Measurement of Tear Film Thickness Using Ultrahigh-Resolution Optical Coherence Tomography

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PURPOSE. To visualize the precorneal tear film with ultrahigh-resolution spectral domain optical coherence tomography, enabling quantification of tear film thickness in healthy subjects.

METHODS. A custom-built spectral domain optical coherence tomography system comprising a broadband titanium:sapphire laser operating at 800 nm and a high-speed charge coupled device (CCD) camera with a read-out rate of 47 kHz was used for measurement of precorneal tear film thickness. The system provides a theoretical axial resolution of 1.2 µm in tissue. The signal-to-noise ratio close to the zero delay was measured with 94 dB. A total of 26 healthy subjects were included in this study. Measurement was started immediately after blinking and averaged over a period of 1 second. In a subset of eight healthy subjects, the reproducibility of the approach was studied by measuring the tear film thickness every 10 minutes over 1 hour.

RESULTS. The average central tear film thickness of the measured population was 4.79 ± 0.88 µm. Reproducibility was very high, with an intraclass correlation coefficient of 0.97. A breakup of the tear film was observed in one subject after 14 seconds.

CONCLUSIONS. Our data indicate that the human precorneal tear film can be measured with excellent reproducibility using ultrahigh-resolution optical coherence tomography. This technique may be a valuable tool in the management of dry eye syndrome. (ClinicalTrials.gov number, NCT01746602.)

Keywords: optical coherence tomography, tear film thickness, dry eyes

Dry eye syndrome (DES) is a highly prevalent disease affecting the ocular surface with potentially sight-threatening potential.1 A major challenge in DES is that the association between the symptoms of the disease and the signs that can be objectively measured are weak.2 One of the most widely used techniques is the measurement of breakup time (BUT), which is employed in clinical routine as well as in clinical trials. However, this technique provides poor reproducibility and significant intra- and interobserver variability.

Traditional methods used for estimation of tear film thickness have an invasive character and include the application of absorbent paper to the cornea,3 the placement of glass fibers against the cornea,4 and the measurement of fluorescence after instilling fluorescein.5 Studies using these methods have reported tear film thickness values between 4 µm and 8.5 µm.5,6 Later, noninvasive approaches such as confocal microscopy and interferometry have been used to quantify the tear film thickness and have shown a large variation of results ranging from 3 µm to 46 µm.6,7

Optical coherence tomography (OCT) is a noninvasive in vivo imaging modality capable of generating images of biological tissues with high axial and transverse resolutions. To date, OCT has made its most significant impact in the field of ophthalmology. It has been successfully employed for obtaining high-resolution images of both anterior11–13 and posterior segments14 of the human eye. In tissues such as skin,14 OCT is mainly limited by strong scattering and absorption of incident light. By performing OCT imaging at 1300 nm, indirect measurement of tear film thickness has yielded values of 3.4 ± 2.6 µm.15–17 With the advances in broadband light sources, direct visualization of the normal tear film became possible and some effort was directed towards the measurement of tear film thickness by means of OCT.19,16,18–21

In this paper, we report on measurement of tear film thickness using a custom-built ultrahigh-resolution OCT system, where central tear film thickness is determined in a fully automated way, and provide data on reproducibility.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of the Medical University of Vienna and was carried out in accordance
with the tenets of the Declaration of Helsinki. A total of 26 healthy female and male subjects aged between 19 and 35 years participated in this study. The nature of the study was explained to all subjects, and they gave written consent to participate. Each subject passed an ophthalmic screening examination including slit-lamp biomicroscopy, indirect funduscopy, and applanation tonometry. Inclusion criteria were normal ophthalmic findings and IOP < 20 mm Hg.

Spectral Domain (SD)-OCT System

The cornea was imaged by a spectrometer-based ultrahigh-resolution SD-OCT system operating at 800 nm. As light source, a broadband titanium:sapphire laser (Integral OCT; Femtolasers Produktions GmbH, Vienna, Austria) was used. The spectrum of the laser was centered at 800 nm with a full width at half maximum (FWHM) bandwidth of 170 nm, resulting in a theoretical axial resolution of 1.2 μm in the cornea. A fiber-optical coupler with an asymmetric splitting ratio of 90:10 was used to divide the light coming from the source into the sample and reference arm. The free-space pathway of the reference arm contained a variable neutral density (ND) filter and a dispersion compensator (LSM04DC; Thorlabs GmbH, Dachau/Munich, Germany) for balancing dispersion due to optic components in the sample arm. In the sample arm, light was collimated by means of a fiber collimator (f = 12 mm; Schäfter+Kirchhoff GmbH, Hamburg, Germany), passed two galvanometric mirrors (GVS002; Thorlabs GmbH) for scanning in two dimensions, and was focused onto the sample by an OCT scan lens (LSM04-BB; Thorlabs GmbH). The power of the incident light focused onto the cornea was lowered to 800 μW, which is 10 times below the maximum permissible exposure as specified by the American National Standards Institute (ANSI), to ensure the safety of the eye. The light-delivery system of the sample arm was mounted on a modified slit-lamp headrest. The interference spectrum returning from the interferometer was directed onto a 50 × 50-mm transmission grating with λ = 1200 lines per mm (1200 l/mm; Wasatch Photonics, Logan, UT) using a collimator with a focal length f = 100 mm (OZ Optics, Ottawa, Canada). The dispersed light emerging from the transmissive grating was imaged onto a high-speed CCD camera (e2v EM4CL 2014; Aviva, Essex, UK) by means of an objective with a focal length f = 85 mm (ZEISS PLANAR T 1.4/85 ZF-IR4; Carl Zeiss AG, Oberkochen, Germany). The system was operated at an acquisition rate of 24,000 A-scans/s. Each OCT B-scan comprised 1024 pixels in depth and 512 or 1024 A-lines, respectively. The transverse resolution of the employed OCT system was 21 μm at the front surface of the cornea.

Experimental Paradigm

In all subjects, the right eye was chosen for OCT imaging of the cornea and tear film. Tomograms were recorded at the center of the cornea directly above and out of the central specular reflex of the probe beam at the corneal apex (Fig. 1). The correct alignment of the probe beam onto the desired measurement location was controlled by means of a CCD video camera. The subjects were asked to look straight forward onto an internal fixation target and to avoid blinking during the recording period, but to blink normally during the alignment procedure. After a short resting period, subjects were asked to blink, and imaging started immediately after opening of the eyes. For measurements of central tear film thickness, 20 tomograms, each comprising 1024 A-lines, at the corneal apex, were recorded. This led to a measurement time of approximately 1 second. In a subgroup of eight participants, imaging of the tear film was repeated every 10 minutes over a period of 1 hour to assess reproducibility of our approach. Furthermore, in one subject, tear film thickness was recorded over 16 seconds to examine the change in thickness over time. To do so, 16 three-dimensional (3-D) volumes with a size of 4 mm × 4 mm × 1 mm (horizontal × vertical × depth), each containing 512 × 128 × 1024 pixels were acquired within 16 seconds. For measurement of central tear film thickness, in each
volume, the 10 frames above the central reflex were postprocessed and gave the thickness value for a certain point in time.

Data Processing and Analysis

Before segmentation of the layers at the anterior eye, the stack of 20 images was preprocessed using the freeware software ImageJ (National Institutes of Health, Bethesda, MD; available in the public domain at http://rsbweb.nih.gov/ij/). This procedure comprised the adjustment of brightness and contrast, application of a median filter for smoothing of the edges, and registration of the images by vertical and horizontal translation. Thereafter, the image stack was loaded into software written in National

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**Figure 2.** Detection scheme for tear film thickness. (a) Line-wise contrast enhancement and automatic detection of air-tear film interface by means of Dijkstra algorithm. (b) Flattening of the tear film. (c) Definition of the tear film in the linearized image. (d) Intensity profile of an A-scan and definition of the tear film thickness as the distance between tear film front surface (blue arrow) and cornea front surface (green arrow). The additional sharp peak arises from the linearization of the tear film using the Dijkstra algorithm.

**Figure 3.** Precorneal tear film thickness as obtained in 26 healthy subjects. The mean ± SD is shown.

**Figure 4.** Time course of precorneal tear film thickness in a healthy subject, over 16 seconds. A significant decrease in tear film thickness is observed.
Instruments LabView 2011 (Austin, TX) for automatic segmentation of precorneal and corneal layers. First, the front surface of the tear film (i.e., the highly reflective surface at the air-sample interface) was detected by means of the Dijkstra algorithm (see Fig. 2a). Thereafter, the curvature of the tear film layer was used to flatten the corneal structures (see Fig. 2b). For each A-scan (i.e., each vertical line within the image), the intensity profile was plotted, and tear film thickness was defined as the distance between the strongest peak arising from the interface air-tear (blue arrow in Fig. 2c) and the peak caused by scattering and reflection of the incident probe beam at the interface tear-epithelium. Finally, the average tear film thickness was calculated as the mean value over the entire central cornea. All axial distance values for the tear film obtained with OCT were divided by the average group refractive index for the tear film of 1.339 in order to obtain geometrical distances (value was interpolated from the values given for 400 nm, 588 nm, 700 nm, and 1300 nm).

In all subjects, the tear film was evaluated as the time mean over the 1-second measurement period. If, in an OCT image, the automatic segmentation algorithm was not capable of identifying the tear film–cornea interface, the image was excluded from further analysis.

To quantify reliability of measurements, intraclass correlation coefficients (κ) were calculated (28). The calculation of κ is based on a repeated-measure ANOVA model using the variance among subjects (σS2), the variance among measurements (σM2), and the residual error variance (σe2) and is given by

$$\kappa = \frac{\sigma_S^2 - \sigma_e^2}{\sigma_S^2 + \sigma_M^2 + 2\sigma_e^2}$$

The higher the intraclass correlation coefficient is, the better the reproducibility of the method. A κ of 1 reflects perfect reproducibility. In addition, the coefficients of variation (CVs) were calculated. For this purpose, the standard deviation (SD) was calculated for each subject individually. By dividing the SD by the individual mean of tear film thickness, a CV was calculated. As a measure of reproducibility, the mean and SD of these individual CVs are presented.

**RESULTS**

In Figure 1, an exemplary ultrahigh-resolution OCT image of the human cornea is depicted. Precorneal tear film—the topmost highly reflective layer—and all corneal layers (i.e., corneal epithelium, Bowman’s layer, corneal stroma, Descemet’s membrane, and corneal endothelium) can be distinguished. Central tear film thickness data as obtained in 26 healthy subjects are presented in Figure 3. The SD over the 1 second of measurement time was typically 1 μm. In one subject (subject number 16), only one image fulfilled the quality criteria, and as such no SD could be calculated. The average tear film thickness in all participating subjects was 4.79 ± 0.88 μm. The range was relatively narrow with values between 3.8 and 6.8 μm.

The CV over the seven measurements within 1 hour was 3.8% ± 3.2% in the eight subjects that participated in the reproducibility studies. The maximum CV that was seen in a subject was 10.6%. The intraclass correlation coefficient was 0.97.

The time course of precorneal tear film thickness in a healthy subject over 16 seconds is presented in Figure 4. A significant decrease in tear film thickness of approximately 3 μm was observed over the observation period. Furthermore, in this subject, a breakup of the tear film at the periphery of the cornea could be observed after 14 seconds. An exemplary image showing this breakup is depicted in Figure 5. As can be seen, the tear film thickness gradually decreases from the left-hand side of the apex to almost zero and then increases again towards the periphery.

**DISCUSSION**

The present study indicates that the precorneal tear film can be quantified in a reproducible way using ultrahigh-resolution OCT based on titanium:sapphire laser. The average thickness of the tear film was found to be 4.79 μm, which is in the same range as the values observed during previous OCT studies. This is very similar to the thickness values (4.7 ± 1.6 μm) for one subject, obtained with an ultrahigh resolution OCT system using a super-contiuum source centered at 812.5 nm with an FWHM bandwidth of 375 nm and comprising a spectrometer based on Czerny–Turner configuration.

In the present study, the used scanning pattern yielded tear film thickness values of a location close to the apex of the cornea. When thickness measurements were performed over a longer...
time, a decrease in central tear film thickness could be observed (Fig. 4). The measured decrease of 3 μm is in accordance with values detected by an interferometric method. 29-30 This decrease in tear film thickness is most likely related to tangential flow. 30 Contrary to a dry eye, where evaporation contributes by a larger extent to thinning of the tear film, in the case of a healthy subject with a normal lipid layer, this effect is negligible when considering an evaporation rate 31 of 13.57 × 10^-7 g cm^-2 s^-1 (resulting in a thinning rate of 1.357 nm s^-1), which is well below the resolution of the present OCT system. The evaporation rate, however, changes dramatically when the lipid layer is ruptured, which results in a fast breakup of the tear film only a few seconds after blinking.

The approach of recording 3-D volumes over the entire cornea enables examination of tear film thickness at different locations and, thus, might allow for both detection of the tear film breakup in patients with DES and the recording of tear film thickness maps as well. Operating the system at higher acquisition speeds and recording several corneal volume data sets enable the generation of tear film thickness maps and the study of tear film dynamics within the time period between two blinks.

Recent studies suggest that measurement of tear film thickness using OCT may be used to quantify treatment success in DES. Employing a superluminescent diode with a center wavelength of 840 nm and an FWHM bandwidth of 100 nm, Wang and coworkers achieved a resolution of approximately 3 μm and were able to show that the cyclosporine treatment increased upper and lower tear meniscus volume. 32 As such, the International Workshop on Meibomian Gland Dysfunction listed OCT as one of the promising technologies to quantify signs of DES. 33-34

In conclusion, we presented a promising approach using ultrahigh-resolution OCT for imaging the precorneal tear film and made use of a fully automated algorithm for evaluating the tear film thickness. Furthermore, we were able to image breakup of the tear film in a healthy subject. This technique may have considerable potential in following patients with DES.

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