{m}$ , which may be an overestimation, according to recent studies.<sup>32,33,40</sup> Our result, determined in this study, was 3.4  $\mu\text{m}$ . There are uncertainties in the assumptions of this model including the viscosity, surface tension, and upper eyelid velocity and in the assumption that the upper tear meniscus is not depleted during its upward motion. Having calculated the static TFT from the tear meniscus radius, and having found that static TFT was associated with tear breakup time, Creech et al.<sup>8</sup> predicted there would be a critical tear breakup thickness for the tear film on each individual. However, the assumption of a critical TFT at the time of tear breakup was never confirmed. In this study, no significant correlations were established between TFT and any other variables of tear menisci. It may be that the changes in TFT are very small during normal blinks, and the OCT would not be able to detect such subtle changes with the sample size in this study. Further studies with large sample sizes, and/or with prolonged eye opening, and/or with artificial tears are warranted.

Correlations between variables within tear menisci and between upper and lower tear menisci were established in this study. Of note, significant correlations between upper and lower tear menisci were found for the first time, although there were no significant differences between the mean values. In patients with dry eye, Oguz et al.<sup>42</sup> found a significant correlation between LTMC and LTMH. The differences of the upper tear menisci between the right and left eyes may be due to differences in the lid margin structure. The negative TFTs for some subjects are probably due to calculations based on measurements from slightly different locations on the cornea. In addition, corneal thickening after the instillation of the drops may also contribute to the negative findings.

The relatively poor agreement of results between days may be due to the variability of the dynamic tear system in addition to measurement error. As indicated in Table 1, the repeatability of the differences between blinks (short term) was slightly better than the results obtained between days. Of interest, the mean of the differences was fairly small between blinks and between days, and this may indicate random variation rather than any tendency. The differences of the tear meniscus variables (excepted for UTMC) between days were greater than the differences between blinks. The averaged differences between blinks and between days were less than 10% of all tear meniscus variables compared with the large variability reported in Table 1. In addition to measurement error, random factors like different space configuration formed by the eyeball and eyelids, palpebral apertures, tear secretion, drainage, and other unknown factors may have contributed to the measurement variations. The mean value may have some clinical implications, which we do not know yet, especially for the upper tear meniscus. Further studies are warranted in comparison between patients with dry eye and normal population.

Because of the dynamic nature of the tear system, it is unlikely that we can obtain two exactly identical images of the upper and lower menisci. Therefore, repeatability of the measurements was not tested between blinks, days, and eyes. However, we could estimate the repeatability of the measurements by looking at the differences between blinks since the variation would be small in such a short period. We found that the repeatability between blinks was slightly better than the results between days, as shown in Table 1. Johnson and Murphy<sup>45</sup> found that the repeatability of five methods measuring tear meniscus height was better over the short-term than on different days. They suggested the variability of the true tear meniscus was responsible for the poor repeatability between days. The blink itself alters the tear distribution, resulting in the variability of the tear menisci measured in this study. The measurement error induced by the study settings would be

smaller than the repeatability between blinks. Even including the results between blinks as a component of the system error, with a sample size of 20, the system is able to detect one standard deviation of the true difference of these tear meniscus variables according to a software program (Gpower, Ver. 2) developed by Erdfelder et al.<sup>46</sup> This could be enough to monitor the changes of tear menisci, which showed increases by a factor of 2 to 10 after the instillation of artificial tears (Fig. 1). Johnson and Murphy<sup>45</sup> found a 150- $\mu\text{m}$  increase in tear meniscus height after 5  $\mu\text{L}$  of tears were instilled in the eye.

Several factors may contribute to the measurement error. The vertical scan plane during blinks and on different days may be altered by horizontal motion, although the subject was fixating on a central target and a specular reflex was viewed on a TV monitor. The error may be small, since the tear menisci may be approximately the same in the central region of the eye lid. This issue appears to exist in all previous reported methods in which the cross-section technique is used in the measurement of the tear meniscus.<sup>45</sup> In our system, en face and vertical motions would not affect image processing since all light beams are parallel because of the telecentric optical design of the probe. Another factor introducing measurement error could be the palpebral aperture. The palpebral aperture, the distance between upper and lower lids, varies even if the subject was asked to maintain a naturally opened eye at primary gaze during OCT imaging. This contributes to the variation of tear meniscus variables reported in this study, and no method was used to control the palpebral apertures during blinks. The variability was expected, and the results between blinks and between days are a component of the baseline data. Further studies investigating the relation between tear menisci and palpebral apertures could address the magnitude of the error that may exist in this study. Manual inputs outlining the tear menisci would also cause some error in the measurements. Further development of the software is needed to detect the borders and junctions of the tear meniscus automatically, to possibly enhance the repeatability of the measurements. The whole tear meniscus around the lower eyelid due to a big palpebral aperture was not always visualized in some subjects during study screening with the OCT shallow scan depth setting (2 mm in this study). These subjects were excluded in this study. The limitation could be eliminated by increasing the OCT scan depth.

In summary, OCT is a versatile technique for noninvasive, real-time and high-resolution imaging of the tears of human eyes as demonstrated for the first time. The method described herein may lead to a new era in studying the tear system and the diagnosis of tear-related disorders, such as dry eye. The objective evaluation of dry eye treatments, such as the instillation of artificial tears and punctal occlusion, could be made with the OCT. Upper and lower tear menisci seem to be identical in their dimensions and to correlate well with one another. Further studies will be conducted to characterize tear dynamics and study the relationships between them and other tear tests such as tear breakup time and Schirmer's test. This method will also be used to study the impact of tear drying on vision and ocular comfort.

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