Tear Film Interferometry and Corneal Surface Roughness

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PURPOSE. Previous studies of optical interference from the whole thickness of the precorneal tear film showed much lower contrast than from the pre-contact lens tear film. It is hypothesized that the recorded low contrast is related to roughness of the corneal surface compared with the smooth contact lens surface. This hypothesis is tested, and characteristics of this roughness are studied.

METHODS. Reflectance spectra were recorded from 20 healthy individuals using a silicon-based sensor used in previous studies (wavelength range, 562–1030 nm) and an indium-gallium-arsenide (InGaAs) sensor responding at longer wavelengths (912–1712 nm). Interference from the whole thickness of the precorneal tear film caused oscillations in the reflectance spectra.

RESULTS. Spectral oscillations recorded with the InGaAs sensor were found to decay as a Gaussian function of wave number (1/wavelength). This is consistent with a rough corneal surface, whose distribution of surface height is also a Gaussian function. Contrast of spectral oscillations for the InGaAs sensor was, on average, approximately four times greater than that for the silicon sensor.

CONCLUSIONS. For the Gaussian roughness model based on InGaAs spectra, the corneal surface was characterized by a surface height SD of 0.129 μm . Spectral oscillations recorded with a silicon-based camera can have higher contrast than expected from this Gaussian roughness model, indicating some reflectance from a smoother or more compact surface. The results also indicate that InGaAs cameras could provide whole-thickness interference images of higher contrast than silicon-based cameras.

Keywords: tears, interference, corneal epithelium, infrared spectroscopy, corneal surface

Optical interference has been used to study the thickness of layers of the tear film and for imaging of those layers. Some investigations involve interference between reflections from natural surfaces and interfaces such as the anterior (air) and posterior (corneal) surfaces of the tears, as well as the interface between the lipid and aqueous layers. These natural interference effects can be used to measure the thickness of the lipid layer^{1,2} and, with more difficulty because of low interference contrast, that of the precorneal tear film.^{3–5} Natural interference effects also provide good images of the lipid layer,^{6–11} but interference images from the whole thickness of the precorneal tear film have relatively low contrast and therefore poor signal-to-noise ratios.¹² In this study, the low contrast of whole-thickness interference in the precorneal tear film will be related to low reflectance from the corneal surface.

Placing a contact lens in the eye introduces two artificial surfaces and divides the tear film into pre- and post-contact lens tear films. It is notable that interference from the whole thickness of the pre-contact lens tear film is readily observed^{7,9,13,14} and has much higher contrast than for the precorneal tear film.^{4,12} Interference from the post-contact lens tear film is more difficult to study because of low contrast¹⁵ presumably related to the low reflectance from the corneal surface noted above. However, images of whole-thickness fringes from the post-contact lens tear film have been obtained by specular microscopy¹⁶ and slitlamp microscopy with oblique illumination.¹⁷ These images demonstrate an aspect of the roughness of the corneal surface, namely, that the

surface height of superficial epithelial cells varies from cell to cell.

A major objective of this proof-of-concept study is to elucidate why the contrast of whole-thickness fringes from the precorneal tear film is so low. In particular, evidence is presented that this low contrast is related to the roughness of the corneal surface. The contrast of interference fringes is reduced by surface roughness¹⁸ and is inversely related to the ratio of (root-mean-square) roughness to wavelength. As an example, consider reflections from two points on the cornea having different surface heights so that the reflections are 180° out of phase and will cancel each other by destructive interference. If the wavelength is doubled, the two reflections will now be 90° out of phase and so will not cancel, leading to increased reflectance at this longer wavelength. If interference contrast is low because of corneal surface roughness, contrast should be an increasing function of wavelength; in this study, this prediction was tested by using a spectrometer responding at longer wavelengths than sensors used in previous interference studies.^{4,7,9,12,14} The effect of corneal surface roughness can be evaluated by studying how contrast depends on wavelength.¹⁸

METHODS

Reflection spectra were recorded from 20 healthy individuals (12 female), with a mean age 28 years. The method of recording and processing reflection spectra was similar to that described

previously^{4,15} except that, by inserting a mirror to divert the measurement beam (reflected from an individual's eye), spectra could be recorded on a spectrometer (BTC 261E; B&W Tek, Newark, DE, USA) using an indium-gallium-arsenide (InGaAs) detector responding over a range of wavelengths from 912 to 1712 nm. By removing the mirror, spectra could be recorded on the original silicon-based spectrograph with a spectral range of 562 to 1030 nm. For each individual, four recordings of 210 reflection spectra were made using the InGaAs system with an exposure duration of 0.095 seconds and a nominal measurement tear film area of $25 \times 33 \mu\text{m}$. Individuals were asked to blink normally. In addition, four recordings of 200 spectra with an exposure duration 0.1 second were made with the silicon-based system using the same measurement area. Details of the analysis of the InGaAs reflection spectra will be presented in the Results section.

The study adhered to the principles of the Declaration of Helsinki and was approved by the Biomedical Institutional Review Board of The Ohio State University. Informed consent was obtained from all participants after explanation of the procedure. Individuals were eligible for the study if they were older than 18 years and had not worn contact lenses for 3 months before the study.

RESULTS

Figure 1 shows a reflection spectrum recorded with the InGaAs system. This will be used to illustrate the analysis performed on the reflection spectra.

Reflectance at a surface (R) is defined as the ratio of reflected to incident intensity. It is also convenient to define amplitude reflectance (r) as the ratio of reflected to incident amplitude. Because intensity is proportional to the square of amplitude, then $R = r^2$.

Amplitude reflectance at a surface is given by Fresnel's equation as follows¹⁹:

$$r = (n_i - n_r) / (n_i + n_r) \quad (1)$$

where n_i and n_r are the refractive indices of the incident (first) and reflecting (second) media. Amplitude reflectance is negative when light is reflected from a denser medium ($n_i < n_r$) such as reflection from the anterior and posterior surfaces of the tear film; however, the analysis below is unchanged if amplitude reflectance is considered to be positive, so the negative sign of amplitude reflectance will be ignored.

The solid line in Figure 1A is an example of (intensity) reflectance of the tear film (R_t) as a function of wave number ($1/\text{wavelength}$) (γ). Reflectance from the tear film involves interference between reflections from the anterior surface (including the effect of the lipid layer) and the posterior surface. Total reflectance of the tear film (R_t) is given by the following equation¹⁹:

$$R_t = r_1^2 + r_2^2 + 2r_1r_2\cos\delta \quad (2)$$

where r_1 is the amplitude reflectance from the anterior surface of the tear film (including the effect of the lipid layer), r_2 is the amplitude reflectance of the posterior surface, and δ is the phase difference between reflected waves from anterior and posterior surfaces. This is given by the following equation:

$$\delta = 4\pi n t + \varepsilon \quad (3)$$

where n is the refractive index of the tear film, t is tear film thickness, and ε is the initial phase, which is approximately zero for the precorneal tear film.¹⁹ The first two terms on the right side of Equation 2 correspond to the sloping baseline given by the dashed line in Figure 1A, whereas the final term

FIGURE 1. Processing of a reflectance spectrum from a 25-year-old white female recorded with the InGaAs spectrometer. The calculated precorneal tear film thickness was $2.62 \mu\text{m}$. (A) The solid line is measured reflectance as a function of wave number ($1/\text{wavelength}$), and the dashed line is the fitted sloping baseline (Equation 7). (B) Reflectance divided by the sloping baseline in (A). Solid black line corresponds to measured value. Blue curve (partly obscured by the magenta curve) gives fitted reflectance based on Gaussian decay of amplitude reflectance from the posterior tear surface (Equation 8). The magenta curve gives fitted reflectance based on exponential decay of amplitude reflectance from the posterior tear surface (Equation 9). (C) Blue and magenta solid curves show in-phase amplitude reflectance of the posterior tear surface for Gaussian and exponential decay models, respectively. Dashed curves show corresponding amplitude reflectance. (D) Extrapolation of (C) to zero wave number.

corresponds to the spectral oscillations⁴ given by the difference between solid and dashed curves in Figure 1A.

Equation 2 may be rewritten as follows:

$$R_t = (r_1^2 + r_2^2)(1 + C\cos\delta) \quad (4)$$

where C is the contrast of the spectral oscillations. By comparison of Equations 2 and 4, C is given by the following

equation:

$$C = 2r_1 r_2 / (r_1^2 + r_2^2) \quad (5)$$

The term $(1 + C \cos \delta)$ in Equation 4 may be derived by dividing the measured reflectance by the sloping baseline in Figure 1A and is shown by the black line in Figure 1B (partly obscured by magenta and blue lines). In that example, the maximum contrast (C) was approximately 0.08, and in most examples it was less than 0.1. Substituting the latter figure into Equation 5 implies that r_2 is typically less than $0.05r_1$; thus, r_2^2 is typically less than $0.0025r_1^2$. Therefore r_2^2 will be ignored in comparison to r_1^2 in this analysis. Equation 2 can therefore be simplified to the following:

$$\begin{aligned} R_t &= r_1^2 + 2r_1 r_2 \cos \delta = R_1 + 2(\sqrt{R_1})r_2 \cos \delta \\ &= R_1 + 2(\sqrt{R_1})r_2 \cos(4\pi n \chi t + \varepsilon) \end{aligned} \quad (6)$$

where R_1 is the (intensity) reflectance of the anterior tear surface (including the lipid layer).

Equation 6 was therefore used for fitting the measured reflectance spectrum, as shown in Figure 1. The least squares fitting procedure was similar to that used in previous investigations.⁴ Functions or parameters that were adjusted to obtain the least squares fit were R_1 (reflectance of the anterior tear surface) and r_2 (amplitude reflectance of the posterior tear surface) (both functions of wave number), tear thickness t , and initial phase angle ε . The refractive index of tears (n) was based on the reported value²⁰ corrected for wavelength using the dispersion of water for visible light and infrared.²¹ Anterior tear film reflectance (R_1), the dashed line in Figure 1A, was assumed to be a cubic function of wave number as follows⁴:

$$R_1 = a + b\chi + c\chi^2 + d\chi^3 \quad (7)$$

where a , b , c , and d were varied to obtain the least squares fit.

Two different functions were considered for the amplitude reflectance of the posterior tear surface (r_2). One, represented by blue curves in Figure 1, was a Gaussian function of wave number of the following form:

$$r_2 = \rho_g \exp\{-k_g(n\chi)^2\} \quad (8)$$

where ρ_g and k_g were varied to obtain the least squares fit.²² As discussed later, this function was chosen because it corresponds to a corneal surface roughness represented by a Gaussian distribution of surface height.¹⁸ Based on the least squares fit of Equations 6 through 8, a good agreement is seen in Figure 1B between the measured spectrum (black curve) and the least squares fit (blue curve) (both curves are partly obscured by the magenta curve). Figure 1C shows the agreement between measurement and fit for the in-phase amplitude reflectance (i.e., the component of amplitude reflectance of the posterior surface that is in phase with the anterior reflectance) given by $r_2 \cos(4\pi n \chi t + \varepsilon)$ (see Equation 6). The dashed blue curve is the corresponding amplitude reflectance of the posterior surface (r_2). Figure 1D shows an extrapolation of the curves of Figure 1C back to zero wave number ($\chi = 0$). For this individual, the blue dashed curve at zero wave number gives the following: $r_2 = \rho_g = 1.27\%$.

For comparison, the amplitude reflectance of the posterior tear film surface was also modeled by an exponential decay of the following form:

$$r_2 = \rho_e \exp(-k_e n \chi) \quad (9)$$

where ρ_e and k_e were varied to obtain the least squares fit. Corresponding least squares fits are shown by the magenta curves in Figures 1B through 1D. The fits within the spectral

measurement range in Figures 1B and 1C are very similar to those for the Gaussian function of Equation 8. However extrapolation back to zero wave number (Fig. 1D) shows a considerable difference between the exponential and Gaussian fits, with an increased amplitude reflectance of $r_2 = \rho_e = 3.59\%$, almost three times greater than that for the Gaussian function.

Figure 2 shows plots of amplitude reflectance, as in Figure 1D, for all 20 individuals; Figure 2A is based on the Gaussian fit of Equation 8, whereas Figure 2B is for the exponential fit of Equation 9. For each individual, median values of ρ_g and k_g (Fig. 2A; Equation 8) and ρ_e and k_e (Fig. 2B; Equation 9) were used in plotting these curves. Vertical dashed lines indicate the lower wave number limit (maximum wavelength) of the spectrometer. The vertical axes in Figure 2 are logarithmic so that Gaussian functions (Fig. 2A) plot as inverted parabolas and exponential functions (Fig. 2B) plot as straight lines. Amplitude reflectances ρ_g and ρ_e are given by the intercepts of the curves at zero wave number; they are expected to be related to the refractive index of the corneal surface (Equation 1) and should be independent of the roughness of the corneal surface. Decay constants k_g and k_e are given by the downward slopes of the curves or lines and should depend on corneal surface roughness¹⁸ and should be independent of the refractive index of the corneal surface. Thus, independence of the intercepts (at zero wave number) and slopes in Figure 2 might be expected because they depend on different characteristics (refractive index and roughness) of the cornea. This expected independence of intercept and slope seems better obeyed in Figure 2A (Gaussian fit) than in Figure 2B (exponential fit); in the latter case, for example, lines with steeper slope tend to have larger intercepts at zero wave number.

The independence of intercept and slope in Figure 2 is analyzed more quantitatively in Figure 3, where the amplitude

FIGURE 2. Amplitude reflectance as a function of wave number for all 20 individuals. (A) Gaussian model (Equation 8). (B) Exponential decay model (Equation 9).

FIGURE 3. The relation between amplitude reflectance at zero wave number and decay constants. (A) Gaussian model (Equation 8). (B) Exponential decay model (Equation 9).

reflectance at zero wave number for the 20 individuals is plotted as a function of decay constants. Figure 3A corresponds to the Gaussian decay model (Fig. 2A; Equation 8), whereas Figure 3B corresponds to the exponential decay model (Fig. 2B; Equation 9). Regression lines through the data points are shown. In Figure 3A, the correlation coefficient 0.126 between amplitude reflectance at zero wave number (ρ_g) and Gaussian decay constant (k_g) was not significant ($P > 0.05$); this is consistent with the expected independence of these two quantities. In Figure 3B, the correlation coefficient 0.899 between amplitude reflectance at zero wave number (ρ_e) and exponential decay constant (k_e) was strongly significant ($P < 0.001$); this shows a lack of the expected independence between these two quantities. Thus, the Gaussian model is in better agreement than the exponential model with the prediction that amplitude reflectance at zero wave number (which should depend on refractive index of the surface of the corneal epithelium but not corneal surface roughness) should be independent of the decay constant (which should depend on surface roughness but not refractive index of the surface of the corneal epithelium).

Figure 2A shows how the contrast of interference fringes in the infrared region of the spectrum is inversely related to wave number and therefore is an increasing function of wavelength. Thus, the contrast of interference fringes for the InGaAs spectrometer should generally be greater than that for the silicon system, which responds to shorter wavelengths. This was confirmed by comparing the contrast of interference fringes for the two systems derived from Fourier transforms of the reflection spectra.⁴ For the 20 individuals, the mean contrast for the InGaAs system was 4.48% compared with 1.10% for the silicon system. By this measure, the contrast for the InGaAs system was thus approximately four times greater. The correlation coefficient between Fourier contrast for InGaAs and silicon-based systems was 0.806 ($P < 0.001$).

DISCUSSION

The results shown in Figures 1 and 2 show that, for the infrared spectral range of the InGaAs sensor, the contrast of interference fringes is inversely related to wave number and so is an increasing function of wavelength. This supports the proposal that the low contrast of interference fringes from the precorneal tear film at shorter wavelengths^{4,12} is related to corneal surface roughness.

The analysis of Figures 2 and 3 indicates that the Gaussian relation between the amplitude reflectance of the corneal surface and wave number (Equation 8) is more consistent with experimental results than the exponential model (Equation 9). Sinha and Tippur¹⁸ showed that the Gaussian function of wave number in Equation 8 is to be expected from a Gaussian distribution of corneal surface height b (deviation from the mean height) of the following form:

$$p(b) = p_0 \exp\left(-\frac{b^2}{2\sigma^2}\right) \quad (10)$$

where $p(b)$ is the probability of height (b), p_0 is a constant, and σ is the standard deviation of surface height. With this assumption, they show that the amplitude reflectance (r) from such a rough surface should be given by the following equation:

$$r = r_0 \exp\{-8\pi^2 \sigma^2 (n\chi)^2\} \quad (11)$$

where r_0 is a constant. Equating this expression with the Gaussian function of Equation 8 gives the following:

$$k_g = 8\pi^2 \sigma^2 \quad (12)$$

Equation 12 was used to estimate a corneal surface height mean (SD) of 0.129 (0.010) μm between individuals. For the Gaussian distribution of the model, approximately 95% of the surface height may be expected to be within 2 SDs of the mean height or a total range of approximately 0.5 μm . The measurement area of $25 \times 33 \mu\text{m}$ (probably larger because of the effects of diffraction, aberrations, and defocus) is similar to the area of superficial corneal cells.²³ Therefore, this estimate of the roughness of the corneal surface presumably includes both the effects of roughness within individual cells such as microplacae^{24–26} and the difference in surface height between cells.^{16,17} The above estimate of total range of approximately 0.5 μm is of the same order of magnitude as the height of human microvilli of approximately 0.5 to 0.75 μm from the transmission electron micrographs by Ehlers.²²

Limitations of this estimate of corneal surface roughness should be noted. The anterior (air) surface of the tear film tends to be pulled flat by surface tension and so is probably quite smooth relative to the posterior (corneal) surface. However, variability in lipid thickness¹⁰ may be expected to contribute to the variability of overall tear thickness and so could contribute to the above roughness estimate. In an unpublished study (Powell DR, Chandler HL, King-Smith PE, unpublished observations, 2013) of more than 1400 high-resolution color micrographs of the lipid layer, we found a lipid thickness SD of 0.014 μm over an area of 180- μm diameter (larger than the test area in the present study), which is only 11% of the estimated corneal surface height SD of 0.129 μm . Given that corneal surface roughness and lipid thickness variability are expected to be uncorrelated, the overall variance (square of the standard deviation) in tear film thickness will be given by the sum of corneal surface variance and lipid surface variance, so the contribution from lipid surface variance is expected to be only approximately 1% of the total variance. Another possible complicating factor is that the glycocalyx may

have a refractive index that is intermediate between that of the tear film and corneal epithelium and could therefore act somewhat like an antireflection coating, whose effect would vary with wave number; alternatively, it is possible that the glycocalyx may have a higher refractive index than the corneal epithelium, which would tend to increase observed reflection, again in a manner dependent on wave number. Perhaps the most important limitation of the Gaussian roughness analysis is that reflectance spectra in the wavelength range of the silicon detector (562–1030 nm) are often not consistent with this model. For example, the reflectance spectrum of Figure 2 in the study by King-Smith et al.⁴ shows a fairly constant contrast of spectral oscillations over a wide range of wave numbers (approximately $1.1\text{--}1.7\ \mu\text{m}^{-1}$), which is inconsistent with the strong decay predicted from Equation 8 and extrapolated from Figure 2A. The interference at shorter wavelengths (e.g., visible light) is therefore often greater than the prediction of Equation 8, suggesting a weak contribution from a smoother or more compact component of the corneal surface. In summary, the Gaussian roughness model predicts quite well the characteristics of spectral oscillations in the spectral range of the InGaAs camera (above approximately 900 nm) but is less satisfactory at shorter wavelengths.

Liu and Pflugfelder²⁷ have shown that corneal surface irregularity (roughness), studied by corneal topography, is increased in aqueous-deficient dry eye. The present study was limited to healthy individuals and concerns roughness on a much smaller scale; it is planned to study whether the surface roughness observed by the methods herein is affected by dry eye disorders.

A final conclusion of the study is that the contrast of interference fringes from the whole thickness of the precorneal tear film is considerably higher in the wavelength range of InGaAs sensors compared with that of silicon-based sensors. Reported images of whole-thickness fringes from the precorneal tear film using silicon-based cameras have been noisy because of their low contrast,¹² so the present study indicates that better images could be obtained by using InGaAs cameras rather than silicon-based cameras.

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