Mapping the Lipid Layer of the Human Tear Film

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Purpose: To describe a new method to distinguish between normal versus lipid-deficient dry eye using a Tear Film Imager (TFI).

Methods: Two groups of study subjects, controls versus lipid-deficient dry eye, were tested using the TFI. This instrument provides an accurate measurement of the thickness and spatial distribution of the muco-aqueous and lipid layers of the tear film. The nanometer thickness resolution of the TFI enables the creation of detailed maps of the lipid layer thickness (LLT) across the corneal surface. These maps are captured with a large field of view of 6.5 mm diameter.

Results: A LLT map taken at 1 second from a blink end in the controls appears uniform, whereas a nonuniform layer was measured in the lipid-deficient dry eye. Lipid map uniformity can quantify the spatial variation of lipid across the cornea. A case study showed the ability to distinguish between controls [lipid map uniformity (LMU) = 14 nm^2] and lipid-deficient dry eye (LMU = 125 nm^2) through characterization of the LLT distribution.

Conclusions: High-resolution lateral LLT maps demonstrate the significance of the lipid layer uniformity, which may play an important role in maintaining tear film health. LLT maps and the quantitative LMU could be used to diagnose and treat patients with dry eye.

Key Words: tear film measurement, muco-aqueous thickness, lipid layer mapping, dry eye

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The Definition and Classification Subcommittee of the Tear Film and Ocular Surface (TFOS) International Dry Eye Work Shop (DEWS) defined dry eye as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film."[1,2] Homeostasis can be evaluated by measuring both temporal stability and spatial uniformity of the tear film and its separate lipid and muco-aqueous layers. Evaluating both temporal stability and spatial uniformity is a highly demanding task, which requires the measuring instrument to provide high precision. As an example,[3] the thickness of a typical lipid layer is 30 nm, and it may change by several nanometers from pixel to pixel.[4] Therefore, to measure variations in this film, a precision of the order of a nanometer is required.

The newly developed Tear Film Imager (TFI) measures the tear film inner layers with a nanometer resolution and enables evaluation of both temporal stability and spatial uniformity. The TFI combines spectrometry and imaging to analyze the structure of the tear film layers. In addition, high-resolution measurement of the lipid layer thickness (LLT) over a large field of view provides an assessment of the spatial uniformity of the lipid layer.

It is common clinical practice to use a variety of imaging devices to document and analyze ocular structures. A colored interference pattern is often seen on the corneal surface when photographing the anterior segment. It is possible to determine the thickness of the interfering lipid layers[5,6] that create this pattern by analyzing its spectral variation. A qualitative evaluation shows that there is color variation within the interference pattern. A simplified method to measure the LLT extracts the thickness from the interference pattern color.[7] However, the color depends not only on the LLT but also on many other variables of the optical path that generated the image. This includes instrument properties, such as the physical characteristics of the light source and the spectral sensitivity of the imaging sensors. The colors further depend on the physical properties of the examined eye, such as scattering or back reflections from underlying layers. These factors will affect the accuracy of the thickness estimate based on the color alone.

Hwang et al[4] recently described a technique to measure the LLT in which analysis of color information is based on statistical scatter plots, which uses all pixels in the image to determine the average thickness. The authors report the meibomian glands hyposecretory and hypersecretory states and their connection to the average values and distribution characteristics of the lipid layers interference in large areas. Clinical evaluation of this method shows a correlation between average LLT and meibomian gland grading as clinically evaluated by an ophthalmologist. The technique of Hwang et al is limited about the uniformity information of

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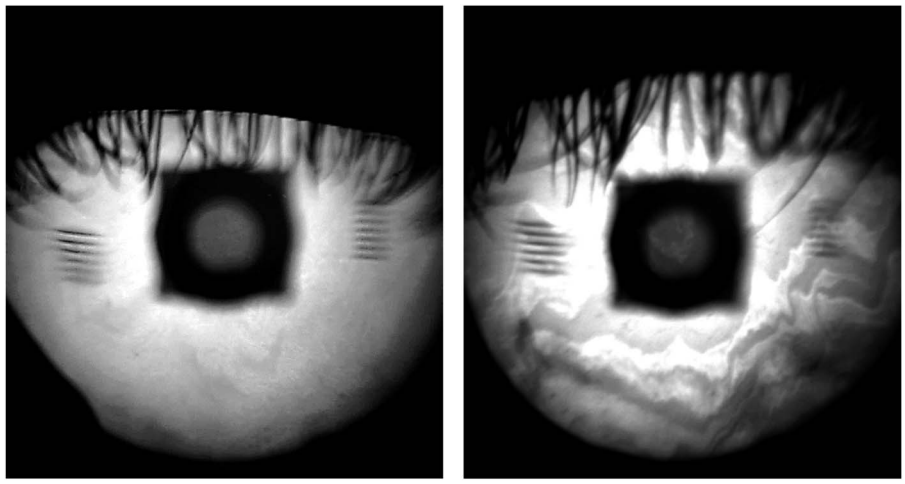

FIGURE 1. Raw images of the lipid layer reflections as captured by the TFI one second after blinking: A, An image of a control patient's eye. The low contrast in the image results from high level of uniformity of the LLT. B, An image of a lipid-deficient dry eye patient's eye. Notes: 1) The central black square is instrumental shadowing. It is not related to the eye and contains no data. 2) The central circle within the square is imaged and processed using the device. This circle is not related to the pupil and contains surface tear film data. 3) The 2 blocks of horizontal stripes on the left and right of the square are patterns projected by the optics of the device and are not related to the eye.

the LLT because it is based on statistical analysis and alters the spatial information of the gathered reflections.

The importance of time stability and spatial distribution of LLT was demonstrated by Goto and Tseng.[8] In their study, the authors used a high frame rate camera (DR-1) to present qualitative differences between healthy patients and lipid tear-deficient patients. Szczesna-Iskander[9] further used projected interferograms to study the tear film surface quality kinetics. The author argues that the dynamics after a natural blink are faster on dry eyes than on healthy eyes, while emphasizing the importance of imaging with "natural blink conditions."

In this study, we report a technological solution that combines the strengths of all aforementioned approaches by incorporating time-resolved spectrometric measurements, color camera images, and image processing. The resulting high-resolution lipid thickness maps present spatial representation of LLT and enable assessment of lipid layer uniformity and dynamics.

THE TFI

The TFI, a device developed and produced by AdOM Advanced Optical Technologies Ltd. (Lod, Israel), measures the static and dynamic properties of different layers of the tear film by creating an image of the cornea that overlies a spectral analysis of the reflected light. The detailed optical scheme of the device has been described elsewhere.[10,11] In this device, the cornea is illuminated by a broadband light source with a large angular distribution. The specular reflection from this beam is collected from each point on the ocular surface using an objective lens aligned normal to the corneal apex.

The design guarantees that specular reflection from the large field of view will be imaged to 2 conjugate planes: one is the camera imager and the other is the spectrometer sensor. In this configuration, points on the corneal surface are imaged to specific pixels of the camera. The spectrometer is located at the equivalent point of the image plane referenced to the center of the cornea. This scheme ensures that the light recorded by the camera and spectral sensor will be mostly specular reflectance from the cornea. The optical design minimizes the collection of scattered and specular light from other locations on the corneal surface.

The TFI measures the thickness of both muco-aqueous and lipid layers of the tear film with nanometer resolution. The measurement is performed as frequently as 10 times per second over 40 seconds. Natural blinking continues throughout the measurement. In addition to the time-resolved measurements at the center of the eye, the TFI produces a 3-dimensional map of the lipid layer over a large field of view (40 mm^2) with a sampling resolution of 5 μm/pixel.

The light levels of the TFI are safe, measuring less than 1 mW/cm^2 in the visible. For each measurement, a representative lipid layer image is chosen. This image is transformed to a LLT map, using TFI's image processing algorithms and the accurate spectrometric data that were obtained during the frame imaging time.

The LLT map is then used to estimate the lipid layer uniformity. Five regions of interest (ROI) are selected, clear of the eyelid image and of TFI internal shadowing. The ROIs consist of 5000 pixels each, with no overlap between the regions, to avoid statistical correlations. The same ROIs are used for every map of the TFI. The average and the variance of the LLT in each ROI are calculated separately as follows:

$$Ave_{ROI} = \sum_{k=1}^{n} \frac{LLT_k}{n},$$

$$Var_{ROI} = \frac{\sum_{k=1}^{n} (LLT_k - Ave_{ROI})^2}{n},$$

where n is the number of pixels in each ROI and LLT_k is the LLT at the *k-th* pixel.

The lipid map uniformity (LMU) was defined as the average LLT variance of 5 ROIs,

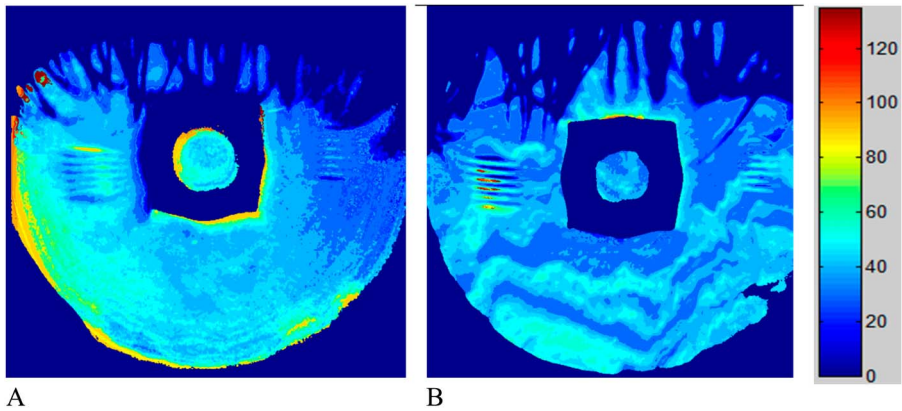

FIGURE 2. LLT maps based on the reflected images. A, A control patient's lipid map processed from image 1 in Figure 1A with a LMU of 14 nm^2, and (B) A lipid-deficient dry eye patient's lipid map processed from Figure 1B with a LMU of 125 nm^2. Each color level represents a 5-nm step in thickness.

$$\text{LMU} = \frac{\sum_{i=1}^{5} Var_{ROI_i}}{5} \quad 5 = \text{Number of ROIs.}$$

RESULTS

The TFI was evaluated in a study (TFI-LH16) conducted by Inflamax Research Inc., Canada, as approved by the local institutional review board. The study focused on 2 groups of patients: The control group of 9 subjects was defined by a tear breakup time (TBUT) greater than 7 seconds, no corneal fluorescein staining (CFS), and a Schirmer test greater than 10 mm in 5 minutes in both eyes. Twenty subjects with lipid-deficient dry eye were defined by a combined score of meibum quality and the number of expressible glands (meibomian gland dysfunction [MGD]) greater than 3 and 1 or more of the following: Schirmer test less than 10 mm in 5 minutes, TBUT less than 7 seconds, and CFS $\geq$ 1 in at least 1 eye.

Figure 1 shows 2 typical images as recorded by the camera from 2 different patients, 1 from the control group (32 mm Schirmer, 9.6 seconds TBUT, 0 CFS, 0 MGD) and 1 from the lipid-deficient dry eye group (3.5 mm Schirmer, 3.6 seconds TBUT, 0.5 CFS, 6 MGD). The frames present a typical image approximately 1 second after the blink ends. At this time, the tear fluids have mostly stabilized. The interference patterns of the lipids can be seen in both images; however, the contrast of the patterns is very low in the image of the control subject, where the lipid layer is more uniform.

The nanometer resolution of the TFI thickness measurement is essential for the evaluation of each pixel's thickness within the lipid layer map. The independent thickness measurement per pixel, in turn, enables assessment of the LMU.

Figure 2 shows the subsequent lipid thickness maps of the 2 images of Figure 1. The control patient presents a uniform LLT map with a LMU of 12 nm^2. The lipid-deficient dry eye patient, by contrast, presents a highly variable LLT map with a higher LMU value of 125 nm^2.

DISCUSSION

Accurate spatial and temporal mapping of the lipid layer of the tear film has been achieved by combining 2 measurement methods in parallel in a full-field camera that simultaneously captures the image of the lipid reflectance in a high-definition format and performs a spectrometric analysis of the image. This combination allows accurate evaluation of each sublayer thickness of the tear film with nanometer resolution. This technique tolerates the natural eye state of blinks, saccadic motions, and defocusing events. The ability to measure the lipid layer at nanometer levels is essential to estimate the variations within the lipid layer maps. This, in turn, can provide a quantitative analysis of the LMU, which consecutively can differentiate specific dry eye subgroups and allows objective assessment of the therapeutic efficacy of dry eye therapies.

In this case study, the lipid-deficient dry eye lipid layer is nonuniform and presents with a larger variance than did the normal eye. We report the "lipid map uniformity" assessment to quantify the LLT map variance and the dry eye differences. The current case study shows similar trends, as reported previously.[4,8] These results may indicate that in patients with lipid-deficient dry eye, disturbance or blockage of the MGD channels may affect the distribution of the lipid layer and, as a result, may increase evaporation of the aqueous layer below.

These LLT maps and the quantitative LMU parameter could play a significant role in the physician's ability to diagnose and treat patients with dry eye disease. Clinical usage of this technique requires larger studies to evaluate the ability of the TFI-measured parameters to distinguish many elements involved in the clinical diagnosis of dry eye.

High-resolution lateral lipid thickness maps reveal that lipid layer uniformity plays an important role in maintaining tear film health in normal eyes and when deficiency contributes to the dry eye disease complex. A clinical assessment of the tear film homeostasis with a quantified parameter can be made by assessing the LMU.

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