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In vivo thickness measurement of the lipid layer and the overall tear film by interferometry

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Abstract

New approaches for the simultaneous measure of the thickness of the lipid layer and overall tear film *in vivo* are needed. In the current research, the interference signal from the ocular surface was analyzed and simplified as the superposition of two sinusoidal functions. One function with low frequency was modulated by lipid layer (LL), and the other function with high frequency was modulated by precorneal tear film (PCTF). Two sinusoidal functions were separated and analyzed to retrieve the thickness of LL and PCTF. To validate the feasibility of the approach, a customized fiber-based interferometer with related data processing program was developed and tested *in vivo*.

Keywords

(310.0310) Thin films; (120.3180) Interferometry; (170.4460) Ophthalmic optics and devices; (030.1640) Coherence

The lipid layer (LL) of the human tear film serves as a barrier to retard the evaporation of aqueous tears [1–3]. Since the thickness dynamics of the tear film (e.g., tear film thinning, breakup) closely relates to ocular surface disease, studies of the thickness of the tear film and LL facilitates understanding of many fundamental properties in ocular health and dry eye disease [4–6]. Interferometric approaches have emerged as one of the most promising and contemporary methods to investigate the thickness of precorneal tear film (PCTF) *in vivo*, due to its considerable advantages of being noninvasive, accurate, highly resolved, and rapid [7–10]. Though the PCTF thickness can be retrieved from the fringes of spectral oscillations, current interferometers lack sufficient resolution to directly measure the LL thickness from spectral oscillations. Specifically, the thickness of LL is typically less than 200 nm thick while one cycle of interference oscillation in the visible or near-infrared light corresponds to at least ~320 nm [11]. Alternatively, conventional methods estimate the LL thickness from the reflected intensity ratios among the different wavelengths (usually red, green, and blue, according to the RGB model of camera) [12–15]. These techniques are based on the fact that the interference signal from a thin film is a sinusoidal function of wave number (inverse of wavelength) for any given thickness. Thus, the ratio among R/G/B channels of the detecting camera could be fitted to a sinusoidal function to estimate the related LL thickness. One recently reported method is to fit the measured spectrum to the

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spectral profiles simulated by interferometric principle to retrieve LL thickness [16]. While the fitting of the overall spectral profile may provide higher accuracy than the merely three-point fitting, one common drawback of these techniques is that the LL is assumed to be an isolated single-layer thin film. However, as the LL comprises a very small fraction of the overall PCTF in thickness (20–200 nm of LL to 3–5 μm of PCTF), the interference signal from the LL always intertwine with the signal from the PCTF [13]. Without an effective way to eliminate the signal of PCTF from that of LL, the accuracy of the LL determination is inevitably decreased. In addition, along with the evaporation of the PCTF, the noise of PCTF imposed on the LL varies over time, which induces more uncertainty in the LL thickness calculation. So far, there still lacks an effective way to deal with the drawback. To differentially diagnose dry eye diseases and meibomian gland dysfunction, characterization of the thickness of LL and PCTF simultaneously is essential, particularly when understanding tear film dynamics and evaporation [5, 17–19]. Unfortunately, the current proposed methods are lack sophistication in design, are heavily time-consuming and have insufficient accuracy [8, 15, 20].

The methodology proposed in this research is an approach to measure the thickness of the lipid layer and overall tear film simultaneously. The interference signal from the ocular surface was analyzed and simplified as the superposition of two sinusoidal functions. One function with low frequency was modulated by LL, the other function with high frequency was modulated by PCTF. Two sinusoidal functions were separated and analyzed to retrieve the thickness of LL and PCTF. A customized fiber-based interferometer with related data processing program was developed and tested *in vivo*.

The tear film is mainly composed of lipids, aqueous fluid, proteins, and mucins. Besides the LL, the rest of tear film can be considered as one aqueous layer resting anterior to the corneal epithelium [20]. For simplicity, suppose a low-coherence light source is applied, i.e., a light source with coherence length shorter than the thickness of the corneal epithelium ~ 50 μm [21]. Thus, the reflections posterior to the tear film do not involve with the interference of the tear film since their optical path difference (OPD) is longer than the coherence length of light source. As shown in Figure 1A, the interference signal is mainly composed of the three reflections, which are from the interface of air/lipid, lipid/aqueous and aqueous/epithelium. R_{total} is given by interferometric principle as follows:

$$R_{total} = E_{total}E_{total}^* = R_{\frac{air}{lipid}} + R_{\frac{lipid}{aqueous}} + R_{\frac{aqueous}{epithelium}} + 2\sqrt{\frac{R_{\frac{air}{lipid}}R_{\frac{lipid}{aqueous}}}{\cos\Delta\varphi_{12}}} + 2\sqrt{\frac{R_{\frac{air}{lipid}}R_{\frac{aqueous}{epithelium}}}{\cos\Delta\varphi_{13}}} + 2\sqrt{\frac{R_{\frac{lipid}{aqueous}}R_{\frac{aqueous}{epithelium}}}{\cos\Delta\varphi_{23}}} \quad (1)$$

Where E_{total} represents the total interference wave, $E_{\frac{air}{lipid}}$, $E_{\frac{lipid}{aqueous}}$ and $E_{\frac{aqueous}{epithelium}}$ represent the reflectance wave from the air/lipid, lipid/aqueous and aqueous/epithelium interfaces respectively. R_{total} is the total interference intensity, $R_{\frac{air}{lipid}}$, $R_{\frac{lipid}{aqueous}}$ and $R_{\frac{aqueous}{epithelium}}$ are the reflectance intensities from the interface of air/lipid, the lipid/aqueous and aqueous/epithelium respectively. $\Delta\varphi_{12}$, $\Delta\varphi_{13}$ and $\Delta\varphi_{23}$ represent the phase difference between the reflected waves from the interfaces of air/lipid, lipid/aqueous and aqueous/epithelium. When

the angle of incident and reflected light is normal, the phase difference of $\Delta\varphi_{12}$, $\Delta\varphi_{13}$ and $\Delta\varphi_{23}$ are described as follows:

$$\Delta\varphi_{12} = 4\pi n_{lipid} \chi t_{lipidlayer} + \pi \quad (2)$$

$$\Delta\varphi_{13} = 4\pi n_{tearfilm} \chi t_{tearfilm} \quad (3)$$

$$\Delta\varphi_{23} = 4\pi n_{aqueous} \chi t_{aqueous} + \pi \quad (4)$$

Where $t_{lipidlayer}$, $t_{tearfilm}$ and $t_{aqueous}$ represent the thickness of lipid layer, tear film and aqueous layer respectively. n_{lipid} , $n_{tearfilm}$ and $n_{aqueous}$ represents the refractive index of lipid layer, tear film and aqueous layer respectively. χ represents the wavenumber. Equation 1 may be rewritten as follows:

$$\begin{aligned} R_{total} = & R_{lipid}^{air} + R_{aqueous}^{lipid} + R_{epithelium}^{aqueous} + \\ & 2\sqrt{\frac{R_{lipid}^{air} R_{aqueous}^{lipid}}{R_{lipid}^{lipid} R_{aqueous}^{aqueous}}} \cos(4\pi n_{lipid} \chi t_{lipidlayer} + \pi) + \\ & 2\sqrt{\frac{R_{lipid}^{air} R_{epithelium}^{aqueous}}{R_{lipid}^{lipid} R_{epithelium}^{epithelium}}} \cos(4\pi n_{tearfilm} \chi t_{tearfilm}) + \\ & 2\sqrt{\frac{R_{lipid}^{lipid} R_{epithelium}^{aqueous}}{R_{aqueous}^{lipid} R_{epithelium}^{epithelium}}} \cos(4\pi n_{aqueous} \chi t_{aqueous} + \pi) \end{aligned} \quad (5)$$

Based on Fresnel's equation, the intensity of reflectance in normal incidence is written as follows:

$$R_{12} = \left| \frac{n_1 - n_2}{n_1 + n_2} \right|^2 \quad (6)$$

Where R_{12} represents the intensity of reflectance at the interface of medium 1 and 2. n_1 and n_2 represent the refractive index of medium 1 and 2 respectively. Given the refractive index of air, lipid, aqueous and epithelium are 1.0, 1.482, 1.337 and 1.401, there is a reflective index abrupt at the air-lipid interface [22, 23]. Thus, the intensity of the reflectance from the air/lipid interface is far greater than that from the lipid/aqueous and aqueous/epithelium. Specifically, $R_{lipid}^{air} : R_{aqueous}^{lipid} : R_{epithelium}^{aqueous} = 1:0.069:0.015$. Due to the rough interface of aqueous/corneal epithelium, $E_{epithelium}^{aqueous}$ is epithelium scattered and the reflected $R_{epithelium}^{aqueous}$ at normal direction is even epithelium weaker than the calculated value [7]. Thus, the component of $2\sqrt{\frac{R_{lipid}^{lipid} R_{epithelium}^{aqueous}}{R_{aqueous}^{lipid} R_{epithelium}^{epithelium}}} \cos(4\pi n_{aqueous} \chi t_{aqueous})$ is negligible in the equation(5), the total reflectance R_{total} can be simplified as the superposition of two sinusoid functions.

$$\begin{aligned}
 R_{total} \approx & R_{lipid}^{air} + R_{aqueous}^{lipid} + R_{epithelium}^{aqueous} \\
 & + 2\sqrt{\frac{R_{lipid}^{air} R_{aqueous}^{lipid}}{}} \cos(4\pi n_{lipid} \chi_{lipidlayer} + \pi) + \\
 & 2\sqrt{\frac{R_{lipid}^{air} R_{epithelium}^{aqueous}}{}} \cos(4\pi n_{tearfilm} \chi_{tearfilm})
 \end{aligned} \tag{7}$$

Given the refractive index of the epithelium, stromal anterior and posterior surfaces were 1.401, 1.380, and 1.373, the variation of the refractive index within the corneal is small [24]. Therefore, the intensity of reflectance from these interfaces is much lower than R_{lipid}^{air} . From the above analysis, a main outcome is achieved. The interference without R_{lipid}^{air} involved is relatively negligible since R_{lipid}^{air} is far greater than any other reflectance. In other words, the interference between and reflections from other interface are predominant though reflections from any pairs of interfaces can cause interference effects. Because the calculation is based on the refractive index of air, tear film and cornea, the outcome is applicable to any kind of light source. For a laser with a long coherence length of ~several mm, the reflections beneath the tear film layer are involved with the interference of the tear film. Given the thickness range of tear film and individual layers of cornea (LL in 40–200 nm, PCTF in 2–5 μ m, epithelium layer in 40–60 μ m, Bowman's layer in 8–10 μ m, stroma in 500–600 μ m), the interference signal from various interfaces underlying the tear film yield high frequency oscillations overlapping on the detected spectra [25]. For the purposes of LL and PCTF measurement, this interference noise can be eliminated by using a low pass digital filter. Thus, the reflected spectra can still be simplified as the superposition of two sinusoid functions.

A new fiber-based interferometer was developed based on a previous free-space reflectometer [7, 10, 23]. Briefly, the source is a broadband Tungsten Halogen lamp. A multimode fiber coupler delivers the light to an objective lens, which focuses the light onto the central cornea in a diameter of ~30 μ m. The reflected beam is separated from the illuminating beam via a beam splitter, and transferred to a custom-made spectrometer via a fiber coupler. The spectrometer was developed previously, and composed of a line scan and a transmission grating with central wavelength of 600 nm [15]. The acquisition time is set to be 3 ms. To aid in aligning eye, a real-time, auxiliary lipid layer imaging system is co-registered with the interferometer via a beam splitter [15]. To separate the light between the interferometer and auxiliary system, the spectral range of the interferometer is set to be 500–750 nm, while the auxiliary system's spectral range is set to be 400–500 nm via a couple of short pass and long pass filters. During the measurement, the head of subject is positioned on a chin and head rest that allow a freedom of movement in three dimensions. After the lipid layer was focused in the auxiliary imaging system, the subject was instructed to gently blink three times and to keep their eyes open until they were instructed to close them. The spectral data that were in focus collected between 6.0 s to 7.0 s after blinking were selected for analysis.

As illustrated in Figure 2, the data processing algorithm was developed from our previous work [7, 10]. Step 1: Spectra from the eye and from a reflectance standard (STAN-SSL, Ocean Optics, Largo, FL) were read from the spectrometer. Figure 2A shows one spectra from the eye, R_{eye} , and one from the reflectance standard, R_{std} . Step 2: the interference oscillation was derived from the difference between the spectra of the eye and that of reflectance standard, as shown in Figure 2B. Step 3: The derived spectral profile were decomposed into two components. One component with low frequency contains the thickness information of LL, the second component with high frequency contains that of PCTF. Step 4: From the two separated spectra, the curve-fitting analysis was carried out to retrieve the thickness of LL and PCTF individually. The resulting fit is shown in Figure 2C where the original spectra is shown by the dashed line and the simulated curves shown by the broken line. The thickness of PCTF is $1.54\ \mu\text{m}$ and the thickness of LL is $108\ \text{nm}$ in Figure 2.

To calibrate and test the accuracy of the system, one thickness standard was measured (TS-SiO₂-Multi, Filmetrics, San Diego, CA). The standard is a Si substrate wafer that contains multiple separated SiO₂ films with different thickness, 25 nm, 50 nm, 100 nm, 500 nm, and 1000 nm. On each single SiO₂ films, the interferometer measured 9 different positions. The averaged thickness values were $27 \pm 2.2\ \text{nm}$, $48 \pm 3.4\ \text{nm}$, $106 \pm 4.8\ \text{nm}$, $492 \pm 7.3\ \text{nm}$ and $1007 \pm 9.7\ \text{nm}$. The representative original spectra are shown with the solid line and the fitted curves are shown with the broken line in Figure 3.

A human subject with and without contact lens was tested *in vivo* (IRB approval was not required under the current circumstance per local policy allowing for case reports to include up to three human subjects). At the beginning of test, a drop of artificial tears was instilled onto the eye. The subject was asked to blink three times before being asked to keep the eyelids open. Figure 4A shows the reflectance curve on the apex of the cornea at 6.5 s after the final blink. The decomposed LL and PCTF spectral curves are shown in solid lines while the fitting curves in broken lines. The thickness of PCTF and LL in Figure 4A is $4.51\ \mu\text{m}$ and $113\ \text{nm}$ respectively. In the same way, the result of the artificial tears on the contact lens is shown in Figure 4B. The thickness of pre-contact lens tear film (PCTF) and LL is $5.14\ \mu\text{m}$ and $175\ \text{nm}$ respectively.

From our previous studies, the aqueous layer was thought to have a rough interface with the corneal epithelium and its microvilli and microplicae, and a Gaussian decay about the reflectance amplitude was included into the curve-fitting process [7]. The plots of PCTF in Figure 4A reflects the same phenomena while the plot of PLTF indicates a smoother surface. The curve-fitting analysis was based on the assumption that PCTF and LL thickness were uniform within the measurement spot and the refractive index were constant within the spectral range. By assuming combinations of different PCTF thickness in the measurement spot and a refractive index varying along the wavelength, better fits to the PCTF will possibly be obtained in the future. Near-perfect fits to LL thickness might imply that the assumption of a uniform LL thickness and a constant refractive index of LL are applicable. Good fits of LL thickness also imply that the interference noise beneath the lipid layer minimally affect the spectral function of LL. There are two possible reasons. One is that the magnitude of this noise is negligible compared to that of the LL. Another reason is that the

oscillating frequency of this noise is much higher than that of LL, which can be completely removed by a low-pass filter.

In summary, a superposition model of two sinusoidal functions was implemented to interpret the interference signal from the ocular surface. The model overcame the drawbacks in the conventional LL measurements. The thickness of LL and PCTF were retrieved from the interference signal separately and simultaneously. The new interferometer may offer potential to characterize many physiological and clinical aspects of the tear film.

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Figure 1.

A two-layer tear film model. The interference signal is mainly composed of the three reflections, which are from the interface of air/lipid, lipid/aqueous and aqueous/epithelium. To a light source with a limited coherence length, the reflections beneath the tear film layer do not involve with the interference of the tear film.

Figure 2. Schematic setup of a new fiber-based interferometer. Light path of the auxiliary lipid layer imaging system is patterned in gray to distinguish from that of the interferometer.

Figure 3.

Data processing from a precorneal tear film in vivo. (A) Recorded spectra from the eye, Reye, and from the reflectance standard, Rstd. (B) The interference oscillation was derived from the difference between the spectra from the eye and from the reflectance standard. (C) The derived spectral profile was decomposed into two components. The curve-fitting analysis was carried out to retrieve the thickness of LL and PCTF individually. The original spectra are shown by solid line, and the simulated curves are shown by the broken line. The plots of LL are vertically shifted.

Figure 4. Data processing of data from one commercial thickness standard, which contains multiple separated SiO₂ films on Si substrate. The original spectra are shown by the solid line, and the fitted curves are shown by the broken line.

Figure 5.

(A) Data from the PCTF of a human subject with artificial tears at time of 6.5 s after three full blinks. (B) Data from the PLTF of a human subject with artificial tears at time of 6.5 s after three full blinks. The original spectra are shown by the solid line, and the fitted curves are shown by the broken line.