Thickness of the Pre- and Post–Contact Lens Tear Film Measured In Vivo by Interferometry

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PURPOSE. To evaluate an interferometric method for measuring the thickness of the pre- (PLTF) and postlens tear film (POLTF) in subjects wearing hydrogel contact lenses. The precision and accuracy of measuring postlens tear thickness is compared with a previous method based on optical pachymetry and mechanical measurement of contact lens thickness.

METHODS. Reflectance spectra (562–1030 nm) from the front of an eye wearing a contact lens were measured at normal incidence. Interference between reflections from four surfaces—the front of the tear film, the front and back of the contact lens, and the front of the cornea—can give rise to as many as six oscillations in the reflectance spectra, three from simple layers (layer A: PLTF; B, POLTF; C, contact lens) and three from composite layers, (layer D, A+C; layer E, C+B; layer F, A+C+B). The thickness of any layer is derived from the frequency of the oscillations. The principle of the method was tested with a rigid contact lens, which was designed to give distinct thicknesses for all six layers. Twenty spectra were then recorded from each of 12 subjects wearing hydrogel contact lenses.

RESULTS. The PLTF thickness averaged 2.31 μm. There was good agreement between a direct estimate from layer A and an indirect estimate: layer D minus layer C. For POLTF, an indirect estimate—layer F minus layer D—averaged 2.54 μm and was more satisfactory than the direct estimate from layer B. There was no correlation between PLTF and POLTF thickness, showing that these are independent measurements, despite the similarity of their means.

CONCLUSIONS. Prelens tear thickness was in reasonable agreement with prior measurements. Postlens tear thickness was much less than the 11 to 12 μm found by the pachymetric method. It is argued that the current method avoids some of the systematic errors of the pachymetric method and also has much higher precision. (Invest Ophthalmol Vis Sci. 2003;44:68–77) DOI:10.1167/iovs.02-0377

For successful contact lens wear, the prelens tear film (PLTF) is important for several reasons. First, the outer layer of the tears provides a uniform coating over the contact lens, making it a smooth optical surface. If this outer layer becomes rough or irregular, as it may during tear drying and break-up, it leads to light scatter and reductions in image quality.1,2 Another function of the PLTF during contact lens wear is to provide comfort and lubrication to the palpebral conjunctiva, especially during the blink. In addition, the superficial lipid layer of the tear film reduces evaporation of the film, maintaining contact lens hydration.3,4 If the lipid layer is altered by the presence of a contact lens, increased evaporation of the PLTF probably occurs, followed by contact lens dehydration and depletion of the postlens tear film (POLTF) by absorption into the contact lens.5 This may be the mechanism of contact lens–related dry eye.

In previous studies, investigators have examined the thickness characteristics of the PLTF, and there is relatively good agreement in the results among these studies.5,6 Guillon3 used an optical system and high- and low-magnification photography to examine "thickness-dependent fringes" associated with the PLTF. The resultant interference patterns were used to characterize the thickness of the lipid and aqueous layers of the PLTF. On hydrogel lens materials, Guillon described prelens thicknesses of the aqueous layer up to 5.5 μm.7 Fogt et al.12 have measured similar PLTF thicknesses by using "wavelength-dependent fringes" (i.e., spectral oscillations), which averaged 2.7 μm in five hydrogel lens wearers (range, 0.5–5.0 μm). Thus, the results of different studies are in relatively good agreement regarding the thickness of the PLTF.

Just as the PLTF is important during contact lens wear, so too is the POLTF. Along with the oxygen permeability properties of the lens material itself, oxygen transmission to the cornea depends on the thickness of the POLTF.7 The POLTF also provides ocular comfort during contact lens wear, cushioning the lens on the corneal and conjunctival epithelium while the lens moves.8–10 The POLTF, through tear exchange, removes cellular and inflammatory debris from this layer and further lubricates the epithelial layers.11 If the POLTF is diminished, as it may be during contact lens–related dry eye or the overnight wearing of contact lenses (i.e., lens adherence), the accumulation of debris and inflammatory mediators may pose a significant threat to the ocular surface involving ocular infection, inflammation, or mechanical desiccation.8,9,12 In fact, depletion of the POLTF is thought to be the mechanism of inferior arcuate staining seen in soft contact lens wearers.13,14

Investigators have observed colored interference effects from the POLTF when illuminated in white light, indicating that the POLTF is particularly thin in these cases.8,15 These colored fringes are similar in principle to those seen in "Newton's rings" which are formed in the tiny air gap between two glass surfaces.16 With white illumination, approximately seven complete cycles of colored fringes can be observed in a Newton's rings setup.16 This limit corresponds to a POLTF thickness of 7λ/(2n cos δ′) = 1.60 μm,17 where the wavelength, λ, is assumed to match the peak of the luminosity function, 555 nm, n is the refractive index of the tears, assumed to be 1.337,18 and δ′ is the angle of refraction in the POLTF—25° in the conditions used to observe colored fringes.8

We are aware of one reported attempt to measure the thickness of the POLTF.19 Indirect measurements of thickness were made by combining pachymetry and mechanical measurement of contact lens thickness, finding an average POLTF thickness of 68–77 μm.10
thickness of 11 to 12 μm — considerably thicker than might be expected from the occurrence of colored interference effects observed in the POLTF. Although there is relatively good agreement regarding the thickness of the PLTF, measures of POLTF thickness are difficult, and a consensus has not been reached regarding the thickness of this layer.

This laboratory has developed an interferometric method for measuring the thickness of layers of the tear film and cornea. This system has a number of advantages over previous methods, including versatility, noninvasiveness, precision, rapidity, and a low noise level. We have adopted the interferometric technique to examine the thickness of the PLTF and POLTF in vivo. The purpose of this study was to describe the thickness of both the PLTF and POLTF in hydrogel lens wearers, by using wavelength-dependent fringes.

**METHODS**

Some general principles of our method of measuring the PLTF and POLTF are presented herein, whereas some specific details of the analysis are described in the Results section. Figure 1A shows an incident light wave and, beneath it, reflections from four surfaces at the front of an eye wearing a contact lens. Reflections from surfaces 1, 2, and 4 are out of phase with the incident wave, because the reflecting medium has a higher refractive index than the incident medium. The ratios of reflected to incident amplitudes for the four surfaces, \( r_1 \) to \( r_4 \), may be derived, theoretically, from Fresnel’s equation:

\[
r_i = \frac{(n_i - n_0)/(n_i + n_0)}{n_i - n_0}/H2084\text{(i.e.,}
\]

where \( n_i - 1 \) and \( n_i \) are the refractive indices of the incident and reflecting media.

Reflections from any pair of these four surfaces can cause interference effects. For example, reflections, \( r_1 \) and \( r_2 \), from the boundaries of the PLTF, are seen to be in phase for the wavelength of Figure 1A, causing constructive interference and hence a large amplitude for the combination of these two reflections. For a different wavelength, these two reflections can be out of phase (destructive interference) so that the combination of the two reflections would be relatively weak. Thus, a reflectance spectrum should show peaks at some wavelengths and troughs at other wavelengths corresponding to constructive and destructive interference, respectively. These peaks and troughs alternate, giving rise to spectral oscillations. For normal incidence, oscillations in reflectance corresponding to interference between reflections from surfaces \( i \) and \( j \) can be fitted by:

\[
R_n = R_0\left[1 + \gamma_n\cos(2\pi t' + \phi_n)\exp(-\frac{\lambda o}{\lambda})\right]
\]

where

\[
R_0 = r_1^2 + r_2^2 + r_3^2 + r_4^2
\]

\[
t' = \sum_{k=i}^{k=j} n_k d_k/n_1
\]

\[
\alpha = 2n_1/\lambda
\]

and \( \gamma_n \) is the contrast of the spectral oscillations, \( t' \) is the effective thickness between surfaces \( i \) and \( j \) based on refractive index of \( n_t \) (i.e., tears), \( f_\lambda \) is the thickness of layer \( k \), \( \lambda \) is the vacuum wavelength, and \( \phi_n \) and \( \lambda o \) are constants. The wavelength variation of refractive index (dispersion) of tears, \( n_t \), was assumed to equal that for water. The term \( \cos(2\pi t' + \phi) \) corresponds to the spectral oscillations, whereas the decay term \( \exp(-\lambda o/\lambda) \) is empiric, providing a better fit to the reflectance spectrum. It follows from equation 2 that the effective thickness, \( t' \), is given by the frequency of the oscillations, when plotted as a function of \( \alpha \). In theory, contrast is given by:

\[
\gamma = \text{abs}(2\pi r)/R_0
\]

where \( \text{abs}(2\pi r) \) is the absolute value of \( 2\pi r \). In practice, the measured contrast can be reduced by any of the following conditions: If the two surfaces are not parallel, if there is an eye movement during the exposure that changes the thickness of the layer, if either surface (particularly the corneal surface, surface 4) is rough, if the micro-projections (microvilli and microfolds) on the corneal surface act as an antireflective coating, or if the modulation transfer function of the spectrograph is less than unity. These factors presumably contribute to the decay term in equation 2.

Six layers that could give rise to interference effects are shown in Figure 1B. Layers A, B, and C are simple layers, whereas D, E, and F are composite layers containing two or three components. The expected contrasts of the spectral oscillations are illustrated by the thickness of the double-headed arrows. These predictions are based on equations 1 and 6, assuming refractive indices of 1.337 for the tear film and 1.40 for a hydrogel (Acuvue; Vistakon, Jacksonville, FL). It may be noted that contrasts involving surface 4, the epithelial surface, are always much weaker than predicted by equations 1 and 6. For example, in the conditions of the current measurements, the maximum contrast we have yet observed for the precorneal tear film (PCTF) is 4.25% (at 800 nm; Nichols JJ, King-Smith, PE, unpublished results, 2000, from more than 3000 spectra), which is much less than the 31.6% predicted from equations 1 and 6 based on a refractive index of 1.401 for the epithelium.
roughness of the corneal surface and the presence of microprojections on the epithelium. When this low contrast from surface 4 is taken into account, layers A and D are expected to give the strongest contrast, then C, then F, whereas B and E should be the weakest. In practice, the high-frequency oscillations due to layers C, D, E, and F are attenuated, in comparison with A and B, by the limited modulation transfer function of the spectrograph (approximately 0.3–0.75 for the range of contact lens thicknesses studied). Thus, the order of observed contrasts tends to be, from strongest to weakest: A, D, C, F, B, and E.

The optical system is shown in Figure 2A and has been modified from earlier versions. In outline, the tungsten filament source (T) is focused on a horizontal slit (S) which is then refocused on the cornea. The reflected beam is then refocused on the vertical entrance slit of an imaging spectrograph (SpectraPro-150; Acton Research, Acton, MA) with a charge-coupled device (CCD) image sensor (S5769-1006; Hamamatsu, Bridgewater, NJ). The circular stop (S) is used to limit the diameter of the beam. The subject is asked to fixate the cross hairs (X). The white screen (W) is used to view the reflected beam when initially aligning the subject’s eye. The shutter (Sh) is used to control spectrograph exposure. The measurement area at the cornea is shown in Figure 2B. The dotted horizontal lines correspond to the image of S at the cornea, and the dotted vertical lines indicate the region sampled by the entrance slit of the spectrograph. The measurement area is therefore the intersection of these two slit images.

The beam reflected by beam splitter B2 passes through a modified optical system used for eye alignment. For alignment along the axis of the interferometer, the image of horizontal slit S at the cornea is reimaged, first onto the split image-focusing screen (Sp) (model 1-14; Olympus, Tokyo, Japan) and then onto the video camera. The split imaging focusing screen contains two adjacent prisms on either side of a sharp vertical edge. The left and right prisms deviate the beam upward and downward, respectively. The spherical front surface of the focusing screen, (Sp) focuses S onto the horizontal slit S. Thus, the beams through the left and right prisms, which pass through S, sample light from near the top and bottom of S, respectively. The left half of the video display of Figure 2C (focus) shows the image on the video monitor, when S is focused in front of the cornea. To focus S on the cornea, lens L4 is moved axially by a rack-and-pinion movement until that the two halves of the slit image are aligned. Alignment along the other two axes (laterally and vertically) has been described previously.

As noted previously, retinal irradiance is below the maximum permissible exposure for continuous viewing for both thermal and photothermal effects. Wavelength calibration of the spectrograph and checks on the accuracy of thickness measurements with an interferometer (model OS-9255A; Pasco, Roseville, CA) have been described previously. As a further check, the mean thickness of eight microscope coverslips agreed to within 0.5%, with the mean thickness measured with a dial lens gauge (model GA-715; Vigor, Japan).

A computer program for processing of the reflection spectra, based on Fourier transforms (e.g., Fig. 4) and least-squares fits (e.g., Fig. 3), has been described previously. For the current studies of PLTF and POLTF, the program was modified so that the thickness of a number of layers, as in Figures 1, 3, and 4, could be determined simultaneously. For hydrogel lenses, spectral oscillations from layers A, C, D, and F were commonly detectable. Layer A (the PLTF) is much thinner than the other three layers and so is easily distinguished. To analyze layers C, D, and F, the largest peak in the Fourier transform in thickness range 50 to 150 μm was used as a starting condition for least-squares fits for layer D (which typically gives much higher contrast than layers C and F). Starting conditions for both layers C and F were varied systematically, in steps of 1 μm. For each starting condition, least-squares fits were eliminated if either the estimate of D – C or F – D was less than 1 μm (experience indicates that these fits often correspond to a single layer rather than to two separate layers). The least-squares fit with the lowest mean square error was then used for tentative estimates of C, D, and F. (Additional criteria will be described later).

A similar procedure was used for studying possible oscillations from layer B. The main peak in the Fourier transform below 20 μm was used for starting values for least-squares fits for layer A, while starting values for layer B were in 1-μm incremental steps.

Rigid Lens Study

Because this study is the first application of interferometry to measuring thickness of the POLTF, it is helpful to present some results from a preliminary experiment conducted to test and illustrate the method. For this experiment, the back surface curvature of a PMMA lens was calculated in one subject to provide a POLTF thickness (approximately 30 μm) that was considerably greater than the PLTF thickness (approximately 3 μm) and considerably less than the contact lens thickness (approximately 100 μm). Thus, all six layers should have different thicknesses, and it should be possible to detect spectral oscillations from all layers.

Clinical Study and Statistical Procedures

Experiments were approved by our institutional review board and the tenets of the Declaration of Helsinki were followed. Informed consent was obtained from each subject. In vivo measures of PLTF and POLTF thickness and contact lens thickness were made using interferometry for 12 hydrogel contact lens wearers (mean age, 35.0 ± 11.7 years, nine men). Twenty reflection spectra were recorded from each of the 12 subjects wearing hydrogel contact lenses for at least 15 minutes before commencement of the experiment. All subjects were current hydrogel contact lens wearers without a history of ocular disease, trauma, or surgery. Each spectrum was captured approximately 2 seconds after a blink, and the exposure duration was 0.5 second with...
of Lin et al. for all experiments was 23.6°C. The average room temperature during yielded a small correction: The average from six layers, A through F.

wearing a rigid contact lens, showing contributions from oscillations of contrast (\(\alpha\)) based on the refractive index of tears\(^{19}\) and assuming that the dispersion constant\(^{17}\) of tears is the same as for water. (For clarity, only half the recorded spectrum is plotted.) The top plot in Figure 3 is the measured reflectance spectrum, and the bottom six curves show the contributions of spectral oscillations from the six layers (cf. equation 2), determined by a least-squares fit.\(^{21}\) The weak oscillations for layers B, E, and F have been magnified 10 times to make them more visible. The second plot is the sum of the oscillations from the six layers and is very similar to the measured reflectance spectrum at the top.

Figure 4 shows the Fourier transform of the reflectance spectrum in Figure 3. The abscissa is thus the frequency of Fourier components in the reflectance spectrum and so shows the effective thickness, \(t'\), of layers (see equations 2 and 4; assumed refractive index of tears, \(n_1 = 1.337\) at 589 nm).\(^{16}\) The six peaks, A through F, are clearly seen. A logarithmic scale of contrast has been used to make the smaller peaks more visible. For example, the contrast of B is less than 1% of the contrast of A. In practice, a Fourier transform such as that in Figure 4, was used for preliminary estimates of thickness (\(t\)), contrast (\(\gamma\)), and phase (\(\phi\)). More precise thickness estimates were then derived from a least-squares fit as in Figure 3.\(^{21}\) For estimating contact lens thickness, a correction for the difference between refractive indices of contact lens and tears would be needed.

Table 1 shows a comparison of direct and indirect estimates of the effective thickness, \(t'\), of the six layers for the PMMA lens in Figures 3 and 4. Agreement is good—with 0.15 \(\mu m\) or 0.4% in all cases—confirming the interpretation of the six
spectral oscillations shown in Figures 3 and 4. In this example, the thickness of all six layers can be estimated directly. However, as discussed later, the thickness of the POLTF (layer B) for hydrogel lenses was often similar to that of the PLTF (layer A), and so the weak oscillations from layer B were hard to detect in the presence of strong oscillations of similar frequency from layer A. In this case, the thickness of the POLTF could still be derived from the indirect estimate, \( F - D \).

Table 1 also gives a comparison of observed and maximum predicted contrasts (MPCs) for the PMMA lens. For layers A, C, and D, the MPCs were derived from equations 1 and 6. The refractive index of PMMA was assumed to be 1.49 at 589 nm, the dispersion constant of all layers was assumed to be the same as for water, and the central wavelength of our spectrophotograph, 800 nm, was used. For layers B, E, and F, which involve reflections from the corneal surface, the MPCs were based on a maximum observed contrast (MOC) of 4.25% for the PCTF. An effective reflectance for the corneal surface, \( r_c \), was then derived by applying equation 6 to the PCTF, and the MPCs for layers B, E, and F were derived from equation 6 by using this value of \( r_c \). Table 1 shows that the MOCs were all less than the MPCs, as expected. The use of MPCs in identifying layers will be described later.

**Clinical Study**

Figure 5 is the Fourier transform of a reflection spectrum for a subject wearing a 3.00-D hydrogel lens. Four peaks corresponding to layers A, C, D, and F (see Fig. 1) were identified. Justification for this interpretation comes from Table 2, which lists the MPCs for interference from the six possible layers, calculated as in Table 1 (but for a hydrogel lens). The peak contrast for the thinnest layer has a greater contrast than the MPC for layer B and so must come from layer A. The contrast for the large peak near 75 \( \mu \)m exceeds the MPCs for layers C, E, and F and so must come from layer D. The contrasts for the two neighboring peaks both exceed the MPC for layer E and so must come from layers C and F, as indicated. As a check on this interpretation, the direct estimate of prelens tear thickness, \( A = 2.24 \mu \)m, was in good agreement with an indirect estimate, \( D - C = 2.26 \mu \)m. An indirect estimate of POLTF was \( F - D = 2.55 \mu \)m. It may be noted that the estimated thickness of B is \( F - D = 2.55 \mu \)m, and so oscillations from layer B are masked by the much stronger oscillations of similar frequency from layer A. For similar reasons, oscillations from layer E are masked by the strong oscillations from layer D. The contributions from layers B and E to the observed oscillations from layers A and D cause errors in the estimates of A and D. Assuming that the ratio of contrasts from layers B (or E) to that of layer A (or F) is 2.1% (derived from the ratios of MPCs in Table 2), simulations showed that the maximum error in A or D from this cause was 0.004 \( \mu \)m.

Table 2 also shows the MOCs for layers A, C, D, and F (using the strict criterion described later) and for layer B (criterion described later). As expected, the observed contrasts never exceeded the MPCs. Because we found little evidence of oscillations from layer E (probably because they would be expected to be weak and often masked by the strong oscillations from layer D) the bracketed value in Table 2 was calculated by noting that, from equation 6, the ratio of the MOC for layer E to that for layer F should be \( r_E/r_F \).

Criteria used for the identification of satisfactory oscillations from thick layers, C, D, and F were as follows: Capital letters, (e.g., C, D, F) are used for thicknesses and lowercase letters (e.g., x, y, z) represent contrasts. An initial criterion is that all peaks must exceed eight times the calculated photon-noise level. (This signal-to-noise criterion was determined empirically to give reliable results for thick layers. Noise was defined

**Table 1. Effective Thickness and Contrast Values for a PMMA Lens**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Direct Estimate (( \mu m ))</th>
<th>Indirect Estimate (( \mu m ))</th>
<th>Indirect—Direct Estimate (( \mu m ))</th>
<th>MPC (%)</th>
<th>Observed Contrast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.814</td>
<td>D - C = 2.818</td>
<td>0.004 (0.14%)</td>
<td>58.6</td>
<td>45.2</td>
</tr>
<tr>
<td>B</td>
<td>30.452</td>
<td>F - D = 30.338</td>
<td>-0.114 (-0.38%)</td>
<td>1.25</td>
<td>0.30</td>
</tr>
<tr>
<td>C</td>
<td>122.159</td>
<td>D - A = 122.163</td>
<td>0.004 (0.003%)</td>
<td>22.0</td>
<td>10.1</td>
</tr>
<tr>
<td>D</td>
<td>124.977</td>
<td>A + C = 124.975</td>
<td>-0.004 (-0.003%)</td>
<td>58.6</td>
<td>26.3</td>
</tr>
<tr>
<td>E</td>
<td>152.748</td>
<td>B + C = 152.611</td>
<td>-0.137 (-0.09%)</td>
<td>1.25</td>
<td>0.28</td>
</tr>
<tr>
<td>F</td>
<td>155.315</td>
<td>A + B + C = 155.425</td>
<td>0.110 (0.07%)</td>
<td>3.31</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* See Figure 4.

**Table 2. Maximum Predicted and Observed Contrasts for Hydrogel Lenses**

<table>
<thead>
<tr>
<th>Layer</th>
<th>MPC (%)</th>
<th>MOC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30.4</td>
<td>27.2</td>
</tr>
<tr>
<td>B</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td>C</td>
<td>4.86</td>
<td>2.51</td>
</tr>
<tr>
<td>D</td>
<td>30.4</td>
<td>19.9</td>
</tr>
<tr>
<td>E</td>
<td>0.65</td>
<td>(0.32)</td>
</tr>
<tr>
<td>F</td>
<td>4.04</td>
<td>1.99</td>
</tr>
</tbody>
</table>
as the calculated, root-mean-square [RMS] noise for a Fourier component in either cosine or sine phase, so that the RMS noise for the Fourier amplitude would be $\sqrt{2}$ times greater. In addition, any two peaks had to differ in thickness by at least 1 $\mu$m (otherwise it was found that the two oscillations might be derived from the same rather than different layers). If three thick layers exceeded these initial criteria, they were designated X, Y, and Z in order of increasing thickness. Then if $x$, $y$, and $z$ all exceed $e_{\text{max}}$, the maximum contrast for $e$, layers X, Y, and Z must correspond to layers C, D, and F. If only two peaks exceeded the initial, signal-to-noise criterion, they were designated U and V in order of increasing thickness; if $u$ exceeded the initial, signal-to-noise criterion, they were designated U and V in order of increasing thickness. In each layer, the contrast from this layer had to exceed the initial, signal-to-noise criterion, then the two peaks must be from layers D and F, whereas if $u > e_{\text{max}}$, $v > e_{\text{max}}$, and $v > e_{\text{min}}$, then the two peaks must be from layers C and D. For identification of a thin layer such as layer A, the contrast from this layer had to exceed $b_{\text{max}}$, the maximum contrast for layer B. An additional criterion for a satisfactory estimate of $D$ was that contrast $d$ should exceed a certain minimum value ($d_{\text{min}}$) taken to be 1% of the corresponding MOC (Table 2). Other criteria ensured that the contrast for the other layers also exceeded 1% of their MOCs. Two estimates for $F$ were excluded because the values of $F - D$ were over 50 $\mu$m, indicating that these oscillations corresponded to reflection from the back, rather than the front of the epithelium.

Three different criterion levels—lax, medium, and strict—were used for identifying layers A, C, D, and F, and are summarized in Table 3. The strict criterion for $b_{\text{max}}$, $e_{\text{max}}$, $e_{\text{min}}$, and $f_{\text{max}}$ corresponds to the MPCs from Table 2. The medium criterion is based on the MOCs from Table 3. The lax criterion differed from the medium criterion in that $e_{\text{max}}$ was set to eight times the photon-noise level (which was typically approximately 0.013%). The number of thickness estimates (out of a possible 240) for layers A, C, D, and F and for each criterion level is given in Table 3.

A test of the effectiveness of these criteria is shown in Figure 6. The 204 indirect estimates of PLTF thickness ($D - C$) using the lax criterion, are plotted as a function of the direct estimates (A). Good agreement was found between the two estimates, with correlation, $r = 0.995$ and a regression line slope of 0.9988; the mean difference between indirect and direct estimates, $(D - C) - A$, is 0.001 ± 0.089 $\mu$m (SD). There was no significant difference in PLTF thickness between the three powers of contact lenses (based on subject means of direct estimates, $F_{(2,7)} = 0.12$, $P > 0.05$). However, a significant difference between subjects was found (direct estimates, $F_{(11,105)} = 37.5$, $P < 0.0001$). A summary of direct and indirect estimates of PLTF thickness using strict, medium, and lax criteria is given in Table 4. Good agreement was found between all the estimates for the subject mean thickness and for the standard deviations between and within subjects. The bottom row shows direct PLTF measurements for the same 204 spectra used for lax indirect estimates in the next to bottom row (and in Fig. 6). Particularly good agreement was found between these data. It may be concluded that PLTF thickness can be determined reliably using the indirect estimate, $D - C$, even when using the lax criterion in Table 3. These findings help to justify the use of an indirect estimate for the thickness of the POLTF.

Table 5 summarizes indirect estimates of thickness of the POLTF ($F - D$) using strict, medium, and lax criteria. Use of the strict criterion should eliminate any misinterpretation (e.g., layer E oscillations interpreted as layer F) but only 20% of the spectra (and no spectra for two subjects) satisfied the strict criterion. Mean thickness estimates for medium and lax criteria agreed with the means for the strict criterion within 0.05 $\mu$m, indicating that reliable POLTF thickness estimates can be obtained with these weaker criteria. These criteria allow many more spectra to be used, and means were derived for all subjects (Table 5). There was no significant difference between the means for the three powers of contact lenses (with strict, medium, and lax criteria, $F_{(2,7)} = 0.04$, $F_{(2,9)} = 1.35$, $F_{(2,9)} = 1.36$, all $P > 0.05$). A significant difference between subjects was found (e.g., medium criterion, $F_{(11,110)} = 16.67$, $P < 0.0001$). Although the mean POLTF and PLTF thicknesses were remarkably similar, the scatterplot of subject means (lax criterion) in Figure 7 shows no correlation between POLTF ($F - D$) and PLTF ($A$) thickness ($r = 0.10$, $P > 0.05$). Thus $F - D$ and $A$ are independent measurements which is consistent with $F - D$ as an estimate of POLTF rather than PLTF thickness.

**Table 3. Criterion Levels Used and Number of Thickness Estimates Obtained for Strict, Medium, and Lax Criteria**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>$b_{\text{max}}$</th>
<th>$e_{\text{max}}$</th>
<th>$e_{\text{min}}$</th>
<th>$f_{\text{max}}$</th>
<th>Number of Thickness Estimates for Each Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strict</td>
<td>0.65</td>
<td>4.86</td>
<td>0.199</td>
<td>0.65</td>
<td>4.04</td>
</tr>
<tr>
<td>Medium</td>
<td>0.42</td>
<td>2.51</td>
<td>0.32</td>
<td>1.99</td>
<td>233</td>
</tr>
<tr>
<td>Lax</td>
<td>0.42</td>
<td>2.51</td>
<td>0.199</td>
<td>8 × noise</td>
<td>204</td>
</tr>
</tbody>
</table>

**Figure 6.** Plot of indirect, $D - C$, versus direct, $A$, estimates of PLTF thickness for 204 spectra. The lax criterion was used. Diagonal line: corresponds to equality of the two estimates.
A limitation of the current method is that POLTF thickness of less than 1 μm could not be determined reliably. Inclusion of these values would reduce the mean thickness in Table 5. To estimate the error caused by this omission, a histogram of POLTF thickness (based on the medium criterion) is shown in Figure 8. This histogram does not differ significantly from a normal distribution (Kolmogorov-Smirnov test, \( P > 0.05 \)), and so a normal distribution has been fitted to the histogram. The hatched area in Figure 8, for POLTF less than 1 μm, thus gives an estimate of the number of missing data points (1.4/128; 1.1%). Adding this hatched tail to the histogram would reduce the calculated mean by 0.02 μm. Estimates of standard deviations would be increased by approximately 3%. This analysis is based on the assumption that most of the 112 missing values in Figure 8 are due to the low contrast of the F oscillations (cf. Table 2). An alternative extreme assumption is that all missing data points correspond to binding of the lens to the cornea so that POLTF thickness = 0. On this assumption, a considerably bigger correction would be needed—for example, using the lax criterion, the mean thickness would be reduced from 2.37 μm (Table 5) to 1.82 μm. We think this extreme assumption is unlikely—for example, with a lax criterion, only 204 indirect estimates of PLTF thickness were obtained compared with 233 direct estimates (Table 4). At least in this case, most of the missing estimates were not due to zero PLTF thickness, but probably related to low contrast of spectral oscillations from layer C.

A further test of the validity of POLTF thickness measures is shown in Figure 9 which plots indirect estimates \( F - D \) (lax criterion) versus direct estimates \( B \). Equality of direct and indirect estimates is shown by the solid line and the fitted regression line is the dashed line. Criteria used for identification of layer B were that the contrast \( (b) \) should be greater than a criterion value (0.125%), that the absolute value of \( B - A \) should be greater than 0.8 μm (otherwise the two oscillations may be derived from the same layer), and that \( B \) should be in the range 1.5 to 4.4 μm. The reason for the last criterion is that misalignment of the subject’s eye caused artifacts in the observed reflectance spectrum, which gave rise to artificial peaks in the Fourier transform below 1.5 and above 4.4 μm. Although these criteria limited the data to 14 spectra in Figure 9, reasonable agreement between indirect and direct estimate of POLTF thickness is obtained, with \( r = 0.964 \) (\( P < 0.001 \)).

The mean difference between the two estimates is \( (F - D) - B = -0.05 \pm 0.18 \) μm (SD).

### In Vitro Measurements of Contact Lens Thickness

The mean contact lens center thickness was 91.5 μm by interferometry and 75.3 μm by the Rehder Gauge (mean difference = 16.1 ± 3.1 μm, paired \( t \)-test = 16.7, \( P < 0.001 \)). Center thicknesses measured with the Rehder gauge were much lower than the manufacturer’s nominally stated thickness of 90 μm (–2.00-D lens; Acuvue; Vistakon). We also found a significant negative correlation between contact lens center thickness and temperature (–0.50-D lens: \( r = -0.998 \), Pearson correlation, \( P < 0.001 \); –2.00-D lens: \( r = -0.988 \), Pearson correlation, \( P < 0.025 \)). Regression lines were plotted and their slopes correspond to –0.109% and –0.113% per degree Celsius.

### DISCUSSION

Estimates of PLTF thickness are given in Table 4. The direct estimate, \( A = 2.31 \) μm (first row of Table 4), is preferable to the indirect, \( D - C \), for the following three reasons: first, the indirect estimate is probably less precise, because it is a small difference between two relatively large values, \( C \) and \( D \); second, the contrast from layer \( C \) is considerably less than from layer \( A \) (Table 2), which limits the precision of the indirect estimate, \( D - C \); third, there are more direct than indirect estimates –97% of all spectra (first row of Table 4). The remainder of Table 4, together with Figures 6 and 7, illustrate that the indirect estimate, \( D - C \), yields very similar results to the direct estimate \( A \).

Our estimate of PLTF thickness is not significantly different from the value of 2.7 μm reported by Fogt et al.\(^9\) who used a similar method. The maximum PLTF thickness that we observed, 5.46 μm (Fig. 8), is in reasonable agreement with the maximum of 5.5 μm reported by Guillon,\(^3\) using a different interferometric method.

For the POLTF, direct estimates of thickness, \( B \), are much less satisfactory than for the PLTF, because the oscillations from layer \( B \) are relatively weak (Table 2) and tend to be masked by the strong oscillations from layer \( A \). Only 6% of spectra yielded acceptable direct estimates of POLTF thickness.

### Table 4. Estimates of PLTF Thickness

<table>
<thead>
<tr>
<th>Layer</th>
<th>Criterion</th>
<th>Subject Mean (μm)</th>
<th>SD between Subjects (μm)</th>
<th>SD within Subjects (μm)</th>
<th>Number of Subjects</th>
<th>Number of Thickness Estimates</th>
<th>Estimates per Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Strict</td>
<td>2.31</td>
<td>0.82</td>
<td>0.53</td>
<td>12</td>
<td>235</td>
<td>17–20</td>
</tr>
<tr>
<td>D – C</td>
<td>Strict</td>
<td>2.36</td>
<td>0.78</td>
<td>0.55</td>
<td>11</td>
<td>163</td>
<td>0–20</td>
</tr>
<tr>
<td>D – C</td>
<td>Medium</td>
<td>2.34</td>
<td>0.77</td>
<td>0.54</td>
<td>12</td>
<td>189</td>
<td>5–20</td>
</tr>
<tr>
<td>D – C</td>
<td>Lax</td>
<td>2.356</td>
<td>0.776</td>
<td>0.537</td>
<td>12</td>
<td>204</td>
<td>11–20</td>
</tr>
<tr>
<td>A</td>
<td>Lax*</td>
<td>2.355</td>
<td>0.776</td>
<td>0.533</td>
<td>12</td>
<td>204</td>
<td>11–20</td>
</tr>
</tbody>
</table>

* These estimates correspond to the same 204 spectra yielding indirect estimates, \( D - C \), with a lax criterion.

### Table 5. Estimates of POLTF Thickness (\( F - D \))

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Subject Mean (μm)</th>
<th>SD between Subjects (μm)</th>
<th>SD within Subjects (μm)</th>
<th>Number of Subjects</th>
<th>Number of Thickness Estimates</th>
<th>Estimates per Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strict</td>
<td>2.33</td>
<td>0.52</td>
<td>0.36</td>
<td>10</td>
<td>47</td>
<td>0 to 14</td>
</tr>
<tr>
<td>Medium</td>
<td>2.34</td>
<td>0.51</td>
<td>0.40</td>
<td>12</td>
<td>128</td>
<td>5 to 16</td>
</tr>
<tr>
<td>Lax</td>
<td>2.37</td>
<td>0.46</td>
<td>0.47</td>
<td>12</td>
<td>184</td>
<td>9 to 20</td>
</tr>
</tbody>
</table>
vides many more estimates than the strict criterion is less likely than the lax criterion to give any consistent estimates averaged for each of 12 subjects.

(Fig. 9). Thus the indirect estimate, \( F - D \), is preferable—for example, using the lax criterion, 77% of spectra yield acceptable estimates (Table 5). The justifications for the validity of indirect estimates are threefold: first, as discussed, it can be successfully applied to the PLTF; second, it agrees reasonably well with direct estimates when those can be obtained (Fig. 9); and third, data for strict, medium, and lax criteria are reasonably consistent (Table 5), which would be unlikely if the data contained appreciable artifacts. The mean thickness estimate for the medium criterion, \( F - D = 2.34 \) \( \mu \text{m} \) will be used; this criterion is less likely than the lax criterion to give any contamination from layer E, noise and other artifacts, but it provides many more estimates than the strict criterion—acceptable estimates were obtained in 53% of spectra and in all subjects (Table 5). It is important to reiterate that, despite the similarity between PLTF and POLTF mean thickness, these two measurements are uncorrelated (Fig. 7), consistent with the analysis that \( F - D \) give POLTF thickness rather than PLTF thickness (see Fig. 1).

The pachymetric method of Polse et al. and Lin et al. has been used to study the effects on POLTF thickness of the base curve radius of contact lenses, palpebral aperture size, corneal curvature, ethnicity, and contact lens material. The current method for estimating POLTF thickness has notable advantages compared with their pachymetric method. Although both methods calculate a small difference between much larger values, the current method takes advantage of the higher precision obtainable with interferometry. For example, for one subject, the SD of contact lens thickness, \( C \), was \( 0.036 \) \( \mu \text{m} \) (\( n = 20 \)), which is approximately 100 times less than the SD of corneal thickness that can be obtained by skilled optical pachymetry. One estimate of measurement error of POLTF thickness, \( F - D \), is the deviation from the direct estimate, \( B \), shown in Figure 9. The SD of \( (F - D) - B \) is 0.18 \( \mu \text{m} \), so that the measurement SD of \( F - D \) is probably less than this, given that the standard deviation of \( B \) also contributes to this value. A different estimate of measurement standard deviation of \( F - D \) is the within-subjects standard deviation in Table 5: \( 0.40 \mu \text{m} \) for the medium criterion. For comparison, from Figure 6 of Lin et al., we estimate a short-term measurement standard error, for means of 40 measurements, of 2.45 \( \mu \text{m} \). To reduce this SE to 0.40 \( \mu \text{m} \), equivalent to the within-subjects standard deviation of the current method, would require some 1500 measurements with the pachymetric method.

An additional concern with the pachymetric method is that it may be susceptible to various systematic errors, as follows: First, our results indicate that the Rehder mechanical thickness gauge may compress the contact lens, thus causing an overestimate of POLTF thickness by as much as 16 \( \mu \text{m} \). (It may be noted that POLTF thickness for lotrafilcon lenses, measured by the pachymetric method, are considerably less than for etafilcon lenses, and this may be due to greater mechanical compression of the etafilcon A lenses, which were also used by Lin et al. This interpretation is supported by the finding that the disparity between pachymetric and interferometric measurements is smaller for balafilcon lenses, with a Young’s modulus five times greater than for etafilcon.) Second, an error may be
caused by an artifact in optical pachymetry with contact lenses in situ. This artifact could amount to approximately 3 μm for the lenses used by Lin et al. Third, an error of approximately 1 μm may be caused by changes in lens thickness due to the increased temperature of the lens in the eye. In addition, it may be noted that, although both pachymetric and current estimates of POLTF thickness use the difference between relatively large values, in the current method, the two values, F and D, are measured at the same moment, whereas, in the pachymetric method, a considerable time interval occurs between the various measurements (pachymetry with and without the contact lens, and mechanical measurement of contact lens thickness). Changes in corneal and contact lens thickness during these intervals causes errors in estimates of POLTF thickness.

Although the current method is also susceptible to systematic errors, we believe that these are considerably smaller than for the pachymetric method. Thickness estimates have been checked with a calibrated Michelson interferometer, and shown to be within 1%. Measurements of coverslip thickness described in the Methods section help to confirm this accuracy. The maximum error due to uncertainty in the refractive index of the tears is below 0.4%. The maximum error due to obliquity of rays through the POLTF is calculated to be less than 0.1%. Error due to omission of mean thicknesses of less than 1 μm, which are too small to measure with the current system, is estimated to be 0.02 μm or approximately 1% (see discussion of Fig. 8). Another error is due to the fact that oscillations from layers A and D are often contaminated by the weaker oscillations from B and E, respectively. As noted previously in reference to Figure 5, this error would be at most 0.004 μm if the relative contrast b/a (or e/d) is given by the corresponding MPCs (Table 2), but could be greater when oscillations from layer A or D are relatively weak (e.g., a maximum error of 0.04 μm if b/a (or e/d) is 10 times greater than the ratio of MPCs). In summary, we propose that systematic errors in the pachymetric method are considerably larger than any of the errors in the current method and that this is a major contribution to the discrepancy between the pachymetric estimate of POLTF thickness, 11 to 12 μm, and our estimate, 2.34 μm.

Little and Bruce9 and Bruce and Brennan10 have developed a method of qualitative assessment of POLTF thickness based on the appearance of the POLTF in white light from a slit lamp. As noted in the introduction, a colored POLTF is due to a white light interference effect, which indicates that the POLTF is less than 1.6 μm thick. In normal wearing conditions, only a minority of POLTFs for hydrogel lenses (Acuvue; Vista-kon) yielded colored fringes.8,9 and this is consistent with our finding that relatively few POLTFs are less than 1.6 μm thick (Fig. 8). Thickness changes in the POLTF have been monitored by the percentage of subjects who show colored POLTFs. In this way the thickness of the POLTF correlates with lens binding,13 lens movement,14 lens thickness, lens wearing time, inferior arcuate staining,14 ambient air flow,15 hypo-osmotic solutions,9 and eye closure.10 The current method has two advantages for studies of this sort. First, POLTF thickness can be determined quantitatively rather than qualitatively. Second, it is more objective, in that the operator is “blind” to the results before data processing (which, for our measurements, took place after the recording session). For comparison, it may be difficult for an observer to be completely objective in detecting color in the POLTF, when the observer can view one of the parameters (e.g., contact lens thickness) that is to be correlated with POLTF thickness.14

In conclusion, the current method can be used to estimate PLTF and POLTF thickness simultaneously. Evidence has been presented that the current method is more precise and accurate than previous methods of estimating POLTF thickness. It has potential for elucidating the factors that determine the thickness of the POLTF and the effects of that thickness on the health of the ocular surface.

References


34. Little SA, Bruce AS. Hydrogel (Acuvue) lens movement is influenced by the POLTF. *Optom Vis Sci.* 1994;71:364–370.