Computer-Synthesis of an Interference Color Chart of Human Tear Lipid Layer, by a Colorimetric Approach

Eiki Goto,1,2,5 Murat Dogru,1 Takashi Kojima,1,4 and Kazuo Tsubota1,2

PURPOSE. To synthesize an interference color chart for the specific tear lipid layer interference camera, DR-1, for the conversion of the tear film lipid layer thickness into color graphic information—that is, for the quantification of the tear interference image—by a colorimetric approach.

METHODS. Because the color of the tear lipid layer interference image is visualized by a white light source interference phenomena, to produce γ-corrected red, green, and blue (RGB) values of a specific interference color at a certain tear lipid film thickness, XYZ tristimulus values of the Commission Internationale de l’Éclairage (CIE) were obtained. XYZ tristimulus values were calculated from the light source spectrum of the DR-1 camera, the color-matching function of CIE, and the reflectance of the tear interference image in wavelengths ranging from 380 to 780 nm. These calculated interference colors were synthesized ranging from 0 nm to 1000 nm of lipid film thickness to produce a color chart. The applicability of the new color chart in the analysis of the lipid layer thickness was tested on a healthy control subject with normal tear function and a patient with dry eye who had aqueous tear deficiency and meibomian gland obstruction.

RESULTS. The specific tear interference color chart for the DR-1 camera was obtained with RGB and XYZ tristimulus values. The interference chart ranged from 0 to approximately the 5th interference order. The interference colors from clinical DR-1 images could be converted to lipid thickness data by using the color chart system.

CONCLUSIONS. A new tear interference color chart was developed in this study, which may be of benefit in converting tear interference color information to data describing the thickness of the tear film lipid layer. (Invest Ophthalmol Vis Sci. 2003;44:4693–4697) DOI:10.1167/iovs.03-0260

Tear lipid layer interferometry is a noninvasive method to visualize the lucent surface lipid layer of the tear film.1–5 Tear interference images have been reported in observing the surface phenomena of the tear film,4–8 using the principle first reported by Sir Isaac Newton.3 Existence of the interference phenomena indicates the presence of the optical path difference.10 The interference phenomena inferred from the tear film indicate the presence of a thin film, which is the superficial lipid layer.5,11 The coloration of these interference images has been reported to depend on the lipid film’s thickness.5,11 This thickness has been considered to affect tear evaporation and lubrication in blinking.12–14 Thus, the quantification of the lipid layer’s thickness is essential in tear interferometry, for objective assessment of tears. However, the translation of the interference color data into film thickness data has not been successfully applied quantitatively, nor has the quantification of the tear interference images itself been achieved.

During examinations for dry eye, interferometry has been used with a color-comparison table to assume lipid layer thickness15–17 or with the semiquantitative severity grade scoring system for Sjögren’s syndrome18 and dry eye syndrome.19 Thus far, with the color table and the severity grading system, there has been some confusion in interpreting the results of the image. Also, semiquantitative grading was unsuitable for evaluating other than aqueous-deficient dry eye status and might not detect mild cases of dry eye.

Several trials of quantification of interference images have been performed.11,20,21 However, because these trials were not initiated using the interference camera with a normal incident specular angle, the background iris coloration and the image defect on the important central corneal area have always interfered with the quantification itself.

To obtain clear interference images, we used the DR-1 tear interference camera (Kowa Co., Nagoya, Japan), because it is a sophisticated system that clearly presents the image while eliminating background iris color and central image defects, while maintaining a normal incidence specular angle, leading to a clear image.19,22–30 Thus far, the correlation of the image from the DR-1 camera to lipid film thickness has been reported only rarely.29,30 Furthermore, the colorimetric approach has not been applied to lipid layer interference colors.

Considering the physics principle of a white light source (broadband) thin film interference with colorimetry and recent advances in computer technology, it should be possible to achieve the quantification of the tear lipid interference image to obtain exact lipid film thickness information.11,21,31,32 Before the conversion of color information to thickness information, a primary requirement would be to convert thickness information to color information.

In this study, we produced a computer-synthesized color chart of a human tear lipid interference image by using the DR-1 camera and the colorimetric approach for the conversion from tear lipid film thickness into interference color information.

METHODS

Synthesis of the Color Chart

Color consists of three primary hues: red (R), green (G), and blue (B). To synthesize interference color at a certain film thickness (Fig. 1, d, in nanometers), γ-corrected RGB values (R’G’B’, equation 1) of the color—that is, corrected for human retinal response to colors—were required, because the DR-1 interference images were output into...
Air   Lipid   Aqueous
\n(n = 1.0) 1.48 1.33 )

**Figure 1.** Intereference caused by reflection from the two surfaces, lipid and aqueous layers: \( r_1 \) and \( r_2 \), the reflections from the lipid and aqueous layers; \( R \), the amplitude reflectance of interference from these two reflected waves, \( d \), lipid film thickness; \( \phi_1 \), angle of refraction in the layer. In the DR-1 assembly, \( \phi_1 \) approaches 0. \( n \), refractive indices of the air, lipid layer, and aqueous layer. (This is an illustration of a simplified model. The actual tear lipid layer causes interference by multiple reflections.\(^{18,19,33,35}\) Assumptions: DR-1 was originally assembled so that all light from the DR-1 light source through the convex lens would be reflected at the surface and at the back of the tear lipid layer with the normal incidence specular angle within a range of the 8-mm diameter of the cornea.\(^{19}\) Because individual corneal curvatures are variable, it would not always be possible to achieve a normal incidence. However, under the practical usage of the DR-1, we think that these problems would be negligible.

general National Television Standards Committee (NTSC) signals\(^{19,34,35}\)

\[
\begin{align*}
R' &= R'1/2.2 \\
G' &= G'1/2.2 \\
B' &= B'1/2.2
\end{align*}
\]

To obtain RGB values, the following matrix was used to transform \( X, Y, \) and \( Z \) tristimulus values, which are sets of three linear light components that conform to the Commission Internationale de l’Eclairage (CIE) color-matching functions\(^{22,34,36-39}\).

\[
\begin{bmatrix}
R \\
G \\
B \\
\end{bmatrix} = 255 \times 
\begin{bmatrix}
3.5064 & -1.7400 & -0.5441 \\
-1.0690 & 1.9777 & 0.0352 \\
0.0563 & -0.1970 & 1.05711
\end{bmatrix} \begin{bmatrix}
X \\
Y \\
Z \\
\end{bmatrix}
\]

CIE \( X, Y, \) and \( Z \) tristimulus values of the color of the reflection were obtained as follows\(^{32,36}\):

\[
\begin{align*}
X &= K \int_360 S(\lambda)\tilde{x}(\lambda)R(\lambda)d(\lambda) = K \sum_{360} S(\lambda)\tilde{x}(\lambda)R(\lambda) \\
Y &= K \int_360 S(\lambda)\tilde{y}(\lambda)R(\lambda)d(\lambda) = K \sum_{360} S(\lambda)\tilde{y}(\lambda)R(\lambda) \\
Z &= K \int_360 S(\lambda)\tilde{z}(\lambda)R(\lambda)d(\lambda) = K \sum_{360} S(\lambda)\tilde{z}(\lambda)R(\lambda) \\
K &= 100/ \int_360 S(\lambda)\tilde{y}(\lambda)d(\lambda) = 100/ \sum_{360} S(\lambda)\tilde{y}(\lambda)
\end{align*}
\]

where \( S(\lambda) \) (in microwatts/square centimeter) is the spectrum from the DR-1 light source at the wavelength \( \lambda \) (in nanometers). The DR-1 camera was assembled with a halogen bulb of 3000 K of color temperature through a heat-absorbing filter.\(^{17}\) Each \( x, y, \) and \( z \) (no unit) is the color matching function of XYZ standard colorimetric system of CIE 1931.\(^{32,56,37}\) \( R(\lambda) \) (no unit) is the energy light reflectance at a light wavelength \( \lambda.\(^{55,56}\) \) was obtained as follows (Fig. 1).

The amplitude reflectance of interference: \( R \) (no unit), in multiple reflection at a thin film monolayer is expressed as \( \ell \) (an imaginary number)

\[
R = \frac{r_1 + r_2 e^{-2\ell N}}{1 + r_1 r_2 e^{-2\ell N}}
\]

where \( r_1 \) and \( r_2 \) are Fresnel’s indices of reflection at each lipid layer surface and aqueous layer interface (Fig. 1) and are given as follows by Fresnel’s equation, when the angle of specular reflection is normal incidence (DR-1 assembly)\(^{11,19,35,40}\).

\[
r_1 = \frac{n_0 - n_1}{n_0 + n_1} \quad r_2 = \frac{n_2 - n_1}{n_1 + n_2}
\]

Also, \( n_0, n_1, \) and \( n_2 \) are the refractive indices of air, lipid layer, and aqueous layer and are reported as \( n_0 = 1, n_1 = 1.48\(^{11,42}\) \) and \( n_2 = 1.33.\(^{12}\)

Noting that energy is proportional to the square of amplitude, here \( R(\lambda) \) is expressed as\(^{11,35,45}\)

\[
R(\lambda) = R_0 \times R^* = |R|^2
\]

where \( R^* \) is the conjugate complex numbers of \( R \).

Thus, using Euler’s equation,

\[
R(\lambda) = \frac{r_1^2 + r_2^2 + 2r_1 r_2 \cos \phi_1}{1 + r_1 r_2 + 2r_1 r_2 \cos \phi_1} \\
= \frac{8n_0 n_1 n_2}{(n_0^2 + n_1^2)(n_1^2 + n_2^2) + 4n_0 n_1 n_2 (n_0^2 - n_1^2)(n_1^2 - n_2^2) \cos \phi_1}
\]

A phase difference of two waves, \( r_1 \) and \( r_2 \)—that is, \( 2\phi_1 \)—is expressed as:

\[
2\phi_1 = \frac{4\pi}{\lambda} n_1 d \cos \phi_1
\]

where \( \phi_1 \) is the angle of refraction (Fig. 1) and is assumed to be 0 in the DR-1 optical system. Thus, \( \cos \phi_1 = 1.\(^{11,19,35,40}\)

Thus

\[
R(\lambda) = 1 - \frac{8n_0 n_1 n_2}{(n_0^2 + n_1^2)(n_1^2 + n_2^2) + 4n_0 n_1 n_2 (n_0^2 - n_1^2)(n_1^2 - n_2^2) \cos \phi_1}
\]

At this point, \( R(\lambda) \) can be obtained only from the actual numbers, \( n_0, n_1, \) and \( n_2, d, \) and \( \lambda, \) and could be calculated to obtain the \( X, Y, \) and \( Z \) tristimulus values in the broadband light source for RGB and R’G’B’ values, which were transformed into the synthesized interference color chart. Computer programs (Excel X; Microsoft, Redmond, WA, and ImageJ; image processing and analysis software; http://rsb.info.nih.gov/ij; image developed by Wayne Rasband of the National Institutes of Health, Bethesda, MD) were used to obtain RGB profiles of the interference colors and to synthesize the interference color chart.

Actual DR-1 images were also obtained from a healthy control subject with normal tear function and a patient with dry eye who had aqueous tear deficiency and meibomian gland obstruction. Interference image data from random pixel points were then converted to lipid layer thicknesses data using the principles of the new color chart.

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RESULTS

A synthesized interference color chart for the DR-1 camera (Fig. 2C) was obtained along with H9253-corrected RGB (R G B) profiles (Fig. 2A) and X, Y, and Z tristimulus values (Fig. 2B), for the range of tear lipid film thickness from 0 to 1000 nm (0 to approximately the 5th interference order).

For tear films thinner than 92.5 nm (interference order lower than 0.5), there was a gray-brown monochromatic increase in intensity. Then, for the film thickness of approximately 185 nm (interference order 0.5–1), a brown color appeared. After that, a cyclical pattern of blue, green-yellow, and red was observed with the change of color intensity according to increasing lipid layer thickness.

Figure 3 and Table 1 show the result of the conversion of interference colors on the DR-1 image to lipid layer thickness. The central, top, right, bottom, and bottom left points on the cornea were selected, and the corresponding lipid layer thicknesses were displayed as shown in the figure.

DISCUSSION

In this study, the tear lipid interference color chart for the specific interference camera, DR-1, which visualizes clear interference images, was synthesized in a colorimetric approach. The colors in the chart were apparently similar to the reported interference colors using the DR-1. This chart is expected to contribute to the exact quantification of the interference images.

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Figure 3 and Table 1 show the result of the conversion of interference colors on the DR-1 image to lipid layer thickness. The central, top, right, bottom, and bottom left points on the cornea were selected, and the corresponding lipid layer thicknesses were displayed as shown in the figure.
The principles of tear interference colors including the meaning and specifics of each color have not been discussed satisfactorily until now. King-Smith et al.11 first showed the principle of tear interference in detail using a simulated equal-energy spectrum or the spectrum of the Tear Scope (Keeler, Windsor, UK), with fundamental retinal spectral sensitivity. However, usage of the Tear Scope was impractical, because the image always overlapped with the background iris color, producing an unclear image, because it could not achieve the normal incident specular reflection.

Goto and Tseng29,30 reported the trial quantification of the lipid layer thickness with the color comparison method using the DR-1 and the color chart of King-Smith et al.11 based on a simulated equal-energy spectrum, which was not based on the spectrum from the DR-1. However, progress and changes in physical optics and colorimetry stimulated us to develop the current color chart system, which would be more suitable for DR-1 camera optics. The DR-1 camera, having superior specular angle capabilities, was the only camera system appropriate for the quantification of the interference images through its sophisticated optics.19

We believe that our efforts in devising the current chart are the first steps in accurate quantification of the interference images. Our chart differs from that of King-Smith et al.11 in color intensity and interference orders, which makes it more suitable for the DR-1 system. Quantification of RGB color intensities at specific spots on the precorneal tear film may pave the way for the development of the quantification of tear film lipid layer thickness in the near future.

Although tear interferometry has been designed principally for the study of the tear lipid layer, up until now it has been used to investigate the changes in the aqueous layer status, lipid–aqueous layer interaction,19,22 and combined aqueous tear and lipid deficiency in certain patients with dry eye.26 Although real-time topographical interference displays in the DR-1 using our color chart have not been achieved at present, conversion of interference colors to lipid layer thickness data based on our logical color chart system has been realized. It is our belief that conducting further clinical studies to determine repeatability of results obtained with our color chart in patients with dry eye and ocular surface disorders would be highly interesting. Our method can also have application in the evaluation of objective and quantitative parameters with different therapeutic modalities.

In conclusion, we developed a new color chart that we believe will be of benefit in converting tear interference color information to tear lipid layer film thickness data.

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### References

### Table 1. Actual Calibration and Conversion of Color Data from Random Pixel Positions of Figures 3A and 3B to Lipid Layer Thickness

<table>
<thead>
<tr>
<th>Location</th>
<th>Pixel Position</th>
<th>RGB</th>
<th>cXYZ</th>
<th>Lipid Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Fig. 3A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>320, 240</td>
<td>241, 190, 190</td>
<td>0.056, 0.051, 0.024</td>
<td>80</td>
</tr>
<tr>
<td>Upper</td>
<td>320, 124</td>
<td>231, 198, 184</td>
<td>0.055, 0.053, 0.025</td>
<td>70</td>
</tr>
<tr>
<td>Right</td>
<td>220, 240</td>
<td>219, 179, 163</td>
<td>0.048, 0.046, 0.019</td>
<td>60</td>
</tr>
<tr>
<td>Lower</td>
<td>325, 386</td>
<td>192, 158, 138</td>
<td>0.040, 0.039, 0.016</td>
<td>60</td>
</tr>
<tr>
<td>Left lower</td>
<td>466, 351</td>
<td>201, 163, 135</td>
<td>0.042, 0.040, 0.016</td>
<td>60</td>
</tr>
<tr>
<td>Dry eye (Fig. 3B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>320, 240</td>
<td>230, 164, 138</td>
<td>0.047, 0.045, 0.016</td>
<td>130</td>
</tr>
<tr>
<td>Upper</td>
<td>320, 168</td>
<td>159, 152, 109</td>
<td>0.034, 0.034, 0.013</td>
<td>250</td>
</tr>
<tr>
<td>Right</td>
<td>220, 240</td>
<td>190, 144, 105</td>
<td>0.037, 0.035, 0.012</td>
<td>160</td>
</tr>
<tr>
<td>Lower</td>
<td>325, 386</td>
<td>143, 113, 67</td>
<td>0.027, 0.027, 0.0094</td>
<td>220</td>
</tr>
<tr>
<td>Left lower</td>
<td>466, 351</td>
<td>137, 83, 56</td>
<td>0.025, 0.025, 0.0087</td>
<td>180</td>
</tr>
</tbody>
</table>

Pixel position, DR-1 image consists of 640 × 480 pixels. Numbers on the horizontal and vertical axes of Figures 3A and 3B indicate pixel position; RGB, RGB values; cXYZ, calibrated XYZ tristimulus values.